Physiological Factors Affecting Nutrient Uptake and Distribution and Fruit Quality in 'Honeycrisp' and 'WA 38' Apple (*Malus* ×*domestica* Borkh.)

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***Abstract*. 'Honeycrisp' is among the most widely grown apple cultivars in the United States and 'WA 38' is a new apple cultivar released in Washington State. 'Honeycrisp' is highly susceptible to bitter pit and other physiological disorders; however, 'WA 38' is not susceptible to bitter pit but little is known about its susceptibility to other disorders. Bitter pit is a calcium-related disorder that has been associated with localized calcium deficiencies in fruit in addition to the proportions of calcium relative to the presence of other nutrients like potassium and magnesium. The objective of this study was to compare physiological differences and fruit quality between 'Honeycrisp' and 'WA 38' to determine how these differences might correspond to differences in mineral nutrient composition and bitter pit susceptibility. Here, 'Honeycrisp' and 'WA 38' elemental composition in leaves, fruit, and xylem sap was measured every 20 days starting 30 days after full bloom and compared with leaf gas exchange and stem water potential. 'Honeycrisp' had greater foliar transpiration rates that corresponded with greater calcium in the leaves and lower leaf K+Mg/Ca ratio, when compared with 'WA 38'. In contrast, fruit calcium concentrations were higher for 'WA 38' with lower K+Mg/Ca ratios. Xylem conductance was higher during late summer in 'WA 38' compared with 'Honeycrisp'. 'WA 38' fruit was denser than 'Honeycrisp' and more research is needed to determine whether differences in fruit structure may affect susceptibility to bitter pit in apple.**

'Honeycrisp' apples are among the most grown in Washington State (USDA NASS, 2017). This cultivar is recognized for its exceptionally crisp texture, juiciness, and is popular with consumers (Luby and Bedford, 1992). Furthermore, it has provided premium economic returns for growers (Gallardo et al., 2015). Despite its popularity, 'Honeycrisp' is difficult to grow because it is susceptible to physiological disorders like bitter pit and sunburn (Luby and Bedford, 1992). 'WA 38' is a new cultivar released by the Washington State University Apple Breeding Program in 2017. It is a cross between 'Honeycrisp' and 'Enterprise' (Evans et al., 2012). 'WA 38' fruit rarely exhibits bitter pit incidence (Evans et al., 2012) but the underlying causes behind its low susceptibility to bitter pit have yet to be identified.

Bitter pit is a physiological disorder responsible for large postharvest fruit losses. This disorder is characterized by dark depressions in the calyx end of the fruit caused by localized cellular calcium deficiencies (de Freitas and Mitcham, 2012; Pavicic et al., 2004). Calcium plays a structural role in the cell, and when calcium concentrations are low in the cell wall, cell permeability increases. Cell permeability contributes to cell death, which produces dark lesions associated with bitter pit. High incidences of calcium-related disorders are associated with rapid cell expansion such that the demand for calcium exceeds the supply to the fruit because calcium uptake and translocation lags behind rapid increase of fruit mass. There is also increased competition between leaves and fruit for available calcium (Ho and White, 2005; Saure, 2005; White and Broadley, 2003). Calcium is translocated via the transpiration stream almost exclusively through the xylem (Ho and White, 2005; White and Broadley, 2003). Because of the relatively low transpiration rates in fruit compared with leaves, most xylem water, carrying calcium and other mineral nutrients, is mostly allocated to leaves (de Freitas et al., 2011; Falchi et al., 2017). The interaction of other elements like potassium and magnesium have been implicated in the induction of bitter pit (de Freitas and Mitcham, 2012). Potassium is directly involved in cell expansion and corresponds to rapid plant and fruit growth, and is far more abundant and easily translocated into fruit tissue when compared with calcium. An increase in potassium causes the fruit cells to swell, possibly affecting cellular structural integrity (de Freitas et al., 2010; de Freitas and Mitcham, 2012; Saure, 2005). Both potassium and magnesium are known to compete with calcium for binding sites at the plasma membrane (do Amarante et al., 2013). Calcium binding at the plasma membrane delays phospholipid and other compound catabolism, which preserves membrane integrity by limiting senescent-related lipid changes at the membrane. Due to ionic similarities between calcium and magnesium, and to some extent potassium, high potassium and magnesium may outcompete calcium for sites at the membrane for processes like activation sites for enzymes and binding on phosphorylated cell membranes without serving the same structural role of calcium (do Amarante et al., 2013; Saure, 2005; White and Broadley, 2003).

Calcium delivery is also dependent on conductive xylem vessels within developing fruit (Song et al., 2018). During fruit growth and development, xylem conductance decreases for some fruit species like apples (Dražeta et al., 2004; Miqueloto et al., 2014). Several hypotheses suggest that losses in xylem conductance are due to vessel stretching during fruit expansion and increasing vessel conductance is due to formation of new, functional, vessels (Song et al., 2018), although some reports show no physical damage to xylem vessels, which indicates that decreases in conductance are reversible (Keller et al., 2006). Miqueloto et al. (2014) reported that 'Catarina', a bitter pit–susceptible apple cultivar, lost xylem conductance earlier in the season than 'Fuji', a cultivar that is less susceptible to bitter pit. However, these patterns have not been clearly described for other fruit cultivars, including 'Honeycrisp' and 'WA 38'. Because of the dependence on the transpiration stream for calcium delivery to the fruit, water transport and nutrient dynamics in developing fruit should be a function of water relations and leaf gas exchange. In this study, we sought to understand how 'Honeycrisp' and 'WA 38' apple cultivars, with differing bitter pit susceptibility, may differ in functional leaf and fruit traits to understand how this might influence nutrient uptake and fruit quality. We hypothesized that differences in nutrient uptake into fruit and overall fruit quality between 'WA 38' and 'Honeycrisp' apple could be attributed to differences in nutrient and water transport within the whole tree.

Materials and Methods

This study was conducted in separate blocks in a 'Honeycrisp' and 'WA 38'

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Table 1. Shoot length, fruit yield, and fruit quality of 'Honeycrisp' and 'WA 38' apple trees at harvest. Symbols denote significance (•$P < 0.10$, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) between fruit of the same year determined by Tukey mean separation test ($\alpha = 0.05$).

	2018			2019		
Harvest Quality	Honeycrisp	WA 38	*P* value	Honeycrisp	WA 38	*P* value
Mean yield (kg)	6.51 ± 0.59	5.18 ± 0.65	0.18	13.8 ± 0.65	19.0 ± 1.70	*
Final shoot growth (cm)	42.7 ± 3.35	44.4 ± 4.43	0.77	35.7 ± 2.42	39.5 ± 0.92	0.25
Background color class (%)						
1	2 ± 2.23	0 ± 0.00	0.36	22 ± 6.22	0 ± 0.00	*
2	40 ± 7.32	0 ± 0.00	**	70 ± 5.48	13 ± 7.04	***
3	57 ± 6.55	29 ±16.9	0.17	8 ± 3.80	82 ± 7.68	***
4	0 ± 0.00	71 ± 16.9	**	0 ± 0.00	5 ± 2.62	0.09
% Red class						
1	1 ± 1.04	0 ± 0.00	0.36	6 ± 1.20	0 ± 0.00	**
2	21 ± 4.70	0 ± 0.00	**	43 ± 3.13	0 ± 0.00	***
3	52 ± 1.59	3 ± 2.00	***	36 ± 2.00	35 ± 11	0.89
4	26 ± 6.18	97 ± 2.00	***	15 ± 2.69	65 ± 11	**
Sunburn intensity (%)						
Clean	1 ± 1.04	54 ± 12.5	**	6 ± 3.61	77 ± 5.24	***
Y1	7 ± 2.62	44 ± 11.0	*	26 ± 2.30	22 ± 4.30	0.64
Y2	46 ± 7.38	0 ± 0.00	***	52 ± 5.24	1 ± 1	***
Y3	37 ± 5.63	0 ± 0.00	***	6 ± 2.69	0 ± 0	0.06
Tan	7 ± 2.13	2 ± 2.23	0.14	10 ± 1.04	0 ± 0	***
Black	0 ± 0.00	0 ± 0.00	1	0 ± 0.00	0 ± 0.00	1
Diameter (mm)	83.6 ± 1.150	86.3 ± 0.52	•	87.9 ± 0.29	87.73 ± 1.28	0.88
Weight (g)	242 ± 10.71	319 ± 5.65	***	286 ± 0.96	310.0 ± 11.0	0.08
Bitter pit incidence (%)	40	0	***	15	4	*
Firmness (kg)	8.25 ± 0.10	9.67 ± 0.057	***	6.61 ± 0.04	7.58 ± 0.08	***
Soluble solid concentration (%)	14.79 ± 0.30	15.73 ± 1.641	0.59	13.2 ± 0.16	13.01 ± 0.26	0.64
Starch index (%)						
1	0 ± 0.00	6 ± 3.61	0.13	0 ± 0.00	11 ± 4.30	*
2	0 ± 0.00	22 ± 8.86	*	1 ± 1.04	35 ± 6.66	**
3	0 ± 0.00	45 ± 10.3	**	0 ± 0.00	32 ± 4.62	***
4	6 ± 2.69	26 ± 4.20	**	9 ± 3.94	17 ± 3.0	0.19
5	43 ± 7.12	5 ± 3.24	**	48 ± 5.51	5 ± 4.69	***
6	51 ± 5.90	4 ± 3.16	***	42 ± 7.01	0 ± 0.00	**

