FINAL REPORT
WTFRC Project Number: TR-16-101

Project Title: Calibration development for nutrient analysis using a handheld XRF

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Cooperators: Jeff Cleveringa (Oneonta Starr Ranch), Glade Brosi (Stemilt), Rob Lynch (Redox), Lee Drake (Bruker Instruments)

Total Project Request: Year 1: $32,754 **Year 2**: $33,818

Percentage time per crop: Apple: 80 Pear: 15 Cherry: 5 Stone Fruit: 0

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1
Organization Name: WSU **Contract Administrator:** Kim Rains
Telephone: 509-335-4564/509-663-8181 **Email:** kim.rains@wsu.edu

Item	2016	2017
Salaries[1]	16,000	16,640
Benefits[2]	5,610	5,834
Wages[1]	4,800	4,992
Benefits[2]	115	120
Supplies[3]	5,840	5,840
Travel[4]	392	392
Total	32,757	33,818

Footnotes:
[1] Salaries for a 33% FTE research intern (Kalcsits) and summer wages for a M.S. student (Corina Serban).
[2] Benefits at 35.1% for research intern and 2.4% for M.S. student.
[3] Goods and services include lab consumables cost for nutrient analysis and service fees in Pullman and California for elemental analysis.
[4] Travel to collect fruit and to Kennewick, WA to meet with Bruker for calibration analysis.

RECAP ORIGINAL OBJECTIVES

1. Identify how correlations between x-ray and lab analysis differ among apple and pear varieties with known differences in skin thickness.

2. Develop cultivar-specific and skin-thickness specific calibrations for non-destructive analysis of calcium and potassium in apple and pear.

3. Incorporate quantitative calibrations into the Bruker software for industry-friendly instrument use.

SIGNIFICANT FINDINGS

- Other research groups are working on using XRF for non-destructive analysis of leaf tissue, apple roots, stems, and fruit. This is a general trend for using this technology for making these types of measurements. This instrument will have its primary utility as a research tool but for larger operations or consulting, this could contribute to assigning risk assessment for bitter pit incidence for commercial Honeycrisp orchards

- Significant linear regressions were obtained for Honeycrisp, Pink Lady, Fuji, D'Anjou pear, Bartlett pear, Starkrimson pear and sweet cherry. These will be put into a calibration software in the instrument. The scientific support at Bruker has had a turnover of scientists in the last year. It is now in less flux and we are working to input the calibrations into our instruments using their calibration software. Testing in a commercial orchard returned estimates that agree with ranges expected for mineral analysis.

- Skin thickness was not related to measurements between cultivars. However, within cultivars, there was a weak correlation between skin thickness and calcium concentrations.

- A calibration for fruitlets and mature fruits was developed for Honeycrisp because of differences in flesh density and nutrient concentrations.

- Commercial orchards were tested in 2018 for the use of PXRF for bitter pit risk assessment. Measurements made six weeks before harvest and at harvest were significantly correlated to bitter pit incidence after storage. However, as expected the relationship was not perfect but is a useful tool to assign risk.

- Newer PXRF systems come with existing calibrations but they would need to be verified as they are not the same calibrations that I have developed. The calibrations developed within this project could be easily incorporated into the newer model instruments.

Objective	Activity	Completed or Anticipated Completion Date
1	Looked at how peel and flesh differ in nutrient concentrations in Honeycrisp	Completed 2016
1	Looked at how the relationship between lab analysis and PXRF differs between fruitlets and fruit at harvest	Completed 2016
1	Analyzed groups of apples, pears and cherries using PXRF and then lab analysis	Completed 2016
2	Calibration sampling for Anjou pear	Completed 2017
2	Look at how lab sampling depth affects the relationship between PXRF and lab analysis	Completed 2017
2	Calibration sampling of Honeycrisp and Pink Lady	Completed 2017
2	Skin thickness measurements of Honeycrisp and Pink Lady	Completed 2017
2	Calibration development for Honeycrisp and Pink Lady	Completed 2018
1	Fruitlet and cherry sampling	Completed 2017
2	Calibration sampling for Gala apple and Bartlett pear	Completed 2017
2	Skin thickness measurements for Gala apple and Bartlett pear	Not necessary
2	Calibration sampling for Fuji	Completed 2017
2	Skin thickness measurements for Fuji	Completed 2017
3	Calibration input into PXRF device and open source for industry use	Incorporated into excel but not instrument

