2014 Apple Horticulture Postharvest Research Review

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FINAL REPORT

Project Title: Method development and validation for analysis of morpholine on apples

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Cooperators: Deborah Carter and Michael Willett, Northwest Horticultural Council

Total Project Request: Year 1: **$20,000**

Other funding sources: None
WTFRC Collaborative Expenses: None

Budget 1
Organization Name: UC Davis **Contract Administrator:** Matt Hengel
Telephone: 530-752-2402 **Email address:** mjhengel@ucdavis.edu

Item	2013
Salaries	$6,000
Benefits	$3,000
Supplies	$1,000
Total	**$10,000**

Budget 2
Organization Name: Pacific Agricultural Laboratory **Contract Administrator:** Steve Thun
Telephone: 503-626-7943 **Email address:** sthun@pacaglab.com

Item	2013
Salaries	$4,500
Equipment Operation	$3,000
Supplies	$1,500
Miscellaneous	$1,000
Total	**$10,000**

Footnote: The expense under Equipment Operation does not purchase new equipment.

No report submitted

FINAL PROJECT REPORT

Project Title: Testing biomarker-based tools for scald risk assessment during storage

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Cooperators: Dr. Jinwook Lee, Dr. Bruce Whitaker

Other funding sources

Agency Name: NIFA, USDA (Grant no. 2010-51181-21446**)**
Amt. awarded: $1,483,438 (federal total over 4 years)
Notes: Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle. David Rudell (Project Director) will manage and participate in the project and James Mattheis (Co-PI) and Yanmin Zhu (Co-PI) will also participate. This is a multi-state, multi-national project. The proposed project extends and compliments the activities of this SCRI project.

Agency Name: AgroFresh, Inc.
Amt. awarded: $232,253 (for Rudell and Mattheis role in SCRI project over 4 years)
Notes: Cash donation to support activities and objectives outlined in the Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle (see above).

Total Project Funding: **Year 1:** $24,750 **Year 2:** $24,750 **Year 3:** $24,350

Budget History:

Item	2011	2012	2013
Salaries			
Benefits			
Wages	$15,038	$15,038	$5,263
Benefits	$4,962	$4,962	$1,737
Equipment			
Supplies[1]	$1,000	$1,000	$1,000
Travel			
Miscellaneous[2]	$3,750	$3,750	$3,750
Miscellaneous[3]			$12,600
Total	$24,750	$24,750	$24,350

Objectives:

1. Determine if scald risk assessment tools indicate when delayed CA imposition leads to high scald risk and high scald incidence.
2. Indicate if and when scald risk is high during CA storage based on risk assessment tools and determine if storage conditions can be changed to alter biomarker levels and scald incidence.
3. Assess effectiveness of scald-risk assessment tools in pilot scale and commercial CA storages.

SIGNIFICANT FINDINGS:

1. Changes in scald risk assessment biomarkers (SRAB) levels directly reflect how the fruit is reacting to the CA and DPA or 1-MCP chemical conditions imposed.
2. Validated 21 of 25 scald risk assessment biomarkers (SRABs) as predictors of scald risk.
3. Levels of 13 SRABs most effectively reflected scald risk altered by delaying CA imposition.
4. SRAB levels correlated with scald risk as early as 3 months after CA storage imposition. Scald was first detected at 9 months.
5. Changes in SRAB levels can detect scald risk early in fruit stored under suboptimal atmospheres and storage outcome improved (reduced scald) by subsequently adjusting CA conditions.
6. Storage monitoring using SRABs can rank different lots by risk during later phases of storage.
7. Spectrophotometric method for SRAB evaluation produced results similar to more expensive techniques.
8. How full a room is may impact SRAB levels.

RESULTS & DISCUSSION

The current report has evaluated and implemented scald risk assessment biomarker (SRAB)-based tools designed to monitor the impact the storage environment has on superficial scald risk**.** SRAB-based storage monitoring is one facet of risk assessment (Fig. 1). Discovery of other SRABs that indicate risk at harvest and just after harvest provide an earlier indication and perhaps a more accurate risk assessment. This work has been funded by our SCRI project.

Scald risk assessment following delayed CA storage and DPA treatment

Granny Smith scald was reduced or eliminated by DPA drenches. Scald symptoms developed on air-stored fruit at 6 months and on CA stored fruit at 9 months. In our small experimental chambers, delayed (up to 1 month) CA had little relationship with final scald incidence and severity after 10 months in 1% O_2 plus 7 days at 68 °F. Levels of 22 out of 25 scald risk assessment biomarkers (SRABs) increased at least 3 months prior to scald symptom development at 9 months in CA storage. Levels of 13 SRABs were highly reflective of scald final incidence and severity; very little increase in fruit that received treatments that didn't develop scald, and increasing levels as final scald incidence increased. For example, elevated scald risk was detectable by measuring SRAB N24 at 2 months in air, air+DPA, and CA fruit not treated with DPA, all treatments which developed scald by 10 months.

Rather than relying on changing levels of just 1 or 2 SRABs, we envision monitoring multiple SRABs to provide a more complete risk assessment. Using multiple SRABs may be a useful way to monitor many different indicators of the causes and effects of scald, painting a more complete picture of the condition of the peel during storage. For instance, many of the SRABs that closely reflect final scald levels may reflect oxidative stress that ostensibly leads to scald development. By

monitoring these SRABs, we are actually providing a direct assessment of damage caused by adverse storage conditions that lead to scald.

Real-time scald assessment (experimental chambers)

Experiments were conducted to determine whether detection of high scald risk can be used to indicate if storage environment should be changed and whether this change can extend the symptom free life of the product. Experimental CA chambers containing 'Granny Smith' apples were held in air, 0.5 or 5% O_2 at 33 ^oF. SRABs were monitored monthly. Changes in levels of 3 SRABs indicated scald risk in fruit held at 5% O_2 at 2 months and in 6 SRABs after 3 months. O_2 in one of the 5% O_2 chambers was lowered to 0.5% O_2 at 3 months + 2 weeks. Scald was present on air stored fruit starting at 3 months and on 5% O_2 stored fruit starting at 6 months + 7 days at 68 °F. Scald incidence and severity was substantially reduced at 6 months + 7 days and less so at 9 months + 7 days by altering the storage conditions when risk was detected (3 months + 2 weeks). Results indicate that scald risk monitoring reflects fruit response to the storage environment. Also, scald outcome can be improved, once risk is accurately assessed, by optimizing conditions farther into the CA storage period than previously considered.

Real-time scald assessment (pilot and commercial rooms)

SRAB monitoring of multiple lots of fruit under pilot (Stemilt RCA rooms) and commercial conditions was performed to indicate whether risk assessment tools work in larger rooms and can rank risk among different lots of fruit. Results from the "real-time" risk assessment/storage monitoring were confirmed by monitoring research rooms set at 2% O_2 (rather than 5%) and reducing O_2 to 0.5% if SRAB levels suggest there is a risk. Elevated risk was detected in fruit from all 4 orchards stored in air or 2% O_2 (and not in fruit stored in 0.5% O_2) at 1 month (air) and at 2 months (2% O_2) (Fig. 2). O_2 levels were reduced in one of the 2% rooms after 3 months improving the storage outcome (reducing scald) after 9 months CA + 7 days at room temp (Fig. 3). Storage outcome may have been improved more had the O_2 levels been reduced when risk was first detected at 2 months. All orchards eventually developed scald when storage conditions were conducive to scald development. Spectrophotometry, an affordable and accessible means (platform) of measuring SRABs, was investigated alongside measurements using our other instrumentation. This platform provided similar estimations of scald risk (Fig. 4). SRAB levels ranked orchards according to risk starting by 3 months indicating storage risk monitoring could provide useful information about further supply chain performance of a lot compared with other lots from the same room, although we expect other SRABs, which can be evaluated earlier in the storage period, may provide the most accurate ranking. SRAB levels in fruit from the same orchards stored in the commercial room increased more rapidly than those in the RCA rooms that were set at a higher O_2 level. This indicates that ripening and stresses associated with the amount of fruit in these rooms compared with the others and the length of room loading and O_2 pull down may impact SRAB levels. This result is the subject of further investigation in our subsequent project.

Other related findings

In collaboration with Dr. Bruce Whitaker, a group of unknown candidate SRABs and other apple peel chemicals in our data base have been identified as *p*-coumaryl acyl esters. These apple peel chemicals are part of the waxy surface layer and are likely part of the wax structure. A few of these compounds have potential as SRABs.

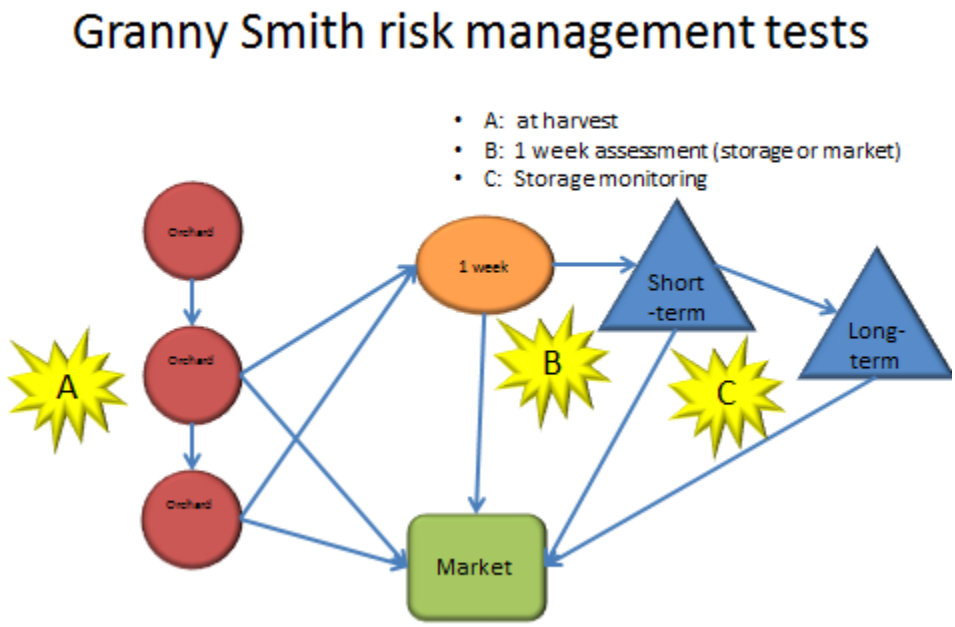

Fig 1. SRAB testing scheme for Granny Smith storage. The tools evaluated by this project primarily cover the storage monitoring phase (C) once postharvest treatments (DPA, SmartFresh) have been applied and storage conditions imposed. At-harvest and early storage (1 week) SRABs have been discovered using samples from this project and will be evaluated using samples for our continuing project (A and B). Assessing scald incidence during the latter phase of storage is another possible outcome of this project.

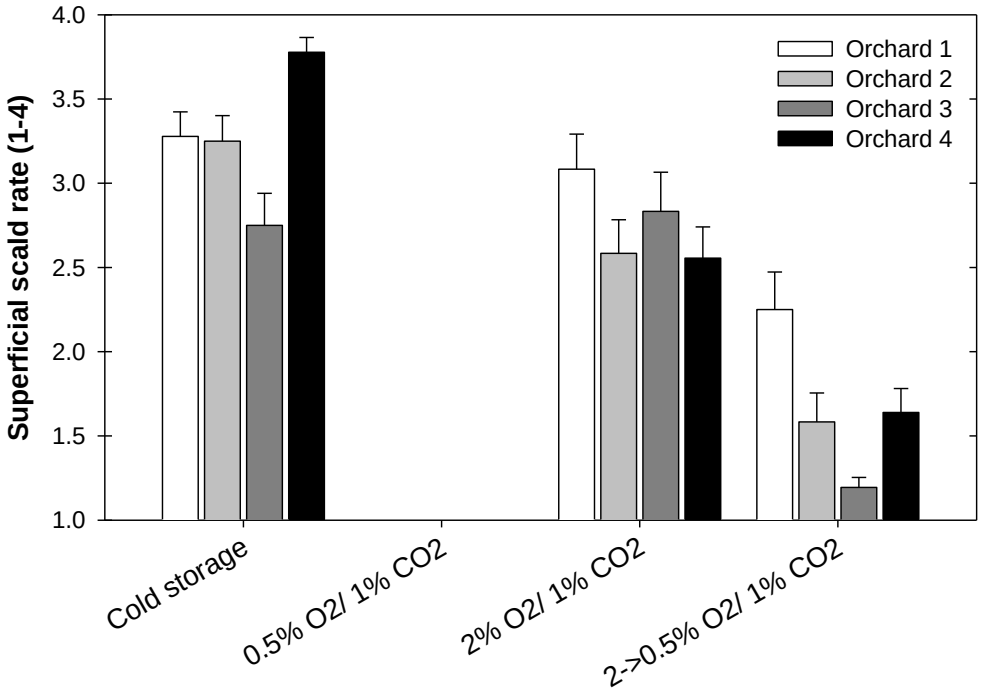

Fig 2. Superficial scald levels of 4 organic Granny Smith lots after 9 months (+ 7 days at 68 °F) at 33 °F in air or CA storage (0.5% O_2, 2% O_2, or 2% → 0.5% O_2). Superficial scald was evaluated by estimating the percent fruit peel with superficial scald symptoms: 1=0%, 2=1-25%, 3=26-50%, and 4=51-100%. SRAB levels were higher in the 2% O_2 rooms by 2 months, however the O_2 levels were not adjusted to 0.5% until after 3 months. Storage outcome may have been improved had the atmosphere been corrected in a more timely manner.

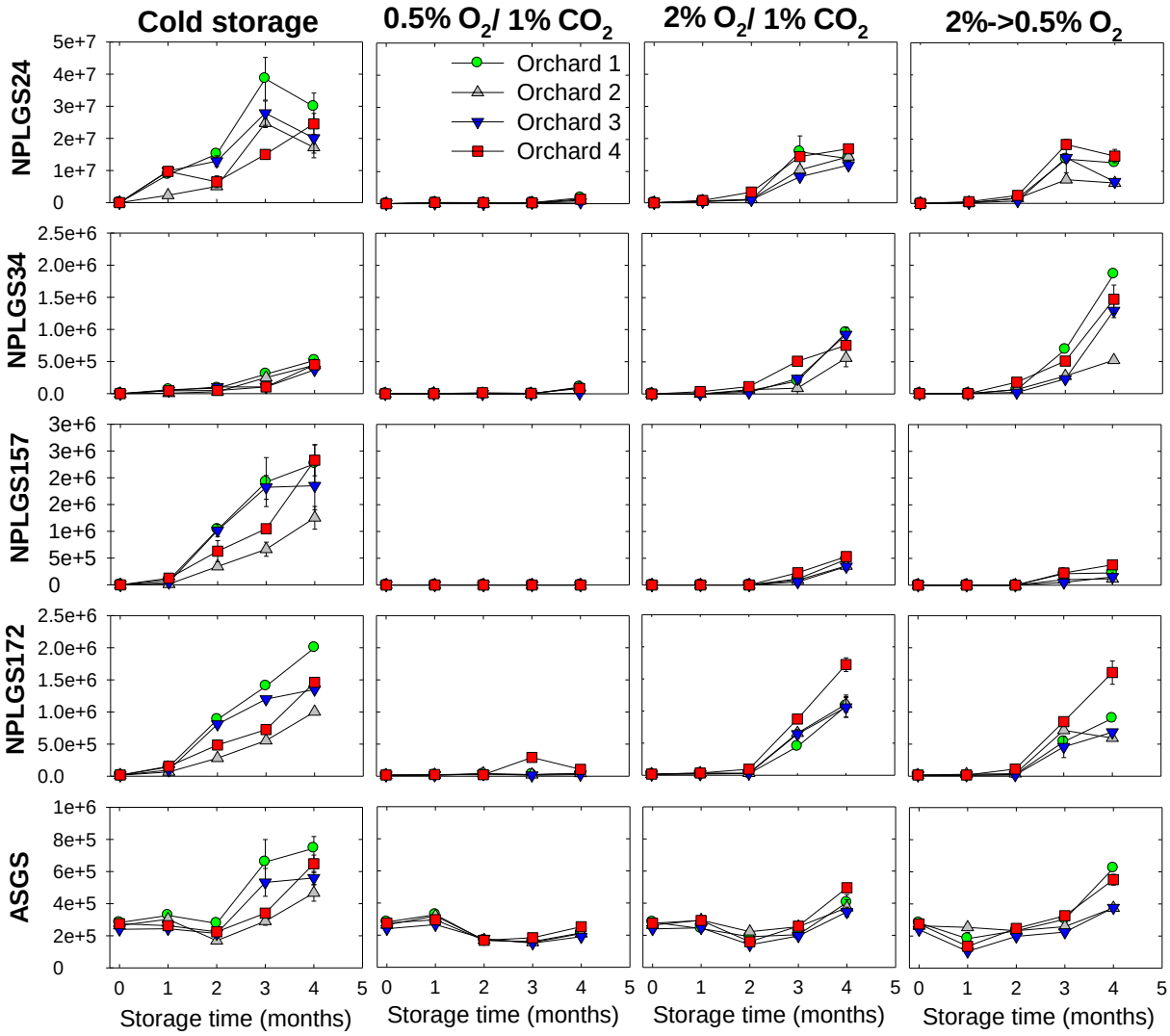

Fig 3. SRAB levels in fruit stored in Stemilt RCA rooms in air, 0.5% O_2, or 2% O_2. SRAB levels began to increase in 2% O_2 rooms beginning between 1 and 2 months storage. One 2% room was adjusted to 0.5% after 3 months. NPLGS34 levels most represented final scald outcome beginning at 3 months and ASGs at 4 months.

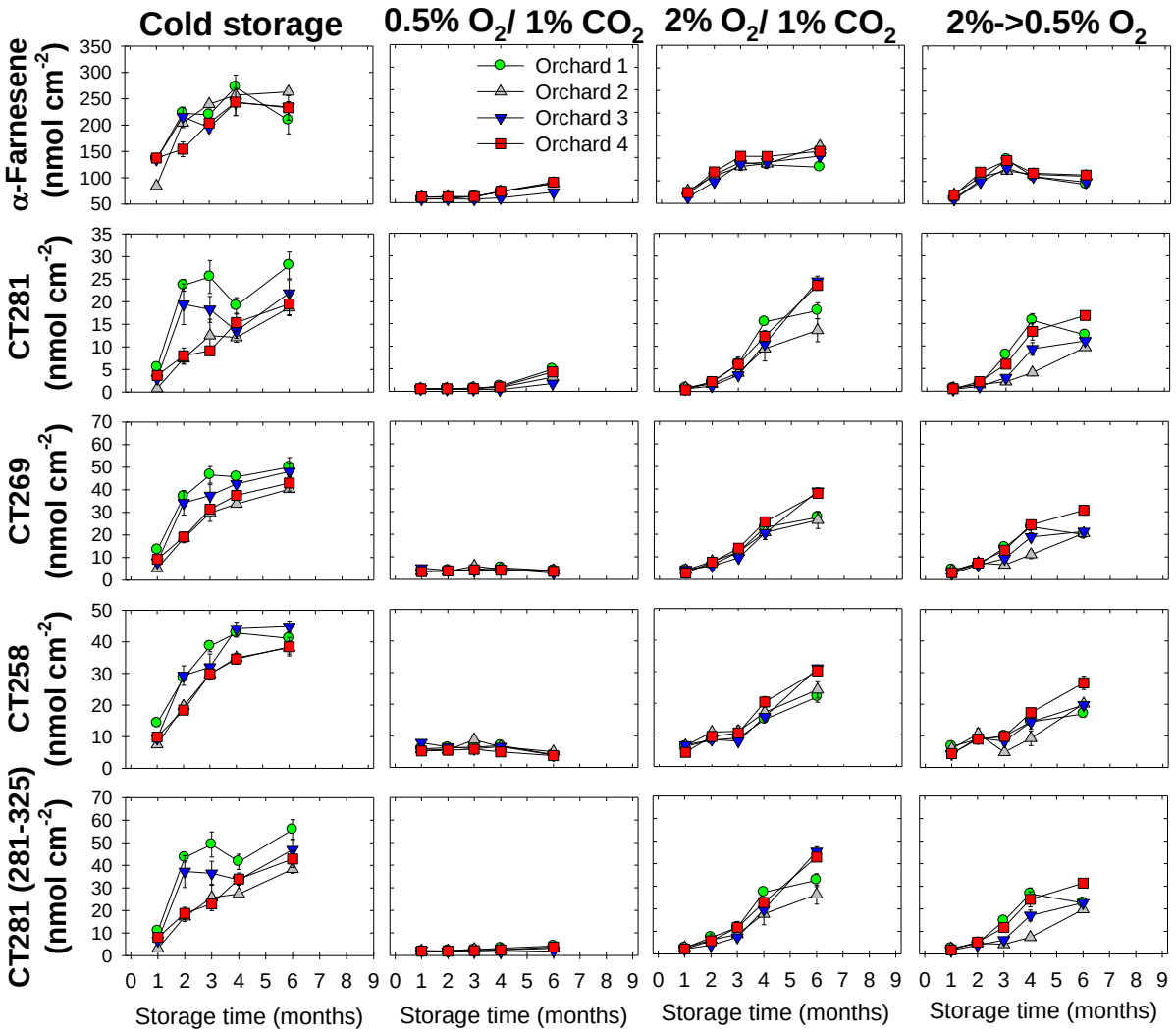

Fig 4. SRAB levels measured using spectrophotometry in RCA rooms under 3 different atmospheric conditions; air, 0.5% O_2, 2% O_2. SRAB levels were estimated using less expensive, more user friendly protocols compared with biomarker discovery instruments used in our laboratory, where CT269 estimates N24 and CT281 estimates N34.

Project Title: Testing biomarker-based tools for scald risk assessment during storage

Executive Summary

Background: Our previous work screening hundreds of natural chemicals in apple peel during scald development has revealed many with potential for use as biomarker-based scald risk assessment tools. Further validation was needed to reveal whether tools will aid in commercial storage and supply chain management decisions by monitoring whether CA storage conditions or crop protectant usage is sufficient to prevent superficial scald. We initially developed and validated this technology by measuring peel chemistry changes related to storage stress during the scald development period of fruit stored in both air and CA. We continued this work by testing additional CA conditions in a laboratory setting as well as in pilot or commercial settings. Our objectives complemented our Specialty Crops Research Initiative (SCRI) project to continue to develop biomarker-based storage management tools for superficial scald and other key postharvest disorders.

Project outcomes:

1. Scald risk assessment tools to monitor risk during storage.
2. Less expensive platform to monitor scald risk assessment biomarkers (SRABs).
3. Potentially new recommendations for room loading.

Significant Findings:

1. Changes in SRAB levels directly reflect how the fruit is reacting to the CA and DPA or MCP chemical conditions imposed.
2. Validated 21 of 25 scald risk assessment biomarkers (SRABs) as predictors of scald risk.
3. Levels of 13 SRABs most effectively reflected scald risk altered by delaying CA imposition.
4. SRAB levels correlated with scald risk as early as 3 months after CA storage imposition. Scald was first detected at 9 months.
5. Changes in SRAB levels can detect scald risk early in fruit stored under suboptimal atmospheres and storage outcome improved (reduced scald) by subsequently adjusting CA conditions.
6. Storage monitoring using SRABs can rank different lots by risk during later phases of storage.
7. Spectrophotometric method for SRAB evaluation produced results similar to more expensive techniques.
8. How full a room is may impact SRAB levels.

Future Directions:

1. Continued validation of storage monitoring SRAB-based tools and defining their utility.
2. Testing and employment of tools for other superficial scald susceptible cultivars.
3. SRABs that provide scald risk assessment at harvest and for all points in the supply chain.
4. Similar risk assessment systems for other disorders such as Honeycrisp soft scald.
5. Biomarker-based tools for other fruit production uses.
6. New, better storing cultivars, with reduced postharvest disorder risk.

FINAL PROJECT REPORT

Project Title: Greater system efficiency and fruit quality via soil microbiology

PI:	Mark Mazzola	**Cooperator:**	James Mattheis
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Address:	1104 N. Western Ave	**Address:**	1104 N. Western Ave
City:	Wenatchee	**City:**	Wenatchee
State/Zip	WA 98801	**State/Zip:**	WA 98801

Other funding sources

Agency Name: USDA-NIFA
Amount awarded: $517,798
Notes: Field trials established and maintained with funds from the USDA-NIFA funded program were used in the studies noted below. These resources were also utilized to conduct metagenome analysis of soil microbial communities.

Total Project Funding: $208,547

Budget History:

Item	2010	2011	2012	2013
Salaries[a]	47,690	49,120	50,594	
Benefits	15,737	16,210	16,696	
Wages				
Benefits				
Equipment				
Supplies	3,500	4,500	4,000	
Travel			500	
Miscellaneous				
Total	66,927	69,830	71,790	0

The objective of this program was to obtain insight into the role of soil microbiology on resource utilization within orchard systems and to determine potential impacts of fertility management programs on the function of soil biology. The resulting effects of fertility inputs on tree growth, with particular emphasis on root development, and orchard production were also to be examined.

Specifically, this program:
1.) Assessed the rooting behavior of apple as affected by different resource inputs.
2.) Examined the effect of orchard management options on soil microbial communities and orchard productivity.
3.) Quantified the key genes driving microbial nitrogen cycling in the apple rhizosphere under different resource input programs
4. Determined the effect of altered soil biology on fruit quality characteristics including coloring.

SIGNIFICANT FINDINGS

2010:

- In a sandy texture orchard soil, root development of M9 rootstock was enhanced or depressed in a nitrogen amendment-dependent manner

- In this same orchard soil it was apparent that the effect of nitrogen amendments on M9 lateral root development were indirect, being determined by the orchard soil biology

- Bacteria belonging to the genus *Streptomyces* initially isolated from the rhizosphere of M9 apple rootstock were capable of enhancing root and overall plant development

2011

- The type of nitrogen amendment significantly altered abundance of microbial genes involved in N cycling, and thus could result in altered retention or loss of N from orchard soils

- The effect of N amendment on microbial cycling gene abundance was influenced by the long-term soil management system; that is organic and conventional systems differed.

- In extension of initial year findings, in an additional orchard soil the effect of nitrogen amendments on rootstock lateral root development were similarly shown to be indirect, apparently being determined by the orchard soil biology

- Through examination of a larger bacterial population, root stimulation induced by *Streptomyces* bacteria was found not to be linked directly to nitric oxide production, and could be replicated using other bacterial species.

2012

- Metagenome analysis (microbial community genomic analysis) provided new insight into the structure and function of orchard soil microbial communities. It served to effectively identify novel microbial species with the potential to affect soil health and orchard productivity.

- Yield performance and rhizosphere microbial communities were similar for both Gala/M9 and Gala/G11 rootstocks in non-treated replant orchard soil.

- In contrast, yield performance exhibited a rootstock-dependent response in Brassicaceae seed meal (BSM) amended soil; Gala/G11 outperformed Gala/M9; however, yields for trees on both rootstocks were superior to that in fumigated soils (23% increase on M9; 24% increase on G11).

- Application of BSM soil amendment did not alter fruit quality as assessed by firmness, relative to control or fumigation treatments after 4 months cold storage in air.

- Contrary to common belief, ammonia oxidation activity, the rate limiting state in the nitrification process, is dominated by the activity of archaea, rather than bacteria, in Central Washington orchard soils and these two microbial groups respond differently to the type of nitrogen input.

2013

- Communities of archaeal ammonia oxidizers differed significantly between organic and conventional orchard systems highlighting the need for increased focus in research to understand and improve specificity of fertilizer application for orchard production systems.

- Long-term lesion nematode suppression in seed meal amended soils was associated with a sustained shift in soil microbial community composition. Rapid nematode re-infestation of fumigated soils was associated with a simultaneous reversion of the soil microbial community to one indistinguishable from non-treated orchard soil.

- This difference in nematode re-establishment typically corresponded with enhanced tree growth and yield in seed meal amended soils. Thus, it is plausible that the beneficial effects of Brassicaceae SM amendment in terms of overall growth and yield will persist leading to enhanced orchard economic viability.

- Overall diversity of the microbial community in the disease suppressive seed meal amended soils was reduced relative to the disease conducive non-treated or fumigated soil. This finding brings into question the widely held view that enhanced "biodiversity" is instrumental in achieving system resilience and/or pathogen suppression.

- Abundance and diversity of potential foliar and fruit pathogens, including *Penicillium* spp., was significantly lower in seed meal amended soils demonstrating that an understanding of below ground/above ground interactions may enhance orchard sustainability and fruit quality.

Results and Discussion

1a. *Effect of fertility inputs on rooting behavior.* Studies were conducted in a Sandy soil, WSU Sunrise orchard (SR), a Sandy Loam soil, RF orchard, Chelan and a Gravelly Sandy Loam, GC orchard Manson. Experiments were conducted in both the native orchard soil and pasteurized orchard soils in order to obtain an understanding of the role of soil microbiology in the observed plant growth response. Plant rooting responses were virtually identical between the orchard soils. In pasteurized orchard soils, amendments had no effect on M9 rootstock root development and there were no significant differences in the number of lateral roots per length of primary root. In contrast, when assays were conducted in the native (natural) orchard soil, the type of fertility input had significant effects on rooting behavior of M9 apple rootstock regardless of the orchard soil. Changes in relative root proliferation were associated with qualitative differences in soil microbial communities. Fertility inputs inducing enhanced root development also elevated populations of total fungi, and bacteria belonging to the genera *Pseudomonas* and *Streptomyces*.

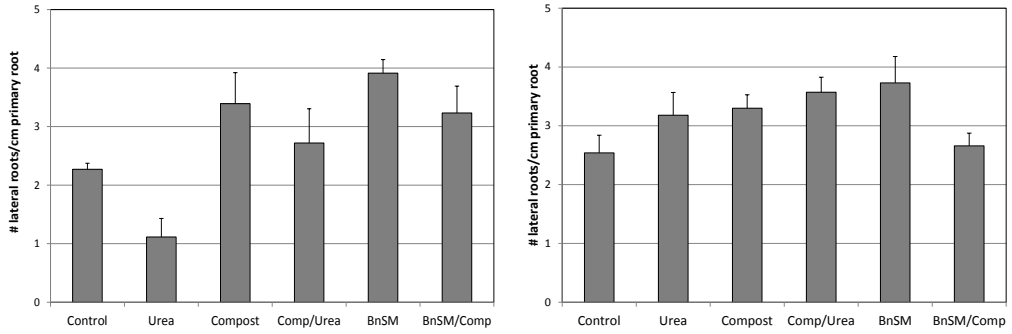

Figure 1. Effect of fertility amendments on M9 rootstock lateral root development in control (left panel) or pasteurized (right panel) sandy soil (WSU-Sunrise). BnSM=canola seed meal.

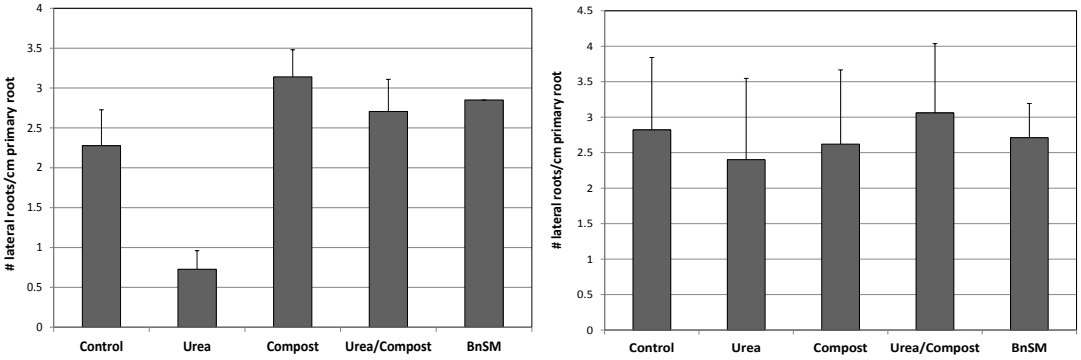

Figure 2. Effect of fertility amendments on M9 rootstock lateral root development in control (left panel) or pasteurized (right panel) sandy loam soil (RF orchard, Chelan) . BnSM=canola seed meal

Significance: Based upon the similar findings in disparate orchard soils, tree root development in response to organic/mineral amendments appeared to be consistent across soil systems, which indicates that the materials are likely to have predictable outcomes for use to enhance tree establishment and long-term performance. The findings also demonstrate that the positive and negative effects of these fertilizer inputs are not direct but likely function through their effects on orchard soil biology.

1.2 *Effect of bacterial strains on root development.* Root development in response to soil amendments was associated with a significant increase in the density of bacteria belonging to the genus *Streptomyces*. In initial studies we demonstrated that among four individual *Streptomyces* isolates, only those producing nitric oxide (NO) stimulated apple root development in sterile orchard soil. However when a larger bacterial population composed of *Streptomyces* and *Pseudomonas* isolates were screened using the model plant *Arabidopsis thaliana* (Fig. 3), no association was detected between NO production and induction of lateral root development. In addition isolates were further characterized for the root induction hormone IAA and again there was no association between IAA production and capacity to stimulate lateral root formation.

Figure 3. Effect of bacterial isolates on root proliferation by *Arabidopsis thaliana.*

2.1. *Effect of soil management practice on orchard microbiology.* The effect of soil treatment and rootstock on composition of the rhizosphere fungal and bacterial community was assessed through metagenome analysis. The study utilized trees (Gala on G11 or M9) that were planted at WSU-Sunrise in May 2010 into replant soil that was not treated (control), fumigated (Telone-C17) or amended with *Brassicaceae* mustard seed meal (*Brassica juncea/Sinapis alba*). Samples were collected in October, 2011 and DNA isolation and sequence analysis was conducted through 2013. The analysis yielded approximately 1 million fungal sequences representing 568 different fungal species and greater than 1 million bacterial sequences which represented 1219 different bacterial species.

There were clear associations between bacterial/fungal community composition and relative tree growth and yield performance as influenced by soil treatments. For both rootstocks, yields attained in seed meal amended soils were greater than that attained in fumigated soils (**Fig**. 4, left panel). Differences in growth and yield were associated with rapid re-infestation of fumigated soil by replant pathogens (e.g. lesion nematode; **Fig. 4**, right panel), while seed meal amended soils were suppressive toward soil-borne pathogens. Correspondingly, within two years of planting the bacterial/fungal communities detected in the rhizosphere of trees cultivated in fumigated soil were indistinguishable from the no treatment control while the same communities in seed meal treated soils were highly dissimilar (**Fig. 5**).

A significant finding relative to long-term orchard resilience to disease development was the observation that members of the genus *Oidiodendron* were only detected in the rhizosphere of apple cultivated in seed meal amended soil (**Fig. 6**). The importance of these fungi resides in the fact that they have been reported to provide biological control of oomycetes including *Pythium* spp. and *Phytophthora cactorum*, causal agent of Phytophthora crown and root rot of apple. In addition, soil treatments also displayed significant effects on populations of multiple fungi known to incite foliar and fruit diseases of apple including species that incite apple scab, fly speck, and blue mold (*Penicillium*; **Fig. 6**). This result indicated that the seed meal amendment suppressed potential inoculum of these aerial pathogens which may reduce disease incidence.

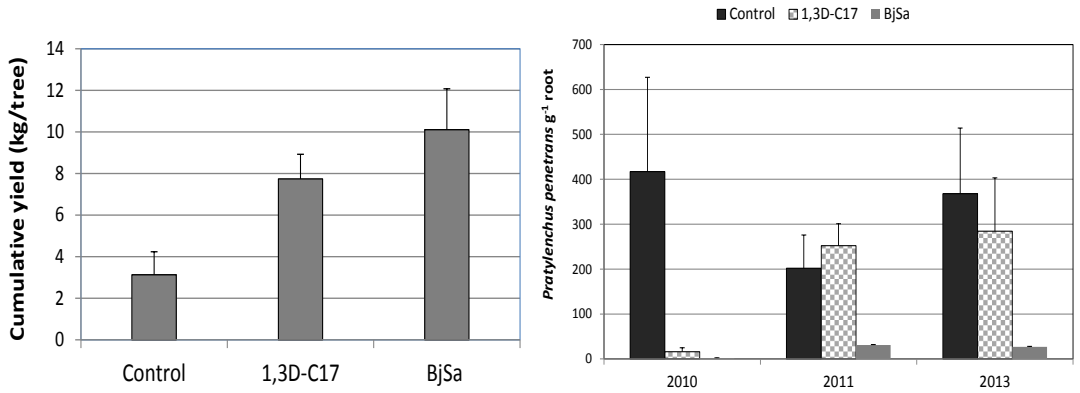

Figure 4. Cumulative yield (2012-13; left panel) and lesion nematode root densities (right panel) of Gala/G11 planted at the WSU Sunrise orchard in May 2010. 1,3D-C17=Telone-chloropicrin soil fumigation; BjSa=*Brassica juncea/Sinapis alba* seed meal.

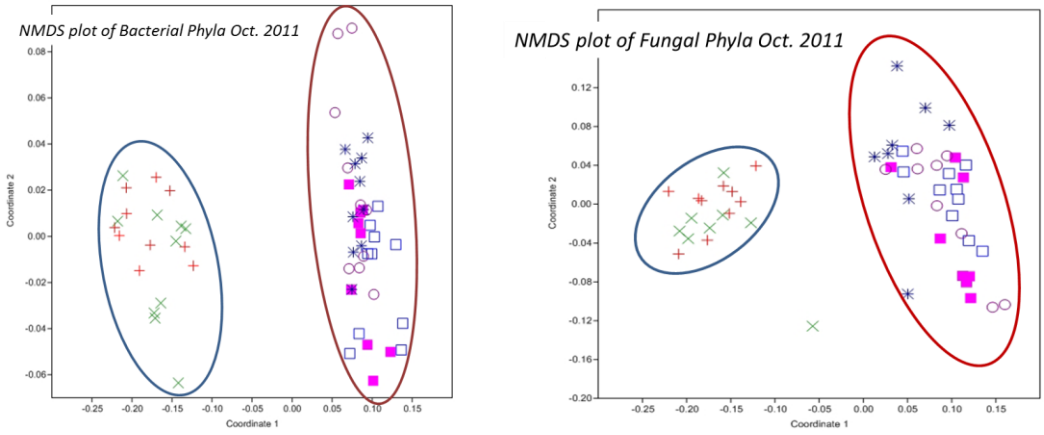

Figure 5. Effect of soil treatments on bacterial community (left panel) and fungal community composition (right panel). Treatment symbols: Control: M9 = ○; G11 = □; Telone-chloropicrin fumigation M9 = *; G11 = ■; *Brassica juncea/Sinapis alba* seed meal M9 = ×; G11 = + .

2.2. *Effect of nitrogen input on dynamics of nematode communities.* Nematode communities are commonly reported to serve as indicators of soil health and composition is influenced by orchard practices including tree row management systems. In the current study, type of nitrogen input had only a short-lived effect on nematode community composition as determined by examination of nematode community DNA using Terminal-Restriction Fragment Length Polymorphism (T- RFLP) analysis. Although the nematode communities from soils treated with nitrogen in the form of *Brassica juncea* or *Brassica napus* seed meal appeared to form a cluster at three weeks after fertilizer application (**Fig. 7**), no such trend was observed at 6, 9, 12 or 15 weeks after application.

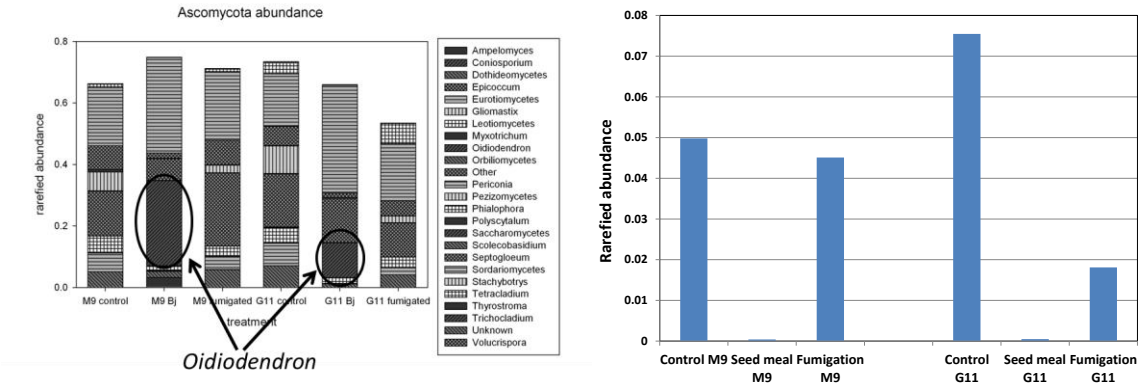

Fig. 6. Effect of soil treatments on composition of the ascomycete fungal community (left panel) and relative abundance of *Penicillium* spp. (right panel) detected in the rhizosphere of apple at the WSU-Sunrise orchard after two growing seasons as influenced by rootstock and soil treatment.

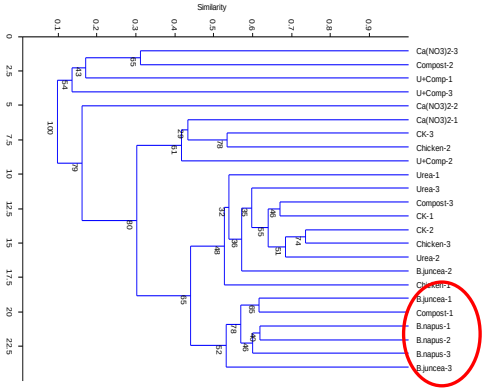

Figure 7. Cluster analysis of T-RFLP derived data for the nematode community in RF orchard three weeks after treatment with different nitrogen inputs.

Significance: These studies demonstrate that specific pre-plant soil treatments can modify the resident soil microbial community in a manner that limits re-infestation of pre-plant treated orchard soils by soil-borne pathogens resulting in significant benefits to long-term orchard health and productivity. The findings also indicate that rapid re-infestation of fumigated soil by "replant" pathogens likely is responsible for the limited (1-year) benefit of soil fumigation that has been commonly reported.

