FINAL PROJECT REPORT **YEAR: 4 of 3 (+1 yr NCE)**

Project number: PR14-108A

Project Title: Improving quality and maturity consistency of 'D'Anjou'

PI: Stefano Musacchi
Organization: Washington State University/ TFREC
Telephone: 509-663-8181 x236
Email: stefano.musacchi@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (1): David Rudell
Organization: USDA, ARS
Telephone: 509-664-2280 x245
Email: david.rudell@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (2): Jim Mattheis
Organization: USDA, ARS
Telephone: 509-664-2280 x249
Email: james.mattheis@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Sara Serra (WSU/TFREC), Glade Brosi (Stemilt)

Total Project Funding: **Year 1**: $ 65,992 **Year 2**: $ 67,272 **Year 3**: $ 68,602

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget History 1

Item	2014	2015	2016	2017 (NCE)
Salaries[1]	24,000	24,960	25,958	0
Benefit[1]	7,992	8,312	8,644	0
Travel[2]	500	500	500	0
Goods and Services[3]	3,000	3,000	3,000	0
Total	35,492	36,772	38,102	0

Footnotes:

[1]Salaries and benefits for 50% Ag. Research Assistant (Musacchi).

[2]Travel to different orchards and farm where the different trials will be conducted (Musacchi).

[3] Consumable lab ware and mineral analyses.

Budget History 2

Item	2014	2015[2]	2016[2]	2017 (NCE)
Wages[1]	15,000	15,000	15,000	0
Goods and Services[2]	15,500	15,500	15,500	0
Total	30,500	30,500	30,500	0

Footnotes:

[1] $12,500 for 25% annual instrument service contracts. $3,000 for consumables

[2]Add proposed same amount for year 1 if work is to be performed in years 2 or 3.

OBJECTIVES

1) *Determine maturity and quality variation as impacted by tree and orchard management regimes.*
2) *Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.*

SIGNIFICANT FINDINGS

Overall

- Considerable variability in fruit maturity exists within the large canopy of an open vase tree.
- The use of DA meter in pre-harvest on selected trees helps to be more aware of the maturity stage and variability within the canopy to address the harvest time.
- From year to year fruit maturity distribution (accordingly to the DA meter) at 2 weeks before harvest is variable. This indicated a potential use of this tool to determine the harvest time.
- The DA meter values (I_{AD}) for internal and external canopy fruit were different at harvest. External fruit on average tend to have lower I_{AD} values compared to Internal fruit.
- At harvest, external fruit had less green background, higher red blush coverage, higher dry matter %, and higher soluble solid content than internal fruit.
- Internal fruit tend to be greener than External up to 8 months of storage.
- Crop inconsistency resulting from pear canopy position impacts most postharvest supply chain decisions.
- Fruit ripening and potentially flavor is different depending upon canopy position.
- Canopy position impacts postharvest behavior including superficial scald risk. This can affect the need to repack fruit boxes.
- Levels of natural peel chemicals we have linked with light exposure may be exploited to develop in-field or warehouse sorting tools to reduce crop variability.

1) Determine maturity and quality variation as impacted by tree and orchard management regimes.

Pre-harvest assessment and fruit maturity distribution

To assess the maturity on the 11th of August 2016 (18 days before harvest) a total of 677 fruit (included 640 good fruit and 37 of <60 mm size and/or with defects) were harvested. Total yield per tree was 121 kg and the average fruit weight was 179 g. Sunburned incidence was 1.8%, cork was 0.44% and no frost damaged fruit were observed.
By measuring I_{AD} before harvest, we determined the maturity stage of the fruit population, in fact, in 2016, more than 2 weeks before harvest, more than 95% of fruit were classified in the least mature I_{AD} classes (above 2.00 I_{AD}) and only a small percentage (0.2%) of fruit were classified in the more ripe classes (below 1.80 I_{AD}, Fig. 1).
From year to year the maturity distribution of fruit accordingly to the DA meter at 2 weeks before harvest is variable.