RESULTS & DISCUSSION

We have worked to develop calibrations that provide quantitative measurements of calcium concentrations in apple, pear and cherry. The measurements made appear to be independent of skin thickness within each cultivar and is not a covariate in our calibrations which simplified calibration development. Through either quantitative or semi-quantitative analysis, comparisons among orchard lots or individual fruit can be made for calcium and potassium to provide some avenue at assigning risk in terms of the development of calcium related disorders. These types of measurements can be used to measure many fruit in a single tree to better understand how variation in nutritional distribution is related to bitter pit incidence. These differences can be expressed as semi-quantitative like in Figure 1 or translated to quantitative values using calibrations described later in this report.

Applications of PXRF Technology

Figure 1. Relative calcium concentrations (top), potassium concentrations (middle), and potassium: calcium concentration ratios (bottom) of fruit with changing relative canopy vertical position (y-axis) and relative radial distance from the trunk (x-axis) acquired using a portable x-ray fluorometer.

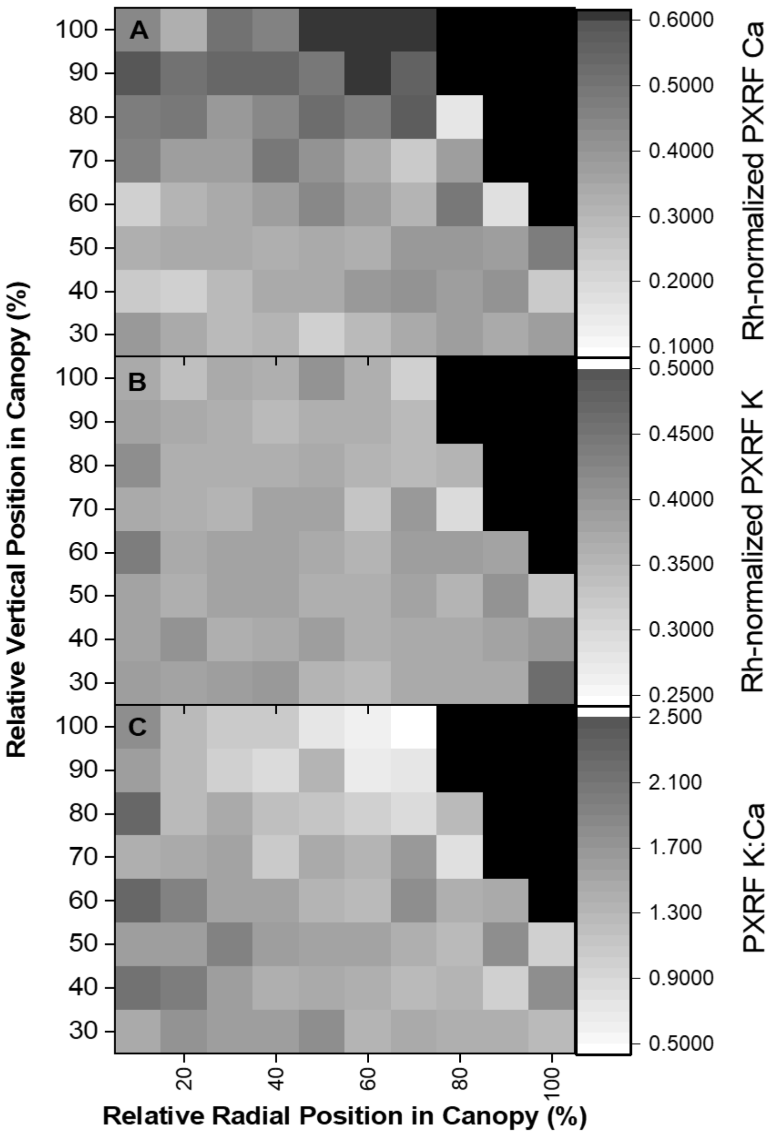

Figure 2. Two-dimensional distribution of bitter pit incidence (%) taken from different relative vertical and radial positions in the tree canopy of 78 'Honeycrisp' apple trees from nine commercial orchards.