3.1 *Effect of fertility inputs on N cycling gene abundance*. The three orchard soils of diverse texture yielded varied responses in terms of N-cycling gene abundance; outcomes which appear to have a greater relationship with long-term management practices/soil type rather than the consequence of short-term fertility amendments. Of particular note were observations concerning the size of the denitrifying bacterial communities based upon abundance of the *nirK* gene; this gene encodes an important step in the denitrification process leading to loss of nitrogen from orchard soils through volatilization. Abundance of bacterial *nirK* was 1 to 2 orders of magnitude lower in the high organic matter (OM) RF orchard soil than in the low OM content SR orchard soil (**Fig. 8**). In addition, fertility amendments had no significant impact on bacterial *nirK* gene abundance detected in the RF soil but urea amendment resulted in a significant (two orders of magnitude) increase in *nirK* abundance in the SR soil. Co-application of nitrogen as either urea or *B. napus* seed meal with

compost significantly reduced *nirK* abundance in the SR orchard soil. In terms of orchard soil and inputs, fungal *nirK* gene abundance exhibited the same pattern. Thus, adding carbon as a strategy to retain N thru decreased volatilization may be of significant benefit in low organic matter orchard soils.

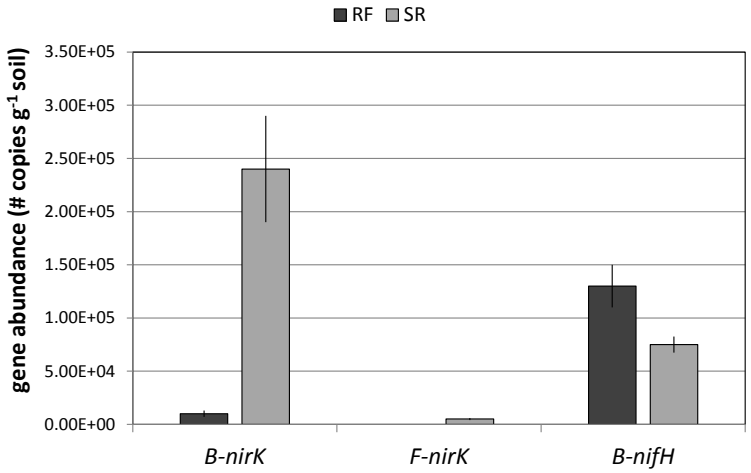

Figure 8. Relative abundance of the bacterial (B) and fungal (F) denitrification gene *nirK* and nitrogen fixation gene *nifH* in the RF and SR orchard soils.

Despite the large additions of N to these soils, bacterial N_2-fixation genes (*nifH*) were present in both soils (**Fig. 8**), though regardless of amendment, they were significantly more abundant in the high organic matter RF than SR orchard soils. At the SR orchard, the free-living N_2-fixing bacterium *Azospirillum brasilense* was commonly detected in the apple rhizosphere and abundance did not differ among soil treatments.

Soil amendments had significant effects on the abundance of the ammonia monooxygenase gene (*amo*) detected in the apple rhizosphere. This gene encodes the enzyme involved in the first step in the process of nitrification, resulting in the conversion of ammonia to nitrate. Initial soil geochemistry appeared to influence the ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) community response to fertilizer treatments. The GC soil microbial community appeared to be more limited by inorganic N than the RF soil microbial community as AOB abundance significantly increased only when additional inorganic N in the form of urea was added. This limitation is not surprising given the initial low soil inorganic N concentration of the GC orchard soil compared to the RF orchard soil. In the RF soils, AOB abundance increased compared to the no-treatment control only with the addition of both urea and compost. In contrast to AOB, fertilizer additions had little effect on AOA gene abundance in the orchard soils, thus illustrating again the complexity and variety of responses of the nitrogen cycling microbial community to fertilizers. Most short-term, fertilizer applications had no significant effect on genetic composition of the AOA or AOB community. The sole exception was observed in the conventionally managed GC orchard soil where a significant shift in genetic composition of the AOB community was detected in response to application of urea (**Fig. 9**).

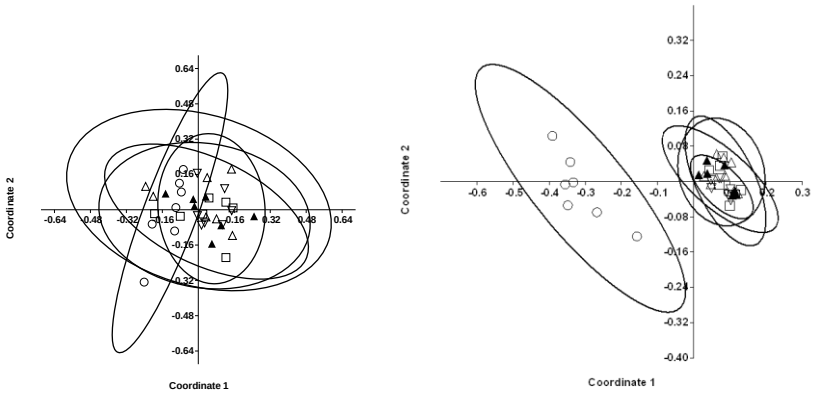

Fig. 9. Effect of fertilizer treatments on ammonia-oxidizing bacterial community composition in RF (left) and GC (right) orchard soils as assessed by non-metric multidimensional scaling of T-RFLP data. Treatments include non-amended control (Δ), *Brassica napus* seed meal (▲), plant-based compost (□), urea (○), and urea with compost (▽). Ellipses represent the 95% confidence region.

Significance: Overall, the relative abundance of the NifH, AOB, and AOA genes compared to the NirK genes indicates a potentially greater capacity for N retention, and thus plant availability, in the high OM RF than the low OM SR orchard soil system. The relative differential abundance of AOB and AOA in soil systems is of significance because AOA have been reported to respond preferentially to organic nitrogen inputs over inorganic N fertilizer. Fumigation will adversely affect the process of nitrification in soil systems and could lead to significant N losses.

3.2 *Effect of physical/chemical properites on community of nitrogen cycling bacteria and archaea.*
Initial composition and activity of the ammonia oxidizer community was evaluated in three orchard systems. Interestingly, AOA abundance was higher in all systems, but the difference was amplified in soils of higher organic matter content (GC and RF orchards). While ammonia oxidizing archaea (AOA) were marginally higher than bacteria (AOB) in the SR soil, AOA abundance was over 10 times greater than AOB in the RF soil system and nearly 100 times greater in the GC orchard soil. (**Fig. 10**). The abundance of ammonia oxidizers in the low OM SR soil was 1 to 2 orders of magnitude lower than that in the high OM content GC and RF soils.

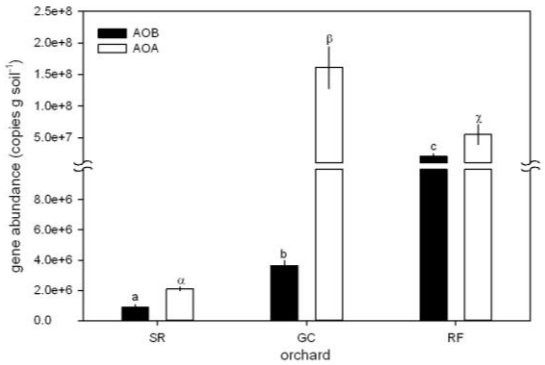

Figure 10. Bacterial (AOB) and archaeal (AOA) ammonia-oxidizing *amoA* gene abundance in non-amended SR, GC and RF orchard soils. Values are mean ± SE (n = 7). Means with different letters are significantly different at $P < 0.05$ based upon Tukey's honest significance test for comparisons between soils.

Significance: AOA abundance was greater than abundance of AOB in orchard soils and genetic composition of AOA communities differed significantly between the organic (RF) and conventional (GC) orchard (**Fig. 11**). These microbial groups largely determine levels of nitrate in soils, the preferred N form for plant uptake. Previous studies indicate that archaea respond less favorably to inorganic forms of N. Correspondingly, in this study, AOA abundance increased in response to the organic N input in the form of brassica seed meal but not urea. This result would suggest inefficient use (and potential loss) of N from these orchard systems. These findings highlight the need for an increased focus in agricultural research to understand and improve the specificity of fertilizer application for orchard production systems.

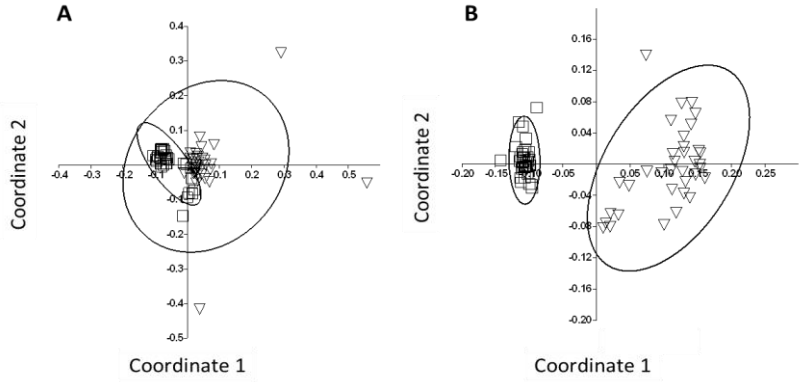

Figure 11. Composition of the ammonia-oxidizing bacterial community (A) and archaeal community (B) in the RF (▽) and GC (□) orchard soils as assessed by non-metric multidimensional scaling analysis of T-RFLP data. Ellipses represent the 95% confidence region.

4.1. Effect of altered soil biology on fruit quality. The effect of soil treatments on fruit quality was assessed. Seed meal amendment at planting did not alter fruit quality in terms of firmness. After 4 months storage under normal atmosphere at 40 F, no significant differences in Gala fruit firmness were observed among soil treatment or rootstock (**Table 1**). Differences in fruit color (hue high to low = green to yellow; chroma high to low = increased red) were observed. Gala fruit from trees grown in the non-treated control replant soil exhibited enhanced color development relative to fruit from either the fumigated or seed meal amended soil. This finding indicated that the difference in fruit color may have resulted from a stress response or differences in canopy development rather than differences in fertility management as fumigated and non-treated plots received the same fertility input. Ongoing studies are examining the potential effects continued urea, calcium nitrate, compost and seed meal fertility inputs on nematode community structure and fruit quality.

Table 1. Effect of soil treatment on Gala fruit quality parameters

Soil treatment/rootstock	lbs	hue	chroma
Control M9	16.33±1.6	68.5±15.1a	33.9±2.6a
Seed meal M9	16.20±2.1	71.3±23.3ab	35.5±3.9b
Fumigation M9	16.39±2.0	76.2±17.6b	35.5±3.3b
Control G11	16.71±1.9	62.0±26.4a	36.1±4.0a
Seed meal G11	16.21±1.8	72.6±20.2b	35.1±3.4a
Fumigation G11	16.78±1.5	80.3±18.8c	34.8±3.5a

Values are means ± one standard deviation; within a rootstock, means followed by a different letter are significantly different.

Executive summary

The unseen realm of soil microbiology has not been widely examined when considering contributions of the overall orchard biology to the function and productivity of orchard ecosystems. This study identified previously overlooked microorganisms including archaea, which were shown to have a dominant role in nitrogen cycling in orchard soils, as well as fungal elements that may contribute to long-term system resilience to pathogen infestation. Our findings indicate that there exist numerous opportunities to obtain benefits of the soil microbiological community that will 1) enhance utilization of fertility inputs, 2) promote tree root development, 3) improve initial tree growth and orchard productivity relative to soil fumigation, 4) suppress re-infestation of orchard soils by soil-borne pathogens and 5) enhance long-term orchard resilience and productivity.

Long-term management programs and soil physical properties were shown to influence efficiency of nitrogen utilization and loss from orchard systems. In general, abundance and function of nitrogen cycling microbial communities were superior in high organic matter soils and those under organic management. The relative abundance of the *nif*H, AOB, and AOA genes compared to the *nir*K genes in these soils indicated a potentially greater capacity for nitrogen retention, and thus plant availability, in the organic relative to the conventionally managed orchard soil system. In addition, it was demonstrated that ammonia oxidizing archaea, rather than ammonia oxidizing bacteria, dominated in all orchard soil systems regardless of management system. As AOA have been reported to respond primarily to organic nitrogen inputs, it demonstrates the need to obtain a greater understanding of how these communities function in order to efficiently utilize fertility inputs.

Based upon findings obtained in orchard soils of very dissimilar texture and organic matter content, tree root development in response to organic/mineral amendments appears to be consistent across soil systems, which indicates that soil amendments are likely to have predictable outcomes for use to enhance tree root development and establishment. In terms of root initiation, the findings also demonstrate that the positive and negative effects of these inputs are not direct but rather function through their effects on orchard soil biology. It was apparent that various bacteria could enhance root development and it is likely that multiple mechanisms contributed to the response. The input of carbon to the soil system was critical to achieving enhanced rootstock root development.

Metagenome analysis of orchard soil bacterial and fungal populations demonstrated that the soil microbial community could be efficiently managed to obtain a more resilient and productive orchard system. Specific pre-plant soil treatments, a Brassicaceae seed meal formulation, transformed the resident soil microbial community in a manner that limited re-establishment of soil-borne pathogens. Interestingly, the altered soil microbial community in seed meal amended soils was maintained over multiple growing seasons and created a soil environment that suppressed pathogen re-infestation. This altered soil microbial environment resulted in significant benefits to long-term orchard health and productivity. In contrast, fumigated soils were highly conducive to re-infestation by soil-borne pathogens. In particular, lesion nematode populations increased dramatically in the second growing season to root densities that were as high or higher than that detected in non-treated orchard soil. The findings also indicate that rapid re-infestation of fumigated soil by "replant" pathogens likely is responsible for the limited (~1-year) benefit of soil fumigation that has been reported. Such a response indicates that continued management of the soil microbial community beyond the initial application of soil fumigation, will significantly improve orchard productivity. Our continued studies will be directed by this question with a focus on reduced seed meal inputs in concert with rootstock genotype as a means to improve orchard system resilience and productivity.

FINAL PROJECT REPORT

Project Title: Reduction of generation cycle in apple breeding

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Cooperators: Cameron Peace, Fred Bliss, Jim McFerson, Ralph Scorza and ARS team at the Ag Station in Virginia

Total Project Request: Year 1: $25,211 **Year 2:** $0

Other funding sources

Agency Name: National Science Foundation
Amt. awarded: $16,000
Notes: The funds support an Undergraduate Research Intern for 10 weeks and cover a stipend, travel and lodging for two years

Organization: WSU **Contract Administrator:** Carrie Johnston
Telephone: 509.335.4564, **Email address:** carriej@wsu.edu

Item	2013	2014
Salaries		
Benefits		
Wages [a]	10,500	
Benefits	4,211	
RCA Room Rental		
Shipping		
Supplies[b]	3000	
Travel	1000	
Plot Fees[c]	1500	
Miscellaneous[d]	5000	
Total	**25,211**	

Note: Project was funded for one year and budget reduced to $22,942

No report submitted

FINAL PROJECT REPORT

Project Title: 'WA 2' plant variety rights applications

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Cooperators: Clean Plant Network, Prosser; Tom Auvil, WTFRC

Other funding sources: None

Total Project Funding: **Year 1:** 9,150 **Year 2:** 10,650 **Year 3:** 3,440

Budget History:

Item	2011-2	2012-3	2013-4
Supplies	250	250	250
Travel	0	0	0
Miscellaneous	0	0	0
- **Quarantine costs**	8,000	0	0
- **PVR application fees**	900	10,400	3,190
Plot Fees	0	0	0
Total	**9,150**	**10,650**	**3,440**

OBJECTIVES

1. To establish certified virus tested (CVT) material of 'WA 2' in the Plant Variety Rights (PVR) process in selected territories as a prelude to applying for PVR in those territories, which is the only way to protect 'WA 2' outside the USA and control its possible release.
2. To apply for PVR for 'WA 2' in the EU and in the countries mentioned below, as is feasible, by project's end.

SIGNIFICANT FINDINGS

- The EU Community Plant Variety Rights application was completed and is ready for submission in spring 2014.
- Australian Plant Variety Rights application is in preparation and is planned for submission in early 2014 (trees due for release in 2015).
- NZ: wood was released from quarantine in March 2013, PVR application planned for March 2015.
- 'WA 2' is established in quarantine in South Africa and Chile.

RESULTS & DISCUSSION

WSU (as WSURF) has licensed agents within each of the territories noted above to oversee the quarantine process and PVR application for 'WA 2'.

Propagating wood from the Clean Plant Network in Prosser was distributed to the territories in winter 2011 as requested. Grafts failed in Australia so further propagating wood was sent winter 2012/13. Wood from Sunrise orchard was sent to the EU as requested in 2011 and 2012.

The application for PVR in the EU was submitted to our agent in spring 2013, but both parties (agent and WSU) decided to delay until 2014 to coordinate virus testing results with supply of the trees to the European CPVO for examination. PVR applications for Chile, South Africa, Australia and New Zealand are expected to be submitted in a timely manner to meet the relevant deadlines in each territory.

'WA 2' was released from quarantine in New Zealand in March 2013. Propagating wood is being bulked up for trialing.

EXECUTIVE SUMMARY

The Washington apple breeding program released 'WA 2' in 2009 for Phase 4 grower evaluation giving participating growers the ability, in the future, to convert their evaluation agreements to Phase 5 commercialization licenses as of January 1, 2011. 'WA 2' is protected in the USA by a plant patent (#PP21,710), but in order to control the variety beyond the territory of the USA, Plant Variety Rights (PVR) applications need to be filed in each territory deemed worthwhile, i.e., necessary to protect the commercial interests of WA growers. For most territories, there is a tight timeline regarding application for PVR. For example, on the Community (EU) Plant Variety Office's website, it is stated that "A variety shall be deemed to be new (and therefore protectable), if, at the date of application...variety constituents or harvested material of the variety have not been sold or otherwise disposed of to others, by or with the consent of the breeder...for purposes of exploitation of the variety:

(a) earlier than 1 year before the abovementioned date, within the territory of the Community;

(b) earlier than 6 years (for trees) before the said date, outside the territory of the Community."

This means that, as of the first date of sale of the apple cultivar in the U.S., you have a six-year window to apply for PVR (in most countries, or, in some cases, in groups of countries, such as in the EU) or forever lose the right to do so. Phase 4 invoices issued in early 2010 could be regarded as the first date of sale for 'WA 2,' which means that any applications for PVR must be submitted no later than early 2016. During that 6-year period, plant materials need to be imported into the country by an agent living in the country (pre-export tests/certificates are required, and requirements vary by country), get through the quarantine period (which is up to two years or more, and varies by country), and come out of quarantine and produce fruiting trees in order to complete the application for PVR protection. Within the timeframe of this project, we have established CVT material of 'WA 2' in the EU, South Africa, Australia, New Zealand and Chile using agents in each country and applied for PVR in the EU. Applications are being prepared in the other territories. Eventually, we expect to license 'WA 2' in the countries where protection is being sought with the goal of controlling release of the cultivar so as to mitigate competition with WA grower interests. Securing protection in a given country must be accompanied with licensing, not only for purposes of controlling the release of a cultivar, but also to comply with compulsory licensing laws within the countries where protection is obtained.

FINAL PROJECT REPORT

Project Title: WA 38 rootstock and systems trial

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Cooperators: Tom Auvil, WTFRC; Jay Brunner, WSU-TFREC; Roger Adams, Willow Drive Nursery

Other funding sources: None

Total Project Funding: **Year 1**: $36,254 (revised to 91% of original as requested)

Budget History:

Budget 1

Item	Year 1: 2013
Salaries	0
Benefits	0
Wages	0
Benefits	0
Equipment	0
Supplies[1]	10,898
Travel[2]	186
Plot Fees	1,865
Miscellaneous[3]	2,099
Miscellaneous[4]	5,781
Total	**$20,829**

Footnotes:
[1]Supplies includes irrigation and trellis system
[2]Travel to Sunrise orchard as required
[3]Contractor to put in posts and anchors
[4]Labor for planting and trellis installation

Budget 2

Item	Year 1: 2013
Supplies - Trees	15,425
Total	**$15,425**

Footnotes:
2158 trees are expected on G.41 and M9 rootstocks, available for planting spring 2013.

ORIGINAL OBJECTIVES

The WSU apple breeding program has recently released a very promising red variety, WA 38, characterized by an unusually firm, crisp and juicy texture. The interest in this apple is widespread within the Washington industry. Once sufficient trees have been propagated they will be available to purchase in 2017. WA 38 has been studied in Phase 3 trials of the breeding program, which primarily focuses on the generation of higher volumes of fruit for different purposes, but a more complete investigation of different training systems should be carried out to determine optimum production systems for this variety. We believe that a training system and rootstocks trial on an experimental scale is necessary to assess the variety and collect as much information as possible about its behavior on different combinations. This one-year project established a WA 38 orchard at the Sunrise orchard (Wenatchee, WA) on two rootstocks (G41 and M9-Nic29) each of them trained to a different system. The same planting was repeated at the Roza orchard (Prosser, WA). This dual-site comparison can also address the suitability of WA 38 under different growing conditions since the two locations differ in soil fertility and structure (Prosser is more fertile than Sunrise). We consider this trial valuable for the industry because it will provide answers that will lead to the development of management guidelines for the future growers of WA 38.

SIGNIFICANT FINDINGS

- Sunrise orchard:
  - During spring 2013, soil in Sunrise was prepared for the new planting (the ground was fumigated, fall 2012).
  - Wooden posts and three wires were set up and anchored prior to planting. Wooden posts were placed every 33 ft within the row and this space was covered with different number of WA 38 trees: 11 for spindle and 20 for the V system. The inclination angle of planting for the trees trained at V is 15˚-20˚ from the vertical. The total number of trees per training system regardless of rootstocks is 308 for spindle and 560 for V (Fig.1).
  - The WA 38 orchard was planted in June 2013 with two training systems, spindle and V system and on two rootstocks, M9-NIC29 and Geneva41.
  - Plots for the two rootstocks were planted with a randomized block experimental design in each row and within each training system. For the spindle and V system there are two plots per rootstock in each row and a total of seven rows per training system intersected by walkways (Fig. 2 and 3).
  - WA 38 spindle trees were planted at 3 ft x 10 ft (1498 trees/acre), while those planted with V system are 1.5 ft x 10 ft (2997 trees/acre, Fig. 3). One-year-old trees from Willow Drive nursery were planted in plots according to the trunk caliper sorting (5/8", ½", ¾").
  - An irrigation system was set up with drippers to optimize the water and fertilizer application. No cooling system net cover has yet been established.
  - The inter-rows are covered with grass.
  - A further four rows of trees with the same scion-rootstock combinations have been planted adjacent to the others.

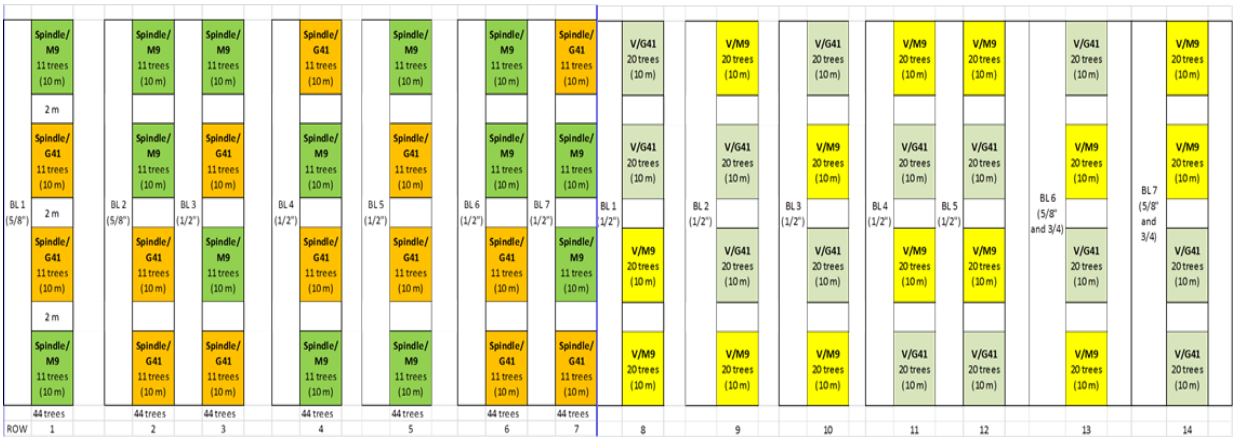

Figure 1: Sunrise and Roza orchards: first seven rows WA38/M9-NIC29 or WA38/Geneva41 trained as spindle and other seven rows with same rootstock combinations trained as V system.

Figure 2: Sunrise orchard in September 2013; detail of the walkways (on the left) and of the trellis in V system and spindle (on the right).

Figure 3: Sunrise orchard in September 2013; plot of WA38/M9-NIC29 at spindle (on the left) and the same combination in V system (on the right).

❖ Roza orchard:

The same experimental design within each row and training system describe above was used in Roza orchard and the planting was made in June 2013 with trees with the two rootstocks mentioned above and trees coming from the same nursery. The trellis structure in Prosser was completed with six wires at equal distance (Fig. 4).

Figure 4: WA38 V trained, Roza, Prosser (September 2013).

RESULTS & DISCUSSION

The two rootstocks were chosen among all those available for their different features. M9-NIC29 is known as the most vigorous clone of M9 and is suggested for Honeycrisp and other low vigor varieties. Geneva41 is more dwarfing, tolerant to root rot, resistant to cold, replant disease, fire blight and woolly aphid (Robinson et al., 2003).

WA 38 is a type 4 habit apple (according to the Lespinasse scale): it produces in the outer part of the canopy mainly in brindle and 2-3 year old branches, Fig. 5 (Sunrise). We have noticed that this variety tends to have "blind wood" after planting. This aspect can be managed but needs to be optimized.

Figure 5: Pictures of WA 38: detail of over 4" long "blind wood" (September 2013).

- Long feathers developed at Sunrise 4 months after planting.
- In this case of bi-axis, a head back cut at 2 ft from the ground was made in order to build a two leaders system by selecting two new branches to become the axes of this trellis. The bi-axis trees planted along the row will produce a flat canopy or fruiting wall, which will more easily allow the use of mechanized management in the orchard (Fig. 6).

Figure 6: WA 38 trained as a bi-axis in Sunrise (September 2013).

Literature:

- Lespinasse J.M**.,** 1980. La conduite du pommier II- L'Axe Vertical, La Rènovation Des Vergers. I.N.V.U.F.L.E.C. Paris.
- Robinson T., Aldwinckle H., Fazio G., and Holleran T., 2003. The Geneva Series of Apple Rootstocks from Cornell: Performance, Disease Resistance, and Commercialization. Acta Hort., 622:513-520.

EXECUTIVE SUMMARY

Two one-acre blocks of WA 38 have been planted at the Sunrise (Wenatchee) and Roza (Prosser) orchards, using two different rootstocks (M9-NIC29 and Geneva41). Trees were established as spindle, V trellis and to make bi-axis trees in randomized replicated blocks for each WA38/rootstock combination. With these two orchards, it will be possible to evaluate the territorial suitability of WA 38 in different levels of soil fertility. A training systems and rootstock trial for this newly released and promising variety will provide information to the growers of Washington on how to manage WA 38 and optimize fruit quality and profitability.

FINAL PROJECT REPORT

Project Title: Support systems to deliver elite new cultivars for extended storage

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Cooperators: Julia Harshman (PhD student, WSU Dept. Horticulture, Wenatchee), Paul Sandefur (PhD student, Dept. Horticulture, Pullman), Lisa Brutcher (Washington Apple Breeding Program), Jerry Tangren (WSU TFREC), Dorrie Main and Sook Jung (WSU Dept. Horticulture, Pullman), Jim McFerson (WTFRC), Fred Bliss (Davis, California)

Other funding sources

Agency Name: WTFRC Apple Review
Amt. awarded: $642,160 (2012-2014)
Notes: "Apple scion breeding program" The foundational program on which the current WTFRC project builds

Agency Name: Winston Churchill Memorial Trust
Amt. awarded: $2,000
Notes: C. Hardner's airfares from Brisbane, Australia to Hawaii

Agency Name: Queensland Alliance for Agriculture and Food Innovation
Amt. awarded: $10,000
Notes: Two weeks of C. Hardner salary

Agency Name: USDA-CSREES, Specialty Crop Research Initiative
Amt. awarded: $7.2 mil plus same matched by universities and industry (Sep 2009 – Aug 2014)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". RosBREED project datasets being generated will be mined further for DNA test development for storability traits

Total Project Funding: $77,724

Budget History:

Item	**Year 1:** May 2013- Apr 2014
Salaries	$58,832
Benefits	$ 3,960
Wages	$ 9,464
Benefits	$ 918
Equipment	
Supplies	
Travel	$4,550
Plot Fees	
Miscellaneous	
Total	$77,724

RECAP ORIGINAL OBJECTIVES

Focusing on extended storage, our overall goal is to deliver routinely implementable methods to the Washington Apple Breeding Program (WABP) for revealing genetic potential for commercial performance. This project is to improve the WABP's prospects for developing superior new cultivars that provide exceptional fruit quality like Honeycrisp but without the storage flaws of that cultivar.

Specific objectives:

1. Optimize resource allocation in the WABP
2. Implement software for routine prediction of genetic potential

SIGNIFICANT FINDINGS

Optimize resource allocation in the WABP:

- Opportunities were identified to optimize resource allocation in the WABP.
- The WABP's Phase 1 and Phase 2 have been thoroughly dissected for their operational activities, costs of each activity, and appropriateness of traits evaluated at that phase. Complete datasets of historical WABP data for these two Phases have been compiled. Only with such dissection and dataset preparation can efficiencies be identified and alternatives objectively compared.
- The greatest proportion of costs for Phases 1 and 2 are in harvesting and fruit processing, especially labor, indicating that any reduction in fruit processing time while achieving the same or better genetic outcomes would substantially save costs.
- An experiment is underway to identify efficiencies that might be gained in Phase 1 fruit quality evaluations. As this one-year project runs from May 2013 to Apr 2014 with extended storage of 2013 season fruit, the final datasets and their analyses are not yet complete.
- In Phase 2 trials, efficiencies were identified in identifying selections superior in their genetic potential under extended storage: fruit quality evaluations after both normal storage (2 months) and extended storage (4 months) are unnecessary; conducting only one is sufficient. After a regular 2-month storage evaluation, a supplemental 4-month duration could be used just to reveal storage disorder fatal flaws.
- DNA testing capability was advanced for storability-related traits by refining the predictiveness and technical efficiency of previously available DNA tests, developing new DNA tests, and identifying new genomic regions to target.
- Deployment of DNA tests is enhancing efficiency, accuracy, and creativity in the WABP.

Implement software for routine prediction of genetic potential:

- New software was developed, delivered, and implemented in the WABP for routine prediction of genetic potential among Phase 2 selections.
- The first routine use of this software, *Elite Advance*, in 2013 by WABP staff supported decision-making by the breeder for advancement of certain selections from P2 to P3.
- *Elite Advance* was also useful in identifying outlier data points that could unduly bias selection decisions.

RESULTS & DISCUSSION

Activity 1. Optimize resource allocation in the WABP

The efficiency of current selection methods in Phase 1 and Phase 2 trials to identify selections with elite performance under extended storage is being compared with alternative methods, using a cost-benefit ratio by incorporating economic information into models previously developed.

Phase 1 trials

An experiment is underway to identify opportunities to enhance the design of Phase 1 trials, targeting extended storage. This experiment involves (i) economic modeling of Phase 1 operations, (ii) collating all available historical Phase 1 data, (iii) assembling a new dataset of fruit quality performance for a large set of current Phase 1 seedlings followed by statistical analyses in quantitative genetics and economics to compare cost-efficiency of alternative, logistically feasible Phase 1 designs, and (iv) developing recommendations for the 2014 harvest season. Fruit quality evaluations after extended storage will not be conducted until around Feb 2014. Therefore, subsequent statistical analyses to calculate cost-efficiency among alternative Phase 1 trial designs have not yet taken place. As originally planned, this part of the project is expected to be completed by May 2014 so that recommendations for the 2014 harvest season can be developed and subsequently implemented. Progress in steps (i) to (iii) is described below.

i) The cost structure of Phase 1 activities, developed in previous years for marker-assisted seedling selection cost-efficiency estimates, has been updated to include personnel time and a more detailed cost inventory of consumables. The collated cost structure information is in a spreadsheet with an output of cost per seedling. The spreadsheet is easily manipulated to allow comparisons of costs of different Phase 1 structures. Detailed notes have been included to make future updating or altering the sheet easier. The largest proportion of Phase 1 costs are in nursery growing (42%), then fruit harvesting and processing (34%) (Figure 1). A reduction in fruit processing time would reduce costs, such as by using a streamlined fruit sampling protocol that efficiently gathers required information to confidently identify genetic potential without redundancy (addressed in the Jan 2013 report for the "Increasing decision confidence in cultivar development and adoption" WTFRC project). WABP resources can also be more efficiently allocated by using DNA testing to cull inferior seedlings in Phase 1, especially prior to nursery budding and field planting (as addressed by Edge-Garza et al. 2010 and in various previous WTFRC projects with Peace as PI, 2007-2012).

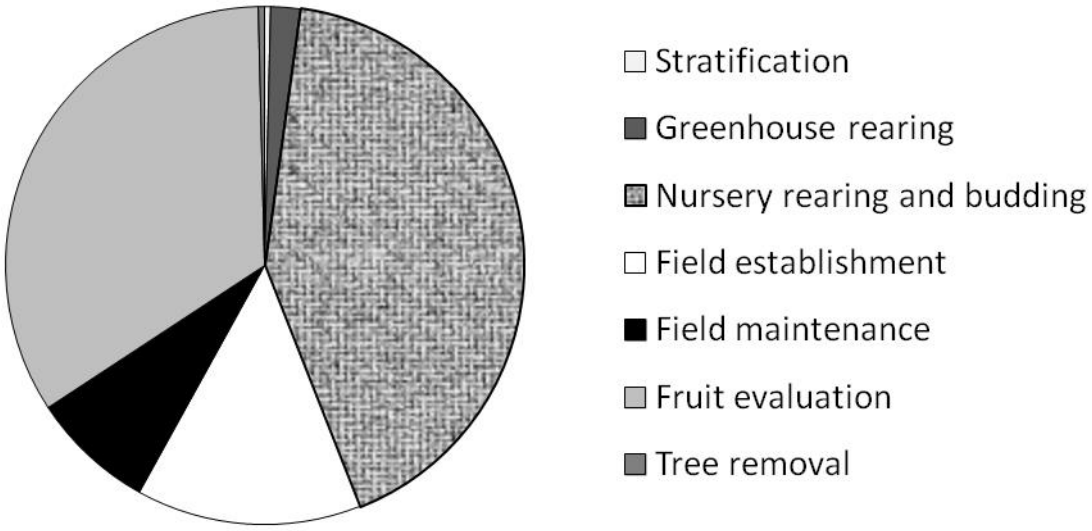

Figure 1: Cost structure of Phase 1 operations in the WABP. Phase 1 is the first stage of seedling evaluation, lasting approximately eight years from pollination.

ii) Existing Phase 1 data has been collated into a single spreadsheet to facilitate subsequent statistical analyses.
iii) 750 Phase 1 seedlings from 29 families were harvested in 2013 from both WSU Sunrise and Columbia View orchards using the typical WABP protocol of starch/iodine assessment of maturity (Cornell stage 3-5). Evaluations of appearance and maturity were completed at harvest. Texture components, soluble solids content, titratable acidity, appearance components, and disorder incidence evaluations after two months of regular atmosphere storage at 36 °F (2-3 °C) plus one week at room temperature have been completed. Fruit quality evaluations after four months of storage under the same conditions plus one week at room temperature are currently underway (i.e., fruit are in storage as of early Jan 2013).

Phase 2 trials

Similar to the economic dissection of Phase 1 trials above, an experiment is underway to identify opportunities to enhance the design of Phase 2 trials, especially for their ability to identify elite selections with superior genetic potential for fruit quality performance after extended storage. This experiment involves (i) economic modeling of Phase 2 operations, (ii) collating all available historical Phase 2 data and analyzing that data with the new software of *Elite Advance* (from Activity 2) to determine the effect of extended storage on identifying superior genetic potential for various fruit quality traits, (iii) combining the previous two elements into a single model that enables comparison of alternative Phase 2 trial designs, (iv) developing recommendations for improved Phase 2 evaluation protocols following the 2013 harvest season, and (v) identifying knowledge gaps and collecting additional Phase 2 trial data to refine Phase 2 evaluation methods for performance after extended storage. Progress in these steps is described below.

i) Phase 2 costs were collected and include personnel costs. The collated spreadsheet has a current output of cost per selection, although this can be easily manipulated to provide other outputs. Harvesting and fruit processing account of the largest proportion of costs (74% together) in Phase 2, the majority of this being labor (Figure 2).

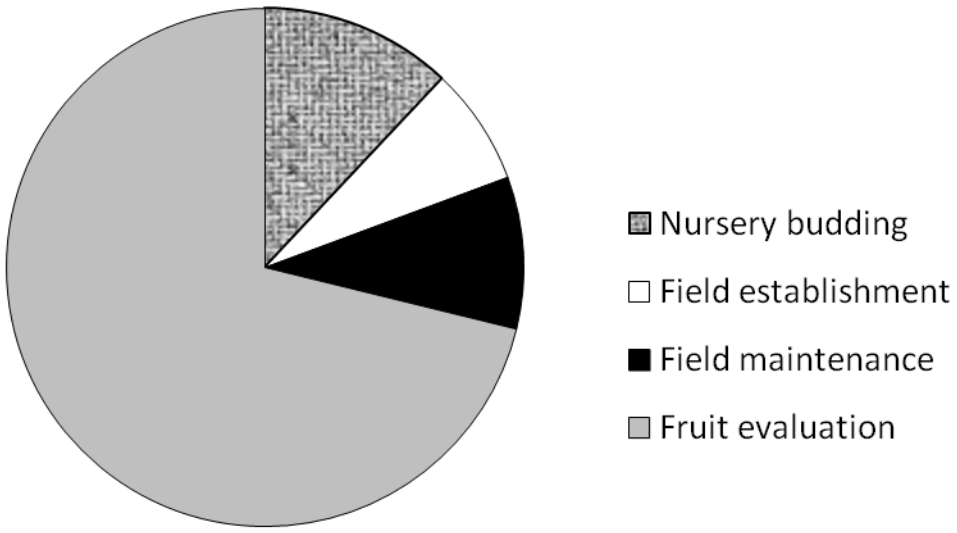

Figure 2: Cost structure of Phase 2 operations in the WABP. Phase 2 is the first evaluation of replicated selections, with multiple trees planted at multiple locations and lasting approximately six years.

ii) Extended storage data was available and collated for 2010-2012 Phase 2 trials, for the following traits:

17 sensory traits: starch rating, ground color, type of color, proportion of red color, extent of lenticels, extent of russeting, shape, size, appearance summary, crispness, hardness, juiciness, aromatic taste, sweetness, tartness, eating quality, and overall quality

8 instrumental traits: weight, diameter, soluble solids content, titratable acidity, pH, and Digi-Test texture measures of M1, M2, and Cn)

Analysis of this Phase 2 data indicated that on average there was a significant difference between 2 months storage and 4 months extended storage for many traits (Table 1).

Table 1. General effects of storage duration in Phase 2 trials on fruit quality traits. Analyses were conducted on Phase 2 selections each evaluated for 1-3 years over the 2010-2012 seasons.

Trait category	Significant differences observed between 2 and 4 months storage evaluations	
	Higher at 4 months extended storage	Lower at 4 months extended storage
Sensory	Starch rating, ground color, extent of lenticels	Size, crispness, hardness, aromatic taste, tartness, eating quality, overall quality
Instrumental	None	Weight, diameter, pH, titratable acidity, M1, M2, Cn

Between the storage conditions of 2 months and 4 months, there was no significant change in ranking of Phase 2 selections for almost all fruit quality traits (Table 2). These results suggest that there is no differential response among Phase 2 selections to extended storage. Therefore, both normal storage and extended storage treatments may not be necessary because performance under extended storage can be predicted from performance under 2 month storage and vice versa. However, extended storage has a better ability to reveal fatal flaws in the form of too-high incidences of storage disorders.

Table 2. Effect of storage duration in Phase 2 trials on identifying Phase 2 selections with superior genetic potential for fruit quality traits. Dataset was the same as used for Table 1.

Trait category	Significant re-ranking of Phase 2 selections observed between evaluations	
	Harvest and 2 months storage	2 months storage and 4 months storage
Sensory	Starch rating, hardness, eating quality	Starch rating, aromatic taste
Instrumental	M1, M2	None

For just a few texture traits (hardness, eating quality, M1, and M2), significant re-ranking of some selections was observed between harvest and 2 months storage evaluations (Table 2), indicating that evaluations at harvest for those traits cannot be used to predict performance after 2 months storage. We may have found the DNA information explaining some of this phenomenon (see *DNA information* – Ma-indel section below) which would allow us to exploit DNA tests in Phase 2 evaluations for increased efficiency.

iii) Alternative designs were modeled. Metrics for comparing alternative designs were chosen – the two methods being pursued are “acceptance interval” and “advance/discard errors”. The

acceptance interval metric measures the effect of the design on the genetic potential of the seedling and the confidence with which one could separate a seedling from a standard. The advance/discard errors measure the probability of an error of advancing (to P3) or discarding a Phase 2 selection. Nested within the equations for these two metrics is an equation to maximize the design of Phase 2 trials. Due to the nature of modeling, both of these metrics are written in the programming language of R.

iv) Evaluation of alternative Phase 2 designs is still underway.

v) Identification and filling of knowledge gaps to refine Phase 2 evaluation methods is still underway.