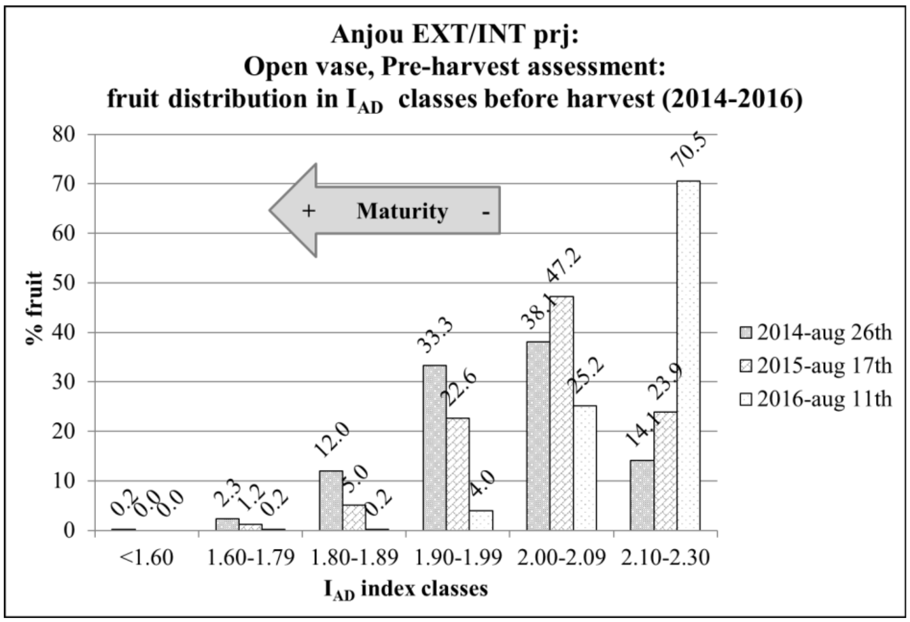

Figure 1: Pre-harvest assessment of fruit maturity distribution across the canopy of an open vase tree in 2014, 2015 and 2016 (≈2 weeks before harvest). Fruit % in each I_{AD} class of ripening is represented.

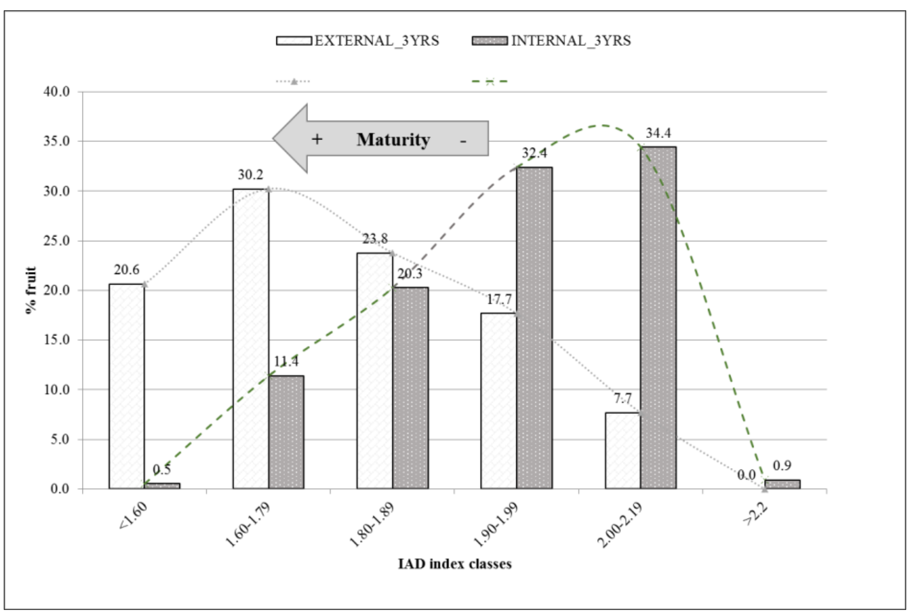

Figure 2: Distribution of fruit picked categorized by canopy position (external and internal) and I_{AD} class as well as in the 3 years, percentage are calculated on all fruit harvested in 3 yrs.

Fruit maturity distribution within I_{AD} classes at harvest divided by canopy position confirmed the observations done in the previous year where Internal fruit tend to be more unripe than the External one (Fig. 2). Looking at the distribution as all fruit harvested in 3 years, ≈34% of Internal fruit fell in the least ripe classes (I_{AD} <2.00), while only ≈8% of External fruit belonged to that class (Fig. 2). Almost 21% of the External fruit were classified in the most ripe categories (I_{AD} <1.60), while only 0.5% of the Internal ones resided in the same classes (Fig. 2).
This represents a strong example of how different are fruit belonging to those two extreme canopy positions. Harvesting as strip pick and collect all fruit in the same bin does not allow anymore to investigate canopy positions variations.

PAR measurement per single fruit and light in the canopy (2016)

PAR measurements of fruit marked for sampling allowed us to accurately choose fruit from the two canopy positions. The percentage of light intercepted by External fruit averaged 92.1% while only 1.4% by Internal fruit (Fig. 3A). Fruit belonging to light interception range from 30% to 70% were discarded. This type of precise harvest allowed us to track the behavior of the two type of pears in postharvest.
A qualitative measure of the light spectrum by a spectroradiometer (measure of photon flux in µmol s^{-1} m^{-2}) was done on 21st of July 2016 underneath one large canopy. A huge variability of light spectra hitting the trees in the four possible inner quadrants (South-West, North-West, North-East, South-East) was observed (Fig. 3B). Three quadrants on four showed lower radiation from 300 to 700 nm (PAR range) while the North-West quadrant was illuminated by direct sunlight and the trend looked similar to a full sun light spectrum (approx. External situation, Fig. 3B). Leaves in the inner part of the canopy have less energy available for photosynthesis so they may be subjected to a shortage of photo assimilates to translocate to the fruit.

