Rootstock and Nutrient Imbalance Leads to “Green Spot” Development in ‘WA 38’ Apples

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Abstract. ‘WA 38’ is a new apple (*Malus domestica* Borkh.) cultivar, released by Washington State University (WSU) in 2017. An unknown disorder, “green spot” (GS), dark green halos in the epidermis, with necrotic, corky, and oxidated cortical tissue underneath the damaged epidermis, leads to unmarketable fruit and has become a threat to the adoption and profitability of ‘WA 38’, with young and mature orchards exhibiting up to 60% incidence in 2020. Given the apparent susceptibility of ‘WA 38’ to GS, this research investigated GS relation with nutrient levels in fruit. Research was carried out in 2018 and 2019 in a ‘WA 38’ apple block planted in 2013, on ‘Geneva 41’ (‘G.41’) and ‘M.9-Nic 29’ (‘M.9’) rootstocks. In both years, fruit number per tree, fruit weight, and fruit diameter were evaluated in 18 trees per treatment, from both rootstocks. From each tree, fruit were classified for presence or absence of GS, and subsequently analyzed for nutrient concentration in the peel and in the flesh, nutrient extraction, and total nutrient content, on an individual apple basis. Apples with GS had higher nitrogen (N) and magnesium (Mg) levels in the peel, regardless of year and rootstock. Apples grown on ‘G.41’ rootstock exhibited higher GS incidence and reduced crop load in both years; reduced size and fruit diameter were exhibited only in 2018. Fruit on ‘G.41’ had higher N, potassium (K), and Mg in the flesh and higher N and Mg in the peel, with lower levels of calcium (Ca) in the flesh and peel; however, only in 2018, with no differences in 2019. GS in ‘WA 38’ apples appears to be another Ca-related disorder in which excessive vigor, rootstock, and N and Mg excess are predisposing factors for its development.

‘WA 38’ (‘Enterprise’ × ‘Honeycrisp’) is a new apple (*Malus domestica* Borkh.) cultivar released by the WSU apple breeding program in 2017 (Evans et al., 2012). The fruit are noted for their excellent eating quality, large size, attractive appearance, and long storability (Evans et al., 2012). By the end of 2020, Proprietary Variety Management reported that

more than 15.5 million ‘WA 38’ trees had been planted in Washington State; an unprecedented rate of planting for an apple cultivar.

After the first commercial harvest in 2019, an unknown disorder was observed in several experimental and commercial orchards of ‘WA 38’. This disorder has been named “green spot” (GS) for the characteristic symptoms observed in the fruit peel. Preliminary evaluations by Sallato and Bishop (2018) described GS as dark green halos in the epidermis, with necrotic, corky, and oxidated cortical tissue underneath the damaged epidermis. In severe cases, GS can further develop into a split through hypodermal layers (Fig. 1). Although GS resembles other apple physiological disorders, such as bitter pit (BP), blotch pit, and other cork-type disorders, GS is distinguished for its green color in early stages and that it develops exclusively preharvest, as opposed to BP, which develops essentially during storage, after 40 to 60 d (Faust and Shear, 1968).

With more ‘WA 38’ plantings starting to bear fruit, the disorder quickly became a great concern to growers in Washington State, and

a high-rated research priority for the Washington tree fruit industry (WTFRC, 2020). There is a wide range of GS incidence among ‘WA 38’ orchards, with severe cases having greater than 60% incidence in the field, in either mature or young orchards, although incidence appears to vary across years (B. Sallato, unpublished data). In a preliminary assessment of culls in the first commercial packing of ‘WA 38’, Torres and Gomez (2020) reported that GS was rated within the top three cullage categories, accounting for $\approx 12.4\% \pm 8\%$ in 4-year-old orchards and $13.9\% \pm 6.9\%$ in 3-year-old orchards.

Many disorders in apple have been associated directly or indirectly with Ca deficiency (De Freitas and Mitcham, 2012; Faust and Shear, 1968; Perring, 1986; Silva and Rodríguez, 1995). BP is one of the most common Ca-related disorders in apples worldwide, and has been extensively investigated (Faust and Shear, 1968; Ferguson et al., 1979; Ferguson and Watkins, 1989; Lang, 1990; Perring, 1986; Shear, 1975). The primary cause(s) of BP remain unknown; however, there is general agreement on the role of Ca deficiency at a cellular level, and the influence of several orchard factors that may predispose fruit to developing BP (Bedford, 2001; De Freitas et al., 2010; De Freitas and Mitcham, 2012; Telias et al., 2006). Ca-related disorders are also strongly associated with the genotype, with varying susceptibilities among cultivars (De Freitas et al., 2015; Lang, 1990; Miqueloto et al., 2014; Volz et al., 2006), with ‘Honeycrisp’ being among the most susceptible cultivars (Telias et al., 2006; Watkins et al., 2004). Thus, given the genetic background of ‘WA 38’, and the close resemblance of GS to BP, we sought to explore the nutritional composition of fruit with and without GS, and its relationship with Ca content and concentration.

Nutrient analyses of whole fruit or its distinct components (e.g., flesh vs. peel) have been used widely in studying Ca-related disorders (Ferguson and Watkins, 1989; Sallato et al., 2016; Telias et al., 2006; Turner et al., 1977). In ‘Honeycrisp’, Baugher et al. (2014) found that peel nutrient content was useful to predict BP incidence. In contrast, Amarante et al. (2013) reported that cultivars are different in which fruit tissue correlates better with BP susceptibility; for example, they found that peel analyses were effective for ‘Fuji’, whereas the flesh nutrient content was better correlated for ‘Catarina’. Further, total fruit Ca does not always relate to Ca-deficiency disorders (Baugher et al., 2014; Chamel and Bossy, 1981; Sallato et al., 2016). For example, Chamel and Bossy (1981) demonstrated that Ca concentration can even be higher in damaged tissue. As a result, different methods and nutrient content calculations have been evaluated to better understand and predict Ca-deficiency disorders. Fruit nutrient ratios N:Ca, K:Ca, and Mg:Ca have also been reported as better predictors of BP in apples than Ca content alone (Amarante et al., 2013; Cheng, 2016; De Freitas et al., 2010; Ferguson and Watkins, 1989), and have been used broadly in diagnosing Ca-related disorders. Volz et al. (2006), by studying 25 seedling

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Fig. 1. Green spot symptoms in 'WA 38' apples (A) in the peel, and (B) in the flesh.

families, indicated that Ca concentration in fruit was a good indicator of BP susceptibility, within, but not across families.

