2009 Apple Research Review
January 27-29, 2010
Confluence Technology Center
Wenatchee, WA

Wednesday , January 27

Time	Page	PI	Project Title	Funding period
8:00		T. Schmidt	Welcome and introduction	
			Final Reports	
8:15	1	Shetty	Washington apple varieties for management of type 2 diabetes	08-09
8:30	12	Ross	Sensory profiles & consumer acceptance of apple breeding selections: **videoconference**	08-09
8:45	22	Zhu	Functional genomics and marker development for apple sensory qualities: **extension**	07-08
9:00	33	Elfving	Management of vegetative growth in apple trees with bioregulators	08-09
9:15	39	Wisniewski	Mapping *M. sieversii* : A valuable genetic resource for apple breeding: **videoconference**	08-09
9:30	48	Dhingra	Apple genome project	09
9:45			**Break**	
Group #			**Continuing Projects/Poster Session 10:00-12:30**	
1	52	Castillo	Chemical thinning of apple	internal
1		McFerson	WTFRC technology projects: see technology reports in appendix	internal
1	59	T Schmidt	Modeling Washington apple bloom phenology and fruit growth	09-11
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2	79	Auvil	Apple rootstock and scion evaluation	internal
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FINAL PROJECT REPORT

Project Title: Washington apple varieties for management of type 2 Diabetes

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Cooperators: None

Other funding sources: None

Total Project Funding: **Year 1: $ 45,000** **Year 2: $ 47,850**

Budget History:

Item	**2008**	**2009**
Salaries (50% Research Associate)	20,000	21,000
Benefits 35% of Salary	7,000	7,350
Wages	NA	NA
Benefits	NA	NA
Equipment (HPLC Columns & Maintenance)	3,500	4,000
Supplies (Reagents and Enzymes)	7,500	8,000
Travel (Part of Travel Support for Conferences and Meetings to Present Research from this Project)	NA	NA
Miscellaneous (Apple orchard needs & preparation of materials & management for enzyme studies**)**	6,000	6,500
WFTRC (Fruit Samples & UPS)	1,000	1,000
Total	$ 45,000	$ 47,850

RECAP ORIGINAL OBJECTIVES:

1) To evaluate the health benefits of major fresh varieties of apples grown in Washington State for combating early stages of Type 2 diabetes and to better advance a fruit and vegetable rich healthy diet based on this information (Year 1).
2) To evaluate the health benefits of post-harvest stored apples under CA and 1-MCP conditions of important varieties grown in Washington State to determine if the potential evaluated under Objective 1 for combating early stages of Type 2 diabetes is maintained during storage (Year 2).

SIGNIFICANT FINDINGS:

The rationale for this study is to better biochemically define the well-known health benefits of apple that have been attributed in part to their polyphenolic metabolite content and related antioxidant capacity. The consumption of apple could provide health benefits by lowering the risk for chronic diseases such as metabolic syndrome diseases including type 2 diabetes. This chronic diet-linked metabolic disease affects 25 million Americans and 205 million globally and is projected to increase to 400 million globally by 2030. The biochemical objectives of this study are to investigate the **phenolic-linked anti-hyperglycemia** (Figure 1) **bioactive factors in apple** varieties.

Overall, <u>whole apple consumption including peel and pulp</u> has more complete health benefits potential relevant for dietary support for potentially managing early stage type 2 diabetes and its complications. These cannot be obtained from apple juice or other common fruits such as banana and orange which are devoid of skin component unlike whole apple.

The results of this WTFRC study clearly provide the biochemical rationale for clinical studies for integration of whole apple consumption in community food systems for managing early stages of type 2 diabetes when diet and exercise can counter this chronic metabolic syndrome disease. This is now being written for 2 community food systems projects to NIH, one submitted (NIH Pioneer Project) and is pending and a second NIH Proposal to study the effect 2 apples a day and exercise on managing hyperglycemia will be submitted in 2010.

Ten different apple varieties were analyzed in relation to peel and pulp fractions of each variety separately and extracted in distilled water and 12 % ethanol. These extracts were analyzed using *in vitro* biochemical and critical enzyme (alpha-amylase and alpha-glucosidase the target of commercial anti-diabetes drug acarabose-Figure1) analysis in the context of relevance and benefits for managing early stages of type 2 diabetes.

1) **Peel** sample was shown to have **higher total soluble phenolic content** and related antioxidant activity than pulp sample (Figures 2 & 3). These bioactivities are potentially important to combat cellular oxidation reactions that are high during hyperglycemia stages of type 2 diabetes**.**
2) **Overall all apples have a good baseline phenolic content and Honeycrisp and Delicious varieties have the highest total phenolic content (>800 ug/g FW)** this is correlated well to high (>70%) antioxidant activity (Figures 2 & 3).
3) All 10 varieties evaluated showed **moderate to high (>70%) α-amylase inhibitory activity in the pulp but low bioactivity in the peel** (Figure 4) which is sufficient to potentially control breakdown of starch but not strongly inhibit as the drug acarabose (which results in undigested starch that may cause diarrhea).
4) **All 10 varieties evaluated showed moderate to higher baseline alpha-glucosidase activity (also target of drug acarabose), which is significant and 4 varieties Honeycrip, Jonagold, Golden Delicious and Red Delicious have marginally higher bioactivity than other varieties.** All varieties show good dose dependent response (Figure 5), which is very important in any further food and clinical study design and optimization.

5) This study provides the biochemical rationale that if whole apple (peel and pulp) is consumed with a soluble carbohydrate (high glycemic index) diet it has the potential to slow digestive process and reduce degradation of starch (alpha amylase inhibition) or sugar and slow down glucose absorption (alpha-glucosidase inhibition) and therefore slow accumulation of high levels of glucose in the blood that can otherwise result in the condition of hyperglycemia relevant to increased type 2 diabetes (Figure 1 below for **summary of mechanism of action**).
6) **For complete bioactive benefits whole apple consumption (peel and pulp) is superior and deliver hyperglycemia managing benefits and antioxidant benefits and bioactive functions is retained in long term stored grocery store apples treated with 1-MCP (Figure 6 & 7)** and more studies are on-going on how 1-MCP treated apple phenolic-linked antioxidants and alpha-gluosidase and alpha-amylase inhibitory activities change every 2 months following 1-MCP treatment (study will be completed in June 2010).
7) Some of alpha-amylase inhibitory (resistance to starch breakdown) potential may be due to insoluble polysaccharide and oligosaccharide (fiber) fractions of apple pulp and similar to standard drugs (acarabose) with **whole apple likely having potential of resulting in less side effects of undigested starch than drugs.**
8) The **additional benefits of high phenolic-linked antioxidant activity in the apple peel** has potential to contribute to the reduction of microvascular complications of late stages of type 2 diabetes such as wound healing, macular degeneration and kidney dysfunction.
9) **Quercetin (Table 1) and related flavanoids** are important phenolics from apple peel that are linked to bioactive benefits. However, overall profile of phenolics and fiber are likely more important. **A 200 gram apple with peel can provide between 2 mg to 8 mg quercetin.**
10) The **bioactive benefits are clear based on *in vitro* biochemical and enzyme assays** and this study provides the strong biochemical rationale for consumption of whole apples to further community food systems needs & clinical studies to meet 2-3 servings per day of US per capta fruit and vegetable consumption will be targeted. **A single 200 gram whole apple can provide up to 40 mg to 50 mg of total soluble phenolics.**

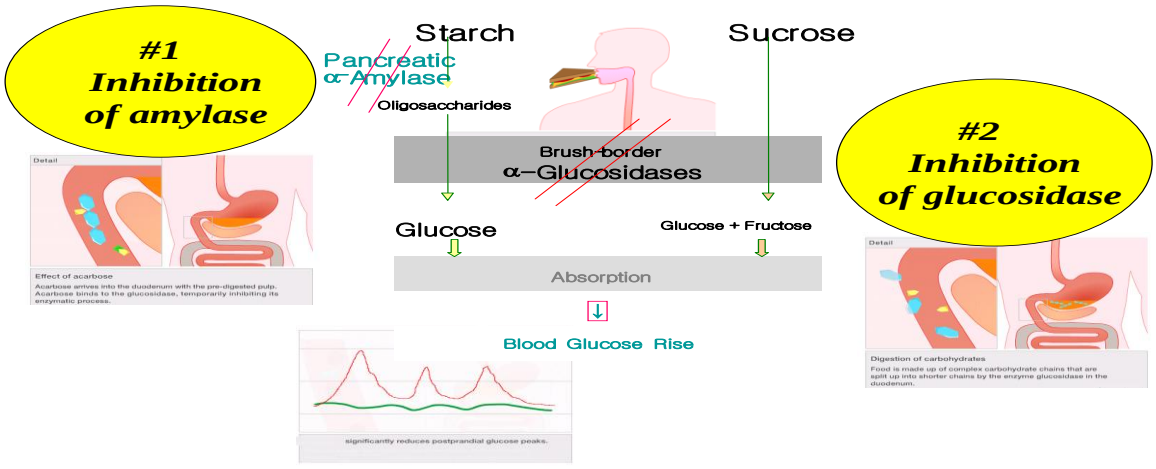

Figure 1: How alpha-amylase and alpha-glucosidase from apple work? Apple polyphenolics and especially from skin (peels) target these same enzymes as the drug acarabose which inhibits both enzymes strongly and therefore has side effects. Whole apples can deliver strong inhibition of alpha-glucosidase and milder inhibition of alpha-amylase which is preferred. The number of whole apples per day (2-3) and what stages of early stage of type 2 diabetes and combination of exercise will be determined by further studies proposed to NIH.

RESULTS & DISCUSSION:

Year 1: Objective 1: Apples were harvested from August 28, 2008 (Ginger Gold) to October 23, 2008 (Braeburn) at the University of Massachusetts Orchards in Belchertown, MA under supervision of Dr. Duane Greene based on standard harvest index standards (data available) based fruit weight, red color, circumrance, firmness, starch, soluble solids and percent water core.

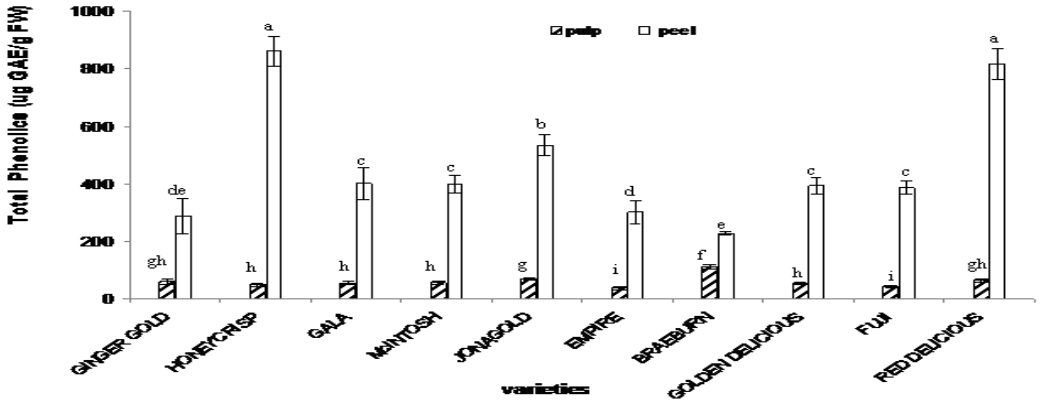

Figure 2: Total soluble phenolic content of freshly harvested apple varieties

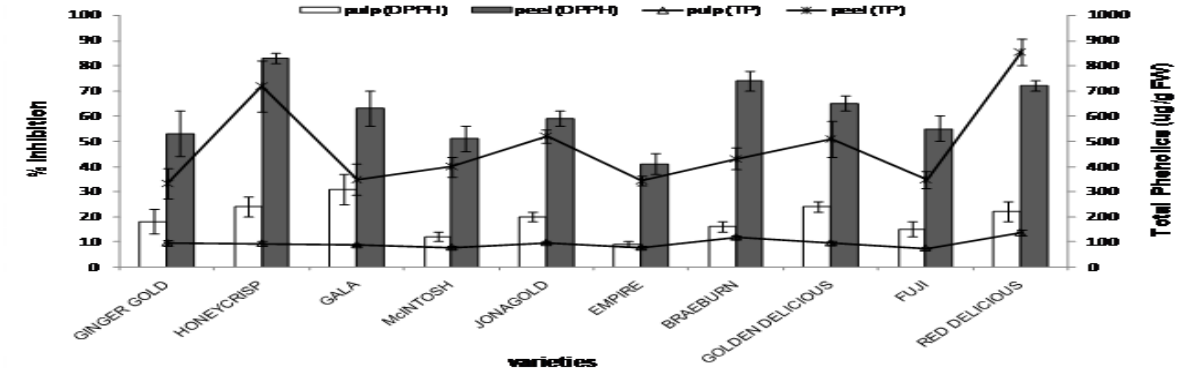

Figure 3: Total Phenolics & Free radical scavenging-linked antioxidant activity of freshly harvested apple varieties