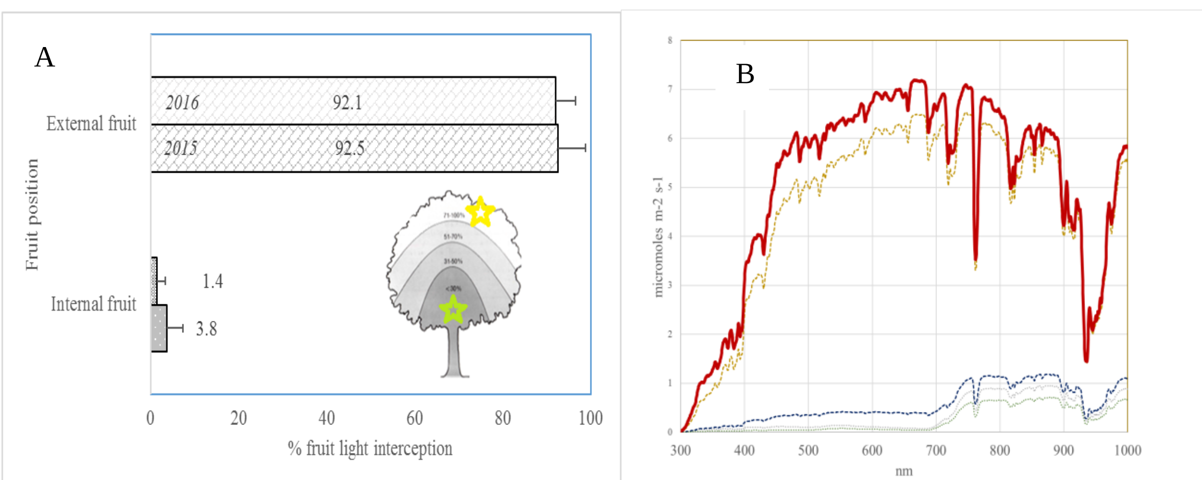

Figure 3: A) Percent of light interception of fruit harvested from the two canopy positions as determined by PAR measurement using the Q53292 quantum sensor in 2015 and 2016 (Li-Cor). Values are average ± stdDev. B) Photon flux measured in the large canopy on 21st of July 2016 between 10 am and 12 pm. Solid line is the light spectra of full sun measured above the canopy at 3.5 m from the ground, four different dashed lines are the four light spectra in the four quadrants (south-west, north-west, north-east, south-east) of a large tree at 40 cm from the trunk and 130 from the ground.

2014 fruit storage and quality assessments

Fruit quality analysis at harvest (T0) showed that External fruit were significantly heavier, larger, and had higher titratable acidity and soluble solids compared to Internal fruit at harvest. Internal were greener. No difference in chroma and firmness.

Regarding I_{AD} index decrease in storage, Internal fruit reported always higher values (less ripe fruit) than External fruit from harvest to 8 months of storage and they showed a slower I_{AD} index decrease (without any ripening post-storage) than external one where each pullout registered a significant drop in this index, suggesting a faster kinetics of ripening of those fruit. The same behavior was noticed after 7 days of ripening at room temperature, where differences between Internal and External were maintained (Fig. 4).

Regarding firmness and storage duration, we did not find differences between External and Internal fruit from harvest up to 6 months, only after 8 months. Internal fruit were firmer than external immediately after removal from cold room. After 7 days ripening, Internal fruit were firmer than External except for no difference at 6 months of storage (Fig. 5).

Dry matter % was always higher in External fruit than Internal at both stages from 3 to 8 months of storage duration. In general, no big dry matter difference found among pullouts. Similar trend was reported for Soluble Solid content (SSC, Brix): External fruit showed higher SSC than Internal with or without ripening time. Correlation between dry matter % and SSC improved along storage moving from R^2=0.677 at 3 months (day 0) to R^2=0.782 at 8 months (day 0). Titratable acidity was significantly higher in the Internal fruit than External at day 0 only after 8 months, while exogenous ethylene was higher in the External than Internal at day 7 after 6 and 8 M.

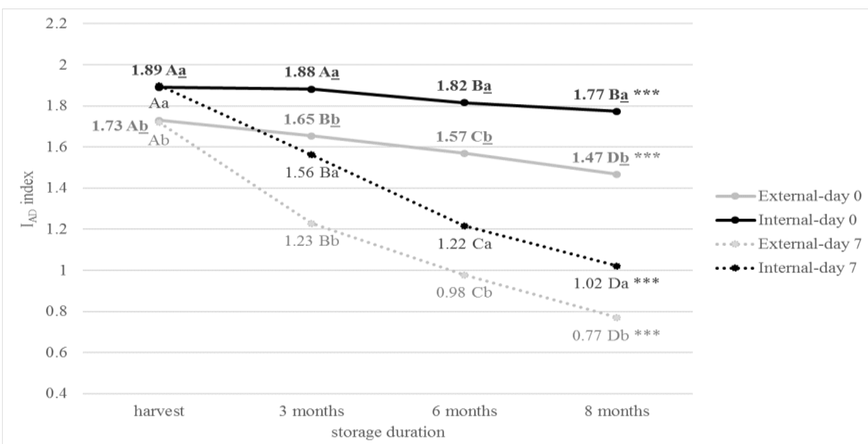

Figure 4: I_{AD} index decrease in storage (fruit harvest 2014). Significance: p<0.05, *; p<0.01, **; p<0.001, *; ns, not significant Capital letters discriminate means among storage duration (horizontally), small letter between canopy position in pairs (vertically).**