Given the unprecedented scale of 'WA 38' plantings in Washington State, and its apparent susceptibility to GS, this research investigated GS incidence in trees grown on two rootstocks, 'G.41' and 'M.9', and studied the nutritional differences between fruit with and without GS symptoms. We tested the nutrient levels in peel and flesh and calculated nutrient concentration, nutrient content, and nutrient extraction as a first step in identifying the underlying factors leading to GS in 'WA 38' apple fruit.

Materials and Methods

This research was conducted during the 2018 and 2019 growing seasons in a 'WA 38' apple orchard located at the WSU Irrigated Agriculture Research and Extension Center, Roza farm, near Prosser, WA (lat. 46°17'35.2"N, long. 119°43'48.9"W). The orchard is in a semi-arid region with ≈220 mm annual average precipitation. The experimental block was planted in 2013 and trained as a single leader spindle architecture with 1 m between trees and 4 m between rows in an N-S orientation. Trees were grown on two different apple rootstocks, Geneva 41 (G-41) and 'M.9-Nic 29' ('M.9'), randomly distributed in 24 blocks of 11 trees each. Standard orchard management practices were followed across all replicated units, including dormant pruning; pest and disease monitoring; and control, irrigation scheduling, and nutrient management. The block received one application of 100 kg per hectare of monoammonium potash (MAP: 12N-61P-0K) that was broadcasted in the tree row during spring. No other fertilizers were used.

Initial soil conditions were analyzed for chemical parameters by collecting four random samples from across the experimental orchard site that were sent to a commercial laboratory for standard nutrient analyses according to Gavlak et al. (2005). Soil pH and levels of K, Ca, Mg, and Na were adequate for apple orchard requirements according to Sallato et al. (2019) (Table 1). Soil pH at the site is slightly alkaline, and organic matter (OM) is low, although normal for Aridisols, representative of the region.

Soil and fruit nutrient evaluations. Three representative trees from each rootstock, and from six randomly selected blocks were selected for whole tree and fruit nutrient analyses. Fruit number per tree, tree yield, and GS incidence (percentage of fruit with visual

symptoms) were evaluated at commercial harvest, defined as the point at which fruit starch degradation index was between 2.5 and 3.0 (Hanrahan and Galeni, 2019). From each experimental unit and rootstock, six representative fruit free from GS (GS-) and six fruit with clear GS (GS+) symptoms were selected for laboratory analyses (i.e., 36 individual fruit per symptom category and rootstock, 144 fruit total). Each fruit was individually evaluated for fresh weight and diameter. Each fruit was then separated into peel, flesh, core, and seeds. The peel was obtained with a fruit peeler and sharp knife. The complete peeled fruit was cut in half to remove the seeds and the core, and the remaining portion corresponded to the flesh. Each tissue type was subsequently weighed to determine the proportion of each tissue part (fresh %). Subsequently, each tissue was dried at 60°C until there was no further weight loss and weighed again to determine % of dry matter (DM %). Each sample of dried peel and flesh was then ground to dust, homogenized, and sent to a commercial laboratory for total N, phosphorous (P), K, Ca, and Mg analyses following the method recommended for total tissue analyses (Gavlak et al., 2005).

Fruit nutrient calculations. Tissue nutrient concentration is reported as mg per 100 g of fresh tissue (TC) and takes into account the dry matter (or fresh matter) of the whole fruit. This makes data relevant when size and moisture content can affect the value.

$$TC \text{ (mg/100 g of tissue)} =$$

$$(\% \text{ Nutrient} \times DM \% \text{ of the tissue}) \times 1000$$

Nutrient extraction, which considers the weighed contribution of each tissue to the total nutrient content of the fruit, expressed in a 100 g basis. Here it considers the nutrient contribution of the peel and the flesh (E), and was calculated as follows:

$$E \text{ (mg/100 g fruit)} =$$

$$(\text{Peel TC} \times \text{peel F \%}) + (\text{Flesh TC} \times \text{flesh F \%})$$

Total fruit content (FC), refers to the total amount of nutrient of each individual fruit, which will be affected by fruit weight, was

calculated by multiplying E by fruit fresh weight.

$$FC \text{ (mg fruit)} = E \times \text{fruit fresh weight (mg)}$$

Data collected were subjected to analysis of variance and Tukey media comparison when at significant level above 95% ($P < 0.05$), and correlation analysis was determined between growing parameters and GS incidence using XLSTAT software (version 2021.1.1; Addinsoft Inc, Paris, France) for each rootstock and between rootstocks within each experimental year.

Results and Discussion

GS incidence. Incidence of GS varied by rootstock and year, ranging from 1% on 'M.9' in 2018 to 45% on 'G.41' in 2019 (Table 2). A similar range of GS incidence has been observed in commercial orchards, with a range from no GS up to 60% of fruit affected (L. Kalcsits and B. Sallato, unpublished data). GS incidence was consistently higher in 'WA 38' trees on 'G.41' compared with 'M.9'. In 2018, GS incidence on 'G.41' was ≈98% higher than on 'M.9'. In 2019, GS incidence on 'G.41' was 36% higher than on 'M.9'. Differences between years were only significant on 'M.9', where GS levels in 2018 were very low (1%) compared with 29% in 2019 (Table 2). Thus, rootstock and year were important determinants of GS incidence in 'WA 38' apples. The influence of rootstock on the development of physiological disorders in apple have been previously reported (Fazio et al., 2018; Lordan et al., 2018; Valverdi and Kalcsits, 2021). Resent research on 'Honeycrisp' has shown higher levels of BP development on 'G.41' rootstock compared with less vigorous rootstocks such as 'Budagovsky 9' ('Bud 9') or 'M.9-337' (Valverdi and Kalcsits, 2021). However, BP development in apples is also variable among years, and across scion and rootstock genotypes (Amarante et al., 2020; Volz et al., 2006), suggesting the influence of several environmental and genetic factors in development of these disorders (De Freitas and Mitcham, 2012; Ferguson and Watkins, 1989).

Fruit load (fruit/tree) ranged between 51 and 67 across years and rootstocks. In 2018, fruit load was 27% higher on 'M.9' com-

Table 1. Initial soil physico-chemical analyses for the 'WA 38' experimental block.

Soil test	Soil test	Unit	2018	2019
pH	pH	—	7.5	7.1
Organic matter	OM	%	1.6	1.4
Exchangeable	Potassium (K)	meq/100 g	0.40	0.47
	Calcium (Ca)	meq/100 g	4.85	5.45
	Magnesium (Mg)	meq/100 g	2.07	2.05
	Sodium (Na)	meq/100 g	0.14	0.11
	CEC	meq/100 g	7.30	7.80
Cation exchange capacity	Potassium (K)	%:TB	5	6
	Calcium (Ca)	%:TB	67	70
	Magnesium (Mg)	%:TB	28	26
	Sodium (Na)	%:TB	2	1
	USDA		Silt loam	Silt loam

TB = total bases.

