FINAL PROJECT REPORT

Project Title: Testing a portable, non-destructive measure of Ca, Mg and K in apple

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Percentage time per crop: Apple: 80 Pear: 15 Cherry: 5

Other funding sources: None

Total Project Funding: $76,632

Budget History:

Item	2014	2015
Salaries[1]	$12,000	$12,480
Benefits[2]	$3,588	$3,732
Equipment[3]	$39,000	$0
Supplies[4]	$2,400	$2,400
Travel[5]	$508	$532
Total	$57,496	$19,136

OBJECTIVES

1. **Test if the concentration of calcium, potassium and magnesium measured non-destructively using handheld x-ray fluorescence (XRF) corresponds to traditional lab analysis of fruit.**

2. **Determine if there is a relationship between K+/Ca2+ ratios and the presence of bitter pit in apple**

3. **Explore applied applications of the instrument in the field and lab**

SIGNIFICANT FINDINGS

- The instrument arrived at the end of September, 2014 and was set up in the lab (Figure 1).
- Magnesium is too light of an element to detect with any precision using the handheld XRF. Calcium and potassium are measurable.
- There is a strong relationship between potassium and calcium measured using the XRF and calcium and potassium concentrations measured using traditional lab analysis (Figures 3-6)
- Presence of bitterpit is associated with high ratios of K/Ca measured using the XRF (Figure 7-9).
- There is a high variability in calcium and potassium within a tree and is likely related to the location on the tree (Figures 10 and 11).
- The effect of crop load on calcium and potassium ratios can be measured using the XRF (Figure 12)
- Calibrations need to be developed for different tissues (skin and flesh of different tree fruit) to be able to quantify potassium and calcium concentrations in ppm. Currently, values are semi-quantitative and allow for relative comparisons among treatments.

RESULTS & DISCUSSION

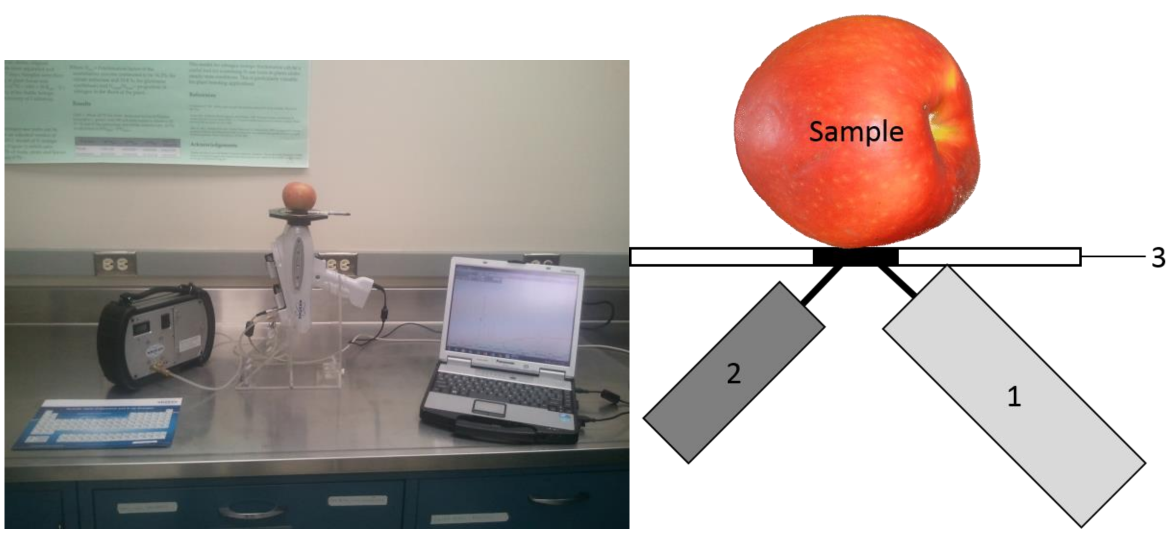

Figure 1. Non-destructive measurement of an apple fruit using a Bruker Tracer III SD portable x-ray fluorometer. 1. X-ray source (rhodium tube); 2. Detector (Si(Li) X-ray detector); 3. Platform
Semi-quantitative, non-destructive measurements using a hand-held PXRF are correlated with MP-AES analysis of Ca and K