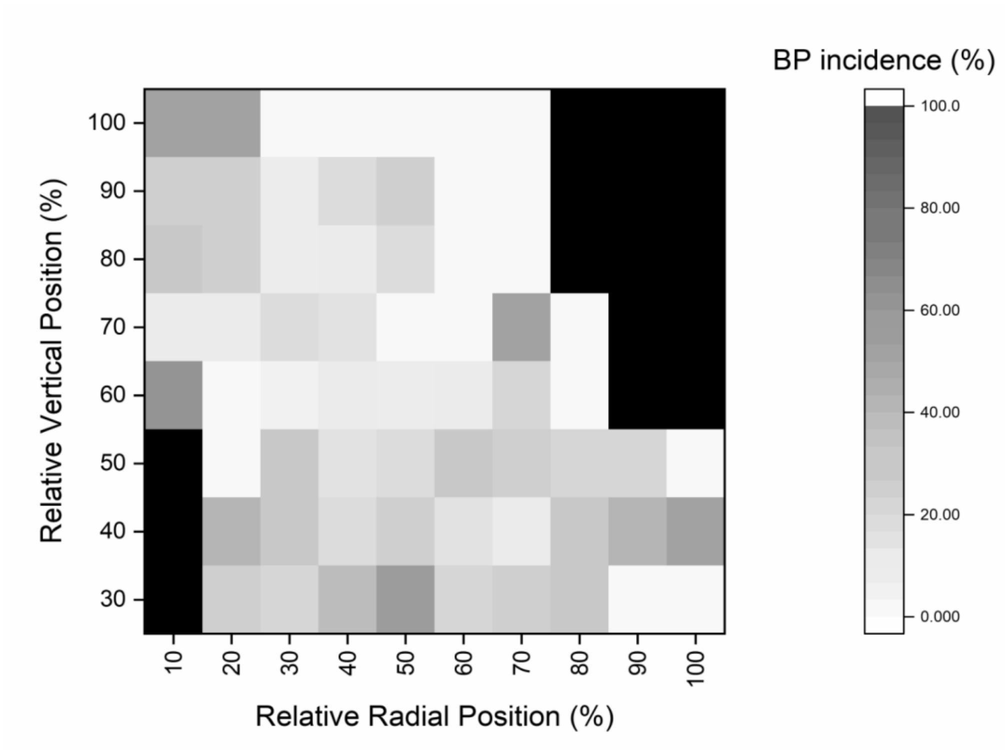

Figure 3. Different applications of PXRF measurements to work towards answering questions related to calcium and potassium in apple, pear, and cherry using top left: field measurements; top right: lab measurements of fruit; bottom left: matrix measurements; bottom right: pelletized ground tissue.

Are cultivar specific calibrations enough?

Across several fields at equal points of maturity, the slope of the lines remain similar indicating that one calibration could be used for a single cultivar if the sampling protocol is clear and uniform. Additionally, skin thickness did not contribute to variability in measurements within individual cultivars. Across cultivars, changes in both peel and cortex density would likely contribute to variability in the readings that may or may not be related to bitter pit incidence. This would be a testable hypothesis for future work with segregating populations.

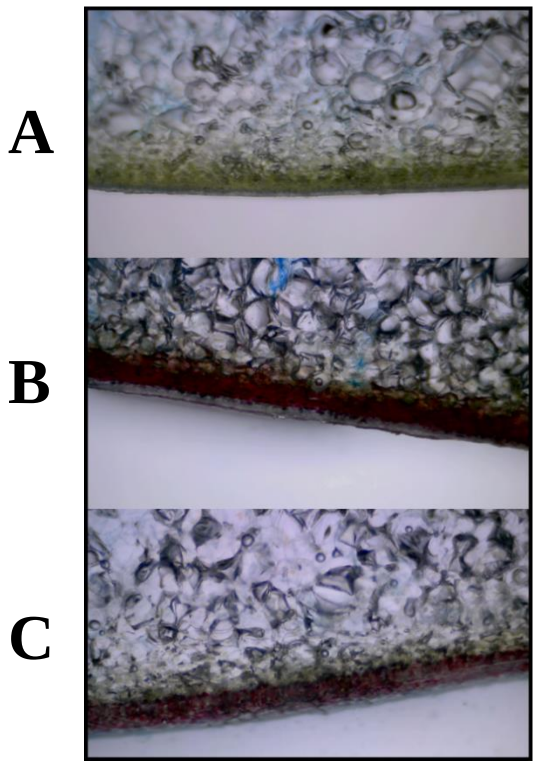

Figure 4. Images of peel thickness in Honeycrisp (A), Fuji (B), and Pink Lady apples harvested at maturity in 2016.

