Washington Tree Fruit Research Commission
Technology Research Review
March 24, 2009
WA Cattlemen's Association, Ellensburg

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CONTINUING PROJECT REPORT **YEAR**: 1

Project Title: Rosaceae micropropagation and tissue culture platform

PI: Amit Dhingra
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Previous Cooperators: Tom Auvil, WTFRC; Nnadozie Oraguzie, WSU; Gennaro Fazio, USDA-ARS; Herb Aldwinckle, Cornell, Geneva; Bill Howell, Northwest nursery improvement institute;
New cooperators: Tye Fleming and Todd Ericksson

Total project funding request: Year 1: 30,000

WTFRC Collaborative expenses:

Item	2009	2010	2011
Travel	500	500	500
Total	500	500	500

Footnotes:

Budget 1
Organization Name: WSU **Contract Administrator: Betty Musick**
Telephone: 5093359505 **Email address: musickb@wsu.edu**

Item	2009	2010	2011
Salaries	28,000	29,120	30,285
Benefits	11,480	11,939	12,417
Wages			
Benefits			
Equipment			
Supplies	2500	2500	3500
Travel	1500	1500	1500
Total	43,480	45,059	47,702

Footnotes:
Note: This project was funded for $30,000 instead of $43,480 that was originally requested. We have revised the goals, milestones and timeline based on the revised budget. This continuing report will recapitulate the original objectives and provide revised goals, milestones and a timeline based on revised funding.

The term "in vitro" repeatedly used in this project means "in tissue culture"; Magenta boxes are tissue culture vessels made of clear polypropylene material. It is a trademark and does not represent a magenta colored box.

JUSTIFICATION

Major goal of the project: This project addresses the ever-increasing time gap between development of new rootstock or scion genotypes by several breeding programs and their commercial utilization by the growers. This delay represents a financial burden both to the program that develops them and the fruit industry.

Rootstocks have revolutionized production of fruit in the PNW. However, problems remain with the existing rootstock genotypes that were adopted several decades ago especially in apple. New genotypes of rootstocks have been developed in different breeding programs but their commercial adoption has been delayed due to a simple yet vital factor of efficient multiplication. Liner bed multiplication is the traditional way of multiplying the rootstocks, however the new genotypes have been found to be hard to root and multiply. Multiplication of rootstocks in tissue culture is an alternative approach that has been implemented even for the older rootstocks with success.

The objectives of the proposal were:

1. Refine or formulate micropropagation protocols for Geneva rootstocks (apple), Pear rootstock OH x F and Polish Quince (Pear)
   - Objective 1A: Finalize the protocol for obtaining rooted rootstocks of G41 and develop similar protocols for G935 apple, OH x F and Pear Quince rootstocks.
   - Objective 1B: Third-party validation of the micropropagation protocols.
   - Objective 1C: Perform a cost-analysis of agar-based or temporary immersion system protocols to assess implementation of the methods in a commercial setting.
2. Define special light conditions for micropropagation of rootstocks and scions.
   - Objective 2A: Identify the most efficient light wavelength combinations for apple and pear rootstocks.
   - Objective 2B: Assess the cost-benefits of utilizing specialized growth chambers in micropropagation.
3. Transition the micropropagation research to the field – Sustaining the Rosaceae Micropropagation Platform

Based on the revision of budget and limitation to one year of funding, the objectives of this project are entirely focused on taking the apple rootstock G-41 to rooting in collaboration with partners from the nursery.

Revised Objectives

Objective 1: Rootstock production: Finalize the protocol for obtaining rooted rootstocks of G41

Objective 2: Rootstock acclimatization: Establish protocols for transfer of material from the lab to the green house

SIGNIGFICANT FINDINGS

A recalcitrant rootstock such as Geneva 41 is a complete black box in terms of its growth potential in tissue culture. Over the last year several media formulations were developed based on close physiological observation of G-41's performance in vitro. While this discovery period was ongoing, it was hard to set timelines or milestones. Now, micropropagation protocols have been established in our laboratory and more concrete timelines to achieve the objectives outlined in this project are provided.

Apple rootstock:

Geneva 41 remains one of the desirable apple rootstock. Our goal was to first develop a good multiplication system. Over the past year, we have developed specific media formulation that accelerates G-41 growth in vitro.

a. **Agar-based media**: The solid, agar-based media enabled us to identify the right mix of macro and micro nutrients in plant tissue culture media for G-41. In this system we are able to achieve 2 to 4X multiplication in 8 to 10 weeks. The plants are healthier and perform consistently as compared to media formulations provided to us by Phytacell Inc.

b. **Liquid media with temporary immersion system**: For multiplication of G-41 rootstocks, we employed a liquid media-based temporary immersion system. This equipment provides recurrent desiccation stress to the in vitro liner and produces 4 to 6X multiplication in 2 weeks. Some of the multiplied shoots (5%) can be vitrified but the higher rate of multiplication adequately compensates for any losses.

Pear rootstock:

Based on prior year of research, a similar multiplication platform is available for OH X F rootstocks. There is an interest for propagating some cold-hardy quince rootstocks as Todd Einhorn (OSU) identifies them after field trials. However, due to revised objectives, we will focus on the G-41 system in this project.

Cherry rootstock:

Gisela rootstocks are in demand but the supply is not enough to satisfy the need. Discussions with the nursery industry indicate that multiplication is a recognized bottleneck. These rootstocks are also a good candidate for in vitro multiplication. In our lab, we have established good tissue culture system for sweet cherry. We intend to include Gisela rootstocks in our micropropagation system during the next year contingent on availability of funding.

METHODS

Objective 1: Rootstock production: Finalize the protocol for obtaining rooted rootstocks of G41.

An in vitro liner consisting of 4 nodes is placed horizontally on the tissue culture media. After 6-8 weeks when each node develops into one or two individual shoots, the basal liner tissue is excised. Individual shoots are moved to rooting media placed in square transparent boxes called Magenta boxes. Figure 1 shows the preliminary results of rooting in G-41 using two methods. The normal light conditions are the conditions used in tissue culture room with 30 micro moles per m2 per sec with 16h day and 8 h dark periods. Under these conditions the rooting media used was supplemented with IBA and non-sucrose sugar.

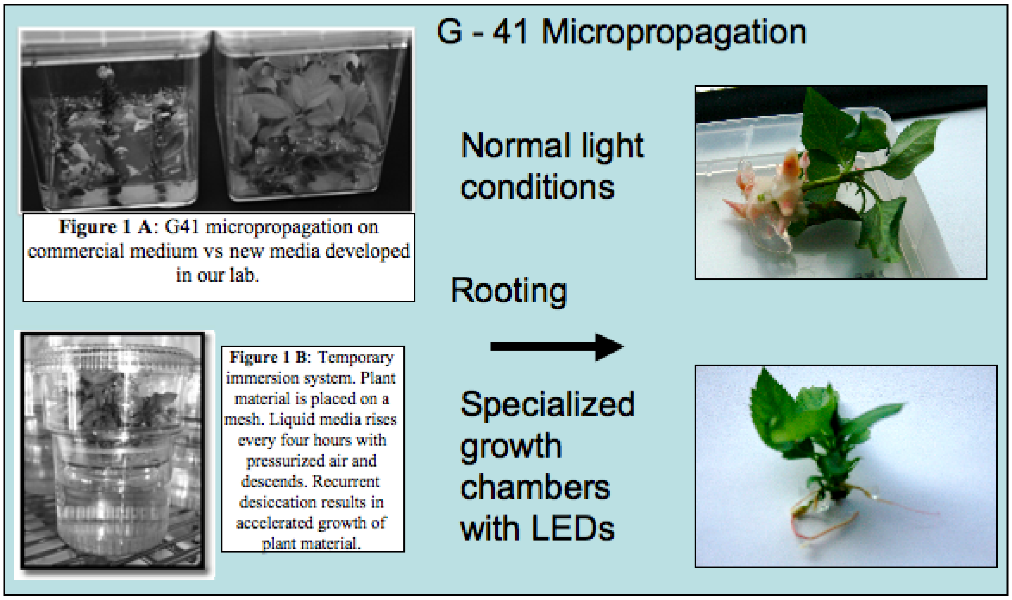
Rooting under special light conditions involves growing individual shoots placed in Magenta boxes under specific intensity of Red, Blue and Green wavelength of light. These are preliminary results obtained recently and we are repeating and standardizing the rooting parameters.

Objective 2: Rootstock acclimatization: Establish protocols for transfer of material from the lab to the green house.

One of the major reasons for tissue culture derived plant mortality is sudden drop in relative humidity. To avoid humidity related mortality, the explants will be moved towards rooting while enclosed in Magenta boxes. In cooperation with Tye Fleming and Todd Erickson, multiplied rootstock shoots in tissue culture will be directly moved to mist beds and their performance will be monitored. Depending upon mortality rates, frequency of misting will be varied to enable high rate of survival.

RESULTS AND DISCUSSION

Preliminary results of successful rooting are shown in the figure below. The explants are either derived from solid media or liquid media based temporary immersion system. Note the thick roots developed in agar media supplemented with IBA and non-sucrose sugar.



Timeline:

Objective 1: Rooting is currently being standardized. The duration for agar-based rooting experiment is 4 weeks. This activity will continue from April to July 2009. The rooted plants will be moved to WSU and Todd Erickson's green house for acclimatization as they get established in rock wool or perlite.

Objective 2: The acclimatization of tissue culture multiplied rootstocks will initiate April 2009 to standardize survival of more than 90% plantlets in the mist beds.

We expect to establish rooting and acclimatization of G-41 rootstocks by September 2009 barring any unforeseen hurdles.

Milestones:
There are two milestones we want to accomplish with this project in this year.
1. Establish a streamlined protocol for G-41 multiplication and rooting in tissue culture
2. Develop a streamlined protocol for transitioning tissue culture derived G-41 into green house.

Role of Cooperators:
Previous Cooperators: These cooperators were listed for the original project. However, in the currently revised framework we will only work with Tom Auvil and Gennaro Fazio.

Tom Auvil, WTFRC – Coordinate tissue culture activities with the nursery industry and enable acclimatization of tissue culture derived plant material.
Nnadozie Oraguzie, WSU – Identify scions and rootstocks that should be multiplied in vitro to support the breeding program activities.
Gennaro Fazio, USDA-ARS – Implementation of standardized protocol to commercial nurseries.
Herb Aldwinckle, Cornell, Geneva – Validation of protocols established in our laboratory.
Bill Howell, Northwest nursery improvement institute – Supporting the research activities based on micropropagation and utilizing in vitro multiplied rootstocks in orchards.

New cooperators: Tye Felming and Todd Erickson
Tye Fleming and Todd Erickson will utilize in vitro multiplied G-41 rootstocks and help in greenhouse based rooting and acclimatization. The cooperation has already been initiated.

CAHNRS Undergraduate Research funding:
There are two undergraduate students working on this project under supervision of Amit Dhingra and Scott Schaeffer. The project was selected for CAHNRS Undergraduate Research Fellowship and will specifically support establishment of rooting under RBG light spectra. The results will be presented at the annual CAHNRS awards banquet on April 4th 2009.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: TR-700A

Project Title: Developing flavor gene markers for the WA tree fruit industry

PI:	Cameron Peace	**Co-PI(2):**	Jim Olmstead
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Cooperators: Yanmin Zhu (USDA-ARS, Wenatchee), Carolyn Ross (Dept Food Science & Human Nutrition, WSU Pullman), Dorrie Main, John Fellman (Dept Horticulture & LA, WSU Pullman), Amy Iezzoni, Wayne Loescher, Randy Beaudry, Steve van Nocker (Dept Horticulture, Michigan State University), Eric van de Weg, Marco Bink (Plant Research International, Netherlands), Fred Bliss (Davis, California), Jim McFerson (WTFRC, Wenatchee), Nnadozie Oraguzie(Dept Horticulture & LA, WSU Prosser), Kate Evans (Dept Horticulture & LA, WSU Wenatchee)

Total project funding request: **Year 1:** 27,035 **Year 2:** 17,200 **Year 3:** 4000

Other funding Sources

__Awarded__:

Agency Name: WTFRC Apple Review
Amount awarded: $87,500 in 2009
Notes: Peace PI and Olmstead co-PI. Synergistic project on marker-assisted breeding application for the WSU apple breeding program.

Agency Name: WTFRC NW Cherry Review
Amount awarded: $45,000 in 2009
Notes: Peace PI and Olmstead co-PI. Synergistic project on marker-assisted breeding infrastructure for the WSU sweet cherry breeding program.

Agency Name: WTFRC Apple Review
Amount awarded: $77,616 in 2009
Notes: Peace, Olmstead, and Evans co-PIs (PI: D. Main). Synergistic project – bioinformatics support for WSU apple and sweet cherry breeding programs.

Agency Name:	WTFRC Technology Review
Amount awarded:	$50,000 in 2009
Notes:	ABI 3730 DNA Analyzer to augment tree fruit breeding and research Peace PI. Matched with $50,000 from Washington Wheat Commission (separate award, PI: D. See) to obtain refurbished ABI 3730 DNA Analyzer ($100,000) for high-throughput genotyping of tree fruit and cereals, based in Pullman.
Agency Name:	WSU Agricultural Research Center
Amount awarded:	$100,000 in 2009
Notes:	Additional support to Dr. Peace and the "Pacific Northwest Tree Fruit Genotyping Laboratory" for high-throughput DNA extraction and genotyping equipment, complementing the ABI 3730 and removing technical bottlenecks for routine tree fruit genotyping.
Agency Name:	WTFRC Apple Review
Amount requested:	$169,210 in 2009
Notes:	Apple Scion Breeding. Evans PI, Peace co-PI. Synergistic project and beneficiary of flavor gene advances for apple.
Agency Name:	USDA-CSREES, NRI Competitive Grants Program
Amount awarded:	$400,000 in 2008-2010
Notes:	Peace PI. Synergistic project – fruit texture genetic control in apple with emphasis on ethylene.

<u>Pending</u>:

Agency Name:	National Science Foundation
Amount requested:	$2,818,331
Notes:	"Genome Database for Rosaceae". Peace and Olmstead co-PIs (PI: D. Main). Synergistic project to develop broad bioinformatics support for Rosaceae crops.

Budget 1:
Organization Name: Washington State University **Contract Administrator**: M.L. Bricker
Telephone: (509) 335-7667 **Email address:** mdesros@wsu.edu

Item	**Year 1 (Jul07-Jun08)**	**Year 2 (Jul08-Jun09)**	**Year 3 (Jul09-Jun10)**
Salaries	4552[a]		
Benefits	1548[a]		
Wages	4395[b]	4484[d]	897
Benefits	505[b]	516[d]	103
Equipment			
Supplies	10000[c]	7200[e]	3000
Travel			
Miscellaneous		5000[f]	
Total	21000	**17200**	4000

Footnotes: [a] Activity 1a; [b] Activities 1b, 2a, 4a, 4c; [c] Activities 1b, 2a, 4a; [d] Activity 4d; [e] Activities 4d and 4b; [f] Activity 4e

Budget 2:
Organization Name: Michigan State University **Contract Administrator:** Bob Rock
Telephone: **(517)355-5040 x242** **Email address:** rock@cga.msu.edu

Item	**Year 1 (Jul07-Jun08)**		
Salaries			
Benefits			
Wages	2500		
Benefits	1535		
Equipment			
Supplies	2000		
Travel			
Miscellaneous			
Total	6035[a]		

Footnotes: [a] Activities 1a and 4c

Objectives:
Understanding the key control points for traits of interest is very valuable for improving crop production. This project seeks to develop a generic system that identifies genes controlling traits of importance to the Washington tree fruit industry, and to implement this system using the example of fruit flavor. This knowledge can then be put to practical use, such as in marker-assisted breeding, controlled sport induction, or chemical genomics. The proposed project uses the candidate gene approach and capitalizes on expanding genomics databases and a large international network of tree fruit genomics, genetics, and breeding researchers.

Specific objectives are to:
1. Develop DNA tests of flavor useful for Washington tree fruit breeding programs
2. Establish a temperate-tropical fruit genomics channel through linkages between the Rosaceae and papaya genomes
3. Identify tropical fruit flavor genes having potential value for the Washington tree fruit industry

This is the second continuing report for the second year of the project. In Year 2, (July 2008 – June 2009), 100 genes on our developed Master List of tree fruit flavor candidate genes will be placed on the *Malus* and *Prunus* genomes and compared to locations of reported flavor trait loci. Sequence variation in these "flavor genes" will be surveyed within germplasm sets that represent the PNW apple and sweet cherry breeding programs. The compositions of these sets of individual have been determined within 2008 WTFRC apple and sweet cherry projects, and refined and placed in a national and international context with our involvement in the NRI project "Functional gene markers for Rosaceae tree fruit texture" and the SCRI proposal "RosBREED: Enabling marker-assisted breeding in Rosaceae". Gathered flavor performance data from collaborating projects and programs will be combined with genotypic and pedigree data for statistical analysis. The Pedigree-Based Analysis approach will be conducted on this combined dataset using the software FlexQTL and support from developer and collaborator, Dr. Marco Bink. Development of ready-to-apply breeding tools (markers for selection) and connections with tropical fruit genomics efforts are slated for Year 3.

Significant Findings:
- The "Flavor Gene Map" has been improved from several sources. This map shows all known locations in the *Malus* (apple) and *Prunus* (sweet cherry) genomes of regions correlated with fruit flavor genetic variation as well as candidate genes for fruit flavor. Genomic regions influencing sweetness, acidity, and aroma, and genes that we postulate control these traits were obtained from published reports, collaborators in other institutions, and our own laboratory work on promising targets.