Calcium concentrations in apple peel tissue ranged by a factor of 10 from approximately 150 mg kg-1 DW to 1500 mg kg-1 dw. Semi-quantitiative measurements ranged by equivalent orders of magnitude in the apple samples. Out of 54 samples, semi-quantitative measurements of calcium using the portable x-ray fluorometer measured on four spots around the equator of the fruit were significantly correlated with the calcium concentrations in the entire peel (See figure 2 for a description of sampling) surrounding the equator of the apple (Figure 3) ($r=0.941$, $P<0.001$). Similarly, when potassium concentrations were measured using MP-OES in apple tissue measured with the PXRF, there was a significant correlation (Figure 4) ($r=0.986$, $P<0.001$). For these 54 apples analyzed in this way, 15 showed symptoms of bitterpit, a calcium-related physiological disorder, on the calyx end of the fruit. Apples showing bitterpit had a higher potassium to calcium ratios than healthy apples (data not shown). High potassium:calcium ratios in fruit has been previously used as an indicator of bitterpit susceptibility (Ferguson and Watkins, 1983; Perring and Pearson, 1986). Individual fruit analyzed show high variations in the presence of potassium and calcium. Perring and Pearson (1986) demonstrated that calcium is lower on the calyx end of the fruit than the stem end. Furthermore, Xiaoyan and Chenglian, 2010 reported that calcium was greater on the sun-exposed portion of the apple and was again, higher on the stem-end than the calyx end of the fruit. In pears, calcium and potassium concentrations in individual fruit were strongly correlated with non-destructive PXRF measurements. The pearson correlation coefficients were 0.958 and 0.977 for PXRF measurements of calcium and potassium compared to digestion analysis using MP-AES (Figure 5 and 6).

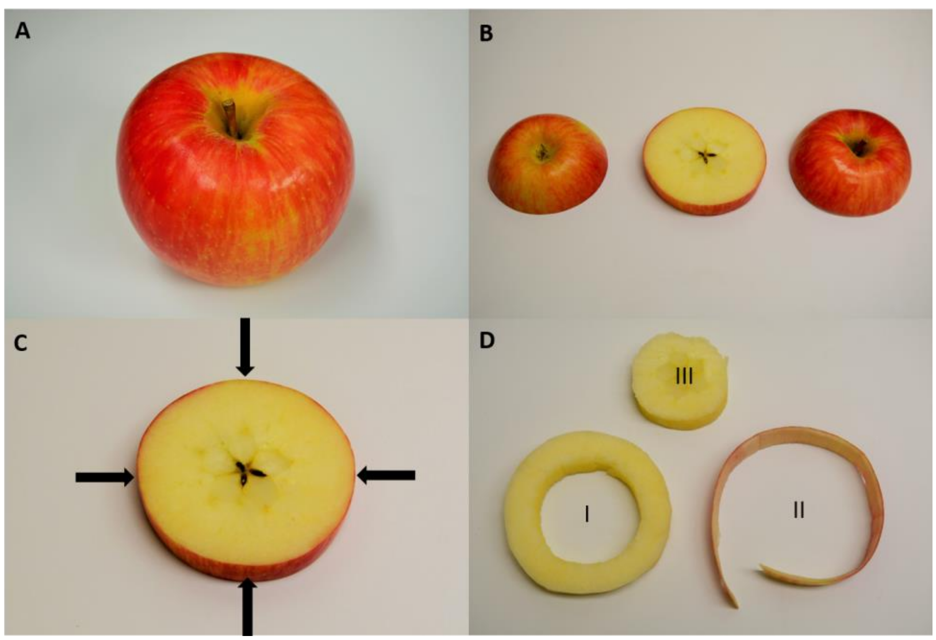

Figure 2. Sampling protocol of a 'Honeycrisp' apple for handheld x-ray fluorescence (XRF) and destructive lab sampling. A. Whole apple where 4 measurements were made with the handheld XRF along the equator of the fruit. B. To compare handheld XRF, an equatorial slice was removed from the apple. C. A representation of the sampling locations for handheld XRF analysis. D. Destructive sampling for elemental analysis of I) the outer cortex of the apple used for analysis of homogenized pellets using the handheld XRF and then digesting for lab elemental analysis, II) apple peel to compare with handheld XRF measurements made along the equatorial region of the fruit while it was whole, and III) the core of the apple which was discarded.

