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ARTICLE

Fruit canopy positioning affects fruit calcium and potassium concentrations, disorder incidence, and fruit quality for 'Honeycrisp' apple

Lee Kalcsits, James Mattheis, Luca Giordani, Michelle Reid, and Katie Mullin

Abstract: One advantage of high-density apple orchard systems is homogeneity in fruit maturity and quality. However, even in modern orchard systems, variation in fruit quality occurs. 'Honeycrisp' apple is susceptible to numerous disorders including bitter pit, soft scald, and poor colour development. Heterogeneity in fruit quality and nutrient distribution can lead to variation in fruit storability. Here, we tested the effect of within-canopy position on fruit calcium and potassium concentrations, quality, and disorder development for fruit across nine representative high-density orchards. Calcium concentrations were greater in the upper part of the canopy compared with the lower part. Potassium was more evenly distributed within the canopy. Calcium concentrations and potassium-to-calcium ratios were significantly correlated with mean bitter pit incidence, which was between 20% and 30% in the lower half of the tree and <15% in the upper half. Fruit quality was significantly affected by the position in the canopy and was not constrained to only colour, but also other quality metrics such as dry matter, size, and firmness. Additionally, the internal ethylene concentrations (IECs) of fruit in the upper canopy were approximately 50% of the IECs for fruit from the lower canopy. With an increased emphasis on uniformity and predictability of fruit for long-term storage, these results underscore the importance of understanding variation within the canopy. Even for high-density systems, significant variation in fruit quality can occur and fruit from the upper canopy has lower disorder incidence and higher fruit quality than fruit from the lower canopy.

Key words: bitter pit, nutrition, distribution, *Malus* × *domestica*, elemental concentration.

Résumé : Un avantage des pommeraies denses est que les fruits obtenus ont une qualité et un degré de maturité homogènes. Toutefois, les vergers aménagés de façon moderne eux-mêmes peuvent donner des fruits de qualité variable. Ainsi, la pomme Honeycrisp est sujette à divers problèmes, parmi lesquels la fossette amère, l'échaudure molle et une piètre coloration. Une qualité et une répartition des oligoéléments inégales affecteront la durée de conservation. Les auteurs ont examiné les effets de l'emplacement du fruit dans le feuillage sur la concentration de calcium et de potassium et le développement de carences dans neuf vergers à haute densité représentatifs. La concentration de calcium est plus élevée dans les fruits situés dans la partie supérieure du couvert que chez ceux situés dans la partie inférieure. Celle de potassium est répartie plus uniformément dans le couvert. La concentration de calcium et le rapport potassium:calcium présentent une corrélation significative avec l'incidence moyenne de la fossette amère, qui affecte vingt à trente des fruits pour cent dans la moitié inférieure de l'arbre et moins de quinze pour cent dans la moitié supérieure. La qualité du fruit est aussi sensiblement affectée par la position de ce dernier dans le feuillage, pas seulement au niveau de la coloration, mais également d'autres paramètres comme la quantité de matière sèche, le calibre et la fermeté. D'autre part, la concentration d'éthylène à l'intérieur des fruits situés dans le haut de l'arbre correspond environ à la moitié de celle relevée dans les fruits se développant plus bas. Puisqu'on insiste davantage sur des fruits uniformes et sur la capacité de prévoir leur durée de conservation, ces résultats soulignent combien il importe de comprendre les variations survenant dans le couvert. En effet, la qualité du fruit peut varier sensiblement, même dans les vergers très denses, et les fruits situés dans le haut de l'arbre présentent moins de défauts que ceux poussant plus bas, donc leur qualité est supérieure. [Traduit par la Rédaction]

Mots-clés : fossette amère, nutrition, distribution, *Malus* × *domestica*, concentration des oligoéléments.

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Introduction

Fruit size and colour development are two of the major factors that determine apple (*Malus* × *domestica*) fruit quality for the consumer (Musacchi and Serra 2018). Furthermore, the susceptibility of specific apple cultivars such as 'Honeycrisp' to postharvest disorders can lead to large losses in marketable fruit (Rosenberger et al. 2001). While it is well known that there can be significant variation in fruit quality among and within orchards, less is known about the variability in fruit quality within individual trees from high-density orchard systems. In large pear trees, Zhang et al. (2016) identified significant variability in fruit quality related to canopy position. Variability with canopy position has also been reported in figs (Trad et al. 2013) and peaches (Wu et al. 2005). Much of this variability has been associated with light penetration into the canopy (Robinson et al. 1983; Zhang et al. 2016). Furthermore, interior canopy positions have been shown to be more sensitive to chilling injury disorders in peaches (Lurie and Crisosto 2005). In apple, the adoption of high-density orchard systems has led to simpler tree canopies with more uniform light penetration (Palmer et al. 2002). High-density orchard systems were developed to increase labour efficiency, productivity, and uniformity in fruit quality. Increased light interception and smaller canopies produce greater amounts of carbohydrates to allocate to developing fruit (Barritt et al. 1987; Palmer et al. 2002). More uniform canopy light environments result in more uniform fruit quality (Musacchi and Serra 2018). Nevertheless, for trees grafted on dwarfing rootstocks, there can be high variability in fruit quality and disorder incidence within orchards that can lead to elevated losses and makes predicting storability more difficult.

One of the most prevalent physiological disorders in apple is bitter pit, which is analogous to physiological disorders in other crops such as blossom end rot in tomato, cork spot in pear, leaf-burn in lettuce, and senescent breakdown in melons (Ho and White 2005; de Freitas and Mitcham 2012; Hocking et al. 2016). There is strong variation in bitter pit susceptibility among apple cultivars (Volz et al. 2006). The development of disorders such as bitter pit has been linked to low bulk calcium concentrations in fruit (Ferguson and Watkins 1989; Telias et al. 2006). In particular, Rosenberger et al. (2004), reported a close relationship between fruit calcium concentrations and bitter pit incidence for 'Honeycrisp' apple. However, this relationship can be inconsistent (Cheng and Sazo 2018). Disorder incidence has been more closely associated with the balance between calcium and other nutrients such as potassium, magnesium, and nitrogen (Ferguson and Watkins 1989; Kalcsits 2016).

Bitter pit incidence has also been associated with other factors such as variability in crop load (Serra et al. 2016), environment (Perring 1979), and vegetative vigor (Bramlage 1992). Specifically, calcium uptake and distribution to fruit has been attributed to fruit transpiration rates (de Freitas and Mitcham 2012; Hocking et al. 2016). High transpiration rates in leaves, low transpiration rates for fruit, or a combination of both can create imbalances in the distribution of calcium to developing fruit. Variability in transpiration rates of fruit can affect fruit calcium accumulation and has been reported in apricot (Montanaro et al. 2010) and kiwifruit (Montanaro et al. 2014). Light intensity and relative humidity can affect transpiration rates and, therefore, calcium accumulation rates (Montanaro et al. 2006). Regions of the tree with high transpiration rates will have greater calcium deposition than regions with low transpiration. These differences are magnified due to the poor mobility of calcium within the plant and strong mobility of other nutrient like potassium, magnesium, and nitrogen (Hill 1980). Because of this we can hypothesize that, even in high-density orchard systems, there can still be significant inter-tree variability in calcium transport that can have implications for fruit susceptibility to disorders like bitter pit.