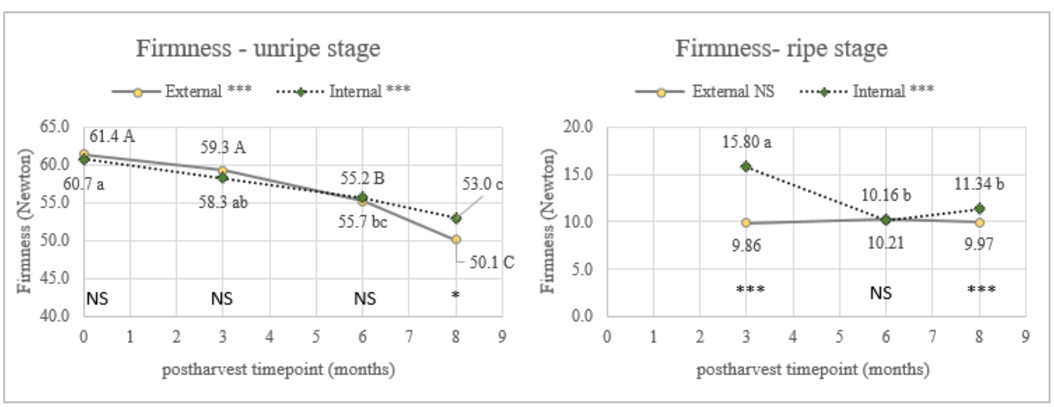

Figure 5: Firmness decrease at unripe and ripe stage in storage (fruit harvest 2014). **Significance: p<0.05, *; p<0.01, **; p<0.001, ***; ns, not significant.** **Capital and small letters discriminate means among storage durations within the same canopy position (horizontally), while in a text box below significance between canopy positions in pairs (vertically) within each storage time.**

2015 fruit storage and quality assessments

Fruit from Internal and External canopy regions were picked separately on 31st August 2015. Fruit from each light condition were separated into two bins (containing 460 external and 486 internal pears) and immediately moved to 40°F for fruit maturity distribution analysis and sorting in DA classes.

Within each group, fruit were again classified using I_{AD} into 5 classes (I_{AD}<1.60, 1.60<I_{AD}<1.79, 1.80<I_{AD}<1.89, 1.90<I_{AD}<1.99, 2.00<I_{AD}<2.19). The first was only included in the External fruit (not present in Internal) and Internal fruit in 1.60<I_{AD}<1.79 class were not enough to cover all pullout so harvest and 8 months storage were chosen. Fruit belonging to each class were, then, equally divided into 3 groups for 0 (= harvest), 6, and 8 months CA storage. Fruit were stored in a research CA room (31°F, 2% O_2 and 0.8% CO_2). For each pullout, except for T0 at harvest, fruit were split in 2 subgroups: with or without 7 days of post-storage ripening time. Fruit quality analysis in 2015-2016 pullouts was performed in the same manner as 2014.

At harvest 2015, External fruit had less green background, higher red blush coverage (%), higher firmness, higher dry matter %, and higher soluble solid content (SSC, brix) than Internal fruit (data not shown). As reported in literature, sun-exposed 'Bartlett' pears had higher firmness than pears grown in the shade before and after ripening at room temperature probably due to the direct sun exposure (Raffo et al., 2011). This firmness difference between positions was a variation in comparison to 2014.

Within each canopy position fruit were divided accordingly to the I_{AD} index in classes and differences among them emerged. External fruit belonging to the least ripe class (2.00<I_{AD}<2.19) presented the highest background hue value (tended to more green) and the lowest SSC content (12.9 °Brix), while External fruit belonging to the most ripe class (I_{AD}<1.60) were bigger in diameter, less firm and higher SSC (14.0 °Brix). Similarly, the 2.00<I_{AD}<2.19 class for Internal fruit showed higher background hue and pH, lower SSC (11.0 °Brix), and lower acidity than the most ripe class for the same light condition (data not shown). No differences were detected in terms of dry matter %, total number of seed, viable vs dead seeds, ethylene production and weight. When all ripening classes and canopy positions were compared as combinations, significant differences of fruit weight, overcolor, dry matter %, firmness, diameter, pH and soluble solid contents, were found at harvest (Fig. 6).

After 6 months of storage in CA (T1), without any post-storage ripening time, External and Internal fruit differed for color/blush, firmness, SSC, dry matter % and pH with the most exposed fruit less green, firmer, higher in SSC and dry matter and lower pH. Same comparison done after 7 days of ripening (+6M storage + 7 days at room temperature) confirmed difference for color, SSC and dry matter. Among classes in External fruit without any post-storage ripening, 1.60<I_{AD}<1.79 class showed the highest drop in I_{AD} index, while 2.00<I_{AD}<2.19 class the lowest, confirming variation in ripening rate; similarly, between 1.80<I_{AD}<1.89 class and 2.00<I_{AD}<2.19 class for Internal (1.60<I_{AD}<1.79 was absent for internal at T1). This latter class showed also the lowest SSC among Internal fruit classes (data not shown). Regarding the comparison between combinations of position and DA class after 7 days of ripening followed the 6 months of CA storage, 2.00<I_{AD}<2.19 class for Internal still showed the lowest drop in I_{AD} index in the 7 days of ripening at room temperature, the lowest SSC (13.1 °Brix) and dry matter %, the highest hue (more green), and the highest pH (Fig. 6).