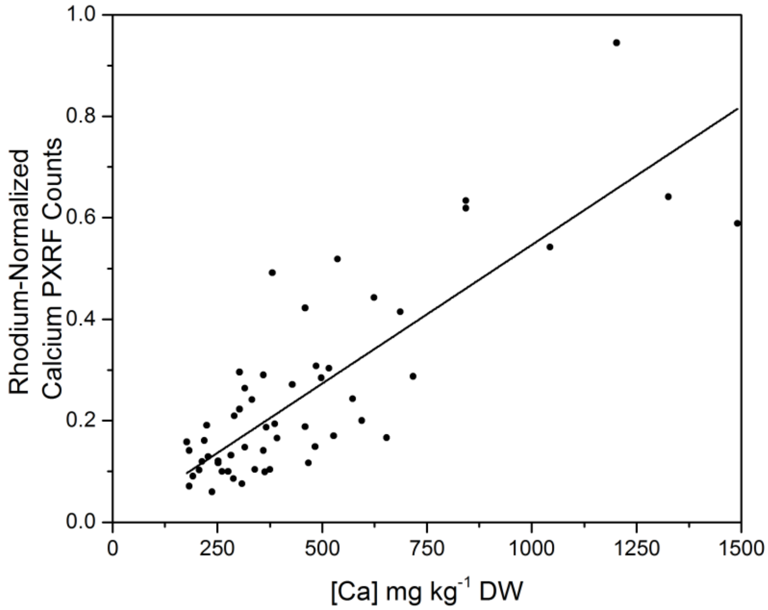

Figure 3. Correlation of non-destructive, semi-quantitative mean calcium content compared to corresponding MP-AES analysis of calcium in apple peel (N=54).

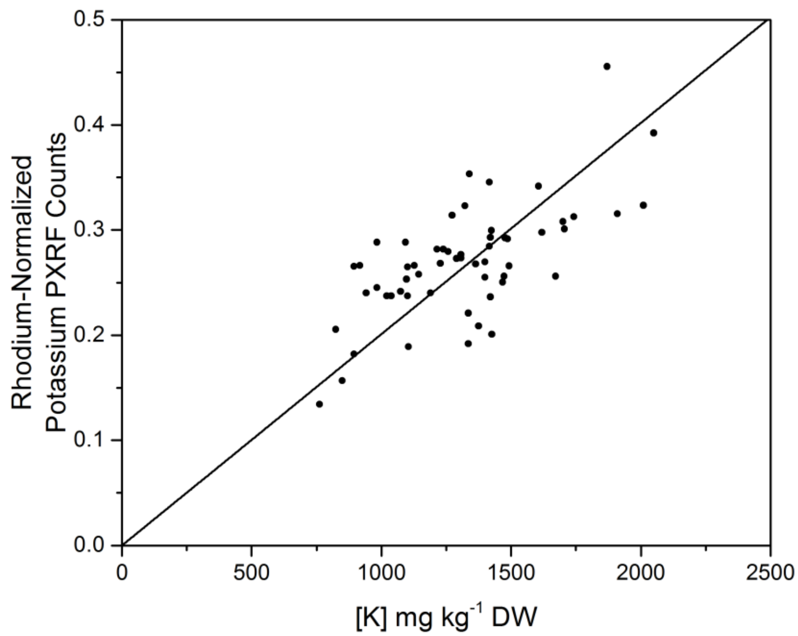

Figure 4. Correlation of non-destructive, semi-quantitative mean potassium content compared to corresponding MP-AES analysis of potassium in apple peel (N=54)

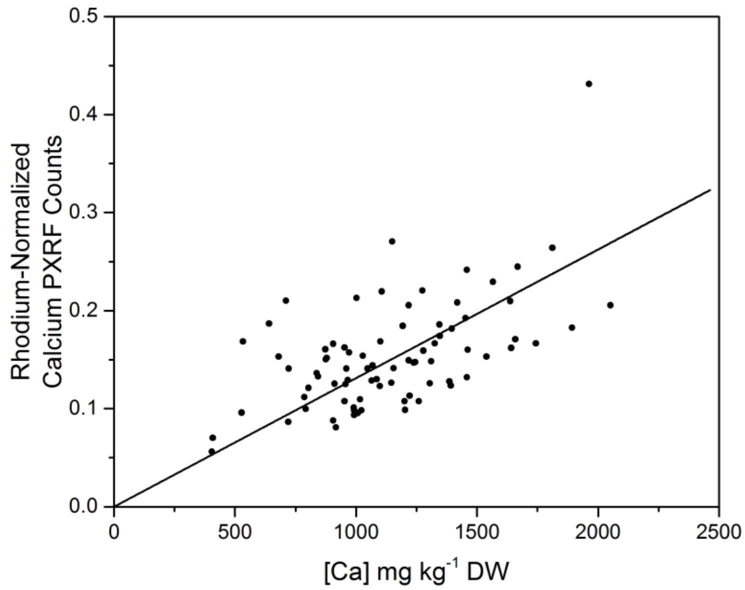

Figure 5. Correlation of non-destructive, semi-quantitative mean calcium content compared to corresponding MP-AES analysis of calcium in pear peel (N=80)

