FINAL PROJECT REPORT

Project Title: Effectiveness of foliar calcium applications in bitter pit management

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Total Project Request: **Year 1**: $69,052 **Year 2**: $63,158

Other funding sources: none

Budget 1:
Organization Name: WSU **Contract Administrator:** Carrie Johnston/Joni Cartwright
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Item	**2015**	**2016**
Salaries[1]	20,624	21,448
Benefits	3,686	3,835
Wages[2]	14,160	14,726
Benefits	1,682	1,749
Travel[3]	10,000	10,000
Goods and Services[4]	18,900	11,400
Total	69,052	63,158

Footnotes:

[1] Salaries for 50% Salary for Luca Giordani (Kalcsits) and 8.25% Research Associates (Sankaran and Khot).

[2] Wages for time slip summer wages for Luca Giordani and undergraduate assistant.

[3] For travel to field sites in Wenatchee, Quincy and Prescott including overnight travel from Pullman, WA.

[4] Goods and services include calcium isotope purchase, calcium isotope analysis, lab consumables and fruit purchase in addition to CT-imaging and FTIR instrument use service charges.

RECAP ORIGINAL OBJECTIVES

1. Examine the relationship between altitude (environment) and bitter pit development in Honeycrisp.
2. Evaluate the effectiveness of different frequencies of calcium applications and reduction in transpiration using ABA on bitter pit incidence.
3. Determine the optimum timing for foliar calcium applications using calcium isotope tracer application in the field.

SIGNIFICANT FINDINGS

1. Additional calcium sprays increase fruit calcium concentrations. For Honeycrisp apple, up to 50 lb/acre of Ca can be applied during the season using frequent, dilute sprays and being careful to avoid hot periods and leaf burn.
2. Environmental factors are not major drivers in bitter pit susceptibility. Horticultural management affects bitter pit the most.
3. When horticultural variables (training system, crop load, spray timing, etc.) are controlled, bitter pit incidence can still be highly variable.
4. Internal bitter pit incidence occurs before external symptoms and is detectable using CT imaging.
5. Within high-density apple trees, bitter pit incidence is greater in the lower half of the tree and this is related to differences in nutrient concentrations
6. Water status appears to be linked to fruit size and bitter pit incidence. More work is needed to see whether this can be used to control bitter pit incidence in Honeycrisp
7. From tracking the amount of calcium spray that can stick to fruit, we can model the potential % increases in calcium from frequent calcium sprays. Earlier in the season, when the surface area to fruit ratio is low and stomata are more active, calcium sprays are more effective. Later on in the season, the impact will be less but absorption should still be occurring. Using the isotope tracing, we will be able to confirm this.

RESULTS & DISCUSSION

1. Examine the relationship between altitude (environment) and bitter pit development in Honeycrisp.

Nine Honeycrisp orchards were selected for sampling in March 2015 that ranged in elevation from 405’ to 1857’. All soils were sandy loam to loam soil with drip or microsprinkler irrigation and had a least weekly calcium applications. All orchards used upright training systems, with planting distances of approximately 3’ x 12’ and all were M-9 rootstock with the exception of one site that was on Bud-9. All orchards were between the 5th and 7th leaf. Fruit from nine trees were counted prior to thinning and then fruit was removed to targeted crop loads of 3, 5 or 7 fruit cm^{-2} TCSA (N = 3 trees). In 2015, there was a large range in growing environments with a range of approximately 850 growing degree-day differences between the coolest sites to the warmest sites (Table 1). Although there were approximately 400 less GDD in 2016 than 2015 (Table 2), the trends among sites were similar with the hottest sites in 2015 also the hottest sites in 2016. Soil temperature and air temperature were approximately 2°F less in 2016 than 2015. 16 fruit from each tree was labelled during thinning in June and the distance from the trunk and from the ground was measured for each fruit. Harvest dates were based upon commercial maturity and fruit was picked just prior to commercial harvest for each site. Harvest dates ranged from August 14-September 6th in 2015 and August 12 and September 4th in 2016. At harvest all fruit was picked from each tree for total weight and size distribution. The 16 tagged fruit was picked and placed into a tray for postharvest and post storage quality analysis. The locations of these fruit were linked to differences in fruit quality, nutrient distribution and bitter pit incidence. Fruit quality data presented in this report represents 2015 data but 2016 data is available upon request and will be included in the final presentation.

Table 1. Site characteristics for each of nine Honeycrisp orchards in 2015. * = sensor failure

Site	Altitude (feet)	Rootstock	Mean Soil Temperature (°F)	Mean (°F) Air Temperature	GDD (50°F) (5/15/15-9/1/2015)
Burbank	405	Bud-9	72.4	71.66	2613
Royal City 1C	1162	M9	70.69	70.55	2389
Royal City 2W	1145	M9	69.89	71.00	2506
Quincy 1M	1369	M9	67.57	70.59	2555
Quincy 2S	1383	M9	*	*	2400
Quincy 3O	1354	M9	*	71.24	2409
Kittitas	1731	M9	65.84	67.1	2033
Chelan	1857	M9	63.82	64.6	1754
Tonasket	900	M9	69.11	68.2	2081

Table 2. Site characteristics for each of nine Honeycrisp orchards in 2016. * = sensor failure