- Several candidate genes were observed to co-locate with genomic regions influencing flavor traits, and warrant closer attention.

- Interestingly, many regions controlling flavor are common between *Malus* and *Prunus*.

- Preliminary "whole genome sequencing" of sweet cherry by Dr. Amit Dhingra is already functionally connecting with marker development within this flavor gene project. The sweet cherry genome sequence of 'Stella' was screened to identify cherry versions of several flavor genes on the Master List. 'Stella' DNA sequences will facilitate the development of flavor gene tests that efficiently screen both cherry and apple, as well as cherry-specific tests where desired, by reducing the occurrence of failed tests.

Methods:
This project involves:

- Molecular genetics, bioinformatics, and molecular biology to choose, test, and analyze the DNA sequences of genes (belonging to known biochemical pathways putatively leading to sweetness, acidity, and aroma for apple and sweet cherry),
- Molecular genetics to locate such candidate genes on the *Malus* and *Prunus* genomes and match with reported locations of controlling chromosomal regions for flavor,
- Physiology, sensory analysis, breeding, and databasing to collect performance data on flavor-related traits (sweetness, acidity, and aroma) for apple and sweet cherry, and
- Statistical approaches to identify significant gene-trait associations that can be exploited for the improvement of flavor characteristics in apple and sweet cherry.

Results and Discussion:
We are testing genes that are likely suspects involved in fruit flavor (sweetness, acidity, and aroma) and collecting flavor-related data, for apple and sweet cherry. We expect that by the end of the project, some of the genes investigated will be found to influence important flavor components of apple and cherry. This knowledge will allow us to better understand the genetic value of existing cultivars, advanced selections, and parents of the WSU apple and sweet cherry breeding programs. The knowledge will also provide a means to manipulate these traits for crop improvement. Within this project, we will develop as many genes as possible into genetic screening tools for breeding, via the marker-assisted breeding approach. With marker-assisted seedling selection, the infrastructure for which we are developing in other projects, we can reduce the proportion of seedlings planted in the field with poor genetic value, to improve the efficiency of breeding operations. Economic analyses within concurrent projects of the PIs are providing bottom line figures that indicate substantial cost savings by implementing even one genetic marker that tags an important trait. Flavor attributes are certainly high priority in the WSU apple and sweet cherry breeding programs.

<u>The Flavor Gene Map</u>
Major flavor trait loci and candidate genes in the *Malus* (apple) and *Prunus* (stone fruit) genomes are summarized in Figure 1. These regions were determined by:

a) A survey of literature on QTL (quantitative trait locus = chromosomal region influencing a trait) analyses in apple, peach, and cherry (Maliepaard et al. 1998; Wang et al. 2000; Etienne et al. 2002; Liebhard et al. 2003; Kenis et al. 2008). In recent scientific conferences, researchers at Plant & Food Research in New Zealand (previously HortResearch) reported the location of a major locus (*2MBAc*) on linkage group 2 of apple controlling levels of the major volatiles contributing to "apple aroma". Most recently, Dunemann et al. (2009) working on different apple germplasm reported 5 major (including *2MBAc*) and 10 minor regions in the apple genome controlling a wide array of volatiles. This report also indicated the positions of two flavor candidate genes.

b) Data kindly provided by colleagues involved in genetic mapping of flavor candidate genes – unpublished work. At UC Davis, Dr. Eben Ogundiwin has recently placed 12 flavor candidate genes on the *Prunus* genome within a study that is developing a "*Prunus* fruit quality gene map" (Ogundiwin, Peace, et al., manuscript submitted). At Plant & Food Research, Dr. David Chagne and students (Mukarram Mohammed and Aurélie Dimouro) mapped 13 flavor candidate genes from a list compiled by their flavor genomics specialists, Drs. Edwige Souleyre, Richard Newcomb, Robert Schaffer, and Ross Atkinson. This work was done to try to identify the gene controlling the *2MBAc* aroma locus, but thus far has not been successful. Nevertheless, the 13 candidate genes may be discovered to control other aroma loci, and thus we include them on our Flavor Gene Map.

c) The map also includes genes involved in the ethylene biosynthesis and perception pathway. Reports on transgenic apple fruit with the ACS and ACO genes knocked out (Dandekar et al. 2004; Schaffer et al. 2007) have shown that while sweetness and acidity development in fruit is apparently independent of ethylene, volatile aroma compounds – particularly volatile esters – are strongly affected by ethylene. The final enzymatic steps of volatile aroma compound synthesis are always affected by ethylene levels, and often the initial enzymatic step is also influenced (Schaffer et al. 2007). The genes for these volatile production enzymes are part of our Master List of flavor genes, and those most affected by ethylene are currently being mapped by our collaborators at Plant & Food Research.

Many cases were observed on the Flavor Gene Map where candidate genes for a flavor trait were at the same region as chromosomal regions influencing the trait (Figure 1). For example, at the bottom end of *Malus* chromosome 16 is located a major QTL for acidity, and a candidate gene for acidity showed up in the same place. We are focusing in on this gene now. Other regions like this are in the middle of *Malus* chromosome 9, towards the bottom of *Malus* 17, the middle of *Malus* 5, and the middle of *Malus* 15. Other comparisons *across chromosomes within apple* are also very interesting. For example, *Malus* chromosomes 9 and 17 have a common ancestral origin, and a QTL for volatile aroma on 9 is at the same equivalent spot as a volatile aroma gene on 17. Several other cases like this were observed. Next, there are ancestral ties between certain *Malus* and *Prunus* chromosomes, and in many cases there were important flavor regions in both crops. For example, *Malus* chromosome 13 and 16 have a common ancestral origin with *Prunus* chromosome 1, and a QTL for volatile aroma on *Malus* 16 is at the same equivalent spot as a volatile aroma gene on *Malus* 13 and *Prunus* 1. Another similarity that will lead us to focused genetic studies is on Malus chromosomes 10 and 5 and Prunus chromosome 4. Still others are apparent in Figure 2. We expect many more exciting opportunities like this once all 100 flavor genes of our Master List are mapped. The Flavor Gene Map will also be continually updated with new QTLs.

<u>Making use of whole genome sequencing of sweet cherry</u>

WSU Pullman collaborator Dr. Amit Dhingra recently performed preliminary whole genome sequencing of sweet cherry ('Stella') using the 454 sequencing machine obtained in 2008 with WTFRC and WSU-ARC support. We have begun to obtain sweet cherry-specific gene sequences for flavor candidate genes on our Master List. 'Stella' DNA sequences will facilitate the development of flavor gene tests that efficiently screen both cherry and apple, as well as cherry-specific tests where desired, by reducing the occurrence of failed tests. Previously, only 1-2% of the DNA sequences we could obtain for flavor genes in Rosaceae were from sweet cherry, and thus we would have been forced to rely mostly on the related crop peach to guess the DNA sequence of each flavor gene of sweet cherry.

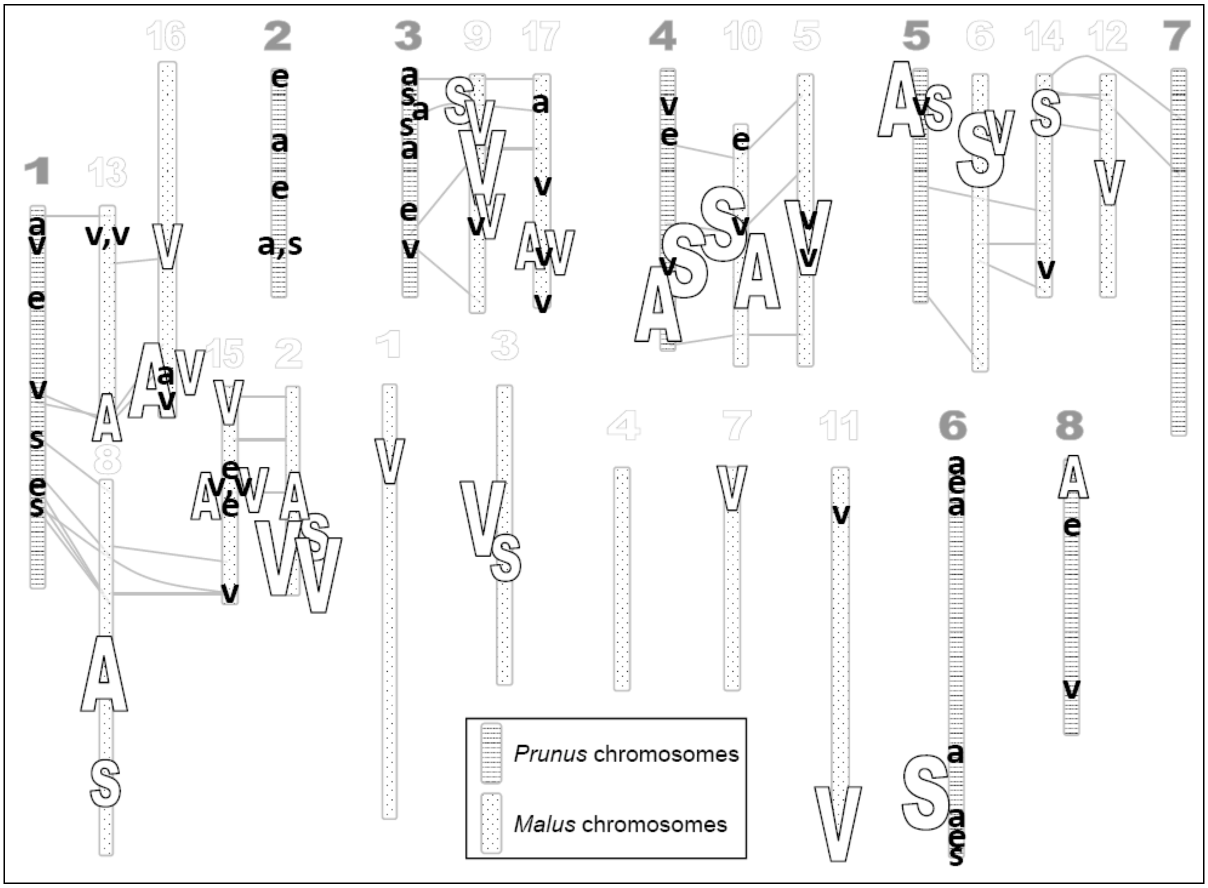

Figure 1: The improved **Flavor Gene Map** of apple and sweet cherry. S,s = sweetness, A,a = acidity, V,v = volatile aroma, e = ethylene. Chromosomal regions (QTLs – quantitative trait loci) influencing fruit flavor in the genomes of pome fruit (*Malus*) and stone fruit (*Prunus*) are indicated in block capital letters (larger letters = larger effect). Locations of candidate genes for flavor (and ethylene synthesis and perception) are shown in lower case letters. Horizontal lines show known connections between the related genomes. The Flavor Gene Map includes locations of genes putatively involved in flavor genetic control.

References:

Dandekar AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, Stow JR, Colgan RJ, James DJ (2004). Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Research 13:373-384

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Liebhard R, Koller B, Gianfranceschi, L, Gessler C (2003). Creating a saturated reference map for the apple (Malus x domestica Borkh.) genome. Theoretical and Applied Genetics 106:1497-1508

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs PM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998). Aligning male and female linkage maps of apple (Malus pumila Mill.) using multi-allelic markers. Theoretical and Applied Genetics 97: 60-73

Schaffer RJ, Friel EN, Souleyre EJF, Bolitho K, Thodey K, Ledger S, Bowen JH, Ma J-H, Nain B, Cohen D, Gleave AP, Crowhurst RN, Janssen BJ, Yao JL, Newcomb RD (2007). A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. Plant Physiology 144:1899-1912

Wang D, Karle R, Iezzoni AF (2000). QTL analysis of flower and fruit traits in sour cherry. Theoretical and Applied Genetics 100:535–544

CONTINUING PROJECT REPORT **YEAR**: 1

Project Title: Investigating flower bud hardiness of new tree fruit cultivars

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Total project funding request: **Year 1**: 35,000

Other funding Sources: None

WTFRC Collaborative expenses: None

Budget 1
Organization Name: WSU **Contract Administrator:** Mary Lou Bricker
Telephone: **Email address:**

Item	**2009**		
Salaries	$12,479		
Benefits	$10,358		
Wages			
Benefits			
Equipment	$12,163		
Supplies			
Travel			
Miscellaneous			
Total	**$35,000**		

Footnotes: salaries include an Associate in Research (@ 42% FTE plus benefits at 83%) responsible for region-wide program coordination, bud collection, data collection and analyses, development of extension material, and equipment maintenance and oversight. Equipment includes a Tenney T2 temperature test chamber with installed humidity control, datalogger, thermoelectric modules and a computer.

OBJECTIVES:

The objectives of this research project directly address the second highest rated research priority of the cherry industry[1], bud hardiness.

1. Establish new fruit bud hardiness standards by phenotyping several genotypes throughout the dormant season and anthesis

2. Partner with DAS to disseminate bud hardiness data to industry as rapidly and conveniently as possible

3. Develop preliminary data and framework for pursuing federally-competitive funding for further research & outreach

SIGNIFICANT FINDINGS:

- differential thermal analysis (DTA) is an effective method for determining cherry and apple bud hardiness
- cherry cultivars exhibit significant variability in hardiness
- DTA is not effective when buds lose hardiness in mid-March
- we can double the capacity for DTA in the freezer from 35 plates to 70 plates
- there is tremendous variability (≈ 20 F) in hardiness among buds on a tree/limb/spur

METHODS:

Objective 1. We propose to assess floral bud hardiness of tree fruit cultivars throughout the dormant season (roughly between November and March) as well as throughout budbreak, to full bloom. Emphasis will be given to sweet cherry and apple. Bud samples will be collected from orchards with weather stations so that temperature variations among sites may be accounted for and to facilitate future modeling efforts. For sweet cherry, samples will be collected from the WSU-Roza farm.

IAREC, PROSSER (2009)
Fully dormant samples will be analyzed for hardiness by differential thermal analysis using programmable freezers (as described in Mills et al., 2006, attached). The freezer will be programmed to hold tissue at 4 C for 1 hr, drop to -40 C in 11 hr, and return to 4 C in 10 hrs. The system will record voltage output at 15 s intervals and the exotherms will be identified manually from a plot of voltage vs. time. Data will be summarized as LTE_{10}, LTE_{50}, and LTE_{90} (i.e., the temperature at which approximately 10%, 50%, and 90% of the buds were killed, respectively). As buds break endodormancy and progress through the stages of flowering, exotherm analysis will not be feasible and bud damage will need to be assessed by dissection and visual inspection for tissue death after 1 hour at room temperature. Most assessments of hardiness at flowering will study pistil damage at 0.5 C increments with temperature changing at 4 C per hour. Additionally, we will evaluate the role of the rate of temperature decline and dew point on bud hardiness. At each sampling, high quality digital images representative of various stages of sampling will be collected for use as visual cues for extension materials and new bud hardiness charts. We propose to build immediately a system similar

[1] Cherry Industry Priority Setting Session, Prosser, WA, 19 August, 2008

to that utilized by the viticulture program at IAREC (http://winegrapes.wsu.edu/frigid.html) to study the potential for differential thermal analysis on apple and cherry.
In 2009 winter, we will evaluate genotypic effects on bud hardiness by assessing hardiness of many cultivars and selections collected from the same orchard (i.e., similar environment). We will include the cultivars of economic importance (e.g., Chelan, Bing, Benton, and Sweetheart) as well as up to 4 advanced selections of the WA/OR sweet cherry breeding and genetics program. Further, the influence of rootstock will be investigated by selecting buds/flowers from these cultivars grown on Mazzard, and Gisela rootstocks. We also will keep record of bloom timing and fruit harvest timing so the influence of these factors on susceptibility or resistance to low temperature damage can also be examined by covariate analysis. Apple cultivars to be included are Fuji, Gala, Golden Delicious, and Red Delicious.

Objective 2. The dissemination of bud hardiness data in a timely, effective manner will be a high priority. We will work with Vince Jones to link hardiness data with the web-based delivery and the decision aid system (DAS).

RESULTS AND DISCUSSION:

We have confirmed the effectiveness of differential thermal analysis for assessing apple and cherry bud hardiness, using the grape physiology program's freezer. Clearly discernible high and low temperature exotherms are observable (data not shown) and up to 5 buds can be measured per analysis plate. With modification however we will double the capacity of the freezer and utilize up to 70 plates in the future. This will allow greater replication and the ability to compare more cultivars at once, up to ca. 350 buds per freezer run. The exotherm data can be analyzed and presented as LT_{10}, LT_{50}, and LT_{90} readily (Fig. 1). We have identified significant differences among cherry cultivars in their minimum hardiness level (Fig. 2). It appears, from our preliminary analyses, that 'Chelan' is hardier than other test cultivars and that 'Sweetheart' is the least hardy. There is about a 12 F (6.5 ºC) difference among the cultivars tested in their LT_{50} (Fig. 2). It is not known whether these relative differences will persist during budbreak and flowering.