It is well known that tree architecture can influence fruit colour development and quality attributes such as soluble solids concentration (SSC), titratable acidity (TA), and dry matter (DM). Furthermore, the effect of branch fruiting position on fruit quality has been reported (Volz et al. 1994). However, the effect of fruiting position within the tree on overall fruit quality, particularly in dwarf apple trees, still needs to be more clearly established. This is particularly important for cultivars like 'Honeycrisp' where, in many cases, variability in fruit quality, colour development, and disorder incidence can be great and efforts to understand the factors contributing to this variability are scientifically and economically important. Since fruit uniformity is required to make accurate storage decisions for apple, a better understanding of intra-tree variability in fruit quality and disorder incidence is needed. Furthermore, the relationship between fruit quality metrics, nutrient distribution, and disorder incidence needs to be more closely explored. The objectives of this study were to understand whether height and radial distance from the trunk are important factors in quality, disorder development, and nutrient composition in fruit from dwarf apple trees.

Materials and Methods

Sampling locations

Nine irrigated commercial 'Honeycrisp' apple orchards were selected for uniform age and productivity (Table 1). These orchards ranged in distance by approximately 380 km. Bloom time ranged from 1 to 20 Apr. 2015. Soil types were medium to coarse-textured and irrigation was applied with a combination of drip irrigation and microsprinklers for all orchards. Within each orchard, nine trees were selected based on uniformity

Table 1. Orchard descriptions for sampling locations of ‘Honeycrisp’ apples in Washington state in 2015.

Orchard location	Elevation (m)	Year planted	Training system	Rootstock	Irrigation type	Spacing
Burbank, WA	123	2007	V-trellis	Bud-9	Drip/microsprinkler	0.45 m × 3.6 m
Royal City, WA	352	2010	Vertical	M9-T337	Drip/microsprinkler	0.9 m × 3.6 m
Quincy, WA	415	2009	Vertical	M9-T337	Drip/microsprinkler	0.9 m × 3.6 m
Royal City, WA	347	2008	Vertical	M9-T337	Drip/microsprinkler	0.9 m × 3.6 m
Tonasket, WA	273	2010	Vertical	M9-T337	Drip/microsprinkler	0.9 m × 3.6 m
Quincy, WA	419	2008	Vertical	M9-T337	Drip/microsprinkler	0.9 m × 3.6 m
Quincy, WA	410	2010	Vertical	M9-T337	Drip/microsprinkler	0.9 m × 3.6 m
Chelan, WA	525	2011	Vertical	M9-T337	Drip/microsprinkler	0.6 m × 3.6 m
Kittitas, WA	563	2009	Vertical	M9-T337	Drip/microsprinkler	0.9 m × 3.6 m

of trunk cross-sectional area (TCSA), height, and crop load to ensure that trees were representative of that orchard location. Then, in early June, trees were thinned to an average crop load of 5 fruit cm^{-2} TCSA with even distribution throughout the tree canopy, as recommended in previous literature (Robinson and Watkins 2003; Serra et al. 2016). To remove sampling bias as a factor, at the time of thinning, 16 fruit were randomly numbered per tree, with two fruit in each quadrant in high and low regions of the tree by placing a small piece of tape around each pedicel. Fruit was picked from each orchard all at one time just prior to the initial commercial harvest. These fruit were then sorted with even-numbered fruit placed in long-term storage (4 mo at 2 °C) to measure disorder incidence, and oddnumbered fruit used for fruit quality measurements and elemental analysis at harvest. This ensured that there was at least one fruit evaluated from each quadrant of the tree from both high and low positions at harvest and after storage. To standardize for small differences in tree radius and height that could add noise to the data, fruit radial and height distances were expressed relative to the longest branch on the tree. This meant that for trees that were conical shaped, there was no fruit sampled from the outer radial locations or the lower 20% relative height where there was no fruit.

Calcium and potassium analysis using portable X-ray fluorescence

For elemental analysis at harvest, fruit were non-destructively analyzed using a Bruker AXS Tracer 3-V portable handheld X-ray fluorometer (PXRF) analyzer (Bruker Elemental, Kennewick, WA). The PXRF was equipped with a rhodium tube from which X-rays are emitted and a Peltier-cooled, silicon PIN diode detector operating at 15 kV and 25 μA from an external power source for 15 s using no filter under a vacuum at <10 torr, as described in Kalcsits (2016). The beam covered an 8 mm diameter area and fruit samples were positioned with as much contact as possible with the instrument's surface. The X-ray counts were processed using the Artax spectra program developed by Bruker and used as a semi-quantitative approach for measuring calcium and potassium. Counts for calcium and potassium were expressed relative to counts for rhodium to account for small differences in fruit density between samples. Each fruit was measured at four different spots along the equator and the mean used as the measure for each fruit.

Fruit quality

Fruit maturity and quality were assessed non-destructively (weight, fruit size, background colour, peel colour) and destructively [internal ethylene concentration (IEC), fruit firmness, starch degradation, SSC, TA, and DM]. The weight of each fruit was measured to the nearest 0.1 g using an analytical balance (Mettler-Toledo, Columbus, OH). Fruit size (height and diameter) was measured using to the nearest millimeter with a VWR caliper (VWR Instrument, Radnor, PA). Peel background hue was measured on each fruit at a disorder-free spot with minimal red colouration with a CR-300 chromameter (Konica-Minolta, Ramsey, NJ) using CIE illuminant C. Values were transformed to hue angle as described by Hunter and Harold (1987).

The IEC was measured on all fruit by acquiring a 0.5 mL gas sample from the fruit core using a gas-tight syringe inserted into the calyx end of the fruit ($N = 10$). To analyze the ethylene concentrations, the gas samples were then injected into a G1530a gas chromatograph (Agilent Technologies, Wilmington, DE) outfitted with a 50 cm long × 0.32 cm internal diameter glass column packed with Porapak Q (Supelco, Bellafonte, PA) and a flame ionization detector. The injector, oven, and detector temperatures were maintained at 100 °C, 35 °C, and 300 °C, respectively. Fruit firmness was measured on opposite sides of each fruit with a MTD2 analyzer (Mohr and Associates, Richland, WA) fitted with an 11 mm diameter probe after the fruit peel was removed. Values reported include maximum firmness in the outer 0.64 cm (M1) and interior (0.64 cm to the core line, M2) regions. Starch degradation was visually assessed on a full-width tissue slice cut from the fruit equator dipped in 30 mmol L^{-1} I-KI (potassium-iodine solution) and rated on a scale of 1–6 (Hanrahan 2012), with 1 = no hydrolysis, all cortex black and 6 = hydrolysis complete, cortex unstained. The SSC and TA were determined using freshly prepared juice extracted from composite samples of segments from all apples used for firmness testing (combined juice from two fruit) using a refractometer (Atago N1, Bellevue, WA) and a TIM850 titrator (Radiometer, Lyon, France), respectively. The TA (expressed as % malic acid) was determined by titrating a 10 mL juice sample with 0.1 mol L^{-1} KOH to an end point of pH 8.2. Dry matter was assessed on equatorial slices by measuring the fresh weight of slices after removing the core and then measuring the fresh weight again after drying in an oven at 60 °C for 7 d.

