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Calcium Absorption during Fruit Development in 'Honeycrisp' Apple Measured Using ^{44}Ca as a Stable Isotope Tracer

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Abstract. **Calcium (Ca) sprays are commonly used to control Ca-related disorders such as bitter pit in apple. Increases in the frequency and the amount of Ca applied directly to the fruit have increased fruit Ca levels and are associated with a reduction in bitter pit incidence. However, the absorption efficiency at different fruit developmental stages is poorly understood. Here, the absorption efficiency was measured using ^{44}Ca stable isotope applied to 30 individual fruit at five different times every 2 weeks between June drop and 2 weeks before harvest in a medium-density 'Honeycrisp' orchard. Fruit size, spray adhesion, and Ca and potassium (K) content were monitored weekly for 12 weeks between 26 May and 13 Aug. 2015. At harvest, the ^{44}Ca-labeled fruit was picked and separated into peel and inner fruit for mass balance analysis of ^{44}Ca absorption to regions of the fruit that are important to prevent Ca-related disorders. As expected, $\delta^{44}Ca$ was greater in the peel than the interior of the fruit. However, there was a significant amount of ^{44}Ca present in the inner fruit at harvest for all five applications applied during the growing season. Using a stable isotope tracer approach, we present evidence that Ca is absorbed throughout fruit development. These findings support current recommendations for frequent Ca applications in low concentrations throughout fruit development to increase fruit Ca levels and reduce the incidence of bitter pit in 'Honeycrisp' apple.**

Calcium (Ca) nutrition and the balance between Ca and other plant nutrients are key factors that affect fruit quality and storability. This is especially important for fleshy crops, such as apple, pear, tomato, pepper, and potato (White and Broadley, 2003). Many agricultural crops are susceptible to physiological disorders that originate from a low concentration of Ca in fruit (de Freitas and Mitcham, 2012), which reduces cell-wall strength, resistance to biotic and abiotic stress, and inhibits necessary cell signaling (Gilliham et al., 2011; Ho and White, 2005). Bitter pit is among these Ca-related disorders and renders 5% to 10% of harvested apples unmarketable each year. In 'Honeycrisp' apple, it is not uncommon to lose up to 50% of the fruit to bitter pit (Rosenberger et al., 2004). These losses represent a major decrease in production efficiency and have a significant impact on the economic viability of the cultivar.

Fruit elemental composition has been consistently linked to bitter pit incidence in apple (Ferguson and Watkins, 1989; Perring, 1986; Peryea et al., 2007; Rosenberger et al., 2004). Elemental balance of Ca relative to other plant nutrients can also affect the susceptibility to bitter pit. Specifically, low Ca or high nitrogen, K, or magnesium have been associated with an increased incidence of Ca-related disorders (Ferguson and Watkins, 1989). Cultivars such as 'Honeycrisp' apple that accumulate less Ca in the fruit (Robinson and Watkins, 2003) are more susceptible to Ca-related disorders such as bitter pit than other cultivars that accumulate higher levels of Ca. Naturally, plant mineral nutrient demand is met by supply from the roots and mineral nutrient uptake is then distributed aboveground. Low crop load and high vegetative vigor can exacerbate Ca-related disorders by altering the balance in Ca distribution between the leaves and fruit (de Freitas and Mitcham, 2012). Because leaves transpire at greater rates than developing fruit, increased leaf area or decreased number of fruits can negatively affect Ca levels in fruit and cause a higher amount of Ca to be distributed to leaves.

Calcium sprays have been used for a number of decades to increase fruit Ca levels and reduce the incidence of Ca-related disorders (Ferguson and Watkins, 1989). Direct Ca spray contact with the fruit is the mechanism that increases fruit Ca levels. Because Ca is immobile in the phloem, Ca that is absorbed by other plant organs cannot be translocated to the fruit. However, phloem mobile nutrients such as K, magnesium, and nitrogen can be remobilized to all parts of the plant in an attempt to compensate for mineral deficiencies. This can lead to Ca imbalances in fruit. The amount, timing, and type of Ca used as foliar sprays have been extensively studied (Biggs and Peck, 2015; Ferguson and Watkins, 1989; Lötze et al., 2008). However, there are questions of how much Ca is absorbed by the fruit and whether the absorption efficiency changes during fruit development.

Rosen et al. (2006) used strontium as a tracer analog for Ca to measure the absorption potential of Ca testing early and late season applications in addition to the frequency of application. There are limits in the use of strontium as a Ca analog that include fractionation between strontium and Ca that changes the ratio in plants relative to the soil (Drouet and Herbauts, 2008). This can complicate the calculation of strontium tracer uptake, particularly when the strontium tracer signal in the plant is relatively low. Although strontium has been shown to be an effective analog tracer for Ca in many natural systems (Rosen et al., 2006; Storey and Leigh, 2004), using Ca instead would provide a more direct measure of Ca spray absorption efficiency.