After 8 months of CA storage (T2), without any post-storage ripening time, External and Internal fruit differed for weight, overcolor percentage and color, firmness, SSC, dry matter % and titratable acidity, with the most exposed fruit bigger, less green, with 15% overcolor, firmer, higher in SSC and dry matter and lower in acidity. In External fruit without any post-storage ripening, differences among classes were less than in shorter storage duration, in fact all destructive parameters like firmness, SSC, dry matter, pH and titratable acidity did not significantly differ. Ethylene production was higher for External fruit class I_{AD}<1.60 than the other classes (less ripe fruit). Internal fruit instead after 8 months and without any post-storage ripening presented differences in the comparison between DA classes with the most ripe class showing lowest firmness and highest SSC and dry matter % (data not shown).

After 7 days of ripening (+8M storage +7 days at room temperature) the comparison between External and Internal fruit reported difference for I_{AD} index drop in the 7 days, overcolor % and color, SSC. Regarding the comparison between combinations of position and DA class after 7 days of ripening followed the 8 months of CA storage, $2.00<I_{AD}<2.19$ class for Internal still showed the lowest drop in I_{AD} index in 7 days at room temperature, but the highest drop in weighs in 7 days (tendency to shriveling without proper ripening), the highest hue (still more green then the others), the lowest SSC and dry matter %, the highest hue (more green), and among the highest pH values (Fig. 6).

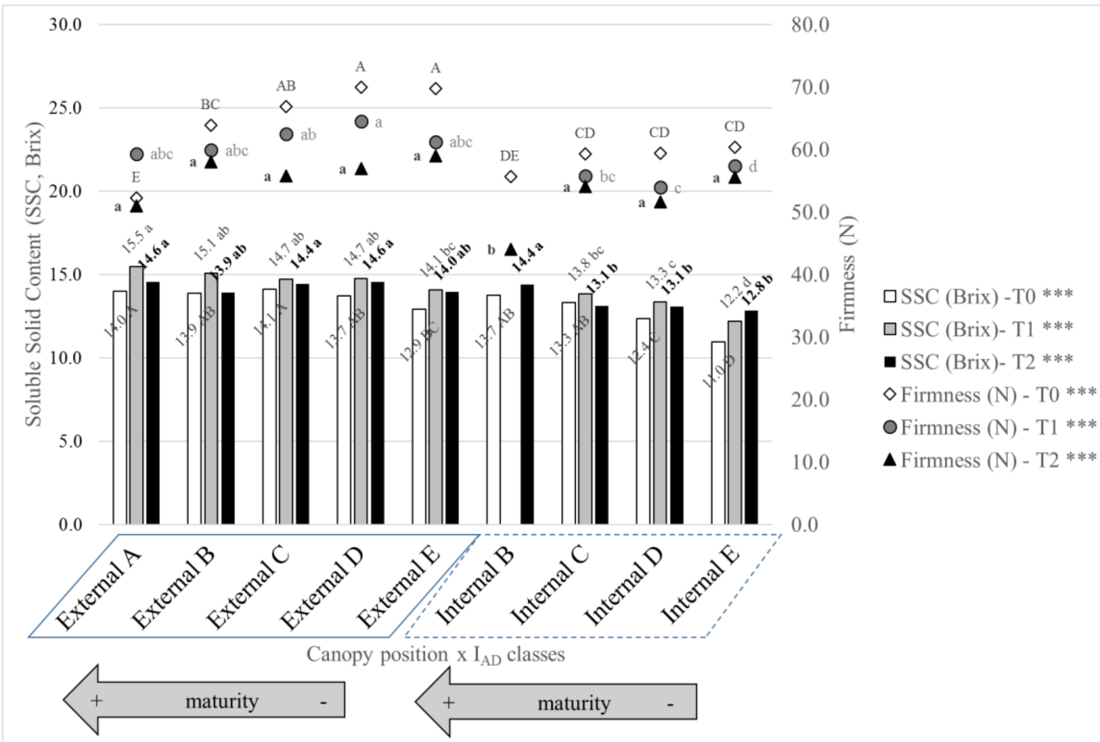

Figure 6: Comparison between combinations of DA classes and canopy position at harvest 2015 (T0), after 6 M of Ca storage (T1) and after 8 M of Ca storage (8M) for Soluble Solid Content and Firmness.

Regarding disorders observed during fruit assessment, cork incidence ranged from 10 to 14% in Internal fruit while for External fruit from 13 to 29%. Scuffing was absent at harvest (T0) in both fruit positions, while increased in the following pullouts, reaching a maximum of 96% of incidence in External fruit after 8 month of storage + 7 days of ripening (88% in the Internal fruit at the same time point). No superficial scald was noticed in the fruit from harvest up to after 8 months of CA without any post-storage ripening (day 0), while after 7 days of ripening at room temperature, superficial scald incidence was 37% in External fruit and 1.5% in Internal fruit (after 6 months) and 48% and 11% respectively (after 8 months). Superficial scald hue tended to get darker longer the storage duration but the affected area was similar approx. around 25% of fruit surface. So, in general, External fruit were more affected by superficial scald.

2) Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.

Peel chemistry changed alongside fruit appearance and other quality traits. Differences of peel chemistry were most dramatic with tree position which changed as fruit ripened during storage (Fig. 7). Results indicate the greatest impact on fruit ripening and chemistry results from tree position more than any other factor in the experiment and, accordingly, it is the greatest source of quality and ripeness variability. Differences were detected at harvest as well as throughout storage indicating the final product on the store shelf may also be different.