orchard that was established at the Washington State University Sunrise Research Orchard in Rock Island, WA. In 2016, scion budwood for each cultivar was top-worked onto 'M.9 T-337' rootstocks with a 'Granny Smith' interstem that were spaced 1.2 m between trees and 3.6 m between rows. Trees were ≈2.7 m tall by the end of 2017 and had filled their canopy space. The experiment was conducted in 2018 and 2019 and was organized as a completely randomized design with four replications of 12 trees each for both cultivars. Within each replication, three trees were selected for physiological measurements and five trees were selected for shoot growth measurements. Ten first-year vegetative shoots were measured on each tree from the start of new growth to the apical meristem. Trees were visually selected for uniformity of bloom density and trees were selected that had filled their canopy space and were 2.7 m tall. Bloom density was determined by conducting cluster counts in both years.

Tree physiology and vegetative vigor. Full bloom was visually assessed to be on 27 Apr. 2018 and 26 Apr. 2019 for both 'Honeycrisp' and 'WA 38' when more than 60% of king flowers had opened on the north side of the tree. In both years, measurements began 30 d after full bloom (DAFB) and continued every 20 d until 125 DAFB, the approximate harvest maturity for 'Honeycrisp'. Fruitlets were thinned in May 2018 and June 2019 to achieve a crop load of four fruit per cm^2 trunk cross-sectional area.

Midday stem water potential. Midday stem water potential was measured on one mature leaf near the base of the trunk for each of three trees per replicate during solar noon (12:00 PM to 2:00 PM). Leaves were enclosed in silver reflective envelopes for a minimum of 1 h to allow the leaf water potential to equilibrate with the stem at the site of attachment. After 1 h, a single cut was made to excise the leaf. Immediately, stem water potential was measured (MPa) using a Model 615D pressure chamber instrument (PMS Instrument Co., Albany, OR).

Stomatal conductance and photosynthesis. Net gas exchange was measured by selecting healthy, fully developed leaves with similar exposure to sunlight and airflow using the Li-COR 6400XT IR gas analyzer fitted with a fluorescence head (Li-COR, Lincoln, NE). Measurements were made before noon on a cloudless day. The reference CO_2 concentration in the chamber was maintained at 400 ppm, photosynthetic photon flux density was adjusted to 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and leaf temperature was set to 25 °C. One to 4 min was allowed for stabilization of gas exchange parameters before values were recorded. In 2018, smoke cover impeded the 90 DAFB net gas exchange measurements and measurements made at 80 DAFB were reported instead.

Xylem sap. In 2018, xylem sap was collected from 6:00 AM to 10:00 AM with a Model 615D pressure chamber instrument (PMS Instrument Co.) using a destructive method starting 30 DAFB and continuing every 20 d thereafter until 110 DAFB. In 2019, xylem sap was collected only at 30, 50, and 90 DAFB. Current year vegetative growth was collected from the bottom half of the canopy and then ≈2 cm of bark and phloem was removed from the cut end. The stems were then rinsed with deionized water to avoid contamination of the xylem sap, and dried using tissue paper to avoid sample dilution. The shoot was then placed into the pressure chamber and sealed, and N_2 gas was slowly released into the chamber until sap emerged from the cut surface. The initial drops of sap were wiped away and the remaining sap droplets were collected using a 20-µL pipette. This process was repeated for at least five shoots or until at least 0.25 mL of sap was collected. After collection, the sap was frozen at –20 °C until analysis. For elemental analysis, sap was thawed and 200 µL of sap was digested in 3 mL of HNO_3. Then, 1 mL from each sample was filtered using a 0.45-µM polytetrafluoroethylene (PTFE) (VWR, Radnor, PA) filter fitted using a Luer-lock syringe. The filtered product was then diluted 10 times in ultrapure water and analyzed for calcium, magnesium, and potassium concentrations using an Agilent 4200 MP-AES (Agilent Technologies Inc., Santa Clara, CA) and run in combination with elemental inductively coupled plasma standards for calibration.

Xylem conductance and mineral analysis. In 2018, three fruitlet samples total were collected from each replicate beginning at 30 DAFB and continuing every 20 d until harvest to estimate xylem conductance. Fruit

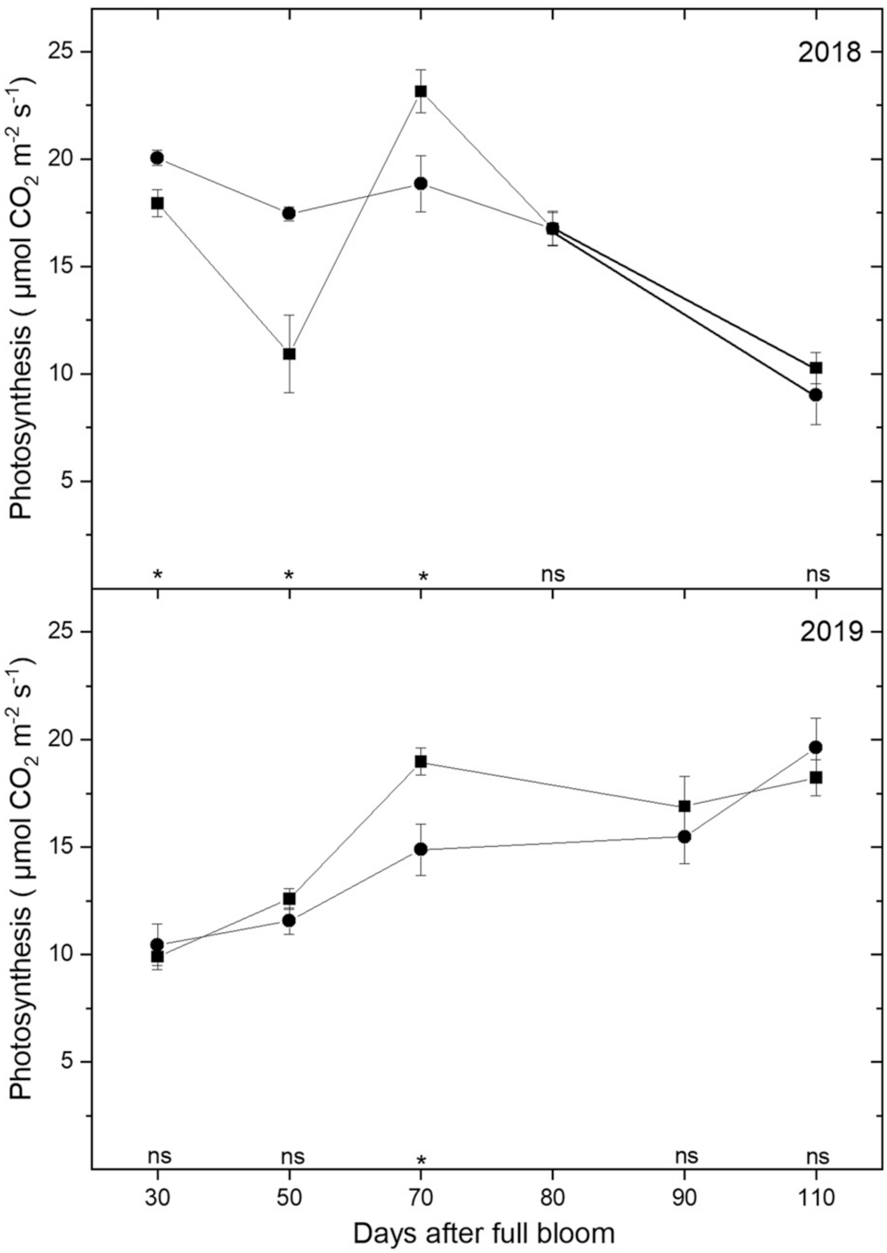

Fig. 1. Mean net photosynthesis of 'Honeycrisp' (square) and 'WA 38' (circle) at various measurement dates in the 2018 and 2019 growing seasons. Symbols denote significance among treatments at each measurement date (ns = not significant, *$P \leq 0.05$).