Table 2. Fruit per tree, fruit weight, fruit diameter, and green spot incidence on 'G.41' and 'M.9' rootstocks. Symbols denote statistical significance between rootstocks, within year (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Rootstock	Fruit load (n/tree)		Fruit wt (g)		Fruit diam (mm)		Green spot incidence (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
M.9	65	67	302	289	88 a	84 b	1 a	29 b
G.41	51	53	262 a ^z	299 b	85	86	40	45
P value	***	***	***	0.417	**	0.072	***	***

^zAny two means within a row not followed by the same letter are significantly different at $P \leq 0.05$ by Tukey's multiple test.

*, **, ***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

pared with 'G.41'. Similarly, in 2019, fruit load was 26% higher on 'M.9' compared with 'G.41' (Table 2). Fruit diameter varied between 84 and 88 mm. In 2018, fruit diameter was 4% larger on 'M.9' compared with 'G.41', with no significant differences observed in 2019. Likewise, fruit weight was 15% heavier on 'M.9' compared with 'G.41' in 2018, with no differences in 2019. Only on 'G.41', fruit weight was different between years, being 14% higher in 2019 (opposite to what we reported for 'M.9'). Fruit weight ranged between 262 and 302 g, and according to Washington Apple Commission standards, these fruits correspond to a box size between 72 and 64 (number of apples in a 40-lb carton box), considered a large-size apple. Fruit count, diameter, and weight are equivalent to those previously reported by Brendon et al. (2019) and Gomez and Kalcsits (2020) for 'WA 38' apples. Gomez and Kalcsits (2020) reported an average fruit weight of 310 ± 11 g and fruit diameter of 87.7 ± 1.28 mm on a fourth leaf 'WA 38' grown on 'M.9-337', and fruit count of 61 fruit per tree. Based on fruit weight, diameter, and fruit number per tree, the estimated yield per hectare in the experimental block was ≈ 59 and 58 tons on 'M.9', and 40 and 48 tons on 'G.41' in 2018 and 2019, respectively, considering a tree density of 3025 trees per hectare. These results are within the same range reported by Brendon et al. (2019) of 28 to 88 tons per hectare for equivalent crop load [2.1 fruit per cm^2 trunk cross-sectional area (TCSA)].

The influence of rootstock on crop load (fruits/ cm^2 TCSA), fruit to leaf ratio, and fruit size have been previously reported in apples (Fallahi, 2012; Fazio et al., 2018; Lordan et al., 2018; Silveira et al., 2012). Fallahi (2012) found higher yield and crop load in 'Gala' apple grown on 'M.9-Nic29' rootstocks when compared with 'Bud 9' and 'Geneva 30'. Russo et al. (2007) found no difference between rootstocks in yield (kg per tree) or fruit weight in 'Gala' grown on 'G.41' and 'M.9-Nic29'; however, differences were significant for 'Honeycrisp' with larger fruit size on 'G.41' compared with 'M.9-Nic29'. These differences suggest that rootstock effect on productive parameters such as yield and fruit size, can also vary depending on cultivar and years (Russo et al., 2007).

In the current study, there was no relationship between GS incidence and fruit weight nor fruit diameter (data not shown). The relation between fruit size and Ca-related physiological disorders is debatable, whereas many

studies have shown a positive relation between BP and larger fruit size (Fallahi et al., 1985; Perring and Jackson, 1975; Reid and Kalcsits, 2020; Valverdi and Kalcsits, 2021), several others have not found a direct correlation (Amarante et al., 2020; Donahue et al., 2021; Ferguson and Watkins, 1992). Recently, Donahue et al. (2021) suggested that the relation with fruit size and BP occurs only with exceptionally large fruit, in 'Honeycrisp' with 48 to 56 size box (equivalent to >89 mm fruit and 340 g fruit), below which threshold there is no correlation. Fruit load, in the current trial, had a strong negative correlation with GS incidence ($y = -0.0078x + 0.88$, $R^2 = 0.662$, $R = -0.814$, $P < 0.0001$) (Fig. 2). Similarly, several authors have reported a strong negative correlation between crop load and the development of Ca-related disorders in apples (Emami et al., 2008; Ferguson and Watkins, 1992; Volz et al., 1993). Valverdi and Kalcsits (2021) reported higher BP incidence on 'Honeycrisp' on 'G.41' compared with 'M.9', and although they did not report on the correlation, they reported higher fruit load on 'M.9'.

Fruit nutrient analysis in fruit with and without GS. Nutrient concentration in the flesh ranged between 23 and 45 mg/100 g of fresh weight for N, 108 and 176 mg/100 g of fresh weight for K, 6.9 and 9.1

mg/100 g of fresh weight for Ca, and 6.2 to 9.0 mg/100 g of fresh weight for Mg (Table 3). In the peel, nutrient concentration ranged between 68 and 98 mg/100 g of fresh weight for N, 119 and 251 mg/100 g of fresh weight for K, 19 and 37 mg/100 g of fresh weight for Ca, and 16 and 25 mg/100 g of fresh weight for Mg. A previous study on nutrient analysis on 'WA 38' fruit flesh by Serra et al. (2016) reported similar N and K concentration (estimated 33 and 142 mg/100 g of fresh weight) but lower levels of Ca and Mg (41% and 11% lower). Peel and flesh levels of Ca and Mg concentration in the current study were also equivalent to those reported by Cheng and Miranda (2018) on 'Honeycrisp' apples (60 and 250 $\mu\text{g/g}$ dry weight in the flesh and in the peel, respectively, equivalent to 6 and 25 mg/100 g fresh weight).

In 2019, overall fruit nutrient concentration in the peel and in the flesh were lower compared with 2018, regardless of their GS incidence or rootstock. In 2019, N, K, Ca, and Mg concentration were 48%, 38%, 20%, and 26% lower in the flesh, respectively. In the peel, K and Ca concentrations were 62% and 114% lower when compared with 2018. Likewise, Gomez and Kalcsits (2020) and Valverdi and Kalcsits (2021) also reported

Fig. 2. Correlation between green spot incidence and fruit load (number of fruits per tree) in 'WA 38' apples grown on 'Geneva 41' and 'M.9-Nic 29' rootstock.