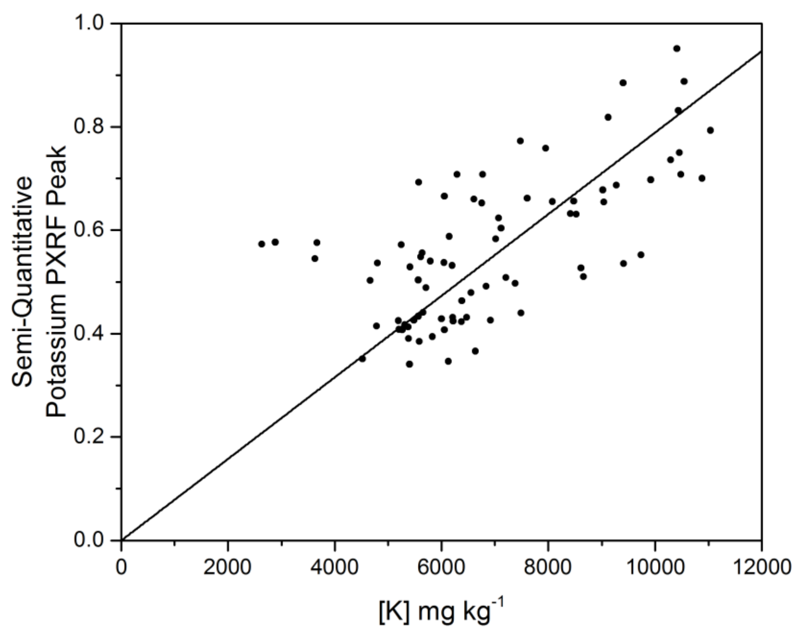

Figure 6. Correlation of non-destructive, semi-quantitative mean potassium content (n=4) compared to corresponding MP-AES analysis of potassium in pear peel (N=80)

Calibration development for quantitative analysis would represent an advancement in the capability to use handheld PXRF for non-destructive analysis. However, heterogeneity in cell type thickness and density among different cell types (i.e. epidermis versus cortex in an apple fruit) can lead to differences in x-ray penetration depth. Using epidermis and cortex as an example, if epidermal thickness was different among varieties of apple, the proportion of epidermis and cortex analyzed non-destructively using the handheld PXRF would also be different. Since the elemental concentrations are different between the epidermis and the cortex, differences in the proportions of each tissue analyzed using PXRF should produce different results. Therefore a calibration developed for one variety would likely be different for another. Calibration development will likely require species and possible even cultivar-specific calibrations for the instrument. Semi-quantitative analysis is still always a possibility but comparisons would have to be among biologically similar samples for valid relative comparisons.

Drying, grinding and pelleting of tissue is also suitable for measuring calcium and potassium concentration of apple flesh

As a more conventional comparison of apple and pear tissue among measurements and similar to Reidinger et al. (2012), homogenized tissue of apple from two different regions in Washington State were analyzed using the handheld XRF. Cortex samples had lower amounts of calcium and potassium than the peel for apple and were less variable than when analyzing the peel. In general, the region of the apple with the lowest concentrations of calcium is in the outer cortex (Wünsche and Ferguson 2005) and is similar to what was measured here using handheld XRF analysis of pelletized outer cortex tissue. Similar to the non-destructive measurements made on the peel, there was a significant relationship between the pelleted homogenized samples and MP-OES lab analysis (Figure 9 and 10). Pearson correlation coefficients were 0.787 and 0.89 for calcium and potassium, respectively. Reidinger et al. (2012) reported high precision in analyzing phosphorus and silicon using this approach. McLaren et al. (2012) also reported high correlations between homogenized samples and lab analysis of leaves from four different species of plants. However, homogenization still represents a destructive approach to sampling. In specific cases, such as calcium, which can be locally deficient within the plant, non-destructive analysis is more appropriate since variation between tissues and organs (in the case of non-destructive handheld XRF) may be more important than a pooled value from completely homogenized tissue. Handheld XRF measurements of in-tact fruit allows for repeated measures of the same spot over time. It also provides opportunities to address different biological questions related to nutrient uptake and mobility that are not possible using traditional lab analysis or destructive homogenization.