Following 4 mo in storage at 2 °C plus 7 d at 20 °C, the quality metrics fruit weight, background colour, IEC, firmness, SSC, TA, and DM were assessed again. External and internal disorders including bitter pit were also rated on each fruit at this time. Fruit disorders (bitter pit, leather blotch, cortex browning, stem rot, greasiness, heat stress, calyx browning, cortex browning, cavities, decay, and shrivel) were reported as incidence per fruit. Bitter pit was determined as absent or present for surface lesions <5 mm in diameter with underlying brown, corky tissue. Irregularly shaped peel areas of rough, brown tissue >5 mm was considered leather blotch. Disorder incidence was assessed as counts of affected fruit per replicate and then calculated as a percentage of fruit per replicate.

Statistical analysis

Data were collected for individual fruit and then classified based on the relative positioning in the tree. Data were analyzed in OriginPro using an unbalanced one-way analysis of variance and tested for homogeneity and equal variances within either the horizontal or vertical relative canopy positions. Linear regression was used to test whether there was a significant difference between relative canopy position, fruit quality, and fruit elemental composition.

Results

Fruit calcium and potassium variation with vertical position

There was significant within-tree variation in the calcium and potassium concentrations of fruit in high-density apple trees (Fig. 1). Fruit calcium was more variable within the tree than potassium, which was mostly evenly distributed throughout the canopy. There was less than a two-fold difference in relative potassium concentrations (Fig. 1B) within the tree but as much as a six-fold difference in relative calcium concentrations (Fig. 1A) between locations in the tree. Calcium concentrations were significantly greater in the upper canopy compared with the lower canopy. The two-dimensional distribution of the potassium-to-calcium ratio (Fig. 1C) for fruit along both the radial and vertical gradients show that the areas where the ratios were lower were in the upper or outer regions of the canopy. Fruit calcium concentrations varied with relative vertical position in the canopy (Fig. 2A) but did not significantly vary according to radial position in the canopy. Calcium concentrations in fruit sampled from the upper 20% of the canopy were approximately twice that of fruit from the lower half of the tree (Fig. 2A). There was a significant positive relationship between relative height in the canopy and relative calcium concentration determined by PXRF ($P = 0.0011$; $r^2 = 0.91$); however, potassium was less affected by vertical canopy position and there was no significant relationship. These differential patterns in concentrations along vertical sampling gradients significantly changed the potassium-to-calcium ratios for fruit (Fig. 2B). The potassium-to-calcium ratio was significantly lower in the upper 20% of the canopy compared with the lower half of the tree.

Fig. 1. Mean semi-quantitative (A) calcium concentrations, (B) potassium concentrations, and (C) potassium-to-calcium ratio of fruit with changing relative canopy vertical position (*y* axis) and relative radial distance from the trunk (*x* axis, where 0 is equal to the trunk and 100% is equal to the end of the branch) acquired using a portable X-ray fluorometer (PXRF). Black tiles indicate where there was no fruit present in that location. [Colour online.]

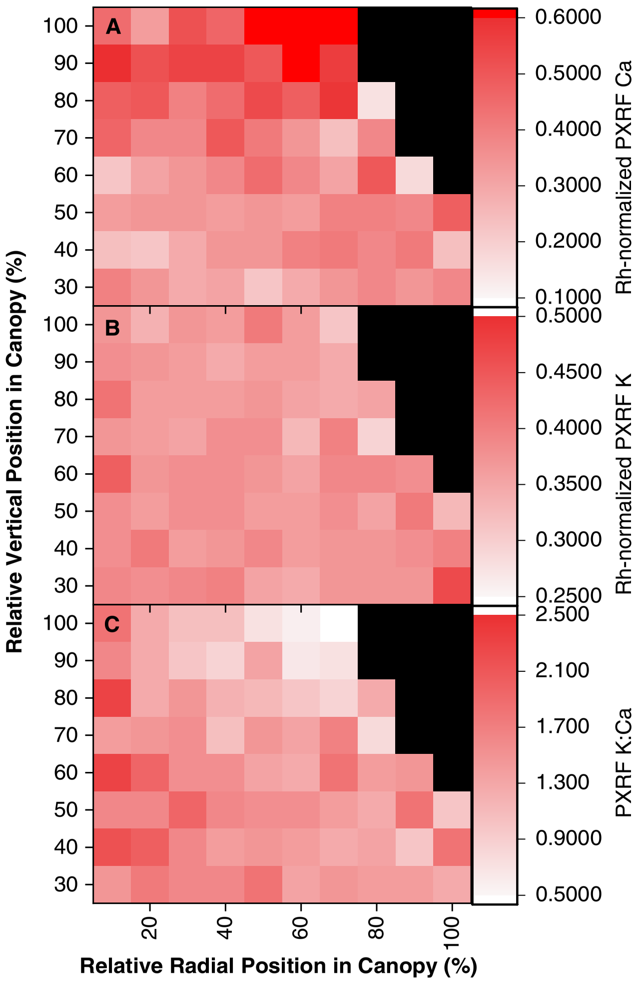


Fig. 2. (A) Rhodium-normalized portable X-ray fluorescence (PXRF) counts for calcium (open circles) and potassium (closed circles) plotted against relative canopy height position (%) for 'Honeycrisp' apple fruit at harvest. Lines represent best non-linear fit for calcium (dashed line) and linear fit for potassium (solid line). (B) Potassium-to-calcium ratios acquired from portable X-ray fluorescence plotted against relative canopy height position (%). Solid line represents best non-linear fit for mean potassium-to-calcium ratio at each relative canopy height position (**, $P < 0.01$; ***, $P < 0.001$)