Site	Altitude (feet)	Rootstock	Mean Soil Temperature (°F)	Mean (°F) Air Temperature	GDD (50°F) (5/15/16-9/1/2016)
Burbank	405	Bud-9	70.32	69.83	2253
Royal City 1C	1162	M9	69.31	68.66	2086
Royal City 2W	1145	M9	68.93	68.32	2015
Quincy 1M	1369	M9	66.46	67.09	1944
Quincy 2S	1383	M9	*	67.02	1877
Quincy 3O	1354	M9	67.89	67.51	1995
Kittitas	1731	M9	62.83	62.87	1531
Chelan	1857	M9	61.85	63.01	1556
Tonasket	900	M9	65.36	65.71	1810

Environmental and horticultural contributors to bitter pit development

Environmental conditions did not significantly affect bitter pit development. There were no notable trends in the incidence of bitter pit and the environmental conditions of each orchard. Cooler orchards were just as likely to get bitter pit as warmer orchards. In 2016, preliminary data indicates that, when crop load is tightly controlled, previous year's bitter pit incidence does not correlate with current year bitter pit incidence. But, more post storage analysis will confirm these observations. Even in an orchard where horticultural variables were highly controlled (training system, crop load, calcium sprays, etc.), bitter pit incidence can vary widely (Figure 1). In 2015, bitter pit incidence ranged from 4% to 38% in the 9 orchards. The second coolest site had the higher bitter pit incidence and one of the moderate sites had the least bitter pit. With many horticultural variables controlled and using similar systems, ages, and all Honeycrisp, the one commonality contributing to higher or lower bitter pit incidence was fruit size (Figure 2). Fruit size was significantly correlated with the plant water status. This was measured using a time-averaged indicator value ($\delta^{13}C$). Therefore, it appears that plant water status may be a significant factor affecting bitter pit susceptibility through changing fruit size within the orchard (Figure 3). Even with correct crop load and calcium sprays, excessive fruit size will lead to high bitter pit incidence.

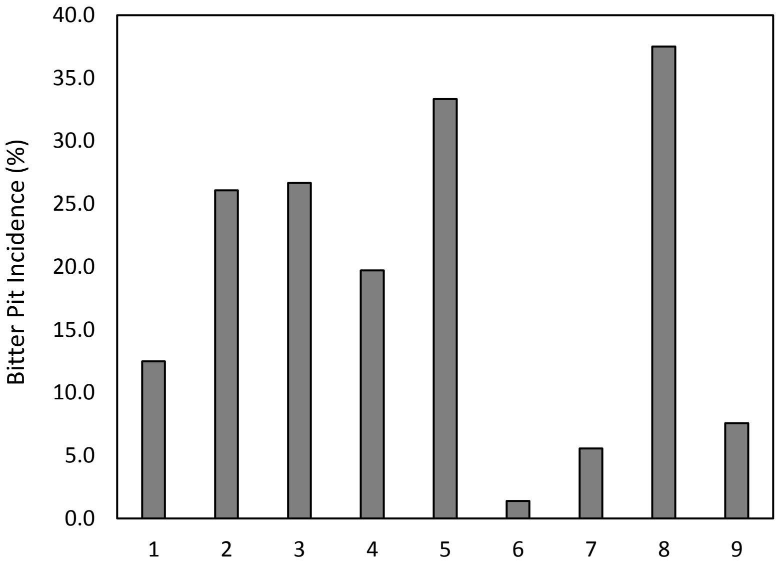


Figure 1. Bitter pit incidence among 9 environmentally diverse 'Honeycrisp' apple orchards in Washington State

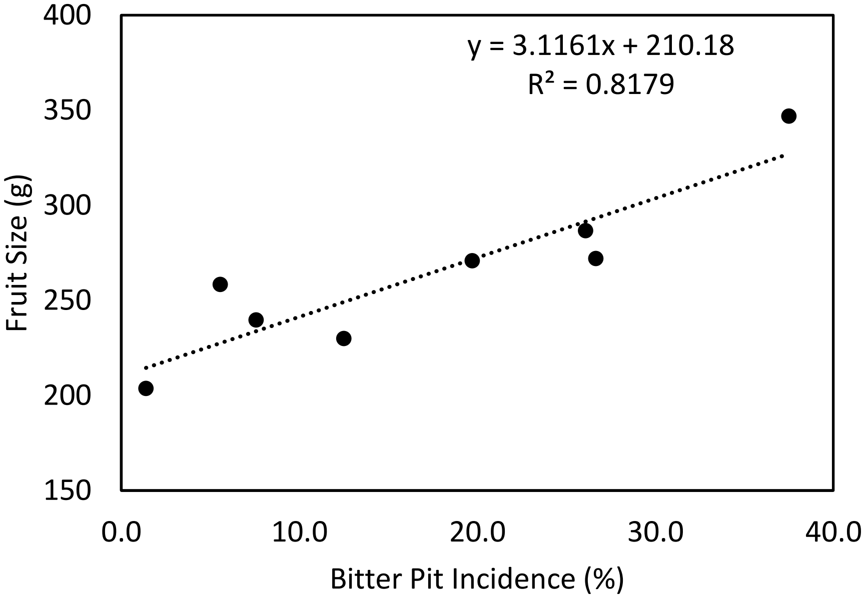


Figure 2. The relationship between average fruit size for nine environmentally diverse Honeycrisp orchards in Washington State and the bitter bit incidence observed after 4 months of cold storage