Calcium and potassium are unevenly distributed within an apple fruit

There is a high amount of variation in calcium and potassium in the peel of apple fruit that was measured using handheld PXRF. Figure 11 shows the potassium to calcium ratio on the surface of a Honeycrisp apple affected by bitter pit and a healthy bitter pit apple. The potassium to calcium ratios were almost twice as high in the bitter pit affected fruit and the variation between different regions of the fruit was also markedly different. The differences in these ratios were driven by both a higher presence of potassium and a lower presence of calcium (data not shown). Calcium and potassium were as much as an order of magnitude different in one location on the fruit compared to another. As concentration increases in fruit, the likelihood of large localized differences increased. The sun-exposed portion of the fruit often had larger concentrations of calcium than shaded portions of fruit (data not included). In the limited number of fruit analyzed here, there were no distinct gradients from the stem end to the calyx end of the fruit. However, Lewis and Martin (1973) reported a decrease in calcium along the longitudinal axis of the fruit. Initial observations on larger amounts of fruit indicate that this trend was also observed when using the PXRF as a semi-quantitative measure of calcium and potassium. Differences not only exist on the peel of an apple but also within the fruit. Ferguson and Watkins (1983) reported that flesh calcium concentrations decrease with increasing distance from the core. This is related to the mode of distribution and proximity to xylem vessels in the fruit (de Freitas et al. 2012).

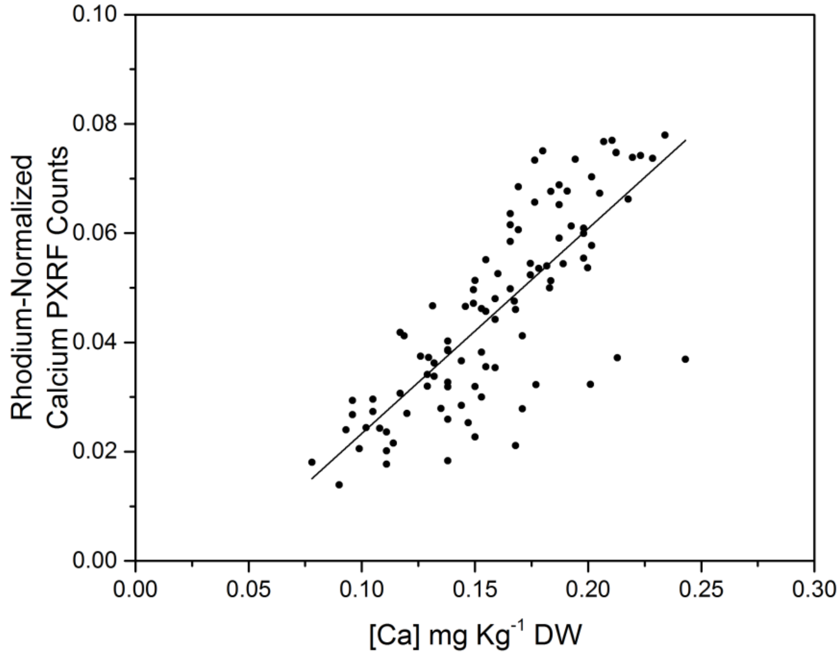

Figure 7. Correlation of non-destructive, semi-quantitative mean calcium content of pelletized, ground apple cortex tissue compared to corresponding MP-AES analysis of calcium. (N=104)

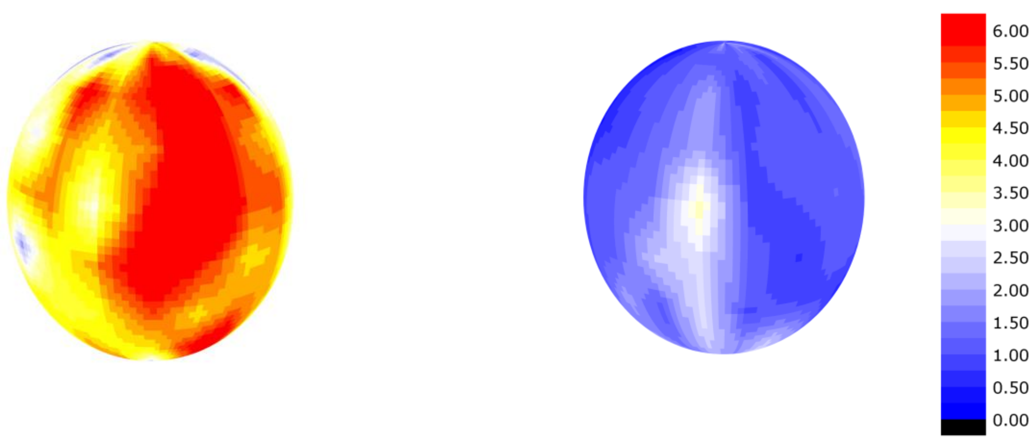

Figure 8. Smoothed semi-quantitative potassium:calcium ratio on the surface of a apple affected by bitter-pit (left) and a healthy apple (right) calculated as the rhodium-normalized PXRF counts for potassium divided by the rhodium-normalized PXRF counts for calcium.
Potassium/Calcium ratios are two times greater in fruit affected by bitter pit and is measurable using the handheld PXRF

In the fall of 2014, 20 apples that were either healthy or affected by bitterpit from three different culativars ('Golden Delicious', 'Granny Smith' and 'Honeycrisp' from two different locations) were analyzed using the handheld PXRF on four locations along the equator of the fruit. Calcium and potassium was measured and then expressed as a potassium:calcium ratio. In all three cultivars, potassium:calcium ratios were more than two times higher in fruit affected by bitterpit. This was a function of both lower calcium concentrations and higher potassium concentrations. There appeared to be differences among cultivars in the threshold for bitterpit development. Granny Smith appeared to have a much higher threshold than Honeycrisp or Golden Delicious. This will need to be examined in more detail in the future.