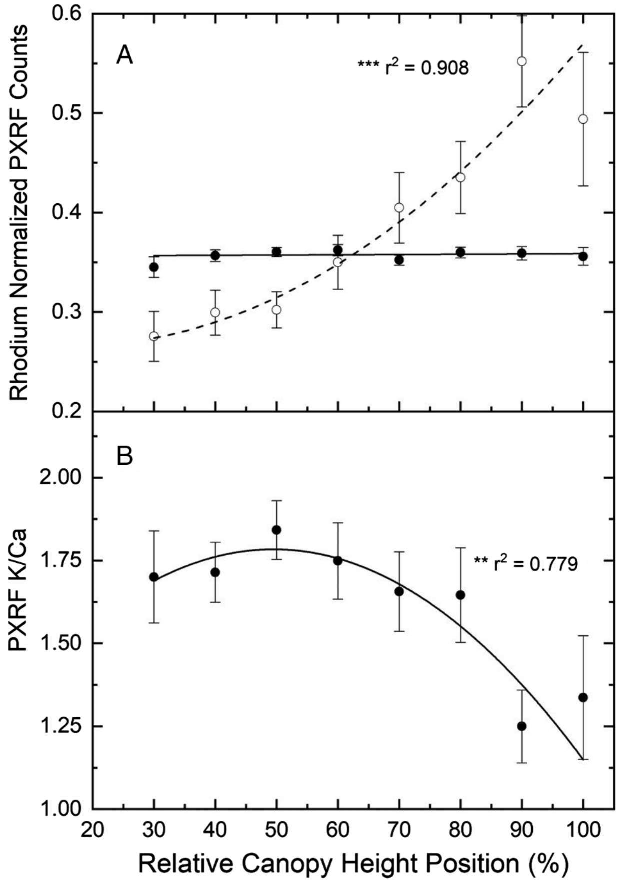
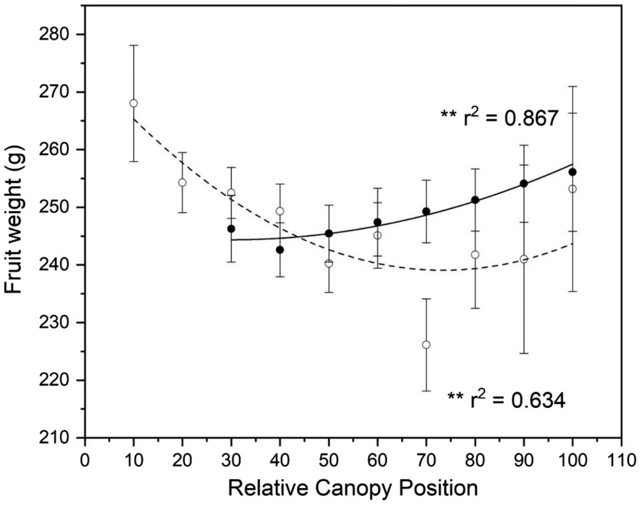


Fig. 3. Mean fruit weight (g) of 'Honeycrisp' apples sampled across vertical (closed circles) and horizontal (open circles) relative canopy positions. Lines represent significant regression relationships for vertical (solid line) and horizontal (dashed line) canopy distribution (**, $P < 0.01$). Low percentages (%) represent either closer proximity to the trunk for horizontal canopy position or at the bottom of the tree for vertical position.

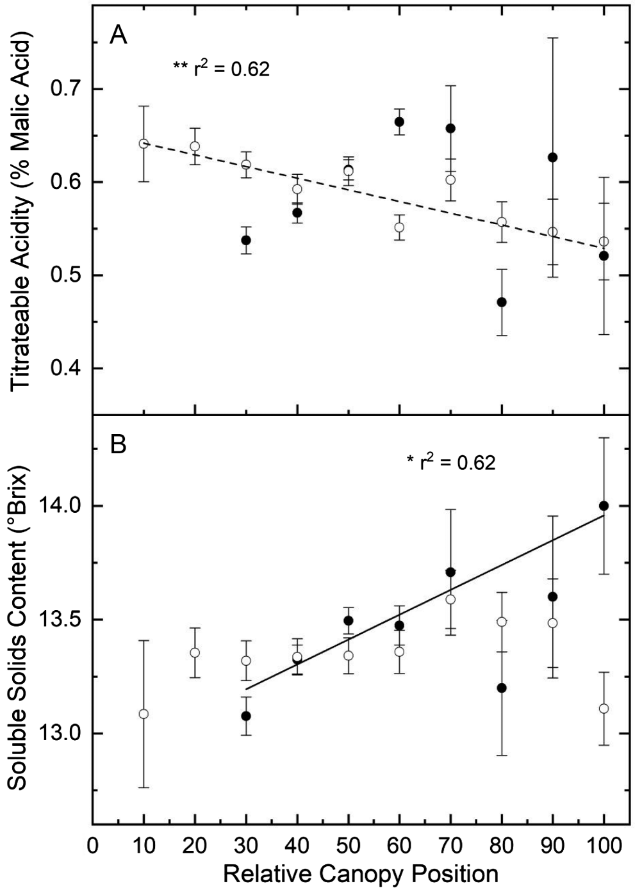


Fruit quality variation by canopy position and maturity

Fruit weight varied more between orchards than within orchards. However, there was as much as a two-fold variation in fruit weight within orchards, where the mean fruit weight ranged from an average of 165 to 342 g (data not shown). Although crop load was reported to be a major contributor of fruit weight at harvest (Serra et al. 2016), crop load was carefully controlled in this study. Fruit weight across all sampled fruit ranged from 122 to 482 g. Fruit weight was significantly affected by canopy position (Fig. 3) and was greater in fruit from the upper canopy compared with fruit from the lower canopy. The average fruit weight for the upper half of the canopy was 252 g, while the fruit weight for the lower half of the trees was 245 g. There was a significant relationship between the relative radial distance from the trunk and fruit weight ($P = 0.012$; $r^2 = 0.63$), where fruit weight was >250 g near the trunk but as the distance from the trunk increased, fruit weight was decreased, with an average fruit weight of approximately 230 g at the outside canopy (Fig. 3). Therefore, the largest fruit size occurred in regions closest to the trunk in the upper half of the canopy.

The SSC was higher in the upper canopy, with a significant positive relationship between SSC and relative canopy height ($P = 0.013$; $r = 0.79$; Fig. 4B). The mean SSC ranged between 13 and 14 °Brix both vertically and radially within the tree. There was no relationship between vertical canopy position and TA, which ranged from 0.45% to 0.67% malic acid and was independent of SSC. However, there was a significant negative relationship between radial canopy position and TA ($P = 0.004$; $r = -0.79$; Fig. 4A). Both DM and M2 fruit firmness followed similar patterns to SSC, where fruit was firmer and DM was greater for fruit from the upper canopy (Figs. 5C and 5E). Internal ethylene concentrations were lower in the upper part of the tree and there was a negative relationship with vertical canopy position ($P = 0.015$; $r = 0.77$; Fig. 5A); however, IEC increased as radial position in the tree increased ($P = 0.005$, $r = 0.77$; Fig. 5A). Fruit starch and M1 firmness were unaffected by canopy position (Figs 5D and 5B). Peel colour development was less clearly impacted by canopy position and overall colour development was well developed across all fruit in these high-density systems; however, there were some significant relationships that were related to canopy position (Fig. 6). Fruit from the outer canopy had greater

Fig. 4. Mean (A) titratable acidity and (B) soluble solids concentration of 'Honeycrisp' apple across vertical (closed circles) and horizontal (open circles) relative canopy position sampled from nine different orchards. Lines represent significant linear relationship for vertical (solid line) and horizontal (dashed line) canopy distribution (*, $P < 0.05$; **, $P < 0.01$). Regression lines are only shown for significant linear relationships. Low percentages (%) represent either closer proximity to the trunk for horizontal canopy position or at the bottom of the tree for vertical position.