The **Peel samples have higher total soluble phenolic content and related antioxidant activity than pulp** sample (Figures 2 & 3). Among these Honeycrisp and Delicious varieties have the highest total phenolic content (>800 ug/g FW) this is correlated well to high (>70%) antioxidant activity (Figures 2 & 3). **Therefore for health benefits derived from phenolics whole apples with peel would be superior.** This is particularly relevant for microvascular complications of type 2 diabetes due to cellular oxidative breakdown such as wound healing, macular degeneration and improved kidney function. In Year 2 when compared to long term stored store varieties the total phenolic content were not only maintained **but in many varieties the total phenolic content and antioxidant activity was enhanced with long term 1-MCP treated storage (Figure 6). This is significant.**

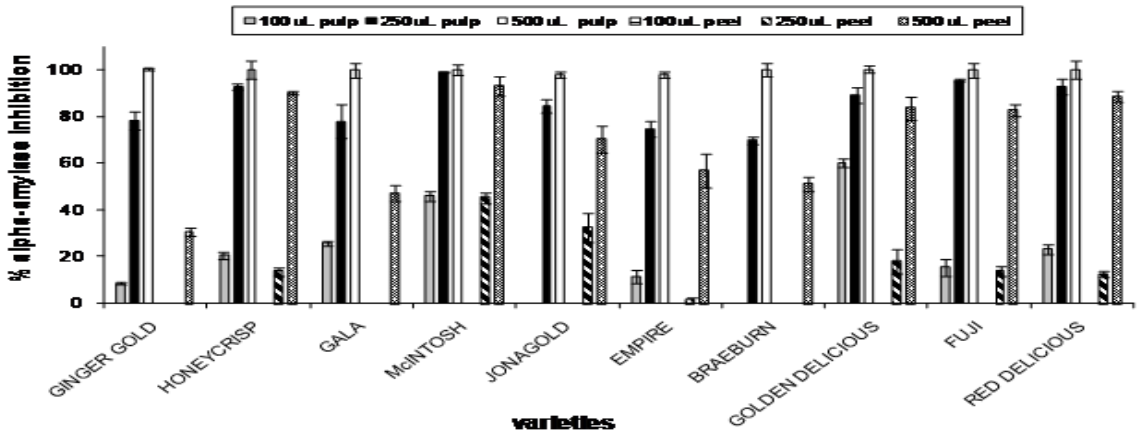

Figure 4: Alpha-amylase inhibitory activity of freshly harvested apple varieties

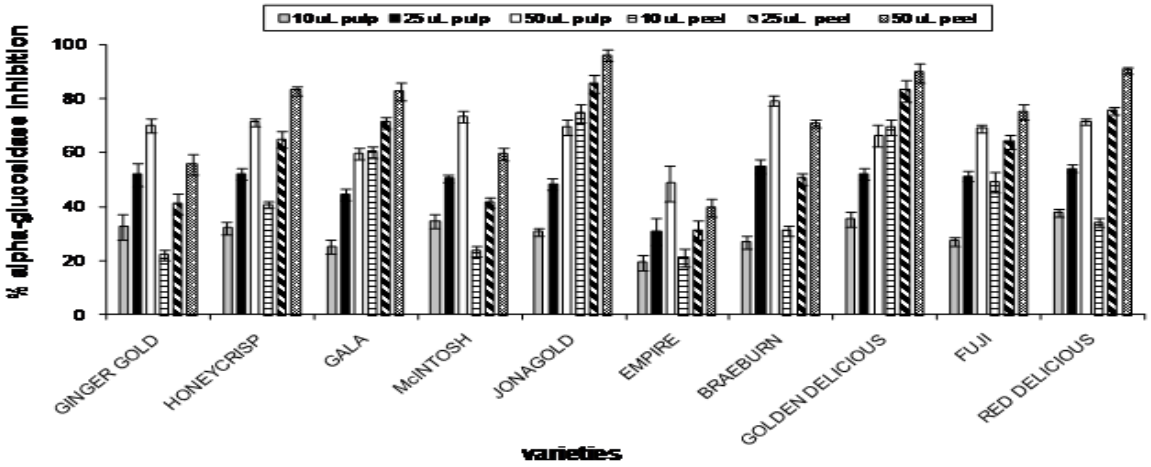

Figure 5: Alpha-Glucosidase inhibitory activity of freshly harvested apple varieties

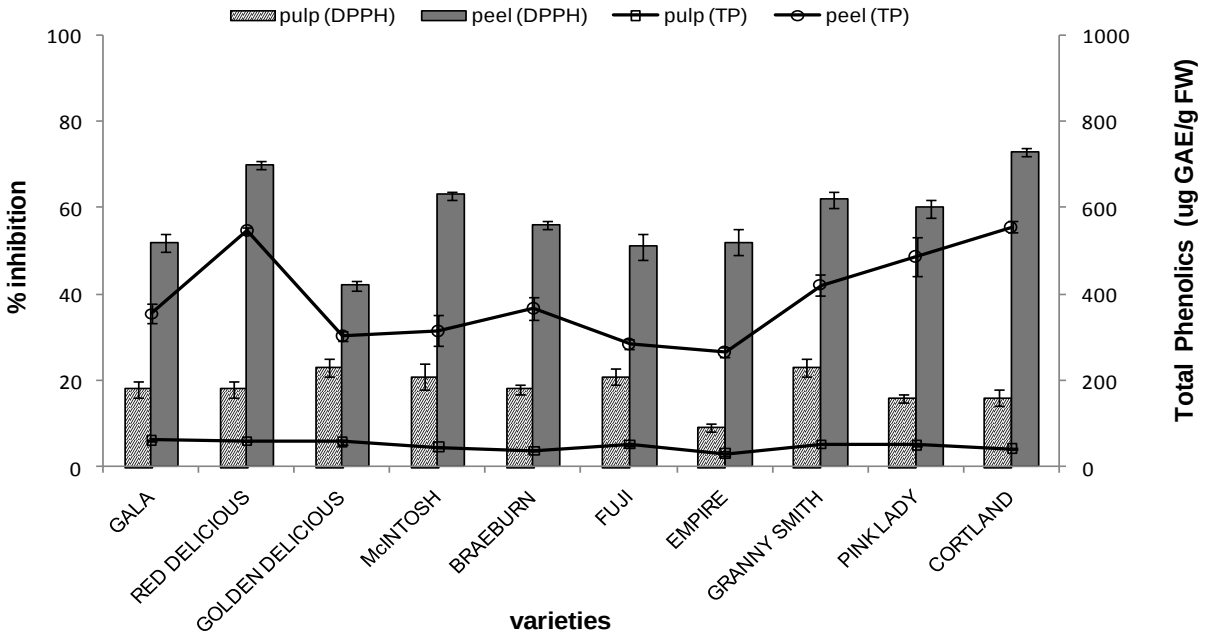

Figure 6: Total phenolics and Antioxidant activity of long term stored apples from Grocery stores **(Levels are maintained and in several cases increased with post-harvest storage)**

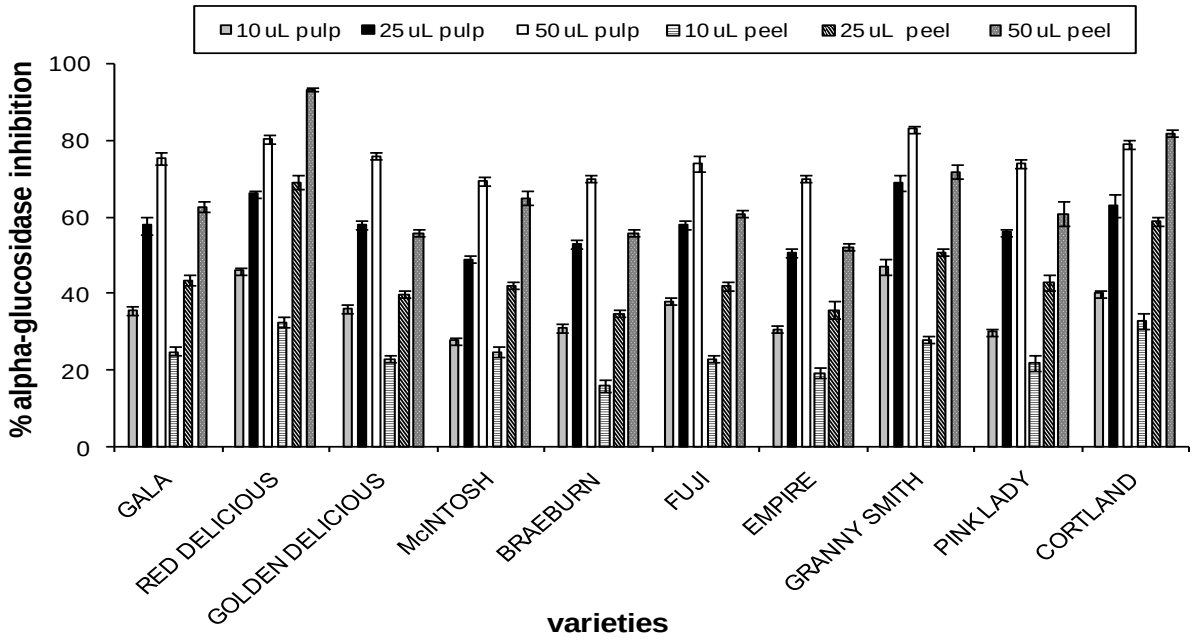

Figure 7: Long term stored grocery store varieties maintain and in some cases increase the alpha-glucosidase bioactive function in proportion to total phenolics and antioxidant activity

Table 1: Quercetin Content as Determined by HPLC: units: micrograms/gram peel)

	Water Extracts of Peel	***Ethanol Extracts of Peel***
Ginger gold	128 ± 5	142 ± 3
Honeycrisp	144 ± 3	554 ± 5
Gala	93 ± 2	68 ± 1
McIntosh	101 ± 2	534 ± 6
Jonagold	286 ± 5	213 ± 2
Empire	41 ± 1	327 ± 5
Braeburn	390 ± 3	419 ± 6
Golden delicious	84 ± 3	119 ± 3
Fuji	136 ± 2	74 ± 2
Red delicious	133 ± 3	171 ± 3

The **peel samples have higher diabetes relevant alpha-glucosidase inhibitory activity ,** which the target of the current pharmaceutical acarabose for this early stages type 2 diabetes target (Figure 1& 5). **All varieties have good baseline and dose dependent activity** and some varieties (Honey crisp, Jonagold, Golden Delicious and Red Delicious) are consistently a little superior. **Among store brought long term stored (1-MCP treated Grocery store apples) varieties the baseline activity is maintained and Red Delicious again is slightly superior.**

Discussions:

1) Whole apple have high phenolic content, related antioxidant activity and good dose dependent alpha-glucosidase inhibitory activity. Therefore there is merit in including 2-3 apples a day to increase daily intake of fruits and vegetables to increase to 5-6 servings needed to manage current fast growing epidemic of metabolic syndrome disease.

2) Whole apple with peel (skin) and pulp with reduced soluble sugar have the best potential.

3) Long term stored apples can retain the bioactive benefits and in case of phenolics-linked antioxidant activity the benefits increase.

4) High quercetin varieties (Table 1) are significant in the context of using apples and exercise to manage early type 2 diabetes and in the context of general endurance where quercetin is being targeted as an important biomolecule for sports and general endurance. In this case also the whole apple with peel is very important.

Note: The results of 2009 harvest of both fresh harvest and 1-MCP treated stored apples comparing to 2008 results is still on-going and will be submitted by end of Spring when the analysis will be completed. Here only select varieties were selected to confirm previous fresh harvest study and to 1-MCP treatments and whether this changes during every two months of storage. The results of long term stored grocery store apples (Figures 6 & 7) clearly indicate no changes in bioactive functions and in many cases increase in phenolic and antioxidant bioactive functions. This increase will be followed in the current 1-MCP studies that are on-going and results available as a publication format in early June 2010. The cultivars that are being investigated are: Gala, Honeycrisp, Golden Delicious, Red Delicious, Jonagold and Braeburn that cover lower to higher phenolic ranges.