DNA information

Refinement of current DNA tests: Three existing DNA tests for storability-related traits were refined in 2013. These DNA tests were "Md-ACS1-indel" and "Md-ACO1-indel" for general storability (as these tests target differences in two genes involved in ethylene biosynthesis) and *Ma* locus markers for the "fresh sensation" traits of crispness, juiciness, and storability. The three DNA tests described above were deployed in 2013 in the WABP in parent selection and seedling selection. Use of all three tests, and others, in 2013 helped guide crossing decisions for better outcomes and help avoid wasteful crosses. In addition, Md-ACO1 and Ma-indel were used in 2013 to discard thousands of young seedlings predicted to be genetically inferior, thereby enriching new generations with superior genetic potential. Furthermore, such trait-predictive DNA information was also obtained in 2013 for all selections advancing into Phase 2.

Md-ACS1-indel: Statistical analyses of RosBREED data in 2013 to refine our understanding of the relative effects of the two variants (alleles) of the *Md-ACS1* gene, as determined by running the Md-ACS1-indel DNA test, confirmed our previous understanding. However, while individuals carrying the "best" genotype for this DNA test are on average firmer than those with the "middle" genotype, differences were more pronounced at harvest than after storage. (Not enough individuals with the "worst" genotype were available in the RosBREED dataset for analysis.) Also, the effects of this DNA test are relatively small in general compared to newer DNA tests (such as Ma-indel and Md-PG1-SSR-10kd). Nevertheless, when used in selection, this DNA test is expected to improve the chances of obtaining superior genetic potential for extended storage.

Md-ACO1-indel: Analyses conducted in 2013 confirmed a small trend in the same direction as previous reports and more pronounced at harvest than after storage, similar to the DNA test above. However, differences between genotypes of this DNA test were even smaller than for Md-ACS1-indel, although there were not enough representatives of the "best" genotype to determine its contrast with the "middle" and "worst."

Ma-indel: In 2013, a previous DNA test for multi-trait *Ma* locus was successfully converted to more reliable test, "Ma-indel." Analyses of this new DNA test with the RosBREED dataset identified some great results for the WABP. Not only were the effects on acidity and crispness confirmed, but also detected were large effects on firmness (both sensory and all instrumental measures) and indeed all textural measures (including the Digi-Test's Cn, instrumental crispness, and Co, instrumental "mealiness"). The most interesting and useful contrasts were between the two alleles at this locus carried by 'Honeycrisp.' One of the alleles is associated with higher firmness at harvest and over storage than the other 'Honeycrisp' allele (Figure 3). The same valuable allele is associated with high crispness that doesn't lose as much crispness over storage as the other 'Honeycrisp' allele and all other alleles from non-'Honeycrisp' lineages (Figure 3). This "jewel in the genome" may explain why some P2 selections perform relatively differently to their peers after storage than at harvest – it may be those with the valuable Ma-indel allele from

the 'Honeycrisp' lineage whose fruit don't lose so much of their crispness and firmness as storage progress – thus better maintaining a "fresh sensation," the moniker given to the suite of traits associated with the *Ma* locus.

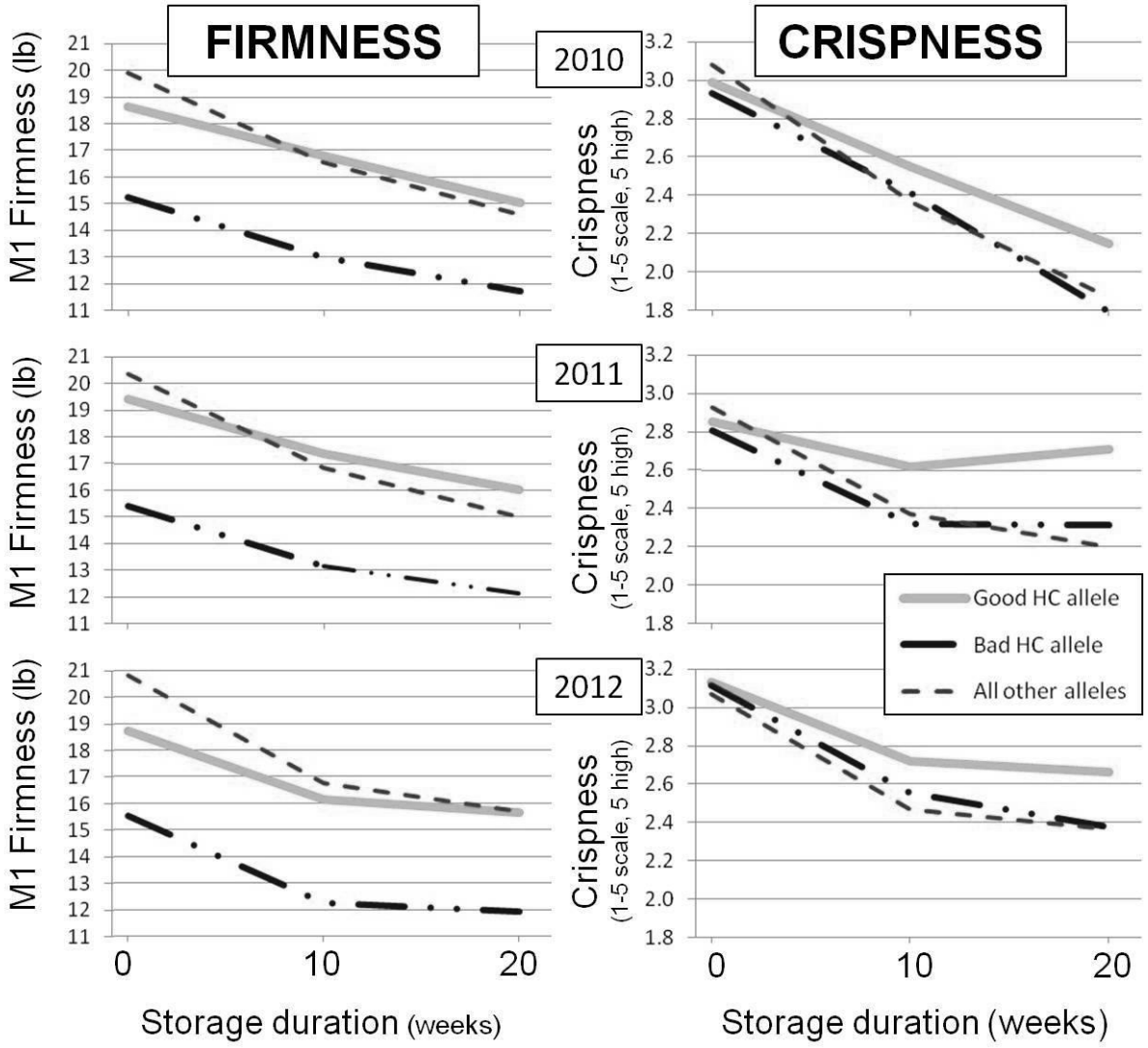

Figure 3: Contrasts among alleles distinguished by the Ma-indel DNA test among seedlings of the WABP in the RosBREED project. Each of the 'Honeycrisp' (HC) alleles is represented by ~40 seedlings while all other alleles are represented by a total of ~125 seedlings. Fruit quality evaluations after storage were over 10 and 20 weeks, each with an additional week of ripening at room temperature (as described by Evans et al. 2012 and Schmitz et al. 2013). Standard deviations are ~3.4-3.9 for M1 firmness and ~0.4-0.8 for sensory crispness.

New DNA tests developed: Two new DNA tests were developed, based on promising RosBREED results previously described in the Jan 2013 report for the "Increasing decision confidence in cultivar development and adoption" WTFRC project. These two DNA tests, "Bp16-indel" and "Bp13-SSR," help predict bitter pit incidence in WABP germplasm. These tests have yet to be used in the WABP

but are expected to be valuable tools in the arsenal against bitter pit. Bp16-indel also provides a useful backup to Ma-indel as it is in the same genomic region.

Future DNA tests: Several additional genomic regions influencing fruit quality after storage were also investigated to determine whether they were promising enough to be developed into new DNA tests for future deployment in the WABP. Three genomic regions were deemed worthy of advancement: the *Md-PG1* gene for firmness, the "*LG8A*" locus for acidity, the "*LG16C*" locus for crispness, and the "*LG1Fru*" locus for sweetness. Other genomic regions are beginning to emerge from analyses of the RosBREED dataset for the storage disorders of internal browning, scald, and shrivel.

Md-PG1: The *Md-PG1* gene, putatively associated with fruit firmness, was previously given attention, together with the original *Md-ACS1* and *Md-ACO1* storability genes, during a 2008-2009 federally funded project with partial WTFRC funding and international collaborations. While *Md-PG1* remained promising for several years, recent analyses with a new DNA test (Md-PG1-SSR-10kd; Longhi et al. 2013a,b) screened on the RosBREED dataset confirmed that there is value for the WABP. Genotype outcomes for this DNA test account for more than 20% of observed variation for firmness, especially after storage, in many WABP families.

LG8A: Characterized by WSU RosBREED grad student Sujeet Verma (Verma 2013). This genomic region accounts for about a third of the observed variation for acidity in WABP germplasm. *LG8A* is highly predictive for fruit acidity differences among parents, seedlings, or selections, at harvest as well as after storage and especially in combination with DNA information at the *Ma* locus. Development and deployment in the WABP of a new DNA test for *LG8A* is recommended.

LG16C: Discovered and characterized by WSU RosBREED grad student Sujeet Verma (Verma 2013). This genomic region appears to explain a contrast in 'Honeycrisp' alleles in crispness after storage. However, this may the same effect already explained by the nearby Ma-indel DNA test. Further research is required to determine whether or not the effects are separate (and additive).

LG1Fru: Discovered and characterized by WSU RosBREED grad student Yingzhu Guan (Guan 2013). This genomic region accounts for about half or more of the observed variation among WABP seedlings for fruit fructose concentration and has almost as much explanatory power for glucose and sucrose concentrations. Development and deployment in the WABP of a new DNA test for *LG1Fru* is recommended.

Expected impact: The DNA tests for storability-related traits already available and soon to be developed for the WABP are valuable for use at various breeding stages. Their use in Phase 1 seedling selection, to eliminate thousands of seedlings predicted to have sub-par genetic potential, remains a powerful strategy for the WABP. The relatively large proportion of Phase 1 operational costs associated with rearing and evaluating seedlings after the greenhouse stage (Figure 1) highlights the value of this strategy. However, the biggest impact on the WABP from DNA tests is expected to continue to come from DNA-informed decisions – by helping avoid less efficient crosses and enabling more creative crosses to achieve target outcomes. (Yet this impact is difficult to quantify unless comparisons are made with hypothetical crosses that would have been made without DNA information.) With new families enriched for superior genetic potential, there is a reduced need for DNA testing of seedlings with the same tests. More opportunity is thereby afforded for selection among Phase 1 seedlings for other valuable attributes (including use of new DNA tests) or simply for identifying more one-in-a-million winners, which becomes more like one-in-a-hundred-thousand. Similarly, use of DNA tests in Phase 1 enriches the genetic potential of selections entering Phase 2.

Better individuals, and more of them, are therefore expected to result from Phase 2, even if operational components of Phase 2 trials themselves remain the same. Furthermore, as additional DNA tests become available, they can be used on Phase 2 selections and combined with performance data to inform advancement decisions. Or, given the proportionally large costs associated with Phase 2 fruit evaluation (Figure 2), new DNA tests can be used as soon as possible to identify Phase 2 selections to avoid evaluating phenotypically, or to chainsaw cull, in subsequent seasons.

Activity 2. Implement software (*Elite Advance*) for routine prediction of genetic potential

New software, called *Elite Advance*, for routine prediction of the genetic potential of candidates from Phase 2 trials was developed by Dr. Craig Hardner based on approaches developed in the 2011 and 2012 WTFRC projects, "Increasing decision confidence in cultivar development and adoption." Programming components and running of the new software are described in the box below.

Elite Advance
New software for routine prediction of genetic potential in WABP Phase 2 trials

Elite Advance utilizes the mixed model program ASReml implemented in R. R is a free software environment for statistical computing and graphics, although a little knowledge of programming in R is required for prediction of genetic potential to facilitate adoption by WABP personnel. The software RStudio is used as the interface for R. While R and RStudio are free, the software requires a valid license for ASReml. *Elite Advance* is run through a "Set_Parameters.R" file that defines:

- the paths for required data files and customized R code
- the traits that are in the data file for which predictions are required
- entries planted in the trials that will be used as standards to compare with Phase 2 selections
- the linear model to be used for the analysis
- subsets of the data to be used for the analysis
- directives to control the analysis.

Elite Advance is run by submitting the Set_Paramaters.R file. This file calls a function that creates separate analysis files for each trait listed in Set_Parameters.R. From this run, parameters are estimated for the linear model, and these parameters are used to predict the genetic potential for each candidate. Post-analysis processing includes testing the significance of the difference each Phase 2 selection and a specified standard. The last function of the single trait analysis is to collate results and output them into Excel files for easier investigation by the operator. Finally, after each trait is run the results are collated across traits.

To facilitate adoption of *Elite Advance* by the WABP, two deliverables were achieved. First, an instruction manual was prepared on the installation and running of the new software. Second, a workshop was presented on 17 July 2013 to Dr. Kate Evans, Lisa Brutcher, Bonnie Konishi, Dr. Cameron Peace, Yingzhu Guan, Sushan Ru, Julia Harshman, Paul Sandefur, and Jerry Tangren. As part of this workshop the alpha version of *Elite Advance* was transferred to the WABP team with a functional example. Further meetings on the following two days provided guidance and identified implementation issues and solutions. Since then, a beta version of *Elite Advance* was delivered and implemented by the WABP for prediction of genetic potential of Phase 2 selections using 2013 at-harvest fruit evaluation data. An updated version will be transferred to the team in Jan 2014, which is likely to be the final version of the software.

Lisa Brutcher (WABP operations team member) completed basic training in R script and has successfully implemented *Elite Advance* on the WABP Phase 2 dataset covering 2005 to 2012. Data output was particularly useful for identification of "outlier" data points which could then be checked

and corrected if erroneous. Outputs from *Elite Advance* of ranking of genetic potential among Phase 2 selections for the various traits were used to support decision-making for fall 2013 tree propagation for advancement to P3.

The WABP team is currently looking at different options in data output display to determine which is the easiest to interpret. The extended storage evaluations of samples from the 2013 season were not completed at the time of writing this report. Once the dataset is complete, data will be uploaded into *Elite Advance* in a timely manner to enable implementation of output analysis in the decision-making for the 2014 propagation season. The WABP operations team expects to use the system routinely from now on.

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EXECUTIVE SUMMARY

Genetic improvement underpins the long-term economic sustainability of the Washington apple industry Focusing on extended storage, our goal was to deliver routinely implementable methods to the Washington Apple Breeding Program (WABP) for revealing genetic potential for commercial performance. The two objectives of this project were to:

1. Optimize resource allocation in the WABP
2. Implement software for routine prediction of genetic potential

By fulfilling those objectives, we expect to improve the WABP's prospects for developing superior new cultivars that provide exceptional fruit quality like 'Honeycrisp' but without the storage flaws of that cultivar. 'WA 38' is an example of such a WABP output, mostly developed using breeding operations established at program's outset in 1994. We believe that we can increase the WABP's development of such superior cultivars – in number and/or performance levels for multiple valuable traits. We believe we can do so by streamlining WABP operations through the routine application of robust statistical genetics calculations and the routine application of predictive DNA tests. Both of these complementary strategies are designed to efficiently reveal genetic potential for superior performance across the spectrum of WABP germplasm and operations. Both strategies were advanced and successfully implemented in this project.

- Opportunities were identified to optimize resource allocation in the WABP.
- The WABP's Phase 1 and Phase 2 have been thoroughly dissected for their operational activities, costs of each activity, and traits evaluated at that phase. Complete datasets of historical WABP data for these two Phases have been compiled. Only with such dissection and dataset preparation can efficiencies be identified and alternatives objectively compared.
- The greatest proportion of costs for Phases 1 and 2 are in harvesting and fruit processing, especially labor, indicating that any reduction in fruit processing time while achieving the same or better genetic outcomes would substantially save costs.
- An experiment is underway to identify efficiencies that might be gained in Phase 1 fruit quality evaluations, to be complete by May 2014.
- In Phase 2 trials, efficiencies were identified in identifying selections superior in their genetic potential under extended storage: fruit quality evaluations after both normal storage (2 months) and extended storage (4 months) are unnecessary; conducting only one is sufficient. After a regular 2-month storage evaluation, a supplemental 4-month duration could be used just to reveal storage disorder fatal flaws.
- DNA testing capability was advanced for storability-related traits by refining the predictiveness and technical efficiency of previously available DNA tests, developing new DNA tests, and identifying new genomic regions to target.
- Deployment of DNA tests is enhancing efficiency, accuracy, and creativity in the WABP.
- New software was developed, delivered, and implemented in the WABP for routine prediction of genetic potential among Phase 2 selections.
- The first routine use of this software, *Elite Advance*, in 2013 by WABP staff supported decision-making by the breeder for advancement of certain selections from P2 to P3.
- *Elite Advance* was useful in identifying outlier data points.

In the remaining months of this project, once fruit of Phase 1 and Phase 2 trees are evaluated after extended storage, we will conduct final comparisons of alternative evaluation methods in early selection phases to improve the efficiency of identifying genetic potential for superior performance after extended storage.

FINAL PROJECT REPORT

Project Title: Design and Development of apple harvesting techniques

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Cooperators: None

Other funding sources: None

Total Project Request: **Year 1:** 53,395

Budget History:

Item	2012
Salaries[1]	$30,534
Benefits[1]	$1,997
Wages[2]	$6,240
Benefits	$624
Equipment	
Supplies[3]	$10,000
Travel[4]	$4,000
Plot Fees	
Miscellaneous	
Total	$53,395

Footnotes:

[1] Salary and benefit for a graduate student
[2] Wages and benefits for hourly help to fabricate sensor platform and collect field data
[3] Cost to purchase materials and build sensor platforms
[4]Travel cost for field data collection and testing

Objectives

Our long term goal was to improve the sustainability and productivity of tree fruit production through reduced labor use and associated costs. Originally the project was proposed for three years with the following specific objectives:

1. Design and develop two prototypes for semi-automated apple harvesting techniques.
2. Characterize the efficiencies of harvesting in two variations of fruiting wall architectures.

The project was funded for only the first year to demonstrate the feasibility of the concept. The scope for the first year for this project involved prototype development and preliminary evaluation in lab and field environments.

Significant Findings

- Two Prototype fruit removal end effectors were developed: Rotational (RAH) and Linear Actuators (LA), and a provisional patent has been filed with WSU (10/2013).
- The RAH and LA prototype mechanisms can remove apples from a limb while retaining fresh-market quality.
- For RAH, the speed of rotation is not a significant factor in resulting fruit removal condition; variety was a significant factor; same direction of rotation achieved the best result.
- Horizontally trained limbs, that are short and stiff, and short spurs are suitable for the rotational harvesting technique.
- Removal rate for RAH was estimated at 2,400 apples per hour; similar to human labor on a mobile platform.
- Removal rate for LA were estimated at 20,000 apples per hour.

Results and Discussion

RAH End-Effector: Results indicate that the rotational apple harvester (RAH) end-effector developed in this project can successfully remove apples from a limb. In this research, the removed fruit was initially classified into three fruit removal conditions (FRC): stem-intact, stempull, and spurpull (figure 1). The fresh-market total (FMT) was defined as the sum of stem-intact and spurpull conditions, while considering that spurpull should be minimized to retain fruiting wood for the next year. Initially, the FMT assumed only the FRC and did not regard other quality issues including bruising downgrades, cuts, and punctures. Damage data relating to bruising was measured in 2013 and will be addressed in further research. Likewise, data relevant to CA fruit storage for some fruit harvested in the fall of 2013 is currently underway. The results will yield supplemental data for these harvesting results and provide further quantifiable data and insight into acceptable fruit removal condition.

Figure 1. Fruit removal condition (L-R): stem-intact; stempull; spurpull.

Ten total varieties of apples were tested using the RAH removal technique: five in 2012 (Jazz, Pacific Rose, Golden Delicious, Granny Smith, Jonagold) plus five in 2013 (Gala, Fuji, Red Delicious, WA-17, Honeycrisp). Four varieties were duplicated in testing, either in location or year (Gala, Granny

Smith, Golden Delicious, and Fuji). A total of 14 tests were conducted for the varieties listed. Table 1 shows the results based on FRC percentage for the varieties tested in 2012 and 2013. Seven out of the fourteen tests resulted in a FMT of 84% or better. The highest percent of apples classified as FMT was for 'Honeycrisp' at 98%. The damage rate associated with 'Honeycrisp' was 32%. All apples that were removed from a limb were allowed to fall to the orchard floor. Damage due to fruit falling through the canopy was not isolated during the first year of testing. It is suggested that a trellising system can improve the "catchability" of RAH removed apples and subsequently lead to a decrease in the damage rate. Research and development regarding localized catching and transport is still needed to move the RAH and LA techniques into complete semi-autonomous harvesting systems.

Variety has a significant effect on the FRC. Two tests with Granny Smith ranked 12th and 14th, out of 14 total tests, as least likely to have a successful FMT. Some consistency is noticeable in table 1, as can be seen with Fuji and Golden Delicious varieties. Two tests with Fuji were conducted in the same year at different orchards, yet ranked 5 and 6 for FMT. Two tests with Golden Delicious were conducted at the same location during two different years and ranked 7 and 8 for FMT. The lack of dispersion for FMT in these varieties make them more suitable, and predictable, for using the RAH technique. However, certain varieties including Granny Smith and Red Delicious were not as suitable for this removal method in both removal condition and horticultural growing characteristics (short stiff limbs).

Table 1 FRC percentage using RAH removal technique for 2012 and 2013.

Year	Variety	***% FMT***	% Stem-intact	% Spurpull	% Stempull
2013	Honeycrisp*	***98***	94	4	2
2013	WA-17 *	***97***	88	9	3
2013	Gala (#1)**	***94***	87	7	6
2012	Pacific Rose**	***92***	86	6	8
2013	Fuji (#1)*	***89***	84	5	11
2013	Fuji (#2)**	***84***	71	13	16
2013	Golden Delicious (#1)**	***84***	58	26	16
2012	Golden Delicious (#2)**	***82***	80	2	18
2012	Jazz*	***80***	70	10	20
2013	Gala (#2)**	***79***	67	12	21
2013	Red Delicious**	***77***	67	10	23
2013	Granny Smith**	***61***	46	15	39
2012	Jonagold**	***60***	58	2	40
2013	Granny Smith**	***36***	36	0	64

* Commercial Orchard

** Research Orchard

Formally trained growing systems achieved a higher removal rate (no. of apples harvested per unit time) than random growing branches. These open systems allowed the user to easily identify an apple and apply the RAH. Three out of the ten varieties tested were grown in formally trained fruiting walls (Gala, Jazz, Pacific Rose) and two of them were in the top five of the FMT ranking. It was observed that fruit was removed better, with less user manipulation, when grown on short branches with a relatively low apple density per spur. Longer, unconstrained, branches are not preferred for use with the RAH technique; so to improve the harvesting results, the length of the branch should be minimized when horticulture permits. Apples growing in clusters were not treated any differently

than single apples and the results showed that the RAH was effective in removing fruit in clusters as well.

The removal rates using the RAH method were similar to a human working on a mobile orchard platform (assumed to be 45 apples/min). The removal rates projected, approximately 2,200–2,400 apples per hour for RAH and 20,000 apples per hour for LA, were almost exclusively calculated on removal rates measured in fruiting wall architectures with no formal training. It is hypothesized that these removal rates will be improved with a formally trained horizontal limb system. Likewise, the horizontal limb system could facilitate the expansion of the RAH technique into a multiple wheel system that allows the user to increase removal rates to an economically justifiable level. The RAH system was not considered to be a selective harvesting method although the multiple wheel system will have the potential to individually activate a section when triggered by a color scheme or human input. The RAH can be used as a type of semi-automated technique with further improvement to aid in labor handling and ergonomics. More research and development is needed to further this type of system.

The materials used for the prototype are all off-the-shelf components. Two 18V electric drill motors (18 ft-lb torque) are suitable for removing an apple from a limb. A soft rubber, TPE type or 30-55 Type A rubber, is sufficient for non-abrasive contact with an apple and provides adequate surface friction during fruit removal. Further work is needed to autonomously control the applied pressure to the surface of the fruit. Year 3 of the initial proposal was to incorporate this mechanism for pressure application.

LA End-Effector: The materials used for the LA prototype include an 18V hand-held linear actuator with a hook that grasped a limb. Preliminary tests conducted in a formally trained horizontal limb system with WA-17 variety showed that actuation can be isolated to a branch with no residual effects, or fruit removed, on upper or lower branches. The precise application of actuation combined with the projected removal rates and FMT rates provide a valuable component for the economic analysis using semi-autonomous fruit removal techniques. Similar to RAH, variety has a significant effect on FRC. However, for Fuji apples tested using LA, FMT averaged 80%. For WA-17, the LA FMT decreased 14% compared to RAH FMT, from 97% to 84%. On the other hand, the removal rate for LA increased by 1,200% from 1,800 apples per hour to 22,000 apples per hour, compared to RAH. Furthermore, a dual motor actuator has been developed to specifically isolate movements to the stem-spur junction by applying rhythmic-patterned actuation. This type of system has the potential to further increase the FMT removal percentages. This research is currently ongoing and expected to continue during Fall 2014 and beyond.

It should be noted that no correlations were made between: a) fruit removal condition and starch-iodine maturity level or b) fruit removal condition and fruit pressure number in either of two prototype evaluations. It is also noted that further research is needed in horizontal limb trellised orchards to incorporate the RAH and LA techniques into a full scale bulk harvesting system.

Executive Summary

This report provides an insight and evaluation of two fresh-market fruit removal techniques developed and tested in apples; a first step in achieving a semi-automatic or fully automatic fruit harvester. Two hand-held devices were compared: a mechanism that rotates fruit vertically and a patterned actuating device. Methods of analysis include fruit removal condition, location, variety, and removal rates, as well as mechanism parameters. Results of data analyzed show that fresh-market quality fruit can be removed from a branch without sustaining significant damage for either technique. Variety is a significant factor on fruit removal condition for each technique. Removal rates for linear actuation were 1,200% higher than rotational. Damage rates ranged 10-50%. Suggestions discussed are:

- that horizontal limb training will improve removal rates and lower damage percentage within the canopy;
- that variety be considered when choosing a technique;
- that actuation removal rates need more economic consideration especially with a modified canopy structure.

The report also discusses the limitations for scaling up these techniques. Some limitations include: horticulture growing characteristics and access to fruit within the canopy. This report also suggests future research and continued development in: rhythmic-pattern actuation, to isolate movement to stem-spur junction; and catching mechanism strategy.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-12-103A

Project Title: Apple scion breeding program

PI:	Kate Evans	**Co-PI (2):**	Cameron Peace
Organization:	WSU TFREC	**Organization**:	WSU-Horticulture
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Cooperators: Bruce Barritt, Professor Emeritus, WSU; Yanmin Zhu, USDA-ARS Wenatchee; Amit Dhingra, Dorrie Main, Carolyn Ross, WSU Pullman; Tom Auvil, Ines Hanrahan, WTFRC; Roger Adams, Willow Drive Nursery, Ephrata; Craig Hardner, Australian Crop Genetic Services, Brisbane, Australia; Fred Bliss, Davis, California.

Total Project Request: **Year 1:** 317,461 **Year 2:** $305,237 **Year 3:** $**239,563**

Other funding sources

Agency Name: WTFRC Apple Review
Amount awarded: $77,724 (2013-2014)
Notes: "Support systems to deliver elite new cultivars for extended storage" PI: Peace. Co-PIs: Hardner, Evans. Synergistic project to improve release and adoption decisions about the WABP's new cultivars.

Agency Name: WTFRC Apple Review
Amount awarded: $22,942 (2013-2014)
Notes: "Reduction of generation cycle in apple breeding" PI: Dhingra. Co-PI: Evans. Synergistic project to implement fast-track breeding to elite selections from the WABP.

Agency Name: WTFRC Apple Review
Amount awarded: $36,254 (2013-2014)
Notes: "WA 38 rootstock and systems trial" PI: Evans. Co-PIs: Lewis, Musacchi. Synergistic project to develop systems for WA 38 production.

Agency Name: WTFRC Apple Review
Amount awarded: $23,240 (2011-2014)
Notes: "'WA 2' plant variety rights applications" PI: Evans. Co-PI: Kelly. Synergistic project to implement PVR protection for 'WA 2'.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $2,000,000 + equal matching from universities, industry (Sep 2009 – Aug 2013)
Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture". PI: Main. Co-PIs include Evans and Peace. Synergistic project for application of bioinformatics to tree fruit crops.

Agency Name: USDA-CSREES, Specialty Crops Research Initiative
Amount awarded: $7,200,000 + equal matching from universities, industry (Sep 2009 – Aug 2013)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace, Main, and Evans. To establish sustainable MAB for U.S. Rosaceae crops.

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries	18,830	18,830	0
Benefits	6,626	7,232	0
Wages[1,2]	52,500	47,250	10,548
Benefits[1,2]	7,650	6,885	7,032
RCA Room Rental	8,400	8,400	8,400
Shipping	0	0	0
Supplies	3,000	3,000	0
Travel[3]	6,875	7,000	5,000
Plot Fees	0	0	0
Total	103,881	98,597	**30,980**

Footnotes:
[1]Lab volume decreased by 50%, Field by 30%.
[2]Lab and Field volume decreased by 10%.
[3] In-state travel to research plots which are spread out across the state.

Budget 1
Organization: WSU-TFREC **Contract Administrator:** Carrie Johnston & Joni Cartwright
Telephone: 509.335.4564, 509.663.8181 **Email address:** carriej@wsu.edu;joni.cartwright@wsu.edu

Item	2012	2013	2014
Salaries[1]	54,642	56,828	57,035
Benefits	21,694	22,562	22,106
Wages[2]	20,800	21,632	22,113
Benefits	3,099	3,223	4,179
Orchard establishment supplies	19,000	20,000	19,000
Genotyping supplies	11,000	13,000	15,000
Travel[3]	15,000	15,000	15,000
Plot fees	8,400	8,800	8,800
Total	153,635	161,045	**163,233**

Footnotes:
[1] Salaries for Agricultural Research Technologist (Bonnie Konishi@ 1.0FTE) and salary for 3 months for genetic screening technician (Terence Rowland @ 0.25FTE)
[2] Wages for time-slip labor for orchard establishment and trait phenotyping.
[3] In-state travel to research plots which are spread out across the state.

Budget 2
Organization Name: Willow Drive **Contract Administrator:** Roger Adams
Telephone: 509 787 1555 **Email address:** roger@willowdrive.com

Item	2012	2013	2014
Seedling propagation	34,720	42,095	41,850
Phase 2 trees	4,425	3,500	3500
Phase 3 trees	20,800	0	0
Plot Fees	0	0	0
Total	59,945	45,595	**45,350**

Objectives:

1. Produce, through conventional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and commercial potential.
2. Using both objective (instrumental) and subjective (sensory) evaluation techniques to develop selections with outstanding eating quality.
3. Use extensive performance evaluation in combination with genetic markers to validate and implement new DNA tests for key fruit and tree traits.

Significant Findings:

1. Nine new crosses were made in 2013 with approximately 9,500 seeds produced in the WSU Apple Breeding Program (WABP).
2. Seedlings from approximately 12,000 seeds from 2012 crosses were grown in the greenhouse.
3. Approximately 6,600 seedlings were screened with DNA markers for fruit quality; just over 2,900 were culled leaving the remaining 3,700 to be transplanted to Willow Drive nursery along with another 2,500 seedlings that were not marker-screened.
4. Seedlings at Willow Drive were propagated on M.9 rootstocks for future orchard evaluation. More than 5,500 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2014, with approximately 20% of them having survived previous screening for fire blight resistance.
5. The final count of new Phase 1 trees planted in 2013 was approximately 4,100.
6. Promising selections already in Phase 2 trials (planted in 2006, 2007, 2008, 2009, 2010, 2011 and 2012) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
7. Bench grafted plants of 43 selections were tested in the greenhouse for resistance to fire blight. Eight selections were equally as resistant as Red Delicious. These tests will be repeated to confirm the data in 2014.
8. Thirteen new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2013.
9. Four promising selections made in 2012 were propagated in 2013 for planting in 2015 Phase 2 trials at three diverse sites in Central Washington.
10. Two new promising Phase 2 selections were propagated onto Geneva 41 rootstock for advancement to Phase 3 initially at the Quincy site only.
11. WSU 36 and WSU 17 were both rejected from Phase 3 trials. The trees of WSU 17 will be top-worked to WSU 68, advancing it to Phase 3, at the Quincy site.
12. The Cultivar Licensing Committee continued to meet to develop a release strategy for WA 38 and future releases from the WABP. Jim Moyer (new WSU ARC Director) has taken this on as a priority.
13. A series of three consumer testing sessions was initiated (October, December and March) to test the performance of WA 38 against ‘Honeycrisp’.
14. New genomics discoveries of value to the WABP were made within the RosBREED project, especially by Dr. Evans’s graduate student Yingzhu Guan and Dr. Peace’s graduate student Sujeet Verma.
15. Trait-predictive DNA information was obtained for all prospective parents using seven DNA tests – for storability, crispness, juiciness, firmness, acidity, skin color, and bitter pit incidence.
16. Trait-predictive DNA information was also obtained for all selections advancing into Phase 2.
17. Genetic identity was confirmed for all mother trees of WA 38 planted in the nursery mother tree blocks. All trees tested as true to type using several DNA markers.
18. The patent for WA 38 was approved.

Methods:

Objective 1 - Breeding:

a. Marker-assisted parent selection will be used to determine the most suitable combinations of parents for crossing to achieve our aim of a portfolio of new improved apple varieties. Using data from the SCRI-funded RosBREED project and the Peace lab, facilitated by the new Breeders Toolbox for breeding database interfacing developed by the Main lab, we will choose the optimum cross combinations from among available germplasm.

b. Crosses will be made each spring, most likely aiming at annual production of around 20,000 seeds. Following vernalization, seedlings will be germinated and grown in the greenhouse at the TFREC. To optimize efficiency and accuracy of sample collection, leaf samples will be collected in the greenhouse from some of these seedling progenies and sent to the Peace lab for DNA testing. Genetic tests used will depend on the particular cross combination. Some progenies will be inoculated with fire blight to enable phenotypic selection for resistance. Only the un-culled seedlings will be planted in the nursery and then budded onto M.9 rootstock for further evaluation.

c. Budded trees will be planted at the TFREC Columbia View orchard for Phase 1 trials (Figure 1) where their resulting fruit will be evaluated. Selection in the orchard will be initially based on fruit appearance (primarily color, uniformity, freedom from defects) followed by eating quality (primarily firmness, crispness, sugar/acid balance).

d. Promising selections will be propagated onto either M.9 or G.41 rootstock and placed in replicated Phase 2 trials (five trees/selection) at three diverse sites in central Washington. Data will be collected on fruit quality, productivity and tree health. DNA samples will be collected from all Phase 2 selections for screening with predictive markers to provide DNA-based information on genetic potential to enhance subsequent selection decisions.

e. Outstanding selections will be propagated as "elites" for Phase 3 trialing with an aim of approximately 75 trees in up to four diverse grower sites in central Washington. A staggered start (planting initially in one or two sites) is also a possibility. Phase 3 is conducted in cooperation with the WTFRC, managed by Tom Auvil. Harvested fruit will be subjected to a range of storage treatments managed by Ines Hanrahan. Certified, virus tested material will be produced for Phase 3 elite selections and distributed to nurseries.

f. Outstanding selections will be proposed for commercialization, patent data will be collected and submitted and the nursery mother trees will be confirmed as true-to-type by genetic fingerprinting.

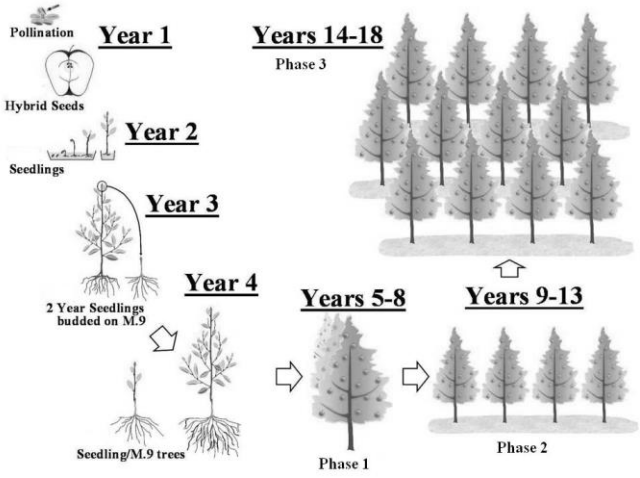

Figure 1: Breeding program schema showing the progression of selections through the program.

Objective 2 - Sensory/instrumental evaluation:

a. Using the labeling system initiated in the Phase 1 orchard, fruit samples collected will be bar-code labeled to correspond to the source tree, the pick number and the harvest date. These labels will then remain with the fruit as it is evaluated in the fruit laboratory thus minimizing mixing of samples and data-entry errors.
b. Ten fruit samples will be divided into five fruit for instrumental evaluation and five for sensory evaluation. The fruit for instrumental evaluation will be tested for maturity using the Cornell starch chart. Texture, size and weight will be recorded with the Mohr® DigiTest and the remaining fruit will be juiced for soluble solids concentration and titratable acidity measurements.
c. Sensory analysis will usually be performed by a team of four, producing a detailed breakdown of appearance and eating quality attributes. The breeding team was trained in sensory profiling by the Ross lab in Pullman in 2010.
d. First-season seedling fruit will be stored in regular atmosphere storage at the TFREC at 34^{o}F for two months prior to evaluation. If a sample achieves the appropriate overall rating, the same seedling tree will be harvested at more than one pick date the following year (subject to fruit availability). Second- and third- season samples will be evaluated instrumentally at harvest as well as after two months storage when a sensory evaluation will also be completed. If sufficient fruit is available, a four month stored sample will also be evaluated.
e. Fruit evaluation will continue as selections move forward through Phases 2 and 3, with samples taken at up to four pick dates and evaluated at harvest and, after two and four months of regular storage. Larger volumes of fruit from Phase 3 will be drenched with a post-harvest fungicide, tested with and without a 1-MCP treatment and stored in regular and controlled-atmosphere storage using the Stemilt facility. Fruit out of storage will be tested in the WTFRC lab as well as the TFREC lab.
f. Fruit from promising selections from Phases 2 and 3 will be sent to the Ross lab in Pullman for trained sensory panel evaluation and consumer panel evaluation when applicable.

Objective 3 - DNA-information development and obtainment:

a. Marker-locus-trait associations will be developed and adapted to the WABP for important fruit quality traits including firmness, crispness, juiciness, acidity, soluble solid concentration and fruit color principally from data collected as part of the SCRI-funded RosBREED project. Genetic tests will be further validated in the WABP using the multi-year data collected to date on advanced and elite selections. Tree traits such as precocity, vigor, disease and insect resistance and cross compatibility will also be targeted.
b. Full marker characterization will be performed on all elite selections to increase decision confidence in advancing selections to commercial release.

Results & Discussion:

Objective 1 - Breeding:

2013 was a challenging crossing season due to weather issues; however, the seeds held in reserve from the 2012 season will amply supplement this years' production. Seedlings from approximately 12,000 seeds from 2012 crosses were grown in the greenhouse.

The new seedling "96-plant" format system was successfully used again for seedlings tested with DNA markers. Approximately 6,600 seedlings were screened with DNA markers for fruit quality; just

over 2,900 were culled leaving the remaining 3,700 to be transplanted to Willow Drive nursery along with another 2,500 seedlings that were not marker-screened.

There was considerable hail damage in the Phase 1 and Phase 2 blocks at Sunrise; however, there was still sufficient fruit to enable Phase 1 selection. Additional harvesting from Phase 1 by the two graduate students (Julia Harshman and Paul Sandefur) as part of the aligned project "Support systems to deliver elite new cultivars for extended storage" (Peace, Hardner and Evans) has maximized the utility of those blocks.

Forty-three Phase 2 & 3 selections were bench-grafted and grown in pots in the greenhouse. No differences were seen between the efficacy of freeze-dried and fresh-produced fire blight inocula; however, considerable differences were seen in the levels of resistance among the selections. Eight Phase 2 selections appeared to be as highly resistant as 'Red Delicious'. These tests will be repeated in 2014 to confirm this season's data.

WSU 17 and 36 were rejected from Phase 3 due to fatal flaws (fruit splitting and tree health, respectively). The first fruit were harvested from the latest P3 selection (WSU 46), two new selections were propagated (WSU 64 and 65) and preparations are underway to top-work the rejected WSU 17 trees with a further new P3 selection (WSU 68) at the Quincy site. Wood of WSU 64, 65 and 68 was all sent to the Clean Plant Center, Prosser in December to initiate virus testing.

Long-term storage of WA 2 and WA 38 continued to show promise with no fatal flaws. Fruit from both received positive feedback when tasted at the Sunrise field day in August. In fact, fruit of WA 38 from 2012 was also favorably received by attendees at the WSU3i event in Seattle in late September!

The patent for WA 38 was approved in September and more CVT material was distributed to Washington nurseries. All CVT trees of WA 38 currently planted in nursery mother tree blocks tested as true-to-type when examined with several markers in the Peace lab.

A release strategy for WA 38 was finalized by Jim Moyer (WSU ARC Director) with advice from the Cultivar Licensing Committee. This strategy was described in the Good Fruit Grower, at the NNII December meeting and also at the Hort Show.

Objective 2 - Sensory/instrumental evaluation:

The fruit evaluation protocol developed in 2012 was continued in 2013. Subjecting the fruit to a week at room temperature after cold storage helped to identify selections with the most potential for market acceptance and we consider this to be a better reflection of the final consumer experience. Julia Harshman (graduate student) completed a small experiment to using Phase 2 fruit to test the potential of the DA meter (T.R. Turoni, Italy) for determining harvest maturity. The results look promising; a pre-proposal was submitted to the WTFRC to expand this testing into a second season looking at a wider range of fruit.