was selected for similar size, color, and approximate location in canopy to avoid possible variation in nutrient distribution within the canopy identified by Kalcsits et al. (2019). Each fruit pedicel was excised from the stem and immediately submerged in water. The pedicel was then recut under water to avoid embolism formation. To stain the fruit, pedicels were recut to uniform length and placed in a 1% acid fuchsin dye solution for 2 h. Afterward, fruitlets were sliced into four sections and digital images were acquired of calyx, middle, and stem end of the fruit. Then, fruitlets were thinly sliced and dried for a minimum of 5 d at 60 °C. After drying, the fruitlets were ground using a VWR homogenizer (VWR); 200 ± 1 mg of ground tissue was weighed into digestion vials and hot-plate digested with 6 mL of HNO_3 for 1 h. Samples were filtered with a syringe fitted with a 0.45-μM PTFE filter (VWR). The filtered product was diluted 100 times and analyzed using an Agilent 4200 MP-AES (Agilent Technologies Inc.) for calcium, magnesium, and potassium concentrations and run in combination with elemental standards at concentrations that bracketed the known concentrations for apple fruit for validation (Kalcsits, 2016).

Leaf mineral analysis. In 2018 and 2019, composite leaf samples consisting of five leaves per tree were collected from random exterior locations from all trees in each replicate starting 30 DAFB and continuing until harvest (125 DAFB for 'Honeycrisp' and 155–160 DAFB for 'WA 38') and dried for a minimum of 5 d at 60 °C for nutrient analysis. The dried leaves were ground to micron size using a VWR high-throughput homogenizer (VWR). Ground leaf tissue was then digested and analyzed for calcium, magnesium, and potassium as described previously.

Fruit quality. 'Honeycrisp' harvest occurred on 30 Aug. 2018 and 3 Sept. 2019. 'WA 38' harvest occurred 26 Sept. 2018 and 7 Oct. 2019. Honeycrisp was harvested based on background color degradation and color development and was similar timing to other nearby commercial orchards. 'WA 38' maturity was determined using a starch index developed for 'WA 38' by the Washington Tree Fruit Research Commission and the starch index was between 2.5 and 3.5 when harvested. All fruit were completely removed from the three selected trees within each replicate and weighed to provide total yield (kg). Then, 16 fruit were randomly selected from each tree in the replicates. Eight fruit were used for fruit quality at harvest (2018 and 2019) and eight fruit were stored in regular atmosphere for 3 months at 1 °C to be used for bitter pit assessment. Fruit diameter (mm) was measured using a digital caliper and weight (g) was measured using a precision digital scale (Mettler-Toledo, LLC, Columbus, OH). Fruit sunburn was scored using an adjusted 'Gala' sunburn severity scale from Schrader et al. (2008), like what Kalcsits et al. (2017) used for 'Honeycrisp' apples. Background color and red coverage were determined using discrete classification variables according to the Washington State Tree Fruit Research Commission's background color and color scale (Hanrahan and Mendoza, 2012a, 2012b). Background color was measured on a ranking system of 1 to 4. A ranking of 1 indicated a background color of green; 2 indicated break, which is a midpoint between yellow and green; 3 indicated yellow; and 4 indicated pink. For red coverage classification: 1 indicated a red cover of 0% to 25%, 2 indicated 26% to 50%, 3 indicated 51% to 75%, and a 4 indicated 76% to 100% coverage. Bitter pit incidence was recorded as either absent or present.

Firmness was tested by first removing a small slice of the peel on both the sun side and the shaded side of the equatorial region fruit, then firmness was measured using a Fruit Texture Analyzer (Güss Manufacturing Ltd., Strand, South Africa) fixed with an 11-mm probe. Soluble solid concentration was measured for each fruit by cutting a longitudinal slice then pressing the juice onto a refractometer (PAL-1; Atago U.S.A. Inc., Bellevue, WA) with a garlic press (OXO, New York, NY). Starch index was assessed using a 1-cm-wide slice cut from the equatorial area of the fruit and sprayed with Lugol's solution (15 g·L^{-1} KI and 6 g·L^{-1} I) with a hand-held spray bottle. Starch content for both 'Honeycrisp' and 'WA 38'and was visually rated within 5 min of spraying on a 1 to 6 scale based on the standard released by the Washington Tree Fruit Research Commission for 'Honeycrisp'. Elemental

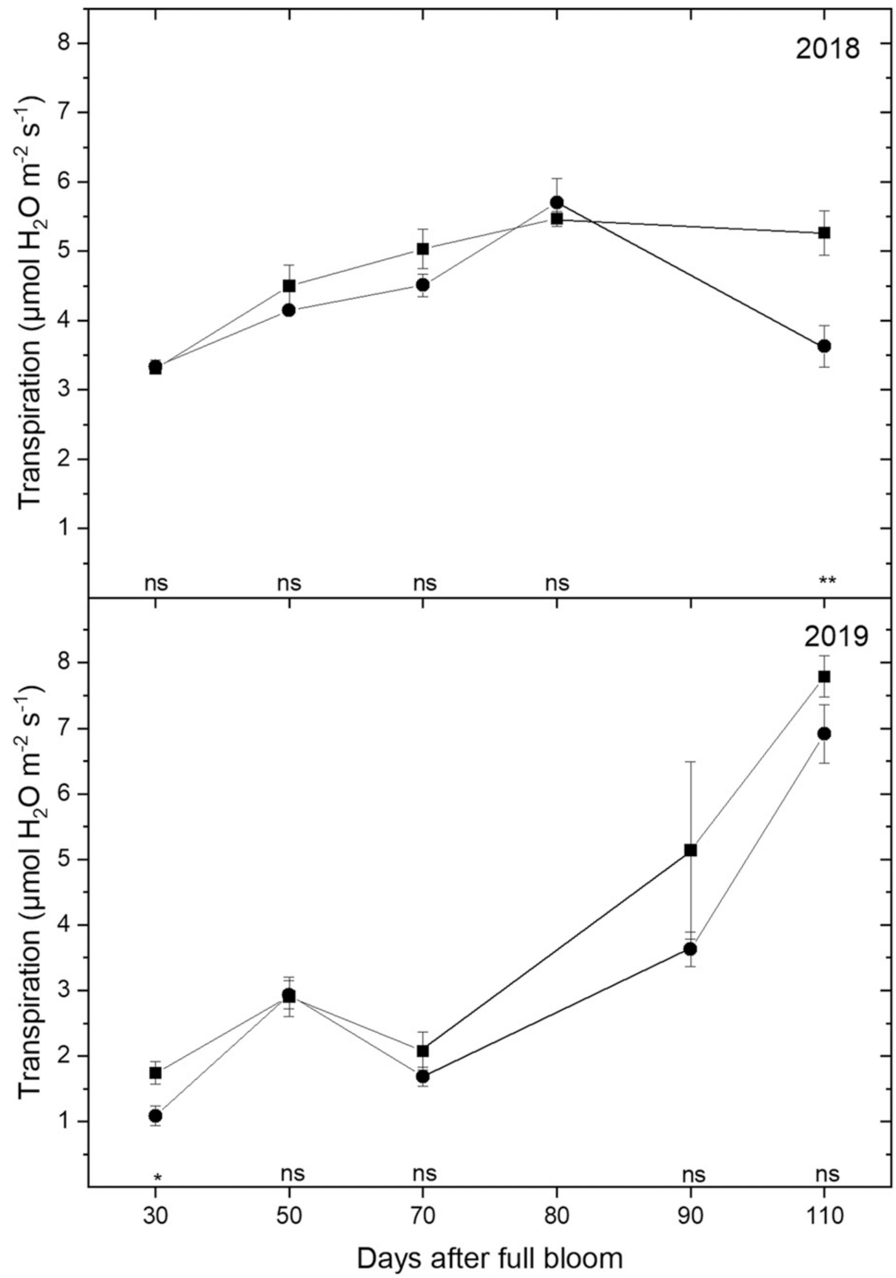

Fig. 2. Mean transpiration of 'Honeycrisp' (square) and 'WA 38' (circle) at various measurement dates in the 2018 and 2019 growing seasons. Symbols denote significance among treatments at each measurement date (ns = not significant, *$P \leq 0.05$, **$P \leq 0.01$).