Significance to apple industry and economic benefits:

Two important 2009 National and Global Reports clearly indicate the benefits of this research and benefits to the apple industry.
1) The States Indicator Reports on Fruits and Vegetables
http://www.fruitsandveggiesmatter.gov/downloads/StateIndicatorReport2009.pdf
2) The rapid growth of global & US type 2 diabetes epidemic
http://www.idf.org/international-diabetes-federation
http://www.cdc.gov/diabetes/

Less than 30% of the US population eats the recommended 2 or more servings of fruit a day and 3 or more servings of vegetable a day. This falls well short of 5-6 servings per day of fruits and vegetable for the population as a whole and even 9-10 servings per day recommended by some for serious management of chronic diseases and chronic diet-linked metabolic syndrome diseases.

One of the consequences of modern refined diet and reduced consumption of fruits and vegetables is the rapid increase in metabolic syndrome diseases including type 2 diabetes which is now affecting 25 million of the US population. This is projected to affect 10% of the population in this decade with minority communities being affected up to 15%-20% of their population. Diet and exercise can prevent and help manage these conditions and this requires more precise definition and biochemical analysis of benefits of fruits and vegetables based cellular and enzyme based structure-function rationale. This research has clearly provided this strong foundation.

Apples showed relatively high levels of total phenolics and antioxidant capacity comparable to those of oranges and their phenolics and antioxidants contribution is the second highest in the American diet (Barbosa et al., submitted to J. Medicinal Food; Chun et al., 2005; *J Sci Food Agric* 85:1715–1724). However, only whole apples (compared to bananas and oranges) have with better defined structure-function characterization of skin and peels as undertaken in this study (Barbosa et al., submitted to J. Medicinal Food), to rapidly help increase US per person intake of fruits and vegetables. If we can increase the average US annual apple production by **100 Billion apples per year** it can increase overall intake by just 1 serving per day per person. This is the most effective antidote for chronic diet and environmental-linked diseases combined with exercise. Therefore not only production of fresh apples has to increase but quality of these apple varieties for chronic disease management has to be better characterized.

Clearly for maximum health benefits in the context of better diet for prevention and early stages of type 2 diabetes management, **whole apple including pulp and peel has best potential when compared to other common fruits such as banana and orange.** These bioactives from whole apple have the potential to influence positively multiple physiological pathways from soluble carbohydrate utilization control to oxidative breakdown of this chronic disease. This biochemical rationale can be basis of better fruit and vegetable diet that could include 1-3 apples per day. The results from these current studies can provide the **key biochemical rationale** for future clinical studies that can be the basis for how many apples/day may be relevant and for what stages of type 2 diabetes such as pre-diabetes, when diet and exercise are most effective. Therefore better defining the biochemical basis of health benefits of apple contributes to enhanced fresh apple consumption globally. Further, based on biochemical rationale from this study and further clinical studies as the next step could show that **whole apple with peel and pulp is potentially superior** to juice from pulp, where high phenolic skin and fiber are discarded. **One 200 gram apple a day can provide 40 mg to 50 mg of total phenolics** which is more than the average total soluble phenolic intake per day in the United States.
Apple industry is the only fruit industry in the United States that has the best prospects to rapidly increase the phenolic-enriched fruit and vegetable consumption needed for managing chronic diseases. It can also be integrated into Community Foods Systems and winter needs.

Publications from this study:

Book Chapter:
Shetty, K., Adyanthaya, I., Kwon, Y-I., Apostolidis, E., Min, B-J., Dawson, P (2008). Postharvest enhancement of phenolic phytochemicals in apples for preservation and health benefits. In: **Postharvest Biology and Technology of Fruits, Vegetables and Flowers** (Paliyath G, Murr D, Handa AK, Lurie S {eds}) 2008, Chapter 16, Pages 341-371. Wiley-Blackwell Publishing, Ames, Iowa, USA.

Manuscript 1:
Barbosa A.C.L., Pinto,M.D.S., Sarkar,D., Ankolekar, C., Greene' D.and Shetty' K. (2010) Varietal Influences on anti-hyperglycemia properties of freshly harvested apples using *in vitro* assays models. Submitted for Publication to **Journal of Medicinal Food (Pre-publication manuscript available on request to WTFRC).**

Manuscript 2*:*
Barbosa A.C.L., Pinto,M.D.S., Sarkar,D., Ankolekar, C., Greene' D.and Shetty' K. (2010) Influence of varietal and pH variation on anti-Hyperglycemia and anti-hypertension properties of long-term stored apples using *in vitro* assay models. In preparation for Publication to **Journal of Food Biochemistry (Pre-publication manuscript available on request to WTFRC).**

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Manuscript 3:
Sarkar,D., Barbosa A.C.L., Pinto,M.D.S., Ankolekar, C., Greene' D.and Shetty' K.. (2010) Phenolic-linked antioxidant and anti-diabetes potential of 1-MCP treated apples during post-harvest storage. On-going research and in preparation for Publication to **Journal of Agricultural and Food Chemistry (Data and pre-publication manuscript will be available in early June 2010).**

Current and Future Proposals from this study:

USDA-Health and Wellness Special Grant:
University of Massachusetts Amherst, Department of Food Science has just been awarded (April 2010 to March 2012) $ 525,000 for Health and Wellness Research. Out of this $ 40,000 has been awarded to PI, Kalidas Shetty to advance inclusion of apples and cucurbits as a part of community food systems for management of metabolic syndrome diseases, including type 2 diabetes. The goal is to enhance inclusion in daily diet of high phenolic fruits (2 apples a day) and vegetables (2 cucurbits a day) with anti-diabetic and anti-hypertension bioactives in several communities of Hampden and Hampshire counties in Western Massachusetts, Based on this community food systems integration a framework and community trust will be built for subsequent clinical studies in collaboration with Baystate Medical Center.

NIH Pioneer:
PI, Kalidas Shetty has submitted a proposal to NIH a 5 year proposal for $ 2.5 million over 5 years to develop "Community Food Systems to Manage Type 2 Diabetes" based on structure-function basis for designing and making available fresh fruits and vegetables. This project provides the framework to develop locally grown fresh foods, high school fresh fruit and vegetable program, collaboration for clinical studies with local hospitals and national network of fresh fruit and vegetables based on bioactive properties for combating chronic metabolic syndrome diseases. In this project type 2 diabetes relevant 2 apples a day is an important part of strategy to increase fruit and vegetable intake to more than 5 serving a day over the next 5-7 years.

NIH-NIDDK
PI, Kalidas Shetty has joined with 2 Exercise scientists to study the interaction of endocrine and stress modulating phenolics and exercise for managing type 2 diabetes and also general endurance in athletes for effective recovery after exercise. In this regard high quercetin apples (Table 1) and few other mono-phenolics in rare apples in Urals, Kazakhstan and some old varieties in New England have high potential. Based on preliminary studies a NIH proposal will be developed in 2010-2011 for use of high phenolic Apples for type 2 diabetes management in combination with exercise and endurance and stress recovery in athletes. Apple, Pear and Cherry with the right varieties have potential for athletic, stress and endurance performance.

NIH-Fogarty International Program
This project proposal in planning stages in Pskov Agricultural College in Russia and Agricultural Ministry in Kazakhstan focuses on developing strategies to maintain and manage apple diversity in Russia and Kazakhstan and especially high phenolic extreme weather tolerating varieties. This diversity project has 2 objectives 1) to preserve rare varieties that have best potential to withstand extreme weather changes based on climate change 2) high phenolic dietary modulators that are relevant for managing metabolic syndrome diseases and daily endurance and stress management.

NSF-Community and Population Ecology Program
Based on the research on understanding type 2 diabetes relevant phenolics, there are specific varieties that have enhanced profiles of phenolics with better post-harvest preservation using 1-MCP (Figure 6) and further have high levels of phenolics like genistein released only in ethanol extracts (Table 1). This provides clues about specific structural linkages of phenolics in peels (skins) dependent on varieties. We will be exploring how specific beneficial bacterial such as lactic acid bacteria change and interact with apple skin and modulate phenolics. This has implications for post-harvest fitness of apples and preservation of apples and use of lower cost fallen apples in community food systems projects. The most exciting part of this project is that there is potential for producing antimicrobials from beneficial bacteria and phenolics from plants to enhance agronomic stages of apples and Rosaceae in terms of stress and disease management. Interactions of beneficial bacteria with probiotic function is not only important for animal function but is being observed as being important for plant functions including stress and reproductive (flowering and fruiting) function. This provides new approaches for organic based production of apples based on stress biology linked to plant phenolic and beneficial bacterial interactions.

NSF-Physiology and Structure
This is a basic research proposal in planning stage by PI and Co-PI and will examine how beneficial bacterial modulate phenolic-linked antioxidant enzyme response in post-harvest preserved apples with and without 1-MCP. This research is based on our recent publication on role of redox biology in post-harvest preservation of apples (Adyanthaya, I., Kwon, Y-I., Apostolidis, E. and Shetty, K. (2009) Apple post-harvest preservation is linked to phenolics and SOD activity. J. Food Biochemistry, 33: 535-556). Understanding from this research will advance new strategies for post-harvest preservation of apples and natural and probiotics to control the right post-harvest biochemistry.

EXECUTIVE SUMMARY:

One 200 gram apple a day can provide an average of 40 mg to 50 mg of total soluble phenolics which is **more than the average total soluble phenolic intake per person per day in the United States compared to Okinawa, Japan (longest living population on earth), who have 600 mg to 800 mg per day per person**. Therefore US Apple industry is the only fruit industry in the United States that has the best prospects to rapidly increase the soluble phenolic-enriched fresh fruit and vegetable consumption needed for preventing and managing diet-linked chronic diseases. This is especially essential for preventing and managing metabolic syndrome disease such as early stages of type 2 diabetes and general chronic disease affiliations linked to obesity. Among various regions Pacific Northwest must be targeted for high value fresh apples with health benefits. Increased fresh apple consumption is one of the most cost effective ways to increase fruit and vegetable consumption from the current levels where more than 70% of the US population DO NOT consume the recommended 2 or more servings of fruit a day and only less than 30% do.

This study indicates that whole apple in general and some varieties like Honeycrisp and Delicious have high phenolics and free radical scavenging-linked antioxidant activity in the peel. Several varieties have high activity profile of α-glucosidase inhibition (enzyme target of anti-diabetes pharmaceutical drugs for managing early stages of type 2 diabetes) and moderate to high α-amylase inhibition in the pulp. This indicates that a **complete whole fruit** offers the best potential for good postprandial blood glucose management linked to hyperglycemia associated with type 2 diabetes and its oxidative stress complications without the common side-effects associated with very high α-amylase inhibition in drugs such as acarbose. Compared to drugs, whole apple also have free radical scavenging-linked antioxidant activity which can help maintain the redox balance in susceptible cells. This study provides a strong biochemical rationale for further clinical studies to include apple as an important part of the overall diet and medicinal therapy for better management of early stages of type 2 diabetes and its complications when better diet and exercise are effective. This also helps basis for breeding of better apple varieties for better health and increase overall fruit and vegetable consumption in the United States.

Overall whole apple with pulp and peel have the best combined bioactives for maximum potential for use in diets for early stages of managing type 2 diabetes and its complications. Bioactive compounds beyond phenolics in the fiber fractions are also important and need to be investigated as part of community food systems project and clinical studies. Based on the *in vitro* biochemical rationale and results of this study, inclusion of 2 apples a day project as a part of community food systems improvement is being undertaken in specific communities in Western Massachusetts to develop a national model.

This project support from WTFRC has resulted in 1 manuscript already submitted for publication in Journal of Medicinal Food and two more in preparation (one to Journal of Food Biochemistry and another to Journal of Agricultural and Food Chemistry). In addition 1 book chapter has been published.

The foundations of this research funded by WTFRC have helped to develop new concepts and project proposals for further advancing health benefits and consumption of whole apples in the American diet. Currently a USDA grant to advance community food systems has been funded ($ 40,000) and a major proposal ($ 2.5 million) to NIH Pioneer program is pending. Additional proposals to federal programs at NIH and NSF are planned for 2010 and beyond with apple as the major focus. Among these proposals the relevance of apple with high phenolics for endurance and stress management in chronic diseases and athletic performance & exercise recovery is a major focus.

FINAL PROJECT REPORT

PROJECT TITLE: Sensory and consumer acceptance of advanced apple breeding selections

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Other funding sources: None

TOTAL PROJECT FUNDING: **Year 1:** $28,580 **Year 2:** $ 28,895

Budget History:

Item	2008	2009
Salaries	14000	22014
Benefits	9380	1881
Wages		
Benefits		
Equipment		
Supplies	4200	4000
Travel	1000	1000
Miscellaneous		
Total	$28,580	$28,895

ORIGINAL OBJECTIVES:
The overall objective of this study is to characterize the sensory properties of newly developed selections from WSU Apple Breeding Program (WABP) and determine the preference of these various apple selections. The sensory properties of these apple selections will then be related to consumer acceptance. Specific objectives are to:

Objective 1: To perform trained sensory panel analysis to characterize new selections of WABP

Objective 2: To perform consumer sensory panel evaluation to determine preference (overall and specific attributes) of new selections of WABP.