Calcium and potassium:calcium ratios measured using the handheld PXRF varied depending on position within the canopy of 'Honeycrisp' apple on a M9 rootstock

As part of another WTFRC-funded project, 16 apples per tree were tagged in June in different regions of the tree. The distance from the ground and from the trunk was measured. At harvest, these fruit were picked and taken back to the lab for PXRF analysis to measure calcium and potassium concentrations. There was a high amount of variability in calcium and the potassium:calcium ratio in the canopy (Figures 9 and 10). In general, calcium was higher on the outer parts of the canopy in the upper half of the tree. Subsequently, the potassium:calcium ratios were lower in the upper and outer parts of the canopy. This implies that there may be differences in bitter pit susceptibility between fruit from the lower-interior parts of the tree and the upper-outer parts of the tree.

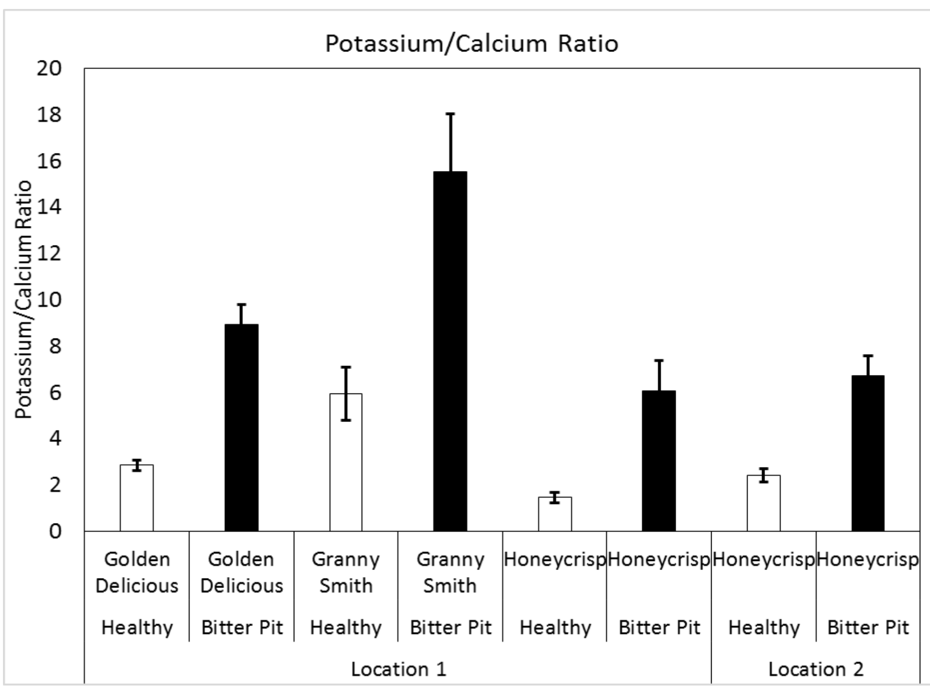

Figure 9. Mean potassium/calcium ratio of Golden Delicious, Granny Smith and Honeycrisp apples either showing bitterpit symptoms or not. Each mean is an average of 20 apples and error bars represent standard error.