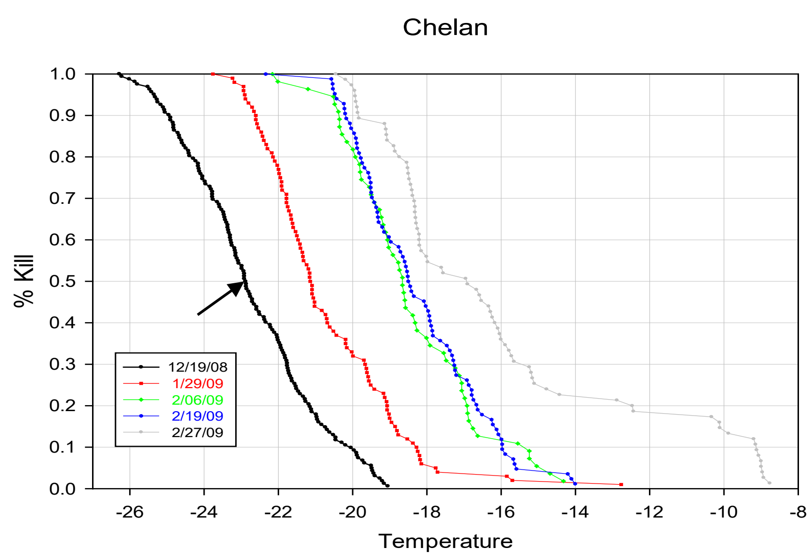

Figure 1. Variability in 'Chelan' fruit bud hardiness over time. Each data point is a recorded low temperature exotherm. Arrow indicates LT_{50} on 12 Dec.

Interestingly, we observed significant variability in hardiness of individual buds within a tree. This hardiness range was as high as 18 F (10 ºC) between the temperature which killed the least hardy flower to the temperature which killed the hardiest flower. Among flowers within a single bud however, there is very little variability in hardiness (i.e., all flowers are killed at a similar temperature, ±0.2 F). This phenotypic diversity in hardiness within a tree/limb/spur is an issue we intend to pursue further.

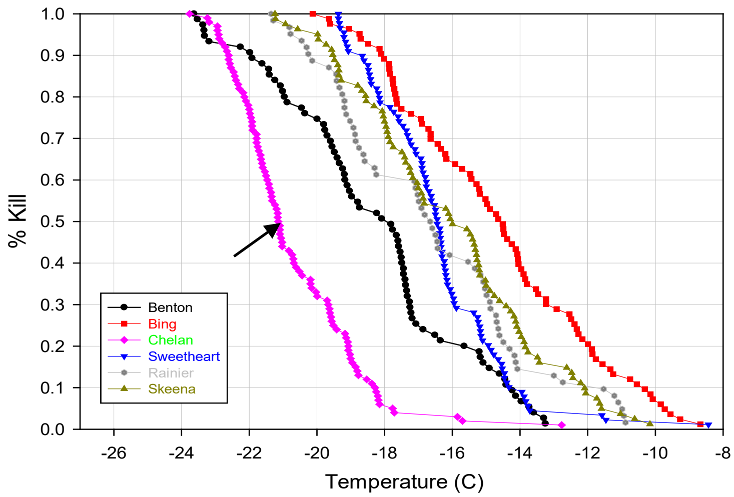

Figure 2. Comparison of fruit bud hardiness among sweet cherry cultivars. Hardiness was assessed on 29 Jan. 2009. Each data point is a recorded low temperature exotherm. Arrow indicates LT_{50} of 'Chelan'.

CONTINUING REPORT **DURATION**: 2 Years

Project Title: A database to aggregate research results and assess technologies

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State/Province/Zip: WA, 98901

Cooperators: Kent Waliser, Sagemoore Farms, Tom Auvil, Tory Schmidt, Ines Hanrahan, WTFRC, Lynn Long, Oregon State University, Matt Whiting, WSU, Paul Tvergyak, Cameron Nursery.

Total Project Request: **Year 1:** $9,078 **Year 2:** $1,000

WTFRC Collaborative expenses:

Item	**2008**	**2009**	
Miscellaneous			
Consultation time[1]	2665		
Total	**2665**	**0**	

Footnotes: 1. Approximately 100 hours to aggregate, explain, and transfer existing datasets from Tom, Tory and Ines.

Budget 1:

Organization: Washington State University	**Contract Administrator:** Jennifer Jansen
Telephone: 509-335-2867	**Email:** jjansen@wsu.edu

Item	**(2008)**	**(2009)**
Salaries		
Benefits		
Wages [1]	$4536	
Benefits (9.6%)	$435	
Supplies	$300	
Travel [2]	$2,307	$500
Miscellaneous		
Database development [3]	$1500	
Database refinement		$500
Total	$9,078	$1,000

Footnotes:
1. Salary for one full time summer person to assist with database entry and surveying.
2. Travel includes mileage and hotel to survey growers in the five fruit growing regions of Eastern Washington and a trip to Oregon.
3. Computer programming of the initial database will be contracted to specialists within WSU.

Objectives:

1. Develop a searchable database that will capture rootstock-variety combinations for apples and cherries, as well as varietal characteristics, management practices, and environmental factors.
2. Aggregate data from existing sources (i.e. projects with OSU, WSU, WTFRC, and nurseries) on rootstock/variety trials and replant practices.
3. Perform a targeted survey of grower and researcher trials that are both geographically and variety robust.
4. Publish to the web under the new tree fruit web portal.
5. Generate reports and analyses to assist collaborators in assessing replant practices and the effects of management practices on production of targeted fruit.
6. Assess the status of the database and identify gaps where incorporation of new variables would assist in development of targeted fruit production.

Significant Accomplishments for the Year:

Relevant rootstock and variety data has been organized and entered for WTFRC, WSU Cherry Breeding Program, OSU Cherry research and most of WSU Apple Breeding Program. Data from select growers is the only information left to aggregate and enter.

A 2-page survey was created to easily extract necessary information for the database in an organized and efficient manner from the growers. We have contacted some growers and plan to start meeting in early spring.

Programmers in Pullman, WA are completing a database with a projected completion by the end of March. The Orchard Conditions Database includes a basic search option with just four criteria to select from—crop, rootstock and/or variety, county, size and/or yield. This data is displayed in a table (figure 1) with basic information.

Figure 1: Output of a "basic search"

Variety	Rootstock	County	Strain	Year	Harvest Date	Soluable Solids	TA	Firmtech (g/mm)
Bing	Mazzard	Yakima		2006		11.00	0.35	
Sweetheart	Mazzard	Chelan		2006		12.50	0.67	
Sweetheart	Gisela 6	Chelan		2006				
Bing		Douglas		2006				
Skeena	Mazzard	Okanogan		2006				
Rainier	Mazzard	Douglas		2006				

The Orchard Conditions Database also includes an advanced search that allows the user to select nearly any of the variables in the database (i.e. crop, rootstock/variety, location, yield and quality, management practices, temperature, and post harvest). The results of this search are exported directly to excel for easy sorting and comparison. Lastly, there is an administration page that allows select users to enter data manually online or upload an excel spreadsheet.

<u>Next Steps:</u>

- For each of the varieties and rootstocks, we will create a page with standard information and pictures if applicable. These pages will be created from the literature and with links from the home and results page.
- Collect and enter grower data.

- By April, begin beta testing the site with researchers and producers.
- Make modifications as suggested by beta users
- Release to public
- Meet with researchers to discuss ways to strengthen the information or data collected. Although some of this is being done now.

Methods:
Objective 1. In year one, we will collaborate with the WSU Extension Communications & Educational Support department to develop a searchable database with a user-friendly interface. The database will account for apple and cherry information on 1) variety and rootstock, 2) environmental conditions, 3) management practices and 4) fruit characteristics.

Objective 2. In year one, we will obtain existing datasets from horticultural trials including as many of the variables as outlined in objective 1. This database aims to capture information from multiple sources on numerous horticultural aspects. Therefore, populating the database with information will incorporate several methods. Initially, the database will be populated with existing data from multiple sources (i.e. WTFRC, WSU cherry and apple breeding programs, and Oregon State University).

Objective 3. In year two, we will perform a targeted survey from a minimum of 3-5 growers in each of the tree fruit growing regions of Washington (i.e. Okanogan, Lake Chelan, Wenatchee Valley, Columbia Basin and Yakima Valley). The goal of this survey is to capture a sample of the information available from the numerous on-farm trials that are not readily accessible to the producer community at large. These data will serve to supplement the existing research trial data.

Objectives 4 and 5. In year two, after the database has been populated, we will publish it to the web for immediate use by growers and researchers. We will also generate reports to collaborate with participants interested in examining the effects of replant practices and the effects of management practices on production of targeted fruit. This information can be inserted into TEAM to determine profitability and feasibility of plantings and employed technologies.

Objective 6. In year two, we will organize two small workshops to demonstrate the database held in conjunction with the Washington Horticultural Association Annual Meeting in Wenatchee (2009) and the Cherry Institute in Yakima (2010). The purpose of these workshops will be to have producers assess the utility of the database for identifying benchmarks for targeted fruit production. As developed, we envision that the database will be useful for generating a snapshot of existing fruit production in multiple geographic locations and could be used as a starting point to assess individual production practices as they relate to production of targeted fruit.

FINAL PROJECT REPORT **WTFRC Project Number:** TR-07-701

Project Title: COS TEAM and TFAM training

PI:	Norman Suverly	**Co-PI(2):**	Karen Lewis
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Cooperators: Clark Seavert - Oregon State University
Tom Auvil – Tree Fruit Research Commission
Tim Smith – WSU Extension
Gwen Hoheisel – WSU Extension

Total project funding request: **Year 1:** 1700 **Year 2:** 1200

Other funding Sources

Agency Name: Risk Management Agency
Amount requested or awarded: $4750
Notes: These funds are managed through the WA Potato Commission and were provided for the project in January 2008 to spring of 2008.

Budget 1:
Organization Name: WSU Extension **Contract Administrator:** Mary Lou Bricker
Telephone: **Email address:**

Item	**2008**	**2009**	
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel	1000 (mileage @ .485/mi)	700 (mileage @ 0.485/mi)	
	500 (lodging/meals)	300 (lodging/meals)	
Miscellaneous	200 (facility rental)	200 (facility rental)	
Total	1700	**1200**	

Footnotes:

OBJECTIVES:

Our objectives are to teach growers, fieldmen, and lenders how to use TEAM to assess economic impact of technology costs and adoption. We are targeting the following topics: wage inflation, orchard establishment sensitivities, establishment time span, yields, packouts and production sensitivities (profit maximization versus cost minimization). Participants in the program will learn how to calculate return on investment (ROI) for specific orchard technologies and practices and the relative impacts of ROI.

METHODS:

Accomplishments to date

July 31-Aug 1, 2007. Norman Suverly and Karen Lewis traveled to Aurora, OR for immersion training by Clark Seavert

December 2007. Washington State Horticultural Annual Meeting, Wenatchee. Team was incorporated in sessions to demonstrate and make economic analysis

December 2007. TEAM demonstration in Pennsylvania

January 11, 2008. Cherry Institute meeting, Yakima. Presentation made to demonstrate TEAM, make economic analysis, and promote workshops

January 24, 2008. Okanogan Horticultural Annual Meeting, Okanogan. TEAM demonstration

January 28-29, 2008. 2008 Fruit School, Wenatchee. Presentations of economic analysis for scenarios related to Competitive Orchard Systems

February 6, 2008. TEAM workshop, Grandview

February, 2008. TEAM demonstration at IFTA meeting in Visalia, CA

March 12 and 13, 2008. TEAM workshops in Wenatchee and Omak, respectively

Various 1-on-1 teaching opportunities with growers

August 5 and 6, 2008. Presented TEAM and the fundamentals of the program to Northwest Farm Credit Lenders in Moses Lake, Pasco, Sunnyside, and Yakima.

October – November 2008 Designed web survey for TEAM workshop participants to evaluate short and medium term outcomes. Results will be summarized in December 2008.

November 20, 2008 TEAM demonstration at Washington State Grape Society annual meeting in Grandview, WA.

December 2008 TEAM demonstrations and economic analysis scenarios for state horticultural meeting and WSU Fruit School.

December to February 2008-09 Hands-on computer workshops were conducted in Pasco (Dec. 16), Grandview (Dec. 17), Yakima (Dec. 18), Wenatchee (Jan. 20), and Omak (Feb. 2).

We provided interactive and step-by-step instruction on the use of A Grower's TEAM software models. Instruction included overview and introduction and then participants could run scenarios using pre-made budgets for various tree fruit crops or participants provided their own budgets.

A spiral bound manual and copy of the software were provided for the workshop attendees.

Outcome: Using an online survey, participants were asked for their level of learning, awareness and knowledge gained.

- 87% stated their knowledge of how inflation, market volatility, and labor efficiencies can increase returns or decrease production cost increased
- 63% stated they understand how to use TEAM to determine the profitability and feasibility of alternative cropping systems, modifying current practices, or implementing technologies
- 88% understand how to use the software as a decision making tool

- 13% stated they can competently set up scenarios to analyze and interpret the output

Another online survey will be e-mailed to evaluate the progress of last year's participants and the outcomes of this year's participants. An evaluation of outcomes will also be mailed to all of those from Washington, who have downloaded the software.

For trademark purposes, A Grower's TEAM is now called AgProfit™ and will be part of a suite of farm management software available through the website named AgTools™ (available in April). AgProfit has some new features along with video imbedded help functions. An online instruction course is being developed and will be piloted this year with Oregon State University. Face-to-face workshops will continued to be provided showcasing the new functions of AgTools™.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2

Project Title: Economic analysis of technology adoption by Washington apple growers

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State/Zip: WA 98801

Cooperators: Tom Auvil – WTFRC
Karen Lewis - WSU Extension
Norman Suverly - WSU Extension

Total project funding request: **Year 1**: $23,368 **Year 2**: $24,075

Other funding Sources: None

WTFRC Collaborative expenses: None

Budget 1
Organization Name: SES-TFREC-WSU **Contract Administrator:** **Ben Weller, Mary Lou Bricker**
Telephone: **(509) 335-5557, (509) 335-7667** **Email address:** **wellerb@wsu.edu, mdesros@wsu.edu**

Item	2009	2010
Salaries	0	0
Benefits	0	0
Wages	15,557	16,179
Benefits	2,111	2,196
Equipment	0	0
Supplies	0	0
Travel	3,000	3,000
Survey	200	200
Extension Support	2,500	2,500
Total	**23,368**	24,075

The purpose of this research project is to determine the factors that impacted technology adoption by Washington apple growers. The focus of the research will be on labor-augmenting technologies, specifically platforms. The motivation for this study was the observation that although automation and mechanization technologies are becoming more readily available to growers and large impacts on profitability may be realized by implementing them, there is a lack of comprehensive understanding of the factors that may affect grower's decisions to invest in these technologies. Hence, the economic analysis aims to provide information that will be useful to Washington tree fruit growers as well as researchers who are focused on the future of the tree fruit industry. Moreover this study will give insights on Tree Fruit Research Commission priorities, by focusing specifically on profitability of technologies augmenting labor and improving worker safety.

Objectives

1. Evaluate the economic and managerial factors that contribute to a grower's decision to adopt automation and mechanization technologies.
2. Use the data collected during this project to support other educational programs and decisions aids focused on technology adoption.
3. Establish a program for continuously collecting production and management data from tree fruit growers.
4. Disseminate research results to tree fruit growers, packing houses representatives, researchers from other disciplines, and interested parties.

Significant Findings

No significant findings have been determined at this point in the project.

Methods

To achieve objectives 1 and 2, we will conduct a growers' survey, attempting to obtain a relevant cross-section sample of growers representing the full distribution of orchard sizes and scopes. We plan to interview a targeted group of growers representing orchards of different sizes and in different regions in the state. We will identify this group with the assistance of the project cooperators, which will allow us to compile a comprehensive sample using minimal resources.

To achieve objective 1 we will use several options to model technology adoption. First, we will use a hedonic model measuring the impact of grower characteristics on technology adoption, which will be useful for understanding differences in management style or perceptions of different technologies. Second, we will use a model of risk aversion that incorporates risk preferences of growers to measure perceived changes in risk from new technologies. Third, we will apply a more complex model of adoption choices in a dynamic decision making framework. A dynamic model will allow for information updating over time and would more accurately reflect the dynamic nature of production and management decisions made by growers in the tree fruit industry. With these models in mind, data collection will be focused on asking questions that will allow for the greatest flexibility in modeling choices.

To achieve objective 4 we will provide periodical reports of this study to the Tree Fruit Commission. Final results from this project will subsequently be submitted for publication as Washington State University Extension bulletins available at the Washington State University Extension website.

Results and Discussion

This early in the project, we thought it more useful to outline the specific activities we will be engaging in during the remainder of Year 1. The timeline listed below reflects information we have obtained since the project was approved regarding producer availability and the amount of time needed to prepare and execute a producer survey.