SIGNIFICANT FINDINGS:

- The project was conducted over 2 years with 3 harvest years of apples: Sensory work performed in January/February 2008 (with 2007 harvest year apples), Sensory work performed in January/February 2009 (2008 harvest year apples) and Sensory work performed in November 2009 (2009 harvest year apples)
- For each harvest year of apples, Objectives 1 (trained panel characterization) and 2 (consumer panel evaluation) were met. Specific findings are listed below.
- For the 2007 harvest year, based on the trained panel and consumer panel results, Allan 2, Fuller 10, 17, 24 and 36 were the most accepted to the consumer panel. The trained panel evaluation of these apples showed that they were high in sweetness and texture attributes.
- For the 2007 harvest year, results showed that flavor intensity and sweetness had a greater influence on the overall acceptance of the apple selection, while the other sensory attributes had a slightly lesser impact.
- For the 2008 harvest year, WSU2 and WSU38 were the most accepted while WSU5 C&C and WSU30 were the least accepted. These consumer results were supported by the trained panel evaluations of the apples in that texture properties (juiciness, crispness and hardness) of these highly accepted selections were high in intensity, along with sweetness. Fuller20 and Fuller7 were the least accepted.
- For the 2008 harvest year, results showed that apple flavor intensity and sweetness had a greater influence on the overall acceptance of the apple selection, while the other sensory attributes had a slightly lesser impact.
- For the 2009 harvest year, based on overall consumer acceptance, the most highly accepted apple selections were WSU38, WSU45, WSU2 and WSU46. From the trained panel evaluations, these apples possessed the sensory properties of high crispness, firmness, juiciness and low mealiness. The apples were also high in sweetness intensity.
- Results from the 2008 and 2009 harvest years were consistent in that WSU2 and WSU38 were the most accepted of the selections evaluated in both years.
- The sensory and consumer data on the advanced and elite selections from the Washington Apple Breeding Program provided useful feedback to the breeding team confirming decisions about which selection or selections to take forward for release and commercialization. The differentiation between the individual sensory attributes and the visual presentation of the range of each attribute was particularly helpful in presenting the qualities of each selection to a wider audience.

RESULTS and DISCUSSION:

Overview of Methods:

Trained Panel Evaluation of Apple Selections: The composition of the trained panel varied: 2007, n=9; 2008, n=10 and 2009, n=9. Each harvest year, the panelists were trained over 11-13 hours using techniques described by Meilgaard et al. (1999). The apple attributes were selected using reported literature (Gomez et al. 1998, Harker et al. 2002; Mehinagic et al. 2004) and previous studies performed in our lab (Chauvin et al. 2007). Panelists were trained to recognize apple flavor (sweetness, sourness, apple flavor intensity and astringency) and texture (firmness, crispness, juiciness and mealiness). Attribute definitions and references are presented in **Table 1**.

Table 1. Attributes and definitions of sensory attributes used by the trained panelists.

Attribute	**Definition**	**Technique**	**Standards**
Flavour and Taste:			
Acidity/Sourness	Sharp, tart or tangy	Will feel on the tongue; tends to be along the sides	Tartaric acid
Sweet	Intensity caused by sucrose	Will feel on the tongue; tends to be at the tip	Sucrose solutions
Apple flavor intensity	Degree to which apple flavors are pronounced and clearly observable.	Notice all over the mouth and tongue	Organic apple juice (high intensity)=15 Diluted juice=6
Mouthfeel			
Astringency	Drying, puckery mouthfeel Found in strong red wines, black teas, banana skins	Will tend to feel at the back of the tongue; may take longer to develop	Tannic acid
Texture:			
1) Crispness	Primarily an acoustic sensation that is detected by the ear during the fracturing on crisp foods	Using a whole 1/8th apple slice (skin on), place the sample between the front teeth and bite down evenly. Measure the amount of sound produced on the first chew of the mechanical mastication.	whole canned water chestnut (~2-3), Gala apple (7-9), carrot (15)
2) Juiciness	Amount of juice released on mastication in the first three chews	Place piece of apple sample between molar teeth and bite down evenly – ensure that piece is skin-side down. Evaluate amount of fluid released on the first chew of the mechanical mastication.	banana (1), Gala apple (5-7.5), canned mandarin oranges (15)
2) Firmness	Force required to completely bite through sample placed between molars	Place apple between molars and press firmly at steady rate – ensure that piece is skin-side down. Evaluate the strength needed to break the sample	banana (1) Gala apple (7.5-9), carrot (15),
3) Mealiness	Degree to which the flesh breaks down to a fine lumpy mass. Consider cohesiveness the opposite of mealiness.	Place sample apple between molars and press down- ensure that piece is skin-side down. Notice the amount of time that the sample takes to break down to a mass. If the sample falls apart quickly, it is considered to highly mealy.	Granny Smith (2) Gala (6-7) Red Delicious (9-10)

Panelists were trained to recognize the attributes using specific evaluative techniques and assign an intensity rating to each attribute using a 15-cm unstructured line scale, with a 1 cm indent at the left end of the scale corresponding to "extremely low" and 1 cm indent at the right end corresponding to "extremely high."

Evaluations took place in individual sensory booths equipped with laptop computers for recording data. Following training sessions, apple selections were presented to each panelist for evaluation in replicate. Panelists were presented with 1/8 of the apple under study. The apple selections were randomly presented to the panelists at room temperature and under white lighting conditions. Panelists were asked to indicate the intensity of the apple attributes described above. Results were collected using Compusense 5.0 software (Guelph, ON) and analyzed using XLSTAT.

Consumer Panel: The panel took place in the sensory evaluation facility at Washington State University. The number of consumers varied with the year: 2007, n=100; 2008, n= 100; 2009, n=80. Evaluations took place in individual sensory booths equipped with laptop computers for recording data. On each evaluation day, consumers were presented with 1/8 apple of the apple selections and a control sample (Fuji). The apple selections were randomly presented to the panelists at room temperature. Consumers indicated their overall acceptance and the acceptability of flavor (sweetness, sourness, astringency and apple flavor intensity) and texture (firmness, crispness, juiciness and mealiness) attributes for each apple selection. The apple flavor and texture attributes were evaluated by the panel using a 7-point scale (1 = dislike very much, 7 = like very much). Results were collected and analyzed as described above.

2007 harvest year:

The apple selections evaluated were: Allan2, Fuller7, Fuller10, Fuller17, Fuller18, Fuller20, Fuller24, Fuller30, Fuller34 and Fuller36.

For the trained panel, results from the analysis of variance indicated that the sensory attributes were significantly influenced by the apple selection. In **Table 2**, the separation of the different apple selections based on specific sensory attributes evaluated by the trained panelists is shown. Results indicated specific attribute differences between selections. Based on range of intensity, the smallest differences between apple selections were observed with astringency, flavor intensity and juiciness while the largest differences between selections were observed with sourness, crispness and mealiness. Fuller 24 was the lowest in sweetness but was the highest in sourness. Fuller 30 was the highest in sweetness but was not significantly different from Allan 2, Fuller 17, 18, 34 and 36. Fuller 20 was the lowest in sourness. Large differences were not observed between the apple selections in astringency. Apples were similar in apple flavor intensity, with Fuller 20 having the lowest flavor. For texture attributes, firmness showed the greatest variation between the apple selections. Fuller 20 had the lowest firmness, crispness and juiciness and the highest perceived mealiness. Fuller 24 was highest in firmness, crispness and lowest in mealiness.

In **Table 3,** the separation of the different apple selections based on consumer acceptance of sensory attributes is shown. Based on the acceptance of all attributes, including overall acceptance, Fuller 17 was the most accepted selection, followed by Allan 2. Fuller17 and Allan 2 were higher in their consumer acceptance of apple flavour intensity and apple texture attributes. Compared to Fuji (control), the apple selections significantly lower in overall acceptance were Fuller34 and Fuller7. This result corresponded to low acceptance of specific attributes in that Fuller34 and Fuller7 were lowest in acceptance of the specific sensory attributes.

Table 2. Mean separation of all apple selections and sensory attributes as analyzed by the trained panel (n=9) along a 15-cm line scale. Within each attribute, different letters indicate a significant difference ($p<0.05$).

Apple Attribute	Allan 2	Fuller 7	Fuller 10	Fuller 17	Fuller 18	Fuller 20	Fuller 24	Fuller 30	Fuller 34	Fuller 36
Sweetness	9.75^{abc}	7.94^{cd}	10.32^{ab}	9.75^{abc}	9.14^{abcd}	8.88^{bcd}	7.05^{d}	11.38^{a}	9.42^{abc}	9.11^{abcd}
Sourness	5.94^{cd}	7.54^{bc}	6.43^{cd}	6.61^{cd}	9.29^{ab}	4.69^{d}	11.71^{a}	7.08^{bcd}	6.59^{cd}	7.26^{bcd}
Astringency	4.79^{ab}	4.23^{abc}	4.29^{abc}	5.34^{a}	4.79^{ab}	2.75^{c}	5.71^{a}	4.59^{ab}	3.45^{bc}	4.32^{abc}
Apple Flavor Intensity	6.39^{ab}	6.33^{ab}	6.85^{ab}	7.73^{ab}	7.12^{ab}	5.12^{b}	6.69^{ab}	8.65^{a}	6.01^{ab}	6.39^{ab}
Firmness	10.84^{abc}	8.11^{de}	11.58^{ab}	11.93^{a}	8.49^{de}	6.07^{f}	12.11^{a}	9.74^{bcd}	6.71^{ef}	9.31^{cd}
Crispness	10.57^{abc}	8.62^{d}	11.84^{ab}	11.59^{ab}	9.35^{cd}	6.24^{e}	12.36^{a}	10.08^{bcd}	6.56^{e}	9.94^{bcd}
Juiciness	8.06^{a}	8.07^{a}	7.99^{a}	7.74^{a}	8.28^{a}	5.09^{b}	8.19^{a}	8.63^{a}	7.14^{ab}	7.89^{a}
Mealiness	3.05^{e}	6.87^{bc}	3.39^{de}	3.35^{de}	5.82^{bcd}	9.68^{a}	3.31^{de}	4.09^{de}	7.21^{ab}	4.49^{cde}

Table 3. Mean separation of all apple selections and sensory attributes as analyzed by the consumer panel (n=162). Values represent acceptance along a 7-pt hedonic scale (1=dislike very much and 7=like very much).

Apple Attribute	Allan 2	Fuller 7	Fuller 10	Fuller 17	Fuller 18	Fuller 20	Fuller 24	Fuller 30	Fuller 34	Fuller 36	Fuji (Control)
Overall Acceptance	5.47^{ab}	4.40^{e}	5.17^{abc}	5.70^{a}	4.57^{cd}	3.32^{e}	5.00^{abcd}	4.95^{bcd}	4.41^{d}	5.36^{ab}	5.24^{abc}
Sweetness	5.40^{ab}	4.47^{de}	5.14^{abc}	5.63^{a}	4.68^{cde}	4.23^{e}	4.73^{cde}	5.11^{abcd}	4.82^{bcde}	5.22^{abc}	5.25^{abc}
Sourness	5.04^{a}	4.36^{cde}	4.80^{abc}	5.09^{a}	4.51^{bcd}	3.95^{e}	4.70^{abcd}	4.54^{bcd}	4.32^{de}	4.94^{ab}	4.60^{abcd}
Flavor Intensity	5.32^{ab}	4.60^{bc}	4.83^{abc}	5.50^{a}	4.34^{cd}	3.68^{d}	4.91^{abc}	4.68^{bc}	4.41^{c}	5.16^{ab}	4.94^{abc}
Hardness	5.77^{a}	4.36^{cd}	5.48^{ab}	5.74^{a}	4.41^{cd}	2.62^{e}	5.70^{a}	4.84^{bc}	3.73^{d}	5.49^{ab}	4.93^{bc}
Crispness	5.75^{a}	4.21^{bc}	5.91^{a}	6.07^{a}	4.55^{b}	2.63^{d}	5.87^{a}	4.83^{b}	3.70^{c}	5.67^{a}	4.83^{b}
Juiciness	5.52^{a}	4.85^{bcd}	5.43^{ab}	5.50^{a}	4.72^{cd}	3.49^{e}	5.21^{abc}	5.37^{ab}	4.39^{d}	5.43^{ab}	5.27^{abc}

From correlation analysis, the results showed that flavor intensity and sweetness had a greater influence on the overall acceptance of the apple selection ($r > 0.70$) , while the other sensory attributes had a slightly lesser impact ($r < 0.70$).