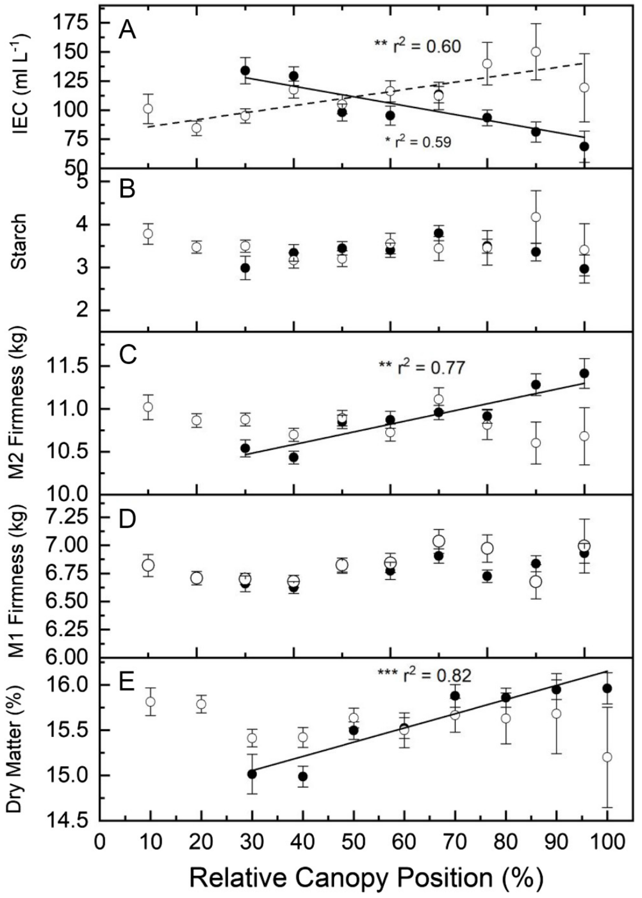


b^* values (more red colour) than fruit from radial positions closer to the trunk ($P = 0.0005$, $r = 0.88$) and lower in the tree ($P = 0.001$, $r = -0.9$). Interestingly, fruit from lower in the canopy on the outer branches had greater Chroma values than fruit from the upper part of the tree closer to the trunk.

Disorder incidence in relation to relative canopy position

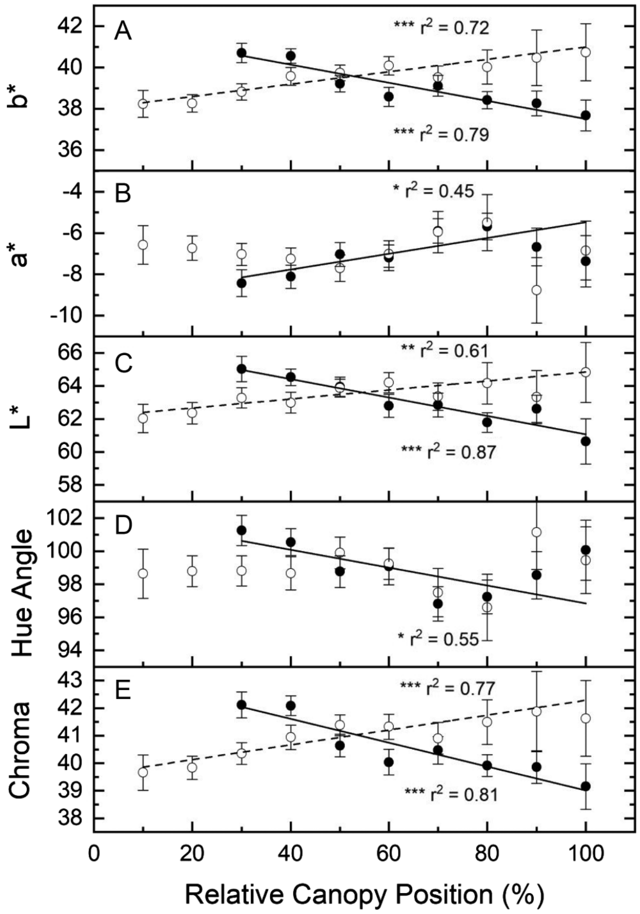
Bitter pit incidence was approximately 20% for fruit sampled across the nine orchards in this study (data not shown). There was significant variability in bitter pit incidence that was dependent on the position of the fruit in the tree. Some regions in the tree did not have any bitter pit while other regions had more than 60% of fruit affected by bitter pit. Bitter pit incidence was the greatest in the lower and inner regions of the tree (Fig. 7, Table 2). Bitter pit incidence varied vertically in the tree and was significantly lower in the upper half of the tree compared with the lower half (Fig. 8). Canopy position also affected the incidence of other disorders during storage (Table 2). Between 8.2% and 17.0% of fruit was greasy in the lower half of the tree compared with between 0% and 2.9% in the upper 20% of the tree. Fruit harvested from lower canopy positions had a higher incidence of soft scald ($P = 0.05$; $r = -0.60$). The only disorder that was related to the radial position in the canopy was fruit rot, where fruit from the inner canopy had a greater incidence of rot ($P < 0.05$; $r = -0.56$).

Fig. 5. Mean (A) internal ethylene concentration (mL L^{-1} IEC), (B) starch index rating, (C) M1 and (D) M2 fruit firmness (kg), and (E) dry matter (%) of 'Honeycrisp' apples sampled across vertical (closed circles) and horizontal (open circles) relative canopy positions. Low percentages (%) represent either closer proximity to the trunk for horizontal canopy position or at the bottom of the tree for vertical position. Lines represent significant linear relationship for vertical (solid line) and horizontal (dashed line) canopy distribution (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Regression lines are only shown for significant linear relationships.



While variable within the canopy, the potassium-to-calcium ratio of the fruit was most closely related to bitter pit incidence; this ratio in the fruit explained 48% of

Fig. 6. Mean (A) *L**, (B) *a**, (C) *b**, (D) hue angle, and (E) Chroma of 'Honeycrisp' apples sampled across vertical (closed circles) and horizontal (open circles) relative canopy positions. Low percentages (%) represent either closer proximity to the trunk for horizontal canopy position or at the bottom of the tree for vertical position. Lines represent significant linear relationship for vertical (solid line) and horizontal (dashed line) canopy distribution (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Regression lines are only shown for significant linear relationships.



the variation observed in bitter pit incidence (Fig. 9C). Within each relative canopy position encompassing a 10% by 10% positioning grid, fruit calcium concentrations explained 29% of the variation observed in bitter pit incidence (Fig. 9A). Potassium, however, was not significantly related to bitter pit incidence (Fig. 9B).