Timeline of Activities for 2009 (Year 1)

1. Obtain IRB approval for use of human subjects
   - Requirements:
     - CITI training for Mykel and Karina (Completed)
     - Draft of survey and cover letter (In progress)
     - IRB Exemption Approval Application form (In progress)
   - Target date for completion: April 1st

2. Develop two versions of a survey instrument based on research objectives of gathering production cost and technology adoption information
   - Requirements for production cost survey:
     - Write questions for survey that will provide the information necessary to answer update AgProfit tools and other production cost-based research
     - Pre-test the survey using a few growers and horticulturists
     - Target date for completion: May15
   - Requirements for technology survey:
     - Conduct a comprehensive review of technology adoption literature
     - Write questions for survey that will provide the information necessary to answer research questions
     - Circulate survey to collaborators for edits/suggestions
     - Pre- test the survey during collection of production cost data using a few growers to detect problems prior to conducting full survey
     - Target date for completion: May 30

3. Collect production cost data via grower interviews using survey
   - Requirements:
     - Identify sample of 20-30 growers representing different apple growing regions Conduct interviews in person when possible (other options such a phone or mail interviews will be used if growers are unable to meet with us)
   - Target date for completion: July 15

4. Summarize and analyze production cost survey
   - Requirements:
     - Data will be cleaned and summarized for use in regression analysis
     - Conduct analysis and write up results
     - Update Grower's Ag Profit online tool
     - Disseminate information via Extension publication and presentations at grower meetings
   - Target date for completion: August 15

5. Send out technology survey to large sample of growers in Washington
   - Requirements:
     - Survey will be sent out in December 2009 and returned by February 2010
     - Survey results will be cleaned, analyzed and summarized for further research and dissemination

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: TR-07-706

Project Title: Mechanized thinning for labor efficient tree fruit cropload management

PI: (PA)	James R. Schupp	**Co-PI (WA):**	Karen Lewis
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Cooperators: Tory Schmidt, WTFRC; Tom Auvil, WTFRC, Katy Ellis, Penn State University; Steve Miller, USDA-ARS; 4-6 Pennsylvania Fruit Growers, Rick Orozco; Rob Valicoff, Travis Allan, Marvin Pitts, WA. State University

Total project funding request: **Year 1:** 25,172 **Year 2:** 26,304 **Year 3:** 27,594

Other funding Sources

WSU – USDA CSREES / SCRI – Innovative Technologies for Thinning of Fruit
WSU – WTFRC – Mechanized Cropload Management with Mueller String Thinner
PSU – USDA CSREES / SCRI – Innovative Technologies for Thinning of Fruit
PSU – PA Peach and Nectarine Board (Objectives 3 and 4)

WTFRC Collaborative expenses:

Item	2008	2009	2010
Crew labor	5,000	5,250	5,500
Travel	3,000	3,250	3,500
Miscellaneous	0	0	0
Total	8,000	**8,500**	9,000

Footnotes: WA Darwin thinning unit will be purchased as a capital expense for the WTFRC internal program (approx. $15,000); inclusion of Darwin treatments increase costs of standard thinning trials by approx. 50%

Budget 1:

Organization: Pennsylvania State Univ.	**Contract Administrator:** Timothy M. Stodart
Telephone: (814)865-1027	**Email:** tms21@psu.edu

Item	**2008**	**2009**	**2010**
Salaries	6,387	6,611	6,842
Benefits	1,648	1,706	1,765
Wages	3,840	3,840	3,840
Benefits	315	315	315
Equipment	0	0	0
Supplies	1,000	1,000	1,000
Travel	0	0	0
Miscellaneous			
Total	13,190	**12,971**	13,262

Footnotes: Estimated salary costs are based on current salary rates (fiscal year 2007-08) escalated approximately 3.5% beginning July 1 of each subsequent year. University policy has been to award salary increases on the basis of merit only.

Fringe Benefits: Rates are computed using the rates of 25.8% applicable to Category I salaries; 15.7%
applicable to Category II graduate assistants; 8.2% applicable to Category III non-student wages and fixed-term II salaries; and 0.4% applicable to Category IV student wages for the current fiscal year of July 1, 2007 through June 30, 2008. If this proposal is funded, the rates quoted above shall, at the time of funding, be subject to adjustment for any period subsequent to June 30, 2008 if superseding Government approved rates have been established. The fringe benefit rates are negotiated and approved by the Office of Naval Research, Penn State's cognizant federal agency.

Budget 2:

Organization: Washington State Univ	**Contract Administrator:** Mary Lou Bricker
Telephone: (509) 335-7667	**Email:** mdesros@wsu.edu

Item	**2008**	**2009**	**2010**
Salaries	0	0	0
Benefits	0	0	0
Wages	1,700	0	2,000
Benefits	282	0	332
Equipment	0	0	0
Supplies[1]	500	2,000	500
Travel[2]	1,500	2,832	2,500
Miscellaneous	0	0	0
Total	3,982	**4,832**	5,332

Footnotes:

[1]Supplies – New Darwin strings (molded, 2 sizes)

[2]Travel – Includes $1500 support for Craig Hornblow – see narrative

Objectives:

1. To evaluate the effect of timing on efficacy of mechanical blossom thinning, relative to peach / nectarine and apple bloom stages. (WTFRC 2008-2010).
2. To evaluate several labor-efficient thinning methods in various combinations. (WTFRC 2008-2010).
3. To evaluate the effect of pruning strategies to influence hanger orientation on peach cropload and on the efficacy of the Darwin vertical string thinner. (PA Peach and Nectarine Bd. 2008-2009).
4. To compare the efficacy of a prototype horizontal mechanical blossom thinner or a rope thinner in traditional vase shaped peach canopies, relative to hand thinning (PA Peach and Nectarine Bd. 2008-2009).

Significant Findings (2008):

- The Darwin string thinner was effective at removing bloom in WA peach, nectarine, cherry, apple and pear blocks.
- The Darwin string thinner and double drum shaker were effective mechanical thinners for a second year in a row.
- In Peaches and nectarines, blossom thinning with the string thinner is more effective between 20% bloom and petal fall than at earlier bud stages.
- Combinations of the string thinner at bloom followed by green fruit thinning with a drum shaker were highly effective thinning combinations.
- The ability to tilt the drum of the single drum shaker so that the rods were perpendicular to the scaffolds was beneficial, and this feature would be desirable in the next prototype. Adapting the drum shaker so that it could be mounted in front instead of behind the driver could also improve performance and operator ergonomics.
- The 1.25 inch diameter nylon rods, while necessary to the original purpose of shaking citrus fruit, are too large for thinning peach blossoms or green peaches, and are prone to damaging the bark of the scaffold limbs.
- Qualitative studies with a smaller drum shaker that was designed by Dr. Don Peterson at AFRS for harvesting raspberries demonstrated that a smaller machine with smaller nylon rods can be very effective for thinning peaches and this design should form the basis of a new drum shaker prototype designed especially for thinning peaches.
- The ability to raise and tilt Darwin thinner is critical to achieving desired bloom removal.
- Green fruit hand thinning costs are reduced with mechanical blossom thinning treatments.
- The horizontal string thinner was effective for thinning vase-shaped peach trees when operated at slower speed (1 mph).

Materials and Methods:

PA. For 2009 we propose to test a new prototype of the mechanical blossom thinner (Darwin PT-250), and a new prototype of the spiked-drum shaker (USDA-ARS) for thinning tree fruit. Thinning with the Darwin will be conducted at bud swell, 80 percent full bloom, and fruit set-shucks on peach, and thinning with the spiked-drum shaker will be conducted ~35 days after full bloom. Trials will be conducted on vertical axe trained apple, and on peach and nectarine trees trained to either perpendicular V or quad V systems. Gala and/or Pink Lady / M.9 apple trees trained to vertical axis will be thinned at early bloom with the Darwin, comparing the old and new style strings, and compared to post bloom thinning with MaxCel and with the combination of Darwin blossom thinning plus MaxCel.

The experimental designs in all trials will be randomized block, with multiple tree replicates in peach and apple blocks at the Penn State Fruit Research and Extension Center, and at four to six commercial peach orchards in Pennsylvania. Mechanical treatments will be compared to hand thinning green fruit thinning. Blossom removal and reductions in fruit set will be evaluated from detailed flower and fruit counts of whole trees, divided into upper and lower canopy sectors. Following physiological drop all trees will be hand thinned to a uniform crop load. Hand thinning time per plot will be recorded to determine potential reductions in labor inputs. At harvest, yield per tree will be assessed, and a sample of fruit evaluated for mean fruit diameter, fruit size distribution, and fruit quality characteristics. Economic cost/benefit analyses will be performed to evaluate the impact of each thinning regime on fruit returns, utilizing the AgProfit (formally known as TEAM). Specific treatments are listed below:

Objective 1:
Treatments:

1. Hand thinned control;
2. String thin, 80% bloom;
3. String thin, bud swell, 1 pass;
4. String thin, bud swell, 2 passes;
5. String thin, fruit set-shucks on.

Proposed PA grower cooperators: V – ACN Sugar Giant, McCleaf Arctic Sweet; OC – BMO Redhaven, Wenk Rising Star

Objective 2:
Treatments:

1. Hand thinned control;
2. Blossom thinned with the string thinner at 60% open bloom;
3. Blossom thinned with the drum shaker at 60% open bloom;
4. Green fruit thinning with the drum shaker ~35 days after full bloom;
5. Blossom thinned with the string thinner at bloom and green fruit thinning with the drum shaker ~35 days after full bloom.

Proposed PA grower cooperators: Quad V – BMO Redhaven; V – BMO John Boy, ACN Sugar Giant

WA. For 2009 we propose to test the Darwin Thinner and the new prototype Mueller Thinner for thinning tree fruit. We will evaluate the new cord system in both stone fruit and pome fruit. Thinning with the Darwin will be conducted at bud swell, 80 percent full bloom, and fruit set-shucks on for peach, nectarines and apricots. Darwin trials in stone fruit will be conducted on trees trained to either perpendicular V. Apple trials will be conducted in 2D systems and Vertical axe.

Results and Discussion:

PA. New Darwin PT-250 blossom thinners for have been ordered. Assembly is complete at the factory in Germany and the units are to be shipped in early March 2009. Materials for the new prototype peach drum shaker have been ordered and assembly is underway. We met with Dr. Steve Miller on March 4th and he assures us that the new drum shaker will be ready before the season starts.

We have identified several grower cooperators for the 2009 projects; have secured their cooperation and selected suitable orchard blocks for conducting the research. Selection of trees, randomization and assignment of treatments, as well as flagging and mapping the plots will be conducted in March 2009.

WA. New strings are in shipment and will arrive for first pink timing. Mueller machine should clear customs on March 24. Drum Shaker fabrication for 2010 trials is on schedule. WA. cooperators have been identified with the assistance of the internal WTFRC staff. Stone fruit trials will be located in Wapato, Basin City and Royal City. Apple trials will be conducted in Tonasket, Brays Landing, Chelan, Royal City and Wapato. Treatment and data collection protocols are in line with the SCRI project for stone fruit and the New Zealand studies in apples. This situation allows for large data sets and pooling of statistical analysis tasks.

In 2008, in collaboration with Neil McCliskie and Craig Hornblow with Heartland Fruit and NZ First respectively, I traveled to New Zealand to establish mechanized thinning trials in apples with the Darwin thinner. With extensive field trials and on site direction from Adolf Betz , owner, Fruit Tec, Darwin manufacturer, we were able to narrow down some of the equipment operation variables that impact optimization of the thinner. Harvest data suggests that we over thinned most blocks and all blocks that we collected data through harvest. Over thinning is somewhat subjective. In our case, the grower – cooperators said that we removed more fruit than they wanted removed. Prior to the marketing/sale of the fruit, the growers did not anticipate the increase in box size would compensate for the lower yields.

In an effort to meet the objectives of this project in a timely manner and to continue this specific collaboration with New Zealand and to stimulate additional bi-hemisphere collaborations I am requesting that my revised 2009 budget be considered. To provide partial financial support for Craig Hornblow to travel to WA this spring, I eliminated expenses for time slip wages and benefits (now covered by SCRI funds) and added that amount to travel. Of the $2,832, I am requesting that I be allowed to allocate $1500.00 towards his travel expenses. This movement of dollars within the budget does not change the total request of $4, 832. Craig and I have outlined specific outcomes for this collaboration and his visit. We will establish field trials building on knowledge gained in Oct, we will conduct 2 field days to demonstrate the equipment and report on NZ trial results and we will meet with appropriate individuals on other joint project opportunities. Travel dates are April 20 – May 1, 2009

CONTINUING PROJECT REPORT **YEAR**: 1 of 1
WTFRC Project Number: TR-08-801

Project Title: Automated picking hand development

PI:	Tony Koselka	**Co-PI:**	Derek Morikawa
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Total project funding request: **Year 1:** $27,500

Other funding Sources
Agency Name: California Citrus Research Board
Amount requested or awarded: $27,500
Notes: The project is being worked on as part of the SCOPE program at Olin College

Budget 1:
Organization Name: Vision Robotics **Contract Administrator:**
Telephone: **Email address:**

Item	**2009**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel	$2500		
Miscellaneous	$25,000		
Total	$27,500		

Footnotes: The $25,000 is the fee to sponsor a SCOPE project at Olin College. The additional $2500 covers VRC out of pocket expenses.

OBJECTIVES

The Washington Tree Fruit Research Commission (WTFRC) has been working with Vision Robotics (VRC) on mechanization for fresh apples. The ultimate goal is a robotic harvester. The picking hand represents one of the remaining unsolved critical systems for the harvester. A successful picking hand must harvest all the fruit from a tree and place each piece into the conveyor that moves the fruit to the bin. The hand must gently hold the fruit of different sizes and work delicately and reliably regardless of whether the fruit is hanging freely; leaning against or partially obstructed by other fruit, branches or leaves. In general, the variability in size, orientation and location of the fruit as well as the delicateness of apples makes the picking hand design challenging. Further complicating the design is that the picking hand is part of the larger harvester and must compensate to work with the system as a whole.

This project is a continuation of one started in 2007 and is a collaboration between the WTFRC, the California Citrus Research Board, VRC and Olin College of Engineering. The technical development is performed through the Senior Consulting Program for Engineering (SCOPE) at the Olin College of Engineering. The SCOPE team consists of five talented engineering students, and the project represents their senior thesis.

Last year's team selected a design direction that uses high flow suction to hold the apples and a pivoting hoop to break the apple stem and remove the apple from the tree. While the team built a working prototype, it was only able to test in the laboratory. This year's objectives are to test and evaluate the design in orchards and refine as appropriate. Specifically, the team is to complete the following tasks.

1. Test the design for:
   - General functionality
   - Long term potential
   - Strengths
   - Weaknesses
   - Operation in all appropriate environmental conditions
2. Improve the design as required

A successful design is one with the potential to pick more than 98% of the apples without damage.

Early in the project, it was clear that the existing prototype had severe limitations. As such, the team was instructed to focus on functionality, not implementation. The plan is to create a working design and a later group would create a production product and not worry much about cost, reliability, design for manufacture, etc. The project goal is a design with the potential to meet the following criteria even if the prototype does not:

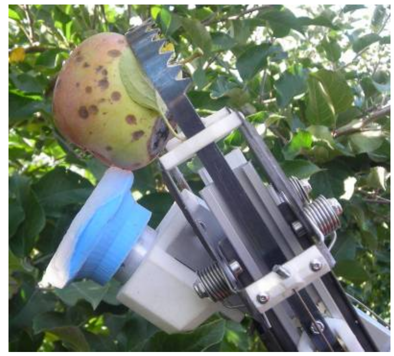
- Effectively pick apples of all sizes and shapes
- Harvest by snapping and/or cutting the apple stems
- Be robust enough to operate for thousands of cycles
- Pick the fruit in approximately ½ second or less
- Place the fruit in the conveyor system in less than ¼ second
- Cause no damage to the fruit it is harvesting
- Cause no damage to other fruit it rubs against when reaching into the tree
- Work around thin twigs and leaves

SIGNIFICANT FINDINGS

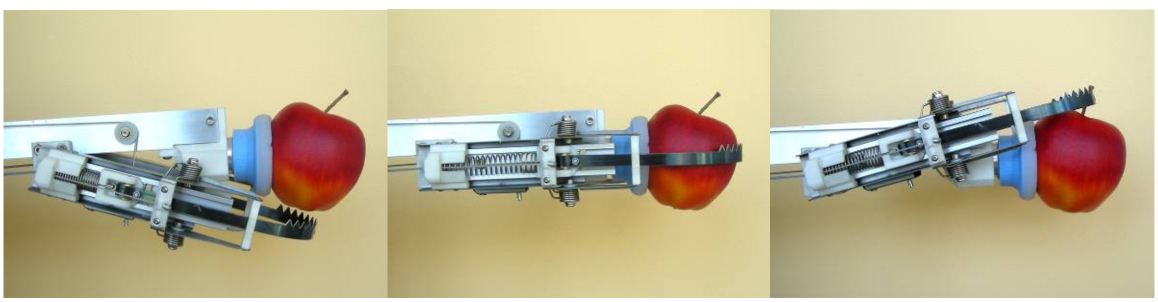
The results of the field testing were reported at the December. In summary:

- Suction is a viable means to hold the apples when picking.
  - High flow suction was strong enough to successfully grab an apple in nearly every case, even when leaves or branches got stuck between the apple and the suction cup.

- The suction cup is a good shape and material and the soft material allows leaves and branches to get caught between the suction cup and the apple without causing bruising.
- Sharp edges anywhere on the picking hand can damage apples.
- A number of apples would come off the tree simply when the suction cup grabbed the apple and the arm moved slightly, i.e., without the hoop. However, the number was not nearly sufficient to use this method exclusively.
- There are a number of problems with the hoop as implemented primarily:



    - The hoop motion as designed does not follow the contour of the apple close enough. In many cases, this causes the hoop to push the apple out of the suction cup and squeeze the apple between the hoop and the suction cup, resulting in much bruising on the sides of the apple.
    - The large gap between the apple and the hoop causes the hoop to hit branches when picking apples with short stems.
    - The hoop alone does not always snap the stem. This is due both to the fact that the hoop does not approach the stem from a perpendicular angle and that the wiping motion does not have an inherent lever point to break the stem.
    - The sharp teeth on the hoop that are designed to cut the stem sometimes cut into the apple or nearby apples, causing great damage and also resulting in the need to clean the hoop before the next pick.