2008 harvest year:

The apple selections evaluated were WSU2, WSU5C&C, WSU5T19, WSU7, WSU17, WSU30, WSU36, WSU38 and Fuji as a control apple.

Results from the trained panel analysis of variance indicated that the sensory attributes were significantly influenced by the apple selection. The separation of the different apple selections based on specific sensory attributes is shown in **Table 4**. Based on range of intensity, the smallest differences between apple selections were observed with sourness and astringency, while the largest differences between selections were observed with sweetness, firmness, crispness, juiciness and mealiness.

Table 4. Mean separation of all apple selections and sensory attributes as analyzed by the trained panel (n=10) along a 15-cm line scale. Within each attribute, different letters indicate a significant difference ($p<0.05$).

Apple Attributes	WSU2	WSU5 C&C	WSU5 T19	WSU7	WSU17	WSU30	WSU36	WSU38	Fuji
Sweetness	8.63^{bcd}	8.79^{bcd}	6.55^{e}	7.74^{cde}	9.01^{bc}	7.90^{cde}	7.44^{de}	9.76^{ab}	11.18^{a}
Sourness	8.57^{a}	7.55^{abc}	7.98^{ab}	8.70^{a}	7.29^{abc}	6.60^{bc}	9.13^{a}	8.87^{a}	5.66^{c}
Astringency	5.37^{ab}	6.41^{ab}	6.03^{ab}	7.26^{a}	5.57^{ab}	7.23^{a}	5.63^{ab}	5.39^{ab}	4.62^{b}
Apple Flavor Intensity	8.89^{abc}	8.45^{abc}	6.15^{d}	7.83^{c}	8.15^{bc}	7.37^{cd}	7.31^{cd}	9.81^{a}	9.68^{ab}
Firmness	10.89^{a}	6.06^{e}	9.65^{abc}	9.19^{abc}	7.98^{cd}	6.64^{de}	8.84^{bc}	10.53^{ab}	10.75^{a}
Crispness	11.88^{a}	6.71^{e}	10.53^{abc}	9.48^{bc}	9.03^{cd}	7.35^{de}	9.53^{bc}	11.81^{a}	11.12^{ab}
Juiciness	10.80^{a}	6.70^{d}	9.65^{abc}	9.01^{bc}	8.83^{bc}	8.03^{cd}	8.47^{c}	11.29^{a}	10.54^{ab}
Mealiness	3.68^{d}	7.87^{a}	4.43^{cd}	4.75^{cd}	5.32^{bc}	6.63^{ab}	5.60^{bc}	3.49^{d}	3.56^{d}

For sweetness, results showed that WSU5 T19 was the least sweet, but was not significantly different from WSU7, WSU30, and WSU36. The sweetest selections were Fuji and WSU38 ($p<0.05$). Based on sourness, the apple selections with the lowest sourness were Fuji, WSU17, WSU5 C&C and WSU30. For apple flavor intensity, Fuji, WSU38, WSU2 and WSU5 C&C had the highest values.

All texture attributes showed variation between the apple selections with a wide range of intensities observed. Fuji was the firmest apple, but did not significantly differ from WSU38, WSU7, WSU5 C&C and WSU2. For crispness, WSU2 was the highest, but was not significantly different from WSU5 T10, WSU38 or Fuji. WSU2, WSU38 and Fuji were the highest in juiciness, while for mealiness, WSU5 C&C and WSU30 were the highest ($p<0.05$). Overall, WSU5 C&C had low firmness, crispness and juiciness and the highest perceived mealiness. WSU2 was high in firmness, crispness, juiciness and one of the lowest in mealiness.

In **Table 5 and 6**, the separation of the different apple selections based on consumer acceptance of sensory attributes is shown. In Table 5 (Day 1), based on overall acceptance, WSU2 and Fuji were the highest and not significantly different from each other. The trend same persisted for acceptance of sweetness, sourness, apple flavor intensity, firmness and juiciness: Fuji and WSU2 had the highest

acceptance ratings for all of these attributes. Based on taste and flavor, WSU5 T19, was consistently rated low for acceptance of these attributes, while for texture attribute acceptance, WSU5 C&C was consistently rated low.

Table 5. Mean separation of all apple selections and sensory attributes as analyzed by the consumer panel. Day 1 selections (n=100): WSU 2, 5 C&C, 5 T19, 7 and Fuji. Values represent acceptance along a 7-pt hedonic scale (1=dislike very much and 7= like very much). Within each attribute, different letters indicate a significant difference ($p<0.05$).

Variables	WSU2	WSU5 C&C	WSU7	WSU5 T19	Fuji Day 1
Overall Acceptance	5.84^{a}	4.17^{b}	4.63^{b}	4.28^{b}	5.77^{a}
Sweetness	5.57^{a}	4.46^{b}	4.37^{bc}	3.89^{c}	5.63^{a}
Sourness	5.00^{a}	3.94^{bc}	4.26^{b}	3.57^{c}	4.79^{a}
Apple Flavor Intensity	5.37^{a}	4.17^{b}	4.41^{b}	3.59^{c}	5.50^{a}
Crispness	6.21^{b}	3.75^{c}	4.90^{b}	5.17^{b}	5.81^{a}
Firmness	6.04^{a}	3.49^{c}	4.59^{b}	4.99^{b}	5.64^{a}
Juiciness	5.71^{a}	4.50^{c}	5.04^{b}	4.85^{bc}	5.87^{a}

In **Table 6**, WSU38 had the highest overall acceptance along with Fuji ($p<0.05$). WSU38 was also high in acceptance of apple flavor intensity, crispness, firmness and juiciness ($p<0.05$). WSU38 and Fuji were highest in acceptance for sweetness, and WSU38 and WSU 36 were highest in acceptance for sourness ($p<0.05$). Consistently low in acceptance of many of the attributes were WSU17 and WSU30.

Table 6. Mean separation of all apple selections and sensory attributes as analyzed by the consumer panel: Day 2 selections (n=114): WSU 17, 30, 36, 38 and Fuji. Values represent acceptance along a 7-pt hedonic scale (1=dislike very much and 7=like very much). Within each attribute, different letters indicate a significant different ($p<0.05$).

Variables	WSU17	WSU30	WSU36	WSU38	Fuji Day 2
Overall Acceptance	4.49^{c}	4.62^{c}	4.96^{bc}	5.75^{a}	5.27^{ab}
Sweetness	4.54^{c}	4.72^{bc}	4.88^{bc}	5.56^{a}	5.07^{ab}
Sourness	4.26^{bc}	4.13^{c}	4.68^{ab}	5.11^{a}	4.21^{bc}
Apple Flavor Intensity	4.15^{c}	4.25^{bc}	4.77^{b}	5.55^{a}	4.69^{bc}
Crispness	4.25^{d}	3.40^{cd}	4.87^{c}	6.35^{a}	5.39^{b}
Firmness	4.06^{c}	3.92^{c}	4.86^{b}	6.25^{a}	5.26^{b}
Juiciness	4.89^{b}	4.98^{b}	5.00^{b}	6.19^{a}	5.30^{b}

From the correlation analysis, the results showed that apple flavor intensity and sweetness had a greater influence on the overall acceptance of the apple selection ($r > 0.70$) , with the other sensory attributes having a slightly lesser impact ($r < 0.70$).

2009 harvest year:

The apple selections evaluated were Fuji (control), WSU 2, WSU 5, WSU 7, ,WSU 37, WSU 38, WSU 39,WSU 45 and WSU 46.

Results from the trained panel analysis of variance indicated that the sensory attributes were significantly influenced by the apple selection. The separation of the different apple selections based

on significant sensory attributes is shown in **Table 7**. Based on the range of intensity, the smallest differences between apple selections were observed for astringency and sweetness, while the largest differences between selections were observed for the texture attributes of firmness and mealiness. Based on texture attributes, the apple selection that showed the greatest difference from the other selections was WSU39. Compared to the other selections, this selection was significantly lower in intensity of crispness, firmness and juiciness, while being significantly higher in mealiness ($p<0.05$). WSU2, WSU38, WSU45 and Fuji were all high in crispness, firmness, juiciness but low in mealiness intensity.

Table 7. Mean separation of apple selections and significant sensory attributes as analyzed by the trained panel (n=10) along a 15-cm line scale. Within each attribute, different letters indicate a significant difference ($p<0.05$).

Apple Attribute	**WSU 2**	**WSU 5**	**WSU 7**	**WSU 37**	**WSU 38**	**WSU 39**	**WSU 45**	**WSU 46**	**Fuji**
Crispness	10.2ab	9.2c	10.5c	9.4bc	10.6a	7.1d	10.6a	10.6a	10.2ab
Firmness	10.2a	8.4cd	7.6d	9.3ab	9.5ab	6.3c	9.2bc	9.7ab	9.6ab
Juiciness	9.4a	8.3b	8.2b	8.3b	9.8a	6.7c	9.7a	10.1a	9.4a
Mealiness	3.3c	4.6bc	5.2b	3.8cde	3.8cde	7.5a	4.3bcd	3.7de	3.7dc
Sweetness	9.0a	8.7ab	7.7cd	6.8d	8.7ab	8.7ab	8.7ab	8.0bc	8.8abc
Sourness	5.6e	6.7cde	6.9cd	9.7a	7.1cd	6.4de	7.8bc	8.4b	6.3de
Astringency	4.0c	4.9ab	4.8abc	5.4a	4.1bc	4.2bc	4.4bc	4.2bc	4.6abc

Based on flavor and taste attributes, the apple selection with the lowest sweetness intensity compared to the other selections was WSU37 ($p<0.05$), which also had the highest sourness intensity. Based on sourness, Fuji, WSU5, WSU39 and WSU2 were significantly lower compared to the other selections ($p<0.05$). While astringency did not show a large range, significant differences were observed between the apple selections. The apple selections with the highest perceived astringency intensity were Fuji, WSU7, WSU5 and WSU37.

In **Tables 8 and 9,** the separation of the different apple selections based on consumer acceptance of sensory attributes is shown. In **Table 8** (Day 1), based on overall acceptance, WSU38 and WSU45 were the most accepted, significantly higher than all of the other selections, including Fuji. Based on acceptance of the texture attributes, WSU38 was significantly higher than the other apple selections and Fuji, except for crispness and firmness where WSU45 was also high. WSU5 and WSU7 were consistently lower in acceptance of texture attributes.

Table 8. Mean separation of apple selections and significant sensory attributes as analyzed by the consumer panel (n=80) along 7-pt hedonic scale (1=dislike extremely and 7=like extremely) on Consumer Day 1. Within each attribute, different letters indicate a significant difference ($p<0.05$).

Apple Attribute	**WSU 5**	**WSU 7**	**WSU 38**	**WSU 45**	**Fuji**
Crispness	5.2b	5.3b	6.3a	6.2a	5.4b
Firmness	5.2b	5.2b	6.2a	5.9a	5.2b
Juiciness	5.4c	5.6c	6.3a	5.9b	5.7bc
Mealiness	5.0c	4.9c	5.8a	5.4b	5.0bc
Sweetness	5.1b	5.3ab	5.6a	5.6a	5.3ab
Sourness	4.8bc	5.0abc	5.4a	5.2ab	4.8c
Apple flavor intensity	4.8c	5.1bc	5.6a	5.5ab	5.1bc
Overall Acceptance	4.9c	5.0bc	5.6a	5.4a	5.0bc

In **Table 9**, apple selections from Day 2 are shown. Based on overall acceptance, WSU2, WSU46 and Fuji were the most accepted ($p<0.05$), with WSU39 being the least well accepted. Based on acceptance of texture attributes, WSU2 was consistently the highest, followed by WSU46 and Fuji. WSU39 was consistently rated the lowest in acceptance for all texture attributes evaluated, with the exception of WSU37 which has also rated low for acceptance of juiciness ($p<0.05$). Based on sweetness, a similar trend to overall acceptance was observed in that WSU2, WSU46 and Fuji were the most accepted and WSU39 and WSU37 being the least accepted. The same trend was observed with astringency.

Table 9. Mean separation (Fisher's LSD) for apple selections and significant sensory attributes as analyzed by the consumer panel (n=80) along 7-pt hedonic scale (1=dislike extremely and 7=like extremely) on Consumer Day 2. Within each attribute, different letters indicate a significant difference ($p<0.05$).