Discussion

Calcium and potassium variation within a high-density apple canopy

Across orchards and trees, there was a significant relationship between tree position and fruit calcium and potassium concentrations, where higher calcium concentrations were seen in the upper part of the canopy. These results contrast the reported results in Jackson and Sharples (1971), where lower calcium concentrations in the upper canopy were reported. The reasons behind these contrasting results could be linked to tree size and vigor, where trees were larger and not densely planted in the earlier study compared with the high-density, low-vigor plantings in the present study. The mechanisms underlying these variations are unclear; however, they may be a result of within-tree variation in plant transpiration and distribution. The association between plant transpiration and the accumulation of calcium in plant organs has been well established (Montanaro et al. 2010, 2014; de Freitas and Mitcham 2012; Hocking et al. 2016). Higher transpiration rates lead to greater deposition of calcium in those organs. These studies and reviews have addressed genetic and treatment differences affecting these rates in whole plants. However, the relationship within individual canopies has been less explored and has significant implications for sampling methods. Transpiration of leaves and organs would be dependent on temperature, wind, relative humidity, and physiological transpiration control such as lenticels and stomata. Transpirational pressure should be greater on the outer parts of trees since these regions would be more exposed to high light and low humidity conditions that would have greater transpirational pressure and therefore, increased calcium deposition in those organs. This was observed in this experiment across many orchards and trees, where the outer regions of the canopy had greater fruit calcium concentrations than fruit from the inner regions of the canopy. In contrast, potassium was less variable and was not related to canopy position. Potassium is plant-mobile and may demonstrate a greater degree of remobilization and plasticity than calcium (Van Goor and Wiersma 1974), and therefore, differences in distribution caused by localized environmental conditions may be less likely to have an impact.

Effect of canopy position on fruit quality for simple, compact apple trees

The relationship between locations within the tree was less clear for fruit quality metrics. Variation in fruit quality is often associated with variability in light penetration into the canopy (Jackson and Sharples 1971; Robinson and Lakso 1988). Fruit quality metrics that are associated with carbohydrate loading such as fruit size, SSC, and DM were positively associated with vertical position in the tree. Similar results were reported in stone fruit by Stanley et al. (2014). Upper regions of the tree would also have higher amounts of light absorption and carbon fixation (Corelli-Grappadelli and Coston 1991). Radially, the trends were often less clear, where radial position was not associated with any fruit quality traits except IEC. Additionally, IEC was not associated with starch degradation for 'Honeycrisp'. This has been reported in other research, where starch degradation is a poor indicator of physiological maturity in 'Honeycrisp' apple (Watkins et al. 2005). Peel colour

Fig. 7. Two-dimensional distribution of bitter pit incidence (%) taken from different relative vertical and radial positions in the tree canopy of 78 'Honeycrisp' apple trees from nine commercial orchards. Black tiles indicate where there was no fruit present in that location. [Colour online.]

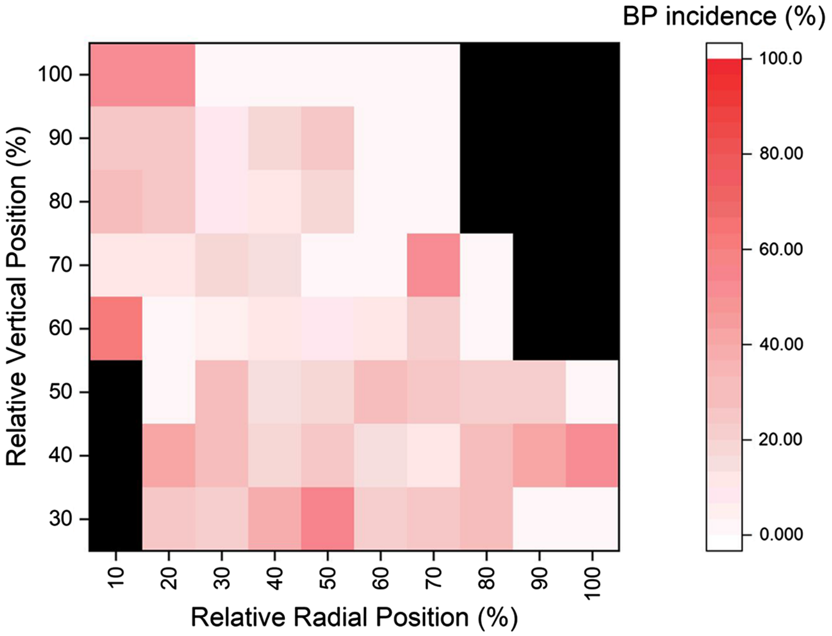


Table 2. Disorder incidence and rot for fruit from relative vertical or radial position in the canopy for fruit from 72 'Honeycrisp' apple trees.

Relative canopy position	Bitter pit	Rot	Greasy	Shrivel	Scald	Soft scald
Vertical orientation						
30	31.1	2.7	8.2	0.0	0.0	4.1
40	23.9	2.5	14.5	0.9	0.0	1.7
50	19.3	0.0	17.0	1.1	1.1	0.0
60	12.9	5.4	15.7	0.0	0.0	0.0
70	12.1	4.2	15.5	0.9	0.0	2.6
80	15.1	4.1	15.1	0.0	0.0	1.1
90	14.7	8.3	2.9	2.9	0.0	0.0
100	14.3	0.0	0.0	0.0	0.0	0.0
r value	−0.79*	0.21	−0.54*	0.22	−0.24	−0.60*
Radial orientation						
10	33.3	0.0	16.7	0.0	0.0	0.0
20	18.6	5.6	16.3	0.0	0.0	3.5
30	15.2	6.0	7.1	1.8	0.0	0.9
40	16.8	3.9	13.7	0.8	0.0	1.6
50	19.0	1.2	17.9	0.0	0.0	2.4
60	15.4	2.5	12.8	1.3	1.3	0.0
70	17.5	2.4	25.0	0.0	0.0	2.5
80	25.0	0.0	5.9	0.0	0.0	0.0
90	7.5	0.0	0.0	0.0	0.0	0.0
100	33.3	0.0	12.5	0.0	0.0	0.0
r value	−0.21	−0.56*	−0.33	0.26	0.02	0.4

Note: Pearson correlation coefficients (Pearson's *r* value) were calculated for each disorder or rot and canopy position (*, $P < 0.05$).

development, like internal quality, was less consistent, but even still, there is consistent variation within the canopy. Fruit produced in dwarfing, simple architecture systems that are just entering their most productive phases of the orchard life would be expected to be relatively uniform in colour development, as light penetration throughout the canopy should also be relatively uniform. However, there were significant negative

Fig. 8. Bitter pit (%) incidence after 4 mo of cold storage at 2 °C for 'Honeycrisp' fruit sampled across vertical canopy position with height.