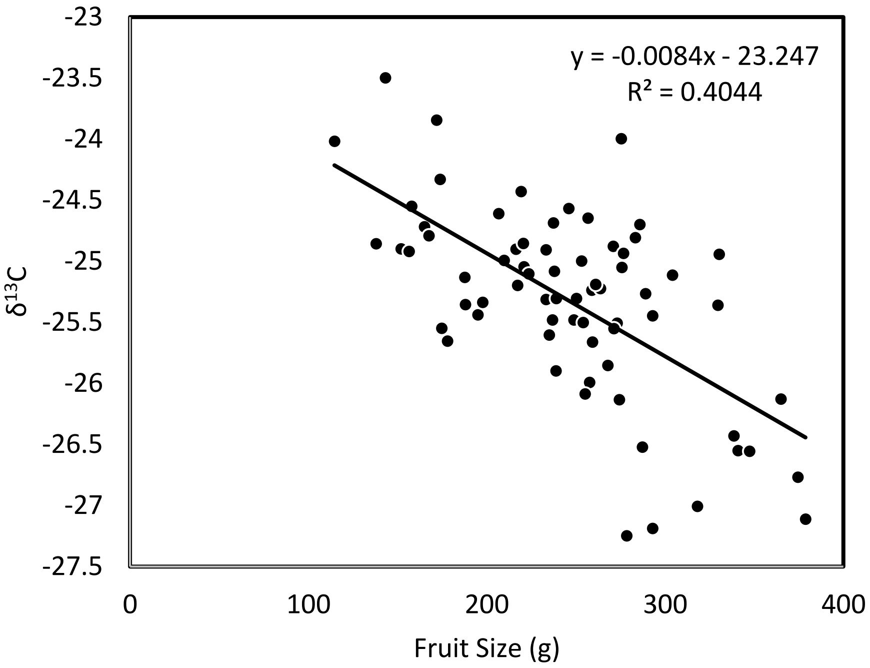


Figure 3. The relationship between δ$_{13}$C (a time-averaged measurement of plant water status) and fruit size for Honeycrisp apples harvested from nine environmentally diverse Honeycrisp orchards in Washington State. More negative values indicate less water stress than plants with less negative values.

Spatial variation within the canopy affecting bitter pit development and overall fruit quality

Fruit quality significantly ranged within the tree canopy, even for simple, high density systems. Dry matter, color development, soluble sugar content, titratable acidity and firmness were greater in the upper parts of the canopy compared to lower parts of the tree canopy. Nutrient distribution as also significantly affected by the position in the canopy. The potassium: calcium measured in June on fruitlets was significantly correlated with the potassium: calcium ratio measured on the same fruit at harvest using a portable x-ray fluorimeter. While the relationship was significant, there was still substantial variation between measurements and June measurements are not entirely predictable indicators of final ratios at harvest, particularly in situations where substantial thinning occurs after this date. At harvest, the potassium: calcium ratios were greater in the top of the tree than the lower half of the tree (Figure 4). However, there was still significant variation within the canopy. When all fruit at each height were pooled, fruit calcium concentration increased significantly as the relative height in the canopy increased and potassium concentrations decreased but not as rapidly (Figure 4). With these two opposing trends in potassium and calcium concentrations in the canopy, the potassium concentration was significantly greater in the lower half of the canopy than the upper and more exposed portion of the tree. These patterns in the potassium: calcium ratios directly correspond to the variation in the bitter pit incidence within the tree canopy. Bitter pit incidence was more than 30% for the lowest portion of the tree and decreased for apples higher in the tree to as low as 12%. It would be worthwhile to test whether segregation of fruit from lower or upper parts of the tree would result in better pack out % for fruit destined for long-term storage.

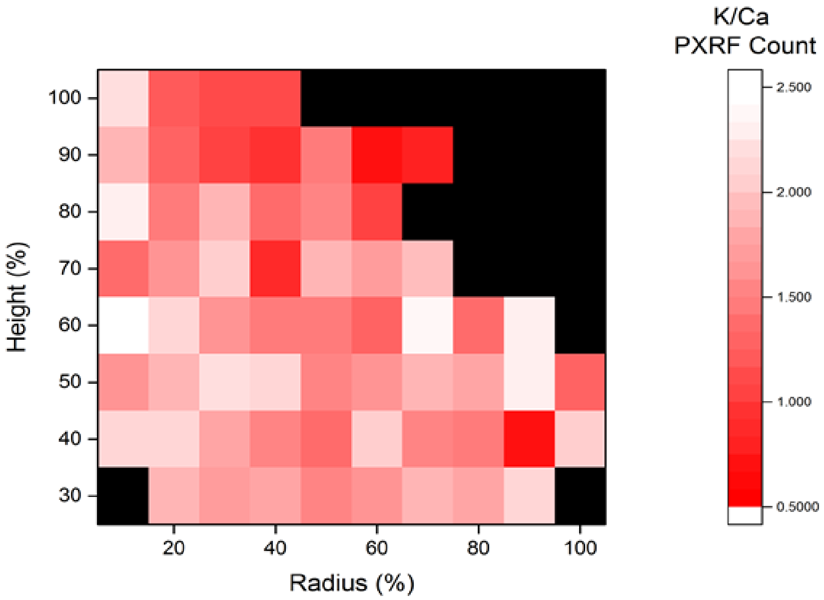


Figure 4. Potassium: calcium ratio measured at harvest using a non-destructive PXRF. Height represents the relative distance from the bottom of the tree where 100% is the top of the tree and the radius is the relative distance from the trunk of the tree.