Calibrations

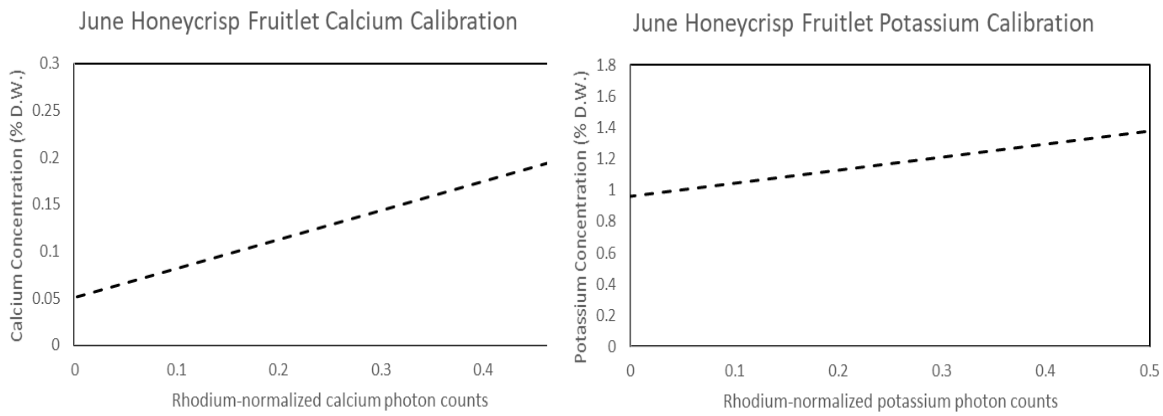

Figure 5. Linear regression for calcium (left) and potassium (right) in Honeycrisp fruitlets analyzed in June. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w.

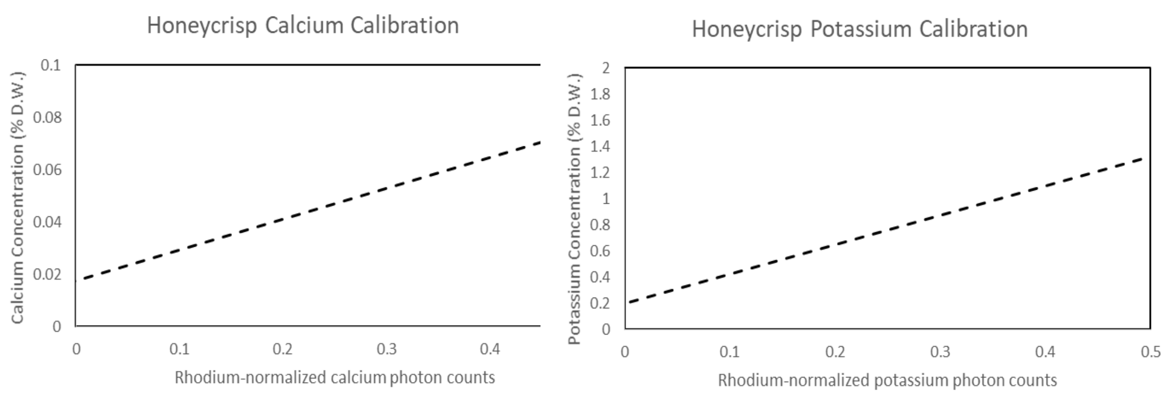

Figure 6. Linear regression for calcium (left) and potassium (right) in Honeycrisp fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