Table 3. Nutrient concentration on the peel and flesh, in 'WA38' apples on 'G.41' and 'M.9' rootstock, with (GS+) and without (GS-) green spot. Symbols denote significance difference between condition within rootstock and year (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Nutrients	Rootstock – yr	Tissue concn (mg/100 g)					
		Flesh			Peel		
		GS–	GS+	<i>P</i> value	GS–	GS+	<i>P</i> value
N	G.41 – 2018	45	44	0.518	78	98	**
	G.41 – 2019	23	31	**	68	80	**
	M.9 – 2019	31	30	0.850	72	84	0.114
K	G.41 – 2018	176	151	**	180	251	***
	G.41 – 2019	115	116	0.864	121	127	0.365
	M.9 – 2019	108	119	0.240	119	136	0.083
Ca	G.41 – 2018	8.1	9.1	*	37	34	0.231
	G.41 – 2019	7.6	7.1	0.493	20	18	0.340
	M.9 – 2019	6.9	7.6	0.209	18	19	0.487
Mg	G.41 – 2018	8.1	9.0	*	16	25	***
	G.41 – 2019	6.2	6.6	0.248	23	25	**
	M.9 – 2019	6.4	6.8	0.234	22	25	**

lower levels of Ca, K, and Mg in the 2019 season compared with 2018, in 'WA 38' and 'Honeycrisp' apple, respectively, grown in WA. Many authors have shown high variation in nutrient concentration between harvest seasons (Amarante et al., 2013; Sachini et al., 2020; Schweitzer and Petri, 2019; Volz et al., 2006), which can be attributed to several agronomic and environmental factors, such as crop load, vigor, fertilization management, and water management, among others (Ernani et al., 2008). In our study, lower levels of nutrient concentration in 2019 coincided with higher incidence of GS (Table 2). In agreement, Amarante et al. (2021) also related the seasonal variability of fruit nutrient concentration with the variation of Ca-related disorders in apples.

In 2018, GS incidence on 'M.9' was less than 1% and, therefore, comparison between fruit condition (with and without GS) was not included for 'M.9' in 2018. Fruit nutrient levels were significantly different in fruit with and without GS regardless of the year and rootstock. Fruit with GS+ exhibited 7% higher Mg in the flesh and 20%, 14%, and 30% higher N, K, and Mg, respectively, in the peel, whereas Ca concentration in the peel was 31% lower (data not shown). In 2018, apples on 'G.41' with GS+ had 14% less K, 13% more Ca, and 12% more Mg in the flesh, whereas in the peel, GS+ fruit had 26%, 39%, and 53% higher N, K, and Mg concentration, respectively, when compared with healthy fruit. In 2019, apples on 'G.41' with GS+ exhibited 36% more N in the flesh, and in the peel, N and Mg were 18% and 7% higher when compared with healthy fruit (Table 2). On 'M.9' rootstock (only evaluated in 2019 for condition differences), there were no differences in flesh nutrient concentration between GS+ and GS– (Table 2), whereas in the peel, only Mg levels were significantly higher (14%) in GS+ fruit compared with healthy fruit. While flesh nutrient levels were highly variable between years and rootstock, and inconsistent between conditions, peel nutrient levels were more consistent and better correlated with GS incidence, with higher N and Mg concentrations in fruit that had

GS. In agreement, Amarante et al. (2013) indicated that peel nutrient levels are better indicators of Ca-related disorders in apples, when compared with flesh nutrient levels. In their experiment, however, reduced Ca concentration in the peel was the most consistent indicator for BP fruit. In agreement with the current results, Faust and Shear (1968) reported higher Mg/Ca levels in BP apples, and later, Shear (1975) also reported higher N/Ca levels in apples with BP and cork spot.

Nutrient extraction (E) incorporates the relative nutrient contribution of the peel and the flesh, in a fresh weight basis, and nutrient FC is the extraction based on the actual fruit weight, incorporating variables associated with tissue proportion (flesh to peel ratio) and fruit size. Nutrient E and FC were positively correlated, and the response to GS was equivalent (data not shown). In 2019, apples with GS+ had 28% and 26% more N extraction and FC compared with healthy fruit, whereas in 2018, only Mg extraction and FC were different, being 23% and 22% higher in GS+ apples on 'G.41'. Similarly, on 'M.9', the GS+ fruit in 2019 exhibited 9% and 8% higher Mg extraction and FC compared with GS– fruit (Table 4). Nutrient contents reported here were similar to those obtained by Palmer and Dryden (2006) for whole fruit N, K, Ca, and Mg on different cultivars

grown in New Zealand. The greatest similarities were with 'Braeburn' and 'Fuji' for N and K, whereas Ca and Mg levels of 'WA 38' were closer to those reported for 'Cox Orange pippin'. Tissue nutrient extraction and content were inconsistent indicators of GS incidence between years and rootstocks.

Ratios between key nutrients have been used to assess and predict Ca-related disorders. In our study, N:Ca ratios were 39% and 29% higher in GS+ fruit flesh and peel, respectively, compared with GS– fruit. The N:Ca ratio of FC and extraction were the same, and 35% higher in GS+. However, these differences were observed only in 2019 and on 'G.41', with no statistical differences in 2018 (data not shown). As reported by Telias et al. (2006), the inverse relation between macronutrients and Ca is frequently found, when Ca is low, which was not the case in our study. In addition, the ratios between macronutrients and Ca were not consistent between years or rootstocks, suggesting it should not be used as an absolute indicator of fruit condition. For example, in 2018, the N:Ca ratio in the peel was 2.9 in GS+, whereas in 2019, the N:Ca ratio was 4.5 in GS+ and 3.5 in GS– fruit.