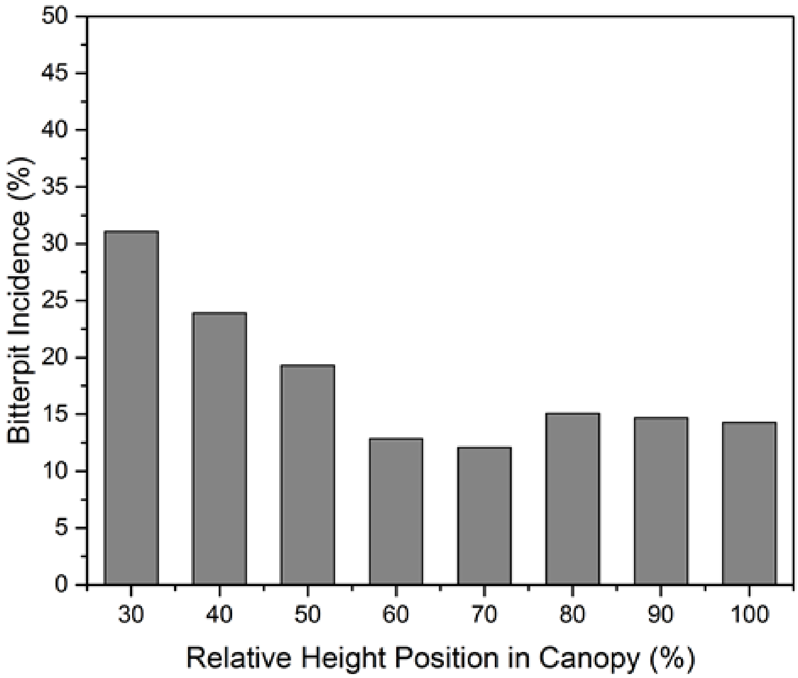


Figure 5. Bitter pit incidence (% of total harvested fruit) in Honeycrisp apples harvested from different heights in the tree canopy where 100% represents the top of the tree.