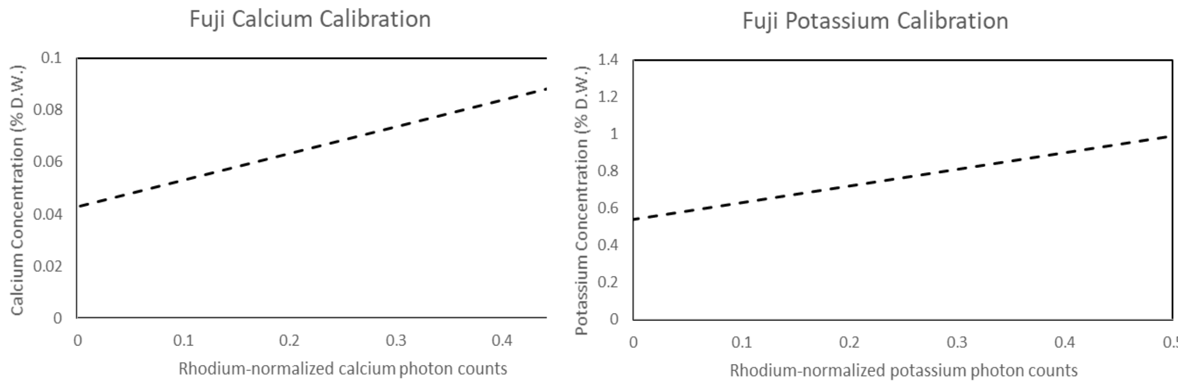

Figure 7. Linear regression for calcium (left) and potassium (right) in Fuji fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

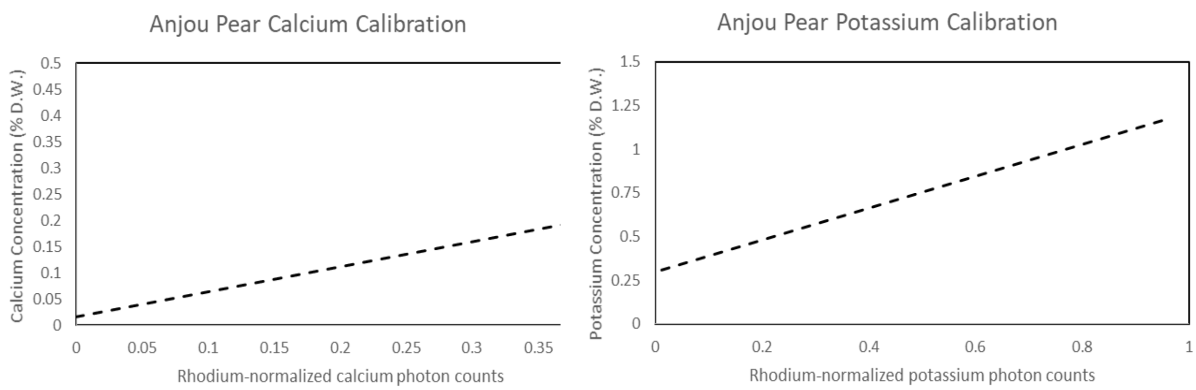

Figure 8. Linear regression for calcium (left) and potassium (right) in Anjou pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

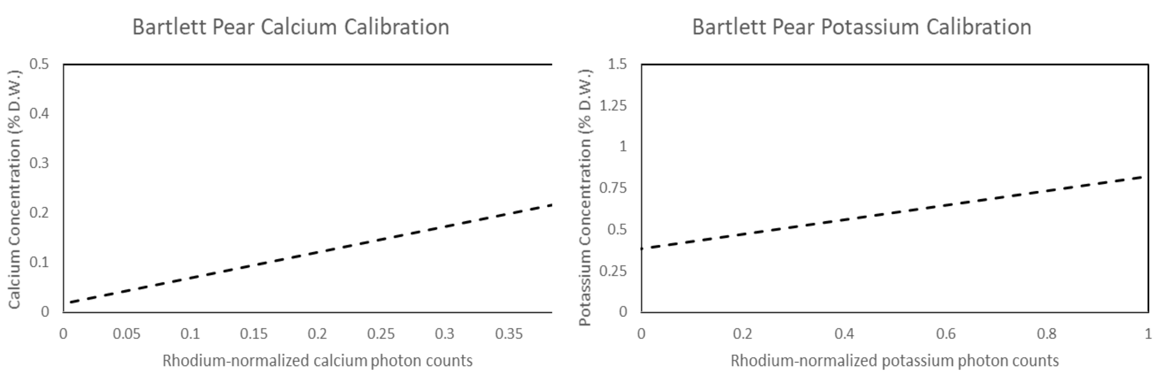

Figure 9. Linear regression for calcium (left) and potassium (right) in Bartlett pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