analysis was performed using a pooled sample consisting of an equatorial slice taken from all eight fruit being analyzed for fruit quality from the same tree. The slices were then cut to exclude seeds and core tissue and dried for a minimum of 5 d at 60 °C. Dry samples were processed and digested using the same procedure described previously. After 3 months of storage, bitter pit presence or absence were assessed as mentioned previously.

Statistical analysis. Cultivar differences were analyzed using a one-way analysis of variance in OriginPro 9.1 software (Originlab Corporation, Northampton, MA). Bitter pit incidence was analyzed using a χ^2 approach in OriginPro 9.1 software. Post hoc mean separation was done using Tukey's honestly significant difference test ($\alpha = 0.05$).

Results

Vegetative growth, leaf physiology, and xylem conductance. Shoot length was not significantly different between the two cultivars ($P = 0.77$ in 2018, $P = 0.25$ in 2019) (Table 1). However, 'WA 38' had a more robust canopy than 'Honeycrisp', indicating differences in the number of vegetative shoots (Supplemental Fig. 1). Net photosynthesis was not different between the two cultivars overall when measurements were pooled for 2018 ($P = 0.75$) or 2019 ($P = 0.39$; Fig. 1). In 2018, there were no differences in leaf transpiration rates between the two cultivars ($P = 0.24$; Fig. 2). However, in 2019, transpiration rates for 'Honeycrisp' were higher than 'WA 38' throughout the season when pooled ($P = 0.06$). In 2019, 'Honeycrisp' had greater stomatal conductance (g_S) compared with 'WA 38' when taking into account the whole season ($P = 0.02$; Fig. 3), but there were no differences in 2018 ($P = 0.46$). Stem water potential was marginally more negative in 'Honeycrisp' compared with 'WA 38' in 2018 ($P = 0.11$) or 2019 ($P = 0.51$; Fig. 4). Xylem conductance, measured by counting the number of stained vessels for the middle cortical section in developing fruit, was higher early in the season for 'WA 38' (10 stained vessels) than 'Honeycrisp' (8.28 stained vessels). For both cultivars, the number of stained vessels decreased dramatically by 70 DAFB and remained nonconductive for the remainder of the season (Fig. 5). Xylem conductance remained higher for longer during fruit development in the middle cortical for 'WA 38' (7.4 stained vessels at 90 DAFB) compared with 'Honeycrisp' (3.9 stained vessels at 90 DAFB). At harvest, neither cultivar showed any vessel staining.

Xylem sap calcium, potassium, and magnesium. In general, sap concentrations for calcium, potassium, and magnesium were not consistently different between cultivars. There were some differences observed between cultivars and sampling dates but were not consistent between sampling years. Sap calcium concentrations were not significantly different for any of the sampling dates in 2018 (Fig. 6). In 2019, sap calcium concentrations were higher for 'WA 38'. Mean seasonal xylem calcium concentrations were higher for 'Honeycrisp' than 'WA 38' in 2018 ($P = 0.02$) but not in 2019 ($P = 0.76$). In 2018, sap potassium concentrations were greater for 'WA 38' at 70 DAFB compared with Honeycrisp ($P < 0.1$; Fig. 6). In 2019, sap potassium concentrations were greater at 30 DAFB for 'Honeycrisp' compared with 'WA 38', but at 50 DAFB and 90 DAFB, sap potassium concentrations were higher for 'WA 38' (Fig. 6). Sap magnesium concentrations for 'WA 38' were significantly greater only at 30 DAFB and 110 DAFB in 2018 (Fig. 6). In 2019, 'Honeycrisp' had greater magnesium concentrations at 30 DAFB than 'WA 38' (Fig. 6). In 2018, sap magnesium concentrations were greater in 'Honeycrisp' throughout the season ($P = 0.01$) but not in 2019 ($P = 0.81$). When looking at the ratio of K+Mg/Ca, the cultivars were similar in 2018 except for 70 DAFB where 'Honeycrisp' had a greater ratio than 'WA 38' (Fig. 6). In 2019, 'WA 38' had a significantly greater ratio at 90 DAFB when compared with 'Honeycrisp' (Fig. 6).

Leaf calcium, potassium, and magnesium. Leaf calcium content was significantly greater in 'Honeycrisp' when compared with 'WA 38' only at 70 DAFB in 2018 (Table 2). Leaf potassium content was consistently higher in 'WA 38' when compared with 'Honeycrisp'

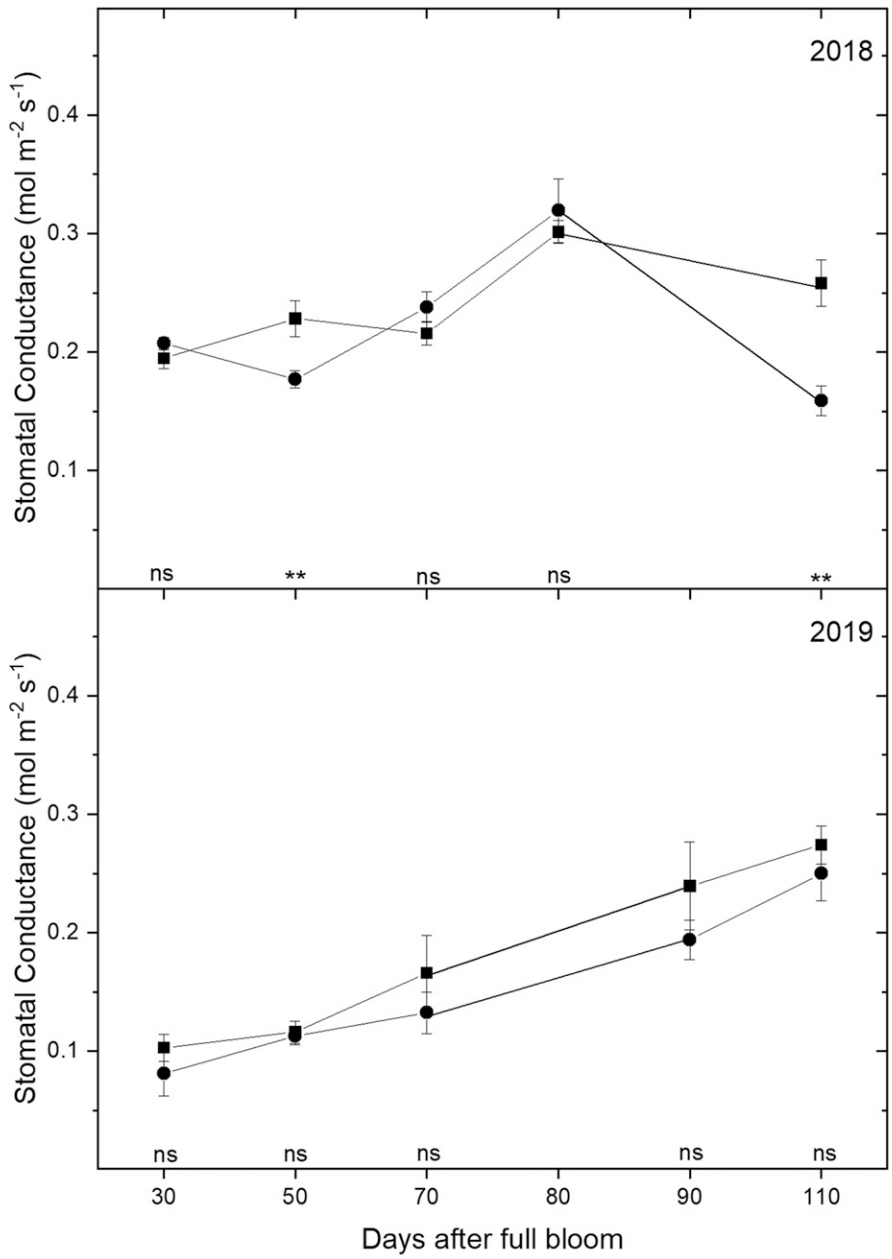

Fig. 3. Mean g_S of 'Honeycrisp' (square) and 'WA 38' (circle) at various measurement dates in the 2018 and 2019 growing seasons. Symbols denote significance among treatments at each measurement date (ns = not significant, $^{**}P \leq 0.01$).