Both stable (^{44}Ca) and radioactive Ca isotopes (^{45}Ca) have been used as a tracer for soil biogeochemical changes (Bedel et al., 2016; Drouet et al., 2005), root uptake (van der Heijden et al., 2013), and entomology (Wanner et al., 2006). ^{44}Ca was also used to measure Ca absorption into the cell wall of apple fruit (Roy et al., 1995). Radioactive ^{45}Ca is another isotope used for tracing Ca movement in plants. Shear and Faust (1970) reported the high effectiveness of ^{45}Ca as a Ca tracer in apple seedlings. However, the use of a radioisotope is currently limited to either laboratory or greenhouse environments because of safety considerations and, therefore, is not practical for in situ apple studies on mature trees in the field. ^{44}Ca is a nonradioactive, stable form of Ca that exists naturally at a concentration of ≈2.086% (Boulyga, 2010). ^{44}Ca is commercially available as enriched calcium carbonate ($CaCO_3$) comprises any of ^{42}Ca, ^{44}Ca, ^{46}Ca, or ^{48}Ca. However, $^{44}CaCO_3$ is the most economical

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form of stable Ca isotope and is, therefore, of greater interest to Ca tracer studies.

During fruit development in apple, there are substantial changes in the cuticle and epidermal layer of the fruit that may contribute to changes in Ca spray adhesion and solution permeability (Ju and Bramlage, 2001). During the early stages of fruit development, the fruit has a greater surface area to volume ratio because of a lower diameter but also a smaller surface area. As fruit matures and the diameter increases, the fruit becomes smooth. Here, we sought to test whether absorption efficiency of Ca sprays changes during fruit development using ^{44}Ca as a stable isotope tracer combined with fruit growth modeling and the quantification of spray adhesion. This will provide direct quantitative information on the effectiveness of preharvest Ca chloride spray applications for 'Honeycrisp' apple, which is a susceptible apple cultivar to Ca-related disorders.

Materials and Methods

Plant material and site descriptions. The experiment was conducted in a 9-year-old 'Honeycrisp' apple orchard located in Quincy, WA (47°10′28″N, 119°57′38″W) grafted on a M9-T337 rootstock and trained to a single leader tree. The planting density was 1.8 × 4.2 m, and the trees were trained to a single axis. The orchard relies on irrigation, and there was only one rain event, on 17 May. Evaporative cooling was used in the orchard starting 16 June 2015. Evaporative cooling was turned on at 10:00 for days that exceeded 30 °C in cycles of 20 min on and 40 min off until 17:00. Bloom occurred on 14 Apr. 2015, fruit thinning was performed on 16 June 2015, and the orchard was harvested on 26 Aug. 2015. Calcium was applied weekly from 15 May to 15 Aug. 2015 at a rate of 725 g·ha^{-1} Ca per application. The mean daily temperatures were 15.1 °C in May and increased to a high of 22.8 °C in August. The mean maximum temperatures during this period were ≈30 °C with a relative humidity of between 40% and 50% (Table 1). Weekly fruit measurements started after the cell division period for apple fruit which is estimated to occur until ≈45 d after full bloom, a time when cell expansion and fruit growth begins to increase (Lakso et al., 1995).

Orchard selection and environmental monitoring. Trees were selected for visual uniformity in height and canopy volume then measured for trunk cross sectional area (TCSA) after bloom was complete. Ten trees were selected to be within 10% of the mean TCSA for a subset of 100 trees measured within the orchard. These trees were thinned to a targeted crop load on 5 May. Fruit growth was monitored weekly from 6 May to 13 Aug. by collecting 50 fruitlets from non-treated trees within the selected region in the orchard. Fruitlets were weighed, measured for diameter and height, and then wet to drip-stage using a 500 mg·kg^{-1} $CaCl_2$ solution. Fruitlets were then weighed again to measure the amount of solution that each fruitlet was holding. Fruitlets were then analyzed on four sides along the equatorial region using a Bruker Tracer IIISD portable X-ray fluorometer (XRF) following the protocols described in McLaren et al. (2012) and Kalcsits (2016) to obtain semiquantitative estimates of Ca and K that were expressed relative to rhodium photon counts. Fruitlets were then pooled into subsamples of 10 fruitlets (N = 5), dried, and then ground using a mortar and pestle for nutrient analysis.