Differences of quality traits, including natural aroma and flavor, are clear within the chemical profile. These include sugars (sweetness), malic acid (tartness), phenolics (bitterness), and aroma volatiles. Pears may have more ripe or unripe aroma depending upon tree position, even at 8 months storage (Fig. 8). I_{AD} classification was reflected in the overall peel chemistry at harvest but this relationship declined with storage duration (data not shown). Peel chemical analysis results to date indicate that tree position will have a major impact on relative storability and eating quality.

Not only are flavor and maturity impacted by tree position but so are critical factors such as appearance. While we expect that external fruit may have more blush or, as fruit appear to ripen differentially, background color would be influenced by canopy position, there are also less obvious factors profoundly impacting finish. For instance, superficial scald incidence was higher in External fruit than Internal fruit, a factor linked with higher levels of key apple scald risk biomarkers detected in Internal peel (Fig. 9). As storage regimes and marketing strategies can be most effectively tailored to a consistent batch of fruit, it is clear that more consistent fruit at the beginning of storage would reduce losses and that these decisions are impacted by canopy position.

Shorter term strategies for reducing inconsistency of fruit going into storage may rely on the ability to "see" and sort fruit according to canopy position as that is the major contributor to inconsistency. Another outcome of our untargeted appraisal of peel chemistry are potential targets for just this task. External fruit have higher levels of compounds associated with light exposure and Internal fruit have higher levels of wax compounds involved in other pathways (Fig. 10). These metabolites associated with sun exposure are part of a fruit's natural defense to increased light exposure that are not apparent with the naked eye but can be detected using devices that focus on portions of the ultra-violet spectrum. This aspect could, potentially, be used to sort fruit in the orchard or warehouse according to tree position yielding a more consistent batch of fruit for tailored supply chain management, reducing downstream losses.

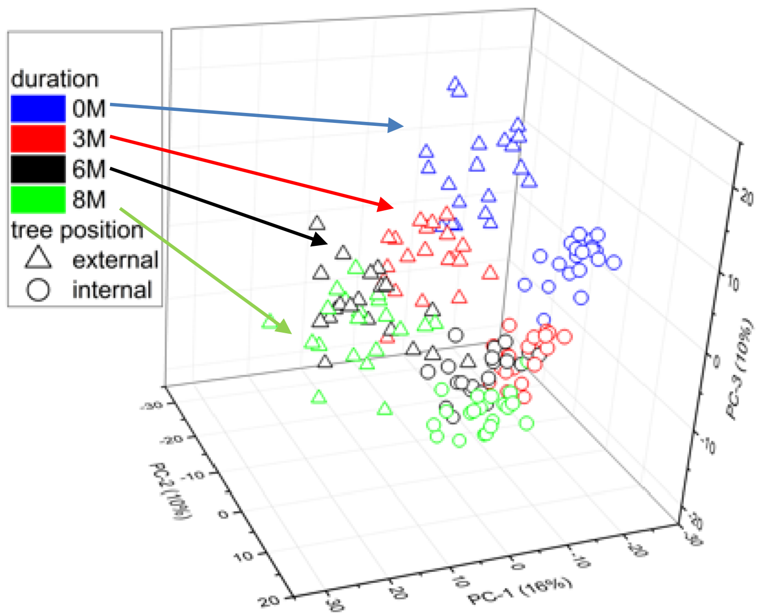

Figure 7: Principal components analysis (PCA) scores plot illustrating differences in overall natural chemical levels from Anjou pears harvested from the external or internal canopy and stored for up to 8 months in CA storage. Each point represents a summary of over 800 natural peel chemicals for a single peel sample. Triangles represent internal and circles represent external fruit peel. Storage duration is indicated by symbol color. Metabolism of internal and external peel changes during CA storage differentially.