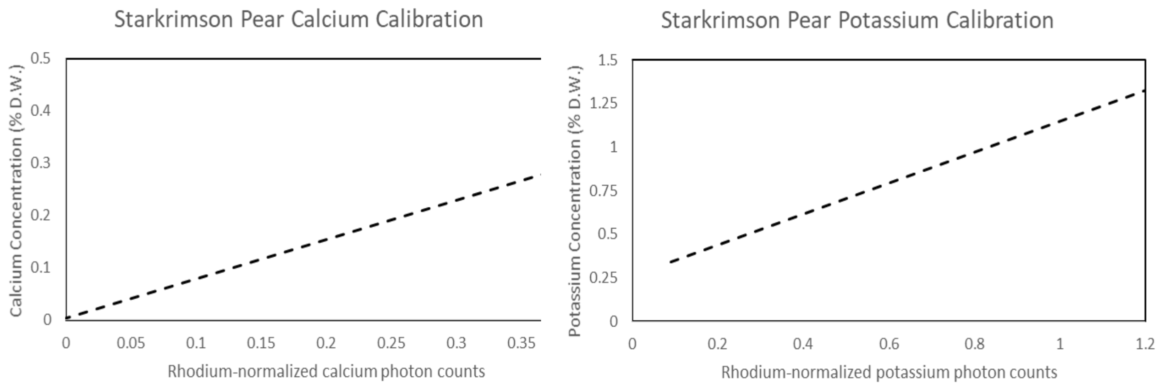

Figure 10. Linear regression for calcium (left) and potassium (right) in Starkrimson pear fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

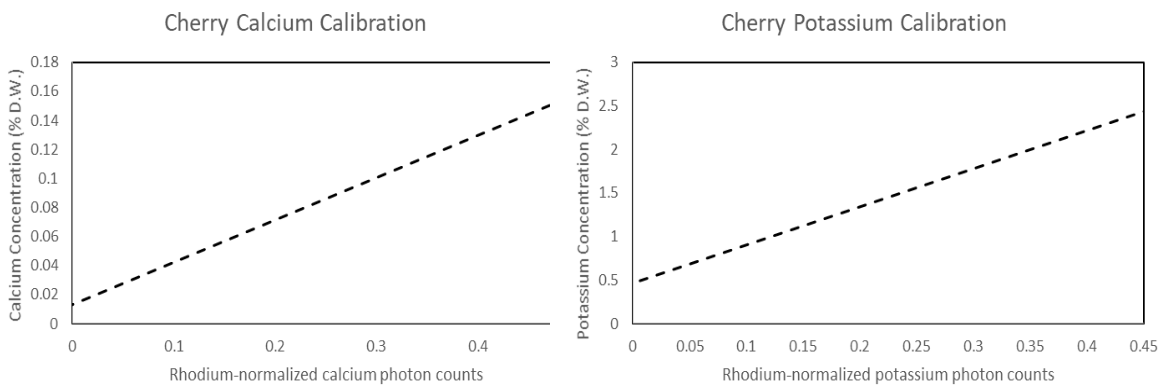

Figure 11. Linear regression for calcium (left) and potassium (right) in sweet cherry fruit measured at harvest. The x-axis represents the PXRF reading normalized for rhodium counts and y-axis is the concentration in d.w. (%)

When the PXRF was tested for risk assessment for commercial orchards, the calculated potassium and calcium concentrations fell within normal ranges observed for Honeycrisp fruit using destructive analysis. Calcium concentrations at harvest ranged from 0.014% to 0.06% dry weight whereas potassium concentrations ranged from 0.2 to 0.73% dry weight. These produced K:Ca ratios that ranged from as low as 5 to as high as 26 (Table 1). These ratios seems a bit lower than normal but were well correlated with bitter pit incidence (Figure 12). Calcium concentrations six weeks before harvest were greater than at harvest. In many cases, the concentrations had dropped by 50 to 80% during that time. This is a key time for rapid fruit growth as well as potassium influx into the developing fruit. Potassium concentrations did not decrease by nearly as much, even though this is a rapid period for fruit growth. This demonstrates that potassium transport to the fruit is much greater than calcium later in the season. There was not a complete agreement between the PXRF readings and bitter pit but that is equal to trends observed for traditional elemental analysis as well.