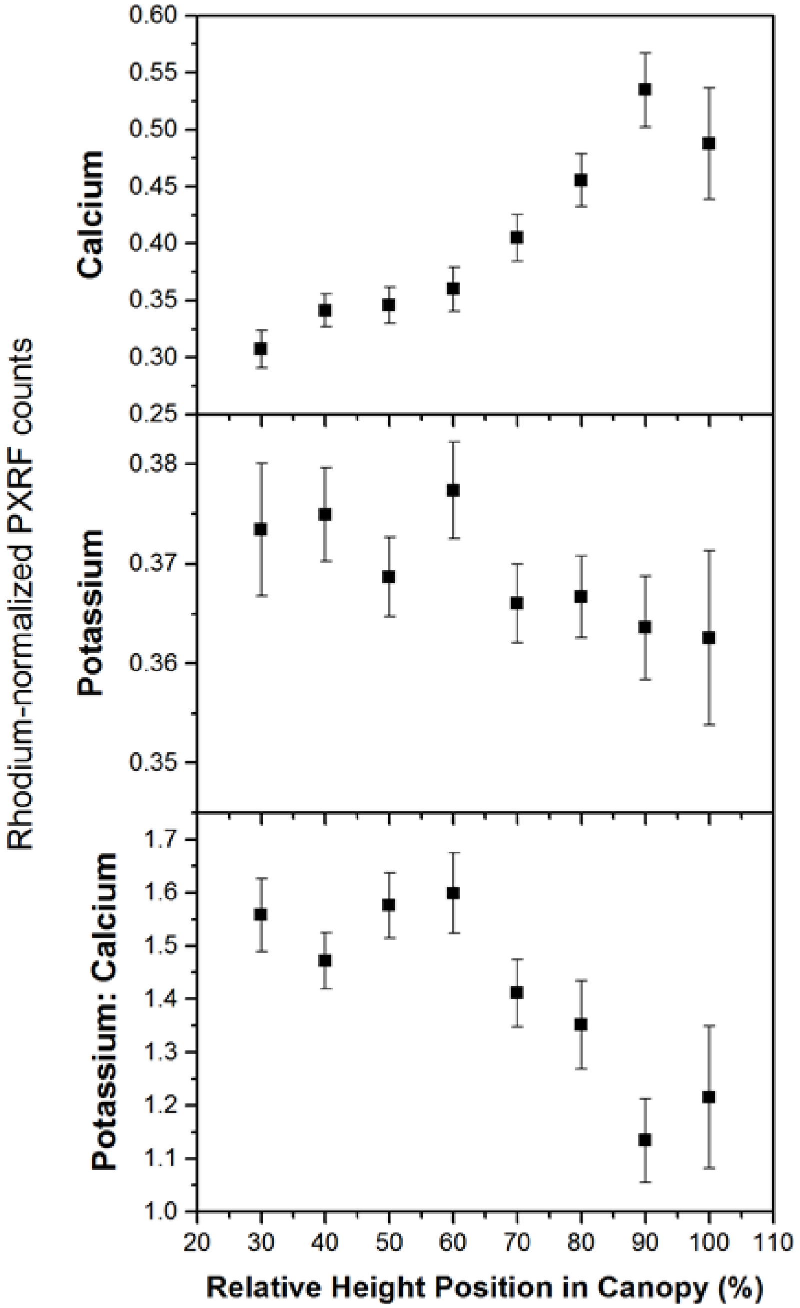


Figure 6. Calcium (top), potassium (middle) and potassium: calcium ratio (bottom) measured using PXRF on fruit from different heights in the canopy (100% represents the top of the tree).

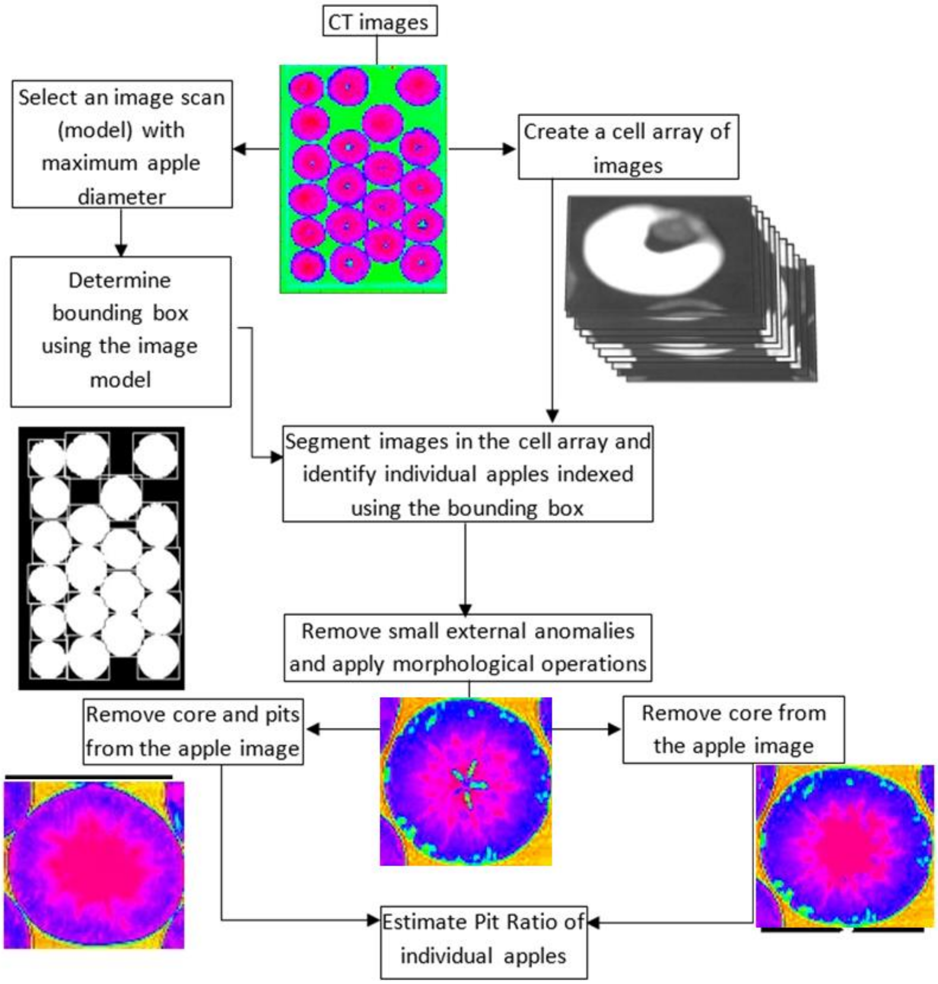


Figure 7. Image processing algorithm used to analyze CT images

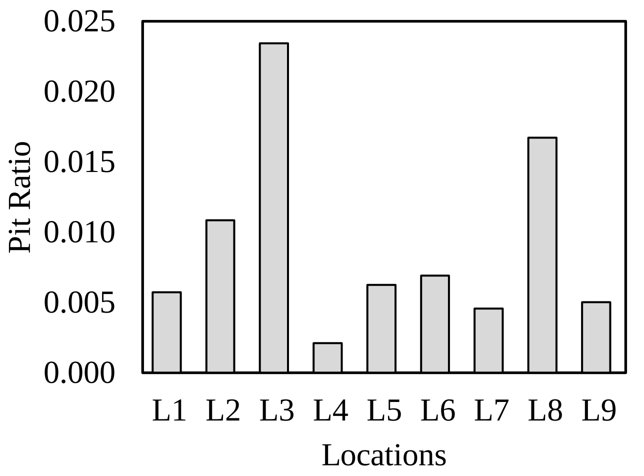


Figure 8. Pit ratio (pit area/total fruit area) of apples from nine environmentally diverse locations in Washington State

2. Evaluate the effectiveness of different frequencies of calcium applications and reduction in transpiration using ABA on bitter pit incidence.