Apple Attribute	**WSU 2**	**WSU 37**	**WSU 39**	**WSU 46**	**Fuji**
Crispness	6.2a	5.9ab	4.8c	6.1a	5.8b
Firmness	6.1a	5.7bc	4.5d	6.0ab	5.6c
Juiciness	5.9a	5.6bc	5.3c	5.9a	5.8ab
Mealiness	5.6a	5.4ab	4.6c	5.5ab	5.3b
Sweetness	5.6a	4.9c	5.2c	5.4ab	5.5ab
Astringency	5.1a	4.6c	4.7c	4.9abc	5.1ab
Overall Acceptance	5.4a	4.8bc	4.6c	5.3a	5.3ab

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EXECUTIVE SUMMARY

Significant Progress/Outcomes

The overall objective of this study was to characterize the sensory properties of newly developed selections from the WABP using a trained sensory evaluation panel and determine acceptance of these apple selections using a consumer panel. Both objectives of the study were conducted over 2 calendar years or 3 harvest years (2007, 2008 and 2009 apples).

- For the 2007 harvest year, based on the trained panel and consumer panel results, Allan 2, Fuller 10, 17, 24 and 36 showed the most promise for commercialization. The trained panel evaluation of these apples showed that they were high in sweetness and texture attributes.
- For the 2008 harvest year, WSU2 and WSU38 were the most accepted while WSU5 C&C and WSU30 were the least accepted. These consumer results were supported by the trained panel evaluations of the apples in that the texture properties (juiciness, crispness and hardness) of these highly accepted selections were high in intensity, along with sweetness.
- For the 2009 harvest year, based on overall consumer acceptance, the most highly accepted apple selections were WSU38, WSU45, WSU2 and WSU46. From the trained panel evaluations, these apples possessed the sensory properties of high crispness, firmness, juiciness and low mealiness. The apples were also high in sweetness intensity.
- Results from the 2008 and 2009 harvest years were consistent in that WSU2 and WSU38 were the most accepted of the selections evaluated.
- Results from two harvest years suggested that apple flavor intensity and sweetness had a greater influence on the overall acceptance of the apple selection compared to the other sensory attributes. The apparent lesser influence of texture may be explained by the high intensity of the texture of the apples pre-selected by the breeding team. As texture is so important to overall sensory quality, the breeding team uses texture to select promising apple selections for sensory testing.
- Flavor and texture groupings were proposed to which trained panelists and consumers assigned apples. These groupings showed promise for providing broad information to both marketers and consumers regarding the taste and texture properties of the apples, particularly important with new selections where the apple name is not recognized.
- The sensory and consumer data on the advanced and elite selections from the WABP provided useful feedback to the breeding team confirming decisions about which selection or selections to take forward for release and commercialization. The differentiation between the individual sensory attributes and the visual presentation of the range of each attribute was particularly helpful in presenting the qualities of each selection to a wider audience.

Future Directions

The future direction of this project is to continue working with the WABP. Because the goal of the breeding program is to commercialize apple selections, sensory evaluation data in the form of trained panel profiles and consumer acceptance data are critical in making decisions regarding which selections to move forward to commercialize. Through the two year involvement of the WSU Sensory Evaluation Facility in the characterization of apple selections developed by the WABP, we feel poised to continue our successful collaboration with the breeding program. We would also like to continue to explore the use of groupings (texture and flavor) to which apples can be assigned based on their intrinsic sensory properties. These groupings may be used to assist in positioning new varieties in the marketplace and as an aid to consumers in making purchase decisions at the point of sale. This is especially important with new apple selections where the name is not familiar to the consumer but the consumer frequently has pre-conceived ideas of how the apple will taste based on its similarity in appearance to well-known varieties for example, all green apples do not taste like ‘Granny Smith’!

FINAL REPORT **PROPOSED DURATION**: 2 years

Project Title: Functional genomics and marker development for apple sensory qualities

PI: Yanmin Zhu
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Co-PI: Bruce Barritt
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Collaborator: Cameron Peace,
Organization: Department of Horticulture and Landscape Architecture, WSU
Telephone/email: 509-335-6899 cpeace@wsu.edu

Budget 1:

Organization: USDA, ARS		**Contract Administrator:** Charles Myers, Extramural Agreements Specialist	
Telephone:(510) 559-6019		**Email:**cwmyers@pw.ars.usda.gov	
Item	**2007**	**2008**	
Salaries	33,000	33,000	
Benefits	10,000	10,000	
Wages			
Benefits			
Equipment			
Supplies	10,000	10,000	
Travel	1,500	1,500	
Miscellaneous	500	500	
Total	$55,000	$55,000	

The **salaries and benefits** are for hiring a postdoc dedicated to this project.
The **supplies** include common reagent for molecular genetics study and gene profiling analysis.
The budget for **travel** includes the cost for visiting Malus germplasm repository at Geneva, New York, for identify the phenotypic extremes on related fruit quality.

OBJECTIVES

1. Continue to apply the tested ethylene molecular markers for ACS1 and ACO1 in segregation populations in the WSU Apple Breeding Program to select for low ethylene production.
2. Test and apply a reported apple fruit peel red color marker in the existing WSU segregation population for selection of red color development capacity.
3. Identify potential candidate genes regulating apple firmness and crispness.
4. Elucidate relationships between expression of apple AAT (alcohol acyl transferase) genes and cultivar differences in volatile ester production.

SIGNIFICANT FINDINGS

1. Tests of two functional DNA markers related to apple climacteric ethylene production in elite breeding parents and advanced selections revealed a close relationship between these markers and fruit firmness.
2. More than 3500 seedlings in WSU breeding program have been genotyped for their allelotypes of both climacteric ethylene biosynthesis genes. The genotype data can be used by for the selection process.
3. Test of a published apple fruit skin color marker among cultivars, and two cross populations revealed a good but less-than-perfect correlation between this marker and apple fruit skin color phenotype.
4. The expression patterns of the AAT gene family were found to be associated with the phenotypic features of aromatic volatile ester generation in two cultivars with extreme aroma production phenotypes.
5. Several functional groups of genes including a short list of cell wall modifying genes and hormone metabolism related genes were identified by a large scale gene expression profiling analysis.. Microscopic examination of fruit revealed a distinguishable feature of cell wall thickness for two cultivars with distinct texture attributes.

RESULTS AND DISCUSSION

1. Test and application of DNA markers for apple ethylene biosynthesis genes Md-ACS1 and Md-ACO1 in WSU breeding parents and suitability for marker-assisted selection. (Collaborators: Dr. Cameron Peace and Bruce Barritt)

Fruit ethylene production genotypes for Md-ACS1 and Md-ACO1 were determined for 60 apple cultivars and 35 advanced breeding selections. Two alleles for each gene are commonly found in cultivated apple. ACO1 plays a minor role compared to ACS1, with homozygous ACO1-1 having lower ethylene production. In this study, ACS1-2 and ACO1-1 homozygotes had firmer fruit at harvest and after 60 days of 32-33^{o}F cold storage compared to other genotypes (Figure 1). This genotype, ACS1-2/2 and ACO1-1/1, was observed for 7 of 95 cultivars/selections including 'Fuji', 'Pacific Beauty', 'Sabina' and 4 breeding selections. Cultivars/selections that were homozygous ACS1-2 but not ACO1-1 were: 'Ambrosia', 'Aurora Golden Gala', 'CrimsonCrisp', 'Gala', 'GoldRush', 'Huaguan', 'Pacific Rose', 'Pacific Queen', 'Pinova', 'Sansa', 'Sonja', 'Sundance', 'Zestar' and 17 breeding selections. Cultivars with the heterozygous ACS1-1/2 genotype were 'Arlet', 'Braeburn', 'Cameo', 'Delicious', 'Delorgue', 'Empire', 'Enterprise', 'Ginger Gold', 'Golden Delicious', 'Granny Smith', 'Honeycrisp', 'Orin', 'Pink Lady', 'Silken', 'Suncrisp', 'Sundowner', 'Sunrise' and 11 breeding selections. No cultivars were detected homozygous for both ACS1-1 and ACO1-1, or for both ACS1-2 and ACO1-2. This study is the first large scale allelic genotyping of both ethylene synthesis genes for a comprehensive set of apple breeding parents used in an ongoing

breeding project. The data reported here are important for informative selection of parent combinations and marker-assisted selection of progeny for breeding low ethylene-producing apple cultivars, which are essential for better storability and improved consumer acceptance.

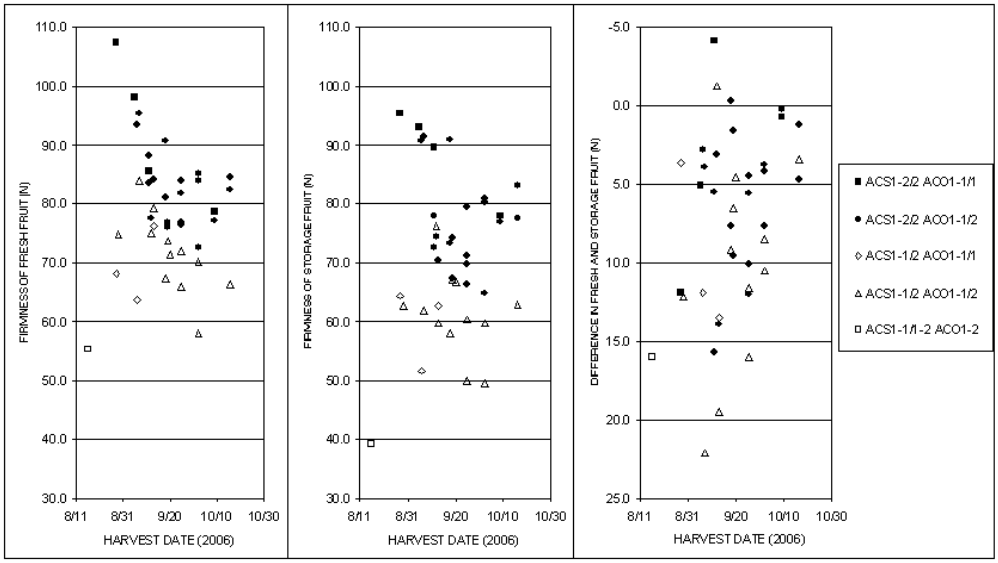

Figure 1. Fresh fruit firmness at harvest (left panel), firmness after 60 days of cold storage (middle panel) and the difference in firmness (fresh minus stored fruit) (right panel) for 40 samples displayed by their ACS1 and ACO1 genotypes across harvest dates.

What this study means to industry:
(1). Among 35 advanced selections from the WSU Apple Breeding Program, a good correlation between fruit firmness and beneficial (low ethylene production, solid symbols in above figure) genotype was observed. None of the advance selections has the otherwise high level ethylene production genotypes (empty symbols in above figure). Therefore, this result supports the implementation of maker-assisted selection to eliminate the high ethylene producer at the early stage of breeding process.

(2). The data reported here are also important for informative selection of parent combinations to produce desirable combination of ethylene production genotypes.

(3). More than 3,000 seedlings from the current breeding pipeline have been genotyped for ethylene production potential through collaboration with Dr. Peace.

2. Utility testing of MdMYB, an apple skin color marker, in two progenies
(Collaborators: Cameron Peace and Kate Evans)

A reported allele-specific dCAPS (derived Cleaved Amplified Polymorphic Sequence) marker, within the gene for the anthocyanin pathway transcription factor *MdMYB1*, associated with apple fruit skin color, was tested in 17 elite breeding parents and two apple seedling progenies. As shown in two tables below, in both progenies, the red skin color phenotype was usually associated with the *MdMYB1-1* allele. This dCAPS marker provided approximately 80% predictability in a 'Golden Delicious' × 'Arlet' and a 'Honeycrisp' × 'Cripps' Pink' progeny. Other potential genetic co-regulators may explain the less-than-perfect association. The specific dCAPS bands associated with

red skin for the latter population were not the same as in the former population or previous reports, and indicates that skin color genotyping based on this marker will require prior association between specific marker alleles and color phenotypes for any given cross. The current form of this marker could be a useful tool for apple marker-assisted breeding, particularly where 'Golden Delicious' is a parent.

Table 1. Association of *MdMYB1* genotype with apple fruit skin color phenotypes in a 'Golden Delicious' × 'Arlet' progeny.

Skin color phenotype	No. of seedlings	*MdMYB1-1* presence:absence	Association consistency
Red	71	60:11	85%
Non-red	26	3:23	88%
Total	97	63:34	86%

Table 2. Association of *MdMYB1* genotype with apple fruit skin color phenotypes in a 'Honeycrisp' × 'Cripps' Pink' progeny.

Skin color phenotype	No. of seedlings	*MdMYB1-1* presence:absence	Association consistency
Red	108	85:23	79%
Non-red	58	11:47	81%
Total	166	96:70	80%

What this study means to industry:
Apple fruit skin or peel color is an important contributor to nutrition, consumer preference, and market value. The current form of this marker has greater than 80% predictability and could be a useful tool for apple marker-assisted breeding, particularly where 'Golden Delicious' is a parent. Similar to ethylene gene markers, this genotype (banding pattern) data are also important for informative selection of parent combinations to produce desirable combinations and to design selection strategies.