Table 1. Calibrated potassium: calcium ratios of Honeycrisp apple measured in fruit either six weeks before harvest or at harvest for 15 commercial orchards. Bitter pit incidence was counted after three months of storage and seven days at room temperature. Bitter pit risks were assigned pre storage to see how risk assessments that included vegetative growth related to bitter pit after storage.

Orchard	BP risk Estimate	K/Ca Harvest	K/Ca 6WBH	BP%
A	High	17.40	5.88	53
B	Moderate	12.61	2.57	27
C	High	18.61	5.68	27
D	Moderate	8.17	3.94	0
E	Low	8.79	3.38	26
F	Low	5.92	2.46	36
G	Low	4.24	1.72	30
H	High	23.71	6.74	70
I	Low	8.83	2.60	4
J	High	8.61	3.39	49
K	High	9.26	5.20	5
L	High	7.20	5.10	13
M	High	9.64	5.89	62
N	Low	8.95	3.68	11
O	Low	11.36	4.83	15

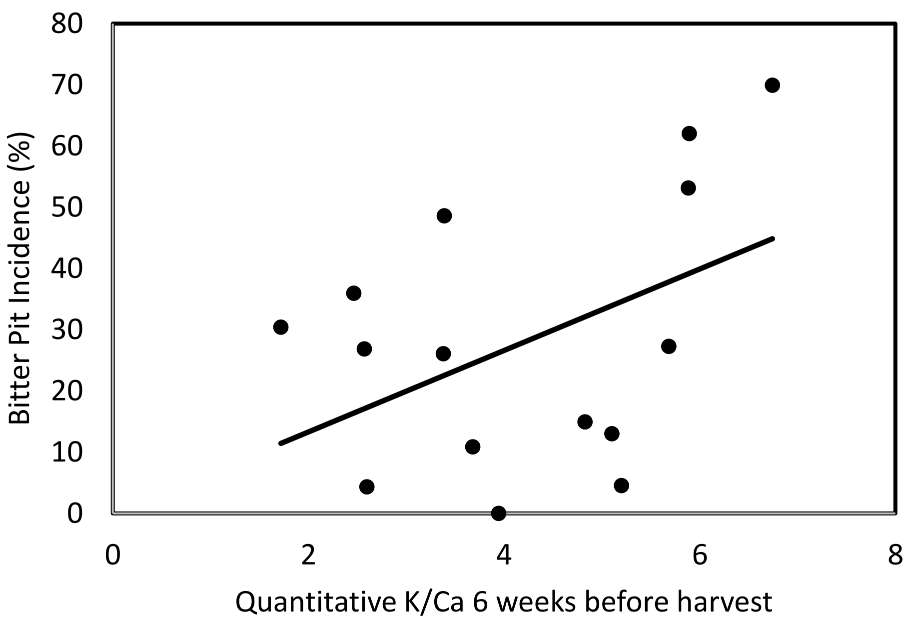

Figure 12. Calibrated potassium: calcium (K/Ca) ratios for Honeycrisp apple measured six weeks before harvest from 15 different commercial orchards related to bitter pit. Line represents best linear fit ($P<0.05$) for this relationship.