Calcium chloride preparation, isotope application. The $^{44}CaCl_2$ (calcium chloride) solution was prepared by weighing 200 mg of 97 atom% $CaCO_3$ (Cambridge Isotope Laboratories Inc, Tewksbury, MA) into an acid-washed glass beaker containing 50 mL 1 M HCl. Calcium chloride was the salt chosen because it is the most widely used Ca spray and previous literature has reported the highest effectiveness relative to other Ca containing compounds (Biggs and Peck, 2015; Ferguson and Watkins, 1989; Sharples and Little, 1970). Then, 1 M HCl was incrementally added until all $CaCO_3$ had dissolved and formed $CaCl_2$ in solution. The pH was measured to be 6.5 then the volume was adjusted using double distilled water to create a 500 mg·kg^{-1} calcium chloride solution made of 97 atom% $^{44}CaCl_2$ for isotope tracing. In the field, fruitlets were selected from a group of five trees. ^{44}Calcium application dates: 11 June, 25 June, 9 July, 23 July, and 7 Aug.. The maximum temperature during the 5 d of applications ranged from 26.8 to 35.2 °C with the warmest temperature occurring on 9 July and the coolest temperature on 6 Aug. (Table 2). ^{44}Calcium chloride was then evenly applied using a paint brush to the point of drip formation. Each fruit was only labeled once. Drips were captured using a cloth to prevent contamination of other parts of the plant with ^{44}Ca. The volume of tracer applied was estimated using a subset of fruit collected from nearby trees with fruit of the same caliper that were weighed before

Table 1. Monthly minimum, average, and maximum temperature, relative humidity, average windspeed and total solar radiation from May to Aug. 2015 in Quincy, WA.

Date	Minimum temperature (°C)	Mean temperature (°C)	Maximum temperature (°C)	Relative humidity (%)	Wind speed (m·s^{-1})	Total solar radiation (MJ/m^2)
May	11.3	17.1	23.5	50	1.9	718
June	17.2	23.3	29.5	40.5	1.8	802
July	18	24.1	30.4	43.7	1.7	799
August	16.3	22.2	28.5	47.8	1.7	625

Table 2. Environmental conditions during the day of application for 5 d in which $^{44}CaCl_2$ was applied to fruit.

Date	Maximum temperature (°C)	Minimum temperature (°C)	Avg temperature (°C)	Wind speed (MPH)	Light intensity (MJ/m^2)
11 June	30.2	18.1	24.2	5.6	29.69
25 June	30.6	17.6	24.1	3.6	28.60
9 July	35.2	21.4	28.1	1.4	26.27
23 July	27.9	15.5	21.5	1.8	26.84
7 Aug.	26.8	13.9	20.4	1.7	25.49

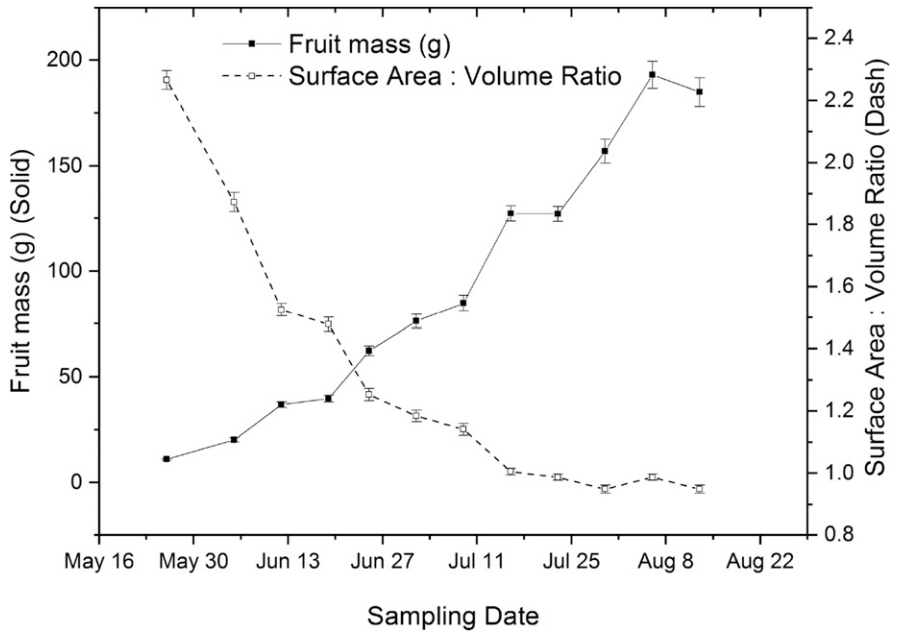

Fig. 1. Fruit mass (solid line) and surface area:volume ratio (dashed line) during fruit development (N = 50 ± SE) of 'Honeycrisp' apple between 26 May and 6 Aug. 2017.

and after Ca application to estimate the volume of spray applied to each fruit. Labeled fruit was then tagged and tracked until the fruit was harvested at maturity on 24 Aug. just before commercial harvest. At harvest, fruit was picked and stored at 2 °C for ≈30 d before sampling for nutrient and Ca isotope analysis. Three subsamples of untreated fruit were collected from control trees to compare the Ca isotope ratio of labeled fruit with untreated fruit.