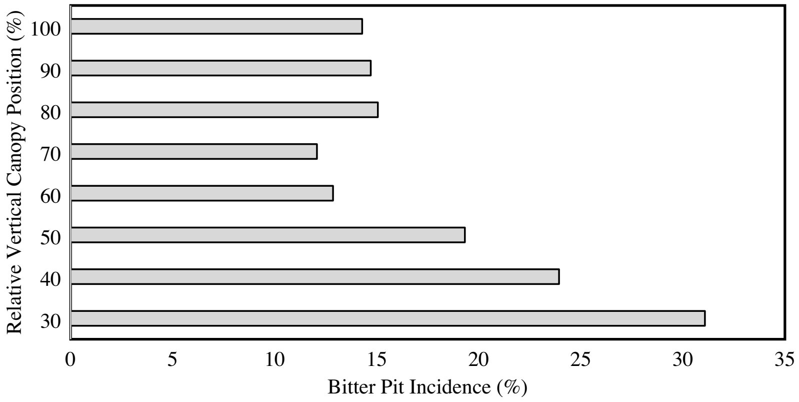
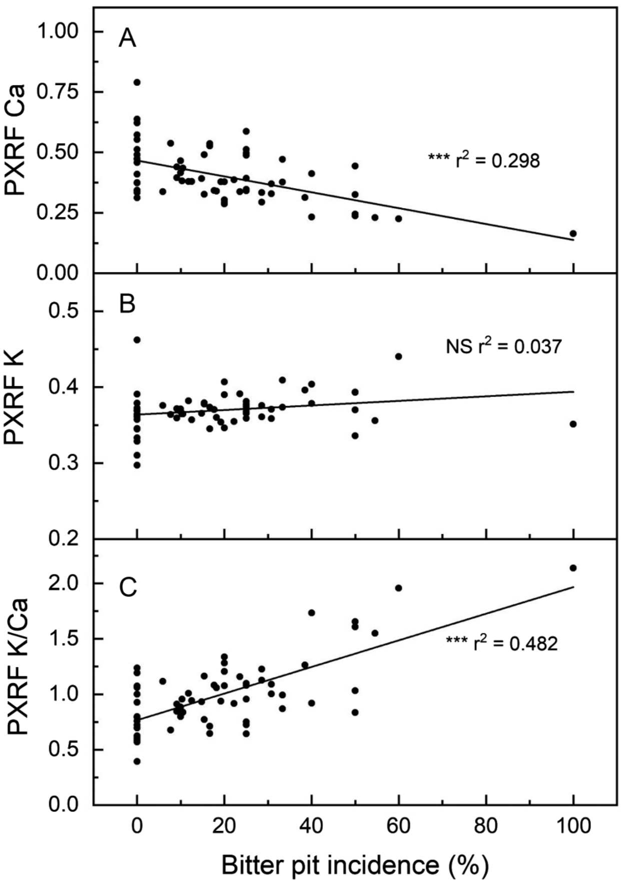


Fig. 9. Linear relationship between relative (A) calcium, (B) potassium, and (C) potassium-to-calcium ratio concentrations measured using PXRF and bitter pit incidence sampled from each classified canopy position from 78 'Honeycrisp' trees from nine commercial orchards.



correlations between both Chroma and hue angle and relative vertical position in the canopy. These values would not have a significant bearing on the colour grading of fruit because the differences in colour ratings using a chromameter were small relative to differences required for grading of fruit into different colour classes (Winzeler and Schupp 2011).

Disorder incidence in relation to canopy position

The distribution of bitter pit in the canopy was strongly related to the distribution of apple fruit calcium in the tree. Although the association between calcium and its ratio with potassium have been well established, the relationship between these factors and bitter pit have not been so clearly identified by location within a tree across a large subset of trees and orchards. The results reported here for 'Honeycrisp' contrast those reported by Jackson and Sharples (1971) for 'Cox's Orange Pippen' in low density apple trees. Here, however, there was also a decrease in greasy fruit and soft scald for fruit harvested from the upper canopy. Since these disorders appear to be somewhat associated with IEC, they may be related to the lower IEC for those same fruit in the upper canopy. Similarly, in stone fruit, internal browning was reported to be lower for fruit from the outer canopy (Crisosto et al. 1997). The greater incidence of rot from inner fruit on the radial axis could also be a function of IEC, where more mature fruit can be more susceptible to some postharvest pathology diseases (Prusky 1996).

Implications for sampling and harvest approaches

Sampling protocols often seek to limit variability in the orchard through even sampling of size, maturity, and location within the tree. However, correcting for these traits can create bias in the sample when attempting to link fruit nutrient status or fruit quality to disorder incidence. This is especially true when equalizing where fruit is collected within the tree. For example, sampling from the sun-exposed portions on outer branches may underestimate the bitter pit risk in the orchard or give a different estimate of maturity and quality than a sample that was entirely random. For the purposes of assessing risk and linking quality metrics to one another, unbiased random samples would be preferred to sorted sampling approaches.

The location on the tree influences nutrient distribution, disorder susceptibility, and fruit quality of 'Honeycrisp' apple, even in dwarfing, high-density trees. Here, we highlight how tree microclimate might contribute to overall nutrient distribution, where greater calcium concentrations are present in the upper canopy, which corresponds to decreased bitter pit incidence in those same locations. The potassium-to-calcium ratio continues to be an important assessment tool for estimating bitter pit risk, not just across an orchard or between trees, but also for segregating fruit within individual trees. These patterns share inter-relatability and have implications for fruit nutrient assessments, fruit quality sampling, and risk assessment in research and commercial scenarios.

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References

Barritt, B.H., Rom, C.R., Guelich, K.R., Drake, S.R., and Dilley, M.A. 1987. Canopy position and light effects on spur, leaf, and fruit characteristics of 'Delicious' apple. HortScience, **22**(3): 402–405.

Bramlage, W.J. 1992. Interactions of orchard factors and mineral nutrition on quality of pome fruit. Acta Hortic. **326**: 15–28. doi:10.17660/ActaHortic.1993.326.1.

Cheng, L., and Sazo, M.M. 2018. Why is 'Honeycrisp' so susceptible to bitter pit? Fruit Q. **26**(1): 19–23.

Corelli-Grappadelli, L., and Coston, D.C. 1991. Thinning pattern and light environment in peach tree canopies influence fruit quality. HortScience, **26**(12): 1464–1466.

Crisosto, C.H., Johnson, R.S., Dejong, T., and Day, K.R. 1997. Orchard factors affecting postharvest stone fruit quality. HortScience, **32**: 820–823. doi:10.21273/HORTSCI.32.5.820.

de Freitas, S.T., and Mitcham, E.I. 2012. Factors involved in fruit calcium deficiency disorders. Hortic. Rev. **40**(1): 107–146. doi:10.1002/9781118351871.ch3.

Ferguson, I.B., and Watkins, C.B. 1989. Bitter pit in apple fruit. Hortic. Rev. **11**: 289–355. doi:10.1002/9781118060841.ch8.

Hanrahan, I. 2012. Starch iodine index for honeycrisp. Washington Tree Fruit Research Commission, Wenatchee, WA. [Online]. Available from http://www.treefruitresearch.com/images/stories/2012_Honeycrisp_starch_scale__COLOR_.pdf [18 Mar. 2019].

Hill, J. 1980. The remobilization of nutrients from leaves. J. Plant Nutr. **2**(4): 407–444. doi:10.1080/01904168009362788.

Ho, L.C., and White, P.J. 2005. A cellular hypothesis for the induction of blossom-end rot in tomato fruit. Ann. Bot. **95**(4): 571–581. doi:10.1093/aob/mci065.

Hocking, B., Tyerman, S.D., Burton, R.A., and Gilliham, M. 2016. Fruit calcium: transport and physiology. Front. Plant Sci. **7**: 569. doi:10.3389/fpls.2016.00569.