Fruit of WA 38 and 'Honeycrisp' (sourced by Ines Hanrahan) are being sampled by consumers, at three different time points through the storage season (October, December and March) in Spokane, for a direct determination of consumer preference.

Objective 3 - DNA-informed selection development:

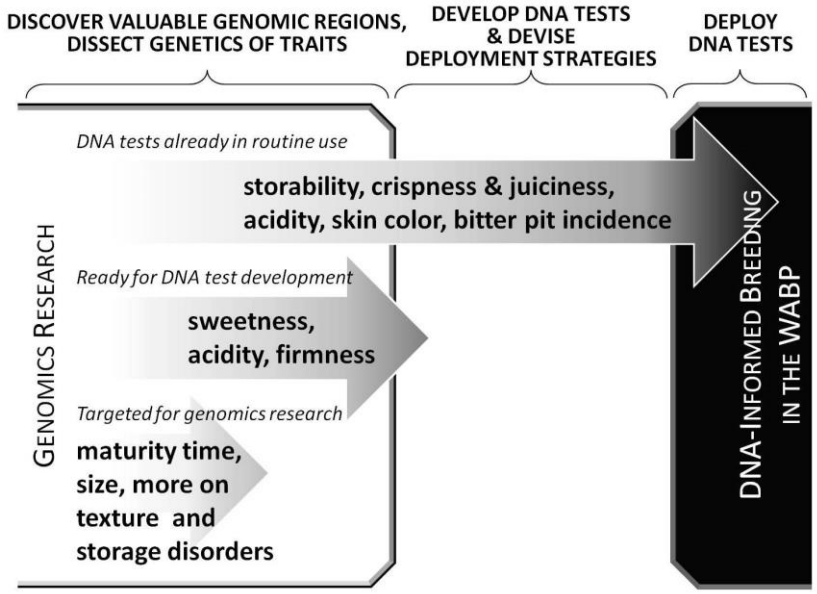

Figure 2: Discovery, development and deployment of DNA tests in the WABP

DNA-informed breeding was successfully implemented again in this program in 2013. Several applications enhanced breeding operations and decisions. The first application involved running DNA tests for most of the new seedlings to cull those seedlings predicted to have inferior genetics, thereby saving on downstream resources. DNA tests were used for "ACS", "Ma-indel", and "Rf-SSR." ACS is one of the original two DNA tests used routinely for seedling testing in this program since 2010, to help identify long-storers. The need for testing ACS on seedlings has reduced in recent years as crosses made have also used ACS information to help avoid seedlings that generate inferior ACS genotypes. The need for the other long-storage DNA test, ACO, was avoided in 2013 because of DNA-informed crossing choices made in 2012. Ma-indel is an improved DNA test developed in 2013. Ma-indel replaces a previous test for the *Ma* locus (influencing acidity, crispness, and as recently discovered, bitter pit incidence) that gave some technical trouble during 2013 seedling testing operations. Ma-indel has proven as informative as the previous markers and more technically reliable. This experience in 2013 emphasized the need to have contingencies for each DNA test, and we have since worked on developing such contingencies. Rf-SSR was a DNA test first developed by the Peace lab in 2012 which has the chief ability of distinguishing between no-cover fruit skin types (like Golden Delicious and Granny Smith) and highly striped or blushed types (like WA 38, Red Delicious, Fuji, Jonathan, etc.).

Other 2013 applications of DNA-informed breeding were in evaluation of breeding value for current and prospective parents, in evaluation of genetic potential for new P2 selections, and in identity confirmation for the WA 38 nursery mother tree blocks. Trait-predictive DNA information was obtained for all prospective parents using seven DNA tests. The DNA tests used for parents included those mentioned above for use on seedlings and several others for firmness, disease resistance, and storage attributes. New P2 selections were also evaluated for the same battery of DNA tests. The information gained on these selections will be used to confirm performance of mother trees and their replicates in P2 trials and will factor into advancement decisions toward commercial release. For WA 38 identity confirmation, leaf samples were collected from all mother trees of WA 38 once planted in the nursery mother tree blocks. All trees tested as true to type using a combination of several informative SSR markers.

In upstream genomics research and DNA test development efforts, some promising new discoveries were made. Dr. Evans's graduate student Yingzhu Guan, whose PhD focused on the genetics of apple sweetness as part of the RosBREED project, identified a very promising genomic region influencing sweetness, the "*G1Fru*" locus. This region was found to have particularly strong effects on fruit fructose levels. Dr. Peace's graduate student Sujeet Verma, whose PhD focused on the genetics of apple acidity, crispness, and juiciness as part of the RosBREED project, further characterized the *Ma* locus and also determined the contribution to apple fruit acidity made by a second genomic region, the "*G8A*" locus. Both the *G1Fru* and *G8A* loci are now being targeted for new DNA test development. Analysis of the RosBREED dataset has resulted in several further promising discoveries, including two genomics regions influencing bitter pit incidence (one being the *Ma* locus). We have since developed two new bitter pit DNA tests.

WABP Publicity - posters, presentations and fruit samples

Fruit was available for tasting at the Sunrise field day (8.07.13). A field day at the Quincy Phase 3 site was open to the public on September 18th. An organic sample of WA 38 was available for tasting as part of an event at the Tilth Producers Conference, Yakima, November 8th.

Talks and posters:

- March 2013 – Dr. Evans and Dr. Barritt published an article in the Good Fruit Grower "Consumer expectations of apple quality: How WA2 and WA 38 measure up?"
- April 2013 – Dr. Evans presented two talks during an invited visit to Chile, "Apple Breeding Program in WSU - Evaluation of new cultivars" at an industry meeting in Santiago and at the INIA apple breeding facility in Chillan.
- June 2013 – Dr. Evans presented the "WSU apple breeding program" following an invitation to meet the international AIGN nursery group, Yakima.
- Examples from the WABP of marker-assisted breeding development and application were used in talks during 2013 at: Plant & Animal Genome Conference, San Diego, CA (Jan), RosBREED annual Advisory Panel meeting, San Diego, CA (Jan), RosBREED 4th annual meeting, East Lansing, MI (Jun), WSU Graduate Student Tour, Wenatchee (Jun), and the American Society for Horticultural Science annual conference, Palm Desert, CA (Aug), as well as in several guest lectures at WSU throughout the year.
- Posters on marker-assisted breeding in the WABP were presented at: the American Society for Horticultural Science annual conference, Palm Desert, CA (Aug, 2 posters).
- March 2013 – Dr. Evans and her team presented a 'hands-on' display about the WSU apple breeding program to middle schoolers and the general public during the UW Gear Up Science and Engineering Festival in Yakima.
- September 2013 – Dr. Evans presented a 'hands-on' display about the WABP at the WSU3i event in Seattle which resulted in a feature on King5 news highlighting WA 38.
- October 2013 – Dr. Evans and Julia Harshman (grad student) presented the WABP to the Post-Harvest class at Wenatchee Valley College. The class also toured the labs and experienced sensory analysis of some selections.
- December 2013 – the WABP presented its usual array of Phase 2 fruit selections for tasting at the WA Hort Show as well as having samples of both WA 2 and WA 38 available.

CONTINUING PROJECT REPORT **YEAR**: 2013
WTFRC Project Number:

Project Title: Apple rootstock and scion evaluation

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Email: auvil@treefruitresearch.com
Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee, WA 98801

WTFRC Staff cooperators: Ines Hanrahan, Felipe Castillo, Tory Schmidt, Jim McFerson,

Collaborators: Dr. Kate Evans, WSU-TFREC, Wenatchee,
Dr. Gennaro Fazio, USDA-ARS, Geneva, New York

Cooperators: Dave Allan, Ron Wilcox, Dale Goldy, Hans Groenke

Total Project Request: **Year 1:** 54,100 **Year 2:** 32,600 **Year 3:** 54,800

WTFRC Collaborative expenses:

Item	(2013)	(2014)	(2015)
Salaries[2,3]	11,500	11,500	15,500
Benefits[2,3]	3,700	3,700	6,200
Crew Wages[3]	20,000	5,000	11,000
Crew Benefits[3]	4,500	1,000	2,200
Stemilt RCA room	8,400	8,400	8,400
Shipping			
Supplies			
Travel[1]	3,000	3,000	8,500
Miscellaneous			
Total	53,100	32,600	51,800

Footnotes:
[1]Fuel and maintenance
[2]Salaries and benefits for Auvil, Hanrahan, Schmidt, and Castillo apportioned to this project.
[3]Harvest, storage and fruit quality lab labor for Phase 3 apple scion

OBJECTIVES:

1. Evaluate apple rootstocks, particularly disease resistant rootstocks, in commercial settings in Washington State.
2. Integrate the processes of evaluation and industry implementation of new rootstocks and scions.
3. Refine protocols and improve efficiency for P3 scion evaluation program.

Scion evaluation activities and findings:

- 40 additional trees at Quincy Phase 3 (P3) site and 43 trees at Prosser P3 site were grafted to WA 38 onto discontinued P3 genotypes for a total of 223 and 209 respectively. A bin of fruit was picked in from the 2012 grafts in Quincy. Color was lacking, no bitter pit or lenticel issues were apparent.
- No MCP treatments were done on fruit from the 2013 crop.
- Hanrahan, Evans and Ross conducted WA 38 and Honeycrisp consumer preferences in Spokane in November and December 2013.
- WSU 36 has been discontinued.
- WSU 17 has been discontinued with the Quincy trees to be grafted to WSU 68, an advancing Phase 2 (P2) genotype.
- Two additional genotypes from P2 will have trees available to plant in 2015 in Quincy. This is a shift in tactics to do initial storage trials from one site in Quincy prior to extending the trial work to other sites. Internal Browning after 4 to 6 months storage has been the most common fatal flaw found in WA 5, WSU 19, WSU 49, WSU 36, WSU 17, WSU 7 and WSU 48.
- BPAC advised no additional storage samples be evaluated on WA 2.
- Held a field day mid September in Quincy and two post harvest report meetings in August.
- WA 38 grafts in Prosser and Quincy produced fruit on 2nd leaf trees that had suitable quality for consumer testing after 2 months of RA storage.
- WSU 46 fruit on second leaf trees were very attractive to birds. Stop Drop will be applied in 2014 to minimize fruit drop.

Rootstock findings and activities:

- G.41, G.890, G.935, G.30 G.210, G.214, G.935 are all replant disorder tolerant and fire blight resistant.
    - G.30 is difficult to propagate and has limited availability.
    - G.935 and G.30 are not woolly aphid resistant.
    - G.210 has performed very well in Wapato replant trial with Gala scion. The unfumigated plots equal to the fumigated plots. It has been released for commercial production. Cornell data indicates this genotype is M.106 in canopy size in eastern trials, the trees are G.935 or M.9 Pajam 2 size in Wapato. **Note:** Only one trial site and one scion has been evaluated in Washington State.
    - G.41 encountered broken unions on budded trees in the nursery in 2012 and 2013.
        - As with many new product introductions, occasional production problems may arise that may preclude full delivery of orders. Production problems were also encountered when M.9 rootstock was first grown and budded in large quantities in the early 1990's.
- Yields of the Geneva replant tolerant genotypes continue to increase compared to static yields of control /commercial standards in replant sites.
- Precocity of the more vigorous Geneva genotypes (G.30, G.210 and G.890) remain high even as the production canopy matures. The crop density of these genotypes are significantly better than the Malling stocks they replace (M.26, M.7 and M.106)

- Production of Geneva rootstocks will increase significantly in each of the next few seasons, with increased volume of finished tree delivery. Tissue culture grown liners and sleeping eyes are now available direct from licensed labs to growers and finished tree nurseries.
- Fumigation in small blocks or rocky soils has become difficult. Replant tolerant, especially the more vigorous and precocious rootstocks may provide a solution to these replant sites. Also, vigorous scions such as WSU 38 may also assist mitigating replant disorders' negative effect on canopy development.

Table 1: Summary of 10 years of rootstock trials by WTFRC

	04 Gala Wapato	**04 Gala Chelan**	**'04 HC Naches**	**03 HC Frenchmn**	**03 HC Chelan**	**06 Fuji Vantage**	**06 Gala Wapato**	**'06 Fuji Brewster**	**08 HC Royal**
Bud 9	Replant	Replant	Replant	Root	Root		Replant		rootstock
3041 = **G.41**	Replant	Replant		Root	Root	PnP	Replant		rootstock
4214 = **G.214**	Replant	Replant	Replant	Root	Root	PnP	Replant	Replant	rootstock
5935 =**G.935**	Replant	Replant	Replant	Root	Root	PnP	Replant	Replant	rootstock
G.11	Replant	Replant		Root	Root	PnP	Root	Replant	rootstock
G.16	Replant	Replant	Replant	Root	Root	PnP	Replant		rootstock
M.26	Replant	Replant	Replant	Root	Root				
Nic.29	Replant	Replant		Root	Root				rootstock
Pajam 2	Replant	Replant		Root	Root				
M.9	Replant	Replant	Replant	Root	Root				
G.65									rootstock
M.27									rootstock
4202 = **G.202**			Replant			PnP		Replant	
G.30			Replant	Root	Root	PnP	Replant		
4011						PnP		Replant	
4019						PnP			
4172						PnP			
4288						PnP			
5046				Root	Root	PnP	Replant		
5202 = **G.222**						PnP	Replant	Replant	
5257						PnP	Root	Replant	
Mark						PnP			
Sup 4						PnP			
M 9 Emla				Root	Root		Replant	Replant	
Pajam 1				Root	Root				
5757							Root		
5890 = **G.890**							Root	Replant	
5012							Replant		
6210 = **G.210**							Replant		
M.7							Replant	Replant	rootstock

Replant = Trial has seperate Fumigated and NON Fumigated units
Rootstock = Only Fumigated plots
PnP = Plant in Place benchgrafts in a well prepared and fumigated site.

Rootstock hypersensitive to virus, not recommended
Replant tolerant.
Discontinued Geneva

Geneva stocks in **Bold** font are commercially propagated

Next rootstock trials:

- A Red Delicious and Gala rootstock trial of 8 Geneva rootstocks plus 3 standards will be planted in unfumigated ground at the WTFRC orchard in Wapato in 2015. New to Washington State is G.969. Cornell rates G.969 as replant tolerant and woolly aphid resistant with a canopy volume similar to G.935 or G.210.
- Three trials with the same rootstocks will be placed with growers in 2015.

Figure 1: 2006 Wapato Gala accumulated yield in bins per acre 2007-2013

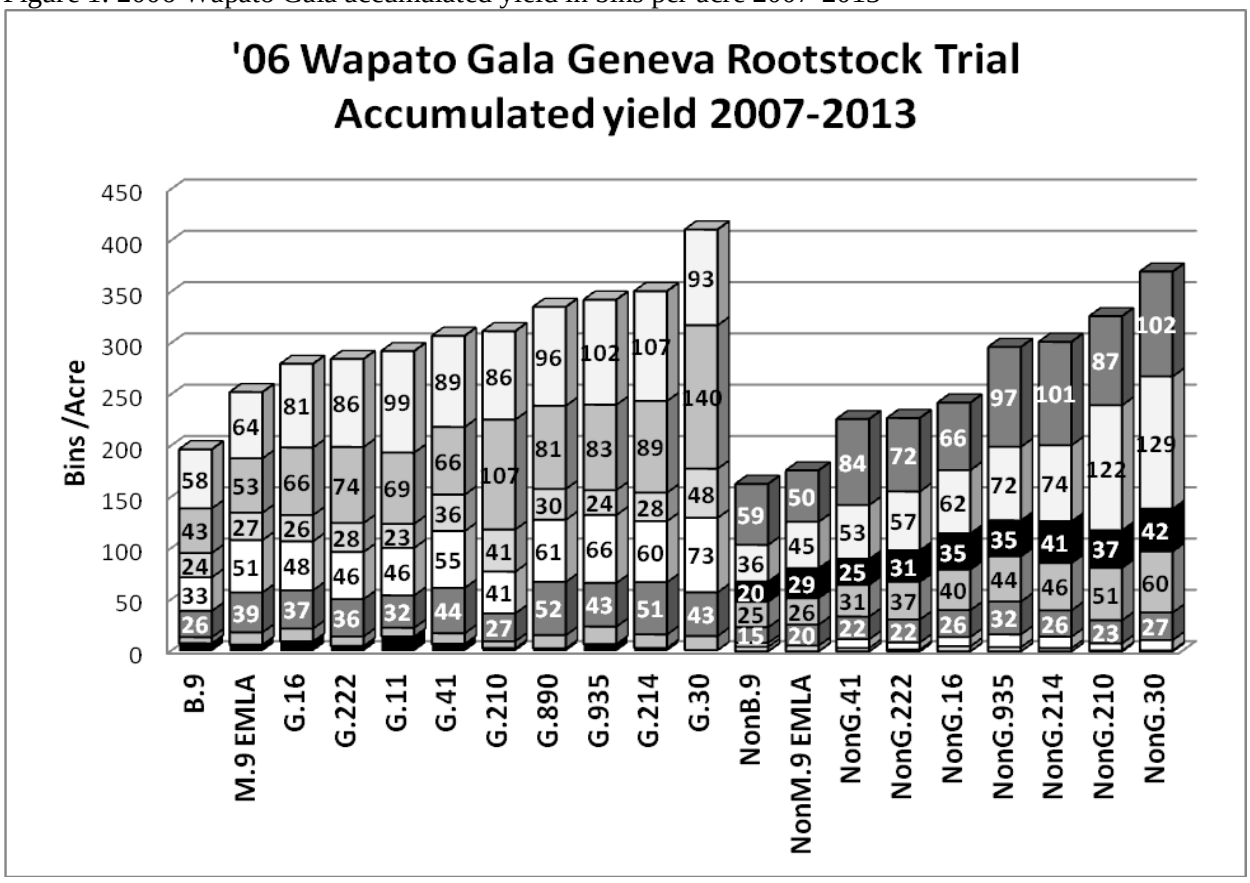


Figure 2: 2006 Wapato Gala Trunk Cross Sectional Area (cm^2) in 2013

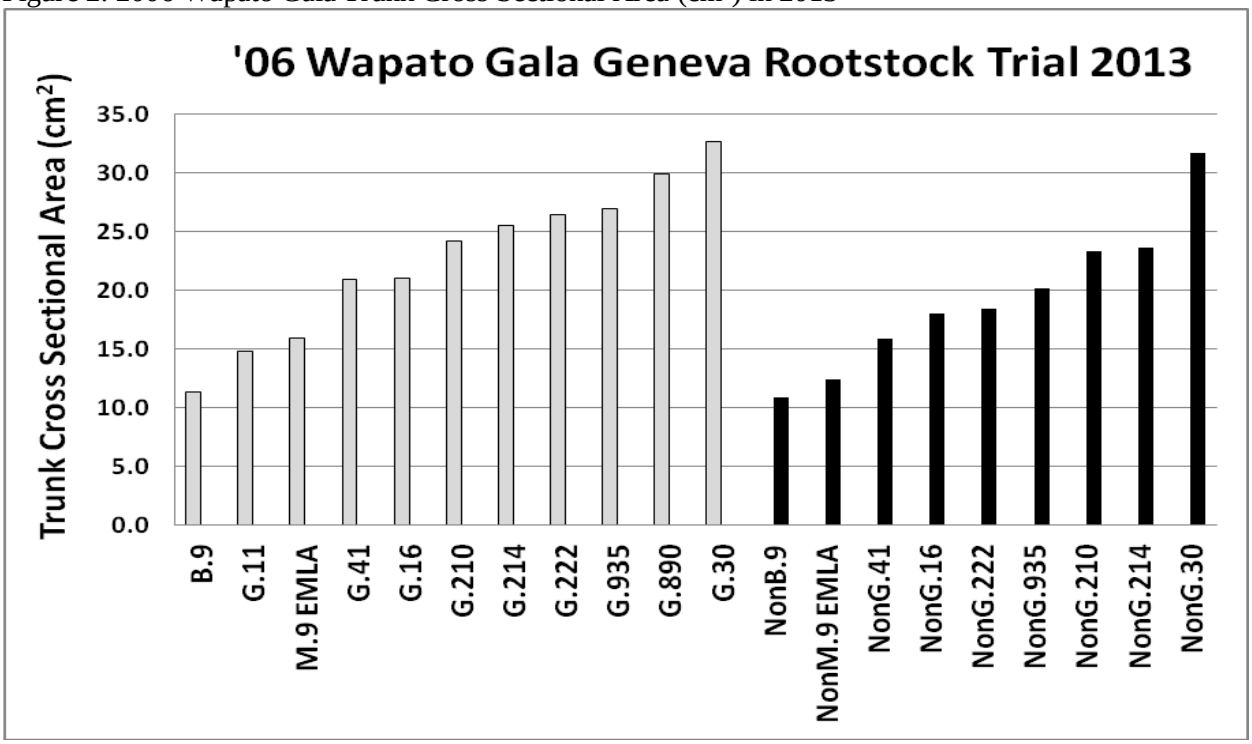


Figure 3: 2006 Vantage Fuji accumulated yield in bins per acre 2008-2013

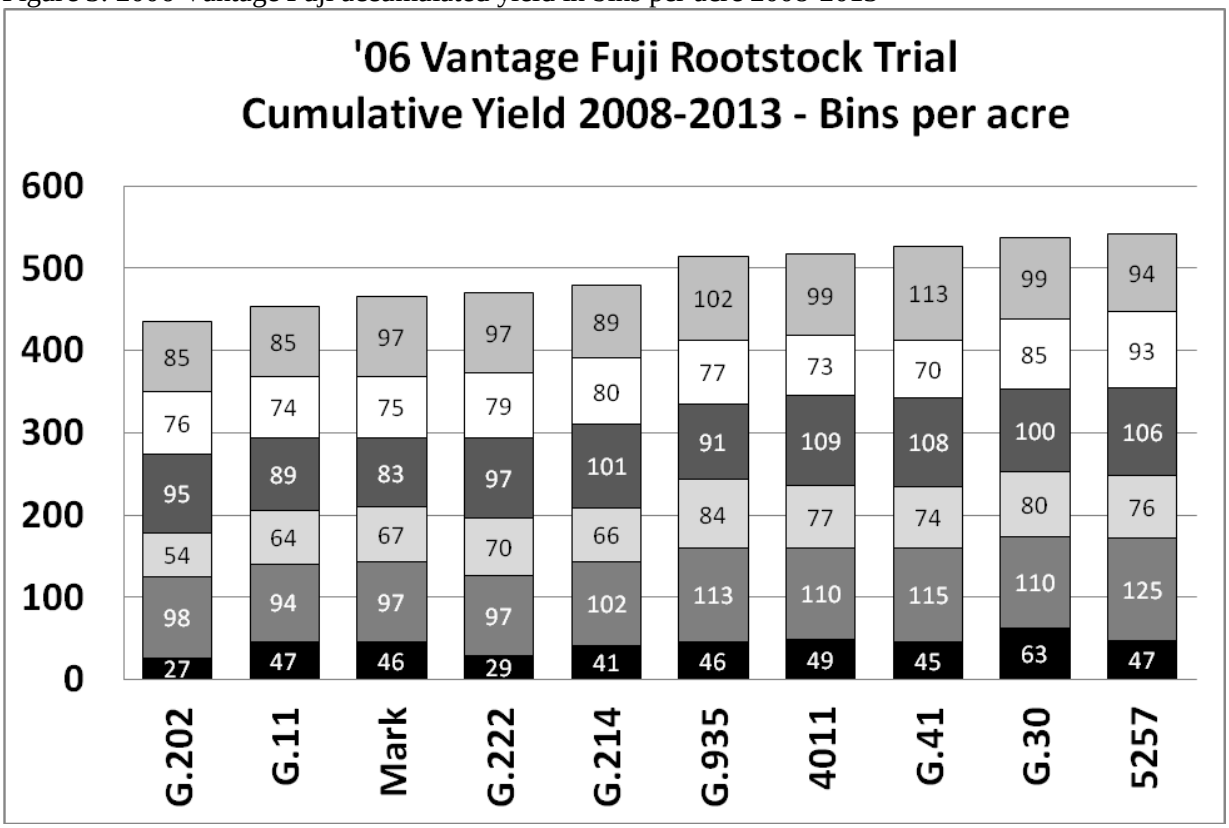
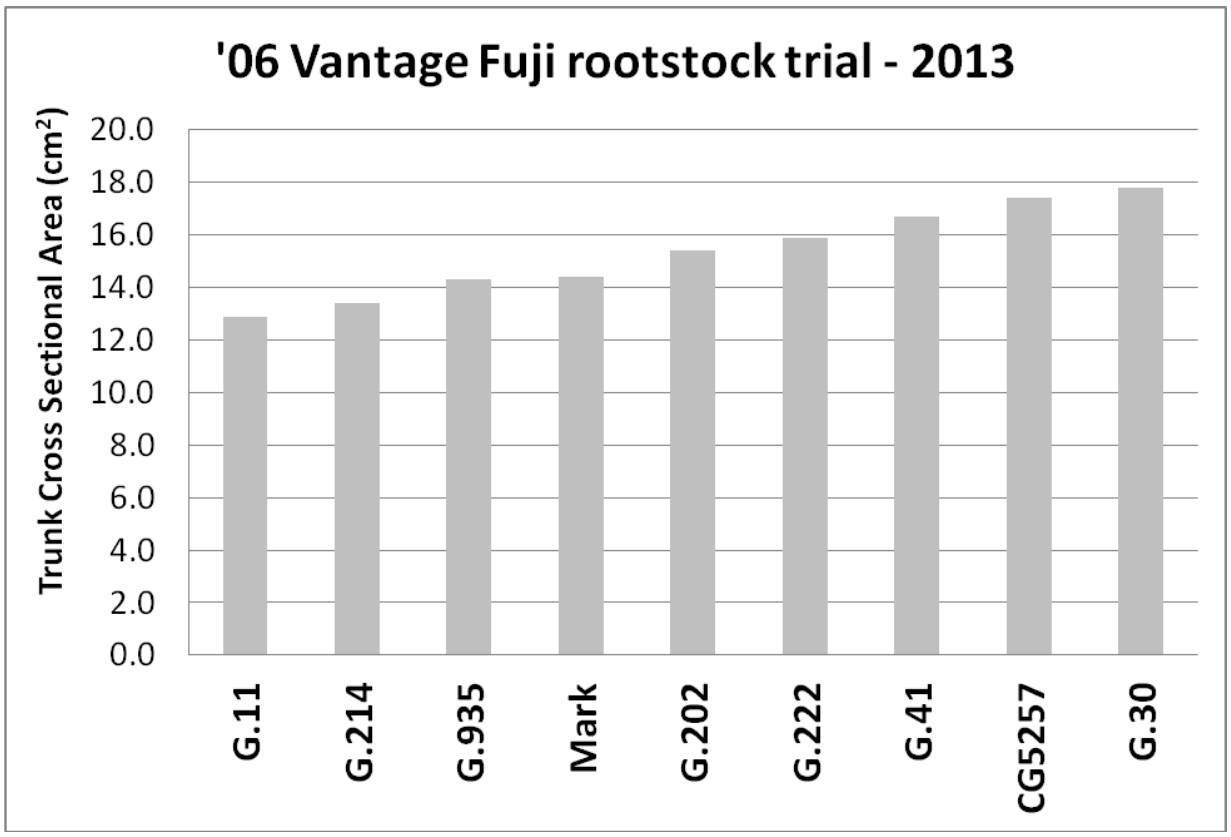


Figure 4: 2006 Vantage Fuji Trunk Cross Sectional Area (cm^2) in 2013



Trunk Cross Sectional Area (TCSA) is a relative guide to dry matter accumulation. TCSA is useful in comparing canopy development in young trees.

2003 to 2013 summary:

- There is not a 'best' rootstock. There are seven replant tolerant rootstocks, each is better than M.9 or Mark or any other rootstock that have been in the trials.

Replant Tolerance:

- The nine trials all demonstrate the genetic potential of disease and insect resistance. Six genotypes(G.41, G.214, G.210,G.935, G.890, G.30), commercially released from the Cornell-Geneva apple rootstock program demonstrate significant improvement in health, growth rate, canopy development and yields when compared to industry standard controls. Four of the replant tolerant rootstocks are woolly apple aphid resistant (G.41, G.214, G.210, G.890).
- Yields of all of the replant tolerant rootstocks were higher than M.9 or Mark.

Liner Production:

- Additional rootstock producers have been licensed to propagate Geneva rootstocks. Included are expansion of tissue culture labs licenses to sell direct to growers and finished tree nurseries.
- Tissue Culture of G.41 and the establishment of stooling beds have substantially increased the production of G.41.
- G.11 has the most liner production. It grows the smallest canopy of the commercial Geneva rootstocks, and is not as robust mitigating replant as G.41 or G.210. It is rated by Cornell as being woolly aphid resistant.
- Increasing production of G.890 and G.210 are being encouraged.

- G.935 is not woolly aphid resistant and has two similar rootstocks that are: G.214 and G.210.
- 2-3 million finished trees on Geneva rootstocks will be planted in 2014. The number for 2015 may double.

Finished Tree Production:

- Some bud unions have been broken. There is not one rootstock + scion combination that is consistently a problem. Different nurseries have different combinations with problems. Honeycrisp is a consistent scion with weak bud unions, Pajam 2 being a noticeable problem.
- Extra large trees (3/4" to 1" caliper) have more breakage medium size trees (1/2" to 5/8").
- Some combinations of rootstock and scion have synergistic response. G.41 and Fuji is vigorous non-bearing combination.

What's next:

- Nurseries are searching for efficient propagation systems for Geneva rootstocks. There are several differing tactics being deployed. Tree buyers will benefit by visiting to the nurseries and observing production practices and results. The range of products (liners, sleeping eyes, bench grafts, one year nursery trees, two year bench graft, two year budded trees, etc.) has never been greater.
- As more sites, scions and Geneva rootstocks are planted, the better and best combinations will be identified. Soil types and scion traits will provide selection preference for rootstocks. It is unlikely that 'one' will be the best in all situations.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-12-105A

Project Title: Implementation and evaluation of apple pollen tube growth models

PI:	Dr. Keith S. Yoder	**Co-PI (2):**	Dr. Greg M. Peck
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Co-PI:	Dr. Gerrit Hoogenboom	**Co-PI**:	Dr. Melba Salazar
Organization:	Washington State University	**Organization**:	Washington State University
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City:	Prosser	**City:**	Prosser
State/Zip:	WA 99350-8694	**State/Zip:**	WA 99350-8694

Cooperators: Leon Combs, Research Specialist, Va. Tech AHS-AREC; Winchester, VA; e-mail: lecombs@vt.edu
Sean Hill, Appl. Systems Analyst/Dev., AgWeatherNet, Washington State Univ., Prosser, WA; e-mail: sehill@wsu.edu
Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA

Total Project Request: Year 1: $113,983 **Year 2:** $104,343 **Year 3: $107,688**

Other funding sources

Indirect support through the existing infrastructure of AgWeatherNet and its network of 137 weather stations.

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries	10,000	10,000	10,000
Benefits	2500	2500	2500
Wages	6000	6000	6000
Benefits	1500	1500	1500
RCA Room Rental			
Shipping			
Supplies	500	500	500
Travel	500	500	500
Plot Fees			
Miscellaneous	10,000	10,000	
Total	$21,000	$21,000	**$21,000**

Budget 1

Organization Name: Virginia Polytechnic Institute and State University (Va. Tech)
Contract Administrator: Kevin Kuether
Telephone: 540-231-7521 **Email address:** tkuether@vt.edu

Item	2012	2013	2014
Salaries*	35,762	37,192	38,680
Benefits	10,282	10,693	11,121
Equipment (laptop-field work)	1,500		
Supplies (lab &field)	1,500	1,500	1,500
Travel (to Wash. St. orchards)	5,000	6,000	6,500
Contractual services & repairs	1,250	1,250	1,250
Total	$55,294	**$**56,635	**$59,051**

***Note:** Salary for Research Specialist Leon Combs.
Virginia Polytechnic Institute and State University (Va. Tech)

Budget 2

Organization Name: ARC-WSU **Contract Administrator:** Carrie Johnson
Telephone: 509-335-4564 **Email address:** carriej@wsu.edu

Item	2012	2013	2014
Salaries	24,699	16,674	17,341
Benefits	9,490	6,534	6,796
Wages			
Benefits			
Equipment			
Supplies	1,000	1,000	1,000
Travel	2,500	2,500	2,500
Miscellaneous			
Total	$37,689	$26,708	**$27,637**

Footnotes: Partial salary support for Research Associate (Dr. Melba Salazar) and for Application Development Programmer (Mr. Sean Hill).

I. Objectives

Our overall goal for 2012-14 is to collaborate with Washington State University and Washington Tree Fruit Research Commission to validate and implement a computer-generated pollen tube growth model for the major commercial apple cultivars on the AgWeatherNet website (www.weather.wsu.edu).

Specific objectives accomplished for 2012:

- Evaluated model at 60 test sites in Washington State.
- Developed cultivar-specific equations for pollen tube growth and interfaced these models with real-time and forecasted weather data on the AgWeatherNet website.
- The website interface allows for generation of site- and cultivar-specific information.
- By using AgWeatherNet weather forecast data, pollen tube growth can be projected 48-hour into the future allowing growers to schedule bloom thinning sprays in advance.
- Model validation included sampling flowers from the test sites to determine the percent of flowers that have been fertilized by using fluorescent microscopy to visualize the pollen tubes.

Specific objectives accomplished for 2013:

- Expanded model test sites in Washington State to more than 150 individual orchard blocks.
- Continued to validate and refine modeling programs cooperatively with WSU-AgWeatherNet (Gerrit Hoogenboom, Melba Salazar, and Sean Hill, WSU).
- Provided two training sessions for beta-testers. Emphasis was on methods for determining when to"start the model clock" by determining desirable amount of king bloom open. (Leon Combs, Virginia Tech and Tory Schmidt, WTFRC)
- Continued beta field testing of models for Gala, Fuji, Golden Delicious and Cripps Pink and began limited field beta-testing of Honeycrisp model. (Leon Combs, Virginia Tech)
- Added plantings of Brookfield Gala and September Wonder Fuji at Virginia Tech for future model development. (Leon Combs, Virginia Tech)
- Started development of a Granny Smith pollen tube growth model. (Leon Combs, Virginia Tech)
- Conducted web-based survey of beta-testers for evaluation of models being tested. (Leon Combs, Greg Peck and Keith Yoder, Virginia Tech)

Specific objectives for 2014:

- Continue developing the Granny Smith pollen tube growth model.
- Provide open access to Gala, Fuji, Golden Delicious and Cripps Pink models through the AgWeatherNet website to all Washington growers.
- Develop training programs for using web-based modeling program.
- Complete model parameters for Granny Smith and Red Delicious.

- Continue beta field testing of Honeycrisp model.
- Begin beta testing of Granny Smith and Red Delicious models in Washington orchards.

SIGNIFICANT FINDINGS

Data shown in the following graphics are results from Washington beta-test sites for the 2013 growing season.

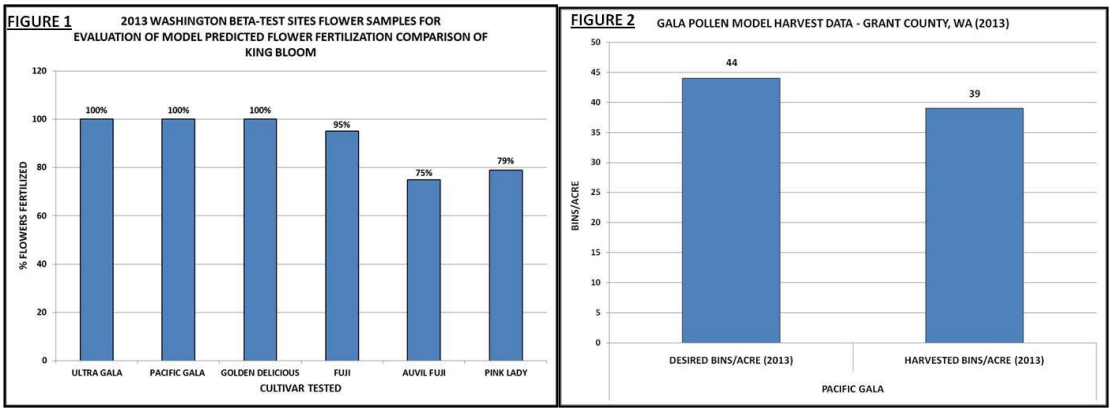
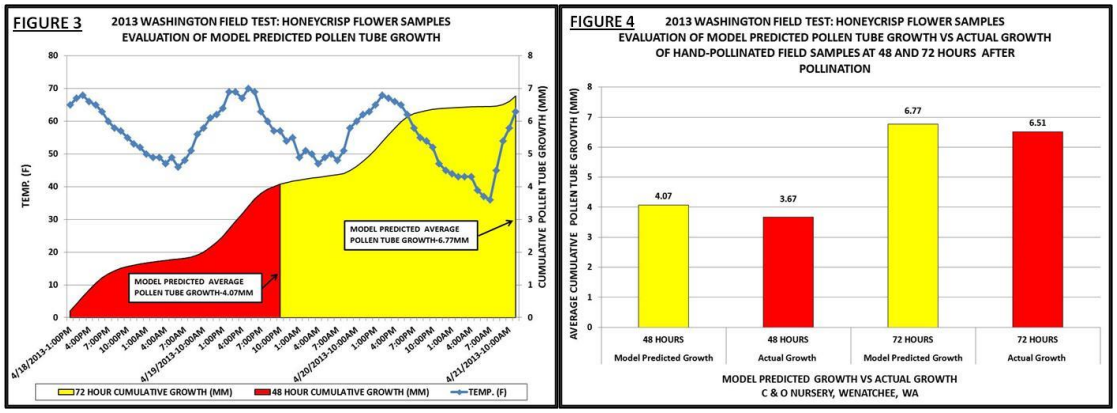


Figure 1: Flower samples taken in field and evaluated under microscope show the percentage of flowers that were fertilized relative to the model predictions. **Figure 2**: Actual yields versus grower desired yields for Pacific Gala at one of the beta test sites where the pollen tube growth model was beta tested.



Figures 3 and 4: Field beta-testing of the pollen tube growth model for Honeycrisp began in 2013. Validation of the model at various stages of predicted growth versus actual growth is shown in Figure 3. Flower samples were hand-pollinated at bloom and covered with insect netting to prevent cross pollinationfrom other sources. Flower samples were taken at 48 and 72 hours, fixed in sodium sulfite solution, and then sent to Va. Tech AHS-AREC in Winchester, VA for evaluation of pollen tube growth after pollination by fluorescent microscopy in Figure 4.

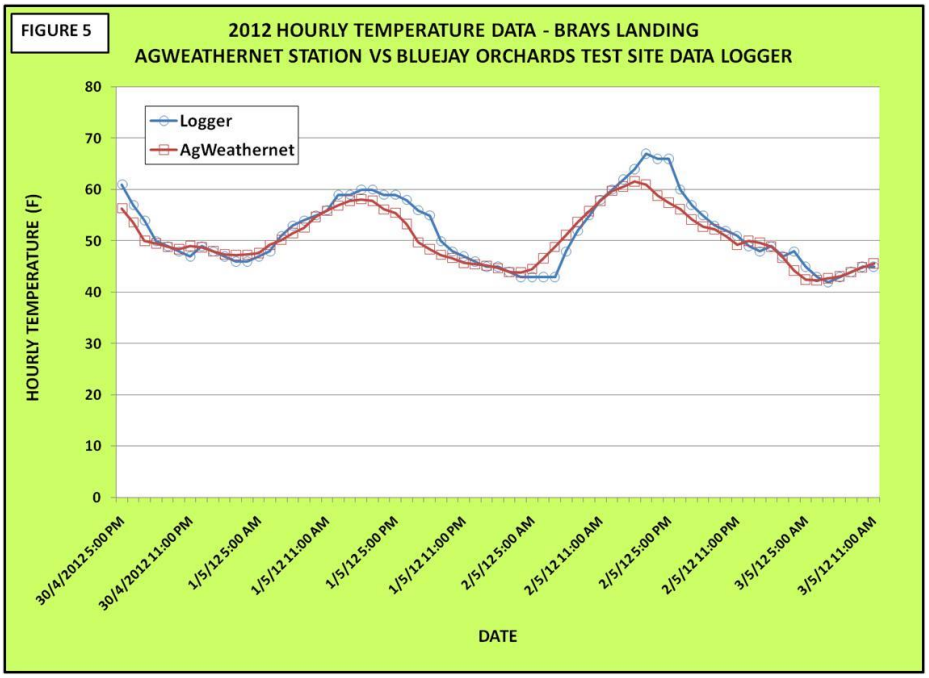


Figure 5. In 2012 and 2013, field tests in selected Washington orchards compared weather station temperature data used by the AgWeatherNet interface to actual in-orchard temperature data-loggers. The above chart compares average hourly temperatures from 30 Apr 2012 to 3 May 2012 between the Bray's Landing AgWeatherNet weather station and a data logger placed at the nearby Bluejay Orchard.