A 9 year-old Honeycrisp orchard spaced at 6' x 14' was selected for this trial. In March 2015, sections of three rows each we marked off and treated with 2 lb/acre Ca either once per month, twice per month, once per week, or twice per week. This results in approximately 50 lb/acre applied in the twice per week treatment, 25 lb/acre in the once per week treatment, 12.5 lb/acre in the twice per month treatment, and 6 lb/acre in the once per month treatment. 10 trees per replicate were selected for sampling and hand-thinned to even crop loads. Fruit was harvested on August 26th, 2015. The average fruit size was 220 g. For this, there was higher calcium in the twice per week treatment compared to the once per week applications when measured with traditional lab analysis and PXRF analysis (Figure 8). However, the incidence of bitter pit after four months of regular atmosphere cold storage was less than 5% across all treatments and there were no differences in bitter pit among treatments, even for fruit that had 6 lb/acre of Ca applied throughout the season.

In 2016, for practicality, calcium treatments were limited to once per week or twice per week because once per week treatments can be tank-mixed with other sprays and do not amount to major additional costs for the industry. However, twice per week sprays require calcium-specific sprays and increase the risk of foliar burn. The additional spray per week amounted to a 10% increase in fruit calcium (Figure 8). However, again in 2016, the average fruit size was about 230 g and bitter pit incidence was exceptionally low at harvest. Fruit will be removed after four months of storage and bitter pit incidence scored but it is expected that the incidence will be low similar to 2015. When looking at the results from Objective 1, orchards that have exceptionally large fruit will likely benefit from additional calcium sprays.

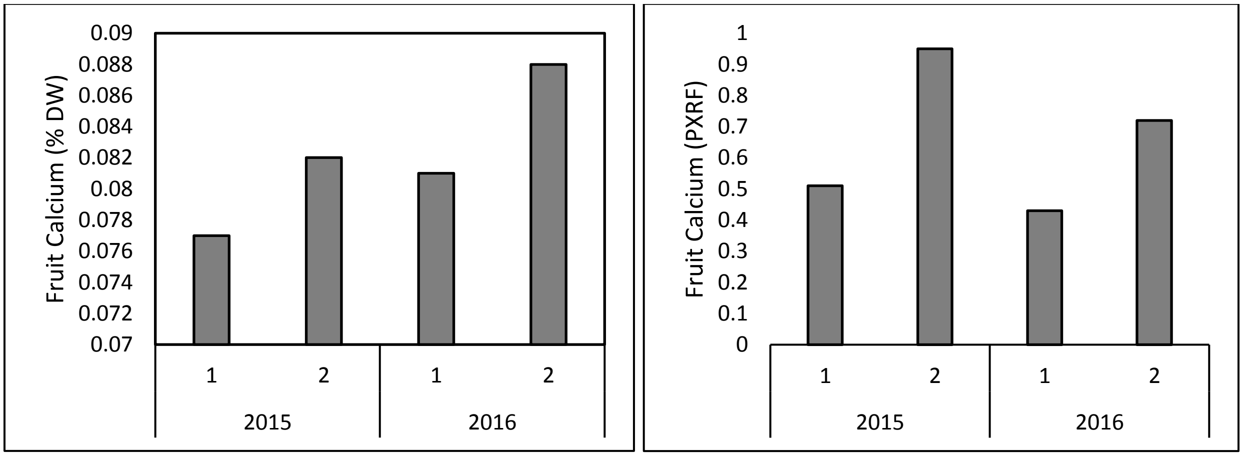
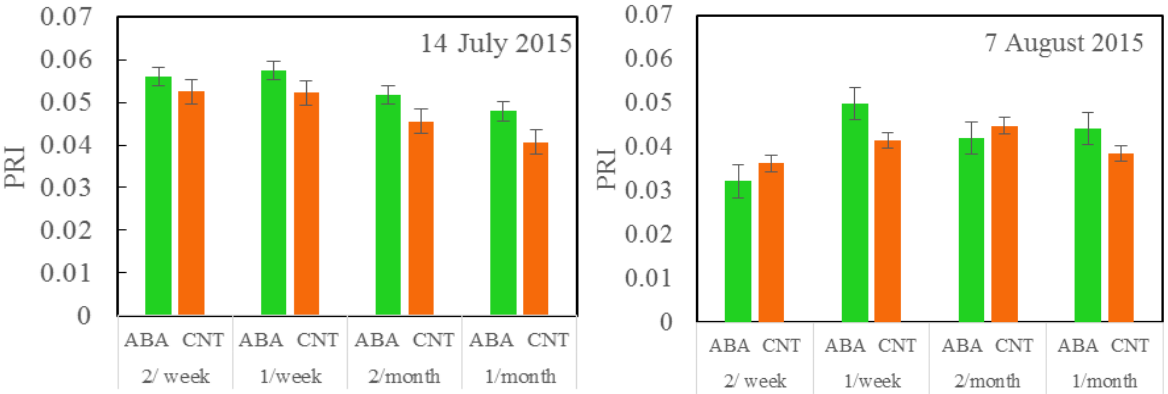


Figure 9. Fruit calcium content measured using traditional lab analysis (left) and PXRF (right) on fruit harvested from trees in 2015 and 2016 treated with 2 lb. /acre Ca once per week or twice per week.