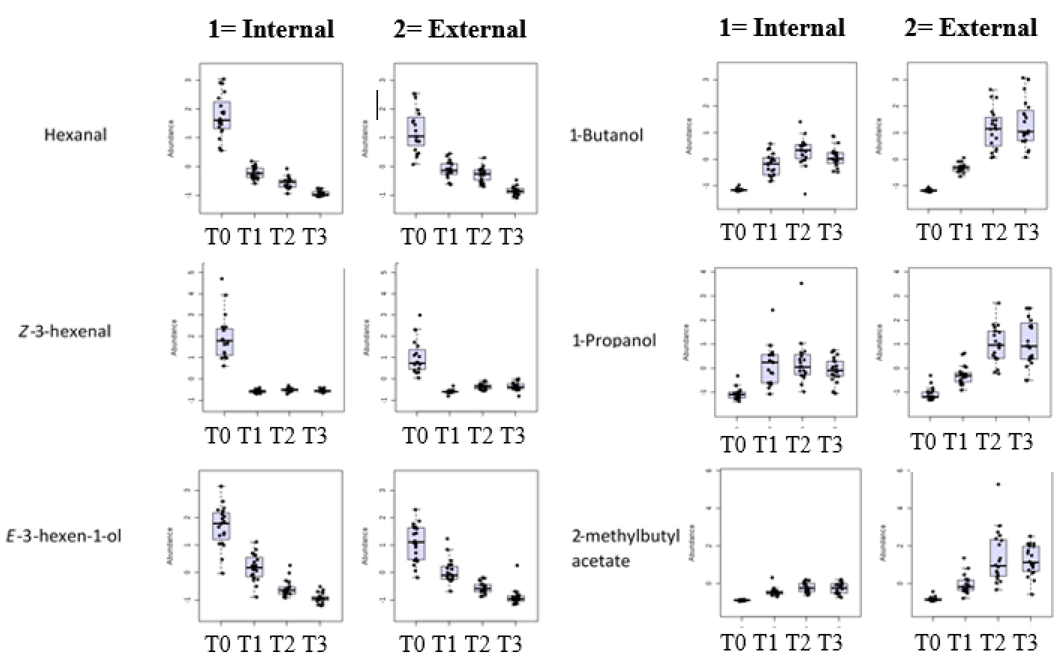

Figure 8: Changes of levels of peel chemicals different in d'Anjou pears from the Internal (1) or External (2) canopy over 8 month CA storage (from T0 to T3). Results suggest that "unripe" flavors (left) are higher in Internal fruit at harvest and are similar by 8 months while "ripe" flavors (right) are more prevalent in External fruit at the end of storage indicating fruit ripeness and quality are different depending upon tree position.

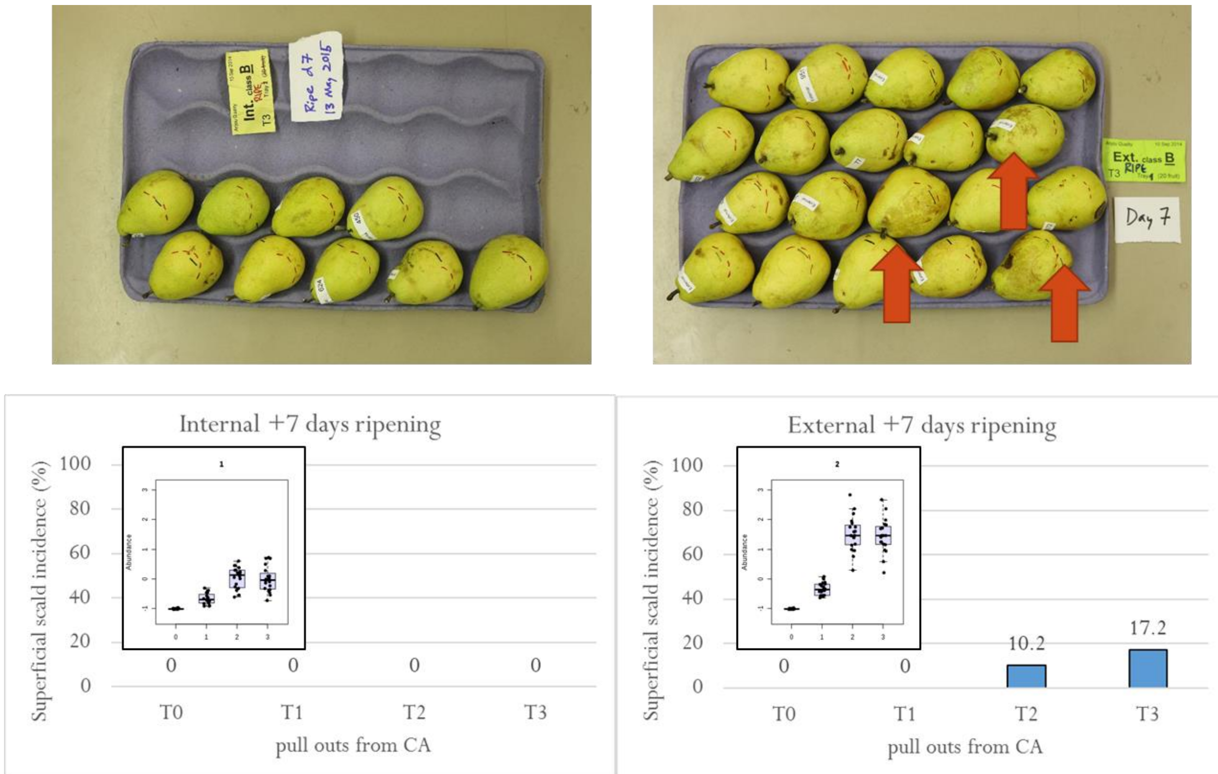

Figure 9: Superficial scald incidence (%) dependent upon canopy position for d'Anjou pears stored in CA for 8 months and left to ripen at 68 F for 1 week. In this case (orchard, year, storage conditions), External fruit (right) developed more scald than Internal fruit (left). Levels of an apple scald risk assessment biomarker (insets) were elevated in External fruit.