3. Characterization of cultivar differences in alcohol acyltransferase (AAT) and 1-aminocyclopropane-1-carboxylate synthase (ACS) gene expression and volatile ester emission during apple fruit maturation and ripening.

(Collaborators: Jim Mattheis and Dave Rudell)

Alcohol acyltransferase (AAT) catalyzes the last step of volatile ester biosynthesis, and ethylene purportedly regulates AAT gene expression. In this study, expression patterns of apple AAT genes and ethylene biosynthesis genes of 1-aminocyclopropane-1-carboxylate synthase (ACS) were investigated in cultivars with relatively high ('Golden Delicious') or low ('Granny Smith') volatile ester production. All four AAT genes expressed stronger in 'Golden Delicious' than in 'Granny Smith'. MdAAT1 and MdAAT2 are the predominant genes expressed in fruit tissues. The expression levels of MdAAT1 and MdAAT2 increased as ripening progressed and were consistent with the total amount of esters detected between two cultivars. The transcript levels of MdAAT3 and MdAAT4 decreased at or after the onset of ripening. The expression of MdACS1 significantly increased at the onset of ripening while the expression of MdACS3 was detected throughout the harvest period. Postharvest 1-MCP exposure had little impact on expression of MdAAT1 and MdACS3 genes, but substantially suppressed the transcript level for MdACS1 in both cultivars and MdAAT2 in 'Golden

Delicious'. The results indicated that: 1) differential expression of AAT genes may contribute to phenotypic variation of volatile ester biosynthesis;, 2) MdACS3 may play a role in induction of AAT gene expression in early fruit development asACS3 is expressed prior to ACS1; 3) climacteric expression of MdACS1 greatly enhanced the expression levels of MdAAT1 and MdAAT2 genes and the emission of aromatic volatile esters; 4) postharvest 1-MCP treatment resulted in selective inhibition of gene expression for specific AAT and ACS family members.

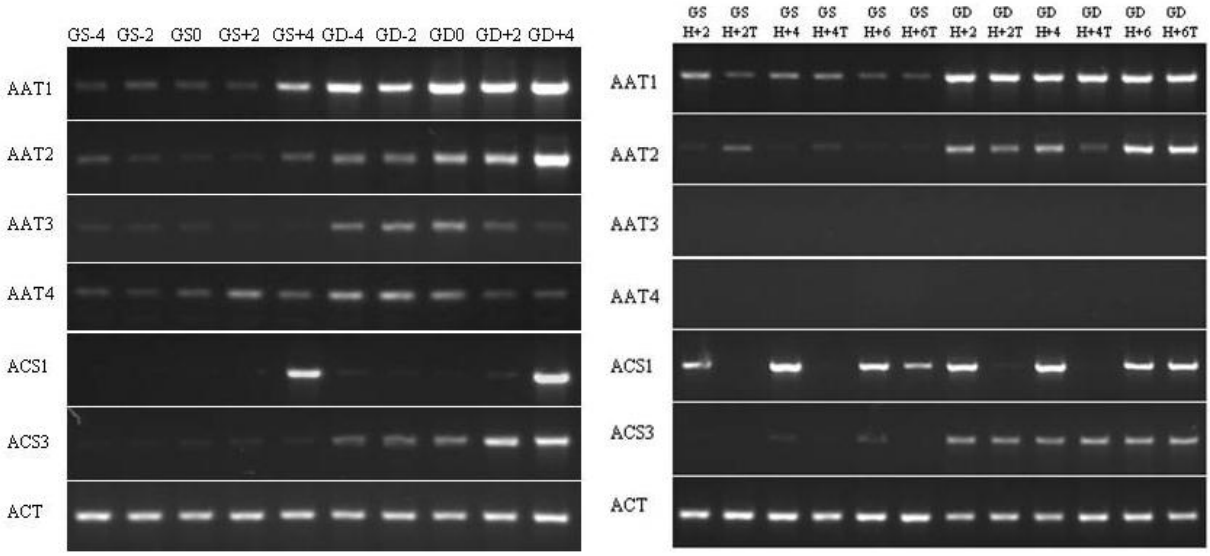

Figure 2. Expression patterns of four AAT genes and two ACS genes in 'Granny Smith' and 'Golden Delicious' apple peel tissue at different ripening stages.

Left panel: Fruit peel tissues with two-week intervals beginning 128 (GD-4) or 149 (GS-4) days after full bloom (DAFB) for 'Golden Delicious' (GD), 'Granny Smith' (GS), respectively. Top axis label indicates cultivar and weeks prior to (designate "-"), at (0), or after (+) physiological maturity was attained. **Right panel:** Expression patterns of four AAT genes in 'Granny Smith' and 'Golden Delicious' apple peel tissue during ripening after harvest. Fruit were harvested 156 (GD0) or 177 (GS0) days after full bloom (DAFB) and held up to 6 weeks at 20 ^{o}C. Top axis label indicates cultivar, weeks after harvest (H+2, H+4 and H+6), and "T" indicates treatment at harvest with 1-MCP.

What this study means to industry?

Production of volatile esters varies significantly among apple cultivars, while the genetic controls underlying these differences have not been elucidated.

Cultivar differences in AAT gene expression intensity, particularly for AAT1 and AAT2, are in principal correlated with the levels of total detected emission of volatile esters. This study represents the first step in assigning function of these genes in aroma production. Further tests of the association between AAT gene expression and phenotypes in wider spectrum of cultivars or germplasm may enable development of a functional DNA marker for practical use in breeding program.

4. Transcriptomic analysis of cultivar-specific apple fruit ripening and texture attributes

(Collaborators: Dorrie Main, Jim Mattheis, Eric Curry)

Molecular events regulating cultivar-specific apple fruit ripening and sensory quality are largely unknown. Such knowledge is essential for genomic-assisted apple breeding and postharvest quality management. In this study, transcriptomic analysis, scanning electronic microscopic examination and systematic physiological characterization were performed on two apple cultivars, 'Pink Lady' (PL) and 'Honeycrisp' (HC), which have distinct ripening behavior and texture attributes. Substantial differences of crispness and firmness in fruit cortex were observed. SEM images of fruit cortex tissues prepared from fruits with similar developmental stage suggest that the cell wall thickness may contribute to the observed firmness and crispness phenotype. A high-density long-oligo apple microarray consisting of duplex 190,135 cross-hybridization-free 50-70-mer isothermal probes, and representing 23,997 unigenes was manufactured on a Nimblegen array platform. The developmental stage- and cultivar-specific expression profiling analysis and QPCR validation indicated that genes in several functional groups express differentially between cultivars and ripening stages.

A. Define ripening stage in both cultivars for selecting fruit cortex tissues transcriptome analysis.

For each cultivar, tissues from three time points representing commercial maturity (0), 4 weeks before (-4) or 2 weeks before (-2) commercial maturity were used for transcriptome analysis (Table 3). Four biological repeats of fruit cortex tissues along with fruit maturity data were collected for each time point.

'Honeycrisp'						
Weeks before commercial harvest	-4	-3	-2	-1	0	+1
Sample date	Aug 5	Aug 12	Aug 19	Aug 26	Sep 2	Sep 9
IEC ($ul \cdot l^{-1}$)	0.11	0.01	<0.01	0.06	0.19	0.18
Firmness (N)	92.3	79.7	75.2	69.8	61.2	60.8
Starch pattern index (1-5)	1.0	1.0	1.3	1.9	3.8	4.5
Cn value	200	273	321	240	268	292
'Pink Lady'						
Weeks before commercial harvest	-4	-3	-2	-1	0	+1
Sample date	Oct 7	Oct 14	Oct 21	Oct 28	Nov 4	Nov 12
IEC ($ul \cdot l^{-1}$)	0.16	0.01	0.24	< 0.01	1.12	0.46
Firmness (N)	103.5	111.6	101.3	97.2	94.5	94.1

Table 3. Physiological characterization of fruit maturity and texture attributes for 'Honeycrisp' and 'Pink Lady' apple cultivars.

All values are means based on a weekly sample of 15 apples. Fruit firmness was evaluated using a Mohr Digitest. Crispness (Cn) is defined as high frequency tearing characteristics of fruit material. Rating of starch pattern index was based on iodine staining and scored using Cornell composite standards. Internal ethylene concentration (IEC) was determined by GC using established methods.

B. Scanning electron microscopy (SEM) of apple fruit cortex cellular and cell wall features of both Pink Lady and Honeycrisp.

Microscopic cellular features in fruit cortex such as cell size, cell number, cell wall properties and intercellular space between adjacent cells may contribute to cultivar-specific fruit texture attributes. As shown in figure above, SEM images revealed no obvious differences on cell size and cell number per unit area from the cortex tissues between cultivars with equivalent maturity (top strip, 400x magnifications). With 2,000x magnification, the images indicated different cell inner face appearance and textural feature between two cultivars, i.e. a fine and smooth inner surface of cell wall from HC and rough and fortified texture of cell walls of PL cortex cells (middle strip in figure x). At 20,000x magnification, the differences on thickness cell walls were readily visible; showing a decreasing trend of cell wall thickness and somewhat increasing thickness in PL were also observed (the bottom strip in figure x). (fruit cortex tissues labeled with stars were used for transcriptomic study).

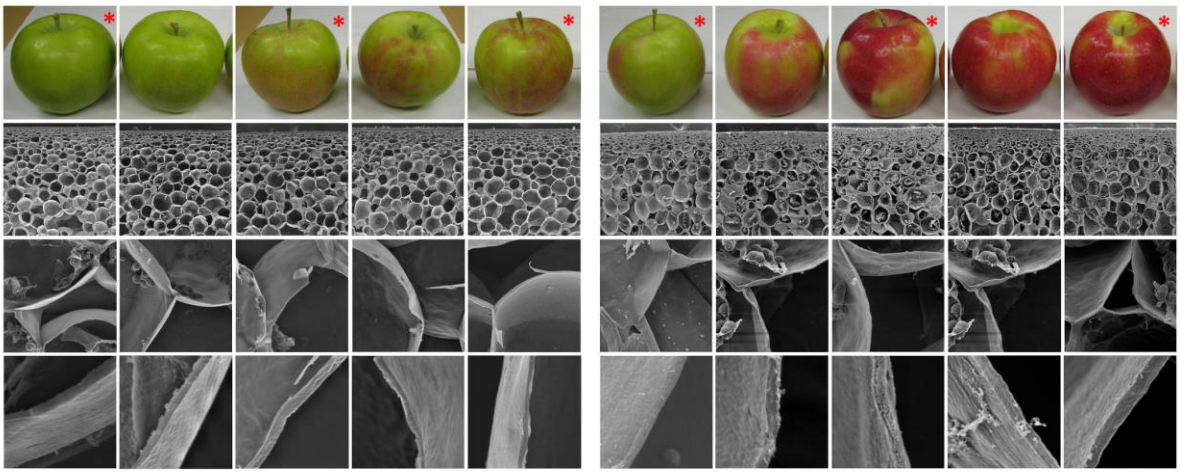

Figure 3. Representing fruit image and SEM images of fruit cortex cells with the maturity, from left to right at -4, -3, -2, -1 and 0 week.

Left panel: 'Honeycrisp'. Right panel: 'Pink Lady'. For both panels, top strip: fruit images represent the developmental stages expressed as week before commercial maturity. Second strip: 400 x magnification showing cellular features of cortex tissues. Third strip: 2000 x magnification showing cell wall. Bottom panel: 20,000 x showing close-up of cell wall.

C. Design of Nimblegen apple long oligo microarray and array hybridization.

A long oligo microarray was designed for apple based on the Malus unigene V4 sequences (Genome Database for Rosaceae, Jung *et al*., 2008). 260,581 Malus EST sequences were downloaded from NCBI dbEST (Benson *et al*., 2007), filtered for contamination and low quality sequence and assembled into 23,284 contigs and 53,200 singletons using CAP3 (Huang and Madan, 1999) with an overlap percentage parameter of 90 (-p 90). The unigenes, comprising of the combined contigs and singlets, were computationally annotated for putative function by pairwise comparison against the Arabidopsis thaliana protein database (www.arabidopsis.org), and the Uniprot Swiss-Prot and TrEMBL databases (Wu *et al.*, 2006; Mulder *et al.*, 2007) using the BLASTX algorithm. Only matches with an E-value of less than 1.0 e-6 were recorded. Based on the similarity search results, 55,960 (73%) of the Malus unigene sequences had significant matches with proteins from these databases. Array hybridization, data acquisition and normalization were done in Nimblegen Lab (Iceland).