Upon completion of the field trials the team brainstormed and tested various design improvements focusing primarily on the hoop. Their findings include:

- Apples do not easily change position in the suction cup, even when the suction cup is covered with slippery tape. This means that a grab and pull of the fruit will not orient the stem in a preferred location, so the hoop must be designed to break the stem anywhere along its length and path.
- It is possible to design a hoop pivoting mechanism that follows a concentric orbit around the apple so that the hoop always approaches the stem perpendicularly.
- It is not practically possible to create a hoop that rolls along the apple surface to ensure the smallest configuration during harvesting.
- The team did not identify a sensor that would enable the system to detect when the hoop is hitting the apple.
- Reversing the suction and blowing air as the arm approaches the fruit may clear leaves and debris that might get caught in the picking hand.
- Active cutters on the hoop can improve performance. Such cutters could include:

  - Sliding a blade along the hoop to cut the stem.
  - A reciprocating cutter.
- The team did not identify other potential hoop features that might improve performance.
- Simple servos and motors are sufficient to test new concepts.

METHODS

As noted, the team tested last year's design on several apple varieties in medium density orchards. Both last year's team and this year's team have determined that it is important to size the hoop to closely match the apple. In order to use a single picking hand, the hoop size must be adjustable on the fly, which requires an estimate of the apple size prior to picking. Including this, the picking process for each apple is as follows:

1. Select apple to pick.
2. Estimate diameter of apple and enter size into control software.
3. Set hoop to starting mode.
4. Turn on vacuum.
5. Maneuver end effector to apple.
6. Secure apple with suction cup.
7. Rotate hoop around apple.
8. Activate the hoop cutting mechanism.
9. Remove end effector from tree, pulling and twisting as necessary to break stem.

The team designed and fabricated a new prototype and just completed testing in California orange groves this week. The new design can be thought of as having several independent systems.

- **Suction** that includes the suction fan, the suction cup and the hose that connects the two.
- **Suction cup positioning** that adjusts the position of the suction cup so that the hoop pivot axis passes through the center of the apple.
- **Hoop width** that adjusts the width of the hoop opening to match the size of the fruit.
- **Hoop length** that adjusts the length of the hoop to closely follow the contour of the apples.
- **Hoop pivoting** system that drives the hoop through its orbit of the apple.
- **Cutting** that actively snaps or cuts the stem.
- **Software** that coordinates all motion and matches actions to fruit size.

The suction system remains principally the same as last year's prototype. The team is using a Shop-Vac to create the suction and the same soft foam suction cup. The frame has been modified to remove some sharp edges that damaged apples during field trials.

The suction cup positioning system is a linear actuator (motor that drives a shaft in and out) that moves the suction cup relative to the hoop. The center of 2" diameter apple is approximately 1 ½" closer to the suction cup than the center of a 5” diameter apple. This is the range of travel for the suction cup.

The hoop width is a simple “Y”-shaped linkage that opens the end of the hoop from 2¼” to 5¼” depending on the size of the apple. The base of the Y lies along the hose and the top of each side is positioned at the center of the apple. The hoop length and pivot systems are mounted at the top of the Y. The team concluded that thin sheets of spring steel are the best material for the hoop. The steel can be bent into almost any radius, but maintains strength and stiffness along its other axis. The hoop is sized to match the contour of a 5” diameter apple, with the length adjusted in and out from either side.

Once the apple is in the suction cup, the hoop is opened to the proper width and sized to match the contour of the apple. The suction cup is positioned relative to the hoop pivot, and the hoop is moved around the apple. When the system detects that the hoop has stopped because it hit the stem, the cutting mechanism is engaged.

The team is completing the design in SolidWorks (computer aided design software) using stock parts as much as possible. Olin has student machine shops and rapid prototyping equipment to enable the team to build the remaining parts. The intent is to independently test each mechanical system and integrate them into the final prototype. The team will travel to California in March to test in orange groves.

Upon completion of the tests, the team will review the design relative to both the new field tests and observations from the tests in apple orchards during the fall. Based on the analysis, the team will refine the design and complete the fabrication of an improved prototype before the end of the school year. The hoop sizing and cutting mechanisms are the two that most likely will require significant improvement. It is also likely that the software will require refinement because the team has limited time between the completion of the hardware and the field tests.

RESULTS & DISCUSSION

As of the writing of this report, the field tests in orange groves were complete this morning and an analysis is not yet available. A preliminary summary is that the prototype shows potential. The picking hand was first assembled the day before it was shipped to California. As would be expected from both a complex system built by students with a relative lack of experience, it still requires significant debugging. Most of the mechanisms should work as designed. However, the hoop itself appears the most critical subsystem and it requires a redesign during the second half of March and April.

The concentric movement of the hoop appears to help minimize the hoop hitting fruit or branches. However, the fact that the fruit is not perfectly spherical means that the hoop cannot closely follow the contour with a pre-programmed path. The team intends to investigate touch less sensors to enable the hoop to follow the contour of the fruit. Also, the active cutting system needs testing, refinement and possible a significant redesign.

Members of the commission are invited to the team’s final presentation on May 12 at Olin College. Vision Robotics will attempt to secure the prototype for testing in Washington orchards next fall.

In addition, the team will create a report that can be used as an aid to help complete a patent application for novel inventions. As such, the commission will retain rights to these inventions.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: TR-09-900

Project Title: Robotic scout for tree fruit

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WTFRC collaborative expenses: None

Other funding Sources

Agency Name: USDA though the SCRI program
Amount requested/awarded: $200,000
Notes:

Total Project Funding:

Budget History:

Item	2009	2010	2011
Salaries	$115,463	$166,926	$167,233
Benefits	$44,037	$63,337	$63,548
Wages			
Benefits			
Equipment			
Supplies			
Travel	$7500	$9500	$8500
Miscellaneous			
Scout Prototype	$8000	$10,237	$10,719
Subcontract to CMU (field expenses for integration)	$25,000	$25,000	$25,000
Total	**$200,000**	$275,000	$275,000

Objectives

The objective of this project is to create a *Robotic Scout for Tree Fruit*, which is the first phase in the development of a robotic system for mechanization of growing and harvesting fresh fruit trees. The Scout scans fruit trees to determine the total crop yield for any portion of medium and high density orchards. The data include an accurate count and size distribution. Ultimately, the data will enable a picker robot to reach critical speeds and efficiency.

Over the three-year project, the goal is to create a market-ready scouting system. VRC will refine the hardware and software to create a pre-production Scout that will map the crop density throughout the block starting shortly after bloom. The system can track growth throughout the growing season and provide data on a year-by-year and tree-by-tree basis.

This collaboration is part of the Comprehensive Automation of Specialty Crops (CASC) project led by Sanjiv Singh at Carnegie Mellon University. The CASC project is part of the Specialty Crop Research Initiative (SCRI) where the USDA matches industry funding, which significantly leverages both VRC and the Commission's funding and technology. In addition to government funding, the project leverages development by other CASC team members. Specifically, the autonomous prime mover (APM) vehicle developed by Carnegie Mellon and Toro will tow the Scout through orchards; the crop data will be incorporated into the GIS system developed during the project; and sensors (multi-spectral, NIR, pest-infestation) to detect plant stress and insect infestations may be incorporated onto the Scout.

The goal for 2009 is to add production functionality and advance both the hardware and software significantly towards a production design. The process begins with creation of detailed specifications that are used to drive both this year's and the production design. For 2009, the largest single technical task is enhancing the lighting system to enable robust operation regardless of the environmental conditions. The worst case is bright sun, which presents problems both when looking into the light and when the light is behind the cameras and parts of the trees are very bright and other parts are cast in shadows. The system must also operate in a wide variety of weather conditions and at night.

The next generation hardware platform will be more rugged and robust like true farming equipment, and will be towed by typical farm machines. This includes a viable power scheme and weatherization. Software refinement will enable faster operation and enhanced apple detection.

The specific objectives include:

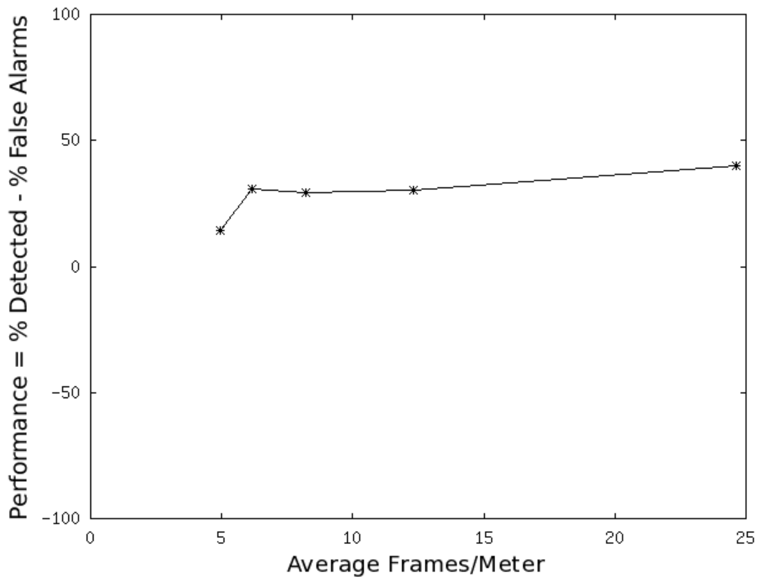
1. Analysis of first generation green apple detection.
2. Product specification.
3. Operation in all lighting conditions from bright sunlight to nighttime and everything in between. The final system may use electrical, mechanical and lighting solutions or a combination.
4. Fabrication of the next generation hardware platform that reflects a production design including integration of electronics and weatherization. The new design will be towed along rows by tractors and other vehicles typically found in orchards. The Scout hardware will be designed for two-sided operation, but will only scan on one side in 2009. The unused scanning mast may not be mounted.
5. Increased operation speed up to approximately 1 – 1½ mph.
6. Scans GPS referenced.
7. Scouting software enhanced to improve detection accuracy and decrease percentage of false positive apple detections.
8. Integrate with CMU, APM and GIS database.

Significant Findings

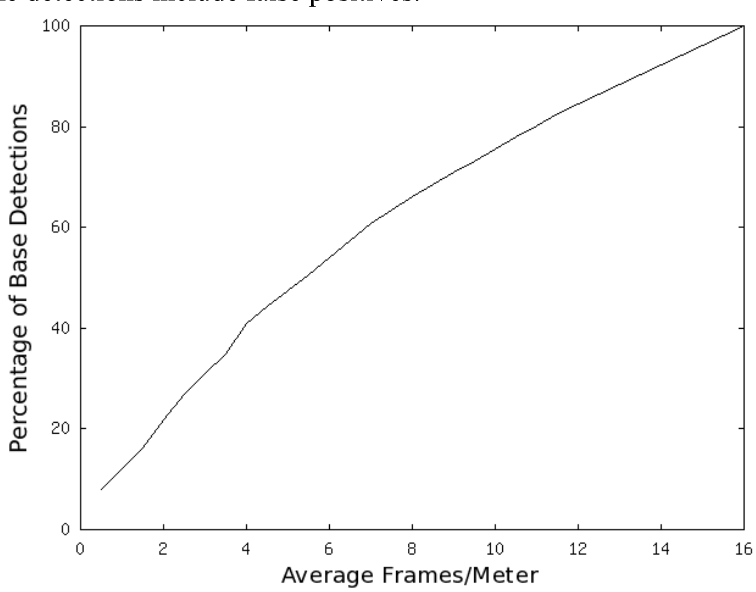
- The data collected by the Scout(see picture to the right) are sufficient to detect green fruit demonstrating that the pictures, frame rates, lighting, odometry and other systems are sufficient for basic performance.

- Boosting algorithms provide a successful basis for detecting green apples and are expected to improve the performance of detection of apples of all colors. The picture below is a single image showing the detected apples.
- Analysis of the runs where hand data were collected for the trees is:
  - Percentage of fruit detected: >71%
  - Percentage of visible fruit detected: >74%
  - Percentage of false positives: 39%
  - Position accuracy: within 4”
  - Size accuracy: 50% of apples sized to within 10% of actual

- The hardest aspect of detecting green fruit is discerning them from other green objects such as leaves and grass. Basic boosting algorithms address the color issue. In real-world conditions, shadows, shape coincidences, and occlusions (due to leaves, stems, branches, tree trunks, and even other apples) greatly affect performance. The detection algorithm must be resistant to large parts of the apple being covered up. Shadows projected on the apple can give rise to spurious edges which distract from the rounded outline of the apple.
- As expected, there are conditions, primarily lighting with bright sun, that significantly degrade performance.
- A sensitivity analysis (using scanned images for which hand measurements were taken) to determine the optimal image frequency (pictures per inch) indicated that performance does not significantly degrade until images are taken at increments larger than every 4” – 8”. The chart shows performance for green apple detection measured as percentage detected - percentage of false positives (which thus ranges between -100 and 100, with larger being better).



- At the anticipated production speed of 2 – 3 mph and an image sampling rate of 4” per set requires a frame rate as high as 13 frames/sec. This rate is faster than what was used during the field tests but less than the 15 frames/sec that the camera system can stream.
- An additional sensitivity analysis (using multiple scans of red apple rows for which no hand measurements were available) demonstrated a moderate detection rate decrease as the sampling rate decreases from the maximum camera system capabilities (of approximately 16 frames per meter at production speeds). The chart below shows the number of fruit detected as a percentage of the number detected when using the baseline rate of 16 frames per meter. Note that the detections include false positives.



In addition to the work done creating the first generation green apple detection algorithms, VRC has been working on Scout specifications. To date, the following documents exist and are available for review.

- Requirements Specification for the High Density Orchard Tree Scout
- 2009 Project Requirements Specification for the High Density Orchard Tree Scout
- High Density Orchard Tree Scout Use Cases
- The Block Diagram for the physical system
- Control and interface diagram

Methods

Scanning

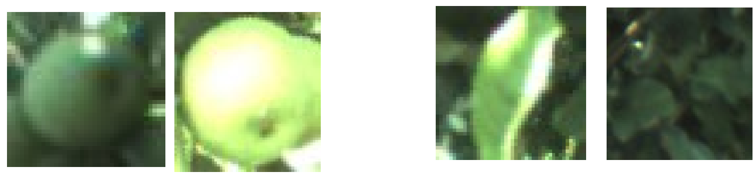
Early scans were run in California orchards to validate the hardware and electronics and to collect a dataset for use during development. In October, VRC ran scans in the Columbia River Basin of Washington State, scanning orchards in Vantage, Orondo, and Othello. The scans included Granny Smith, Fuji, Gala, and Jazz apple varieties. This included scans in angled-V orchards. The system was able to traverse the rows flawlessly and capture images in many lighting conditions, but scanning on hilly terrain in the Wenatchee area required some human intervention to prevent over-rolling on the hills. A total of 222 runs were conducted, with distances covering 1 to 10 m. Some scans used the internal winch to move the Scout, while for other scans the Scout was towed by an ATV. The Scout speed ranged from approximately 0.34 mph to a little faster than 0.68 mph, and the cameras captured data at 6 frames/s from all twelve cameras, or one image about every 2 in. Artificial lighting was mounted to the boom for nighttime scans. A total of 187 gigabytes of data was collected. For one scan, the team made hand measurements of the X, Y, Z coordinates and the size of all apples.

Fruit Detection

As in the 2007 and 2008 projects, the software searches the images for apples; however, new software has been developed to enhance the detection of green fruit. The complete image processing is composed of three consecutive states: boosting, post processing, and tracking. Together, all three stages enhance the performance of the system.

Boosting algorithms form the first stage of the image processing, by classifying each pixel in an image as either a fruit or a non-fruit. Boosting is loosely related to the concept of neural nets. The system, like the brain, considers a very large number of features such as edges, smoothness, texture, color, etc. Fruit generally have characteristics such as round edges, smooth interiors, specific "3-D" appearance, possible stems at the top and holes at the bottom, etc. No single feature by itself can identify the object, but a combination of a great number of such filters, weighted by importance, can. Thus, we can produce a "strong" (good) classifier out of many "weak" (barely better than 50%) classifiers. Boosting refers to a specific class of algorithms that examines training data and picks (a) the feature detectors, (b) the weights, and (c) a threshold. To use the output classifier, one applies the feature detectors to windows in an image, multiples their outputs by their weights, sums the results, and compares the total to the threshold. Windows where the total is greater than the threshold are marked as fruit, and windows where the total is less than the threshold are marked as non-fruit. This test is performed at every possible window position in an image and at a range of reasonable scale factors. To further improve performance, a cascade of such boosted classifiers is used.

The training data used in the boosting algorithm consists of both in-class and out-of-class images. In-class images represent windows which should be classified as fruit. In order for the boosted classifier to be insensitive to occlusions and shadows, this set may also contain pictures of partially obscured fruit, and both shadowed and well-lit fruit. The out-of-class images contain pictures that do not include fruit, but should include pictures that might be mistaken as fruit, such as round leaves, round patches of sky, and random collections of leaves, sky, tree, ground, etc. Examples of in-class and out-of-class images are shown below.