In 2015, ABA was also applied to trees within all treatments three times in June, July and August to reduce plant water-use. Figure 10 shows the photochemical reflectance index after treatment in June and July, 2015. Vegetation indices were extracted from the visible-near infrared spectra. Amongst different vegetation indices, photochemical vegetation index (PRI) that is often used as an indicator of photosynthetic light-use efficiency, showed that ABA affected the physiology of the plant. During the mid-season (maximum canopy vigor and nutrient uptake), the PRI showed consistent differences between calcium treatment as well as effects of ABA. The ABA treated trees had higher PRI values in all four calcium treatments. However, in August, the effects are not as clear. This may be a result of leaf aging and lower responsiveness to the ABA. There were no differences in fruit nutrient concentrations or bitter pit incidence between the treated and untreated trees. There were also no significant differences in fruit quality between the treatments. This orchard had low vegetative vigor. However, this practice may be beneficial in orchards with more vegetative vigor and higher bitter pit risk. Future work trialing out ABA at more frequent doses could be helpful.


(a) Field Location (b) Proximal Sensing (c) NDVI False Color Image (d) Thermal Image



(e) Ground-based Photochemical Reflectance Index (PRI)

Figure 10. Ground and aerial-based sensing for changes in leaf physiological responses to ABA treatments.

3. Determine the optimum timing for foliar calcium applications using calcium isotope tracer application in the field.

For this objective, 15 trees were identified and thinned to even crop loads of 5 fruit/cm^2 TCSA. On five trees, 4 fruit per tree were sprayed with $^{44}CaCl_2$ every two weeks starting at 25 mm fruit size for a total of seven points during the season. At the end of the season, the fruit was harvested, separated into peel and flesh, and digested for isotope analysis (140 total fruit). This analysis will take place in January, 2016 and will be presented at grower meetings throughout WA state and in discussion with industry stakeholders. For the other 10 trees, fruit were subsampled weekly for 12 weeks and fruit weight, diameter and spray holding capacity was measured for each fruit. This was used to calculate theoretical gains and calcium application rates on fruit based on fruit size, adherence of spray to fruit and surface area. Calcium and potassium concentrations were also determined using traditional lab analysis weekly to look at changes in fruit calcium concentrations as fruitlets develop.

The surface area to volume ratio of fruit decreases as the season progresses and the fruit increases in diameter (Figure 11). The water holding capacity per unit of surface area also decreases as the season progresses as cuticle thickness increases and the fruit becomes more hydrophobic (Figure 11). These two factors support that early season applications of calcium are more important than later season applications simply because more calcium per unit volume is sticking to the fruit when it is less developed. The isotope data should provide more definitive data on what % of calcium applied is absorbed into peel and flesh, respectively. The theoretical increase in fruit calcium from calcium applications is all of it was absorbed would approach 60% depending on the frequency of application. However, fruit calcium only increased by 10% when the amount of calcium applied was doubled from once per week to twice per week (Figure 8). For this trial, we expect the isotope data to show that between 15-20% of calcium applied is absorbed by the fruit. However, this will need to be confirmed with the final analysis that will be completed in February, 2017.