Nutrient concentration, extraction, and FC differences between GS+ and GS– vary year to year and between rootstocks. The most consistent indicators of GS+ were higher N and Mg concentration in the peel. For all years and rootstocks, GS+ exhibited N concentrations above 80 mg/100 g (fresh weight) and Mg concentration above 25 mg/100 g (fresh weight) in the peel. Nutrient concentration in the peel should still be used with caution, especially when comparing between growing environmental conditions (different orchards) (Amarante et al., 2013; Telias et al., 2006; Volz et al., 2006). The relation between high N levels in the tree, leading to Ca-related disorders, has been reported extensively. Excessive vigor (normally associated with high levels of N) leads to rapid shoot growth, and the associated increased demand for water and transpiration flow, which comes at the detriment of Ca flow into the fruit (Ho and White, 2005). High levels of N have also been associated

Table 4. Nutrient extraction and content in 'WA 38' apples on 'G.41' and 'M.9' rootstock, with (GS+) and without (GS–) green spot. Symbols denote significance between condition (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Nutrients	Rootstock – yr	Extraction (mg/100 g fruit)			Fruit content (mg/fruit)		
		GS–	GS+	<i>P</i> value	GS–	GS+	<i>P</i> value
N	G.41 – 2018	38.3	39.7	0.385	99	101	0.562
	G.41 – 2019	51.5	65.9	*	77	97	*
	M.9 – 2019	63.8	67.0	0.705	93	97	0.761
K	G.41 – 2018	135	127	0.164	348	323	0.119
	G.41 – 2019	94	95	0.762	139	139	0.987
	M.9 – 2019	92	102	0.227	134	147	0.209
Ca	G.41 – 2018	9.5	9.8	0.425	25	25	0.614
	G.41 – 2019	8.1	7.4	0.369	12	11	0.314
	M.9 – 2019	7.6	8.2	0.205	11	12	0.288
Mg	G.41 – 2018	7.1	8.8	***	18	22	***
	G.41 – 2019	7.7	8.1	0.154	11	12	0.340
	M.9 – 2019	7.9	8.7	*	12	13	*

with greater cell expansion, which can lead to Ca dilution in the fruit and increased development of Ca-related disorders (Saure, 2005). Thus, the absence of a clear relation between Ca levels and GS incidence does not, however, indicate that GS is not another Ca-related disorder. As reported by Shear (1975), Ca levels alone are not a good predictor of BP in apples, although it is common knowledge that BP is a Ca-related disorder (Baughner et al., 2017; Chamel and Bossy, 1981; Sallato et al., 2016). For example, Chamel and Bossy (1981) demonstrated that Ca concentration can even be higher in BP-damaged tissue. Absolute values are often difficult to compare given the diversity of methodologies used for nutrient levels and expressions.

One hypothesis for the lack of correlation between total Ca levels and Ca-related disorders relates to the many forms and functions of Ca in plants (Marschner, 2002; Volz et al., 2006; White and Broadley, 2003). It has been reported that ~40% of the Ca in the fruit tissue is located in the vacuole, as an inactive form of Ca, with most of the remaining 60% located in the cell wall (Bangerth, 1979; White and Broadley, 2003). When we evaluate total Ca in the tissue, we are not segregating between active forms of Ca that might be more closely related to the Ca-related disorders. In addition, Ca sprays used in the orchards to either augment Ca content in fruit or used to prevent sunburn damage can alter Ca readings, especially in the peel.

Nutrient levels between rootstocks. GS levels were 97% and 35% higher on 'G.41' rootstocks compared with 'M.9' in 2018 and 2019, respectively, with higher GS incidence exhibited in 2019 (Table 2). Nutrient levels in fruit with GS+ were not significantly different, regardless of the rootstock. Thus, comparison between rootstock nutrient levels was done only in healthy fruit (Table 5). In 2018, healthy fruit on 'G.41' had higher N, K, and Mg levels, and lower Ca levels compared with 'M.9'. N concentration in the peel and flesh were 19% and 6% higher on 'G.41' compared with 'M.9'. Total N extraction varied between 34 and 64 mg of N per 100 g of fruit (0.34 and 0.64 kg of N per ton of fruit), 13% higher on 'G.41' compared with 'M.9', with no differences in 2019. Concentration of K in the flesh was 26% higher on 'G.41' and K extraction averaged 135 mg per 100 g of fruit, 18% higher on 'G.41' compared with 'M.9'. Flesh and peel Mg concentration were

7% and 6% higher on 'G.41' compared with 'M.9', and Mg extraction varied between 6.9 and 7.9 mg per 100 g of fruit, with no significant differences between rootstocks or years. In contrast, Ca in the flesh, peel, extraction, and content were consistently lower on 'G.41' compared with 'M.9' (~12%, 35%, 26%, and 45% lower, respectively). Ca extraction varied between 7.6 and 12.1 mg per 100 g of fruit, with the highest extraction on 'M.9' in 2018 (Table 5). This extraction is equivalent to 0.076 and 0.12 kg per ton of fruit, thus with a production of 80 tons per hectare, Ca extraction will range between 6 and 9.6 kg per hectare on healthy 'WA 38' apples. Rootstock influence in nutrient uptake and fruit disorders has been previously reported in sweet cherry (Jimenez et al., 2004; Milošević et al., 2014) and apples (Fallahi, 2012; Valverdi and Kalcsits, 2021). The mechanism of these relations can be associated with several canopy factors: crop load (number of fruits per trunk diameter), fruit to leaf ratio, fruit size, nutrient demand, water demand, and plant growth regulator balance, all factors being previously differentiated between rootstock (Ernani et al., 2008; Fazio et al., 2018; Lordan et al., 2018; Saure, 2005; Silveira et al., 2012). The higher amount of N, K, and Mg levels on 'G.41' may be associated with its higher vigor and root growth differences, observed in a parallel study between 'G.41' and 'M.9' root growth in terms of volume of roots and growth rates (B. Sallato, unpublished data). These differences were major in the 2018 growing season, when 'M.9' stopped growing 40 d after full bloom and 'G.41' continued growing throughout the season, for more than 90 d. Likewise, nutrient differences between rootstocks were significant only in the 2018 season, when also GS incidence on 'G.41' was much greater (40-fold), compared with 'M.9'. As reported by Ferguson and Watkins (1989), although Ca uptake occurs early in the season, K, N, and Mg continue to increase until the fruit has reached full maturity. Thus, in roots that can continue to grow throughout the growing season, the uptake of N, K, or Mg can become excessive, leading to Ca imbalance, and consequently, Ca-related disorders. The relation between Ca with K and Mg at a cellular level is still unclear; however, De Freitas et al. (2010) and Miqueloto et al. (2011) proposed that K

and Mg compete with Ca for binding sites in the plasma membrane surface, resulting in the breakdown of the plasma membrane, leading to BP development.