in both sampling years. 'WA 38', at the 30-DAFB date in 2018 had greater magnesium than 'Honeycrisp'. No other date in 2018 or 2019 showed differences in magnesium content. Elevated potassium content for 'WA 38' led to greater (K+Mg)/Ca ratios in leaves compared with 'Honeycrisp'.

Fruit calcium, potassium, and magnesium. Fruit calcium content was greater in 'WA 38' than 'Honeycrisp' in 2018 (Table 3); however in 2019, 'WA38' had greater fruit calcium content than 'Honeycrisp' for 30 and 50 DAFB. Fruit potassium was not significantly different between cultivars at any measurement date in 2018 except at harvest, when 'Honeycrisp' had significantly more potassium than 'WA 38'. In 2019, fruit potassium content was not significantly different at any of the measurement dates (Table 3). 'WA 38' fruit magnesium content was significantly greater than 'Honeycrisp' at each measurement date in 2018 except for 30 DAFB (P = 0.08) and at harvest (P = 0.84). In 2019, fruit magnesium content was not significantly different at any of the measurement dates. Fruit K+Mg/Ca ratio was significantly greater in 'Honeycrisp' at every measurement date in 2018 except for 70 DAFB where the values were not significantly different (P = 0.07). In 2019, fruit K+Mg/Ca ratio was significantly greater in 'Honeycrisp' at 30 and 50 DAFB and nonsignificant at the other measurement dates (Table 3).

Yield and fruit quality. Yield was similar for both cultivars in 2018 and averaged ≈7 and 5 kg per tree in 2018 (P = 0.18) and 14 and 19 kg in 2019 (P = 0.01) for 'Honeycrisp' and 'WA 38', respectively (Table 1). Individual fruit weight was significantly greater for 'WA 38' than 'Honeycrisp' in 2018 but not in 2019. Mean fruit weight was greater for 'WA 38' (P = 0.08). Fruit diameter was similar for both cultivars in both 2018 and 2019 (Table 1). In 2018, 40% and 0% of fruit was affected by bitter pit at harvest for 'Honeycrisp' and 'WA 38' (P < 0.0001), respectively. In 2019, 15% and 4% of fruit showed bitter pit symptoms (P < 0.05), for 'Honeycrisp' and 'WA 38', respectively. Fruit firmness at harvest was greater for 'WA-38' fruit than 'Honeycrisp' in 2018 and 2019, but there were no significant differences for soluble solid content, which averaged between 14 °Brix and 15 °Brix in 2018 and 13 °Brix in 2019. 'WA 38' had significantly greater red color coverage compared with 'Honeycrisp' fruit for both 2018 and 2019. Most of the fruit for 'WA-38' had greater than 75% red coverage, whereas most of the 'Honeycrisp' fruit had less than 75% red color coverage and more than 20% had less than 50% red coverage (Table 1). Sunburn intensity was also different between cultivars. 'WA 38' had more fruit with no sunburn (clean) or minimal damage (Y1) than 'Honeycrisp'. 'Honeycrisp' was more susceptible to sunburn and had significantly greater percentage of apples within the more severe, Y2 and Y3 categories (Table 1) in both 2018 and 2019. True to cultivar, 'WA 38' had significantly more fruit in the starch index categories 2, 3, and 4, indicating lower starch degradation. 'Honeycrisp' apples had significantly more apples in starch index categories 5 and 6, indicating higher starch degradation.

Discussion

Here, we report high disorder incidence of bitter pit and sunburn for 'Honeycrisp' compared with 'WA 38' of the same age under the same environmental conditions, training system, and rootstock. 'WA 38' had elevated fruit calcium concentrations and was consistently less susceptible to both sunburn and bitter pit. Sap elemental concentrations, shoot vigor, and instantaneous leaf level physiological traits did not account for differences in fruit mineral nutrient content or disorder incidence. Xylem conductance was maintained for longer in 'WA 38' than 'Honeycrisp', which may contribute to elevated calcium concentration observed in 'WA 38'. Fruit weight was greater for 'WA 38' than 'Honeycrisp', even though yields were similar. Within susceptible cultivars like 'Honeycrisp', crop load and fruit weight can be significant contributors to bitter pit development (Serra et al., 2016). However, these rules cannot be applied across cultivars. Differences between cultivars in the distribution of mineral nutrients may be caused by other factors, such as differences in xylem conductance or early season calcium uptake.

Comparing sap, fruit, and leaf elemental composition and implications for nutrient

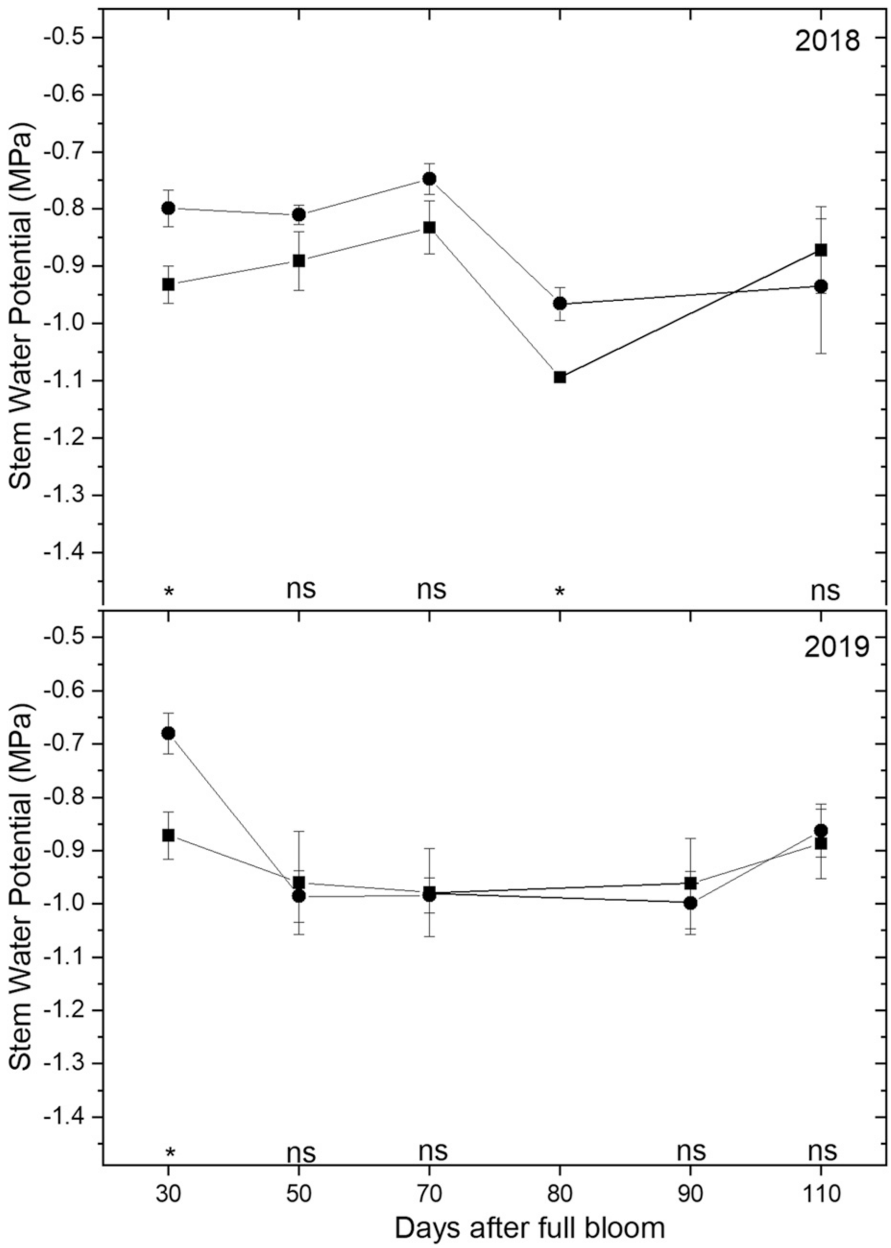

Fig. 4. Mean stem water potential of 'Honeycrisp' (square) and 'WA 38' (circle) at various measurement dates in the 2018 and 2019 growing seasons. Symbols denote significance among treatments at each measurement date (ns = not significant, *$P \leq 0.05$).