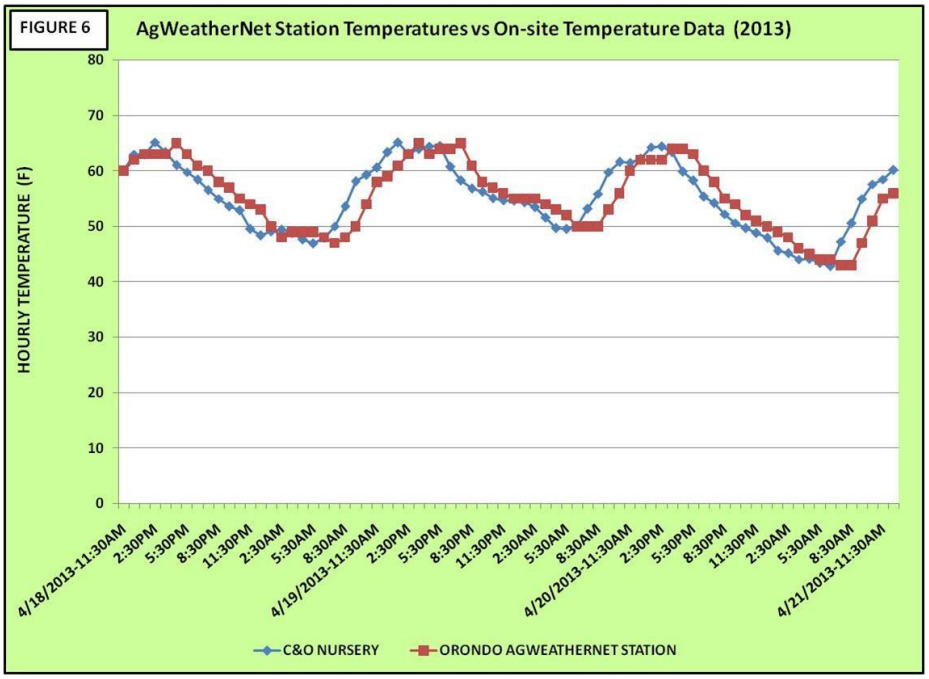


Figure 6. Temperature data recorded from 18 Apr 2013 to 21 Apr 2013 comparing the Orondo AgWeatherNet data to a data logger placed at a nearby C&O Nursery orchard block. Temperature and therefore projected pollen tube growth differences among locations may reflect differences in elevations or distance from AgWeatherNet station for the test sites. At this point the temperature

variations do not appear significant enough to warrant the addition of correction factors on the AgWeatherNet website.

Results and Discussion

As has been stated throughout the project, properly timed bloom thinning optimizes fruit yields and quality and reduces the biennial bearing tendency of apple trees. Understanding the progression of pollen tube growth after pollination is critical in applying bloom thinners at the proper time. In addition, usage of the pollen tube growth model gives the grower a longer amount of time for scheduling bloom thinning sprays. Equipment and labor can be used more efficiently when the grower and/or farm manager has advanced insight regarding application timing.

In 2013, beta-testers used the pollen tube growth models on nearly 2000 acres. Based on a survey conducted in Fall 2013 to evaluate the benefits of using the pollen models, beta-testers stated that they were "very satisfied" with the model (rated 5-points on a 6-point scale). The respondents also stated that they have a "moderate" to "great" understanding of the model and have a "very high" to "extremely high" satisfaction with the AgWeatherNet interface. The following bulleted statements are a few additional comments from our beta-testers:

- A great scientific tool to manage risk and to bring the art and science together regarding chemical thinning.
- Predictive ability (through AgWeatherNet) to sequence sprays and to manage people.
- Makes you look at style / bloom quality.
- Able to communicate predicted spray timing to managers.
- Takes out difference of opinion of what 30% open flowers looks like.
- Able to see different growth rates from one area to another.
- It helps you prioritize.
- If all blocks are in the computer no block is lost / gone over.
- Helps predict growth of pollen tube in bad / different weather.
- It is based upon the actual parameters of growing degrees and style length, which vary from year to year.
- It removed the guesswork out of knowing when to spray.
- It includes the temperature forecast and 'actual' temps within reason based upon real time AWN data, and give the applicator time to plan for the application.
- It is a"TOOL" and must always be thought of in that way. Bloom density, bloom health, cross pollination, frost, etc. will always have to be considered into ones decision process when using the model.

A meeting regarding the release of the pollen models to the general public was conducted in Wenatchee, WA on December 2, 2013. Attending the meeting were three beta-testers [Darin Case (Dovex Fruit Company); Harold Schell (Chelan Fruit Company); and Harold Ostenson (Tree Fruit Consulting)], Dr. Melba Salazar (WSU-AgWeatherNet, Research Associate), Sean Hill (WSU-AgWeatherNet, Application Development Programmer), Tory Schmidt (Washington Tree Fruit Research Commission), and Leon Combs (Virginia Tech, Research Specialist). After reviewing the project's history and results from multiple years of field beta-testing and controlled laboratory testing it was decided by the group to release four of the models to the general public for use starting spring, 2014. The models to be released are: Gala, Golden Delicious, Fuji, and Pink Lady. The Honeycrisp model is scheduled to be released in 2015.

The group discussed the need to have training sessions and on-line information available for educating the growers on how to use the models. It was suggested that the WSU extension department

take an active role in organizing these programs and work with Tory Schmidt, Dr. Keith Yoder, Dr. Greg Peck, and Leon Combs to develop the content of the workshops.

We would like to thank the Washington Tree Fruit Research Commission for its continued support of this project. We would particularly like to thank Tory Schmidt, whose help on the project has been essential to the project's success. Lastly, we would like to thank the beta-testers, growers, and others who are providing critical feedback on the pollen tube growth model.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-12-104

Project Title: Development of apple bloom phenology and fruit growth models

PI: Gerrit Hoogenboom
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Telephone: 509-786-9371
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Organization: WTFRC
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City/State/Zip: Wenatchee, WA 98801

Co-PI (4): Nairanjana Dasgupta
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Email: dasgupta@wsu.edu
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Address 2: Neill 103
City/State/Zip: Pullman, WA 99164

Cooperators: Karen Lewis (WSU-Extension), Felipe Castillo (WTFRC)

Total Project Request: **Year 1:** $84,100 **Year 2:** $97,600 **Year 3: $101,100**

Other funding sources

Indirect support through the existing infrastructure of AgWeatherNet and its network of 150 weather stations

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries	3,000	3,500	4,000
Benefits	1,200	1,400	1,600
Wages[1]	7,500	7,500	7,500
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel[2]	2,400	2,700	3,000
Plot Fees			
Miscellaneous			
Total	$14,100	$15,100	**$16,100**

Footnotes:
[1] Labor calculated as 2 persons at $16.00/hr working 12 hrs per week for 13 weeks during the growth season.
[2] In-state travel to research plots

Budget

Organization Name: ARC-WSU **Contract Administrator:** Carrie Johnston
Telephone: 509-335-4564 **Email address:** carriej@wsu.edu

Item	2012	2013	2014
Salaries	53,936	65,536	67,496
Benefits	12,564	13,464	14,004
Wages			
Benefits			
Equipment			
Supplies	1,000	1,000	1,000
Travel	2,500	2,500	2,500
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	$70,000	$82,500	**$85,000**

Footnotes: The budget that is requested through this proposal includes partial support for an Assistant Research Professor (Dr. Melba Salazar) who will be responsible for the overall evaluation and implementation of the various growing degree models that are applicable for conditions in the Pacific Northwest and partial support for an Application Programmer (Sean Hill) for integration of the model on the web portal of AgWeatherNet (www.weather.wsu.edu). We also have budgeted for a Graduate Student (to be hired) who will be responsible for the development of a physiological fruit growth model. The proposal includes a request for a computer for the graduate student during the first year of the project. Additional budget items include operating expenses for computer software and related costs and travel to participate in field data collection. Finally, this proposal includes support for Professor Dasgupta in the Department of Statistics to complete her statistical model development and evaluation (objective 2).

OBJECTIVES

1. Continue data collection on bloom phenology and fruit growth for selected sites and cultivars to enhance model accuracy and vigor. (Schmidt in collaboration with Castillo)
2. Continue refinement of statistical models for bloom phenology and fruit growth. (Dasgupta)
3. Develop physiological-based models for bloom phenology and fruit growth of apples. (Hoogenboom, Salazar)
4. Implement and evaluate models as decision support aids on the AgWeatherNet portal using industry beta-testers. (Hoogenboom, Salazar and Dasgupta in collaboration with Lewis)
5. Improve model/portal user interface based on feedback from beta-testers and other stakeholders. (Hoogenboom, Salazar in collaboration with Lewis)

Timetable for Project

Activities	**2012-2013**				**2013-2014**				**2014-2015**			
1. Experimental data collection		x	x			x	x			x	x	
2. Statistical model development and evaluation	x	x	x	x	x	x	x	x	x	x		
3. Physiological model development and evaluation	x	x	x	x	x	x	x	x	x	x	x	x
4. Web-based user interface development						x	x	x				
5. Web-based user interface evaluation by WSU Extension and stakeholders; final implementation								x	x	x	x	x

SIGNIFICANT FINDINGS

- Differences among locations and among cultivars for the different phenological stages where found in terms of Growing Degree Days.
- Fruit growth was modeled using Growing Degree Days at each location. Differences among cultivars were found for each location.
- Red Delicious and Gala had significantly larger diameters than Cripps Pink. The final fruit size varied from year to year and location to location.

METHODS

1. Data collection

For the development of robust models, high quality data are needed incorporating a diverse range of environments and annual weather conditions. WTFRC staff will continue collecting bloom phenology and fruit growth data from established sites to augment data sets from the previous project.

2. Continue refinement of statistical models for bloom phenology and fruit growth

For the growth models, data have been compiled for Gala for 2010 and 2011, while for Red Delicious and Cripps Pink data have been compiled for 2010. For the bloom models, data have been compiled for 2010 and an ordinal logit model has been used to fit the data. All data for phenology, growth and temperature will be compiled for 2011. For the growth model the data for 2010 and 2011 have to be combined and new parameters have to be estimated. For the bloom model similar procedures will be followed. Following a successful development of both the statistical bloom phenology model and the statistical fruit growth model, they will be evaluated with the new data that will be collected during the 2012 and 2013 growing seasons.

3. Develop physiological models for bloom phenology and fruit growth

Although statistical models can play an important role in estimating phenology, physiological models are normally more robust as they eliminate the need for the development of a location-specific and

time-specific model. This can be partially accomplished by using physiological time as input, sometimes referred to as Growing Degree Days (GDD) or growing-degree hours. The latter is a more sophisticated model, but also requires additional weather data observations. In order to identify an appropriate model to estimate the GDD requirements for different phenological stages for apple, a comparison between three of the most traditionally methods for GDD accumulation will be used. This includes averaging, standard GDDs and the triangle method. Thermal-time, based on a two-step phenological model will be tested and a simple thermal-time (TT) model based on historical observations for the key phenological stages will be developed. We are planning to use the initial set of data collected as part of the WTFRC Project Number AP-09-908 for model development. Once additional data have been collected as part of the current project, we will use the new data for model evaluation and improvement.

Temperature records from AgWeatherNet will be used for estimating the requirements for the different phenological stages of the most important apple cultivars. Once the models have been applied for the calculation of heat requirements, an evaluation to determine the most accurate model using historical observed dates under different environmental conditions will be performed. The climatic requirements for the beginning of the season, pre-bloom, bloom and the end of the season, will be obtained as a result of this evaluation.

4. Implement and evaluate models as decision support aids on the AgWeatherNet portal
In order to assist the growers for making decisions, an information delivery system and media tool will be developed using the statistical models developed under objective 2 and the physiological models developed under objective. This tool will provide, in an easy and user-friendly way, thermal time, cumulative chilling, and cumulative degree hours in real-time (current) for different environmental conditions where local weather data are available through tables and graphs. An example of a similar tool can be found on the AgWeatherNet portal (www.weather.wsu.edu). This decision support tool will provide information about the current phenological and development stages and the climatic requirements to complete the next stage. In addition, this tool will be able to provide alerts to the growers when the crop can be at risk due to the actual temperatures in excess of the threshold temperatures.

The system will be available through a link created on the AgWeatherNet web portal and other web portals where information for apples is provided, including the Decision Aid System (DAS). We will also explore the development of alternative communication systems to support apple growers through cell phones applications and instantaneous message alerts for critical weather conditions or when threshold values have been reached. Furthermore, this web site and portal will offer a link to access the automated weather station and local weather predictions through tools that are being proposed in a parallel project that has been funded by the Washington Tree Fruit Research Commission for one year.

5. Improve model/portal user interface and release for general use
We will work closely with WSU Extension and industry representatives as beta testers during the second and third year of this project. We will try to incorporate all comments to help improve the tool and decision aid to the benefit of the local apple growers. The overall goal is to develop a web portal that will provide a guideline and advisory for the growers who are monitoring their individual apple orchards in terms of weather conditions and weather predictions. That will ultimately allow for better planning to improve fruit quality, increase yield, more efficient marketing and ultimately result in an increase in net returns.

RESULTS & DISCUSSION

1. Data collection

Observations of bloom phenology were recorded in 2013 by WTFRC staff every Monday, Wednesday, and Friday in 29 blocks clustered around 10 location nodes. Current varieties include Red Delicious, Cripps Pink and Gala. WTFRC staff also collected fruit size data starting at petal fall until final harvest with a brief break during thinning. In addition, AgWeatherNet staff collected similar bloom phenology and continuous fruit size and weight data for Prosser and Finley, with the latter as a new site for 2013 due to its warmer conditions compared to the locations sampled in prior years.

2. *Continue refinement of statistical models for bloom phenology and fruit growth*

Based on the analysis conducted by the AgWeatherNet group, it is evident that there is a substantial difference among the years and the locations in terms of bloom stage for the three cultivars. We had seen this previously and tried to determine why and how these locations and years were different. We modeled bloom stage as an Ordinal variable and determined what the best predictors were for each year, each cultivar and each location. The logic was, if the same model was selected for all locations and all years, then the data could be combined and analysis done on the whole data set.

We used: Day of year, x1; gdd, x2; cumulative average air temperature, x3; cumulative maximum temperature, x4; cumulative minimum temperature, x5; cumulative relative humidity, x6; cumulative average dew point, x7; cumulative average soil temperature, x8; latitude, x9; longitude, x10; elevation, x11. To summarize, the model was: Probability of a given stage, y = function (x1-x11). To select our potential predictors we ran a step-wise selection method using Ordinal logistic regression. Cross validation was done in the following way: for each observation we predicted the most likely stage using our final model. If the most likely stage matched up with the observed stage, we scored it as correct prediction, if not it was considered incorrect.

Most models selected all 11 variables for all models except for three locations. The R^2 were quite good given that we did this over all the locations and used latitude, longitude, elevation and the weather variables as proxy for the location (Table 1). Cross validation was quite good as we went with a straight, correctly predicted or not criterion. If we were off, we were mostly off by one stage. 1. Certain locations performed better than others as far as the model fitting went. East Wenatchee and Wapato had lesser variability than the other locations. Stage 6 (first bloom), was the hardest to predict. Very little data were available for this stage and often there were data sets with no data for this stage. Also, the first green stage was often not present in the data sets.

3. Develop physiological models for bloom phenology and fruit growth

The percentage of buds for each phenological stage was determined for each sampling date, year, location and cultivar. Differences in the phenological stages distribution through time by cultivar year, and location were observed. For Cripps Pink and Gala the first stage (A) started relatively at the same time while Red Delicious started the first stage (A) ten days later. There was significant effect for year, cultivar, location and their interactions as an indication of the dependence among factors; in other words, the pattern of the stage distribution for years depends on cultivar and location. The trend of the distribution for each phenological stage was determined for each cultivar and each location. An example for Cripps Pink for each stage is presented for 2010 -2013 for Brays Landing, Chelan, Prosser and Royal City East (Fig. 1).

The dynamics of the different phenological stages were analyzed using Growing Degree Days (GDD). The duration in GDD was determined for Gala, Red Delicious and Cripps Pink for each season (2010-2013) for each phenological stage, using a base temperature of 43°F, starting January 1

and April 1, as well as using a base temperature of 32°F. An example of the total accumulation for Gala, Red Delicious and Cripps Pink for 2013 is presented for all locations using 43°F as the base temperature and starting on January 1 (Table 2). The analysis showed differences among locations and among cultivars for the different phenological stages. In order to compare the locations and to determine if they were significantly different, an F-test was performed. Brays was different of all the other locations and there were not significant differences ($P < 0.001$) in stage distribution between Chelan and Orondo, East Wenatchee was equal to Royal City East, Sunrise, Wapato, and Prosser; Konnowac Pass was equal to Prosser, Royal City East and Wapato; Prosser was equal to Wapato; Royal City East was equal to Sunrise and Wapato. The elevation of each location varied from 879 to 1580 ft. and the latitude from 46° 10' to 47° 45' approximately. The sampling in Omak was conducted only in 2010 for Red Delicious and Gala and in Naches for the same cultivars from 2010 to 2013.

Additional data were collected in Prosser and Finley for fruit growth analysis. Fruit growth curves showed differences in dry matter among cultivars and locations (Fig 2).Red Delicious had significantly higher dry matter than Gala and Cripps Pink for both locations. However, the rate of growth for Gala was higher in Prosser compared with Finley (Fig 3). The correlation between diameter, length, dry matter and fresh weight was highly significant ($p<0.05$). This illustrated that dry matter can be estimated using the diameter or fruit length. In general for all locations Red Delicious and Gala had significantly larger diameter than Cripps Pink, the final size of the fruit varied from year to year and location to location. Cultivar differences in fruit diameter reflected differences in mean fruit diameter as well as fruit growth period.

4 Implement and evaluate models as decision support aids on the AgWeatherNet portal
This activity will be initiated during the coming months based on the initial models developed under Objectives 2 and 3.

5 *Improve model/portal user interface and release for general use*
This activity will be conducted during the final months of the project.

Table 1. Results from combining all locations for the 4 years: using all the eleven predictors, R^2 and Cross Validation and the variables selected using Step-wise method.

Cultivar	2010		2011		2012		2013	
	R^2	CV	R^2	CV	R^2	CV	R^2	CV
Cripps Pink	.95	.73	.86	.77	.94	.79	.94	.77
	SV=all (GDD, CumRH)		SV=all		SV=all		SV=all (CmMin, CmST)	
Gala	.94	.76	.93	.77	.97	.80	.94	.76
	SV=all		SV=all		SV=all		SV= all	
Red Delicious	.93	.71	.93	.75	.94	.80	.94	.73
	SV=all		SV=all-(GDD)		SV=all		SV=all	

Table 2. Duration in Growing Degree Days for Gala, Red Delicious and Cripps Pink for 2013 for each phenological (A to H) stage, using a base temperature of 43°F starting January 1.

Cultivar	**Location Name**	**Year**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Cripps pink	Brays Landing	2013	113.5	113.5	199.5	231.3	268.8	319.1	384.7	412.8
	Chelan South		99.5	115.9	169.1	221.8	254	330.4	348.2	422.2
	East Wenatchee		138.7	138.7	219.4	219.4	274	309.6	343.4	388.1
	Konnowac Pass		199.8	223.3	288.5	348.3	348.3	405.4	453	501.5

	Orondo		145.3	145.3	238.1	271.7	318.9	349.6	446.2	478.2
	Royal City East		156	175	265.8	265.8	315.4	358.8	418.5	513.3
	Roza		101.9	101.9	166.8	241.8	277.9	305.6	353.1	400.1
	Wapato		171.9	197.6	250.3	325.7	357.6	429.4	429.4	469.3
	WSU Sunrise			181.4	233.3	272.6	330.7	371.6	390.2	431.7
Gala	Brays Landing	2013	113.5	142.6	231.3	231.3	268.8	366.4	384.7	412.8
	Chelan South		104.2	169.1	169.1	221.8	330.4	376.6	422.2	456.7
	East Wenatche		138.7	148.6	219.4	296	325	388.1	406.1	484.5
	Konnowac Pass		199.8	223.3	348.3	381.4	436.7	474.3	501.5	555.5
	Naches		173.5	192.8	301.9	351.7	410.8	449.6	496.8	573.6
	Orondo		145.3	145.3	238.1	293.1	333.1	372.4	446.2	478.2
	Royal City East		156	175	265.8	297.1	344.8	392.4	418.5	463.8
	Roza		101.9	166.8	277.9	277.9	305.6	384.6	408.4	454.2
	Wapato		171.9	197.6	325.7	357.6	382.5	429.4	469.3	537.9
	WSU Sunrise		200.6	215.2	309.3	355.6	390.2	464.4	542	568.2
Red delicious	Brays Landing	2013	113.5	199.5	231.3	248.6	311.4	366.4	384.7	460.1
	Chelan South		104.2	169.1	200.6	254	330.4	348.2	376.6	456.7
	East Wenatchee		138.7	138.7	252.2	252.2	296	343.4	388.1	460.1
	Konnowac Pass		199.8	223.3	348.3	381.4	405.4	474.3	491.4	555.5
	Naches		192.8	211.7	301.9	351.7	391.8	449.6	496.8	573.6
	Orondo		145.3	157.8	238.1	271.7	318.9	372.4	446.2	478.2
	Royal City East		156	175	265.8	297.1	344.8	392.4	447.6	513.3
	Roza		145.3	166.8	185.4	277.9	277.9	353.1	400.1	454.2
	Wapato		171.9	197.6	325.7	325.7	382.5	429.4	469.3	537.9
	WSU Sunrise		174.2	215.2	309.3	330.7	371.6	413.3	484.1	542

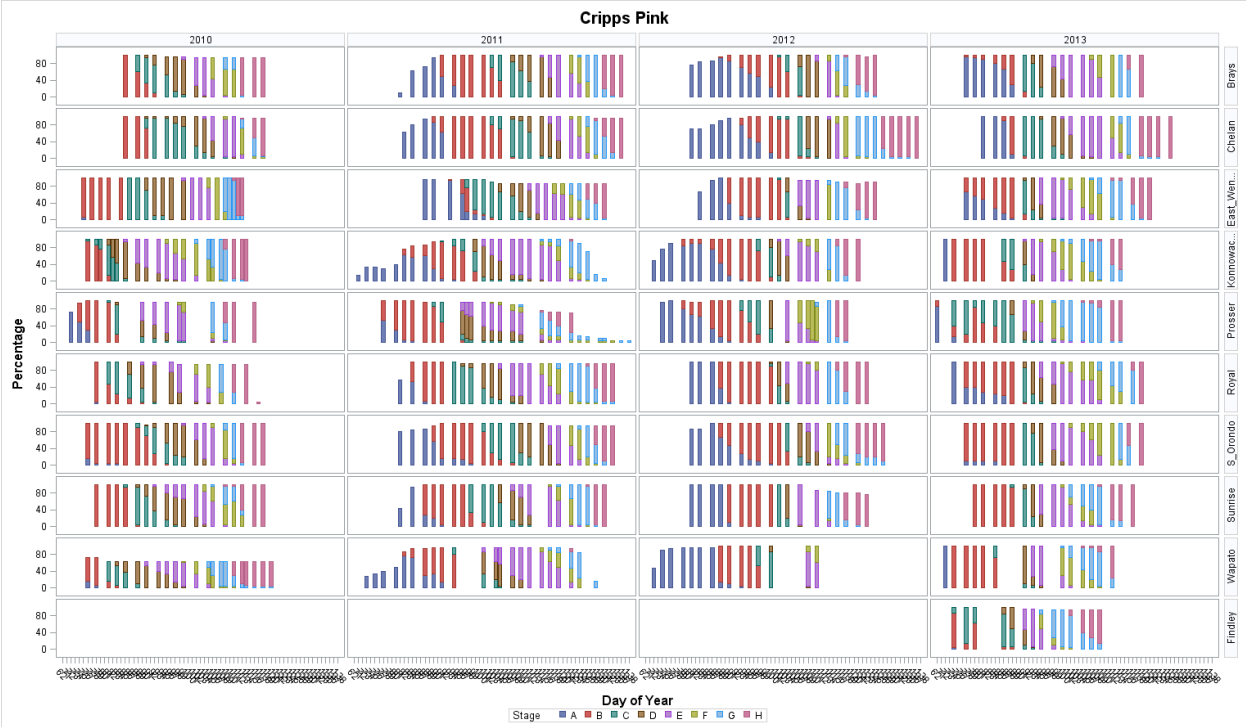

Figure 1. Percentage for each phenological stage for a given sampling date for Gala by year and location.

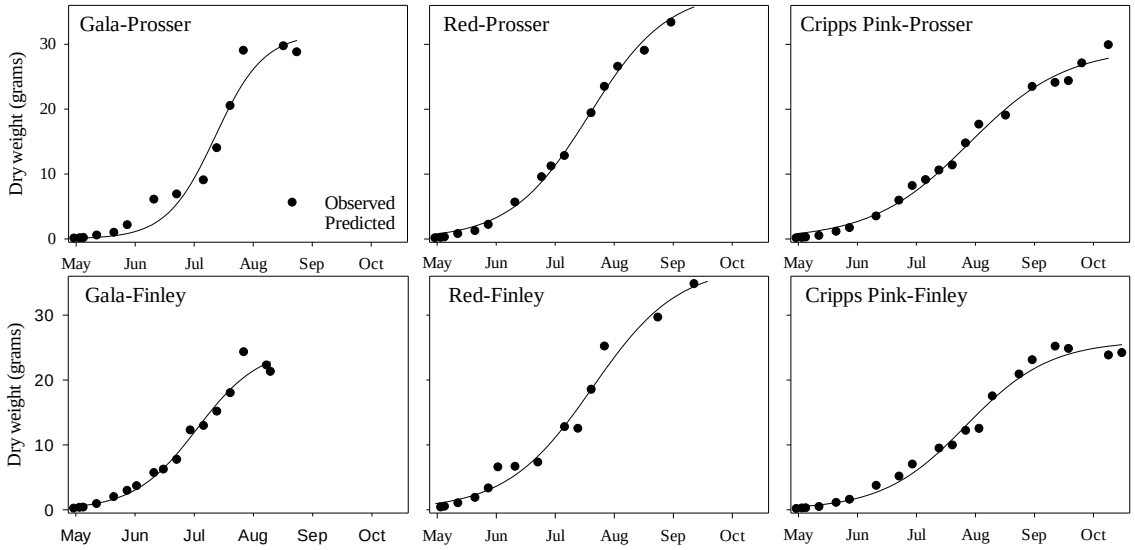

Figure 2. Observed and predict fruit dry matter for 2013 for Prosser and Finley for Gala, Red Delicious and Cripps Pink.

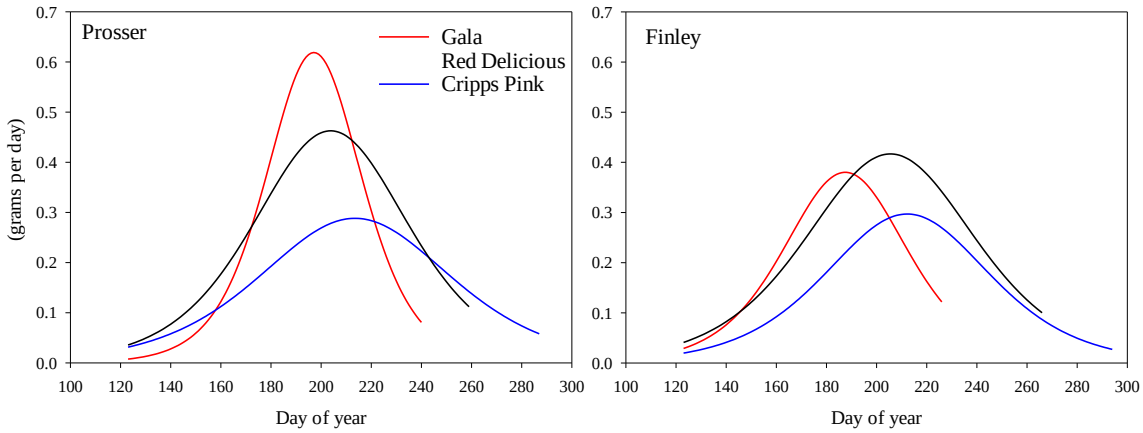

Figure 3. Dry matter growth rate per fruit for 2013 for Prosser and Finley for Gala, Red Delicious and Cripps Pink.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-13-104

Project Title: Glyphosate fate in inland pacific northwest apple orchards

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Total Project Request: **Year 1:** $37,787 **Year 2: $40,536** **Year 3:** $15,000

Other funding sources: None

Budget 1
Organization Name: CSS **Contract Administrator: John Brabb**
Telephone: 509.335.2562 **Email address: jbrabb@wsu.edu**

Item	2013	2014	2015
Salaries	$25,587	$28,149	
Benefits	$ 2,200	$ 2,387	
Wages			
Benefits			
Equipment			
Supplies	$10,000	$10,000	$15,000
Travel			
Plot Fees			
Miscellaneous			
Total	$37,787	**$40,536**	$15,000

Footnotes:

OBJECTIVES:

Objective 1: Recap of Objective 1: Experiment 1.1 and 1.2 will determine the fate of the glyphosate after application without a significant recent glyphosate use history in apple production systems, including fate of glyphosate absorbed through the bark.

Experiment 1.1: Fate of glyphosate in an orchard without a recent glyphosate use history. Assessment of apple injury, determination of yield components and fruit quality from the 2013 field experiment is completed. Residue analysis from soil samples is not completed. Goals and activities for the next year include the continuation of the field experiment at the WSU Sunrise Orchard, as well as the establishment of a second field study in a different orchard without a recent glyphosate use history. The second field experiment will be a replication of the field experiment established in 2013 at the WSU Sunrise Orchard.

	Field Experiment	**Results Timeline**	**Result Type**
Experiment 1.1	Field Experiment 1	First year data by December, 2013	Results of field experiments and residue data.
	Field Experiment 1	Second year data by December 2014	Results of field experiments and residue data.
	[1]Field Experiment 2	First year data by December, 2014	Results of field experiments and residue data[2].
	[1]Field Experiment 2	Second year data by December 2015	Results of field experiments and residue data[2].

[1]The addition of field experiment 2 is a deviation from the original methods of objective 1.
[2]Continuation of residue analysis is dependent of the residue analysis of field experiment 1.

Experiment 1.2: Absorption and translocation of basal-applied and soil-applied glyphosate. Young apple trees have been ordered and are expected to be available for pickup in January 2014. Young cherry trees have not been ordered. The young cherry trees will be ordered post completion of the absorption and translocation of basal-applied glyphosate in the apple experiment. Goals and activities for the next year include completing the absorption and translocation of glyphosate in young apple and cherry trees by basal and soil application.

	Tree Fruit	**Results Timeline**	**Result Type**
Experiment 1.2	Young Apple Trees- Gala/M9	Laboratory experiments completed by March 2014	Detailed knowledge of the absorption and translocation of glyphosate in young apple trees by basal application.
	[1]Young Cherry Trees	Laboratory experiment completed by March 2015	Detailed knowledge of the absorption and translocation of glyphosate in young cherry trees by basal application.
	[2]Young Apple and Cherry Trees	Laboratory experiment completed by March 2015	Detailed knowledge of the absorption and translocation of glyphosate in young apple and cherry trees by soil application.

[1]Postponing glyphosate absorption and translocation by basal application in cherry is a deviation from original objective 1 schedule.
[2]Postponing glyphosate absorption and translocation by soil application in apple and cherry is a deviation from original objective 1 schedule.

Objective 2: Recap of Objective 2: Identify optimum conditions for microbial degradation to mitigate soil adsorption (and potential persistence) of glyphosate in inland Pacific Northwest orchards, and characterize shifts in bacterial and fungal communities in the soil.

Experiment 2.1: Genetic analysis of microbial communities.
Knowledge of the fungal community composition within the nontreated control and the plots treated with glyphosate at 1920 g ae/ha has been obtained. Further analysis of the fungal community composition in the remaining plots treated with glyphosate at 840 g ae/ha, as well as the bacterial community composition analysis, remains to be completed.

	Results Timeline	Result Type
Experiment 2.1	First year data by December 2013	Knowledge of the effect of glyphosate on overall microbial dynamics
	Second year data by April 2015	Knowledge of the effect of glyphosate on qualitative changes in community structure

SIGNIFICANT FINDINGS:

- Following the applications of glyphosate at the WSU Sunrise Orchard field experiment, no visual injury was present.
- Apple yield per tree, apples per tree, and apple weight were affected by the application of glyphosate and the presence of vegetation at the time of application.
- The application of glyphosate or the presence of vegetation at time of application did not affect apple firmness, brix, titratable acidity, or pH.
- The application of glyphosate and the presence of vegetation at the time of application affected apple color.
- Glyphosate had no treatment effect on the fungal community composition.

METHODS:
Experiment 1.1: Fate of glyphosate in an orchard without a recent glyphosate use history.
Field experiment 1 was established on April 24, 2013 in block 3C at the WSU Sunrise Orchard. Trunk diameter measurements as well as notes on trunk, graft, and overall bark condition were recorded for each tree. Field experiment 1 was established with a randomized complete block design with a split-plot treatment arrangement and four replications. Main plots were 2.1 m wide by 24 trees, or ~24 m, in length and consisted of three treatments; 1) no postemergence glyphosate and maintained weed free by hand weeding or with a paraquat application at 140 g/ha, 2) glyphosate at 840 g ae/ha, and 3) glyphosate applied at 1920 g ae/ha. Split-plots were 2.1 m wide by 12 trees, or ~12 m, in length and were either 1) no vegetation facilitated by hand weeding or a directed application of paraquat or 2) a uniform stand of volunteer weeds. Each split-plot used trees 1 and 12 as border trees, which were not included in analysis of yield components or fruit quality. To fulfill the blocked experimental design, the trunk diameter measurements were converted to cross-section measurements of area and the treatment replications were blocked by increasing trunk cross-section area. Additionally, due to variation in weed pressure, care was taken to insure that the experimental layout minimized potential effects of previous weed management studies.

Prior to each glyphosate application, the no vegetation split-plots were hand weeded and the low hanging branches along with any suckers were trimmed. Glyphosate applications were applied to the whole plot and directed at the base of the tree. Glyphosate was applied on May 16, 2013 and July 11, 2013. To supplement the soil residue analysis as well as eliminate any concerns of glyphosate drift into the canopy during application, spray targets were placed systematically throughout the tree canopy and on the ground to document were the spray droplets were landing. Spray targets consisted of Watman #1 filter paper (12.5 cm diameter). After applications were made, the spray targets were

allowed to dry, removed from the field and placed in individually labeled plastic bags, and stored at -20 °C (-4 °F) until further analysis.

To quantify non-adsorbed and adsorbed glyphosate residue, soil samples were collected after each glyphosate application using a zero-contamination system (core diameter of 5 cm) set for 10 cm depth. Following both the May 16th and July 11th application, two soil samples were systematically collected from within the plots 0, 1, 4, 8, and 15 days after application. After sampling was completed, samples were stored at -20 °C (-4 °F). In collaboration with Mark Mazzola and objective 2, the soil samples were removed from the freezer and split in half. One half of the soil was delivered to Mark Mazzola and the remaining half of the soil sample was returned to the -20 °C (-4 °F) storage until further analysis for free and adsorbed glyphosate and AMPA residues. Soils will be processed as described by Alferness and Iwata (1994) with extractions processed as outlined by Eberbach (1999). Free and adsorbed glyphosate and AMPA residues will be quantified by GC-MS/SIM.

In addition to the soil samples that were collected after both the May 16th and July 11th application, tissue samples were also collected 22 days after each application. Tissue samples were stored at -20 °C (-4 °F) until further analysis.

The harvest of field experiment 1 took place on August 28th, 2013. Prior to harvest the farm manager at the WSU Sunrise Orchards confirmed that the apples were mature and ready to harvest. Ten trees from each split-plot were harvested. Each tree was harvested individually. The number of apples and weight of apples per tree were recorded. A subsample of 20-40 apples, sized between 80 and 88, was saved from each split-plot for quality analysis. The sub-samples were stored in a temperature and humidity controlled storage provided by the WSU horticulture post-harvest group.

Fruit quality analysis was performed between September 23rd and October 23rd on 20 apples from each split-plot (480 apples total). Fruit firmness, brix, titrabable acidity, pH, and color (L a*b*) were all recorded. Color a* and b* values were used to calculate hue angle. Fruit quality analysis was performed under the supervision and training of the WSU horticulture post-harvest group.

Experiment 1.2: Absorption and translocation of basal-applied and soil-applied glyphosate.
Two experiments will be conducted to address soil and stem uptake. To quantify stem uptake of glyphosate in juvenile apple and cherry trees, dormant representative apple and cherry saplings will be grown in a growth chamber in cone-tainers containing a common orchard soil. Each plant stem will be treated with 10 uL mixture of radiolabeled glyphosate mixed in water and 0.25% v/v non-ionic surfactant. Each plant will be dosed with approximately 29.29 kBq radiolabeled material. After treatment, plants will be placed in a growth chamber and destructively harvested at 2, 8, and 24 hours after treatment, and at 1 and 3 wks after treatment. Each harvest will consist of 8 replicate plants. Each plant will be divided into six parts: treated stem bark, treated stem cambium (and, if possible, older xylem), stem below the treated stem, roots, stem above treated stem and leaves. Parts will be dried at 40 C, weighed, and larger samples ground and subsampled. The samples or sub-samples will be oxidized and the evolved ^{14}C-CO_2 will be captured and quantified. Absorption of glyphosate will be determined from the recovered radioactivity in the oxidized samples.

Procedures to quantify soil uptake of glyphosate in juvenile apple and cherry trees will be similar to the bark experiment, except the radioactivity will be applied to the soil surface instead of the bark. The soil will be a common orchard soil. Treatment and harvest intervals will be similar to the bark experiment. At harvest, the first 5 cm of soil will be harvested separately from the rest of the soil. The plant will be divided into roots, stem, and leaves and processed as described previously.

Experiment 2.1: Genetic analysis of microbial communities.
A composite apple root sample with adhering rhizosphere soil will be collected from two trees in each treatment plot from a depth of 5-15 cm. DNA will be extracted from duplicate sub-samples (5 g) for

each plot using the MoBio PowerMax Soil DNA extraction kits and resulting DNA will be pooled. Initial examination of microbial communities will utilize a genetic approach to identify quantitative shifts in populations. Bacteria will be quantified from the duplicate soil extracts by real-time quantitative PCR (qPCR) by targeting the 16S gene with the primer set 338F and 518R, and fungi using the primer set NSI1 and 5.8S.. Quantification will be achieved using the StepOne Plus Real Time PCR thermocycler. All reactions will be performed using three technical replicates. The standard curves for PCR quantification will be generated by diluting DNA plasmid containing cloned amplification product. The plasmid used for the bacterial 16S standard curve will be constructed with the 16S gene from *Methylobacterium* sp. amplified from soil using the primers 8F (50-AGA GTT TGA TCC TGG CTC AG-30) and 1406R (50-ACG GGC GGT GTG TRC-30). The fungal standard curve will be prepared from the ITS region of *Mortierella alpina* amplified from soil using the qPCR primers in which the complete and correct plasmid insert was previously verified by DNA sequencing. Qualitative changes in microbial community structure will initially be examined using a coarse genetic approach by employing terminal restriction fragment length polymorphism (T-RFLP) analysis. This method will be used as a cost savings approach and will serve to identify the most appropriate community to target for examination by pyrosequencing. T-RFLP analysis of bacterial and fungal communities will be conducted using the methods previously described and commonly employed by the collaborators (Weerakoon et al., 2012). These data will be utilized to determine what, if any, microbial populations should be targeted for analysis by pyrosequencing. In the conduct of prosequencing analysis. The bacterial 16S gene will be targeted for amplification using the forward primer consisting of the 25-bp 454 A Adapter and an 8-bp barcode followed by the modified universal bacterial primer16S-27F (5´-AGRGTTTGATCMTGGCTCAG-3´). The reverse primer will consist of the 25-bp 454 B Adapter and the modified universal bacterial primer 16S-519R (5′-GTNTTACNGCGGCKGCTG-3′). Fungal DNA will be amplified using the 25-bp 454 A Adapter and an 8-bp barcode followed by the forward primer ITS1F and the reverse primer consisting of the 25-bp 454 B Adapter the universal primer ITS4. Duplicate reactions will be conducted for each primer pair/sample combination and the resulting amplicon population will be purified using a QIAquick Gel Extraction Kit. Purified amplicons will be quantified and pooled within samples for pyrosequencing. Pyrosequencing will be conducted using a 454 sequencer at Washington State University.

RESULTS AND DISCUSSION:

Experiment 1.1: Fate of glyphosate in an orchard without a recent glyphosate use history.

No visual injury was present following an application of glyphosate in Field Experiment 1. We plan to monitor the trees as they break dormancy and begin to grow in the spring. The likelihood for any injury as a result of glyphosate sequestration and remobilization in the tree would be highest when the trees break dormancy.

The components of yield (Table 1) were affected by the application of glyphosate and the presence of vegetation at the time of application. The apple yield for the nontreated plots was 10.96 kg. The apple yield in plots treated with glyphosate at 840 g ae/ha and 1920 g ae/ha was 10.46 and 15.10 kg, respectively. The apple yield in plots treated with glyphosate at 1920 g ae/ha was greater ($\alpha = 0.1$) than the apple yield in plots treated with glyphosate at 840 g ae/ha. The plots with vegetation at the time of application yielded 13.56 kg of apples and were greater ($\alpha = 0.1$) than the plots with no vegetation, which yielded 10.79 kg of apples. Apples tree^{-1} for the nontreated plots was 84. Apples tree^{-1} for the plots treated with glyphosate at 840 g ae/ha and 1920 g ae/ha was 69 and 115, respectively, and was significant ($\alpha = 0.1$). The plots with vegetation yielded 102 apples tree^{-1}, which was greater ($\alpha = 0.05$) than 77 apples tree^{-1} in the plots with no vegetation. Apple weight in the nontreated plots was 141 g. Apple weight in the plots treated with glyphosate at 840 g ae/ha and 1920 g ae/ha was 153.3 and 138.2 g, respectively. The apple weight in the plots with vegetation was different ($\alpha = 0.1$) than the plots with no vegetation at the time of application, 147.5 and 140.7 g, respectively.

Table 1. Glyphosate treatment effects on apple yield (kg per tree, apples per tree, and g per apple) and yield components (color and hue), from Experiment 1.1 conducted in the Sunrise Orchard in 2013.