Other factors associated with rootstocks have also been related directly or indirectly with the presence of Ca-related disorders. Several authors have reported a positive relationship between fruit size and Ca-related disorders (Bangerth, 1979; Bedford, 2001; Ferguson and Watkins, 1992). In this study, in 2018, fruit size was 15% higher on 'M.9', which actually had reduced GS levels compared with 'G.41'. However, the role of fruit size has been debated as a factor associated with BP and other Ca-related disorders (Donahue et al., 2021; Ferguson and Watkins, 1992). Crop load, on the other hand, has more widely been suggested as an important factor associated with Ca-related disorders and nutrient balance in apples (Ernani et al., 2008; Ferguson and Watkins, 1992; Robinson and Watkins, 2003; Serra et al., 2016; Volz et al., 1993). In our study, crop load (as number of fruit per tree) were 27% and 26% higher on 'M.9' in 2018 and 2019, respectively. When calculating crop load as number of fruit per TCSA, in 2018, 'M.9' averaged a crop load of 1.8 fruit/cm², whereas 'G.41' averaged 1.3 fruit/cm² of TCSA. In 2019, 'M.9' averaged 1.9 fruit/cm², whereas 'G.41' was 1.4 fruit/cm², 40% and 39% higher compared with 'G.41' in 2018 and 2019, respectively. According to Serra et al. (2016), crop load levels on 'Honeycrisp' apple influenced fruit quality, nutrient uptake, and BP levels, with higher levels of BP with crop loads of fewer than 2.0 fruit/cm² TCSA.

Conclusion

GS is a disorder that can lead to considerable losses for growers of 'WA 38' apples; we report here nearly half of the fruit being afflicted with GS in 'G.41'. GS in 'WA 38' apple was influenced by the rootstock and the year, with higher levels of GS incidence observed in 2019. The greatest difference between rootstock was observed in 2018, when 'G.41' had 40 times higher GS incidence. The 40-fold difference in GS levels between rootstocks in 2018 might be explained by the significant differences observed between nutrient levels between rootstocks. In 2018, N, K, and Mg levels in the peel were higher in fruit with GS, whereas in 2019 only N and Mg

Table 5. Nutrient concentration, extraction, and content of healthy 'WA38' apples on 'G.41' and 'M.9-Nic29'. Symbols denote significance between condition (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

Nutrient	Yr	Tissue concn (mg/100 g)						Extraction (mg/100 g fruit)			Fruit content (mg/fruit)		
		Flesh			Peel								
		G.41	M.9	<i>P</i> value	G.41	M.9	<i>P</i> value	G.41	M.9	<i>P</i> value	G.41	M.9	<i>P</i> value
N	2018	45	38	***	78	73	*	38	34	***	99	100	0.770
	2019	23	31	0.085	68	72	0.543	51	64	0.079	77	93	0.112
K	2018	176	139	***	180	179	0.796	135	114	***	348	337	0.571
	2019	115	108	0.386	121	119	0.755	94	92	0.830	139	134	0.562
Ca	2018	8.1	9.0	**	36.9	50	***	9.5	12.1	***	25	36	***
	2019	7.6	7.1	0.306	20.0	18.1	0.465	8.1	7.6	0.509	12	11	0.396
Mg	2018	8.1	7.5	*	16.4	15	*	7.1	6.9	0.294	18	20	0.083
	2019	6.2	6.4	0.638	22.9	22.2	0.465	7.7	7.9	0.440	11	12	0.734

levels were significantly higher in GS fruit, regardless of the rootstock. Nutrient extraction and content varied between years and rootstocks, and, therefore, are not good indicators of GS incidence. The N:Ca ratio was only significant for 'G.41' in 2019, being above 4.3 and 4.5 in flesh and peel, respectively, on GS apples. However, these differences were inconsistent between years and rootstocks, and thus not recommended as a GS indicator. Although all these growing factors are interconnected and difficult to isolate, it appears that the influence of rootstock on GS development is highly related to vigor and fruit to shoot relationships, similar to other Ca-related disorders (Ferguson and Watkins, 1992). De Freitas et al. (2015), in their review on Ca-related disorders in apples, described in detail the many factors that can lead to Ca-related disorders, many of those have also proven to be related to GS development in 'WA 38' in this study. The use of nutrient levels can be used as indicators within year and rootstock, and might also differ between growing locations and management practices, similar to nutrient indicators for BP development, which have proven to be variable between cultivars, years, location, and rootstocks. GS incidence, and its relationship with nutrient balance and rootstocks, should be further investigated under different growing conditions, especially those conducive to excessive vigor.