Fruit nutrient analysis. To prepare samples for nutrient analysis, isotopically labeled fruit separated into peel (1 mm depth) and inner fruit including the cortex and then weighed for fresh weight. Inner fruit were then sliced into thin segments and the seeds were removed. These samples were, along with the peels, oven-dried at 60 °C for 7 d. When dry, samples were homogenized using a mortar and pestle and then ground to micron size using a tissue homogenizer (VWR, Radnor, PA). For nutrient analysis of fruitlets collected during the growing season and peel and inner fruit samples prepared at harvest, 200 ± 1 mg of ground tissue was weighed into digestion vials and hot-plate digested with 6 mL of HNO_3. After the digestion was complete, the digest was filtered with a 0.45 µM polytetrafluoroethylene filter (VWR). Filtered digests were then diluted 100× and analyzed for Ca and K concentrations at Washington State University soil chemistry service laboratory using an Agilent 4200 microwave plasma absorption emissions spectrometer (MP-AES) (Agilent Technologies Inc., Santa Clara, Ca) and run in combination with Ca and K standards for validation. The concentration of Ca and K standards were chosen to bracket the approximate concentrations of the digests for Ca and K.

Isotope analysis. The ratios of $^{44}Ca/^{40}Ca$ isotope were measured with an 820 MS Analytical Jena inductively coupled mass spectrometer (ICP-MS) (Analytik Jena AG, Jena, Germany) following isotope analysis methods described in van der Heijden et al. (2013, 2014). Methods were validated by instrument intercalibration (van der Heijden et al., 2013). Measured isotopic compositions of samples are expressed in permil (‰) deviations relative to the Ca reference ratios (National Institute of Standards and Technology SRM 915a):

$$\delta^{44/40}Ca = \left\{ \left({}^{44}Ca/{}^{40}Ca \right)_{sample} / \left({}^{44}Ca/{}^{40}Ca \right)_{NIST915a} - 1 \right\} \times 1000$$

Given natural isotope variations and ICP-MS measurement precision, a tracer detection limit was set at 10‰.

µg $^{44}Ca_{peel}$ and µg $^{44}Ca_{cortex}$ is the amount of Ca in the peel or cortex that originates from the tracing solution, respectively. µg $^{44}Ca_{peel}$ and µg $^{44}Ca_{cortex}$ were calculated using the following equation

$$\mu g\,{}^{44}Ca_i = \left(\frac{\alpha_i - \alpha_{control}}{\alpha_{tracer} - \alpha_{control}} \right) \times [Ca]_i \times mass_i$$

where, α_i is the ^{44}Ca isotopic composition of the peel or cortex of isotopically labeled fruits (^{44}Ca atom%), $\alpha_{control}$ is the ^{44}Ca isotopic composition of the peel or cortex of control fruits (^{44}Ca atom%), α_{tracer} is the ^{44}Ca isotopic composition of the tracing solution (97 atom% ^{44}Ca), $(Ca)_i$ is the Ca concentration in the peel or cortex (µg·g^{-1}), and $mass_i$ is the dry mass of either the peel or inner fruit (g). Then, the total amount of ^{44}Ca that was absorbed into each individual fruit was calculated using the following equation:

$$\mu g\,{}^{44}Ca_{fruit} = \mu g\,{}^{44}Ca_{peel} + \mu g\,{}^{44}Ca_{cortex}$$

Statistical analysis. Data were analyzed as a split-plot design using analysis of variance in OriginPro (Originlab Corporation, Northampton, MA) with application time as the main factor for the ^{44}Ca stable isotope analysis (N = 30) and sampling time as the main factor for fruit growth, spray adhesion, PXRF Ca and K analysis (N = 50), and MP-AES Ca and K analysis (N = 10). Mean separation was performed for ^{44}Ca tracer absorption among application times using Tukey's honestly significant difference test (α = 0.05).