delivery. Sap elemental concentrations did not correspond to observed differences between cultivars in leaf and fruit elemental content. Despite there being little difference in shoot growth between cultivars, seasonal leaf nutrient accumulation was similar for calcium and magnesium while potassium was generally higher in 'WA 38' than in 'Honeycrisp'. 'WA 38' had consistently higher leaf K+Mg/Ca ratios than 'Honeycrisp'. In contrast, the fruit K+Mg/Ca ratios were higher for 'Honeycrisp' than 'WA 38'. This corresponds to previous reports of low fruit calcium content and high K+Mg/Ca fruit ratios for 'Honeycrisp' (Cheng and Sazo, 2018). These high ratios have also corresponded to elevated bitter pit incidence in other studies (Cheng and Sazo, 2018; de Freitas et al., 2015). When compared with 'Gala', a cultivar with low susceptibility to bitter pit, 'Honeycrisp' had higher leaf calcium content and lower fruit calcium content (Cheng and Raba, 2009; Cheng and Sazo, 2018), which was similar to differences observed here between 'Honeycrisp' and 'WA 38', which also has low susceptibility to bitter pit.

At times, 'Honeycrisp' had elevated leaf g_S when compared with 'WA 38', but these differences were not consistent. Transpiration rates of fruit were not measured during fruit development, which can affect the delivery of calcium to fruit. This may contribute to nutrient imbalance between leaves and fruit contributing to elevated bitter pit incidence. Because xylem sap concentrations and transpiration rates were similar between cultivars, but there were differences in both leaf and fruit nutrient ratios, whole canopy transpiration rates may affect nutrient delivery to the fruit. Although not specifically measured here, Supplemental Fig. 1 shows differences in canopy size between the two cultivars at the same age. Calcium accumulation in leaves and less in fruit for 'Honeycrisp' compared with 'WA 38' may correspond to the differences in bitter pit susceptibility and fruit and leaf nutrient content between the two cultivars. Vegetative vigor and total leaf area have been attributed to the development of calcium-related disorders in horticultural crops (Watkins et al., 2004); however, when comparing these two cultivars where 'WA 38' has more vegetative growth, this contrasts with other studies that have focused only on susceptible cultivars. Similar to comparisons made here, Cheng and Sazo (2018) reported strong differences in mineral nutrient partitioning between 'Honeycrisp' with 'Gala' apple. Furthermore, conditions that limit water uptake like hot, dry, or saline conditions may exacerbate calcium-related disorders like bitter pit in apples and blossom end rot in tomatoes (Biggs and Peck, 2015; Montanaro et al., 2015; Rosenberger et al., 2001).

Ho and White (2005) identified that environmental and genetic factors that influence the occurrence of blossom end rot in tomatoes do so by affecting the rate of cell expansion or calcium delivery to the fruit. Factors like tree hormone balance, cultivar-specific patterns in growth, and soil water availability should also be considered. Methods targeting the reduction of foliar or whole tree transpiration like regulated deficit irrigation, summer pruning, and antitranspirants could provide more information regarding the relationship of fruit calcium, transpiration, and bitter pit; however, it is clear that there were factors that were unrelated to transpiration rates that contribute to differences in mineral nutrient allocation to fruit between 'WA 38' and 'Honeycrisp'.

Cultivar-specific qualities determine fruit quality. Loss in xylem conductance into developing fruit has been identified across a wide range of horticultural species, and for apple, genotypic variation has been previously reported (Miqueloto et al., 2014; Song et al., 2018). Miqueloto et al. (2014) reported that 'Catarina' apple lost xylem conductance earlier than 'Fuji', and attributed the high susceptibility to bitter pit for 'Catarina' to these differences. The dynamic changes in xylem conductance has been previously associated with fruit calcium content (Dichio et al., 2003; Miqueloto et al., 2014; Song et al., 2018). In our study, xylem sap concentrations remained relatively consistent throughout the season, whereas xylem conductance declined. Leaf mineral nutrient content increased throughout the season indicating continued uptake of calcium into leaves but not fruit. The mobility of potassium and magnesium and immobility of calcium would imply that reductions in conductance could contribute to imbalances

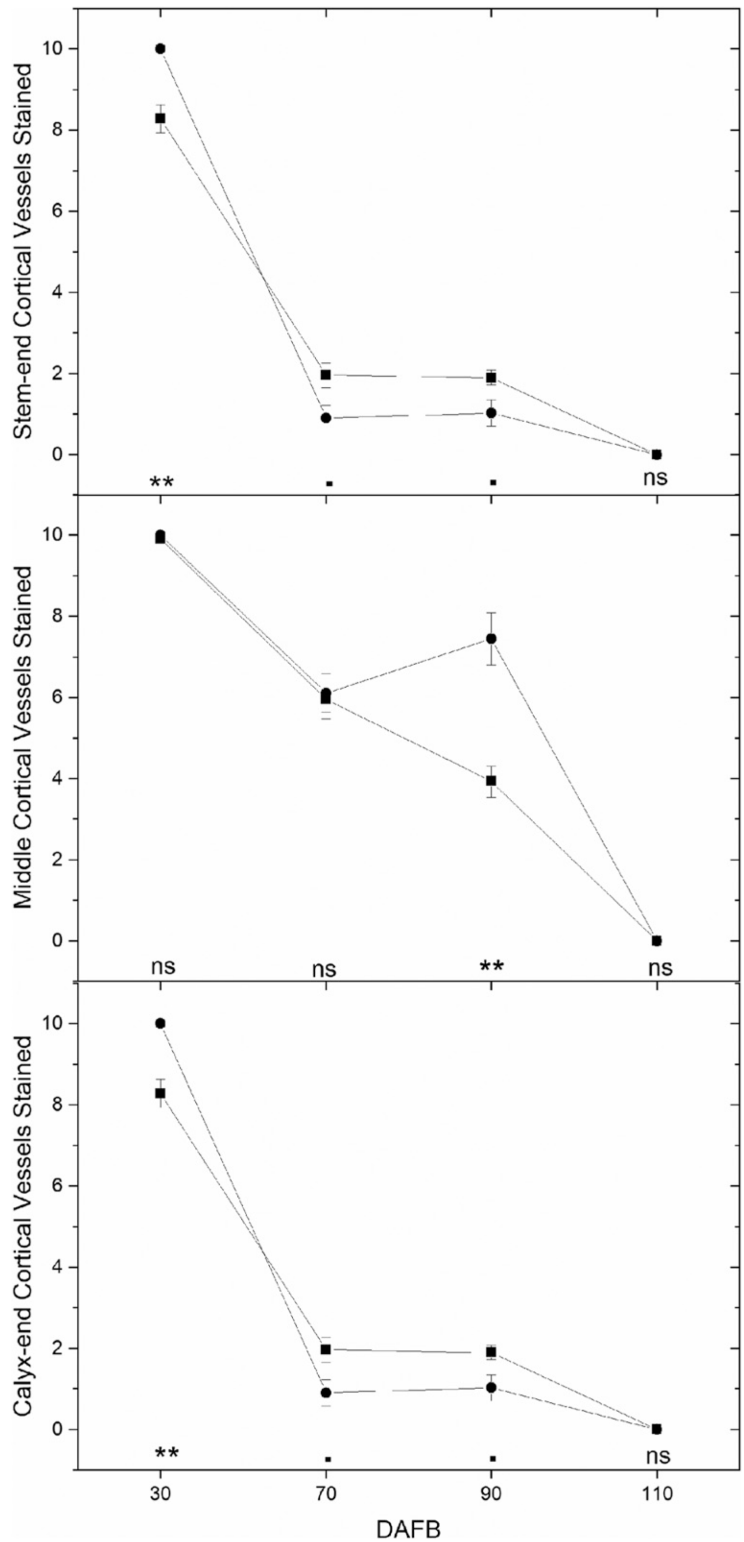

Fig. 5. Xylem functionality as expressed by the number of stained primary cortical vascular bundles in the stem, middle, and calyx ends of ‘Honeycrisp’ (square) and ‘WA 38’ (circle) at various sample dates throughout the growing season after 2 h of fruit peduncle submersion in 1% acid fuchsin sodium salt. Symbols denote significance among treatments at each measurement date (ns = not significant, $^{**}P \leq 0.01$).