Literature Cited

- Amarante, C.V.T., J.P.G. Silveira, S.T. de Freitas, C.A. Steffens, and E.J. Mitcham. 2021. Fruit quality of 'Braeburn' apple trees sprayed at post-bloom and preharvest with prohexadione-calcium and GA (4 + 7). *Rev. Bras. Frutic.* 43(1): <https://doi.org/10.1590/0100-29452021653>.
- Amarante, C.V.T., C.A. Steffens, S.T. de Freitas, J.P.G. Silveira, V. Denardi, and J.M. Katsurayama. 2020. Post bloom spraying apple trees with prohexadione-calcium and gibberellic acid affects vegetative growth, fruit mineral content and bitter pit incidence. *Acta Hort.* 1275:193–200, <https://doi.org/10.17660/ActaHortic.2020.1275.27>.
- Amarante, C.V.T., J.P.G. Silveira, C.A. Steffens, and F.N. Paes. 2013. Tissue sampling method and mineral attributes to predict bitter pit occurrence in apple fruit: A multivariate approach. *Acta Hort.* 1012:1133–1139.
- Bangerth, F. 1979. Calcium-related physiological disorders of plants. *Annu. Rev. Phytopathol.* 17:97–122, <https://doi.org/10.1146/annurev.py.17.090179.000525>.
- Baughner, T., J. Schupp, C. Lara, and C. Watkins. 2014. Crop load and fruit nutrient studies in commercial Honeycrisp orchards to determine best practices for minimizing bitter pit. *PA Fruit News* 94(2):37–40.
- Baughner, T.A., R. Marini, J.R. Schupp, and C.B. Watkins. 2017. Prediction of bitter pit in 'Honeycrisp' apples and best management implications. *HortScience* 52(10):1368–1374, <https://doi.org/10.21273/HORTSCI12266-17>.
- Bedford, D. 2001. Honeycrisp. *Compact Fruit Tree* 34:98–99.
- Brendon, A., S. Serra, and S. Musacchi. 2019. Optimizing crop load for new apple cultivar: "WA 38". *Agronomy (Basel)* 9:107, <https://doi.org/10.3390/agronomy9020107>.
- Chamel, A.R. and J.P. Bossy. 1981. Electron-microprobe analysis of apple fruit tissues affected with bitter pit. *Scientia Hort.* 15:155–163.
- Cheng, L. 2016. Challenges and opportunities for Honeycrisp nutrient management. *Proc. Empire State Expo.* 15 June 2017. <<http://www.hort.cornell.edu/expo/proceedings/2016/TreeFruit.%20Challenged%20and%20opportunities%20to%20optimize%20mineral%20nutrition%20of%20Honeycrisp.Cheng.pdf>>.
- Cheng, L. and M. Miranda. 2018. Why is 'Honeycrisp' so susceptible to bitter pit? *NY Fruit* 26(1):19–23.
- De Freitas, S.T., C.V.T. do Amarante, J.M. Labavitch, and E.J. Mitcham. 2010. Cellular approach to understand bitter pit development in apple fruit. *Postharvest Biol. Technol.* 57:6–13, <https://doi.org/10.1016/j.postharvbio.2010.02.006>.
- De Freitas, S.T. and E.J. Mitcham. 2012. Factors involved in fruit calcium deficiency disorders. *Hort. Rev.* 40:107–146.
- De Freitas, S.T., C.V.T. do Amarante, and E.J. Mitcham. 2015. Mechanisms regulating apple cultivar susceptibility to bitter pit. *Scientia Hort.* 186:54–60, <https://doi.org/10.1016/j.scienta.2015.01.039>.
- Donahue, D.J., G.R. Córdoba, S.E. Elone, A.E. Wallis, and M.R. Basedow. 2021. 'Honeycrisp' bitter pit response to rootstock and region under eastern New York climatic conditions. *Plants* 10(5):983, <https://doi.org/10.3390/plants10050983>.
- Emani, P.R., J. Dias, C.V.T. Amarante, D.C. Ribeiro, and D.A. Rogeri. 2008. Preharvest calcium sprays were not always needed to improve quality of 'Gala' apples in Brazil. *Rev. Bras. Frutic.* 30(4):892–896, <https://doi.org/10.1590/S0100-29452008000400009>.
- Evans, K.M., B.H. Barritt, B.S. Konishi, L.J. Brucher, and C.F. Ross. 2012. 'WA 38' apple. *HortScience* 47:1177–1179, <https://doi.org/10.21273/HORTSCI.47.8.1177>.
- Fallahi, E., T.L. Righetti, and D.G. Richardson. 1985. Predictions of quality by preharvest fruit and leaf mineral analyses in 'starkspur Golden Delicious' apple. *J. Amer. Soc. Hort. Sci.* 110:524–527.
- Fallahi, E. 2012. Influence of rootstock and irrigation methods on water use, mineral nutrition, growth, fruit yield, and quality in 'Gala' apple. *HortTechnology* 22(6):731–737, <https://doi.org/10.21273/HORTTECH.22.6.731>.
- Faust, M. and C.B. Shear. 1968. Corking disorders of apples: A physiological and biochemical review. *Bot. Rev.* 34:441–469, <https://doi.org/10.1007/BF02859134>.
- Fazio, G., J. Lordan, P. Francescato, L. Cheng, A. Wallis, M.A. Grusak, and T.L. Robinson. 2018. 'Honeycrisp' apple fruit nutrient concentration affected by apple rootstock. *Acta Hort.* 1228:223–228, <https://doi.org/10.17660/ActaHortic.2018.1228.33>.
- Ferguson, I.B., M.S. Reid, M. Prasad, and P.B. Levin. 1979. Calcium analysis and the prediction of bitter pit in apple fruit. *N. Z. J. Agr. Res.* 22:485–490, <https://doi.org/10.1080/00288233.1979.10430778>.
- Ferguson, I.B. and C.B. Watkins. 1992. Crop load affects mineral concentrations and incidence of bitter pit in 'Cox's Orange Pippin' apple fruit. *J. Amer. Soc. Hort. Sci.* 117:373–376, <https://doi.org/10.1080/00221589.1993.11516336>.
- Ferguson, I.B. and C.B. Watkins. 1989. Bitter pit in apple fruit. *Hort. Rev.* 11:289–355, <https://doi.org/10.1002/9781118060841.ch8>.
- Gavilak, R.G., D.A. Horneck, and R.O. Miller. 2005. Soil, Plant and Water Reference Methods for the Western Region, 3rd ed. Western Coordinating Committee on Nutrient Management.
- Gomez, R. and L. Kalcits. 2020. Physiological factors affecting nutrient uptake and distribution and fruit quality in 'Honeycrisp' and 'WA 38' apple (*Malus x domestica* Borkh.). *HortScience* 55(8):1327–1336, <https://doi.org/10.21273/HORTSCIENCE15064-20>.
- Hanrahan, I. and M. Galeni. 2019. Developing a cv. 'WA 38' starch scale for the Washington State apple industry. May 2019. <<http://treefruit.wsu.edu/article/developing-a-cv-wa-38-starch-scale-for-the-washington-state-apple-industry/>>.
- Ho, L.C. and P.J. White. 2005. A cellular hypothesis for the induction of blossom-end rot in tomato fruit. *Ann. Bot.* 95:571–581, <https://doi.org/10.1093/aob/mci065>.
- Jimenez, S., A. Garón, Y. Gogorcena, J.A. Betran, and M.A. Moreno. 2004. Flower and foliar analysis for prognosis of sweet cherry nutrition: Influence of different rootstocks. *J. Plant Nutr.* 27(4):701–712, <https://doi.org/10.1081/PLN-120030376>.
- Lang, A. 1990. Xylem, phloem and transpiration flows in developing apple fruits. *J. Expt. Bot.* 41:645–651, <https://doi.org/10.1093/jxb/41.6.645>.
- Lordan, J., G. Fazio, P. Francescato, T.L. Robinson. 2018. Effects of apple (*Malus x domestica*) rootstocks on vigor and yield response on 'Honeycrisp'. *Acta Hort.* 1228: <https://doi.org/10.17660/ActaHortic.2018.1228.22>.
- Marschner, H. 2002. Mineral nutrition of higher plants. 3rd ed. Academic Press, London, UK.
- Milošević, T., N. Milošević, J. Milivojević, I. Glišić, and R. Nikolić. 2014. Experiences with Mazzard and Colt sweet cherry rootstocks in Serbia which are used for high density planting system under heavy and acidic soil conditions. *Scientia Hort.* 176:261–272, <https://doi.org/10.1016/j.scienta.2014.07.020>.
- Miqueloto, A., C.V.T. Amarante, C.A. Steffens, A. Dos Santos, and E. Mitcham. 2014. Relationship between xylem functionality, calcium content and the incidence of bitter pit in apple fruit. *Scientia Hort.* 165:319–323, <https://doi.org/10.1016/j.scienta.2013.11.029>.
- Miqueloto, A., C.V.T. Amarante, C.A. Steffens, A. Dos Santos, T. Miqueloto, and J.P.G. Silveira. 2011. Atributos fisiológicos, físico-químicos e minerais associados à ocorrência de bitter pit em maçãs. *Pesqui. Agropecu. Bras.* 46:689–696.
- Palmer, J. and G. Dryden. 2006. Fruit mineral removal rates from New Zealand apple (*Malus domestica*) orchards in the Nelson region. *N. Z. J. Crop Hort. Sci.* 34:27–32, <https://doi.org/10.1080/01140671.2006.9514384>.
- Perring, M.A. 1986. Incidence of bitter pit in relation to the calcium content of apples: Problems and paradoxes, a review. *J. Sci. Food Agr.* 37:591–606, <https://doi.org/10.1002/jsfa.2740370702>.
- Perring, M.A. and C.H. Jackson. 1975. The mineral composition of apples. Calcium concentration and bitter pit in relation to mean mass per apple. *J. Sci. Food Agr.* 26:1493–1502, <https://doi.org/10.1002/jsfa.2740261008>.
- Robinson, T.L. and C.B. Watkins. 2003. Crop load of Honeycrisp affects not only fruit size but also many quality attributes. *NY Fruit Q.* 11(3):7–10.
- Reid, M. and L. Kalcits. 2020. Water deficit timing affects physiological drought responses, fruit size, and bitter pit development for 'Honeycrisp' apple. *Plants (Basel)* 9(7):874, <https://doi.org/10.3390/plants9070874>.
- Russo, N.L., T. Robinson, G. Fazio, and H.S. Aldwinckle. 2007. Field evaluation of 64 apple rootstocks for orchard performance and fire blight resistance. *HortScience* 42(7):1517–1525, <https://doi.org/10.21273/HORTSCI.42.7.1517>.