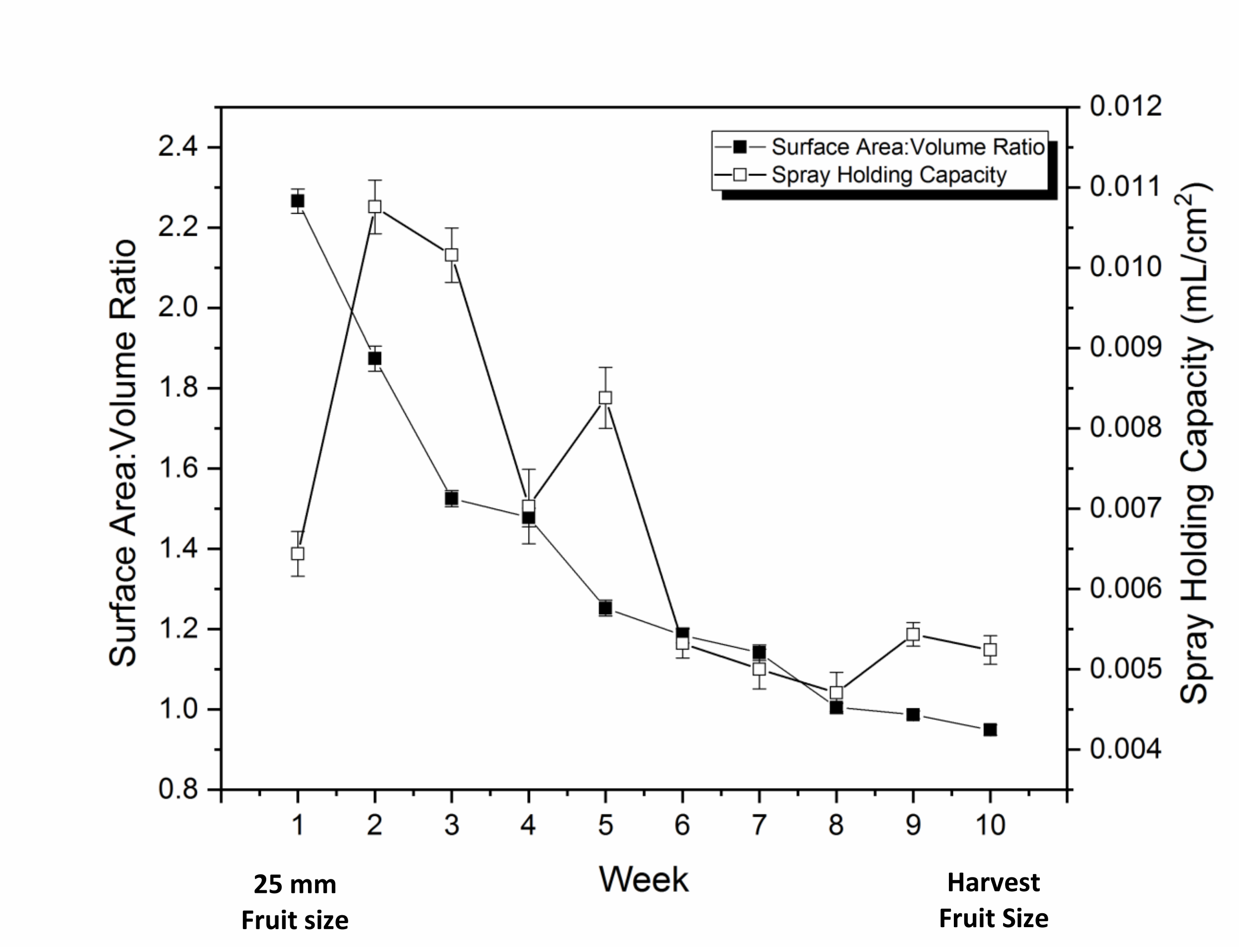


Figure 11. Surface area to volume ratio and spray holding capacity of apple fruit starting at 25 mm fruit (week 1) and ending at harvest (week 10)

EXECUTIVE SUMMARY

In 2015 and 2016, experiments were conducted to look at how environment impacts calcium uptake and distribution in Honeycrisp apple and how effective frequent calcium sprays are in improving fruit calcium levels and the optimum timing for calcium spray applications for Honeycrisp apple. Although there is still some final data to collect in early 2017, we have identified some critical factors contributing to bitter pit development in Honeycrisp apple.

Frequent, low rate calcium sprays are the most useful in improving fruit calcium levels. In situations when fruit calcium concentrations are low, calcium sprays have been shown to increase calcium levels by small amounts. However, even with frequent calcium sprays (even once per week), bitter pit incidence can still be very high (up to 50%). Calcium sprays show only minor increases in fruit calcium content compared to sound horticultural management (crop load, vigor control, irrigation and nutrient management). As fruit matures and the wax layer on the fruit becomes thicker, the amount of spray per unit volume of fruit becomes significantly smaller suggesting that early season application of calcium is more effective. However, because calcium needs to be applied frequently at low rates, late season applications could also be effective. With tank-mixing, applications of once per week are practical and the additional cost is minimal. Isotope data, when it is available, will identify the optimum timing or calcium application and will be shared at grower meetings or through individual grower discussions.

Abscisic acid (ABA) reduced plant transpiration and induced stomatal closure. However, this did not result in increased calcium in the fruit in this trial. However, this orchard was a low vigor situation and high fruit calcium concentrations. In a high vigor orchard, the result may be different and might be worth trialing under Washington State conditions. Frequent ABA applications would likely be needed to have an appreciable effect on calcium distribution within the plant.

While environment can impact the timing of fruit maturity and lead to better color development because of later maturity in cooler regions, there was no relationship between the environment and bitter pit susceptibility. Warm, low altitude environments had incidences of low bitter pit and cooler, high elevation sites had instances of high bitter pit rates. External bitter pit rates were related with the occurrence of internal bitter pit in independent subsamples of apples from the same trees. The main contributor to bitter pit development appeared to be fruit size. Larger fruit size was linearly correlated with bitter pit incidence where bitter pit incidence was under 20% for fruit that was 250 g or smaller (between 72 and 80 box size). Fruit size was also correlated with the time-averaged water status of the tree. Therefore, more work is needed to identify a link between water status and bitter pit in Honeycrisp to develop ways to use this as a tool to manage fruit size and bitter pit incidence in Honeycrisp.

Spatial variation within the tree was also assessed. While variation within the canopy has been identified for lower density orchards, within canopy variation in nutrient balance, bitter pit incidence and fruit quality has been less studied for high density orchards that are assumed to have more uniform canopies. Bitter pit incidence in the lower half of the tree was almost double that of the upper half of the tree. These differences in bitter pit incidence were related to the potassium and calcium concentrations in the fruit from different locations on the tree. This has potential to be adaptable for fruit segregation for harvest and storage management to maximize pack outs for long-term storage and make more informed decisions on handling of fruit in the field.