	Components of Yield					
	Apple Yield (kg tree^{-1})		Apples Tree^{-1}		Apple Weight (g apple^{-1})	
Treatment	**Mean**	**α = 0.1**	**Mean**	**α = 0.1**	**Mean**	**α = 0.1**
Nontreated	10.96	ab	84	ab	153.26	a
Glyphosate 840 g ae/ha	10.46	b	69	b	140.95	ab
Glyphosate 1920 g ae/ha	15.10	a	115	a	138.18	b
Split-plot	**Mean**	**α = 0.1**	**Mean**	**α = 0.05**	**Mean**	**α = 0.1**
Vegetation	13.56	a	102	a	140.72	b
No Vegetation	10.79	b	77	b	147.54	a

	Components of Color			
	Color L		Hue	
Treatment	**Mean**	**α = 0.05**	**Mean**	**α = 0.05**
Nontreated	53.94	b	35.54	b
Glyphosate 840 g ae/ha	56.34	a	40.08	a
Glyphosate 1920 g ae/ha	53.87	b	36.65	b
Split-plot	**Mean**	**α = 0.05**	**Mean**	**α = 0.05**
Vegetation	56.30	a	39.54	a
No Vegetation	53.14	b	35.30	b

Fruit firmness, brix, titratable acidity, and pH were not affected by the application of glyphosate or by the presence of vegetation, but the components of fruit color (Table 1) were affected by both the application of glyphosate and the presence of vegetation at the time of application. In the nontreated plots, the L (lightness) value was 53.94. In the plots treated with glyphosate at 840 g ae/ha and 1920 g ae/ha, the L value was 56.34 and 53.87, respectively. The L value for the plots treated with glyphosate at 840 g ae/ha was different ($\alpha = 0.05$) than the nontreated plots and the plots treated with glyphosate at 1920 g ae/ha. A difference ($\alpha = 0.05$) in the L value was also present between the two split-plots, 56.30 in the plots with vegetation and 53.14 in the plots without vegetation. The hue value for the nontreated plots and for the plots treated with glyphosate at 840 g ae/ha and 1920 g ae/ha was 35.54, 40.08, and 36.65, respectively. The hue for plots treated with glyphosate at 840 g ae/ha was different ($\alpha = 0.05$) than the nontreated plots and the plots treated with glyphosate at 1920 g ae/ha. The hue in the plots with vegetation was different ($\alpha = 0.05$) than the plots with no vegetation at the time of application, 39.54 and 35.30, respectively.

A common practice among growers is to thin the fruit and a unique detail of field experiment 1is that the fruit was not thinned. Thinning the fruit in a field experiment where yield components are being recorded can potentially eliminate treatment effects. Based on the results of the field experiment, we feel that choosing to not thin the fruit was helpful. Glyphosate applied at 1920 g ae/ha resulted in the plots with the highest yield per tree, largest number of apples per tree, and the smallest apples. Glyphosate applied at 840 g ae/ha resulted in the plots with the lowest yield per tree, smallest number of apples per tree, and the largest apples. With no difference in the components of yield between the lowest rate of glyphosate and the nontreated plots, there is a possibility that glyphosate at the lower rate has no effect on the trees, but the higher rate does. In addition, the plots with vegetation at the time of application had a higher yield per tree, larger number of apples per tree, and smaller apples compared to the plots with no vegetation during application. The presence of vegetation at the time of

application may be providing an avenue for glyphosate to interact with the tree roots; resulting in the increased yield and smaller apples found in the plots treated with the higher rate of glyphosate. It will be interesting to see if the trends continue in the second year of field experiment 1.

However, the effects of glyphosate treatment and rate on the color of the apples could be attributed to the lack of fruit thinning, resulting in a variation of exposure to sunlight and other environmental factors. No observable injury due to glyphosate treatment was observed, and a more definitive understanding of the yield and yield components will be gleaned when the various target and tissue samples are processed for glyphosate residue.

Experiment 2.1 Genetic analysis of microbial communities:
No treatment effect of glyphosate on fungal community composition was present between the nontreated plots and the plots treated with glyphosate at 1920 g ae/ha (Figure 1). Although no treatment effect is present between the nontreated plots and the plots treated with glyphosate at 1920 g ae/ha, further analysis of fungal community composition in the plots treated with glyphosate at 840 g ae/ha as well as looking at multiple year effects will be helpful.

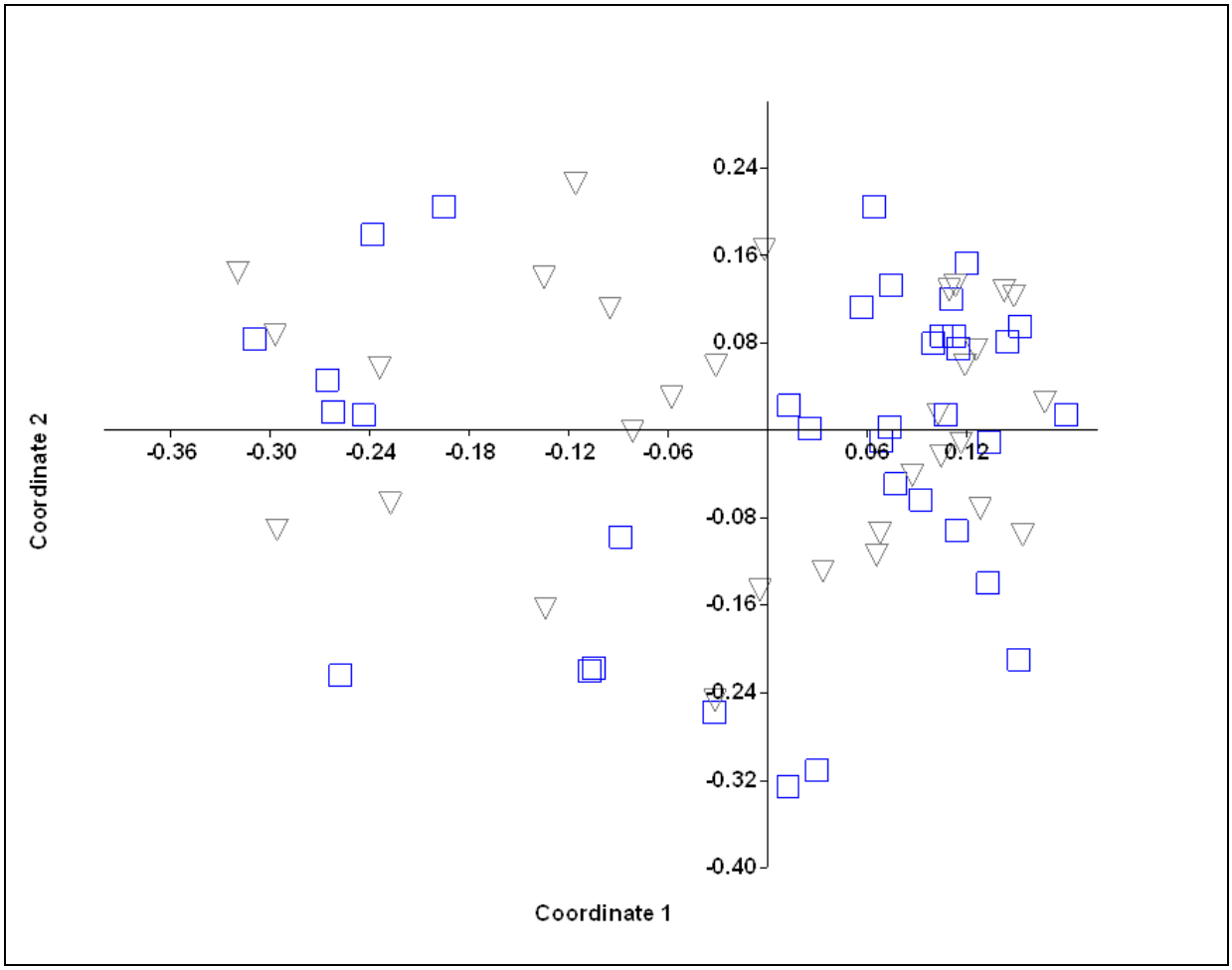

Figure 1. The effect of glyphosate on fungal community composition based upon principal coordinate analysis. Blue squares represent nontreated control and inverted triangles represent data points from plots treated with glyphosate at 1920 g ae/ha.

CONTINUING PROJECT REPORT **YEAR**: Year 2 of 3
WTFRC Project Number: AP-12-102

Project Title: Enhancing apple packing HACCP programs while ensuring fruit quality

PI:	Karen Killinger, Ph.D.	**Co-PI (2):**	Ines Hanrahan, Ph.D.
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Co-PI(3): John Fellman, Ph.D.
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City/State/Zip: Pullman, WA 99164

Cooperators:

Richard Kim, Ph.D., Pace International. Dr. Kim designed and conducted experiments related to fruit quality during a commercial trial. He also reported results related to those experiments in Year 1.

Several chemical suppliers have donated supplies, other resources and time to discuss aspects of this project and ensure relevancy to the apple packing industry. Several apple packing facilities have agreed to or expressed interest for participating in packing plant studies to validate interventions to ensure that laboratory results relate to large scale production treatments.

Total Project Request: **Year 1:** 80,089 **Year 2:** 81,034 **Year 3: 85,571**

Other funding sources

Agency Name: Center for Produce Safety
Amt. requested: $199,784.60
Notes: A proposal requested funding to:

1) Evaluate the potential influence of wax applications and drying temperatures on microbial levels in laboratory and pilot line studies and

2) Evaluate a chlorine stabilizer to enhance apple dump tank chlorine applications in laboratory and commercial settings.

The proposal was not selected for funding.

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries			
Benefits			
Wages[1]	6,221	6,470	6,728
Benefits	1,555	1,617	1,682
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Total	7,776	8,087	**8,410**

[1] Wages and benefits for assistance from WTFRC crew.

Budget 1
Organization Name: WSU **Contract Administrator: Carrie Johnston**
Telephone: (509) 335-4564 **Email address: carriej@wsu.edu**

Item	2012	2013	2014
Salaries[1]	44,856	38,051	41,324
Benefits	6,457	7,966	16,843
Wages		4,080	5,220
Benefits		82	506
Equipment		3,000	0
Supplies[2]	19,000	17,500	11,188
Travel[3]	2,000	2,268	2,080
Plot Fees			
Miscellaneous			
Total	72,313	72,947	**77,161**

Footnotes:
[1] Technical support and undergraduate students in Pullman.
[2] Fruit, chemicals, measurement devices, microbial supplies and analysis fees.
[3] Travel to Wenatchee and Yakima for commercial studies and fruit collection.

Objectives:

1) Evaluate the most effective microbial controls for HACCP systems individually and in combination using indicator organisms under commercial conditions
2) Identify and evaluate potential alternative approaches or novel compounds to enhance microbial control
3) Perform laboratory studies to examine pathogen and fungicide adherence to different apple varieties and examine the effects of storage over several harvest seasons
4) Conduct appropriate food safety extension and outreach with the apple packing industry

Significant Findings:

- Four replications at a commercial facility examined high and low organic loads in a chlorinated dump tank, hyperwash with chlorine dioxide and chlorinated flume system.
    - Antimicrobial activity at different points in the packing system differed depending on organic load.
    - High ORP chlorine levels in the dump tank were less effective in the presence of higher organic loads in the dump tank (similar reduction to the water control). However, significant microbial reductions (almost a 99% reduction) was achieved on apples that were subsequently treated in the hyperwash with chlorine dioxide and the chlorinated flume in the presence of higher organic loads.
    - With low organic load water, the chlorinated dump tank achieved a significant, 90% microbial reduction.
    - With low organic load water, the higher ORP chlorine flume treatment (approximately 850mV) achieved a significant microbial reduction when compared to the lower ORP chlorine flume treatment (approximately 750mV).

- Three replications at a commercial facility examined dump tank applications of water, phosphoric acid, chlorine, chlorine dioxide and PAA were performed using low organic load water.
    - All treatments produced significant reductions (near or greater than 90%; 0.9-1.7 log reductions) in generic *E. coli* compared to the inoculated control. Phosphoric acid (pH 3.1-3.4) and high and low ORP chlorine treatments were statistically similar to the water treatment. High and low ORP chlorine dioxide treatments were slightly, but statistically different from the water treatment. PAA at 80ppm produced 90% bacterial reduction (1.1 $\log_{10}$) compared to the water control.

- Commercial scale PAA spray bar applications resulted in a >90% reduction of generic *E. coli* (1.4 to 1.5 $\log_{10}$ reduction) when 60-80ppm was directly applied to apples for at least 30 seconds with or without soap application.

- Laboratory experiments (3 replications) focused on PAA for dump tank application and lactic acid for spray bar application. PAA treatments included water and 2 concentrations of PAA (60 and 80ppm) applied at 3 application times (2, 3.5, and 5 minutes) representing exposure time in dump tanks. Lactic acid treatments included water and lactic acid (1 and 2%) applied at 3 application times (5, 15, and 30 seconds) followed by a 10 second exposure time.
    - PAA (60ppm and 80ppm) treatments responded similarly producing 1.2 to 1.6 $\log_{10}$ cfu/mL (90%) reduction of generic *E. coli* and *E. coli* O157:H7.

- Pathogenic *E. coli* O157:H7 and generic *E. coli* responded similarly to both 1 and 2% lactic acid with a 90% reduction (0.8 - 1.0 $\log_{10}$); however this response was the same as water.

Methods:

Year 2, Objective 1: The release of proposed rules associated with the Food Safety Modernization Act and the anticipated need for industry data focused research efforts on Objective 1 for Year 2. Commercial trials for PAA spray bar treatments and chlorine dump tank treatments were planned. Due to changes at a cooperating commercial facility, the PAA spray bar trials were not performed. Therefore, all efforts focused on commercial scale dump tank studies.

Commercial Scale Dump Tank Studies.

Examination of microbial reductions in high and low organic loads for a chlorinated dump tank, hyperwash with chlorine dioxide and chlorinated flume system. Four replications were conducted at a commercial packing facility. Project planning and performance involved industry representatives and WSU personnel. Apples were inoculated with non-pathogenic (generic) *E. coli* at WSU and transported in coolers containing ice to the packing facilities. Inoculated controls were measured immediately upon arrival at the packing facility on day 1 and at the beginning and end of day 2.

The following points in the system were examined for antimicrobial reductions: 1) dump tank treated with chlorine 2) hyper-wash treated with chlorine dioxide 3) flume system treated with high and low ORP chlorine levels. The levels of chlorine and chlorine dioxide were measured using stationary and portable meters for pH and ORP (mV). On day 1, inoculated apples were treated in high organic load water using typical production levels for chlorine (ORP range: 873-955 mV) and chlorine dioxide (ORP range: 665-818 mV). On day 2, fresh, low organic load water was used as a control treatment and then chlorine and chlorine dioxide levels were adjusted to typical production levels in the dump tank and hyperwash. Two treatment levels were examined for chlorine in the flume on day 2 with chlorine levels adjusted to reach approximately 750 mV and 850 mV ORP. The portable ORP meter readings were not consistent with the stationary ORP meter readings. It was difficult to accurately establish ORP levels of the chlorine treatments.

Seventy-five inoculated apples were placed into the dump tank and were collected prior to the hyper-wash cabinet (20 apples), after the hyper-wash (20 apples), and after the flume system (35 apples). Apples were sampled immediately after collection, and the diluent was chilled for transport to WSU. Samples were diluted appropriately and plated on VRBA for enumeration of generic *E. coli*.

Year 3, Objective 3. A set of experiments will be performed to examine adherence of relevant bacteria and fungicides to different apple varieties stored for different periods in controlled atmosphere. Factors that will be examined include: apple variety (up to 6 varieties), fungicide type (3 fungicides), and length of controlled atmosphere storage (3 storage periods). Apples from orchards where pre-harvest fungicides were not applied will be utilized. The following varieties will be considered for examination and selected with input from the industry: Golden Delicious, Granny Smith, Gala, Fuji, Honeycrisp and Red Delicious. Factors to consider in variety selection include: wax levels, acidity, economic value to the industry and typical antimicrobial treatment applications, among others. It is anticipated that at least 3 different sources of apples for each variety will be examined (3 replications). Determination of disorders will be conducted at harvest; only fruit free of disorders will be utilized in the experiments described above. It is likely that fruit will be collected at identified orchards and drenched in a laboratory setting using appropriate methods (Errampalli et al., 2005; Xiao et al., 2011).

Untreated apples will be collected for examination of initial bacterial and mold levels and for use as a control group (untreated). To examine fungicide adherence, at least one of the following

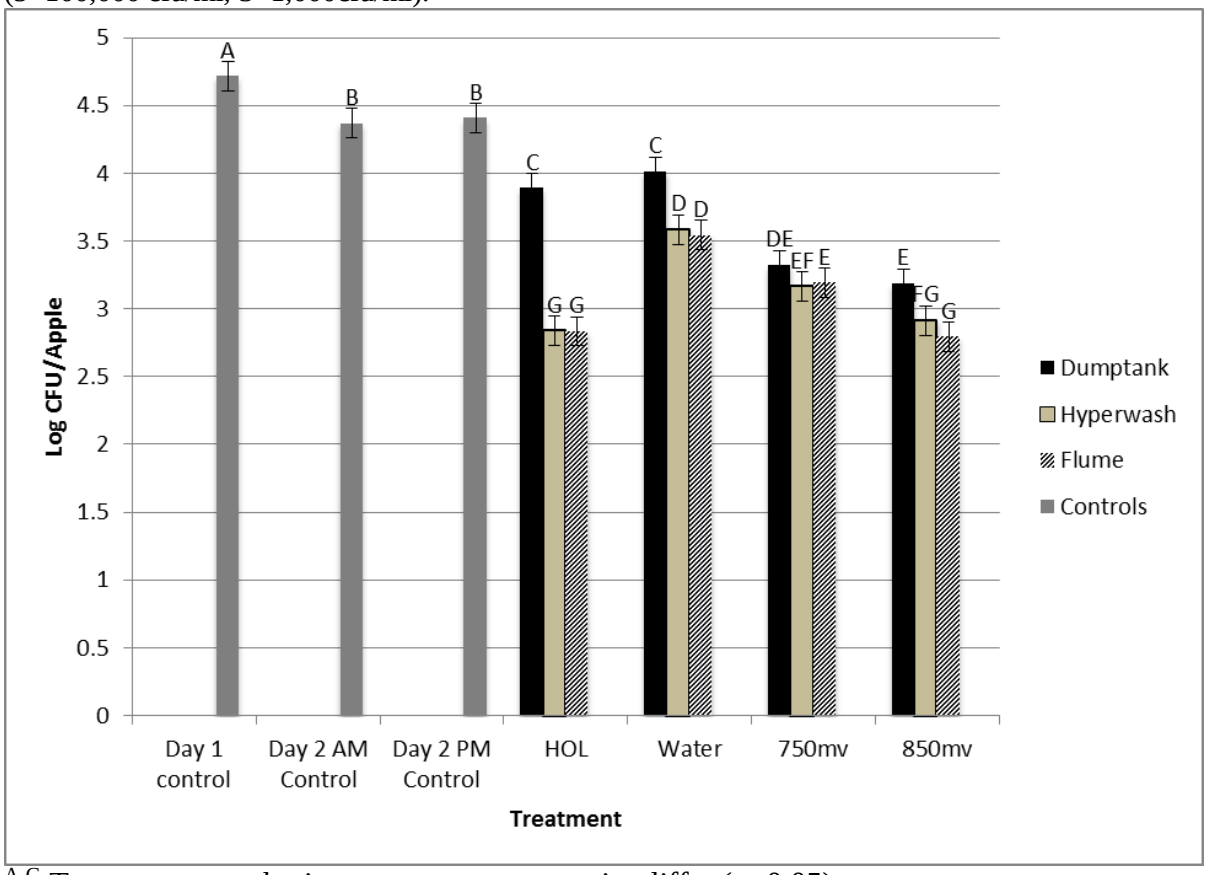
fungicides will be examined in Year 2 with expansion to other fungicides in Year 3: fludioxonil (Scholar), pyrimethanil (Penbotec) and thiabendazole (Mertect). Recommended concentrations by manufacturer labels will be used. After fungicide application, apples will be examined prior to storage as well as after five and eight months of storage in controlled atmosphere. Fungicide residue measurement will be contracted to a qualified laboratory for analysis with a gas chromatograph. To examine bacterial adherence, three strain cocktails of each of the following bacteria will be examined separately: generic *E. coli*, *E. coli* O157:H7, and *Salmonella*. Apples for bacterial attachment studies will be selected and equivalent to apples from the untreated treatment in the fungicide adherence studies to maintain consistency for examination of quality and safety aspects. Each variety will be examined at the time of harvest prior to storage, as well as after five months and eight months of controlled atmosphere storage. To examine the potential influence of factors associated with fruit physiology, characteristics of the apples will also be examined. Quality measurements will include common maturity testing at harvest and after storage. Measurements of natural wax levels (Belding et al., 1998; Schreiber and Riederer, 1996) and lenticel size and density will be performed (Turketti et al., 2011). The need for examination of lenticel breakdown will also be considered (Turketti et al., 2011).

Results and Discussion:

Examination of high and low organic loads in a chlorinated dump tank, hyperwash with chlorine dioxide and chlorinated flume system.

Antimicrobial activity at different points in the packing system differed depending on organic load (Figure 1). For the fresh water treatment, apples collected after the dump tank had a 0.4 log reduction and the entire dump tank, hyperwash and flume achieved almost 90% microbial reduction. In the high organic load treatment, the dump tank chlorine application was similar to the reduction achieved in the dump tank water control treatment. However, a significant microbial reduction (almost a 99% reduction) were achieved for apples collected after the chlorine dioxide hyperwash and chlorine flume in the high organic load treatment. For the low organic load treatments (fresh water with antimicrobials), apples treated in the chlorinated dump tank had significantly lower microbial levels (0.8 log reduction) compared to apples treated in the water control dump tank and a >90% reduction compared to the inoculated control apples. Using higher ORP levels in the flume with low organic load water also achieved greater microbial reductions, apples treated in the dump tank, hyperwash and 850mV ORP flume had a 1.6 log (>90% reduction). It should be noted that similar reductions were achieved in the high organic load treatment, but at different points in the packing system. Therefore, organic load in water tanks or flumes must be carefully monitored to ensure effective microbial control and to achieve microbial reduction.

Figure 1. Average generic *E. coli* levels on apples after inoculation and direct application of water at each collection point, or typical production levels of chlorine in the dump tank and chlorine dioxide in the hyperwash and chlorine (low and high ORP) in the flume system at low organic loads or high organic loads (HOL) in a commercial study. Values reported in $\log_{10}$ colony forming units (cfu)/ml (5=100,000 cfu/ml, 3=1,000cfu/ml).

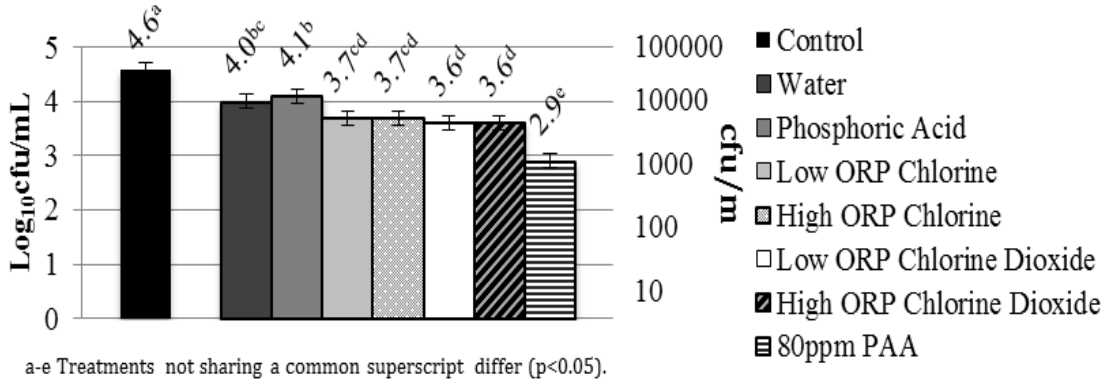


[A-G] Treatments not sharing a common superscript differ ($p<0.05$)

The trends observed in this commercial study differed from results in Year 1at a different commercial facility involving chlorine and chlorine dioxide in a dump tank system involving only low organic load water (summarized below).

Examination of chlorine, chlorine dioxide and peroxyacetic acid in low organic load dump tanks.
A commercial study was performed to examine the effect of chlorine, chlorine dioxide, phosphoric acid, and peroxyacetic acid in a dump tank. Three replications examining generic *E.coli* inoculated apples were performed (Figure 2). All treatments significantly reduced generic *E. coli* levels compared to the inoculated controls. However, chlorine treatments (low and high ORP) were similar to water for reduction of generic *E. coli*, less than a 90% reduction (0.5 to 0.9 $\log_{10}$ reduction). Chlorine dioxide (low and high ORP) produced a slight, but statistically significant reduction of generic *E. coli* compared to water; however, this reduction was similar to that observed for chlorine. Based on the results from this study, chlorine (low and high ORP) and phosphoric acid achieved less than a 90% reduction, chlorine dioxide (low and high ORP) achieved a 90% reduction and 80ppm PAA achieved almost a 99% reduction in generic *E.coli.*

Figure 2. Average generic *E. coli* levels on apples after inoculation and direct application of water, phosphoric acid (pH 3.5), chlorine (low and high ORP) and chlorine dioxide (low and high ORP) and peroxyacetic acid (80ppm) in a commercial study. Values reported in $\log_{10}$ colony forming units (cfu)/ml (5=100,000 cfu/ml, 3=1000cfu/ml).



a-e Treatments not sharing a common superscript differ ($p<0.05$).

Summary:

It appears that the potential for microbial reduction in apple packing systems depends on several factors, including the order of steps within a packing system (dump tanks, hyperwashes, etc), organic load, antimicrobial concentration, tank agitation, among others. Achieving a better understanding of these factors to influence microbial reduction is important. Moreover, the use of antimicrobials to ensure microbial control (limitation of cross-contamination) in any water tank or flume system is essential.

CONTINUING PROJECT REPORT (Extension) **YEAR**: 2 of 2
WTFRC Project Number: AP-12-108

Project Title: Overhead cooling influences on microbial food safety

PI: Karen Killinger, Ph.D.
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Cooperators: Several growers participated in the study, including active participation, discussion of potential sites, and industry organizations assisted to identify interested cooperators.

Total Project Request: **Year 1:** $128,000 **Year 2:** $8,322

WTFRC Collaborative expenses:

Item	2012	2013	2014
Wages[1]	6,400	6,658	
Benefits	1,600	1,664	
Total	8,000	8,322	0

Footnotes:
[1] Wages and benefits for assistance from WTFRC crew.

Budget 1
Organization Name: WSU **Contract Administrator: Carrie Johnston**
Telephone: (509) 335-4564 **Email address: carriej@wsu.edu**

Item	2012	2013	2014
Salaries[1]	24,905		
Benefits	7,671		
Equipment[2]	10,000		
Supplies[3]	15,000		
Travel	2,517		
Total	60,093	0	0

Footnotes:
[1] Graduate student, technical support and undergraduate students in Pullman.
[2] Freezer and laboratory equipment for inoculation methods.
[3] Fruit, chemicals, measurement devices, microbial supplies and analysis fees.
[4] Travel to central Washington for inoculation studies and fruit collection.

Budget 2
Organization Name: UW **Contract Administrator: Julie Tran**
Telephone: 206-543-6959 **Email address: Juliet4@uw.edu**

Item	2012	2013	2014
Salaries	32,828		
Benefits	10,379		
Supplies	14,900		
Travel	1800		
Total	59,907	0	0

OBJECTIVES

1) Select and validate appropriate, non-pathogenic surrogate organisms as well as inoculation methods in the laboratory to evaluate food safety risk associated with overhead, evaporative cooling water application.

2) Investigate foodborne pathogen and surrogate survival in laboratory studies and non-pathogenic surrogate survival in field studies to examine risks associated with standard overhead cooling water application practices.

SIGNIFICANT FINDINGS

- Candidate strains were identified through a literature review and selection of isolates from Washington irrigation water sources. Candidate strains were narrowed for validation of growth characteristics.
    - At least 54 strains of pathogenic *E. coli* O157:H7 strains
    - At least 101 *Salmonella spp.* strains
    - At least 22 non-pathogenic surrogates
- Replicated growth curves of non-stressed (healthy) cells were performed for 13 strains, including pathogenic *E. coli* O157:H7, *Salmonella spp.* and potential surrogate strains of generic *E. coli*.
    - Most strains were similar in growth characteristics when replicated. Some strains exhibited variation that should be monitored closely when experiments under stress conditions are performed.
- For evaluation of potential field sites, over 300 sampling events were performed at 24 sampling locations in three tree fruit growing regions of Washington.
    - Preliminary analysis of generic *E. coli* levels using the most probable number method indicated that of 300 samples evaluated, approximately 13 samples (4.3%) exceeded the currently proposed FDA standard for any single sample (126 MPN/100ml generic *E. coli*) and one site (1%) exceeded the rolling geometric mean standard.

METHODS

Year 1. Objective 1. Strain Selection. Strain selection involved 1) strain selection and acquisition and 2) growth curves performed with non-stressed (healthy) cells. An extensive literature review was performed. Replicated growth curves were performed with 13 strains, including pathogenic *E. coli* O157:H7 and *Salmonella* as well non-pathogenic (generic) *E. coli* strains. For the growth curves, samples were collected at the following time points: 0, 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24 hours. Samples were measured for absorbance at 600nm in a spectrophotometer, and the sample was serially diluted and spread plated on tryptic soy agar plates.

Year 1. Objective 2. Evaluation of potential field sites through examination of fecal coliform and generic *E. coli* levels in open surface waters used for overhead cooling. Sampling locations (24 locations) were identified for preliminary water testing. Sampling locations represented three primary tree fruit growing regions: northern (5 locations), central basin (6 locations) and southern (13 locations). Efforts were made to reflect several irrigation districts (9 districts total). When possible, areas where water quality challenges were known to exist were included.

Water delivery in some cases was complex and collecting water from the original, open surface water body was not possible due to lack of direct access, whereas at some locations, it was possible to sample from several points within the water delivery system (Table 1). Subpart E of the FDA proposed produce rule, Standards Directed to Agricultural Water, does not indicate specific expectations for water sample collection sites. Therefore, a robust sampling strategy at each sampling location was developed. Sampling sites were selected to examine the potential for variation in water quality values based on the original source of the water, the delivery and holding of the water and sampling sites within a point in the water delivery system. For example, when possible, water was collected from the originating, open surface water body (canal, lateral, etc.) as well as from the holding system (pond). Additionally, multiple samples from each sampling site were also collected to evaluate consistency between bacterial levels at multiple points within a sampling site; for example, the minimum number of samples collected from a pond was between two to five samples. At four locations, water was collected from overhead cooling sprinklers. ***The total number of specific sampling sites examined consistently over a three month period was 101 sampling sites.***

Near the end of overhead cooling application season (early September), a more in-depth sampling period was performed at two sampling sites. Sampling at these sites involved sampling the open surface water canal, lateral, water box, ponds and collection from overhead cooling spigots during an overhead cooling event. Apples (68) were collected from two orchards prior to and after overhead cooling water application.

Water samples were tested for total coliforms, fecal coliforms and *E. coli* using the IDEXX Colilert®-18, Quanti-Tray®/2000 system and Most Probable Number method. For the IDEXX examination, two 100ml duplicates were taken from each water sample, and each was mixed with a packet of Colilert®-18. The duplicates were then poured into a Quanti-Tray®/2000 and sealed using the IDEXX Quanti-Tray® sealer. For each water sample, one

Quanti-Tray® was incubated at 35°C for 18-22 hours to test for total coliforms and *E. coli*, while the second Quanti-Tray® was incubated at 44.5°C for 18-22 hours to test for fecal coliforms. After 18-22 hours, the Quanti-Trays® were removed from incubation. Using the IDEXX Quanti-Tray®/2000 MPN table, the most probable number (MPN) of total coliforms, fecal coliforms and generic *E. coli* was generated for each duplicate. Apples were examined using 3M Petrifilm.

Table 1. Summary of water sampling locations and points within a water delivery system that were examined.

Description of Sampling of Water Delivery Systems for Overhead Cooling	**Number of Sites Examined**
Direct from Open Surface Water Source	1
Open Surface Water Source and Pond	9
Open Surface Water Source and Water Box or Cistern	4
Pond only	5
Pump only	4
Spring	1

RESULTS AND DISCUSSION

It is important to note that data analysis for this project is ongoing. Therefore, all results should be considered preliminary and subject to be altered based on further analysis.

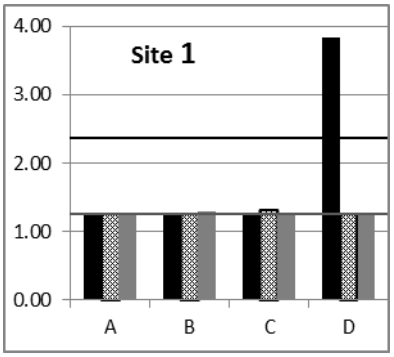
Year 1. Objective 1. Strain Selection. Replicated growth curves were completed for 13 strains (Table 1). In general, the growth curves using healthy cells were fairly similar when averaged over three replications. However, five strains (ATCC 43895, ATCC 23716, KMK180, ATCC 14028, ATCC BAA-664) had more variability among replications that should be noted as experiments under stress conditions are conducted.

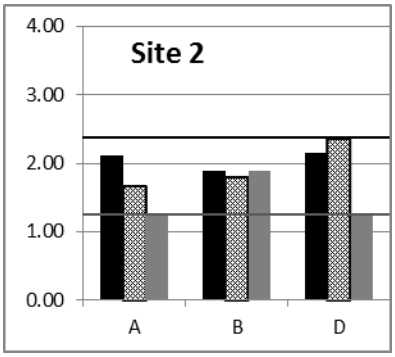
Table 2. Summary of strains examined for growth of healthy cells with strain designation, classification and number of replications.

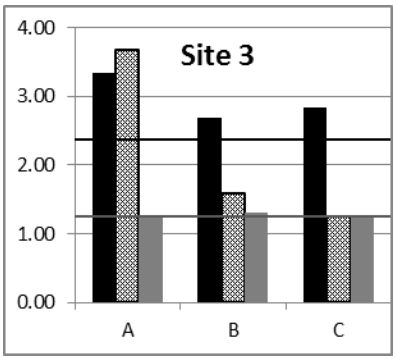
Strain Designation	Strain Classification	Number of Replications
ATCC 43890	*E. coli* 0157:H7	3
ATCC 43895	*E. coli* 0157:H7	3
SEA-13B88	*E. coli* 0157:H7	3
LJH 644 (SEA-13B88, Nalidixic Acid Resistant)	*E. coli* 0157:H7	2
LJH 537 (H1730)	*E. coli* 0157:H7	2
ATCC 14028	*Salmonella* Typhimurium	2
ATCC BAA-664	*Salmonella* Braderup	3
LJH 542 (Nalidixic Acid Resistant)	*Salmonella* Agona	3
LJH 546 (Nalidixic Acid Resistant)	*Salmonella* Montevideo	2
KMK 180 (Environmental Isolate)	*Salmonella* Typhimurium	3
ATCC 11775	Generic *E. coli*	3
ATCC 23716	Generic *E. coli*	2
ATCC 25922	Generic *E. coli*	3

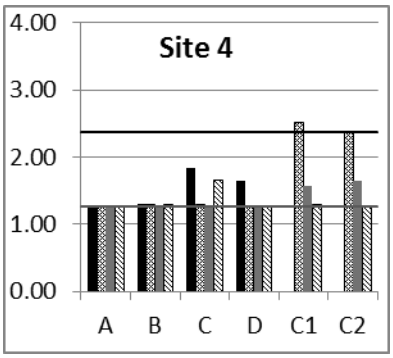
Year 1. Objective 2. Evaluation of potential field sites through examination of fecal coliform and generic *E. coli* levels in open surface waters used for overhead cooling and on apple surfaces.

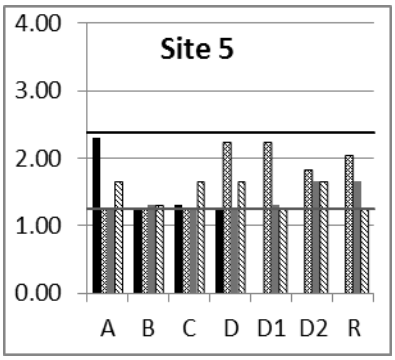
The total number of sampling events (sampling sites multiplied by number of dates sampled) was 300. Of 300 samples evaluated using the MPN method, approximately 13 samples (4.3%) exceeded the currently proposed standard for any single sample (126 MPN/100ml generic *E. coli*). Sites exceeding the single sample standard were observed in June (5), July (4) and August (4). Samples exceeding the single sample standard were collected from the original water source canal/lateral (7) and ponds (6); sample from overhead cooling spigots were not collected from these sampling locations. Only one site (1%) exceeded the proposed rolling geometric mean standard. Analysis of generic *E. coli* level results using the IDEXX Colilert®-18, Quanti-Tray®/2000 system is currently being performed. Additionally, consideration should be given to analyzing the data using average values from multiple sites within a water system. The following pages include graphs of each sampling location and sampling sites within each location. Values are reported in $\log_{10}$ colony forming units (cfu)/ml (5=100,000 cfu/ml, 3=1,000cfu/ml).