- Sachini, R., C.A. Steffens, M. Schlichting, B. Schweitzer, C.L. Fenili, and J.L. Petri. 2020. Mineral content in the skin and flesh of fruits of apple cultivars. *Rev. Bras. Frutic.* 42(2):1–8, <https://doi.org/10.1590/0100-29452020572>.
- Sallato B., T. DuPont, D. Granatstein. 2019. Tree fruit soil fertility and plant nutrition in cropping orchards in Central Washington. Washington State University Extension. EM119E.
- Sallato, B. and G. Bishop. 2018. Preliminary observations on “green spot” symptoms in ‘WA 38’. Jan. 2020. <<http://treefruit.wsu.edu/article/preliminary-observations-on-green-spot-symptoms-in-wa-38/>>.
- Sallato, B., C. Bonomelli, and J. Martiz. 2016. Differences in quality parameters and nutrient composition in Fukumoto oranges with and without creasing symptoms. *J. Plant Nutr.* 40(7):954–963, <https://doi.org/10.1080/01904167.2016.1184278>.
- Saure, M.C. 2005. Calcium translocation to fleshy fruit: Its mechanism and endogenous control. *Scientia Hort.* 105:65–89, <https://doi.org/10.1016/j.scienta.2004.10.003>.
- Schweitzer, B. and J.L. Petri. 2019. Exportação de nutrientes pela produção em pomares de frutas de clima temperado. *Brazilian J. Dev.* 5(8):13408–13414, <https://doi.org/10.34117/bjdv5n8-142>.
- Serra, S., R. Leisso, L. Giordani, L. Kalcsits, and S. Musacchi. 2016. Crop load influences fruit quality, nutritional balance, and return bloom in ‘Honeycrisp’ apples. *HortScience* 51(3):236–244, <https://doi.org/10.21273/HORTSCI.51.3.236>.
- Shear, C.B. 1975. Calcium-related disorders of fruit and vegetables. *HortScience* 10:361–365.
- Silva, H.E. and J. Rodríguez. 1995. Fertilización de plantaciones frutales, p 519. Pontificia Universidad Católica de Chile, Santiago, Chile.
- Silveira, J.P.G., C.V.T. do Amarante, C.A. Steffens, A. Miqueloto, and J.M. Katsurayama. 2012. Inhibition of gibberellin synthesis reduces vegetative growth of apple trees and provides control of bitter pit in the fruit. *Rev. Bras. Frutic.* 34:328–335.
- Telias, A., E. Hoover, C. Rosen, D. Bedford, and D. Cook. 2006. The effect of calcium sprays and fruit thinning on bitter pit incidence and calcium content in ‘Honeycrisp’ apple. *J. Plant Nutr.* 29:1941–1957, <https://doi.org/10.21273/HORTSCI.39.4.780D>.
- Torres, C. and R. Gomez. 2020. ‘WA 38’ first commercial season (2019-2020) storage and packing observations. May 2021. <<http://treefruit.wsu.edu/article/wa-38-storage-and-packing-observations-cs1/>>.
- Turner, N.A., I.B. Ferguson, and R.O. Sharples. 1977. Sampling and analysis for determining relationship of calcium concentration to bitter pit in apple fruit. *N. Z. J. Agr. Res.* 20:525–532, <https://doi.org/10.1080/00288233.1977.10427369>.
- Valverdi, N.A. and L. Kalcsits. 2021. Rootstock affects scion nutrition and fruit quality during establishment and early production of ‘Honeycrisp’ apple. *HortScience* 56(2): 261–269, <https://doi.org/10.21273/HORTSCI.56.2.261>.
- Volz, R.K., P.A. Alspach, D.J. Fletcher, and I.B. Ferguson. 2006. Genetic variation in bitter pit and fruit calcium concentrations within a diverse apple germplasm collection. *Euphytica* 149(1):1–10, <https://doi.org/10.1007/s10681-005-9000-8>.
- Volz, R.K., I.B. Ferguson, J.H. Bowen, and C.B. Watkins. 1993. Crop load effects on fruit mineral nutrition, maturity, fruiting and tree growth of ‘Cox’s Orange Pippin’ apple. *J. Hort. Sci.* 68:127–137, <https://doi.org/10.1080/00221589.1993.11516336>.
- Watkins, C.B., J.F. Nock, S.A. Weis, S. Jayanty, and R.M. Beaudry. 2004. Storage temperature, diphenylamine, and pre-storage delay effects on soft scald, soggy breakdown, and bitter pit of ‘Honeycrisp’ apples. *Postharvest Biol. Technol.* 32:213–222, <https://doi.org/10.1016/j.postharvbio.2003.11.003>.
- White, P.J. and M.R. Broadley. 2003. Calcium in plants. *Ann. Bot.* 92:487–511, <https://doi.org/10.1093/aob/mcg164>.
- WTFRC. 2020. Washington Free Fruit Research Commission 2020 research priorities. Dec. 2019. <<https://treefruitresearch.org/requests-for-proposals/>>.