The second stage of the image processing is responsible for post processing the output of the boosting algorithm to eliminate a large number of the false positives. Algorithms which search for specific types of non-fruit pixels (such as blue sky, clouds, tree trunk, grass, and leaves) are applied to the boosting algorithm output. Fruit center and (2-D) radius information is then extracted from the remaining fruit pixels for use in the tracking system.

The third and final stage of the image processing is a tracking algorithm which matches detected fruit across different images to further eliminate false positives, as well as to improve size and position estimates. By considering multiple views of objects initially classified as fruit, those that do not look like fruit from different perspectives can be eliminated. For example, a leaf which appears round in one image may not appear so in surrounding images.

Performance Evaluation

In order to determine the percentage of fruit detected, the percentage of false positives, the position accuracy, and the size accuracy, the software was run on the scans for which data were collected by hand. To determine the percentages of visible fruit detected, green apple images were scanned manually and the fruit identified by hand. The software was then run to do the tracking and convert them to 3-D co-ordinates. By matching these results to the hand-measured fruit, the visible apples were determined. The system was able to detect a significant number of “Invisible” fruit (apples which human analysis initially failed to identify).

The sensitivity analysis consisted of running the algorithm using every frame, every second frame, up to every fifth frame to simulate a lower frame rate. Because the “performance” depends on both the detection rate and the false positive rate, the performance measure was defined as the percentage detected – the percentage of false positives (which thus ranges between -100 and 100, with larger being better).

Results and Discussion

In summary, the methods employed in the development and testing of the Scout have supported the hypotheses presented in the project plan. It is possible to continuously scan trees and collect data with sufficient detail and clarity for visual analysis. The analysis is able to detect the fruit in the images.

The results stated above are less than the goals at the start. In hindsight, it is clear that the initial goals were overly optimistic; however, the results are very satisfactory and indicate refinement and optimization can bring performance to that required for production. There are several factors leading to this belief.

- As noted, the lighting conditions greatly affect performance. Hand measurements were not collected during the night scans, but analysis indicates that performance is significantly better when lighting is controlled. Specifically, bright sunlight, whether in front or behind the cameras, greatly degrades the performance of the current Scout prototype. Improving the performance in various lighting conditions is the single largest technical task for 2009. VRC’s approach was outlined in the project proposal.
- The trees for which the hand measurements were taken are atypical with the vast majority of fruit growing in a small band. This results in large clumps of apples, which are harder to individually identify than those grown in most higher density orchards.
- Currently, possible apples are only detected in and tracked over images from a single camera pair. Examining the images from different camera pairs along the mast for possible apples

creates significantly different viewpoints of areas that will greatly reduce the number of false positives and improve the accuracy of the data collected for actual apples.

- The scans corresponding to the trees for which hand measurements were taken contain some motion blur and overexposure, making analysis more difficult. Improvement to exposure and camera control will improve image quality.
- The scans corresponding to the trees for which hand measurements were taken have the cameras positioned quite close to the trees, whereas other analysis suggest a greater distance may yield better performance.
- Additional scaling information, which is not currently used, provided by the boosting stage may assist in fruit sizing performance.
- Additional confidence information, which is not currently used, provided by the boosting stage may yield improved detection and false positive rates.
- Using weighting and confidence level techniques in the post processing stage may yield improved detection and false positive rates.

In addition to lighting enhancements, the next development steps include continued creation of the specification. The most noteworthy are the System, Hardware and Software Design Specifications. These describe the overall architecture and specific system requirements. Based on these specifications, fabrication of the next generation Scout will begin in March.

The next Scout will include a GPS receiver to geo-reference the data for inclusion in GIS databases. VRC has already begun work with Carnegie Mellon University to integrate the Scout data into their GIS system and to configure the prototype with their Autonomous Prime Mover. Testing is planned for late July in Washington.

CONTINUING PROJECT REPORT **YEAR**: 1 OF 2 YEARS
WTFRC Project Number: TR-09-05

Project Title: Mobile linear asymmetric fruit transport systems

PI: Randy Allard
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City: Mercer Island
State/Zip: WA 98040

Cooperators: Oxbo International Corporation, California Citrus Research Board, WTFRC, Familigia LLC, Washington State Growers Councils

Total Project Funding Request: **Year 1:** 200,000 **Year 2:** 500,000

Other funding Sources

Agency Name: California Citrus Research Board & SCRI
Amt. requested/awarded: $1,000,000+ requested
Notes: Pending

WTFRC Collaborative expenses: None

Budget 1
Organization Name: Picker Technologies LLC
Contract Administrator: Vincent Bryan III
Telephone: 206-275-0641 **Email address:** v3@pickertech.com

Revised Budget: [1]

Item	2009	2010
Salaries	100,000	N/A
Benefits	28,000	N/A
Wages	30,000	N/A
Benefits	3,000	N/A
Supplies	14,000	N/A
Travel	25,000	N/A
Total	200,000	

Notes: Travel = In-field testing costs in WA and CA. Miscellaneous = 5% Contingency

OBJECTIVE 1

Develop and test one (1) pilot fully integrated mobile fruit transport harvest system that increase the economic efficiency of harvest and post harvest, 2.5 to 6 times over traditional methods.

The goals and activities for the next 9 months are to employ 3 tests:

I. A Shop Test whereby the controls for the bin management system for the fruit handling system will be put 'through its paces' and solve any issues that may arise.

II. A Citrus Field Test will prove out the durability of the components and further delineate any issues and improvements needed in the transport of the fruit during harvest.

III. An Apple Test Track will use fruit from a packing house and further prove out the function of the Fruit Handling System with its intended purpose to 'harvest' apples.

Schedule of activities:

I. The Shop Test will be conducted in the last week of March and will expose any improvements or issues before any field trials.

II. The Citrus Field Test will occur in the month of April 2009.

III. The 'Apple Test Track" will occur in the month of May 2009.

No deviations at this time from original objectives or schedule.

SIGNIFICANT FINDINGS (First 3 Months)

- Transport Tube has been improved to effectively and gently deliver fruit through a 20 foot tube and a 9 foot elevation.
- Flexibility of the tube and the laborers' picking portal has been improved to aide in the efficiency and mobility of the laborer during harvest.

METHODS

for VALUE PROPOSITION

This portion will test and validate labor efficiency gains, culling capture, and damage to fruit in the bin when compared to traditional hand harvest and transport methods. Field observation and testing in 2008 suggest an average picking speed of 30 apples per minute using traditional hand harvest methods. The fruit handling system has been designed to operate at up to 2 apples per second per picker, four times as fast. Culls are currently thrown on the ground or placed in the bin which is shipped to the packing house. Culls may constitute 2% - 25% of an orchard harvest, with an estimated half of the culls created from fruit damaged during harvest, and transport. Use scanning reports to identify percentage of crop culled in field, and test bins to assure fruit placed in bins is of a higher degree of " damage-free" prior to transport to packing house.

for SHOP TEST AND CITRUS FIELD TEST

B. Field test plan and functional evaluations:
    1) Field validation of all items from shop test plan
    2) Dynamic evaluation on all systems, product damage under dynamic operation, core technology performance
    3) Maximum operational angle verification of all systems. 7% grade
    4) Trellis fit, interference analysis
    5) Maneuverability
    6) Turning requirements
    7) Steering
    8) Braking/park brakes
    9) Operator interface
    10) Bin sequencing timing and performance evaluation
    11) Picker load balancing functionality
    12) Machine productivity, Limitations to productivity
    13) Machine transport: field and road, trailer loading
    14) Horsepower usage, Fuel usage and capacity
    15) All fluids: flow and cooling capacity checks
    16) Electrical loading and capacity: 12V, 24V
    17) Endurance test plan: Cumulative testing from California and Washington
    18) Minimum machine hours prior to pilot build: 200 harvest hrs

for APPLE TEST TRACK

Take 12 Apples (Golden Delicious) in the range from 2.5 inches to 4 inches and progressively feed them into the Transport Tube at 2 Apples per second. The Functional areas will be evaluated to make sure that the transition of the Apple occurs efficiently and with minimal bruising. The Impact Recording Device (IRD) will then be passed through the Harvester to see if excessive impacts (which could result in bruising) are recorded or to divulge areas of concern.

A. Functional test plan
    1) OBJECTIVE for APPLE TEST TRACK
        a) Apples/IRD pass through Transport Tube

b) Apples/IRD are decelerated in Vacuum Deceleration Box and pass successfully to Transition Conveyor
c) Apples/IRD are efficiently transported onto Transition Conveyor
d) Apples/IRD move from Transition Conveyor to Scanning Conveyor
e) Apples/IRD are tumbled, scanned, and culled per the Sorting Algorithm
f) Apples/IRD move from Scanning Conveyor to Dry Bin Filling Conveyor
g) Apples/IRD are effectively delivered and filled into the Dry Bin

for BIN PATH

Using a plastic MacroBin and a wood bin, feed the bins into the carriage transport, taking note of cycle times and mechanical interaction. Measure openings and interference to verify fit for all sizes of bins

1) OBJECTIVE for BIN PATH
   a) Bins are loaded onto entry ramp.
   b) Bins are automatically loaded into Cull position
   c) Cull Hopper works properly
   d) Bins are automatically and properly unloaded from Cull Bin position
   e) Bins are shuttled and automatically loaded into Dry Bin position
   f) Dry bin and Cull bin shuttling and filling work properly
   g) Bin unloading occurs effectively

for OPERATION and MECHANICAL CONTROL

This portion will evaluate and address areas of interaction for the stopping, starting, and possible malfunction of the interaction between the vacuum motors, water pumps, loading ramps, Transition Conveyor motor, hydraulic Scanning Conveyor motor, shuttling conveyor motors, scanning "tumbling" mechanism, culling mechanism, lift cylinders for Cull and Dry bins, mobility controls, all STOPS, Picking Platform mobility, and Engine Function.

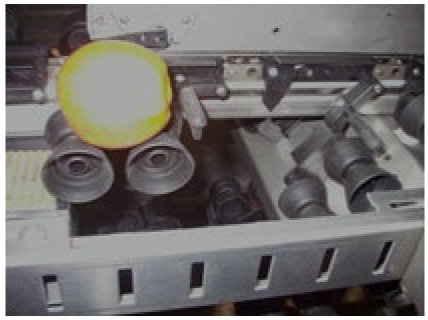
1. MECHANICAL ELEMENTS
   a) Propulsion system functional tests
   b) Engine package: operation, cooling, 12/24 v alternator functionality
   c) Hydraulic package: initial data acquisition
   d) All actuated cylinder functionality
   e) Mechanical integrity of the overall machine

RESULTS & DISCUSSION (first 3 months)

To date we have been designing and implementing improvements learned from field test last October, before we received the grant. Formal findings will be forthcoming in the following 9 months based on the test plans. Focus will be on the evaluation and improvement of the laborer's picking efficiency and gentle filling of the Dry Bin by the download transport system. The Citrus Test Plan and Apple Test Track will provide an indication of harvests' economic benefits to the industry.

OBJECTIVE 2

Develop and test an in-field cull sorter and bin storage system (to decrease storage costs and increase revenue opportunities for the grower).



The goals and activities for the next 9 months are to prove out the mechanical components for culling (Citrus Test) and the reliability and sensing ability to 'cull' apples with the mobile scanner technology (Apple Test Track).

No deviations at this time from original objectives or schedule.

SIGNIFICANT FINDINGS

- Defects in Apples can be determined within the 8" scan zone and before the apple leaves the space underneath the Scanning Hood, leaving ample time to activate culling mechanisms.
- The size of each piece of fruit and a histogram can be successfully logged for each bin of apples.

METHODS

The scanning algorithm should identify 80% of the offending fruit with a 90% confidence level for culling. Packing Houses at best try to achieve 90% of cull identification within a more controlled environment. With graded apples being 10X more valuable than a cull, feedback suggests allowing a few culls into the graded bin would be a better situation than culling a higher quality grade of apple. The Fruit Handling System will allow for logging of different 'cull' defects and the ability to 'tweak' scan settings during harvest, per Grower preferences.

RESULTS & DISCUSSION (first 3 months)

Knowing that a higher quality bin of apples is being provided to the packing house will improve 'pack out' for the growers. The logged information for size will also aide the packing house in filling orders and not having to sample (guess) the fruit bins and having that sample represent the whole bins' demographics for size. When the bin leaves the orchard with a higher quality bin of fruit it will assist in improving transportation and handling for post-harvesting activities, and reduce in-field sorting costs.

OBJECTIVE 3

Develop and test a 'dry bin' filler that minimizes damage to fruit while safely loading a bin in-field at a minimum of 8 apples per second (profitability).

The goals and activities for the next 9 months are to optimize (speed, leveling, corners, minimizing damage) the filling capacity of the Dry Bin during the Citrus Field Test and the Apple Test Track.

No deviations at this time from original objectives or schedule.

SIGNIFICANT FINDINGS

- The in-sync transition of fruit from the Scanning Conveyor to the Dry Bin Filling Conveyor has been optimized.

METHODS

Outlined above.

RESULTS & DISCUSSION (first 3 months)

Observations when watching the Dry Bin Filling Conveyor and subsequent comments appear to be promising. In order to prove out the benefit or pitfalls from this component, the functioning complements of the Fruit Handling System need to be in full operation. The following 9 months will provide a better and more vivid picture of how these objectives are being met.

FOOTNOTES:

1. As originally forecast, project costs will exceed WTFRC grant. Picker Technologies is planning to fund the difference. Below is a table showing project expenses through February 2009. Travel budget will be used for 25 days of April Field test trials in California Citrus groves to simulate apple harvest conditions. A rotating crew of Picker Technology engineers and 4 local field laborers will work 6 days per week in California during the month.

	Picker Technologies, LLC					
	Project Billing Analysis					
	Actual Costs 1/1/2009 - 2/28/2009					
	System	Scan	Dry Bin	Total	**Budget**	Delta
Salaries	$ 70,020.68	$ 11,187.51	$ 17,895.85	$ 99,104.04	**100,000**	$ 895.96
Benefits1	$ 10,087.78	$ 1,927.52	$ 2,309.39	$ 14,324.69	**28,000**	$ 13,675.31
Wages	$ 7,624.34	$ 6,223.42		$ 13,847.76	**30,000**	$ 16,152.24
Benefits					**3,000**	$ 3,000.00
Supplies	$ 22,308.77	$ 6,620.92		$ 28,929.69	**14,000**	$ (14,929.69)
Travel					**25,000**	$ 25,000.00
Total	$ 110,041.57	$ 25,959.37	$ 20,205.24	$ 156,206.18	**200,000**	$ 43,793.82
Remaining to bill					43,793.82	

CONTINUING PROJECT REPORT **YEAR**: 2 of 2
WTFRC Project Number: TR-07-703

Project Title: Expanding and stabilizing WSU-decision aid system

PI:	Vincent P. Jones	**Co-PI(2):**	Jay F. Brunner
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City:	Wenatchee	**City:**	Wenatchee
State/Province/Zip	WA 98801	**State/Province/Zip:**	WA 98801

Co-PI(3): Gary Judd
Organization: Agriculture & Agri-Food Canada
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Address: 4700 Hwy 97
City: Summerland, BC
State/Province/Zip Canada V0H 1Z0
Cooperators: Jerry Tangren, WSU-TFREC; Leo Garcia, Wen. Valley College

Total Project Request: Year 1: $80,965 **Year 2:** $79,960

Other funding Sources

Agency Name: Washington State Commission on Pesticide Registration
Amount awarded: $22,834
Notes: This supplements WTFRC funding for DAS.

Budget 1:

Organization: Washington State University	**Contract Administrator:** Mary Lou Bricker, Kevin Larson
Telephone: MLB 509-335-7667, KL 663-8181 x221	**Email: MLB**: mdesros@wsu.edu, **KL:** kevin_larson@wsu.edu

Item	Year 1: 2007	Year 2: 2008
Salaries[1]	58093	57297
Benefits[2]	20372	20063
Wages	0	0
Benefits	0	0
Equipment	0	0
Supplies[3]	2000	2080
Travel[4]	500	520
Miscellaneous	0	0
Total	$80965	$79960

Footnotes:
[1]Programmer 1 FTE, Web Programmer, 4 mo. 50% FTE, Callie Baker 16.7% FTE
[2]Programmer 35%, Web Programmer 36%, Callie Baker 34%.
[3]Cell phone charges are allowed
[4]Within State Travel

Objectives:

- Stabilize and extend the current DAS program, including extensive documentation, help files, improving the overall interface, and better integration with AWN. We will also add the ability for models to interact to help improve pesticide recommendations when a single pesticide may control multiple pests or diseases at the same time.
- Improve the current disease models and add the model for shot-hole of stone fruit.
- Develop organic control recommendations.
- Once the program is stabilized, implement a bilingual interface for Spanish-speaking users.
- Investigate methods to improve the codling moth model.

Significant Findings:

- DAS will be on-line by 16 March, including new pesticide recommendations (2009 Spray guide).
- We have added an Oriental Fruit Moth degree-day routine to help shippers to Mexico meet quarantine guidelines. Because the OFM model is not validated in Washington, we will not provide recommendations other than times to have the traps out in the spring.
- We have the AWN historic weather database installed on the DAS server, this dramatically speeds up historic data queries and reduces overhead on the AWN server.
- We have a new data summary page that allows the user to see the output for a particular model at all the sites where that model is chosen in their user profile. Graphs are color coded for quick visualization of key periods.
- We have made significant changes in the interface to fireblight, powdery mildew, and scab models with guidance from Tim Smith and Gary Grove.
- We are adding organic recommendations for the disease models in conjunction with Tim Smith and Gary Grove. The insect models already have organic recommendations implemented.
- We are finishing the laboratory experiments on the effect of the pre-chilling period on codling moth emergence. This data should be available in the mid-summer progress report.