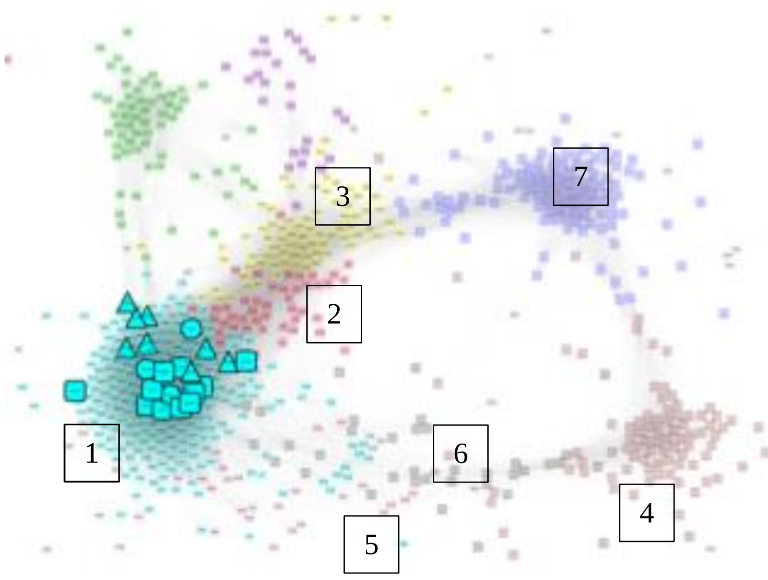

Figure 10: Associations among natural peel chemicals during 8 months CA. Chemicals (shapes) that are closer together indicate that their levels over the storage period change similarly with respect to other factors in the experiment such as tree position. Compounds associated with higher light environment are colored turquoise (1), red (2), and yellow (3), and those linked with lower light are brown (4), pink (5), and black (6). Compounds higher at harvest are blue (7). Turquoise compounds increase with storage more in external fruit. Chemicals we have identified that are associated with higher light conditions include flavonol glycosides with can be detected using UV reflectance imaging and possibly exploited for in-field or warehouse pre-storage sorting.

- **Publications:**
  - Zhang J., Serra S., Leisso R.S., Musacchi S. (2016) *"Effect of light microclimate on the quality of 'd'Anjou' pears in mature open-centre tree architecture".* Biosystems Engineering, 141:1-11.
  - Rudell, D. R., Serra, S., Sullivan, N., Mattheis, J. P., & Musacchi, S. (2017). *"Survey of 'd'Anjou'Pear Metabolic Profile Following Harvest from Different Canopy Positions and Fruit Tissues".* HortScience, 52(11), 1501-1510.

- **Presentations:**
  - Rudell D., Serra S., Sullivan N., Mattheis J., Musacchi S. *"Fruit position within pear trees impacts ripening and associated metabolism after harvest"* (oral presentation by Rudell D.). 12th Annual Conference of Metabolomics Society, Dublin, Ireland (June 2016).
  - Serra S., Rudell D., Mattheis J., Musacchi S. *"Evaluating Fruit Quality and Maturity in Large Open Vase-trained 'D'Anjou' Trees"* (Oral presentation by Serra S.) ASHS annual meeting, Atlanta, Georgia (August 2016).
  - Serra S. "DA Meter and Dry Matter" (oral presentation in IFTA session IV: New Instrument Panel discussion. 2017 IFTA Annual Conference, From bud to bin, Wenatchee WA (February 2017).

Executive Summary

Project Title: Improving quality and maturity consistency of 'D'Anjou'

Background

'D'Anjou has been trained for many years using an open vase. Single trees can reach 17 ft high with a very large canopy volume where fruits are distributed mostly in the upper-medium portion of the canopy. Fruit characteristics inside such a big and vigorous tree can be very different as less light can penetrate into the inside of the canopy and, consequently, light exposure can be quite different. Harvest in those orchards cannot be mechanized and is performed manually without any sorting. Consequently, many fruit quality characteristics, including maturity, can be highly variable within a single bin. This factor can dramatically impact fruit quality and storability often resulting in the need to repack to eliminate over-ripe, spoiled and scalded fruit from packed boxes.
Our preliminary work indicates a non-destructive approach using the DA-meter, which can be adopted to segregate pear fruit according to maturity by estimating associated chemical changes. We have found that fruit picked from the internal part of the canopy ripen more slowly, as estimated using the DA index, but lose weight more rapidly than fruit harvested from the outer part of the canopy. Our long-term goal is to develop tools and protocols that improve uniformity of fruit maturity and quality at harvest. Moreover, one possible long-term outcome is implementation of existing sorting technology to afford storage operators the ability to pre-sort pears by orchard or tree position/maturity. This sorting capacity would allow tailored storage regimes for improved ripening and quality consistency and reduced losses from postharvest disorders such as scald and possibly decay.

Project outcomes:

1. Method to prove that large 'D'Anjou open vase trees show inconsistency in ripening depending on light exposure.
2. Repacking problem and postharvest losses can be improved with fruit sorting at harvest and tailored storage conditions and durations.
3. New potential chemicals targets for sorting fruit accordingly to canopy position in the orchard or warehouse.

Significant Findings:

1. Crop inconsistency resulting from pear canopy position impacts most postharvest supply chain decisions.
2. Fruit ripening and potentially flavor is different depending upon canopy position.
3. Canopy position impacts postharvest behavior including superficial scald risk.

Future Directions:

1. Change 'D'Anjou trees architecture (and rootstocks) toward a narrower canopy and higher density planting and more planar canopy for more consistent crop.
2. Improve the picking process by canopy position and fruit sorting ability in the orchard.
3. Tailored storage duration depending on fruit sorted by maturity levels.
4. Tailored storage duration depending on fruit sorted by non-destructively predicted dry matter %.
5. Imaging to discriminate fruit by position.