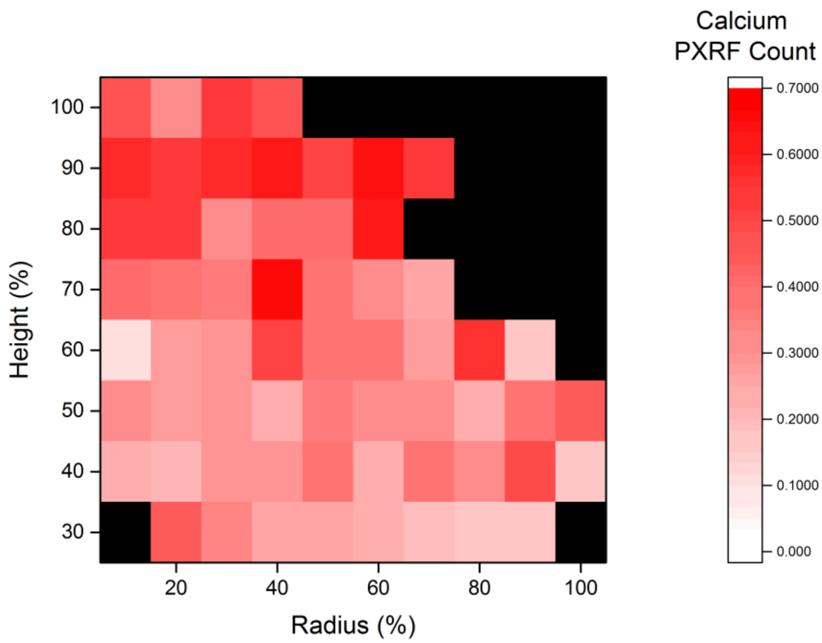

Figure 10. Calcium PXRF counts of apples measured using the PXRF at different heights (y-axis) and distances from the trunk (x-axis). Height and distance is expressed as a relative distance from the top of the tree or tip of the branch where 100% is equal to the top of the tree and tip of the longest branch. 16 Honeycrisp apples were measured from 9 trees at 9 different sites after being tagged in early June.

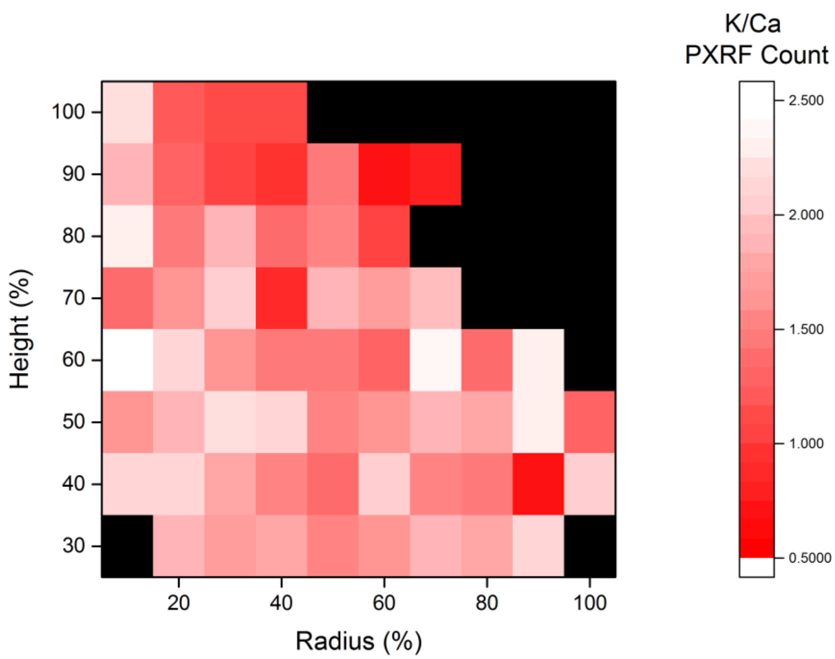

Figure 11. Potassium:calcium (K/Ca) ratios of apples measured using the PXRF at different heights (y-axis) and distances from the trunk (x-axis). Height and distance is expressed as a relative distance from the top of the tree or tip of the branch where 100% is equal to the top of the tree and tip of the longest branch. 16 Honeycrisp apples were measured from 9 trees at 9 different sites after being tagged in early June.

Crop load affects calcium and potassium concentrations in apples measured using the handheld PXRF.

Eight Honeycrisp apples were measured from 78 different trees from 9 different orchards across Washington State that were thinned to different target crop loads in early June. Calcium increased as crop load increased from less than 3 fruit per cm-2 to 5 to 7 fruit cm-2 then decreased as crop load increased further. However, potassium showed a linear decrease in concentration as crop load increased. As such, the potassium: calcium ratios were the highest at low crop loads then gradually decreased as crop load increased. The treatment with the greatest potassium to calcium ratio is also the treatment with the most optimum crop load for quality and storability (Wünsche and Ferguson, 2005; Serra et al. 2016 unpublished).