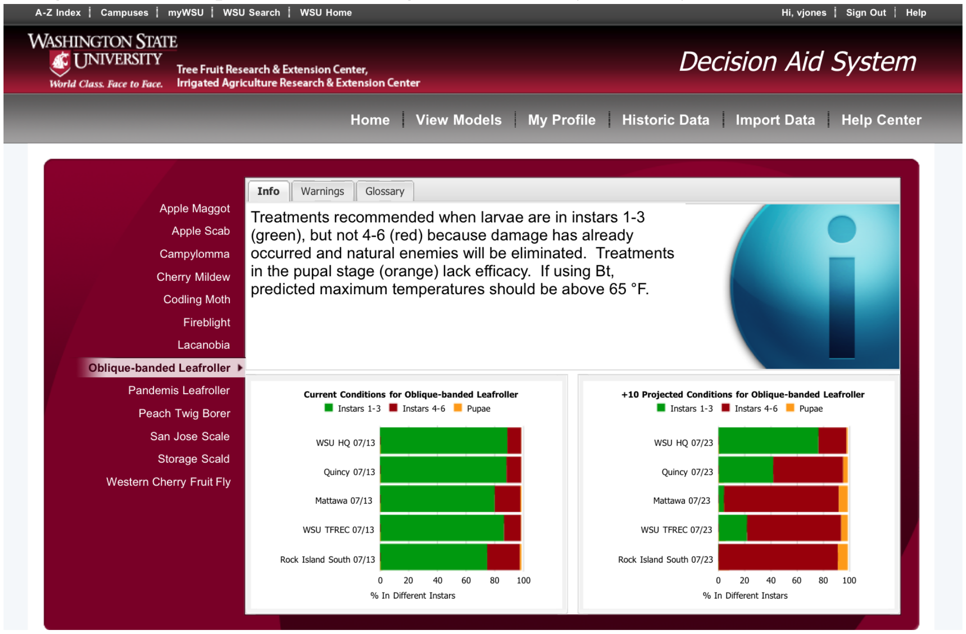
Significant Progress:

Objective 1. The major changes in the user interface were reported at the last meeting. For the last quarter, Brad has been working to fix errors, incorporating new features for managing the system, and improving the AWN–DAS interactions. The changes are nearly complete and the system will be on-line by 16 March for the public.

New features added since the last report include:

1. Addition of an Oriental Fruit Moth degree-day routine. The OFM model is not validated in Washington, so we are not providing recommendations similar to our other models, but instead are providing DD summaries and predictions. The only recommendations we will provide are these for when traps should installed in the orchard before first moth flight. Users will be able to go to the historic weather data module to obtain a text file that lists date and degree-day values for the entire season (which is needed for the Mexico export protocol).
2. The AWN historic weather database is now present on the DAS server, which dramatically speeds up our access for historic data queries and eliminates problems if Internet services to the main AWN servers are affected. We now mirror the AWN database, updating it after midnight every day.
3. We have included a new summary page that allows the user to look at any particular model over all their sites at once (Fig. 1). The graphs are color-coded to help the user quickly pick out patterns and determine whether actions are needed quickly as well as the ten-day projection. For example, looking at the chart on the lower left, the green bars indicate the time

Fig. 1. New summary screen showing the phenology of OBLR at all sites present in the user profile. Graphs are color coded so that green indicates the times to treat, red or orange are times of poor control or high natural enemy mortality.



to spray for OBLR, red are the latter instars where damage has already occurred and natural enemies are active, orange indicates the percentage in the pupal stage. A quick glance indicates that most of the orchards would be in the window to treat for OBLR. However, the 10-day projection (bottom right) shows a completely different picture where only the orchard near the WSU HQ station would be reasonably treated. These charts will be available for all the different models.

Objective 2. We have worked with Gary and Tim on the disease output formats and these have been changed and are being fine tuned at this point. They are already active for beta users and for all beginning on 16 March.

Objective 3. As reported last time, the organic recommendations are complete and on-line for the different insect models. We are currently working with Gary and Tim to provide the organic recommendations for fireblight, scab, cherry powdery mildew, and shot hole. These should be on-line by 1 April.

Objective 4. No direct progress on this objective, although the re-write of DAS has separated the interface part from the scientific subroutines. This makes it easier to make interface changes (language or focus on pda/smart phones) without re-writing program code. Depending on progress in the disease models and new features, we may attempt this section late in the fall of 2009.

Objective 5. We are currently still collecting data on the effects of the length of time that diapause-destined larvae spend at warm temperatures (the “pre-cold period”) on CM emergence. The adults have started to emerge, but emergence is not complete for any of the four different lengths of pre-cold periods that we are testing. This part of the study should be included in the August report.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (Extension)
WTFRC Project Number: TR-06-600

Project Title: Orchard automation and mechanization

PI: Karen Lewis
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State/Province/Zip: WA 98801

Co-PI (3): Jack Maljars
Organization: Vinetech
Telephone/email: 509.788.0900 jackm@vinetechequipment.com
Address: 335 N. Gap Road
City: Prosser
State/Province/Zip WA 99350

Cooperators: Grower Cooperators, Grower Committee

Total project funding request: **Year 1:**108,175 **Year 2:** 5,646

Other funding Sources: USDA - SCRI – CASC $690,000

WTFRC Collaborative expenses:

Item	2007	2008	2009
Stemilt RCA room rental			
Crew labor		$4,646	
Shipping			
Supplies			
Travel		$1,000	
Miscellaneous			
Total		$5,646	

Footnotes: Crew Labor and travel is for OTR operation

Budget 1:

Organization Name: Washington State Univ.	**Contract Administrator:** M.L. Bricker
Telephone: 509.335.7667	**Email address:** mdesros@wsu.edu

Item	**2007**	**2008**	
Salaries	17,190		
Benefits	4,985		
Wages			
Benefits			
Equipment	11,000		
Supplies	1,600		
Travel	16,000		
Miscellaneous			
Total	50,775		

Footnotes: as of 03/2009 – 34,000 remains in WSU PI account

Budget 2:

Organization Name: Vinetech	**Contract Administrator:** Kathy Schmidt
Telephone: 509.665.8271	**Email address:** kathy@treefruitresearch.com

Item	**2007**	**2008**	
Salaries			
Benefits			
Wages			
Benefits			
Equipment	57,400		
Supplies			
Travel			
Miscellaneous			
Total	57,400		

Footnotes: Paid in full

Objectives:

1. **1.** Field evaluate Over the Row (OTR) machine and compare labor efficiencies and quality of work to tasks completed on ladders and mobile platform.
2. **2.** Identify best management practices for multi platform equipment.
3. **3.** Determine optimal number of platforms for OTR machines.
4. **4.** Incorporate OTR in on-going vision studies
5. **5.** Incorporate OTR in application technologies field projects

None of the objectives have been met. OTR machine was delivered to Obrien Orchard for dry run operation. Arrangements are being made for Prosser area grower cooperator evaluation and studies. OTR is currently housed at WSU Prosser.

Significant Findings: OTR travels through the orchard quite well – it is stable and responsive. It can maneuver around obstacles; all functions (hydraulics, E stops, multidirectional and turning mechanisms) were tested and performed as expected.

Materials and Methods:

1. Efficiency measurements:
1) # feet / time unit
2) # trees/ time unit
3) Tops only
4) Complete tree

2. Economic Assessment:
1) Cost per unit (tree/row/block)
2) AgProfit Assessment for IRR/ROI/NPV

3. Quality of work:
1) Subjective / Qualitative

4. Best Management Practices:
1) Number of people per platform
2) Employee interview/survey
3) Ergonomic mitigation

5.Green Fruit Thinning Treatments
1) OTR
2) Mobile Platform (Blueline)
3) Ladder

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: TR-09-901

Project Title: Technology roadmap support

PI: James Nicholas Ashmore
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Address: 400 North Capitol Street, N. W., Suite 363
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State/Zip: District of Columbia 20001

Cooperators: None

Total project funding request: **Year 1**: $30,000 **Year 2**: $33.000 **Year 3**: $33,000

Other funding Sources: None

WTFRC Collaborative expenses: None

Budget
Organization Name: James Nicholas Ashmore & Associates
Contract Administrator: James Nicholas Ashmore
Telephone: (202) 783 6511 **Email address:** nickashmore@cox.net

Item	2009	2010	2011
Salaries	$30,000	$33.000	$33.000
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Total	$30,000	$33,000	$33,000

OBJECTIVES

1. To protect funding for ongoing research programs and to seek funding for new proposals identified as significant and beneficial to the Washington tree fruit industry;
2. To continue cooperative efforts with other specialty crop stakeholder groups and to work to educate the new Administration about the importance of the Washington tree fruit industry and its economic importance to the Pacific Northwest and to the nation;
3. To insure that research activities and requests for research proposals made by the new Administration are constructed in such a way as to address the needs of the Washington state industry and to give the flexibility to the Commission to participate in the process;
4. To keep the Commission informed of developments in both the Congress and the Administration that impact on ongoing or future research funding;
5. To pursue specific activities related to high priority research initiatives
    a. USDA-ARS apple root stock breeding program, Geneva, New York
    b. Expansion of pear research in pear genomics, genetics, and breeding in the Pacific Northwest
    c. Expansion of automation and precision ag efforts in the Pacific Northwest
    d. Expansion of research and extension efforts in sustainable tree fruit production and handling.

ACTIVITIES TO DATE

- Monitored closely developments at the Department of Agriculture in the final days of the previous Administration focusing especially on the efforts to merge USDA agricultural research efforts;
- Discussed with senior staff of the House Committee on Agriculture the continuing interest of the Washington tree fruit industry in insuring that the merger efforts pursuant to the new General Farm Act result in continuing changes in how USDA views and structures research efforts in agriculture;
- Monitored closely how the new Administration and the Congress began efforts to address the ongoing economic crisis in the United States focusing especially on the new Stimulus legislation that was enacted by Congress and also on how the Congress developed Omnibus appropriations legislation funding Federal programs for the remainder of the current fiscal year;
- Monitored and reviewed the President’s announced budget outline for the upcoming fiscal year to determine its implications for USDA programs, especially as they relate to agricultural research;
- Monitored ongoing efforts by the Administration to appoint policy personnel for USDA to determine how those appointments could impact on specialty crops generally;
- Continued to monitor developments in the food safety debate, discussed those developments with leaders of related interest groups such as Northwest Horticultural Council, focusing especially how those developments and the related debate could impact on sustainable tree fruit production and handling;
- Monitored and worked with associated interest groups on a wide range of environmental issues that could have significant implications to the Washington tree fruit industry, focusing especially on steps to insure that Federal regulatory decisions are based on sound science;]
- Discussed and worked with Commission Manager and leaders of the Northwest Horticultural Council different possible approaches to the pear genome, genetics, breeding issue that could

lead to greater movement to achieve the goals of the Washington and Pacific Northwest pear industry;

- Worked with key House Committee staff to insure that the interests of the specialty crops, especially with respect to competitive agricultural research, are made known to the new Administration and to its incoming management team; and,
- Worked to insure that to the extent possible relevant congressional offices are provided with sound information about the advances that have been made to date in the specialty crops area and why it is important to the nation as a whole that those advances remain in place and that further progress continue to be made in these areas.

SIGNIFICANT FINDINGS TO DATE

- Presidential appointments to USDA are proceeding slowly. Tom Vilsak, a former Democratic Governor of Iowa, has been confirmed as Secretary. The President has announced the selection of Kathleen Merrigan to be Deputy Secretary of Agriculture. While as of this date, she has not been confirmed by the Senate, this selection is generally viewed as likely to be favorable to specialty crops interests.
- As of this date, no further announcements have been made regarding Under Secretary and Assistant Secretary posts at USDA.
- The stimulus package that has been signed into law contains monies for USDA, with a majority of emphasis on safety net programs and on rural development and waste water programs.
- The House of Representatives has completed work on an Omnibus Spending Bill funding Federal programs through the end of the current fiscal year. That measure has proven controversial in the Senate, although a clear majority of Senators favor its enactment. Much of the debate has been focused on the extent to which "earmarks" or "congressionally directed spending" appear in the legislation. If there are differences between the House bill and the final version that clears the Senate, those differences will have to be worked out before the measure goes to the President for signature into law.
- The President has released his overall budget outline for the coming fiscal year and has submitted it to Congress. The President is expected in late March or early April to release more detailed budget documents showing specific program designations for the Departments and agencies of the Federal government.
- The President's overall budget document would increase funding for USDA programs. On the plus side, that document suggests increased funding for competitive research efforts. On the negative side, that document suggests certain reductions in the market promotion efforts that are widely supported by the tree fruit industry.
- The most controversial element of the President's overall budget document relates his proposals in the area of payment limitations. The Chairman and Ranking Republican Member of the House Agriculture Committee have expressed strong opposition to these proposals because they would force the reopening of the General Farm Act that was so controversial in the last Congress. Further, the Chairman of the Senate Budget Committee, Senator Kent Conrad, has also expressed strong opposition to this aspect of the President's overall budget.
- The Congress is currently in the process of beginning its debate leading to the development of the Congressional Budget Resolution, which when finalized will provide the framework for appropriations actions. The House Agriculture Committee is expected this week to send its "Views and Estimates" on budget issues under its jurisdiction to the House Budget Committee.

- Based on conversations with senior staff, it appears that there is strong support for the specialty crops research effort and for the President's announced interest in increasing funding for competitive research proposals at USDA.
- The President is interested in and committed to enhancing science and its use by Administration agencies in reaching decisions. That effort is designed to depoliticize science in the decision-making process while insuring that there is transparency and that there is the use of sound science supporting Federal decisions.

Next Steps

- Monitor closely developments as the Congress proceeds to develop its Congressional Budget Resolution to insure to the extent possible that overall research funding for USDA is funded at the highest possible level.
- Monitor and report as necessary how the Congress intends to handle the controversy over the President's proposals for payment limitations and also focus on how the Congress will handle the controversy over the President's proposed reductions in market promotion funding.
- Continue to monitor developments in the public debate over food safety issues and how the Congress proposes to handle those matters, focusing especially on the adequacy of science supporting regulations governing how food is handled as it moves through the marketing chain.
- Continue to work with Commission Manager and officials of the Northwest Horticultural Council in exploring options that could be identified and used to make further progress in ongoing efforts to enhance pear genome, genetics, and breeding research in the Northwest.
- Continue to work with agency officials at USDA regarding the merger of research agencies at USDA and how USDA intends to respond to the President's memorandum governing the use of science in decision-making processes.
- Continue to work with the Northwest Horticultural Council, U. S. Apple Association, and other specialty crop interest groups to insure that we have continued broad-based agreement on going forward with the Administration and the Congress on all of these issues.
- Continue to work with the Northwest House Members and Senators in preparing for development of appropriations legislation for the upcoming fiscal year.
- Continue to work with USDA officials to insure that the views and concerns of the Pacific Northwest and especially the Washington State tree fruit industry are considered carefully within the Department.
- Monitor possible upcoming action by Congress on issues like food safety and water and air issues to determine their implications to the state tree fruit industry and work to insure that there are adequate scientific studies available or that there is a recognition of the need for further research into these areas.
- Continue to work closely with senior professional staff in the House and Senate, especially those directly involved with agriculture legislation or those working closely with Members and Senators who have strong specialty crops interest.

Methods

Based on what we have has happened and the reactions so far, it is clear that the new Administration will be fact-based, that it honors science and seeks to define the proper role of science in reaching final decisions. It appears, therefore, that we should continue to emphasize our own commitment to sound science and where possible and appropriate show how and why our interests coincide with that of the Administration.

It is also clear that the new Administration will continue to support agriculture and that such a support will recognize the importance both of specialty crops but also of competitive research generally. There is likely to be continuing disagreement, however, within agriculture overall and we will have to be cognizant of the conflicts existing between the specialty crops and the traditional program crops and we will have to also recognize that certain of the President's budget proposals could create hard choices within the tree fruit industry. These facts, in my view, further emphasize the importance of continuing to work closely with other specialty crops interests and with the relevant committees of jurisdiction in such a way as to minimize controversy and reach an equitable resolution that meets the needs of the Commission.

In summary, I am recommending that we continue our present course of being cooperative but persistent and that we continue to insure that all parties are informed as well as possible and that we continue to be interested more in getting the research done in a credible manner by the best scientists. We have built a great reputation for being honest, for being transparent, and for being patient and persistent in continuing to make progress toward our stated goals.

Results and Discussion

It appears that the new Administration and new Congress are working to create a climate that could be quite favorable to further movement toward insuring better and more research to address the needs of the Washington tree fruit industry. There are potentially major problems ahead, most of which are associated with overall controversies outside of our control: the extent and depth of the current economic crisis, the political debate over the use of the so-called "earmarks" or "congressionally directed spending" in appropriations legislation.

I believe that we may have to become more creative in approaching our interests in enhancing pear genome, genetics, breeding research so that we present the new Administration with a "menu" of options that might be useful in getting funding for the actual research efforts that we are seeking. My sense is that we have had good movement in this area.