that develop during the late season sampling. Cortical xylem remained active longer for ‘WA 38’ than ‘Honeycrisp’ and this may, in part, explain the elevated fruit calcium content for ‘WA 38’ and its low susceptibility to bitter pit. Other cultivars with similar harvest timing to ‘WA 38’ are also susceptible to bitter pit, including ‘Golden Delicious’ (Lötze et al., 2008). Partitioning of water transport to the fruit between xylem and phloem also may contribute to changes in xylem conductance in apple. Although this was not measured in this study, it has been reported that sap flow into fruit at later stages of development is dominated by the phloem. Keller et al. (2006) reported that fruit xylem vessels remained active but not conductive by applying pressure to the cut stylar end of attached grapes. Even during periods where phloem water transport dominates solute flow to developing fruit, xylem can remain hydraulically connected to the shoot and physical disruption and cavitation were

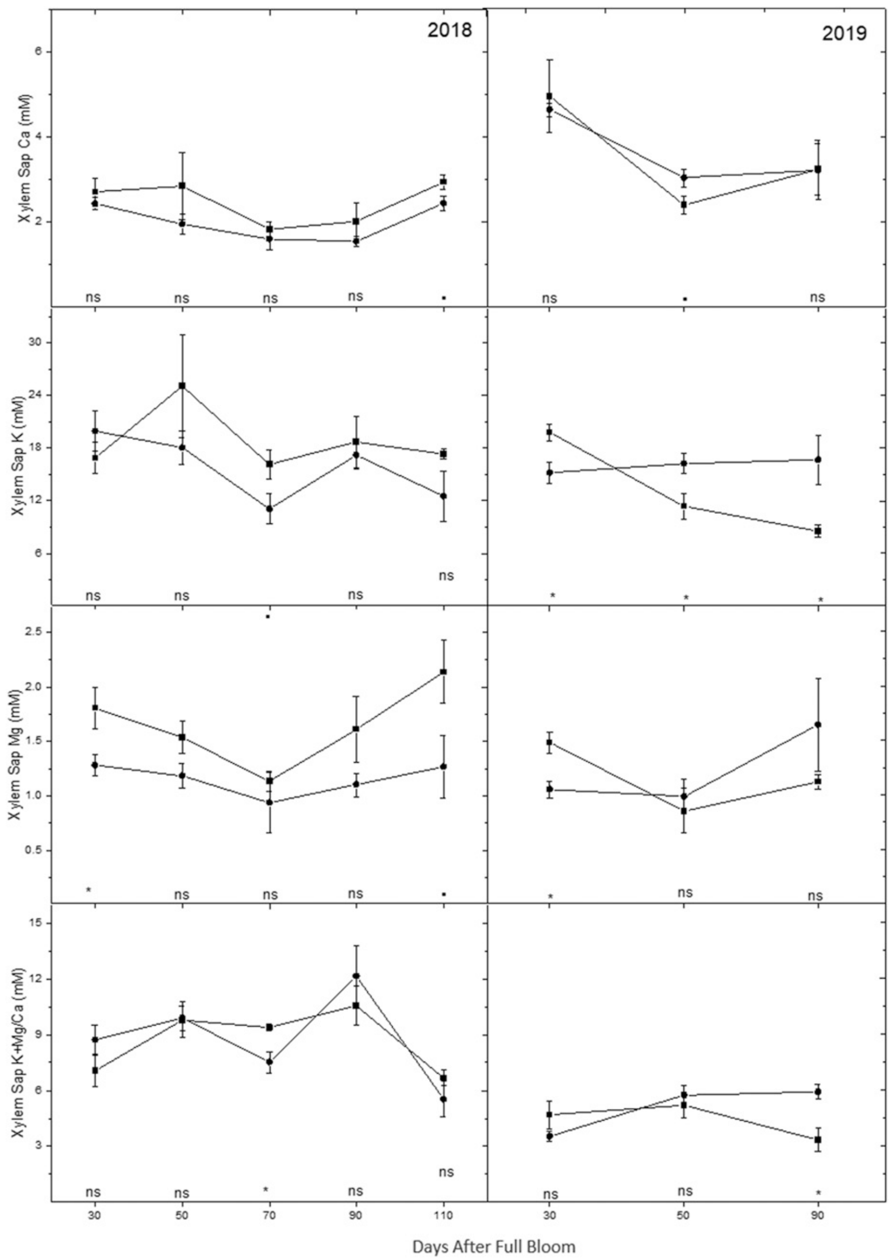

Fig. 6. Calcium (Ca), potassium (K), and magnesium (Mg) concentrations (mM) of 'Honeycrisp' (square) and 'WA 38' (circle) apple tree xylem sap at various sampling dates in 2018 and 2019. Symbols denote significance among treatments at each measurement date (ns = not significant, $*P \leq 0.05$).

ruled out as the explanation for the reduction in xylem transport (Bondada et al., 2005; Keller et al., 2006). In apple, more research is needed to determine if early losses to xylem conductance in 'Honeycrisp' are a factor contributing to its susceptibility to bitter pit.

There were differences in sunburn development between 'WA 38' and 'Honeycrisp'. Fruit sunburn browning is induced under high solar radiation when the apple surface temperature reaches between 46 and 49 °C (Schrader et al., 2008). High light conditions induce anthocyanin production to tolerate photooxidative stress (Li et al., 2008; Makeredza et al., 2015). 'Honeycrisp' has been reported to have among the lowest fruit surface temperature thresholds for the development of sunburn browning (Schrader et al., 2008). Chlorophyll degradation during fruit ripening before harvest leaves fruit particularly susceptible to sunburn development (Morales-Quintana et al., 2020) and earlier maturation in 'Honeycrisp' when daily maximum temperatures are greater may be a contributing factor to the high incidence of sunburn in 'Honeycrisp'. It is also possible that the red coverage can mask sunburn browning in red cultivars like 'WA 38' (Makeredza et al., 2015). Here we found 'Honeycrisp' had significantly less red coverage than 'WA 38'. Fruit coloring differences are already known to affect sunburn development, as has been reported for red 'Anjou' pears and green 'Anjou' pears (Li et al., 2008).

Other fruit quality traits differed between the two cultivars, including starch index and fruit firmness. Flesh firmness is influenced by cell wall strength and turgor pressure (Mignani et al., 1995). The cell wall is the largest pool of calcium in fruit tissues (Harker and Venis, 1991; White and Broadley, 2003) such that previous research has shown that calcium-rich fruit retain their firmness longer because calcium in the cell wall can delay degradation during ripening (do Amarante et al., 2013; Fallahi et al., 1996; Hirschi, 2004). Previous work has reported that 'WA 38' apples have a firmness of ≈8.75 kg (Evans et al., 2012), which is similar to our results (9.67 kg in 2018 and 7.58 kg in 2019). In this study, 'WA 38' was significantly firmer than 'Honeycrisp'. Although firmness is an indicator of maturity, we do not accredit the increase in firmness to immature fruit. We observed that most 'WA 38' apples were in the 2 and 3 starch classes in 2018 and 2019.