Results and Discussion

Calcium concentrations decrease more rapidly than K concentrations during fruit development. As fruit size increased (Fig. 1), dilution was a significant contributing factor affecting both Ca and K concentrations during fruit development (Figs. 2 and 3). Both Ca and K concentrations decreased from the initial sampling of fruitlets on 26 May to the final sampling date on 6 Aug.. Miqueloto et al. (2014) reported similar results where, in 'Fuji' and 'Catarina' apples, Ca and K concentrations decreased during fruit development. In our

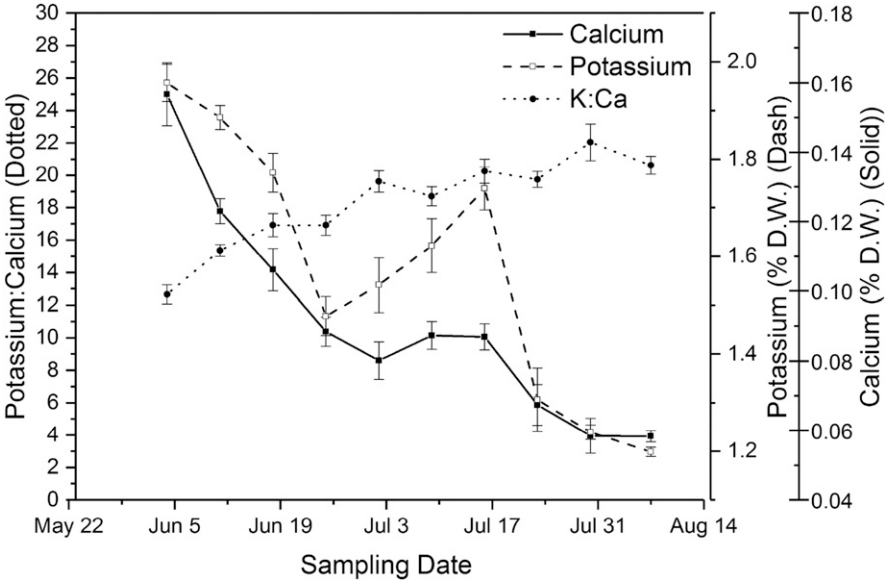

Fig. 2. Potassium (K), calcium (Ca) concentrations (% D.W.) and the K:Ca ratio of developing 'Honeycrisp' apple fruit (N = 5 ± SE) sampled weekly from 4 June to 6 Aug. 2015.

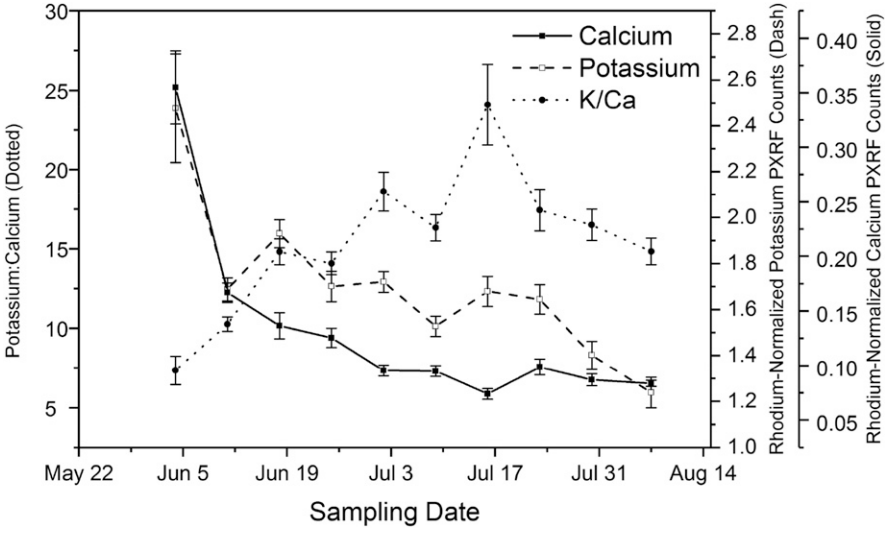

Fig. 3. Potassium (K), calcium (Ca) concentrations (rhodium-normalized PXRF counts) and the K:Ca ratio measured using a portable X-ray fluorometer for developing 'Honeycrisp' apple fruit (N = 50 ± SE) sampled weekly from 4 June to 6 Aug. 2015.

study, however, K concentrations decreased at a slower rate than the observed decrease in Ca concentrations, particularly later in the season. This resulted in a consistent increase in the K:Ca ratio of the apple tissue during the growing season that has also been reported for other cultivars (Nachtigall and Dechen, 2006). Calcium and other cation concentrations are dynamic during apple development. Delivery of non-mobile nutrients, such as Ca, is dependent on xylem transport whereas phloem-mobile nutrients such as K, magnesium, or nitrogen are transported to the fruit through the xylem and the phloem (de Freitas and Mitcham, 2012). Decreases in xylem functionality during fruit development have been attributed to changes in susceptibility to Ca-related disorders because of the inhibition of Ca transport relative to the transport of other cations (Miqueloto et al., 2014; Montanaro et al., 2010). In some situations, the increase in K:Ca in fruit may contribute to increases in bitter pit incidence. For 'Honeycrisp', a K:Ca ratio above 25:1 is considered the threshold value that could lead to an increase in bitter pit development (Cline, 2000). In this study, the mean K:Ca ratio never exceeded 20:1 during the entire sampling period. A small subsample of the fruit stored for 3 months revealed that less than 6% of the fruit developed symptoms of bitter pit (data not shown).