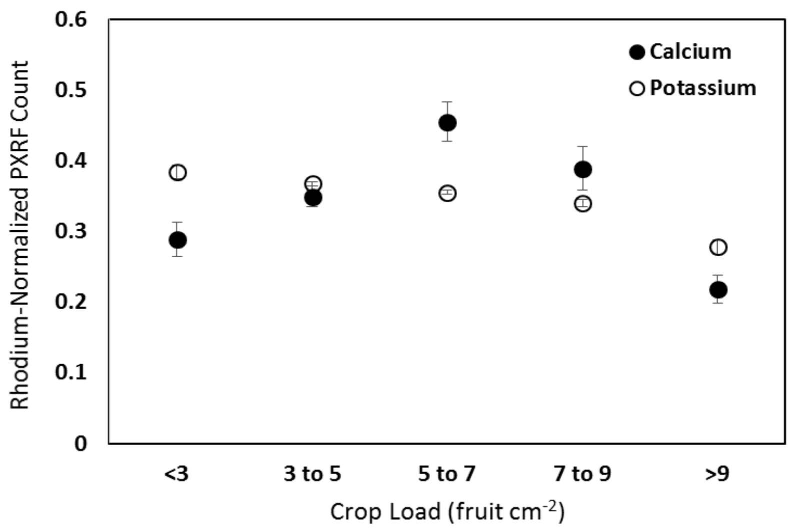

Figure 12. Rhodium-normalized portable x-ray fluorometer (PXRF) counts for calcium and potassium as a function of tree crop load measured on 78 trees across 9 different orchards.

CONCLUSIONS

Handheld PXRF has potential to be used as a semi-quantitative instrument that provides information to make relative comparisons on calcium and potassium concentrations amongst treatments with biologically similar samples. However, as a quantitative measure, there is still a need for the development of species-specific, or even cultivar-specific, calibrations. Even still, here we show the use of handheld PXRF for semi-quantitative, non-destructive measurements. Using this approach, repeated measures are possible on the same biological sample through time and it also permits greater replication, reduced sampling time and more complex data sets that are often not possible with traditional lab analysis techniques. Potential applications include improved precision in estimating changes in elemental concentration over time in plant tissue, analysis of the elemental distribution within an organ, within a tree or within a field when compared with traditional lab analysis. Handheld PXRF is a viable alternative to compliment traditional lab elemental analysis which can improve the understanding of calcium and potassium dynamics in plants and make *in-situ* non-destructive elemental measurements in the field.

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EXECUTIVE SUMMARY

Objectives

1. **Test if the concentration of calcium, potassium and magnesium measured non-destructively using handheld x-ray fluorescence (XRF) corresponds to traditional lab analysis of fruit.**

2. **Determine if there is a relationship between K+/Ca2+ ratios and the presence of bitter pit in apple**

3. **Explore applied applications of the instrument in the field and lab**

In the 16 months that the instrument has been active in the lab, we have been testing it against lab analysis in pears and apples. We have analyzed apples, pears and cherries. Non-destructively, the instrument measures calcium and potassium of a layer that is approximately 1 mm (~1/25 of an inch) thick. This is inclusive of the peel and a small portion of the flesh. Magnesium is too light to precisely measure using the PXRF in plant tissue. There is a strong relationship between potassium and calcium measured using the XRF and calcium and potassium concentrations measured using traditional lab analysis. This was evident in both non-destructive analysis at the surface of the fruit and destructive analysis using dried, ground and pelleted flesh tissue.

The presence of bitter pit is associated with high ratios of K/Ca measured using the XRF. In three apple cultivars, there were consistent trends where semi-quantitative K/Ca ratios measured using the PXRF were almost two times greater in fruit affected by bitter pit than healthy fruit. There is also a high degree of variability on the surface of the fruit for the K/Ca ratio. There is a high variability in calcium and potassium within a tree (Figures 10 and 11). The effect of crop load on calcium and potassium ratios can be measured using the XRF (Figure 12).

Handheld PXRF has potential to be used as a semi-quantitative instrument that provides information to make relative comparisons on calcium and potassium concentrations amongst treatments with biologically similar samples. In a productive day, a user can make approximately 200 measurements in the field or up to 500 measurements in the lab. With replication on individual fruit, a user could measure about 50 fruit in the field (4 measurements per fruit) and 120 fruit in the lab. Only in situations where changes in calcium need to be measured on the same fruit (i.e. calcium applications or change in calcium concentrations during fruit development) does measurements need to be made in the field.

As a quantitative measure, there is still a need for the development of species-specific, or even cultivar-specific, calibrations. Even still, here we show the use of handheld PXRF for semi-quantitative, non-destructive measurements. Using this approach, repeated measures are possible on the same biological sample through time and it also permits greater replication, reduced sampling time and more complex data sets that are often not possible with traditional lab analysis techniques. Potential applications include improved precision in estimating changes in elemental concentration over time in plant tissue, analysis of the elemental distribution within an organ, within a tree or within a field when compared with traditional lab analysis. Handheld PXRF is a viable alternative to compliment traditional lab elemental analysis which can improve the understanding of calcium and potassium dynamics in plants and make *in-situ* non-destructive elemental measurements in the field.