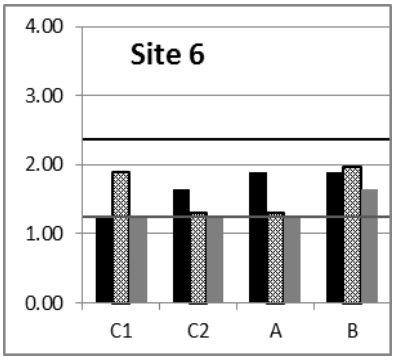


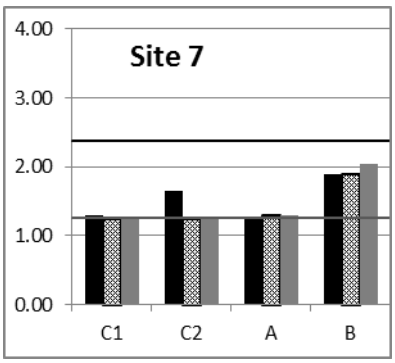


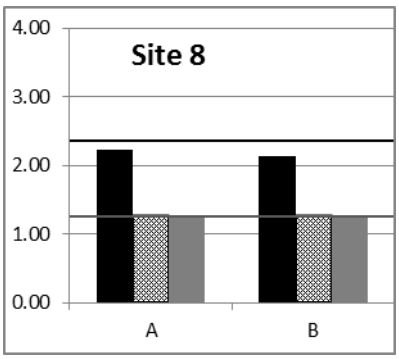


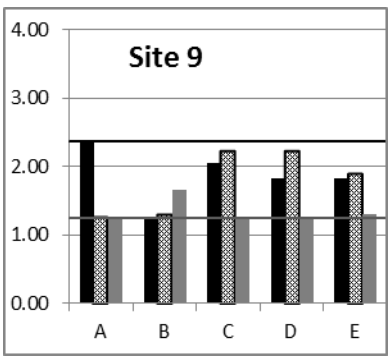


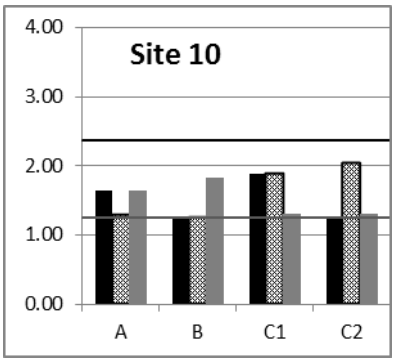


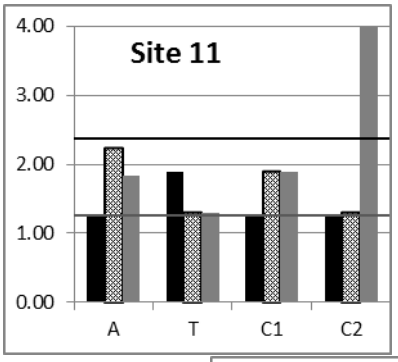


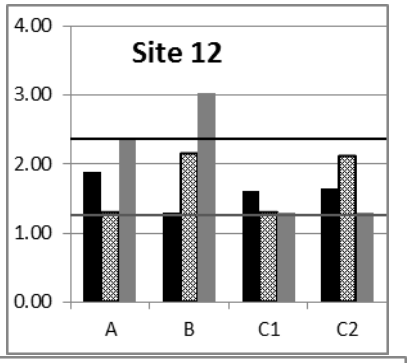




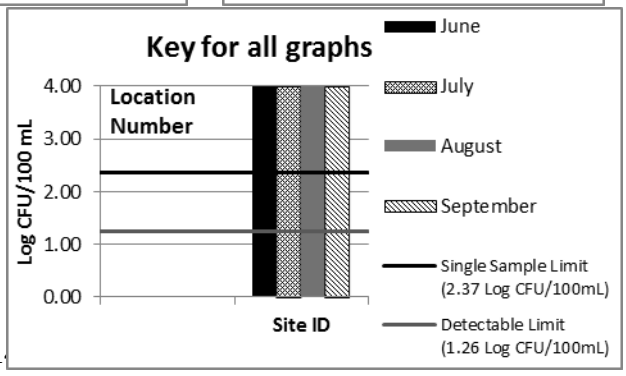


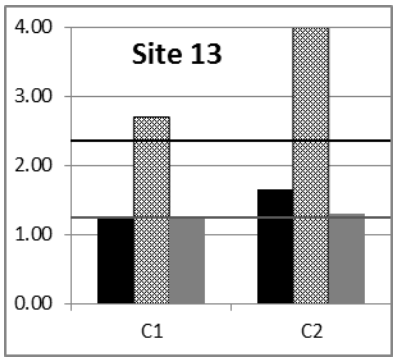


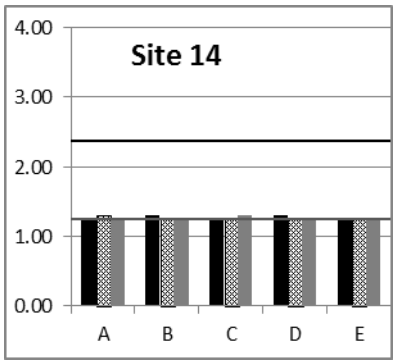


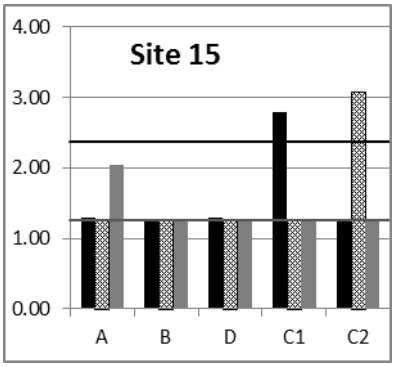


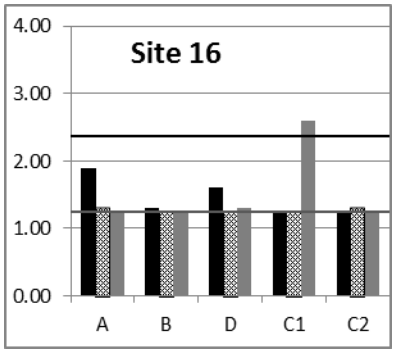
Log CFU/100mL generic *E. coli* at overhead cooling monitoring sites in Central Washington over four months (June-September 2013)

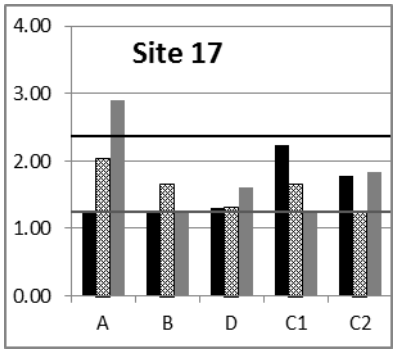


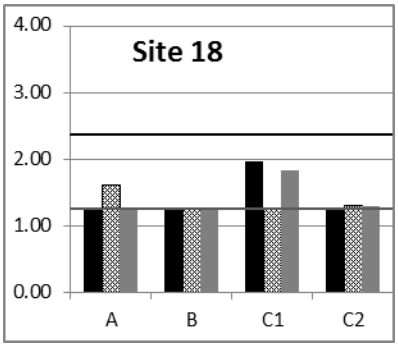


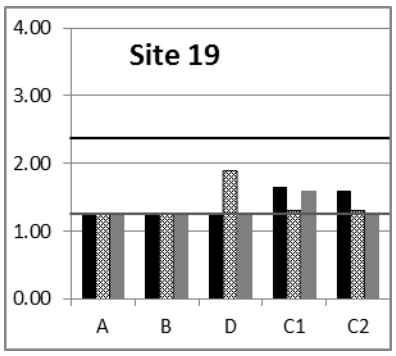


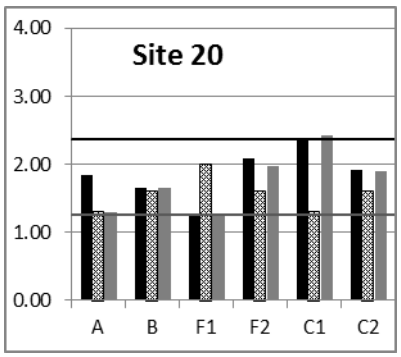


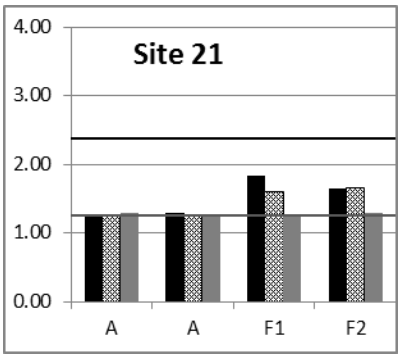


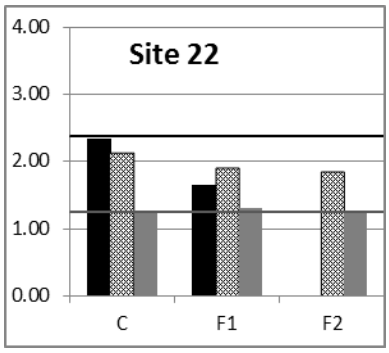


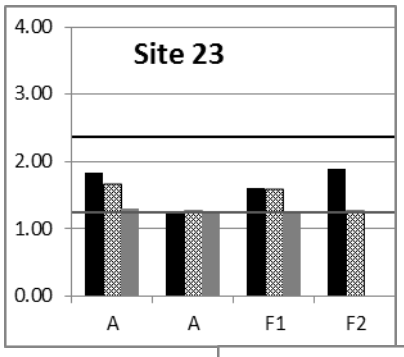


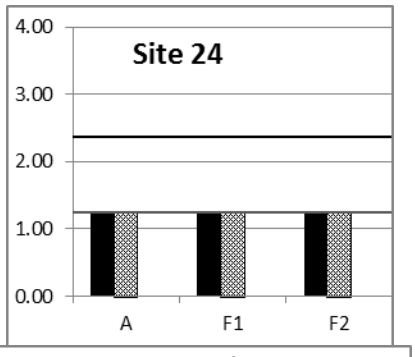




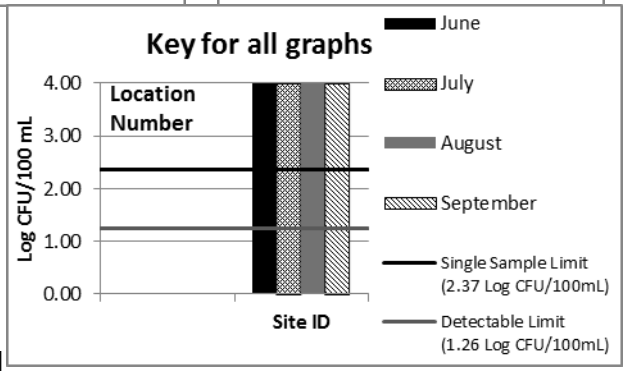








Log CFU/100mL generic *E. coli* at overhead cooling monitoring sites in Central Washington over four months (June-September 2013)



CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: MISC-11-100

Project Title: Apple microbial risk factors

PI: Richard Pleus
Organization: Intertox, Inc.
Telephone: (206) 443-2115
Email: rcpleus@intertox.com
Address: 600 Stewart St., Ste. 1101
City/State/Zip: Seattle, WA 98101

Co-PI (2): Gretchen Bruce
Organization: Intertox, Inc.
Telephone: (206) 443-2115
Email: gbruce@intertox.com
Address: 600 Stewart St., Ste. 1101
City/State/Zip: Seattle, WA 98101

Cooperators: Intertox Decision Sciences

Total Project Request: **Year 1:** $26,994 **Year 2:** $39,388 **Year 3:** $425

Other funding sources

Agency Name: The Center for Produce Safety
Amt. awarded: $66,807

WTFRC Collaborative expenses: NONE

Budget 1
Organization Name: Intertox, Inc.
Telephone: 206-443-2115

Contract Administrator: Barbara Herr
Email address: bherr@intertox.com

Item	2013	2014	
Salaries	20,370.60		
Benefits	9,667.44		
Wages			
Benefits			
Subcontract	1,850.00		
Shipping			
Supplies			
Travel		425.00	
Expert Panel	7,500.00		
Miscellaneous			
Total	$39,388.04	**$425.00**	

Footnotes: The 2013 funded amount of $39,388.04 included a 2012 budget carryover of $9,129. Of the expended funds, $1,850 was spent on subcontractor data collection and analysis work, $7,500 was invoiced for Expert Panel member payments and the remainder was spent on salary and benefits. 2014 funds requested are $425 for travel to present the research results at the Northwest Horticultural Council Meeting in March 2014 and at the Center for Produce Safety Symposium in June 2014.

OBJECTIVES

This project has five objectives. As of December 31, 2013, four of the five objectives have been completed. The final objective is scheduled for completion in January 2014. A review of the objectives and a discussion of the major findings are provided below.

1. **Gather pathogen testing data and information about mitigation measures from apple growers.**

In early 2013 Intertox conducted an industry survey focused on food safety practices growers use to protect against microbial risks. Sixty-eight companies completed surveys. In the survey, growers were asked to identify their water sources, irrigation types and evaporative cooling details. For food safety practices, growers were asked to describe their sanitation and maintenance procedures and worker training. Finally, growers were asked about microbial testing types and frequencies.

2. **Correlate pathogen levels in water used in fresh market apple production and packing operations at different points in the system to levels measured on apples before they leave the packinghouse.**

In addition to the water and microbial test data collected from testing laboratories, Intertox Decision Sciences collected packing line data consisting of pH, ORP, chlorine and temperature readings for various points along individual packing lines. Efforts were made to correlate the available data with environmental and product tests results.

3. **Characterize potential exposure to pathogens from consumption of fresh market apples and describe potential human health effects associated with these exposure levels; combine the results to estimate the risk of becoming ill from eating contaminated apples.**

The exposure assessment was completed in 2013. The exposure assessment was combined with the hazard characterization in an apple-specific quantitative microbial risk assessment (QMRA) model and FDA-iRISK modeling tool to provide risk estimates for illness from eating contaminated apples.

4. **Prepare a written risk assessment report about the findings of Objectives 1-3.**

Intertox completed a written risk assessment in December 2013. Included in the risk assessment is a discussion of the findings from Objectives 1-3.

5. **Submit the risk assessment model and report for review to an advisory panel consisting of the WTFRC, the Northwest Horticultural Council and other experts in the field of quantitative microbiological risk assessment.**

In December 2013, the risk assessment model and report were submitted to representatives from the WTFRC and the Northwest Horticultural Council (NHC) for review. After completion of the WTFRC and the NHC review, the risk assessment model and report will be submitted to an expert panel. The expert panel meeting is scheduled in January 2014.

SIGNIFICANT FINDINGS

- No Foodborne illness outbreaks associated with whole fresh apples were identified in either the CDC's FOOD database or in further Internet research.
- Research studies relating pathogens to apples were identified for *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* (*L. monocytogenes*). However, *Salmonella* and *L. monocytogenes* contamination risk assessments were ruled out at this time due to a lack of data and research related to the behavior of these pathogens on apples.
- In both the Intertox-developed risk assessment model and the FDA-iRISK® model, the risk of illness from *E. coli* O157:H7 is negligible for a given orchard treated with evaporative cooling water at *E. coli* levels found in the Washington state.

METHODS

Submit the risk assessment model and report for review to an advisory panel consisting of the WTFRC, the Northwest Horticultural Council and other experts in the field of quantitative microbiological risk assessment.

Expert panel members were selected based on their experience in the field of quantitative microbiological risk assessment modeling or their experience in the apple packing/processing industry. Panel members are charged with the duty of providing guidance and input regarding the model itself as well as feedback on the final written report. Panel member feedback will be obtained through a conference call scheduled for late January. After the call, the panel comments will be incorporated into the final report for this project.

RESULTS & DISCUSSION

The major finding from this study is that the risk of *E. coli* O157:H7-related illness from the consumption of Washington apples following a water-related contamination event in the orchard (associated with evaporative cooling) is negligible. Intertox came to this conclusion after modeling Washington apple production and packing processes using available literature study data along with data collected from packinghouses and orchards. For the QMRA, Intertox developed a model using Microsoft Excel and Palisade @Risk software to estimate potential exposure levels to these pathogens and the risk of becoming ill from eating contaminated fresh market apples. The model is based on potential contamination and growth/reduction of pathogens occurring in the field, during cooling, storage and packing. The model estimates exposures for adults children, pregnant women and the elderly using national fresh apple consumption rates, and incorporates probabilistic methods to characterize the uncertainty and variability in model inputs as well as outputs (e.g., where possible, input parameters such as temperature, time, etc., are included). Risks are characterized as the probability illness along with the total estimated number of cases. In addition to using the Intertox model, Intertox also assessed the risk of illness using the FDA-iRISK® modeling tool in the same manner as the FDA used this tool to assess the risk of illness from consuming contaminated leafy greens. As in the Intertox model, the FDA-iRISK® model characterized the probability of illness along with the total estimated number of cases. While results from both models are comparable, the Intertox model included parameters that had greater specificity for apples.

As part of the QMRA process, Intertox identified data limitations and needs for further research. In several cases, assumptions regarding pathogen growth and decrease were drawn from studies that may not reflect conditions consistent with commercial packing, (e.g., sanitizer concentrations, available nutrients, characteristics of the apple variety). Studies estimating pathogen growth and decrease in fresh market apples are also extremely limited. Even with the data limitations, however, this study is of value. The QMRA provides a baseline estimate of risk for the industry and indicates where further research efforts are needed.

Further and complete details on the study findings will be available at the end of January following completion of the Expert Panel review.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-13-105

Project Title: Effect crabapple pruning on Sphaeropsis and speck rot incidence

PI: Yong-Ki (Richard) Kim
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Co-PI (2): Mike Willet
Organization: Northwest Horticultural Council
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Co-PI(3): Tom Auvil
Organization: WTFRC
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Email: auvil@treefruitreserach.com
Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Growers: Jeff Cleveringa (Oneonta/Starr Ranch Growers) and Bob Bossen (Northern Fruit Company)

Total Project Request: **Year 1:** $20,984 (funded) **Year 2: $26,756** **Year 3:** $15,451

Other funding sources: None

WTFRC Collaborative Expenses:

Item	2013	2014	2015
Salaries			
Benefits			
Wages[1]	137	137	
Benefits	66	66	
RCA Room Rental[2]	2,100	2,100	
Shipping			
Supplies			
Travel[3]	190	190	
Plot Fees			
Miscellaneous			
Total	$2,493	**$2,493**	

Footnotes: [1]Wages and Benefits @ $15/hour (two trees pruned per hour); [2] Storage for approximately 50 cartons of fruit (1/3 of a RCA room@ $6,300 per room); [3]Travel @ $0.555 per mile for two trips each to 3 sites in central Washington.

Budget 1

Organization Name: Pace International **Contract Administrator:** Micah Dunstan

Telephone: 206-331-4777 **Email address:** micahd@paceint.com

Item	2013	2014	2015
Salaries			
Benefits			
Wages[1]	8,640	12,980	8,986
Benefits[2]	2,851	4,283	2,965
Equipment			
Supplies[3]	4,500	4,500	2,500
Travel[4]	2,500	2,500	1,000
Plot Fees			
Miscellaneous			
Total	$18,491	**$24,263**	$15,451

Footnotes: [1] Wages for a part-time research technician to work 720 hrs in year 1 and 3 and 1040 hrs in year 2 at $12/hr for performing sample collection, pathogen isolation, decay evaluation, and data management. The increase in wages for years two and three reflects a 4% rate increase. [2] Benefits 33%. [3]Supplies include culture media, chemicals, petri-dish plates for isolation of fungi. [4] Travel to orchards for sampling and harvesting is required for sampling and harvesting.

Objectives:

1. Generate practical information regarding the impact on commercial cultivars of removal of inoculum from infected 'Manchurian' crabapple pollinizers in commercial apple orchards as part of a postharvest decay IPM program
2. Understand the in-season incidence of fruit infection by *Sphaeropsis pyriputrescens* and *Phacidiopycnis washingtonensis* following winter pruning of adjacent crabapple trees
3. Evaluate the impact of crabapple pruning on the incidence of Sphaeropsis rot and speck rot in commercial cultivars following storage

Significant Findings:

- In all three orchards, pycnidia of *P. washingtonensis* were observed on crabapple twigs with dieback and canker symptoms and mummified crabapple fruit that had not been harvested in the previous year, indicating that these were the primary sources of fruit infections in the orchards.
- All crabapple trees in these orchards except for one tree that was recently replaced with a young tree in an orchard were infected by *P. washingtonensis*.
- In general, *P. washingtonensis* infections and pycnidial formations were more prevalent on mummified crabapple fruit than twigs with dieback and canker symptoms.
- Detailed pruning that removed all infected twigs, branches, and mummified fruit of crabapple trees significantly reduced the fruit infections of apples by *P. washingtonensis* during the fruit growing season.

Methods:

Orchard and plot design

Three commercial 'Red Delicious' orchards in central Washington with a previous history of either Sphaeropsis rot or speck rot incidence and with unpruned or minimal pruned 'Manchurian' crabapple trees were selected. Four replicated rows, three crabapple trees per replicate in the middle of the orchard with at least one buffer row between the replicate were randomly selected. Treatments were no pruning as a control, chainsaw pruning as a commercial standard, and detailed pruning that removed all twigs and branches with visible dieback and canker symptoms. Pruning 'Manchurian' crabapple trees were performed before bloom (April 8, 2013 in orchards A and B, and March 26, 2013 in orchard C).

Identification of fungi in infected crabapple trees

Three twigs with dieback or canker symptoms and three mummified crabapple fruit per tree from ten trees per orchard were randomly sampled at the time of pruning crabapple trees before bloom. Samples were examined under a dissecting microscope for the presence of pycnidia of the target fungi. Pycnidia similar to that of *S. pyriputrescens* or *P. washingtonensis* were then crushed in sterile water and examined under a microscope. A drop of conidia was streaked on acidified potato dextrose agar (APDA; 4 ml of a 25% solution of lactic acid per liter of medium) to establish cultures of the fungus. To confirm the pathogens on twigs with dieback or canker symptoms, samples were surface-disinfested by immersing 5 min in 0.6% sodium hypochlorite solutions, rinsed twice with sterile deionized water, and air-dried in a laminar hood. Small segments were cut from the margins between the diseased and healthy tissues of the twigs and embedded in APDA. The presence of pycnidia on mummified crabapple fruit was also microscopically examined and the viability of pycnidial spores was tested as described above. Fruit were then gently sprayed with 70% ethanol and allowed to dry in

a hood. The skin of the diseased area on the fruit was peeled off using a sterile scalpel, and then small fragments of fruit flesh were cut and placed on APDA. Plates were incubated at room temperature (22 ± 1°C) for up to 7 days. The fungus was identified based on the morphological characteristics on APDA and confirmed according to the descriptions of *P. washingtonensis* (Xiao et al, 2005).

In-season monitoring of fruit infections

To monitor fruit infections among the treatments during the fruit growing season, ten apple fruit from trees adjacent to the treated (chainsaw and detailed) or control (unpruned) crabapple trees in replicate (four replications per treatment) were collected every 5-6 weeks from 3 weeks after petal fall to harvest. After surface-disinfestation in 0.6% sodium hypochlorite solutions as described above, stems and sepals of the fruit were aseptically excised and embedded on APDA. The presence and absence of *P. washingtonensis* were examined for up to 7 days. All data in percentage were arcsine-transformed before performing analysis of variance. Since there was no significant interaction between the treatments and sampling times in all three orchards, data were pooled and the mean separation was conducted using the least significant difference ($P = 0.05$).

Results and Discussion:

Identification of fungi in infected crabapple trees

In all three orchards, pycnidia were observed on crabapple twigs with dieback and canker symptoms and mummified crabapple fruit that had not been harvested in the previous year, indicating that these were the primary sources of inoculum for fruit infection in the orchard. Pycnidial spores from all samples with pycnidia were microscopically examined and identified as *P. washingtonensis* according to the descriptions of Xiao et al. (2005). The morphological characteristics on PDA also confirmed that the fungus grown out from the pycnidial spores were *P. washingtonensis*. All sampled crabapple trees, except one tree from orchard A that was recently replaced with a young tree, harbored viable pycnidia of the fungus at the time of pruning (Table 1). Percent samples with viable pycnidia ranged from 53 to 57% of the sampled twigs and 77 to 83% of the fruit mummies in these orchards. The fungus was isolated from 73 to 87% of the diseased twigs and 90 to 100% of the mummified crabapple fruit. In general, mummified crabapple fruit had higher percentages of *P. washingtonensis* infections and pycnidial formations than diseased twigs of crabapple trees.

Table 1. Identification and inoculum availability of *Phacidiopycnis washingtonensis* on crabapple trees at the time of pruning before bloom in three 'Red Delicious' orchards in central Washington

Orchard	Type of sample[a]	% Trees with viable pycnidia[b]	% Samples with viable pycnidia	% Samples with *P. washingtonensis*[c]
A	Twig	90.0	53.4 ± 7.4[d]	86.7 ± 5.4
	Mummy	90.0	83.3 ± 10.3	90.0 ± 10.0
B	Twig	100.0	53.3 ± 5.5	80.0 ± 5.4
	Mummy	100.0	76.7 ± 7.1	100.0 ± 0.0
C	Twig	100.0	56.7 ± 5.1	73.4 ± 6.7
	Mummy	100.0	83.4 ± 5.6	100.0 ± 0.0

[a] Three samples each for twigs with dieback or canker symptoms and mummified fruit of crabapple per tree, ten trees per orchard were randomly collected.

[b] Viability of pycnidial spores was assessed by plating conidia on acidified potato dextrose agar and examined for the growth of *P. washingtonensis*.

[c] Isolation of *P. washingtonensis* was made from diseased crabapple twigs and mummified fruit.

[d] Values are mean and standard error based on the samples from 10 trees.

In-season monitoring of P. washingtonensis on apple fruit

Detailed pruning that removed all infected twigs, branches, and mummified fruit of crabapple trees significantly reduced the infections of *P. washingtonensis* on apple fruit compared with the unpruned control and chainsaw pruning in all three orchards (Fig. 1). Although chainsaw pruning of crabapple trees was not as effective as detailed pruning, it significantly decreased fruit infections of apples compared to the unpruned control in two of three orchards. It is apparent that removing infected crabapple tissues by *P. washingtonensis* in the apple orchard can reduce the fruit infections on apple fruit during the fruit growing season. However, we could not demonstrate a complete control of fruit infection on apples by pruning crabapple trees. Since our study was conducted in the middle of the orchards where unpruned crabapple trees are surrounded, it is possible that conidia of *P. washingtonensis* disseminated by wind-blown rain or irrigation water may deposit on fruit in our experimental blocks. Regardless of pruning treatments, the *P. washingtonensis* infection on apple fruit in orchard A was higher than those in orchard B or C (Fig. 1). This might be due to the different irrigation methods; orchard A was irrigated by overhead sprinklers and orchards B and C were irrigated by under-tree sprinklers. However, our study was not designed to test the effect of different irrigation methods.

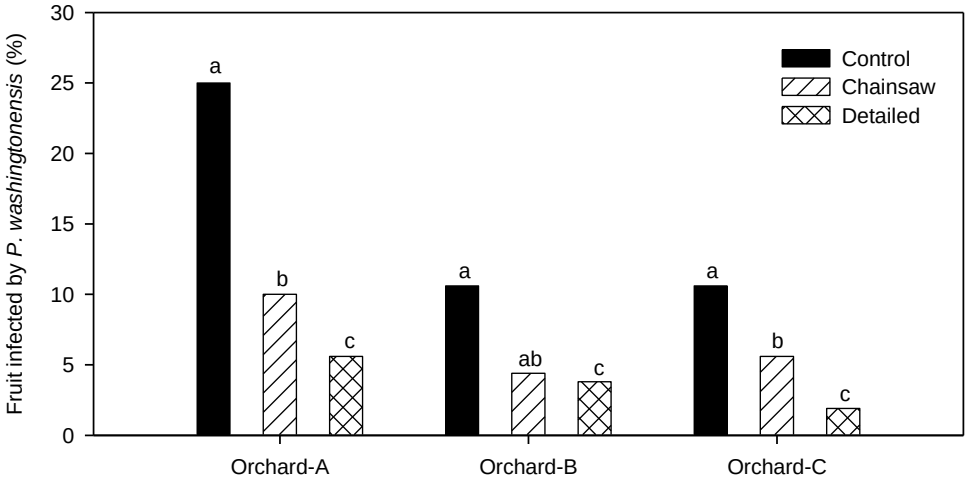

Fig. 1. In-season monitoring of *Phacidiopycnis washingtonensis* on stems and sepals of apple fruit adjacent to crabapple trees that were unpruned (control), chainsaw pruned, and pruned to remove all infected twigs, branches, and fruit mummies in three commercial 'Red Delicious' orchards. Values within an orchard, when followed by a common letter, are not significantly different according to the analysis of variance and least significant difference ($P = 0.05$).

Work next year

All fruit harvested in 2013 will be monitored monthly for the development of speck rot in cold storage. This coming year, we will continue the study at two of the three orchards to investigate the effect of crabapple pruning for two consecutive years on the reduction of fruit infections by *P. washingtonensis* and are seeking to add at least one orchard with a history of Sphaeropsis rot incidence in 2014.

Literature Cited:

Xiao, C. L., Rogers, J. D., Kim, Y. K., and Liu, Q. 2005. *Phacidiopycnis washingtonensis* – a new species associated with pome fruits from Washington State. Mycologia 97:464-473.

CONTINUING PROJECT REPORT **YEAR:** 2013

Project Title: Programs to increase packouts of apples

PI: Ines Hanrahan
Organization: Washington Tree Fruit Research Commission
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
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City/State/Zip: Union Gap, WA, 98903

Cooperators:
WTFRC internal program: Manoella Mendoza, Tory Schmidt, Udel Mendoza, Felipe Castillo
Scientists: James Mattheis, David Rudell
Product suppliers: Valent Biosciences
Others: Grower collaborators, WTFRC seasonal crew, Glade Brosi (Stemilt)

Other funding sources
All supplies and chemicals were donated by industry suppliers.

Budget 1
Organization Name: WTFRC **Contract Administrator: Kathy Coffey**
Telephone: 509 665 8271 **Email address: Kathy@treefruitresearch.com**

Item	2012	2013	2014 (proposed)
Salaries	10,306	9,000	10,000
Wages	23,073	8,336	10,000
Equipment + supplies	0	0	
RCA rental	2,100	1,800	1,800
USDA rental	0	0	
Travel	0	0	
Total gross costs	35,839	19,136	21,800
Reimbursements	(7,000)	(8,000)	(8,000)
Total net costs	**28,839**	**11,136**	**13,800**

Footnotes:
Entire budget is based on fiscal year August 1st– July 31st the following year, i.e. 2013 reflects costs from Aug.1, 2012 until July 31st, 2013.
Salaries: incl. benefits, proportional time spent on outlined projects for Hanrahan, Castillo, Schmidt
Wages: incl. benefits, covers time slip expenses, benefit rate at 42%
Supplies: experimental fruit, storage boxes and trays donated
RCA rental: numbers based on fiscal year (@ approx. $6,300/room/year)
Reimbursements: monetary contributions by chemical suppliers

NOTE: Budget for informational purposes only. Research is funded through WTFRC internal program

OBJECTIVES

1. Determine harvest maturity and storage behavior of red strains of Honeycrisp.
2. Investigate methods to deliver consistent eating experiences of Honeycrisp following long term storage.
3. Determine time course of physiological disorder development of Honeycrisp.
4. Determine the influence of sun damage on harvest maturity and storage potential of Honeycrisp.
5. Expand collaborative efforts with other research programs working on fruit quality management.

SIGNIFICANT FINDINGS

Objective 1: A strain evaluated in 2012 had 100% red color, no bruising and bitterpit, but taste and texture were altered, compared to standard Honeycrisp. Fruit remained very firm in storage. No soft scald developed, but soggy breakdown (8-13%) and cavities (8%) were recorded.

Objective 2: Fruit treated with 1-MCP delivered a more consistent product after 6 months of storage, regardless of storage regime. Best tasting fruit was achieved when held for 4.5 months in CA, followed by 1.5 months in RA.

Objective 3: Fruit with high bitterpit levels could benefit from storage at lower temperatures, especially if chilling disorder potential is low, while fruit with sensitivity to chilling injury should not be stored at 33F for prolonged periods of time.

Objective 4: Fruit with slight sunburn damage at harvest (Y1-2 on McFerson/Schrader scale) had advanced starch degradation at harvest, and developed delayed sunburn in storage. Post storage fruit was firmer and sweeter.

Objective 5: Ongoing collaborative efforts across disciplines, institutions, and regions leverage funding and increase relevance and impact of research.

METHODS

Red strains of Honeycrisp: Fruit was picked by a commercial crew and obtained from a collaborator at the warehouse. It was stored for 1 week at 50F, and then cooled down to 36F in a) four months RA, or b) six months CA with 1% carbon dioxide and 2% oxygen. Standard maturity and defect analysis was performed at harvest, and after 7 days at room temperature following cold storage.

Honeycrisp flavor retention: We utilized second pick fruit from a mature v-trellis. Half the fruit was treated with 1-MCP. After one week at 50F preconditioning, fruit was subjected to 3 storage treatments: 1. RA for 6 months, 2. CA for 6 months (1%CO_2, 2% O_2), 3. CA for 4.5 months + RA for 1.5 months. Final evaluations after 7 days at room temperature included flavor ratings (absent/present) of: apple flavor, no flavor, off flavor.

Disorder development: In 2012 we harvested fruit sequentially (3 picks) from 2 orchards and immediately placed into a) 33F cold storage or b) 50F for one week, followed by 36F for 12 weeks. Fruit was rated for soft scald and bitterpit weekly for 13 weeks.

Sunburn vs. Honeycrisp quality: In 2012 we selected 100 fruit each (clean and slight sunburn (Y1or Y2)) approximately four weeks prior to the anticipated first pick. We followed the fruit color and sunburn development at harvest, determined fruit maturity at harvest, and stored fruit for 6 months in CA (same as red strains).

RESULTS & DISCUSSION

Red strains of Honeycrisp: The strain evaluated in 2012 had 100% red color, no bruising and bitterpit, but taste and texture were altered compared to standard Honeycrisp both at harvest and after four month RA or six month CA storage. Fruit remained very firm in storage and subsequent week at room temperature (Table 1). No soft scald developed, but soggy breakdown (8-13%) and cavities (8%) were recorded. After three days at room temperature, an unnamed skin discoloration developed on some fruit.

Table 1: Fruit maturity development of a red strain of Honeycrisp in 2012.

Treatment	*Firmness (lbs.)*	*SSC (Brix)*	*TA (%)*	*Soft scald (%)*	*Soggy Breakdown (%)*	*Cavities (%)*
At harvest	19.0 ns	12.1 ab	0.639 a	0 ns	0 b	0 b
4 months RA	19.0	12.8 a	0.476 b	0	8 ab	0 b
6 months CA	18.3	12.4 a	0.414 b	0	13 a	8 a

Honeycrisp flavor retention: Although appearance of fruit sold in local stores from late storage is typically good, the flavor can vary dramatically between fruit from the same batch. Most notably are bland tasting fruit and fruit with unpleasant off flavor in the later part of the storage season. In this experiment, fruit treated with 1-MCP retained flavor well and little or no off flavor was developed in 6 month storage (Figure 1). Further, switching CA rooms to RA for several weeks before packing eliminated off flavor and resulted in 90% of fruit with 'apple flavor', the most preferred category (Figure 1).

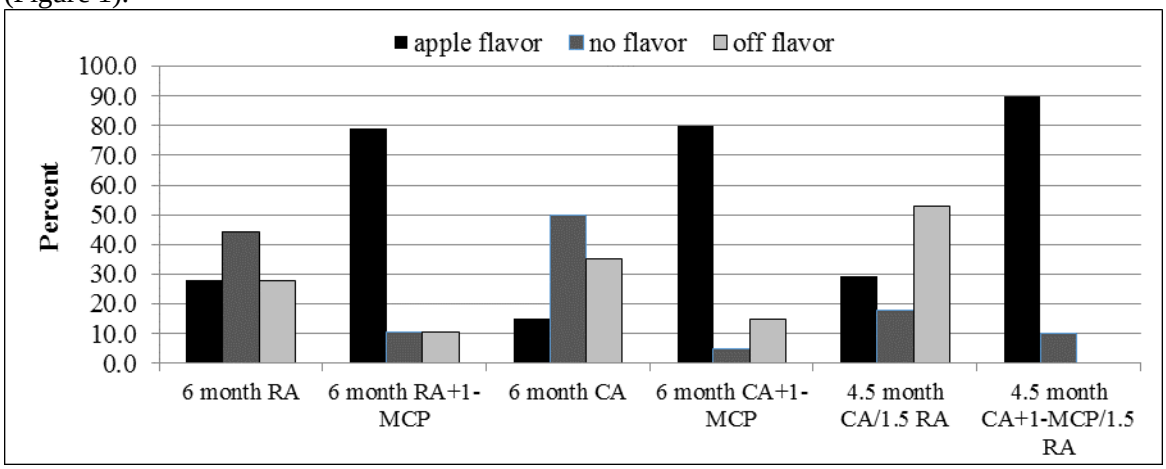

Figure 1: Flavor profile of Honeycrisp apples stored for six months.

Disorder development: Orchards exhibit different levels of sensitivity to develop bitterpit or soft scald in storage and depending on the type of storage regime chosen (Figures 2&3). For example, orchard 1 was not sensitive to chilling disorder development, but exhibited large amounts of bitterpit when placed in a delayed cooling system recommended for Honeycrisp. Orchard 2 was sensitive to chilling disorder development and benefitted from being placed in a storage system utilizing delayed cooling (Figure 2). In summary, if it were possible to determine at harvest, what the likelihood of disorder development for each incoming lot may be, packouts of Honeycrisp could be dramatically improved. In the example shown in Figure 3, fruit from orchard 1 would develop 10-45% bitterpit in the delayed cooling storage, but only 0-15% bitter pit was observed in fruit if placed in 33F common cold storage, without additional losses due to soft scald (Figure 2). Orchard 2 on the other hand would

loose 15-65% of fruit to soft scald without delayed cooling and general storage at higher temperature at a tradeoff of 0-5% of fruit with bitterpit due to higher storage temperatures (Figures 2&3).

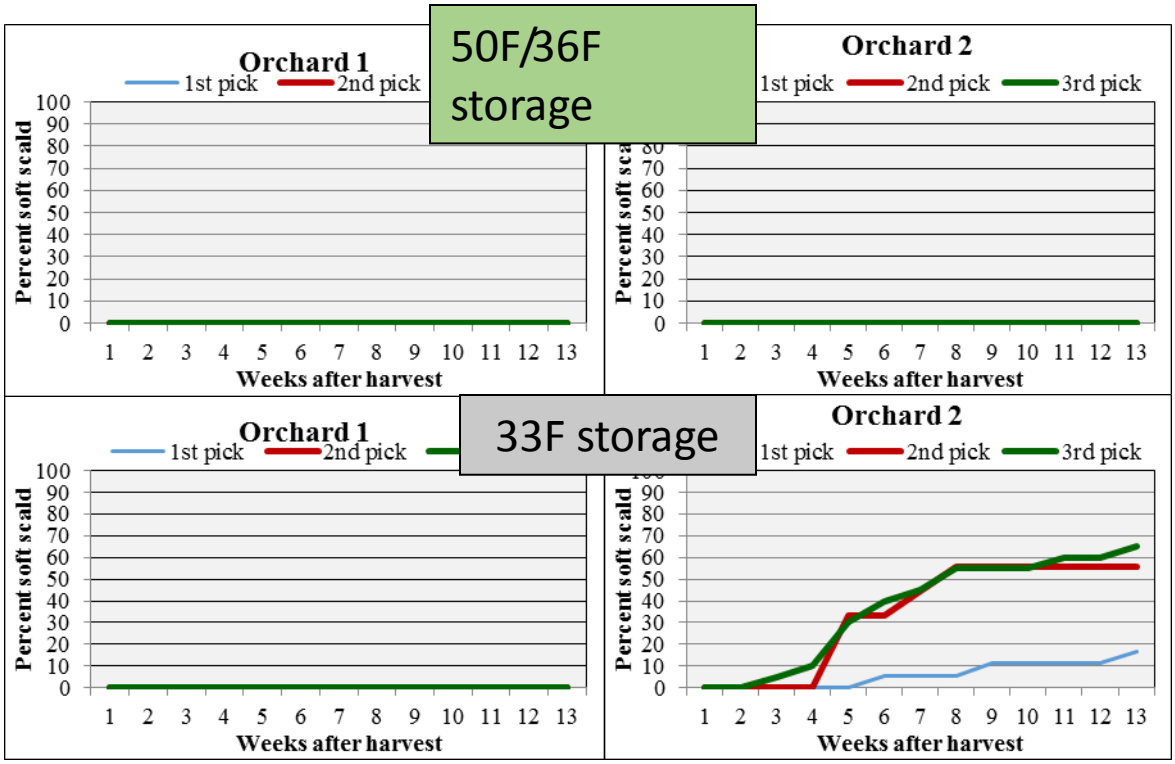

Figure 2: Soft scald development of two orchards.

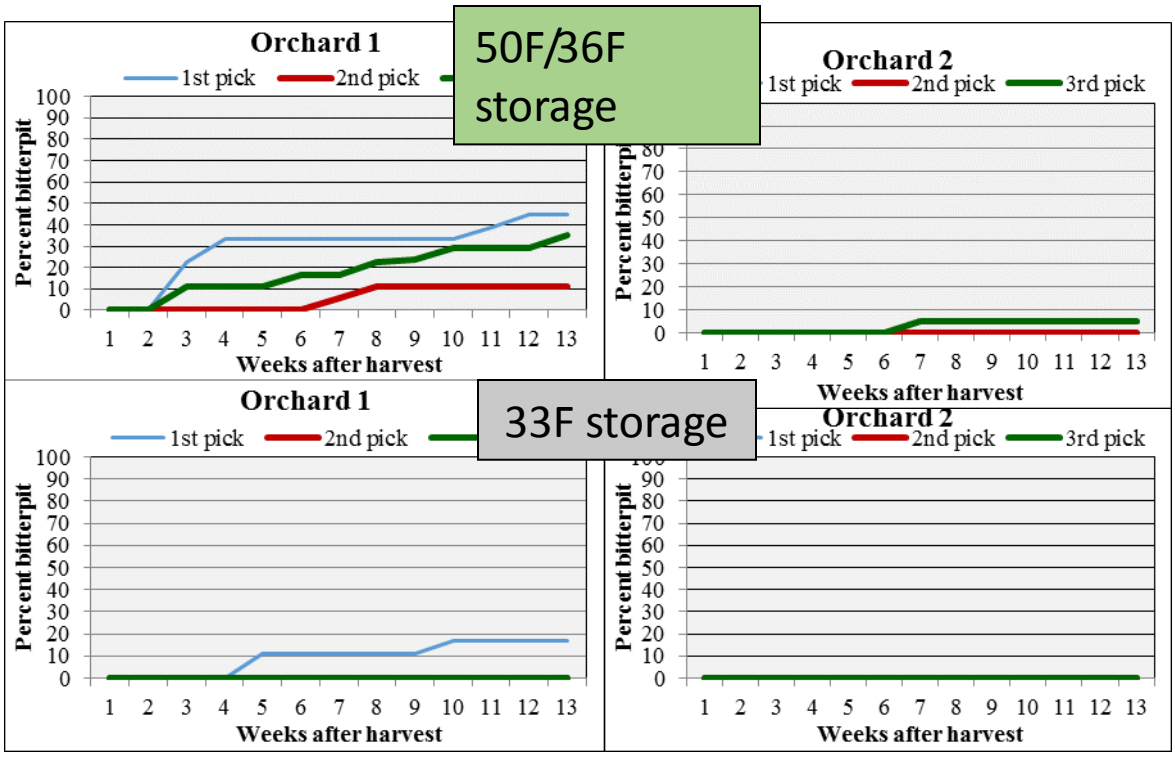

Figure 3: Bitterpit development of two orchards.

In addition, harvest timing (Figure 2) will help minimize chilling disorders such as soft scald.

Sunburn vs. Honeycrisp quality: Fruit with slight sunburn damage at harvest (Y1-2 on McFerson/Schrader scale) had advanced starch degradation at harvest (data not shown). When evaluated at harvest in the laboratory, only 44% of fruit with known sunburn were identified correctly (Table 4). Of that fruit, an additional 22% developed delayed sunburn in storage (Table 2). This would have caused a downgrading of that fruit, because of a darkening of the sunburn. Contrary, fruit without known sunburn at harvest, stayed clean throughout storage. Post storage fruit with preharvest sunburn was firmer and sweeter (Table 2).

Table 2: Delayed sunburn development of undamaged and sunburned fruit after six months storage.

	HARVEST SUNBURN INCIDENCE					
TREATMENT	Sunburn (%)	Y1 (%)	Y2 (%)	Y3 (%)	Tan (%)	Black (%)
Sunburn harvest	44 b	41 a	3 b	0 ns	0 ns	0 ns
Sunburn 6 month CA storage	66 a	38 a	22 a	6	0	0
Control harvest	6 c	3 b	0 b	0	0	0
Control 6 month CA storage	0 c	0 b	0 b	0	0	0

Collaborative research

We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to pre-and postharvest fruit quality management (Table XXX).

Table XXX. 2013 WTFRC collaborations on pre- and post- harvest fruit quality management projects.