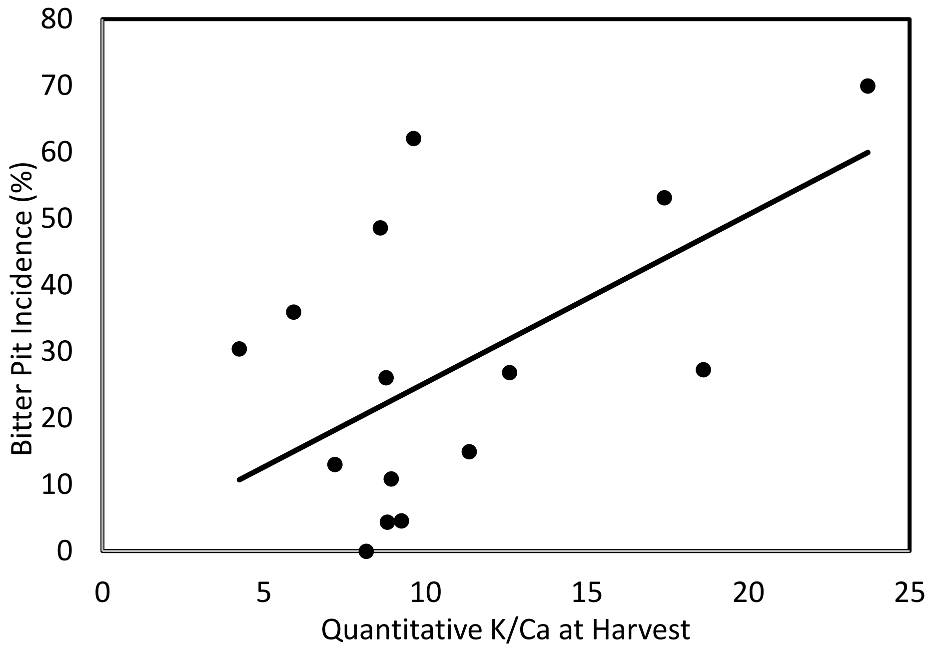

Figure 13. Calibrated potassium: calcium (K/Ca) ratios for Honeycrisp apple measured six weeks before harvest from 15 different commercial orchards related to bitter pit. Line represents best linear fit ($P<0.05$) for this relationship.

Since the start of this project, this information has been incorporated into two peer-reviewed publications and one more is in preparation that will use these approaches developed with this project. Furthermore, this work has been included in 10 state and regional talks, 4 national talks, and 5 international invited presentations. We are also in the process of testing its use for measuring strontium and rubidium uptake which act as tracers for calcium and potassium, respectively. We have found that the PXRF measurements are nicely related to analytical approaches for measuring these elements. This allows us to non-destructively sample the same tissue over the course of the season to measure fluxes into specific plant tissues. This instrument is being used in several labs in the US at least partially based on this project and funding from the WTFRC.

EXECUTIVE SUMMARY

This project had the goal of developing some translation of the semi-quantitative measurements given using PXRF to quantitative measurements of calcium and potassium. Additionally, we sought to identify how surface measurements using a portable x-ray fluorometer related with traditional lab analysis. In the previously funded project, the focus was to validate that the instrument measurements agree with traditional lab analysis. In the current project, we sought to develop calibrations that can be inserted into the commercially available unit for measurements of fruitlet, fruit at harvest, or fruit in storage. However, there is evidence that each cultivar might behave differently with the instrument.

Across several fields at equal points of maturity, the slope of the lines remain similar indicating that one calibration could be used for a single cultivar if the sampling protocol is clear and uniform. Additionally, skin thickness did not contribute to variability in measurements within individual cultivars. Across cultivars, changes in both peel and cortex density would likely contribute to variability in the readings that may or may not be related to bitter pit incidence. This would be a testable hypothesis for future work with segregating populations from breeding material.

Since the start of this project, other research groups have started to integrate PXRF approaches into their research. This includes measurements of leaf tissue, apple roots, stems, and fruit. There is a general trend for using this technology for making these types of measurements since it provides rapid and immediate measurements that can be used non-destructively to track changes in the same sample over time or to measure many more replicates than would normally be feasible in research.

This instrument will have its primary utility as a research tool but for larger operations or consulting, this could contribute to assigning risk assessment for bitter pit incidence for commercial Honeycrisp orchards. There are several models of this instrument available but they only come with generalized calibrations that may not agree with measurements of fruit. Newer PXRF systems that come with existing calibrations would need to be verified as they are not the same calibrations that I have developed. The calibrations developed within this project could be easily incorporated into the newer model instruments and would be available if any industry members in Washington State wish to use this instrument in QC as part of their operations.