Potassium and Ca concentrations in the apple fruit using a destructive and non-destructive approach. Although MP-AES is a quantitative approach using international standards as points of reference, handheld XRF is a non-destructive and semiquantitative approach that produces rhodium standardized photo counts for Ca and K (Kalcsits, 2016). Furthermore, the destructive MP-AES analysis includes the entire fruit whereas for Ca and K, the handheld XRF only analyzes the surface of the fruit to a depth of ≈1 mm which would include the skin and a small portion of the outer cortex of the fruit (Kalcsits, 2016). These two approaches measure different regions of the fruit. However, despite the different measurement regions of the fruit, the trends in K and Ca concentrations were similar between the two different approaches of measuring elemental analysis; MP-AES and handheld XRF (Fig. 4). Calcium and K measured using MP-AES were significantly correlated with measurements made using PXRF ($P < 0.05$, $r = 0.81$ and $r = 0.57$ for Ca and K, respectively) (Fig. 4).

Spray adhesion is a function of fruit growth and development. On 23 May, the mean fruit size was 12 g/fruit (Fig. 1). During the sampling period, fruit growth was nearly linear with an average weekly increase in fruit weight of ≈10 g until 8 Aug. when fruit growth slowed and the developing fruit approached its final mean fruit size of 211 g measured after harvest on 26 Aug. (data not shown). As a function of the increase in fruit size, fruit surface area also increased during the growing season. Using an estimation of surface area and volume based on a sphere, the estimated surface area and volume of the fruit at each sampling period was calculated. The surface area: volume ratio, a factor that may impact the efficacy of Ca sprays, was 2.3 on 26 May, the first application time for the ^{44}Ca tracer and decreased to ≈1.0 in late July as fruit size increased (Fig. 1).

The volume of $CaCl_2$ solution adhered to the fruit was calculated based on unit area (m^2) or as a total volume applied to each individual fruit. Spray adhesion was greater when the fruit was smaller compared with later in the season when standardized based

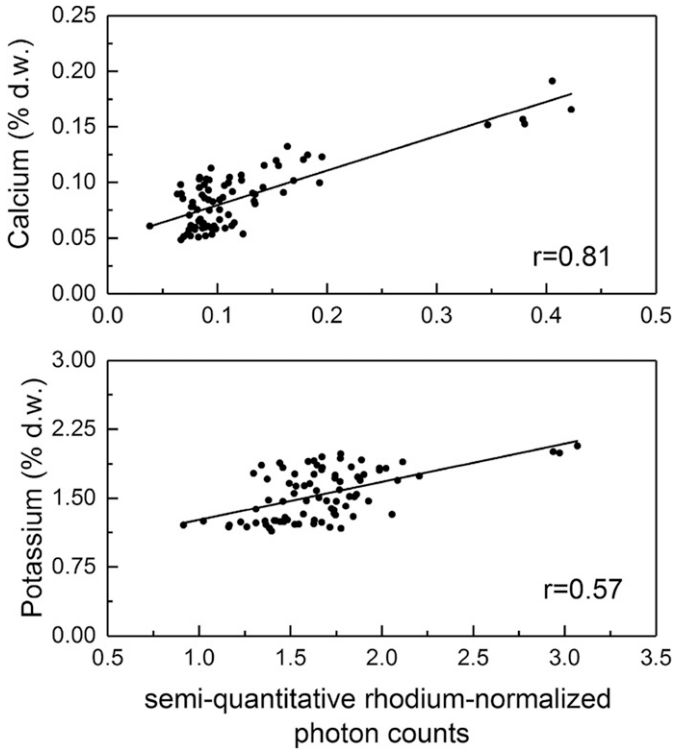

Fig. 4. Scatter plot comparing quantitative calcium (top) and potassium (bottom) concentrations in apple fruit measured using microwave plasma absorption emissions spectrometry and semiquantitative portable X-ray fluorescence ($N = 60$). The solid line represents the best linear fit using linear regression and Pearson's correlation coefficient is indicated for each comparison.