COLLABORATOR(S)	PROJECT	COMMENTS/HANRAHAN ROLE
Rudell	Disorder toolbox	Cooperator on SCRI project
NSure*	Honeycrisp disorder ID	WA field testing
Killinger	Packingline microb. safety	Field support (see AP-09-906)
Killinger	Overhead cooling	Field support (see AP-12-108)
Pleus	Apple microbial risk factors	Cooperator on CPS/WTFRC project
Brunner, MSU	SSCDS	Cooperator on SCRI project
Evans/Auvil	WSU Breeding: P3	Storage evaluation (see Auvil/Evans cont.)
Mattheis/Rudell	Extend HC storage life	CO-PI
Killinger	Microbial safety of bins	Collaborator on CPS project

*project costs covered by companies

CONTINUING PROJECT REPORT **YEAR**: 2013-14
WTFRC Project Number: AP13-103

Project Title: Identification of procedures to extend 'Honeycrisp' storage life

PI: Jim Mattheis
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Co-PI: Ines Hanrahan
Organization: WTFRC
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Email: hanrahan@treefruitresearch.com
Address: 2403 S 18th Street, Suite 100
City/State/Zip: Union Gap, WA 98903

Cooperator: Tory Schmidt, WTFRC

Total Project Request: **Year 1:** $72,714 **Year 2: $68,714** **Year 3:** $68,714

Other funding sources

Agency Name: AgroFresh Inc.
Amt. requested/awarded: $12,000
Notes: Funds represent 25% of project specified for 'Honeycrisp' research

WTFRC Collaborative expenses:

Item	2013	2014	2015
Wages	$8,000	$8,000	$8,000
RCA Room Rental	$630	$630	$630
Miscellaneous	$4,000[1]		
Total	$12,630	**$8,630**	$8,630

Footnotes: [1]Funds for acquisition of a differential absorbance (DA) meter for maturity assessment

Organization Name: USDA, ARS **Contract Administrator:** Chuck Myers
Telephone: (510)559-5769 **Email address:** Chuck.Myers@ARS.USDA.GOV

Item	2013	2014	2015
Salaries	$39,586	$39,586	$39,586
Benefits	$19,498	$19,498	$19,498
Supplies	$1,000	$1,000	$1,000
Total	$60,084	**$60,084**	$60,084

Footnotes: Salary, benefits for GS-6 technician

OBJECTIVES

1. Characterize differences in orchards that produce fruit with a history of disorder resistance or susceptibility.
2. Determine utility of ethylene green life, fruit density, titratable acidity, chlorophyll fluorescence and chlorophyll absorbance as additional indicators of storability.
3. Identify alternatives to the 7 day 50 ^{o}F pre-conditioning protocol.
4. Identify factors contributing to CO_2 injury occurring during the initial 30 days after harvest.
5. Identify CA protocols that maximize quality retention and minimize disorders.

SIGNIFICANT FINDINGS

- The DA meter enabled non-destructive measurements of fruit maturation on the tree and ripening in storage.
- DA meter values did not identify chilling sensitive fruit as evaluated during the 2013 harvest.
- Ethylene green life varies with maturity at harvest and orchard lot.
- Humidity during conditioning did not influence chilling disorder development.
- Conditioning less than 7 days can enhance chilling injury incidence.
- Chlorophyll fluorescence during cooling changes with fruit temperature.
- High CO_2 during SmartFresh treatment did not cause CO_2 injury.

METHODS

1. *Preharvest factors*:

1.1. Orchard location: 12 orchards with a known history of disorder resistance or susceptibility were monitored from bloom to harvest. Data was collected on orchard climate, fruit phenological phases, and horticultural practices. Fruit was picked at the second commercial pick and placed in 33F cold storage for 3 months to force chilling injury symptoms. Common fruit maturity and a full defect analysis was performed at harvest and after storage.

1.2. Fruit position in tree: in a mature v-trellis block 12 trees (6 east, 6 west- facing) were divided into 3 sections: upper (> 7ft.), medium (4.5-6.5ft.), low (<4ft.). Cropload was determined for each tree. In each section 6 fruit were marked and evaluated twice weekly until harvest for: red color, fruit size, sunburn, DA meter. Four trees were harvested at each of 3 harvest dates. Fruit was placed in 33F cold storage and DA meter and disorder readings performed weekly for 3 months.

1.3. DA meter values vs. chilling sensitivity: Fruit harvest was conducted at a commercial second pick timing. Only fruit approx 5' off the ground with a minimum of 50% red color and free from damage or disorders was harvested. At harvest, initial DA meter readings were taken in the field and fruit sorted according to three DA categories, based on readings within this block (class1= 0.40-0.69; class2=0.70-0.84, class3=0.85-1.20). Fruit maturity was determined at harvest and after 3 month in 33F storage. During storage, DA meter and disorder readings were performed weekly.

1.4. Altered light and temperature: a pilot study was performed using single drape nets approx. 30ft long, supplied by Extenday, in 3 orchards. Nets were compared to fruit receiving evaporative cooling (EC) vs fruit receiving Raynox + EC. Temperature sensors were placed under the nets and within the EC section. Fruit conforming to commercial maturity requirements were harvested sequentially, conditioned 7 days at 50F and then stored in CA for 8 months.

2. *Postharvest factors:*

Maturity assessment: Fruit from multiple lots will be harvested over a several week period covering pre- through post- commercial maturity. Fruit from each orchard/harvest will be conditioned 7 days at 50 ^{o}F and then stored in air or CA for up to 8 months. At harvest, starch index, firmness, soluble solids content, titratable acidity, weight, red and background color, internal ethylene content were assesed as was ethylene green life, chlorophyll fluorescence and absorbance.

2.1 Ethylene green life is determined by monitoring ethylene emission over an extended period rather than once at harvest. Green life is the number of days required for a sustained increase in ethylene production compared to the initial value. Chlorophyll fluorescence and absorbance were measured at room temperature using HarvestWatch and a differential absorbance (DA) meter, respectively. Fruit was pre-conditioned at 50 ^{o}F for 7 days then stored in air at 36 ^{o}F, or placed directly into 33 ^{o}F at harvest to assess chilling disorder sensitivity.
2.2 Pre-conditioning protocol: Cooling parameters (cooling rate, duration at initial temperature, cooling rate to final temperature) will be examined using multiple lots. Initial conditioning temperature and final storage temperature will be achieved by either rapid (refrigerated chambers held at desired final temperature) or slow (refrigerated chamber temperature setpoint decreased stepwise) cooling. During this period, fruit temperature and chamber rH will be monitored. CO_2 injury susceptibility: Fruit from multiple orchards was exposed at harvest to up to 4% CO_2 in air at 50 ^{o}F with or without SmartFresh. Following 7 days at 50 ^{o}F, fruit will be stored in air at 36 ^{o}F.
2.3 CA effects on fruit quality and disorders: Fruit from multiple orchards harvested sequentially will be pre-conditioned in air or CA then stored in air or (2% O_2, 1% CO_2). Fruit volatile production and quality will be assessed at regular intervals up to 8 months. Initial results will provide the basis to plan additional CA experiments to assess utility of dynamic CA regimes to impact fruit volatile production capacity. CO_2 injury: fruit was pre-conditioned in air then stored in CA in up to 5% CO_2 with 2% O_2. Rapid CA: fruit was pre-conditioned at 50 ^{o}F in air or CA (2% O_2, 0.5% CO_2) for 7 days, then temperature reduced to 37 ^{o}F. Fruit pre-conditioned in air were stored in air or CA at the lower temperature.

RESULTS & DISCUSSION

Orchard location: Experiments are on-going.

1.1. Fruit position in tree: (preliminary results, experiments in progress)

- DA-value range (Figure 1):
  - **1.31**(0.53-1.68) at beginning of experiment (avg fruit size: 3.2 inches, < 25% color)
  - **0.95** (0.00-1.38) at first pick (avg size: 3.37 inches and 50-75% color)
  - **0.73** (0.00-1.24) at third pick (avg size: 3.58 inches, >75% color)
  - Rate of decrease: 0.14/week until first pick, then 0.12/week
- Fruit position: lower canopy fruit has higher values than fruit in top of canopy, but effects are slight and fruit orientation within canopy has bigger impact (Figure 2).
- Inversely correlated to starch, soluble solids, firmness; no correlation to TA (not shown).
- Best fruit maturity: DA-value 0.7-0.85 (based on mature v-trellis).
- Both red color and sunburn increased in all parts of the canopy until first pick (not shown).
- Sunburned fruit shows lower DA-values, even when red color masks symptoms (not shown).

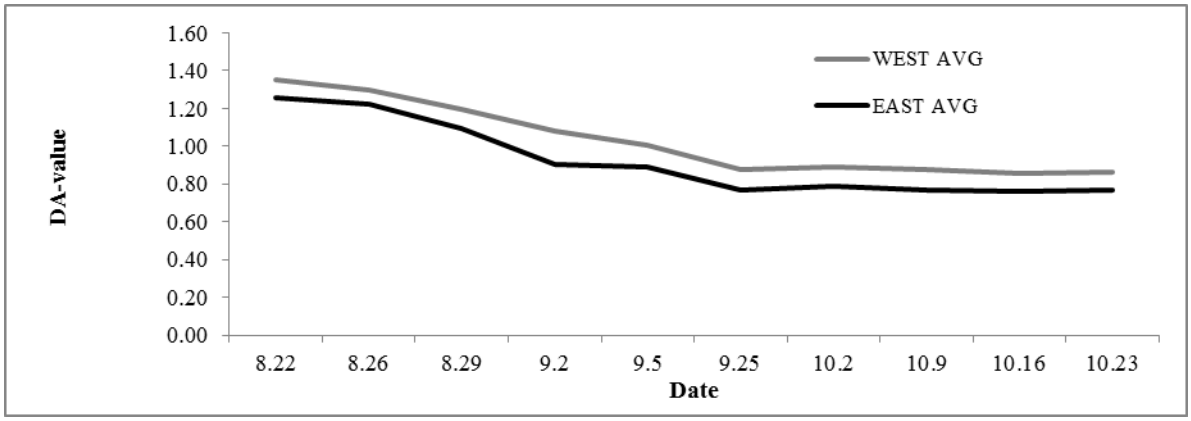


Figure 1: Average DA-value of 108 fruit on the west or east side of the trellis pre- and postharvest.

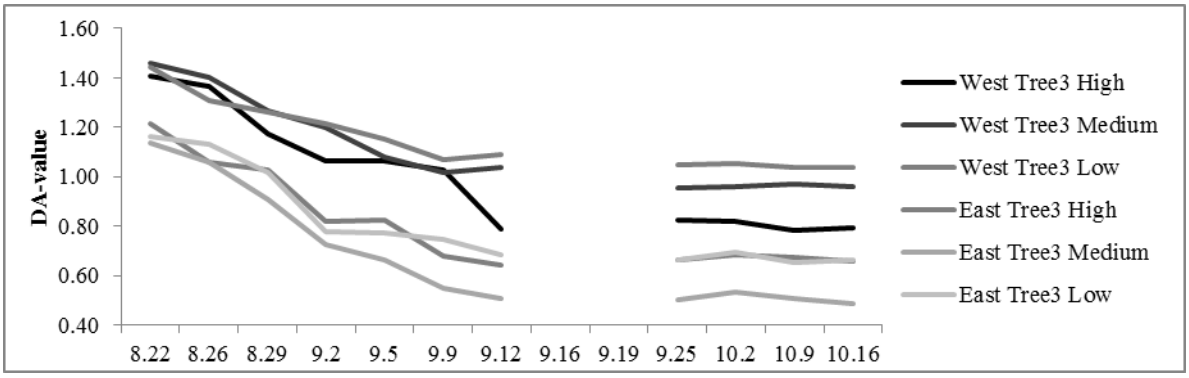


Figure 2: DA-value development on the tree and in storage in three sections and two orientations (east vs. west) of the trees within the v-trellis canopy.

1.2. DA meter values vs. chilling sensitivity:

- Dividing fruit into three classes based on DA meter readings resulted in a spread of maturity at harvest and after storage (Table 1).
- Background color, firmness, soluble solids content and starch degradation increased with decreasing DA values. Titratable acidity not correlated to DA meter readings (Table 1).
- Based on red color alone, fruit in all DA-classes was commercially harvestable, but maturity spread can be further separated by utilizing DA meter values (Table 1).
- Bitterpit, stem punctures, splits and sunburn levels were highest in most mature fruit (Table 2).
- The DA index of damaged tissue ranged between 0.3 and 0.6 (data not shown).
- Fruit developed visible CI symptoms after the DA index declined below 0.5 (Figure 4).

Table 1: Fruit maturity spread within DA categories at harvest and after three month RA storage.

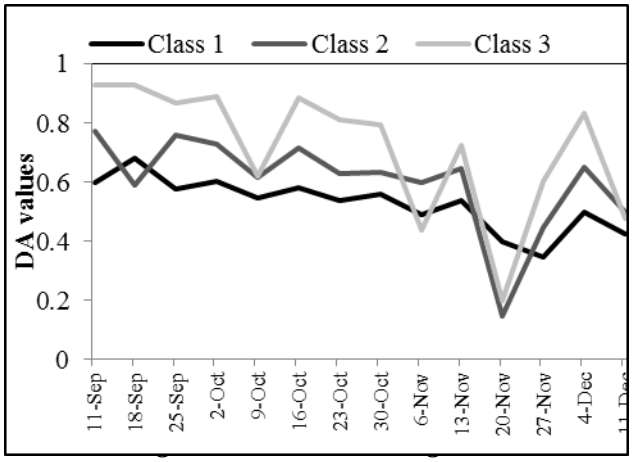
Date	***Treatment***	***Size***	***Weight***	***Color***	***Background color***	***Firmness***	***SSC***	***TA***	***Starch***	***DA***
		(inches)	(grams)	(1-4)	(1-3)	(lb)	(Brix)	(%)	(1-6)	(0-5)
Sept.11	Class 1	3.39	262.9	3.9	3.0	15.1	14.4	0.54	5.4	0.60
	Class 2	3.40	258.5	3.7	2.5	14.2	13.3	0.48	4.9	0.77
	Class 3	3.43	255.4	2.9	1.3	13.8	12.6	0.53	4.1	1.03
Dec. 11	Class 1	3.38	256.8	3.90	2.1	15.0	14.4	0.45	NA	0.43
	Class 2	3.43	261.5	3.79	1.7	14.8	14.2	0.46	NA	0.50
	Class 3	3.40	260.1	3.40	1.4	14.3	13.8	0.48	NA	0.48

Conclusions: fruit with a DA index between 0.40 and 0.84 at harvest was less suited for long term storage while fruit with a DA index of 0.85 and higher had a greater chance of reaching adequate packouts when stored longer. Soft scald risk was not identified by DA-values. DA meter categories should be further adjusted.

Table 2: Defects within DA classes after three months of RA storage at 33F.

Defect	*Class 1*	*Class 2*	*Class 3*
Sunburn (%)	51.3	31.3	15.0
Stem Puncture (%)	17.5	5.0	17.5
Watercore (%)	0.0	0.0	0.0
Cavity (%)	0.0	0.0	0.0
Internal Browning (%)	0.0	0.0	0.0
Bitter Pit (%)	10.0	2.5	3.8
Splits (%)	7.5	2.5	2.5
Softscald (%)	3.8	10.0	1.3

Using non-destructive techniques, such as the DA meter, could help commercial 'Honeycrisp' growers to accurately separate fruit within lots into different maturity classes to establish the length of time in storage prior marketing, when common measurements such as starch, red color, and background color development yield less reliable results.



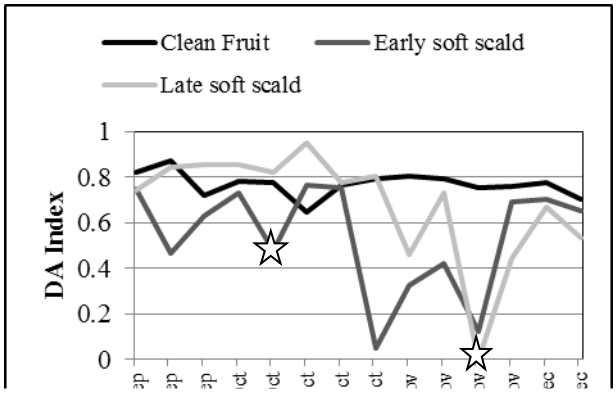
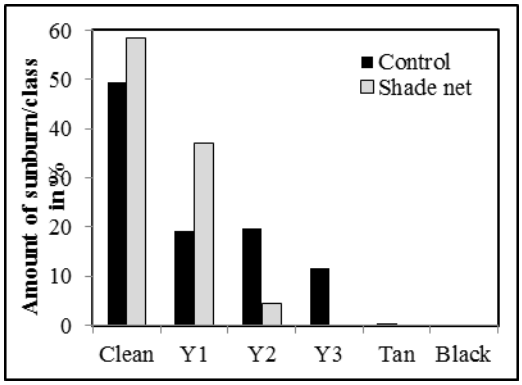


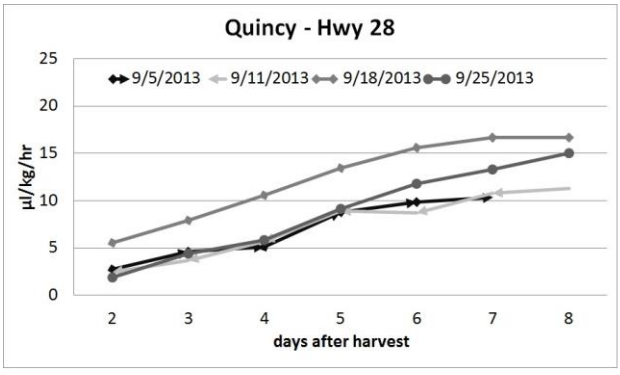
Figure 4: DA values compared to onset of visible soft scald symptoms.
("X" indicates 1st symptoms of CI development)

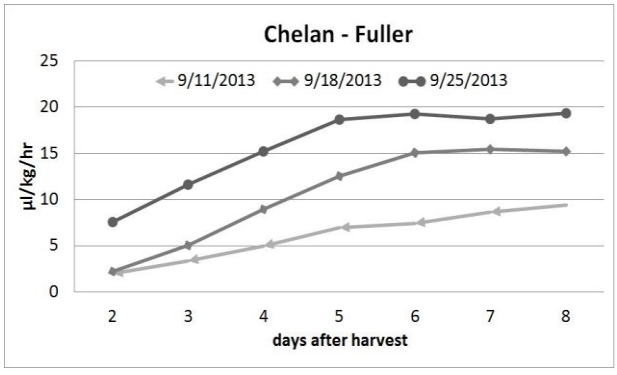
1.3. Altered light and temperature:

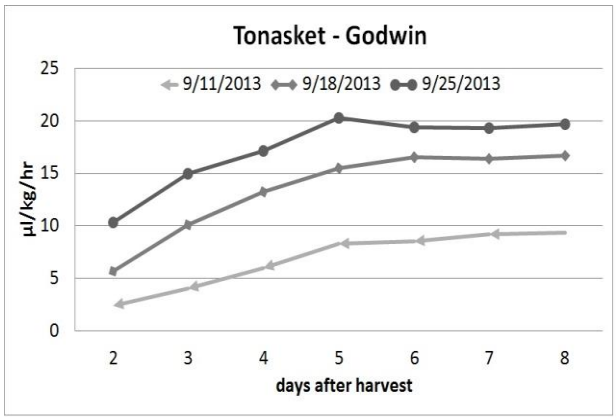
The application of a drape nets from June until September resulted in several changes: a) the maximum air temperature did not exceed 90F even on days with maximum air temperature readings > 100F (not shown), b) maturity of fruit was not significantly changed except for higher TA levels in the first 2 commercial picks (not shown), c) sunburn was reduced both overall and in severity leading to more fruit in the premium categories (35% downgrades or culls in control vs. 5% under shade) (Figure 5). All fruit remains in storage.

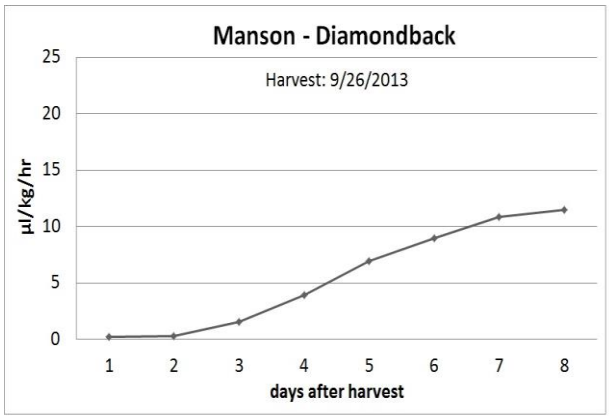


2.1 Ethylene green life. Fruit from 3 orchards were harvested weekly for 1-4 weeks. Ethylene emitted from whole fruit was measured daily for 8 days. Ethylene production varied with harvest date and orchard.



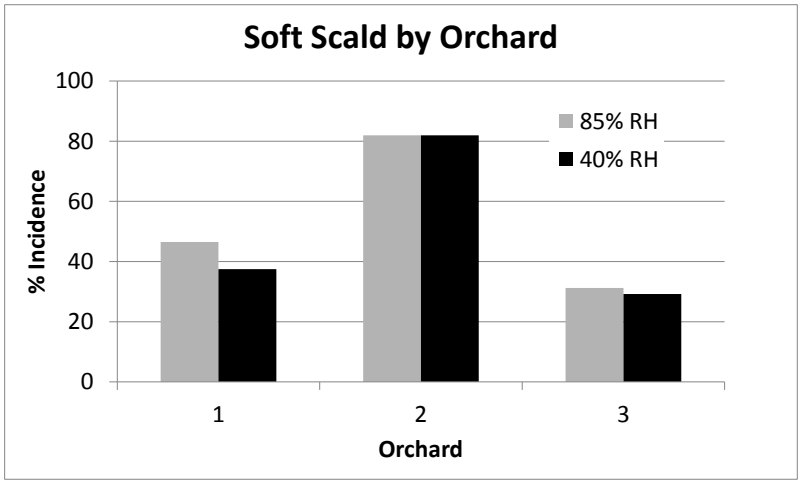




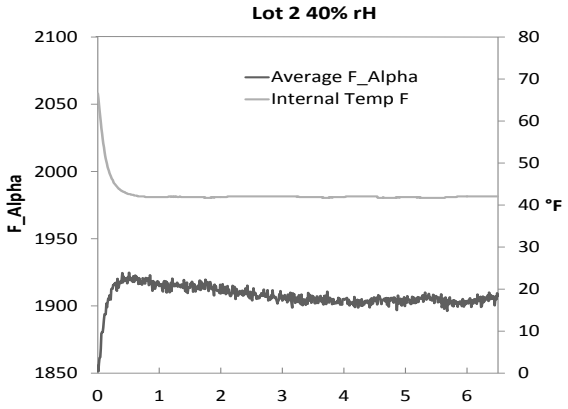


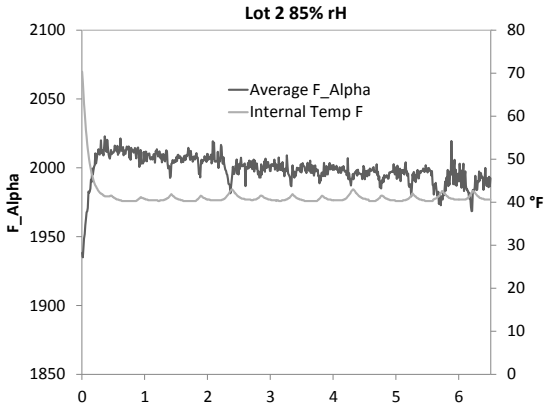
Initial production and rate of production increase may be indicators of storability.

2.2 Humidity during conditioning. Fruit from 3 orchards were conditioned at 50 °F for 7 days in 50 or 95% relative humidity then stored at 37 °F in air for 3 months.

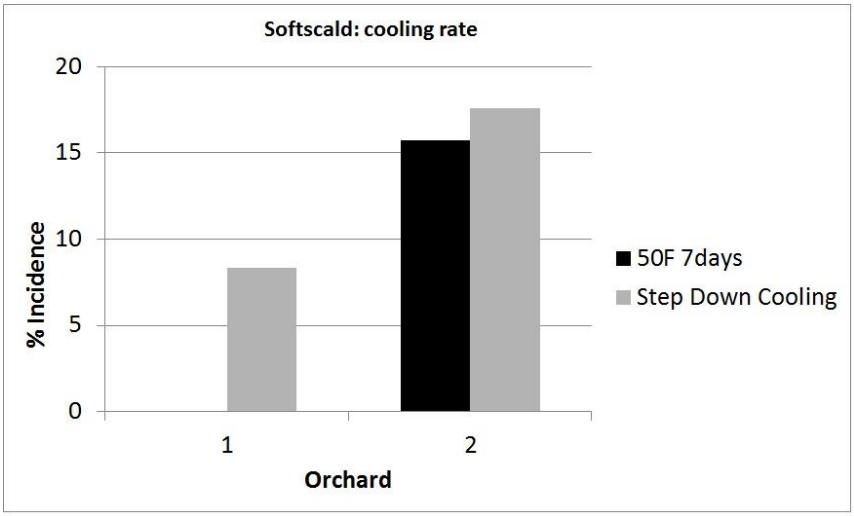


Humidity during conditioning did not impact softscald incidence during 3 months in cold storage. Chlorophyll fluorescence decreased regardless of humidity during cooling.





Short term conditioning**.** Fruit from two lots were conditioned at 50 °F for 7 days or for 2 days then temperature was reduced 5 °F after 2 and 4 additional days then 3 °F to a final temperature of 37 °F.



Less than 7 days at 50 °F enhanced softscald incidence in fruit from one of two orchards.

2.3 Honeycrisp CO_2 exposure during SmartFresh treatment

Fruit were exposed to 0 or 1 ppm SmartFresh at 50 °F with 0, 2, or 4% CO_2 added to treatment chambers. Chambers were opened after 24 hours and fruit held an additional 6 days at 50 °F. Fruit were then stored at 37 °F for 3 months. Incidence of CO_2 related cortex browning was low and was not related to SmartFresh use.

Orchard	% CO_2	% cortex browning	
		Control	SmartFresh
A	0	0	0
	2	0	0
	4	0	0
B	0	0	0
	2	0	0
	4	0	0
C	0	0	0
	2	2	0
	4	2	0

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

Project Title: Commercial testing of early scald risk assessment tools

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

Cooperators: Drs. Jinwook Lee, Bruce Whitaker, and Christopher Watkins

Total Project Request: **Year 1:** $54,881 **Year 2: $56,275** **Year 3:** $57,675

Other funding sources

Agency Name: NIFA, USDA (Grant no. 2010-51181-21446**)**
Amt. awarded: $1,483,438 (federal total over 4 years)
Notes: Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle. David Rudell (Project Director) will manage and participate in the project. This is a multi-state, multi-national project. The proposed project extends and compliments the activities of this SCRI project.

Agency Name: AgroFresh, Inc.
Amt. awarded: $232,253 (for Rudell and Mattheis role in SCRI project over 4 years)
Notes: Cash donation to support activities and objectives outlined in the Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle (see above).

Agency Name: AgroFresh, Inc.
Amt. awarded: $270,000 (estimated over 3 years, beginning 2013)
Notes: Continued development of systems for implementation of biomarker-based tools developed from the above SCRI project as well as finding additional biomarkers

Budget
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510)559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2013	2014	2015
Salaries	$38,417	$39,302	$40,372
Benefits	$16,464	$16,972	$17,302
Wages			
Benefits			
Equipment			
Supplies[1]			
Travel			
Total	$54,881	**$56,275**	$57,675

Objectives:

1. Determine if risk assessment tools accurately represent scald risk in multiple commercial lots of Granny Smith apples.
2. Test scald risk assessment tools using Delicious apples.
3. Validate additional biomarkers for CA storage.
4. Extend search for biomarkers for at-harvest superficial scald risk assessment tools.

Goals and activities for the next year: Repeat Granny Smith and begin new Delicious storage monitoring trials in commercial storages.

SIGNIFICANT FINDINGS:

1. Delaying CA imposition results in enhanced ethylene and scald risk assessment biomarker (SRAB) levels.
2. SRAB levels increase with higher O_2 levels in CA storage.

Methods:

Equipment and Cooperative Summary: Tissue sampling, processing and analysis of biomarkers using analytical instrumentation (gas and liquid chromatography-mass spectrometry, spectrophotometry) will be performed at ARS-TFRL, Wenatchee. Storage experiments will be performed both at ARS-Wenatchee and in commercial storages. Additional chemical identification will be performed in cooperation with Dr. Bruce Whitaker (BARC, USDA-ARS, Beltsville, MD). New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

Procedures:

Year 1

Validating SRAB tools for Delicious apples

Scarlett Spur Delicious apples were harvested 3 weeks prior to commercial harvest. Fruit maturity and quality was evaluated at harvest. Apples are currently being stored at 33 ^{o}F in CA at 0.5% or 2% O_2 (all 1% CO_2), and scald risk assessment biomarker (SRAB) levels are being monitored monthly. Biomarker levels indicate scald risk increased between 2 and 3 months in 2% O_2. One of the 2% O_2 chambers was pulled down to 0.5% O_2 once increasing biomarker levels indicated risk was elevated. Biomarker evaluation will continue. Scald will be evaluated in all treatments at 0 and 7 days (at 68 ^{o}F) after 3, 6, and 9 months storage.

Lot to lot scald assessment in commercial Granny Smith storages

Multiple lots (10 lots in 3 storage facilities) of Granny Smith were commercially harvested and stored in organic CA rooms. Monthly estimation of scald risk is underway using biomarker-based scald risk assessment tools. Scald will continue to be evaluated monthly on all samples and then upon removal of each lot from storage. Tests will indicate whether tools accurately assessed the likelihood of scald development in different commercial lots.

Impacts of rapid CA imposition and ethylene scrubbing on scald development and risk assessment

A single lot of Granny Smith with a high risk for scald development is being stored in 4 research CA rooms (30 bins/room). Room atmospheres of 0.5% O_2, 0.5% CO_2 were imposed either immediately upon loading or after 1 week with or without ethylene scrubbing. Scald development and SRAB

levels are being evaluated monthly as well as room ethylene levels, fruit volatile compounds including volatile SRABs.

Impact of rapid CA imposition of non-superficial scald peel injury development
Granny Smith apples harvested 1 month prior to commercial harvest are being stored in 0.5% O_2, 0.5% CO2 in USDA, ARS experimental storage chambers. Storage atmosphere was imposed while fruit were still warm or after fruit had reached storage temperature. Peel injury and scald development will be monitored monthly from 3 through 9 months storage.

Year 2

Lot to lot scald assessment in commercial Granny Smith and Delicious storages

Repeat of year 1 of 'Granny Smith' study and include 'Delicious' in the test.

Year 3
At-harvest scald risk assessment
Granny Smith apples will be harvested 3 times (1 month prior to commercial, commercial, and 2 weeks following) from 5 locations with varying histories of scald susceptibility. Apples will be stored in air for up to 6 months. Peel tissue will be sampled at harvest and 2 and 4 weeks following harvest. Scald incidence and severity will be assessed monthly.

RESULTS & DISCUSSION

Scald risk assessment for Delicious apples
Scald risk assessment biomarker (SRAB) levels are being monitored in Scarlett Spur Delicious apples harvested approximately 3 weeks before typical commercial harvest. As SRAB levels increased between 2 and 3 months in 2% O_2 CA stored fruit, the atmosphere in one of the 2% O_2 chambers was lowered to 0.5 % O_2 (Fig. 1). SRAB levels in air stored fruit began to increase between 1 and 2 months. Scald has not yet developed on fruit from any of the treatments. Results to date are consistent with previous results with Granny Smith.

Scald risk assessment of Granny Smith apples stored in commercial rooms
10 Granny Smith lots from around the state are stored in 1 of 3 commercial rooms (800 bins +). SRAB monitoring is underway. Extra peel was collected at harvest and following 1 week storage for further validation of new at-harvest and early storage SRABs found last year as an activity of our SCRI project. We are testing both at-harvest and early storage markers as well as storage monitoring SRABs for the ability to rank orchards according to scald risk.

Scald risk assessment of 'Granny Smith' apples as impacted by headspace ethylene and delayed CA imposition
Storage room ethylene levels were highest in rooms were CA imposition was delayed (Fig. 2). Ethylene scrubbers slightly reduced headspace ethylene levels in both treatments at 1 month. SRAB levels are, likewise, higher in rooms were CA imposition was delayed (Fig. 3). Analyses are ongoing. Because initial SRAB levels in commercial storage were higher in all orchards than those in RCA rooms, we are testing whether the prolonged room loading period impacted SRAB levels as a result of enhanced ethylene production and ripening. Results to date support this assertion.

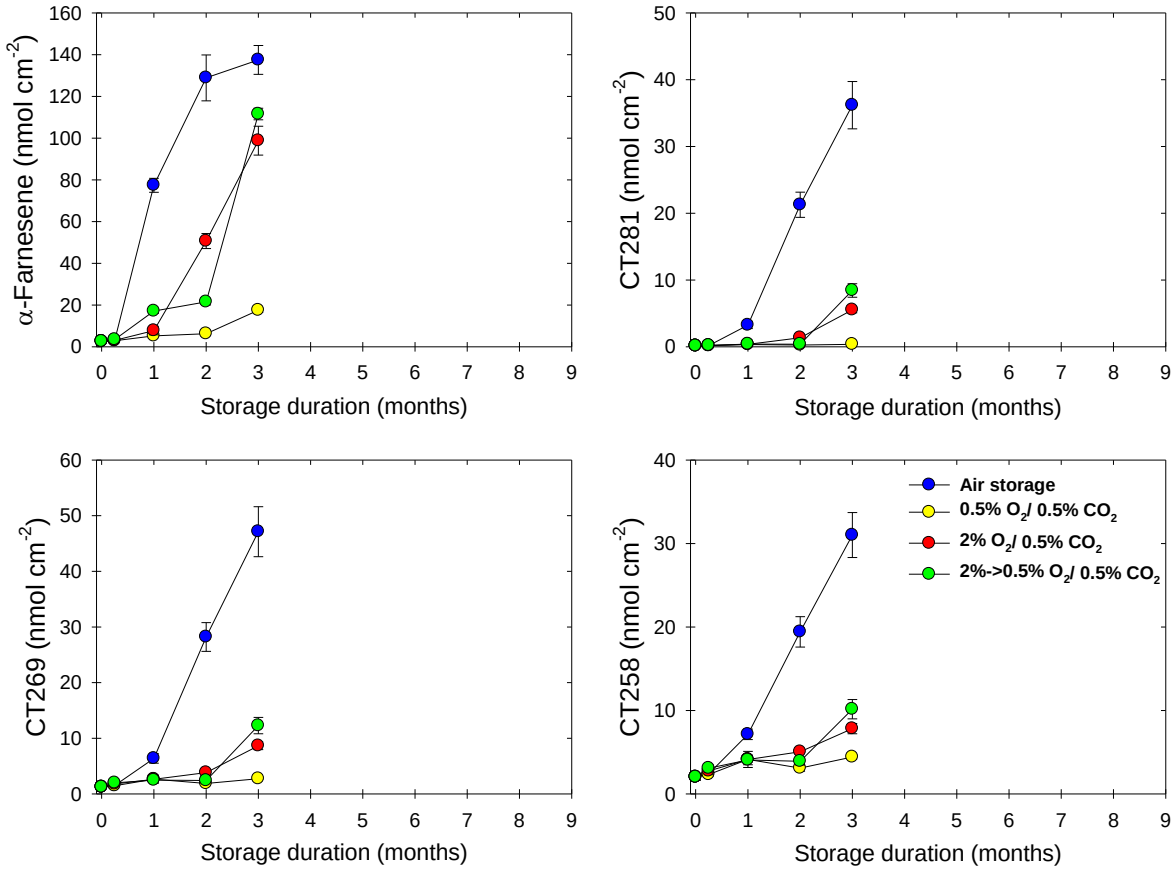


Fig. 1. Peel SRAB levels of Scarlett Spur Delicious apples stored in air or controlled atmosphere regimes at 33 °F. SRABs were measured using relatively inexpensive techniques. Changes in CA stored apple SRAB levels are appreciable between 2 and 3 months. Chamber O_2 reduced from 2 to 0.5% at 3 months in one of the 2% chambers. We will continue evaluating scald incidence and SRAB levels.

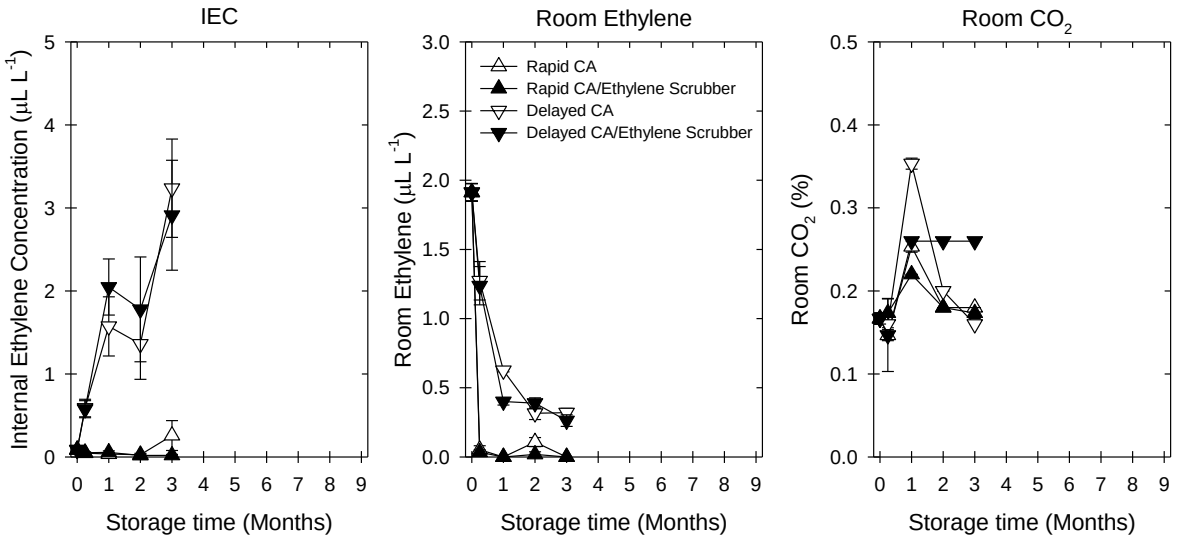


Fig. 2. Granny Smith internal ethylene (core space) concentration (IEC), room ethylene concentration, and room CO_2 concentration. Fruit were stored at 33 °F in Stemilt RCA rooms containing 0.5% O_2/0.5% CO_2. CA was imposed immediately in 2 rooms and delayed 1 week in the others. One room from each treatment is equipped with an ethylene scrubber. CA imposition speed impacted internal ethylene levels indicating room loading/CA imposition speed may impact ripeness. The impact of CA imposition rate on scald development will be evaluated when scald symptoms begin to occur. Ethylene scrubbers had little impact on room ethylene levels and none on internal ethylene levels.

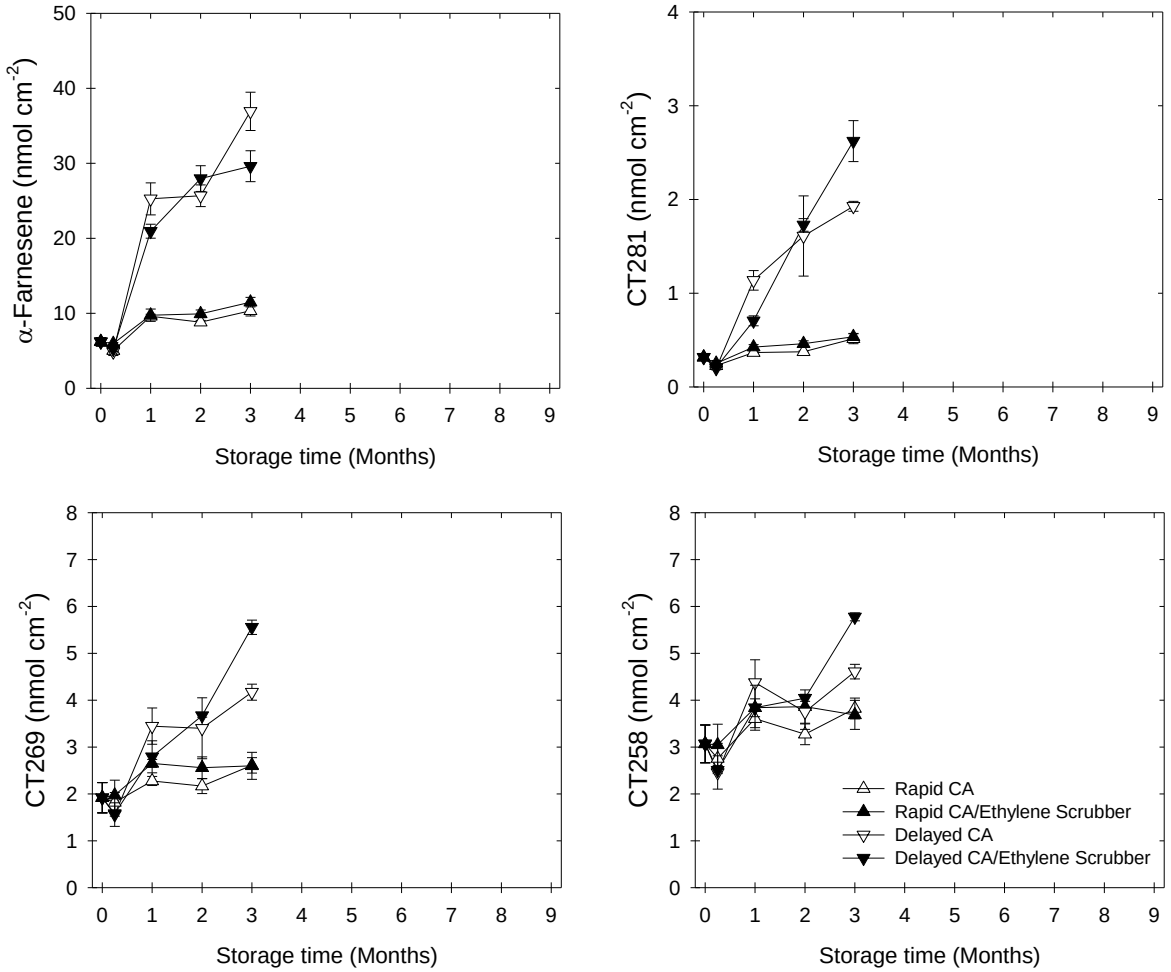


Fig. 3. Granny Smith SRAB levels. Fruit stored at 33 °F in Stemilt RCA rooms containing 0.5% O_2/0.5% CO_2. CA was imposed immediately in 2 rooms and delayed 1 week in the others. One room from each treatment is equipped with an ethylene scrubber. Delaying CA imposition impacted SRAB levels indicating room loading/CA imposition speed may impact scald risk with higher risk in rooms where CA imposition was delayed by 1 week. Its impact on scald development will be evaluated when scald symptoms begin to occur. Ethylene scrubbers had little influence on room ethylene levels and none on internal ethylene levels.