Table 2. Calcium (Ca), potassium (K), and magnesium (Mg) content (mg/g) of ‘Honeycrisp’ (HC) and ‘WA 38’ leaves at various sampling dates in 2018 and 2019 growing seasons. Symbols denote significance among treatments at each measurement date (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

	30 DAFB			50 DAFB			70 DAFB			90 DAFB			Harvest		
2018 Leaves	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value
Ca (mg/g)	12.9 ± 0.37	20.0 ± 3.31	0.07	11.3 ± 0.60	10.8 ± 0.83	0.63	11.4 ± 0.44	8.93 ± 0.67	*	20.2 ± 0.55	20.4 ± 3.22	0.95	16.7 ± 0.077	11.2 ± 0.411	0.24
K (mg/g)	14.5 ± 0.55	47.3 ± 7.75	*	16.7 ± 0.64	21.6 ± 0.76	**	16.6 ± 0.066	22.7 ± 0.066	***	24.3 ± 0.86	36.6 ± 4.44	*	11.4 ± 0.37	19.6 ± 6.99	0.29
Mg (mg/g)	0.24 ± 0.010	0.65 ± 0.132	*	30.8 ± 0.08	31.5 ± 0.04	0.77	3.07 ± 0.20	2.76 ± 0.11	0.22	5.2 ± 0.27	5.7 ± 0.81	0.64	1.84 ± 0.10	3.93 ± 1.38	0.18
K+Mg/Ca	1.32 ± 0.046	2.68 ± 0.0	***	1.76 ± 0.113	2.32 ± 0.118	*	1.73 ± 0.072	2.88 ± 0.136	***	1.46 ± 0.053	2.11 ± 0.089	***	0.80 ± 0.035	2.22 ± 0.126	***
	30 DAFB			50 DAFB			90 DAFB			110 DAFB			Harvest		
2019 Leaves	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value
Ca (mg/g)	5.01 ± 0.32	6.09 ± 0.14	*	6.23 ± 0.46	6.00 ± 0.40	0.72	10.5 ± 1.08	10.0 ± 0.50	0.70	11.4 ± 0.25	10.6 ± 0.3	0.09	21.1 ± 0.95	8.98 ± 0.15	***
K (mg/g)	12.8 ± 0.33	13.7 ± 0.20	0.05	10.0 ± 0.31	14.5 ± 0.80	***	6.5 ± 0.08	11.1 ± 0.52	***	7.01 ± 0.39	10.2 ± 0.40	**	10.8 ± 0.60	9.33 ± 0.55	0.12
Mg (mg/g)	1.57 ± 0.03	1.59 ± 0.08	0.89	1.72 ± 0.02	1.77 ± 0.14	0.75	1.93 ± 0.13	1.73 ± 0.43	0.21	1.66 ± 0.01	1.65 ± 0.01	0.90	2.32 ± 0.05	1.69 ± 0.10	***
K+Mg/Ca	2.89 ± 0.078	2.51 ± 0.078	0.07	1.90 ± 0.138	2.75 ± 0.226	*	0.81 ± 0.070	1.28 ± 0.055	**	0.76 ± 0.048	1.12 ± 0.056	**	0.63 ± 0.03	0.12 ± 0.04	***

DAFB = days after full bloom.

Table 3. Calcium (Ca) potassium (K), and magnesium (Mg) (μg/g) content of ‘Honeycrisp’ (HC) and ‘WA 38’ fruit at various sampling dates in 2018 and 2019 growing seasons. Symbols denote significance among treatments at each measurement date (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

	30 DAFB			50 DAFB			70 DAFB			90 DAFB			Harvest		
2018 Fruit	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value
Ca (μg/g)	1,939 ± 170	6,440 ± 1,172	**	592 ± 64.1	1,532 ± 101	***	742 ± 89.0	1,245 ± 85.6	**	813 ± 131	1,721 ± 130	**	432 ± 15.1	600 ± 77.2	0.08
K (μg/g)	30,378 ± 2,154	30,895 ± 2,729	0.89	12,717 ± 429	14,083 ± 431	0.07	10,725 ± 733	11,319 ± 108	0.45	10,997 ± 459	12,259 ± 555	0.13	13,195 ± 680	9,616 ± 226	**
Mg (μg/g)	2,060 ± 229	3,104 ± 435	0.08	594 ± 24.9	928 ± 55.9	**	467 ± 33.3	612 ± 24.9	*	590 ± 56.0	767 ± 36.7	*	517 ± 32.2	510 ± 8.30	0.84
K+Mg/Ca	16.8 ± 0.341	5.53 ± 0.476	***	23.4 ± 2.687	9.9 ± 0.687	**	16.1 ± 2.908	9.69 ± 0.546	0.07	15.2 ± 2.020	7.75 ± 0.888	*	31.8 ± 1.292	18.1 ± 3.123	**
	30 DAFB			50 DAFB			90 DAFB			110 DAFB			Harvest		
2019 Fruit	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value	HC	WA 38	P value
Ca (μg/g)	2,382 ± 252	3,902 ± 489	*	928 ± 55.0	1,499 ± 43.3	***	518 ± 260	410 ± 29.0	0.70	153 ± 31.5	163 ± 26.3	0.82	295 ± 78.3	240 ± 45.3	0.56
K (μg/g)	22,366 ± 233	23,181 ± 565	0.23	17,694 ± 591	16,028 ± 549	0.08	10,308 ± 204	9,619 ± 510	0.26	10,065 ± 417	9,473 ± 197	0.25	12,944 ± 1,939	9,895 ± 602	0.18
Mg (μg/g)	1,934 ± 114	2,309 ± 222	0.18	999 ± 34.2	949 ± 25.7	0.29	415 ± 17.3	395 ± 9.67	0.35	400 ± 11.8	375 ± 4.79	0.09	465 ± 57.1	409 ± 34.0	0.43
K+Mg/Ca	10.5 ± 0.961	6.85 ± 0.892	*	20.4 ± 1.609	11.3 ± 0.423	**	68.4 ± 38.15	25.04 ± 3.13	0.30	79.5 ± 17.64	64.50 ± 8.07	0.47	60.3 ± 10.1	46.1 ± 5.61	0.26

DAFB = days after full bloom.

These results indicate that ‘WA 38’ apples were harvested at proper maturity, as the marketing standard is that fruit should not be harvested until a minimum of 2.5 starch class is reached to a maximum of 3.5 starch class (Cosmic Crisp, 2019). ‘Honeycrisp’ is different from ‘WA 38’, in which starch degradation is often a poor indicator of maturity. ‘Honeycrisp’ fruit was harvested at a starch index of ≈4.5.

Conclusion

Here, despite a smaller canopy and smaller fruit weight, ‘Honeycrisp’ had greater bitter pit than ‘WA 38’, indicating physiological differences between the cultivars that contribute to the susceptibility of ‘Honeycrisp’ to bitter pit. Leaf calcium content was greater for ‘Honeycrisp’ and fruit calcium content was greater for ‘WA 38’. ‘WA 38’ had higher fruit calcium content and lower (K+Mg)/Ca ratios that corresponded to reduced bitter pit incidence. Instantaneous measures of transpiration and xylem sap mineral nutrient composition did not account for differences in fruit mineral nutrient composition. However, xylem conductance was maintained for longer during fruit development in ‘WA 38’ than ‘Honeycrisp’, indicating a longer duration of calcium uptake into the fruit. Fruit was also denser for ‘WA 38’ than ‘Honeycrisp’. Differences in maturity timing and limited red color development likely contributed to an enhanced susceptibility to sunburn for ‘Honeycrisp’. Furthermore, starch degradation patterns strongly differed among cultivars at commercial harvest, indicating differences in ripening progression that could also contribute to the manifestation of physiological disorders like bitter pit and sunburn browning. This research further supports the possible importance of xylem conductance in fruit calcium transport and susceptibility to bitter pit in ‘Honeycrisp’ apple.

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Supplemental Fig. 1. ‘Honeycrisp’ (left) and ‘WA 38’ (right) apple at harvest on 30 Aug. 2018 and 26 Sept. 2018, respectively, showing a more robust canopy for ‘WA 38’ than ‘Honeycrisp’ apple.