Hunter, R.S., and Harold, R.W. 1987. The measurement of appearance. 2nd ed. John Wiley & Sons, New York, NY.

Jackson, J.E., and Sharples, R.O. 1971. The influence of shade and within-tree position on apple fruit size, colour and storage quality. J. Hortic. Sci. **46**(3): 277–287. doi:10.1080/00221589.1971.11514408.

Kalcsits, L.A. 2016. Non-destructive measurement of calcium and potassium in apple and pear using handheld X-ray fluorescence. Front. Plant Sci. **7**: 442. doi:10.3389/fpls.2016.00442.

Lurie, S., and Crisosto, C.H. 2005. Chilling injury in peach and nectarine. Postharvest Biol. Technol. **37**(3): 195–208. doi:10.1016/j.postharvbio.2005.04.012.

Montanaro, G., Dichio, B., Xiloyannis, C., and Celano, G. 2006. Light influences transpiration and calcium accumulation in fruit of kiwifruit plants (*Actinidia deliciosa* var. *deliciosa*). Plant Sci. **170**(3): 520–527.doi:10.1016/j.plantsci.2005.10.004.

Montanaro, G., Dichio, B., and Xiloyannis, C. 2010. Significance of fruit transpiration on calcium nutrition in developing apricot fruit. J. Plant Nutr. Soil Sci. **173**(4): 618–622. doi:10.1002/jpln.200900376.

Montanaro, G., Dichio, B., Lang, A., Mininni, A.N., Nuzzo, V., Clearwater, M.J., and Xiloyannis, C. 2014. Internal versus external control of calcium nutrition in kiwifruit. J. Plant Nutr. Soil Sci. **177**(6): 819–830. doi:10.1002/jpln.v177.6.

Musacchi, S., and Serra, S. 2018. Apple fruit quality: overview on pre-harvest factors. Sci. Hortic. **234**: 409–430. doi:10.1016/j.scienta.2017.12.057.

Palmer, J.W., Wünsche, J.N., Meland, M., and Hann, A. 2002. Annual dry-matter production by three apple cultivars at four within-row spacings in New Zealand. J. Hortic. Sci. Biotechnol. **77**(6): 712–717. doi:10.1080/14620316.2002.11511561.

Perring, MA. 1979. The effects of environment and cultural practices on calcium concentration in the apple fruit. Commun. Soil Sci. Plant Anal. **10**: 279–293. doi:10.1080/00103627909366894.

Prusky, D. 1996. Pathogen quiescence in postharvest diseases. Annu. Rev. Phytopathol. **34**(1): 413–434. doi:10.1146/annurev.phyto.34.1.413.

Robinson, T.L., Seeley, E.J., and Barritt, B.H. 1983. Effect of light environment and spur age on 'Delicious' apple fruit size and quality. J. Am. Soc. Hortic. Sci. **108**(5): 855–861.

Robinson, T.L., and Lakso, A.N. 1988. Light interception, yield and fruit quality of 'Empire' and 'Delicious' apple trees grown in four orchard systems. Acta Hortic. **243**: 175–184. doi:10.17660/ActaHortic.1989.243.21.

Robinson, T.L., and Watkins, C.B. 2003. Cropload of 'Honeycrisp' affects not only fruit size but many quality attributes. N. Y. Fruit Q. **11**(3): 7–10.

Rosenberger, D., Schupp, J., Watkins, C., Iungerman, K., Hoying, S., Straub, D., and Cheng, L. 2001. Honeycrisp: promising profit maker or just another problem child? N. Y. Fruit Q. **9**(3): 9–13.

Rosenberger, D.A., Schupp, J.R., Hoying, S.A., Cheng, L., and Watkins, C.B. 2004. Controlling bitter pit in 'Honeycrisp' apples. HortTechnology, **14**(3): 342–349. doi:10.21273/HORTTECH.14.3.0342.

Serra, S., Leisso, R., Giordani, L., Kalcsits, L., and Musacchi, S. 2016. Crop load influences fruit quality, nutritional balance, and return bloom in 'Honeycrisp' apple. HortScience, **51**(3): 236–244. doi:10.21273/HORTSCI.51.3.236.

Stanley, J., Marshall, R., Scofield, C., and Alspach, P. 2014. The effects of reflective cloth and adjusted fruit distribution on apricot fruit quality. Acta Hortic. **1130**: 241–248. doi:10.17660/ActaHortic.2016.1130.36.

Telias, A., Hoover, E., Rosen, C., Bedford, D., and Cook, D. 2006. The effect of calcium sprays and fruit thinning on bitter pit incidence and calcium content in 'Honeycrisp' apple. J. Plant Nutr. **29**(11): 1941–1957. doi:10.1080/01904160600927492.

Trad, M., Gaaliche, B., Renard, C.M., and Mars, M. 2013. Inter- and intra-tree variability in quality of figs. Influence of altitude, leaf area and fruit position in the canopy. Sci. Hortic. **162**: 49–54. doi:10.1016/j.scienta.2013.07.032.

Van Goor, B.J., and Wiersma, D. 1974. Redistribution of potassium, calcium, magnesium, and manganese in the plant. Physiol. Plant. **31**: 163–168. doi:10.1111/ppl.1974.31.issue-3.

Volz, R.K., Ferguson, I.B., Hewett, E.W., and Woolley, D.J. 1994. Wood age and leaf area influence fruit size and mineral composition of apple fruit. J. Hortic. Sci. **69**(2): 385–395. doi:10.1080/14620316.1994.11516468.

Volz, R.K., Alspach, P.A., Fletcher, D.J., and Ferguson, I.B. 2006. Genetic variation in bitter pit and fruit calcium concentrations within a diverse apple germplasm collection. Euphytica, **149**: 1–10. doi:10.1007/s10681-005-9000-8.

Watkins, C.B., Erkan, M., Nock, J.F., Iungerman, K.A., Beaudry, R.M., and Moran, R.E. 2005. Harvest date effects on maturity, quality, and storage disorders of 'Honeycrisp' apples. HortScience, **40**(1): 164–169. doi:10.21273/HORTSCI.40.1.164.

Winzeler, H.E., and Schupp, J.R. 2011. Image analysis of blush coverage extent and measures of categorical blush intensity in 'Honeycrisp' apples. HortScience, **46**(5): 705–709. doi:10.21273/HORTSCI.46.5.705.

Wu, B.H., Mimoun, M.B., Genard, M., Lescourret, F., Besset, J., and Bussi, C. 2005. Peach fruit growth in relation to the leaf-to-fruit ratio, early fruit size and fruit position. J. Hortic. Sci. Biotechnol. **80**(3): 340–345. doi:10.1080/14620316.2005.11511941.

Zhang, J., Serra, S., Leisso, R.S., and Musacchi, S. 2016. Effect of light microclimate on the quality of 'd'Anjou' pears in mature open-centre tree architecture. Biosyst. Eng. **141**: 1–11. doi:10.1016/j.biosystemseng.2015.11.002.