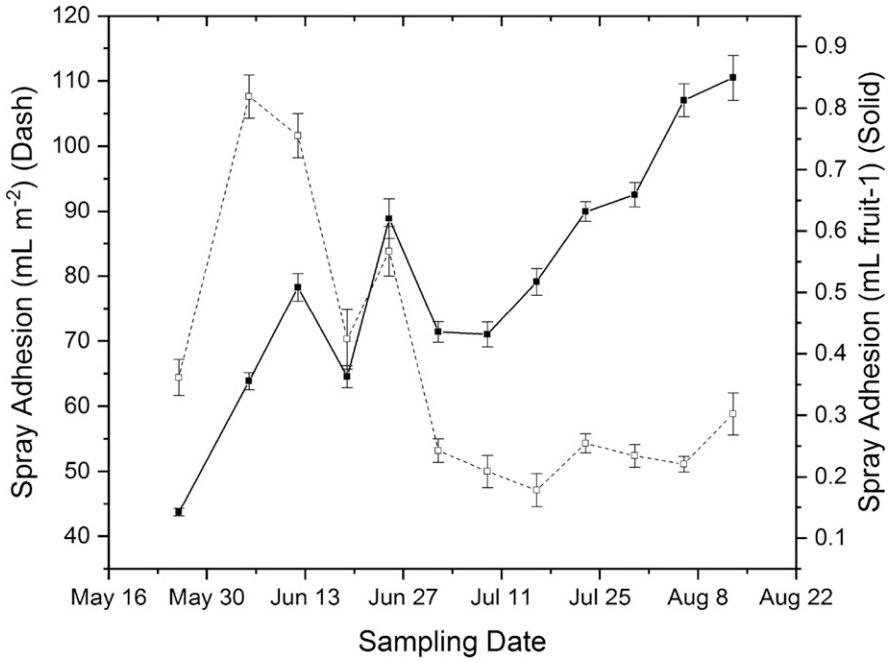

Fig. 5. Estimated spray adhesion [(mL·m^{-2}) (dash) and (mL/fruit) (solid line)] during fruit development of 'Honeycrisp' apple fruit ($N = 50 \pm$ SE) between 26 May and 6 Aug. 2017.

on surface area. On 2 June 2016, spray adhesion was estimated to be 108 mL·m^{-2} (Fig. 5). In late July and early August, spray adhesion had decreased to 50–60 mL·m^{-2}. However, because fruit size increased during the growing season and the surface area of the fruit exponentially increased, the total volume that adhered to the fruit surface at each time point increased during the growing season. The decrease in adhesion capacity may result from changes in fruit surface characteristics that could counteract the increase in absorptive surface areas during fruit development.

In situ quantification of Ca absorption measured using ^{44}Ca, a stable isotope tracer. The δ^{44}Ca was significantly higher in the peel than the flesh (Fig. 6). The δ^{44}Ca of peel ranged from 76‰ to 193 ‰ whereas the δ^{44}Ca of the flesh ranged from 46‰ to 90 ‰. However, the δ^{44}Ca values measured in the fruit flesh were well above instrumental detection limits meaning that significant amounts of tracer transferred to the inner fruit and that Ca diffusion from the epidermis into the cortex occurs when Ca sprays are applied. There was a significant correlation between the δ^{44}Ca of the peel and the flesh indicating that absorption efficiency and distribution of absorbed Ca were related. Similar findings were reported in Rosen et al. (2006), where strontium concentrations were greater on the peel but still moved into the cortex and the core. Previous work has reported that during early fruit development, absorption is greater when there is a thinner cuticle layer and active lenticels (Schlegel and Schönherr, 2002). However, the δ^{44}Ca of peel and cortex was significantly higher ($P < 0.05$) on 9 July (193 ‰) and 6 Aug. (137 ‰) than the first application that occurred on 11 June (76 ‰).

The total amount of ^{44}Ca that was estimated to be present in each fruit was always less than that applied to the fruit at all five application times indicating incomplete absorption of the applied Ca (Fig. 7). Calcium absorption was calculated to be no lower than 30% for any application time. The absorption efficiency on 9 July was greater than the other application dates. Although the amount of tracer was greater on 9 July, much of the enrichment was observed in the peel and did not extend from the peel to the cortex of the fruit (Fig. 6). Interestingly, this was the day with temperatures approaching 37 °C whereas the daily temperature for the other four application times ranged from 27 to 31 °C. During the day, leaf temperatures can be up to 10 °C greater than the ambient air temperature (Ferro and Southwick, 1984). The permeability of the cuticle may have been altered by an increase in the fruit surface temperature that might approach as high as 47 °C on a day when the air temperatures reach 37 °C. Although there is no direct evidence of changes to apple fruit cuticle in this study, there is strong evidence for cuticular changes under high surface temperatures for leaves that increase the permeability of compounds into the leaf (Baur and Schönherr, 1995; Baur et al., 1997). Interestingly, Ca applications with calcium chloride are avoided in high heat conditions due to the susceptibility to leaf burn caused by chloride phytotoxicity in the leaves. Although, only $CaCl_2$ was used in this study, in the future it may be useful to use a similar approach to look at the efficacy of applications of different Ca products commonly used in apple production.