As to other research initiatives, it might be useful to consider how to reframe our requests based on the interests and policies of the new Administration, which at the present time seem to favor environmental and rural development programs aimed toward enhancing the quality of life in rural areas.

As other legislation moves in the Congress (such as food safety or efforts to reauthorize the Clean Water Act, or climate change legislation), it is my hope that we can look at those efforts to determine if the proposed legislation provides greater opportunities to advance the stated interests and needs of the Commission and the Washington tree fruit industry.

Finally, I think that it is essential that we continue to work in concert with other agricultural interest groups and that move through the process moving from the general interest in getting enough funding for agriculture to the specific details of where and how that money will be spent.

I very much appreciate the opportunity to continue to work with the Commission and I look forward to an interesting and what I hope to be a profitable year where we can protect what we have gained to this point and also show continued movement toward our goals.

CONTINUING PROJECT REPORT **YEAR:** 2 of 3
WTFRC Project Number: TR-700A

Project Title: Developing flavor gene markers for the WA tree fruit industry

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Total project funding request: **Year 1:** 27,035 **Year 2:** 17,200 **Year 3:** 4000

Other funding Sources

<u>**Awarded**</u>**:**

Agency Name: WTFRC Apple Review
Amount awarded: $87,500 in 2009
Notes: Peace PI and Olmstead co-PI. Synergistic project on marker-assisted breeding application for the WSU apple breeding program.

Agency Name: WTFRC NW Cherry Review
Amount awarded: $45,000 in 2009
Notes: Peace PI and Olmstead co-PI. Synergistic project on marker-assisted breeding infrastructure for the WSU sweet cherry breeding program.

Agency Name: WTFRC Apple Review
Amount awarded: $77,616 in 2009
Notes: Peace, Olmstead, and Evans co-PIs (PI: D. Main). Synergistic project – bioinformatics support for WSU apple and sweet cherry breeding programs.

Agency Name: WTFRC Technology Review
Amount awarded: $50,000 in 2009
Notes: ABI 3730 DNA Analyzer to augment tree fruit breeding and research Peace PI. Matched with $50,000 from Washington Wheat Commission (separate award, PI: D. See) to obtain refurbished ABI 3730 DNA Analyzer ($100,000) for high-throughput genotyping of tree fruit and cereals, based in Pullman.

Agency Name: WSU Agricultural Research Center
Amount awarded: $100,000 in 2009
Notes: Additional support to Dr. Peace and the "Pacific Northwest Tree Fruit Genotyping Laboratory" for high-throughput DNA extraction and genotyping equipment, complementing the ABI 3730 and removing technical bottlenecks for routine tree fruit genotyping.

Agency Name: WTFRC Apple Review
Amount requested: $169,210 in 2009
Notes: Apple Scion Breeding. Evans PI, Peace co-PI. Synergistic project and beneficiary of flavor gene advances for apple.

Agency Name: USDA-CSREES, NRI Competitive Grants Program
Amount awarded: $400,000 in 2008-2010
Notes: Peace PI. Synergistic project – fruit texture genetic control in apple with emphasis on ethylene.

<u>Pending</u>:
Agency Name: National Science Foundation
Amount requested: $2,818,331
Notes: "Genome Database for Rosaceae". Peace and Olmstead co-PIs (PI: D. Main). Synergistic project to develop broad bioinformatics support for Rosaceae crops.

Budget 1:
Organization Name: Washington State University **Contract Administrator**: M.L. Bricker
Telephone: (509) 335-7667 **Email address:** mdesros@wsu.edu

Item	**Year 1 (Jul07-Jun08)**	**Year 2 (Jul08-Jun09)**	**Year 3 (Jul09-Jun10)**
Salaries	4552[a]		
Benefits	1548[a]		
Wages	4395[b]	4484[d]	897
Benefits	505[b]	516[d]	103
Equipment			
Supplies	10000[c]	7200[e]	3000
Travel			
Miscellaneous		5000[f]	
Total	21000	**17200**	4000

Footnotes: [a] Activity 1a; [b] Activities 1b, 2a, 4a, 4c; [c] Activities 1b, 2a, 4a; [d] Activity 4d; [e] Activities 4d and 4b; [f] Activity 4e

Budget 2:
Organization Name: Michigan State University **Contract Administrator:** Bob Rock
Telephone: **(517)355-5040 x242** **Email address:** rock@cga.msu.edu

Item	**Year 1 (Jul07-Jun08)**		
Salaries			
Benefits			
Wages	2500		
Benefits	1535		
Equipment			
Supplies	2000		
Travel			
Miscellaneous			
Total	6035[a]		

Footnotes: [a] Activities 1a and 4c

Objectives:
Understanding the key control points for traits of interest is very valuable for improving crop production. This project seeks to develop a generic system that identifies genes controlling traits of importance to the Washington tree fruit industry, and to implement this system using the example of fruit flavor. This knowledge can then be put to practical use, such as in marker-assisted breeding, controlled sport induction, or chemical genomics. The proposed project uses the candidate gene approach and capitalizes on expanding genomics databases and a large international network of tree fruit genomics, genetics, and breeding researchers.

Specific objectives are to:

4. Develop DNA tests of flavor useful for Washington tree fruit breeding programs
5. Establish a temperate-tropical fruit genomics channel through linkages between the Rosaceae and papaya genomes
6. Identify tropical fruit flavor genes having potential value for the Washington tree fruit industry

This is the second continuing report for the second year of the project. In Year 2, (July 2008 – June 2009), 100 genes on our developed Master List of tree fruit flavor candidate genes will be placed on the *Malus* and *Prunus* genomes and compared to locations of reported flavor trait loci. Sequence variation in these "flavor genes" will be surveyed within germplasm sets that represent the PNW apple and sweet cherry breeding programs. The compositions of these sets of individual have been determined within 2008 WTFRC apple and sweet cherry projects, and refined and placed in a national and international context with our involvement in the NRI project "Functional gene markers for Rosaceae tree fruit texture" and the SCRI proposal "RosBREED: Enabling marker-assisted breeding in Rosaceae". Gathered flavor performance data from collaborating projects and programs will be combined with genotypic and pedigree data for statistical analysis. The Pedigree-Based Analysis approach will be conducted on this combined dataset using the software FlexQTL and support from developer and collaborator, Dr. Marco Bink. Development of ready-to-apply breeding tools (markers for selection) and connections with tropical fruit genomics efforts are slated for Year 3.

Significant Findings:

- The "Flavor Gene Map" has been improved from several sources. This map shows all known locations in the *Malus* (apple) and *Prunus* (sweet cherry) genomes of regions correlated with fruit flavor genetic variation as well as candidate genes for fruit flavor. Genomic regions influencing sweetness, acidity, and aroma, and genes that we postulate control these traits were obtained from published reports, collaborators in other institutions, and our own laboratory work on promising targets.

- Several candidate genes were observed to co-locate with genomic regions influencing flavor traits, and warrant closer attention.

- Interestingly, many regions controlling flavor are common between *Malus* and *Prunus*.

- Preliminary "whole genome sequencing" of sweet cherry by Dr. Amit Dhingra is already functionally connecting with marker development within this flavor gene project. The sweet cherry genome sequence of 'Stella' was screened to identify cherry versions of several flavor genes on the Master List. 'Stella' DNA sequences will facilitate the development of flavor gene tests that efficiently screen both cherry and apple, as well as cherry-specific tests where desired, by reducing the occurrence of failed tests.

Methods:
This project involves:

- Molecular genetics, bioinformatics, and molecular biology to choose, test, and analyze the DNA sequences of genes (belonging to known biochemical pathways putatively leading to sweetness, acidity, and aroma for apple and sweet cherry),
- Molecular genetics to locate such candidate genes on the *Malus* and *Prunus* genomes and match with reported locations of controlling chromosomal regions for flavor,
- Physiology, sensory analysis, breeding, and databasing to collect performance data on flavor-related traits (sweetness, acidity, and aroma) for apple and sweet cherry, and
- Statistical approaches to identify significant gene-trait associations that can be exploited for the improvement of flavor characteristics in apple and sweet cherry.

Results and Discussion:
We are testing genes that are likely suspects involved in fruit flavor (sweetness, acidity, and aroma) and collecting flavor-related data, for apple and sweet cherry. We expect that by the end of the project, some of the genes investigated will be found to influence important flavor components of apple and cherry. This knowledge will allow us to better understand the genetic value of existing cultivars, advanced selections, and parents of the WSU apple and sweet cherry breeding programs. The knowledge will also provide a means to manipulate these traits for crop improvement. Within this project, we will develop as many genes as possible into genetic screening tools for breeding, via the marker-assisted breeding approach. With marker-assisted seedling selection, the infrastructure for which we are developing in other projects, we can reduce the proportion of seedlings planted in the field with poor genetic value, to improve the efficiency of breeding operations. Economic analyses within concurrent projects of the PIs are providing bottom line figures that indicate substantial cost savings by implementing even one genetic marker that tags an important trait. Flavor attributes are certainly high priority in the WSU apple and sweet cherry breeding programs.

<u>The Flavor Gene Map</u>
Major flavor trait loci and candidate genes in the *Malus* (apple) and *Prunus* (stone fruit) genomes are summarized in Figure 1. These regions were determined by:

d) A survey of literature on QTL (quantitative trait locus = chromosomal region influencing a trait) analyses in apple, peach, and cherry (Maliepaard et al. 1998; Wang et al. 2000; Etienne et al. 2002; Liebhard et al. 2003; Kenis et al. 2008). In recent scientific conferences, researchers at Plant & Food Research in New Zealand (previously HortResearch) reported the location of a major locus (*2MBAc*) on linkage group 2 of apple controlling levels of the major volatiles contributing to "apple aroma". Most recently, Dunemann et al. (2009) working on different apple germplasm reported 5 major (including *2MBAc*) and 10 minor regions in the apple genome controlling a wide array of volatiles. This report also indicated the positions of two flavor candidate genes.

e) Data kindly provided by colleagues involved in genetic mapping of flavor candidate genes – unpublished work. At UC Davis, Dr. Eben Ogundiwin has recently placed 12 flavor candidate genes on the *Prunus* genome within a study that is developing a "*Prunus* fruit quality gene map" (Ogundiwin, Peace, et al., manuscript submitted). At Plant & Food Research, Dr. David Chagne and students (Mukarram Mohammed and Aurélie Dimouro) mapped 13 flavor candidate genes from a list compiled by their flavor genomics specialists, Drs. Edwige Souleyre, Richard Newcomb, Robert Schaffer, and Ross Atkinson. This work was done to try to identify the gene controlling the *2MBAc* aroma locus, but thus far has not been successful. Nevertheless, the 13 candidate genes may be discovered to control other aroma loci, and thus we include them on our Flavor Gene Map.

f) The map also includes genes involved in the ethylene biosynthesis and perception pathway. Reports on transgenic apple fruit with the ACS and ACO genes knocked out (Dandekar et al. 2004; Schaffer et al. 2007) have shown that while sweetness and acidity development in fruit is apparently independent of ethylene, volatile aroma compounds – particularly volatile esters – are strongly affected by ethylene. The final enzymatic steps of volatile aroma compound synthesis are always affected by ethylene levels, and often the initial enzymatic step is also influenced (Schaffer et al. 2007). The genes for these volatile production enzymes are part of our Master List of flavor genes, and those most affected by ethylene are currently being mapped by our collaborators at Plant & Food Research.

Many cases were observed on the Flavor Gene Map where candidate genes for a flavor trait were at the same region as chromosomal regions influencing the trait (Figure 1). For example, at the bottom end of *Malus* chromosome 16 is located a major QTL for acidity, and a candidate gene for acidity showed up in the same place. We are focusing in on this gene now. Other regions like this are in the middle of *Malus* chromosome 9, towards the bottom of *Malus* 17, the middle of *Malus* 5, and the middle of *Malus* 15. Other comparisons *across chromosomes within apple* are also very interesting. For example, *Malus* chromosomes 9 and 17 have a common ancestral origin, and a QTL for volatile aroma on 9 is at the same equivalent spot as a volatile aroma gene on 17. Several other cases like this were observed. Next, there are ancestral ties between certain *Malus* and *Prunus* chromosomes, and in many cases there were important flavor regions in both crops. For example, *Malus* chromosome 13 and 16 have a common ancestral origin with *Prunus* chromosome 1, and a QTL for volatile aroma on *Malus* 16 is at the same equivalent spot as a volatile aroma gene on *Malus* 13 and *Prunus* 1. Another similarity that will lead us to focused genetic studies is on Malus chromosomes 10 and 5 and Prunus chromosome 4. Still others are apparent in Figure 2. We expect many more exciting opportunities like this once all 100 flavor genes of our Master List are mapped. The Flavor Gene Map will also be continually updated with new QTLs.

<u>Making use of whole genome sequencing of sweet cherry</u>

WSU Pullman collaborator Dr. Amit Dhingra recently performed preliminary whole genome sequencing of sweet cherry ('Stella') using the 454 sequencing machine obtained in 2008 with WTFRC and WSU-ARC support. We have begun to obtain sweet cherry-specific gene sequences for flavor candidate genes on our Master List. 'Stella' DNA sequences will facilitate the development of flavor gene tests that efficiently screen both cherry and apple, as well as cherry-specific tests where desired, by reducing the occurrence of failed tests. Previously, only 1-2% of the DNA sequences we could obtain for flavor genes in Rosaceae were from sweet cherry, and thus we would have been forced to rely mostly on the related crop peach to guess the DNA sequence of each flavor gene of sweet cherry.

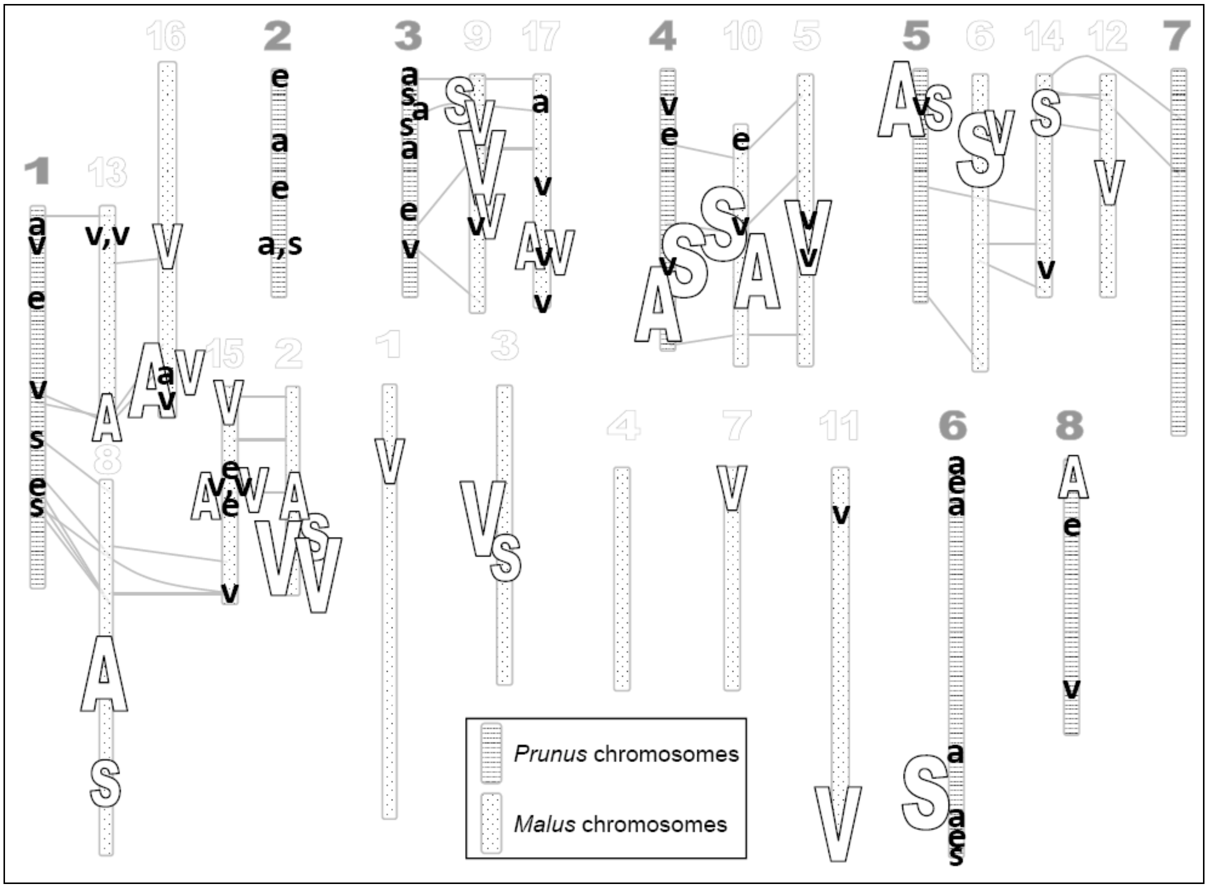

Figure 1: The improved **Flavor Gene Map** of apple and sweet cherry. S,s = sweetness, A,a = acidity, V,v = volatile aroma, e = ethylene. Chromosomal regions (QTLs – quantitative trait loci) influencing fruit flavor in the genomes of pome fruit (*Malus*) and stone fruit (*Prunus*) are indicated in block capital letters (larger letters = larger effect). Locations of candidate genes for flavor (and ethylene synthesis and perception) are shown in lower case letters. Horizontal lines show known connections between the related genomes. The Flavor Gene Map includes locations of genes putatively involved in flavor genetic control.

References:

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