In conclusion, we report that the antagonistic effects of decreasing adhesion capacity and increasing fruit growth result in a consistent pattern of Ca spray adhesion throughout fruit development. Calcium isotope tracing showed that calcium chloride was consistently absorbed by the fruit throughout most of the developmental period. However, there was variation that indicates that there may be application windows that may be more optimal than others. This has been reported in other similar work done in controlled conditions using ^{45}Ca radioisotope tracing (Schlegel and Schönherr, 2002). More work is required to determine how fruit surface characteristics during fruit development affect Ca

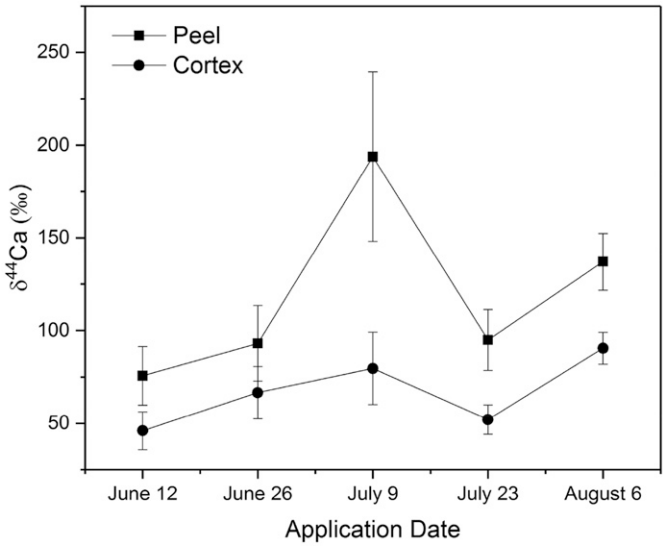

Fig. 6. δ^{44}Ca (‰) of peel and cortex tissue sampled at harvest from 'Honeycrisp' apple fruit ($N = 30 \pm$ SE) covered with 500 mg·kg^{-1}. $^{44}CaCl_2$ on 12 June, 26 June, 9 July, 23 July, and 6 Aug. 2015.

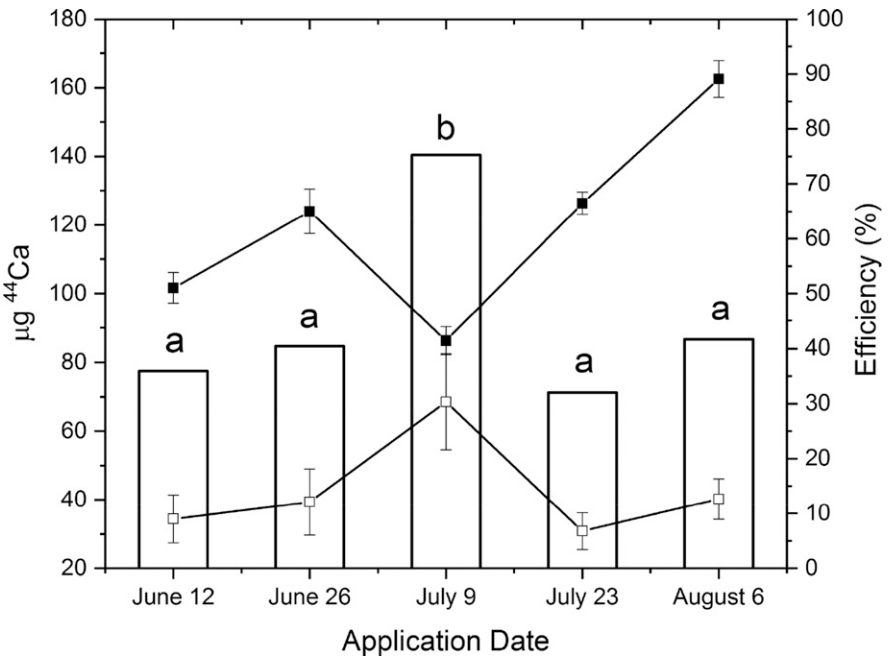

Fig. 7. Estimated ^{44}Ca (μg) applied to (solid squares) and present (open squares) in apple fruit ($N = 30 \pm$ SE) at harvest for five different application dates during the 2015 growing season. Absorption efficiency (%) was calculated as the percentage of applied ^{44}Ca that was present in the fruit at harvest. Letters denote mean separation of absorption efficiency (%) using Tukey's honestly significant difference test ($\alpha = 0.05$).

absorption under different environmental conditions and also for different cultivars. The stable isotope, ^{44}Ca, provides the opportunity for targeted tracer experiments conducted under field conditions and provides a quantitative approach for determining the effectiveness of foliar Ca applications. The findings in this experiment support current recommendations that frequent, low-concentration applications throughout the entire fruit development period result in consistent Ca absorption into apple fruit.

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