D. Transcriptomic changes in apple cortex during ripening

By ANOVA analyses using a non-adaptive false discovery rate (FDR) of 0.01 and a cutoff value of 2-fold change of detected signal strength between any one of the two adjacent time points, a total of 1796 differentially expressed unigenes from HC and 1213 from PL were identified, representing 5% and 7.5% of all unigene deposited on the array, respectively. Unigenes with "unknown function", defined as those sequences with no match against a protein in TrEMBL, Swiss-Prot or Arabidopsis protein database, consisted of an average 33.2% of all identified differentially expressed unigenes. In each cultivar, unigenes showing down-regulated expression patterns greatly outnumbered the ones showing up-regulated expression patterns. A slight higher percentage of unigenes as "up-regulated" was identified from late-ripening cultivar PL (34.7%) than that from early-ripening cultivar PL (31.3%). Based on their functional annotations, three functional groups were apparently regulated during the late stage of fruit ripeing.

Hormonal metabolism and response Transcriptomic changes related to plant hormone biosyntheses and responses are a major characteristic during late ripening stages of apple fruit. Over half of the unigenes (40 out of total 76) in this category are directly related to auxin and ethylene metabolism and response. Even more unigenes if specific transcription factors are included, such transcriptomic change suggests the central roles for these two hormones. Based on the number of unigenes with up- or down-regulated expression patterns, it seems that the roles of auxin were attenuating, while the effects of ethylene were strengthening during the period of 4 weeks before commercial maturity. Unigenes implicated in other hormone functions including gibberellin, brassinosteroid and jasmonate were also identified. Asummarized list is presented in Table x. **Ethylene:** ACS3 showed steadily increased expression patterns in both cultivars as on-tree development progressed, but substantially higher transcript levels of ACS3 were observed in HC. The expression level of ACO1 increased dramatically in HC, but just moderately in PL. Coincident with the low abundance of ACS3 transcript in PL, several unigenes encoding EIN3-binding F-box protein were up-regulated only in PL. Differential gene expression patterns were also observed for unigenes related to auxin metabolism and response between these two cultivars. **Auxin:** Five unigenes annotated as "auxin efflux carrier component" were specifically down-regulated in PL; in contrast, a unigene with similar annotation was only up-regulated in HC. Several unigenes related to the regulation of auxin homeostasis were also differentially regulated during fruit ripening. A unigene encoding indoleacetamide hydrolase functioning in auxin biosynthesis was down-regulated only in PL. However, a unigene annotated as a probable indole-3-acetic acid-amido synthetase GH3.5 is down-regulated only in HC. Conversely, a unigene with similar annotation was up-regulated in PL. More than a dozen "AUX/IAA proteins", the auxin response repressor, were also identified. The data seem to suggest that IAA is more readily available in the fruit cortex tissues of HC, and a positive correlation exists between auxin availability and active ACS3 and ACO1 expression. **Gibberellin**: four unigene encoding "gibberellin 3 (or 2)-beta-dioxygenase" for GA biosynthesis, four unigene for "DELLA proteins" (nuclear repressors of GA-responses) were identified as differentially expressed genes. **Jasmonate:** Four Jasmonate O-methyltransferase encoding unigenes were selectively up-regulated in both cultivars, while in contrast all ten unigenes annotated as "BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor" were selectively down-regulated in both cultivars. In many cases the number of identified unigenes can be reduced pending sequence analysis, as often multiple unigenes are associated with the same protein identity; therefore they may be different alleles for the same gene.

Transcription factors (TFs) TFs are proteins with sequence-specific DNA binding ability which may activate or repress transcription of sets of genes in response to endogenous and exogenous stimuli (Riechmann et al., 2000). Based on available EST data, an estimated 1025 unigenes encode 62 transcription factor (TF) families, and more than 90% of these TFs have orthologs in other species (Guo et al, 2008). A extensive number of TF-encoding unigenes were differentially expressed in both

cultivars, i.e. 82 of them from PL and 142 of them from HC, indicating a dramatic transcriptional regulation at late fruit ripening stages. Consistent with the strong presence of gene activity related to auxin and ethylene biosyntheses and responses, almost a quarter of TFs encoding unigenes belong to those specifically responding to auxin and ethylene. The majority of unigenes encoding TFs were down-regulated as fruit ripening progressed, except ethylene responsive TFs. Transcriptional regulation controls many biological processes, which is achieved primarily through the actions of transcription factors (TFs). A table below summarizes the identity of the classification of identified TFs.

Table 4. No. of differentially expressed unigenes encoding proteins in various transcription factor (TF) families

TF family	Up-regulated in PL	Down regulated in PL	Up-regulated in HC	Down-regulated in HC
Ethylene-responsive family	11	6	4	8
Auxin-responsive TF family	1	10	3	13
Br responsive family	0	3	0	2
bHLH family	1	5	1	3
bZIP	0	3	1	1
Homeobox-leucine zipper	1	7	4	7
Myb protein family	7	3	4	10
NAC domain TF family	2	2	2	10
RING-H2 finger	1	1	3	2
SBP-like	0	2	0	3
Zinc finger family	0	2	1	19
Dof zinc finger protein	0	2	0	10
CONSTANS-like protein	0	1	0	2
WRKY transcription factor	0	0	4	6
MADS-domain protein	1	0	0	0
Other	3	3	5	6

Cell wall metabolisms Unigenes belonging to several gene families encoding proteins that modify specific cell wall components were identified in cortex tissues. While most of these genes are similarly regulated between two cultivars, a few unigenes showed strong cultivar-specific expression patterns. The most noticeable groups of unigenes were those involved in hemi-cellulose degradation. Four xyloglucan endotransglycosylase (XET) encoding unigenes were identified with differentially expressed patterns. All three XET genes identified from HC exhibited increased expression patterns, while only one showed increased pattern and two other exhibited decreasing expression patterns in PL. All of the identified four "probable pectate lyase" encoding unigenes were down-regulated in PL, but only 2 of them exhibited down-regulated expression patterns in HC. A down-regulated "pectinesterase inhibitor" encoding unigene was detected only in PL, but an up-regulated "putative xyloglucanase inhibitor" encoding unigene was only detected in HC. Of three unigenes for "beta-galactosidase", one from each cultivar showed down-regulated expression, while an extra unigene was up-regulated only in PL. A unigene encoding for a 6(G)-fructosyltransferase was significantly up-regulated only in the first two-week period of fruit ripening in HC.

What this study means to industry

Genetic controls and molecular mechanisms of apple fruit ripening and fruit quality are the most critical component in tree fruit genomics study, yet very limited knowledge current exists. These

results represent an initial step to identify genes responsible for unique fruit ripening behavior and fruit quality traits. Specifically, two aspects are expected to contribute to the sustainability and profitability of fruit industry, i.e. developing tools for genomics-assisted breeding and innovative fruit quality management.

Four peer reviewed publications related to this proposal:

Zhu Y and Barritt BH. 2008. Md-ACS1 and Md-ACO1 genotyping of apple (*Malus x domestica* Borkh.) breeding parents and suitability for marker-assisted selection. Tree Genetics and Genomes 4: 555-562.

Zhu Y, Rudell, DR, Mattheis JP. 2008. Characterization of cultivar differences in alcohol acyltransferase and 1-aminocyclopropane-1-carboxylate synthase gene expression and volatile ester emission during apple fruit maturation and ripening. Postharv Bio Technol 49: 330-339.

Zhu Y, Evans K and Peace C. 2010. Utility testing of an apple skin color MdMYB1 marker in two progenies. Molecular Breeding (submitted).

Zhu Y, Zheng P, Varanasi V, Main D, Curry E and Mattheis JP. 2010. Transcriptomic analysis of cultivar-specific apple fruit ripening and texture attributes. BMC Plant Biology (in preparation)

EXECUTIVE SUMMARY

Project Title: Functional genomics and marker development for apple sensory qualities

The essence of genetic studies is to establish gene-traits associations. The knowledge from such studies provides the basis for **genomics-assisted breeding** and **genomics-based quality management**. Breeding for locally adapted apple cultivars requires a long-term commitment due to the crop's perennial nature, long-juvenile phase, and high heterozygosity of the genome and poor predictability of fruiting performance. Implementation of a marker-assisted seedling selection strategy, such as using gene-specific functional molecular markers, is especially beneficial for perennial tree fruit breeding. By implementing genotype-directed selection, less desirable seedlings (such as those with non-red skin fruit) can be culled at an early stage prior to actual fruiting, therefore minimizing future orchard planting costs, tree maintenance, and performance evaluation. Breeding efficiency can also be increased by using DNA information to design crosses that lead to a greater proportion of desirable seedlings, such as all seedlings having fruit with red skin, or at least a predicted proportion of seedling outcomes.

Testing and seeking implementation of three reported apple gene-specific DNA markers, two for **ethylene biosynthesis** potential and one for **apple skin color,** in an on-going Washington State Apple Breeding Program has never been reported. These studies provide guidelines for potential utilization in apple breeding. As current understanding of tree fruit genetics is very limited, more research is required to better understand the molecular mechanisms regulating fruit quality traits. In addition to testing currently available markers, this research also included genetic analysis of apple fruit related to **firmness, crispness** and **aromatic volatile ester production**. Using gene expression profiles, candidate genes were compiled for further examination. The results from this study set the foundation for further functional analysis to associate specific genes with apple firmness, crispness and volatile ester production, with the ultimate goal to develop functional molecular markers for these traits.

The achievements from this project were largely due to the close collaboration among researchers; a total of six labs with different research expertise were cooperatively contributing to the progress including bioinformatics from **Dr. Main**'s lab, physiological characterization in **Dr. Mattheis**' lab, germplasm availability from on-going breeding program managed by **Dr. Barritt** and **Dr. Evans**, scanning electronic microscopic study in **Dr. Curry**'s lab, and high through-put genotyping facility in **Dr. Peace**'s lab. While our study has been closely aligned with the needs of the tree fruit industry, these research activities also generate essential data for scientific advancement in apple genomics as evident by several peer-reviewed publications.

FINAL PROJECT REPORT
WTFRC Project Number: AP-08-807 (WSU Project 13C-3655-4295)

Project Title: Management of vegetative growth in apple trees with bioregulators

PI: Don C. Elfving
Organization: WSU Tree Fruit Research & Extension Center
Telephone: 509-663-8181, ext. 252
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Address: 1100 N. Western Ave.
City: Wenatchee
State/Zip: WA/98801

Cooperators: Tory Schmidt, Research Associate, WTFRC;
Dwayne B. Visser, Agricultural Research Technologist III

Other funding sources

Agency Name: BASF
Amount requested/awarded: $5,000 awarded in 2008

Agency Name: NNII
Amount requested/awarded: $1,000 awarded in 2009

Total Project Funding: **Year 1:** 13,111 **Year 2:** 14,109

WTFRC Collaborative expenses:

Item	2008	2009
Stemilt RCA room rental	0	0
Crew labor	840	550
Shipping	0	0
Supplies	0	0
Travel	520	50
Miscellaneous	0	0
Total	$1360	$600

Budget History:

Item	2008	2009
Salaries	5,442	5,714
Benefits	1,959	2,057
Wages	1,080	1,134
Benefits	130	204
Equipment	0	0
Supplies	500	800
Travel	4,000	4,200
Miscellaneous	0	0
Total	$13,111	$14,109

Objectives:

1. Explore possible methods for improving the efficacy of prohexadione-Ca (Apogee) for control of unwanted vegetative vigor, including application timing, combinations of Apogee with ethephon and/or other bioregulator products, such as abscisic acid (ABA), a bioregulator implicated in the initiation of dormancy.
2. Examine whether ABA can be used either alone or in combination with other bioregulators to force terminal bud set in growing shoots, thus controlling growth.
3. Evaluate the potential benefits for stimulation of latent bud growth on "blind wood" with high concentrations of cytokinins (e.g., chlorfenuron, thidiazuron, 6-benzyladenine) with or without supplemental gibberellic acid (e.g., GA_{4+7}).
4. Compare cyclanilide (Tiberon®) with cytokinin/gibberellin products (e.g., Promalin) for induction of desired shoots at trellis wires during canopy development in sleeping-eye apple trees.
5. Examine fruiting in cyclanilide treated sleeping-eye trees to determine if this approach results in the development of better quality fruiting wood.

Significant findings 2008:

1. Three treatments, 1) Promalin (PR, 20,000 ppm, pure formulation), 2) thidiazuron (TDZ) + ProVide (GA_{4+7}) (5,000 ppm and 2,500 ppm + Pentra-Bark 2% v/v), and 3) thidiazuron (TDZ) + ProVide (GA_{4+7}) (both 5,000 ppm + Pentra-Bark 2% v/v) painted on one-year-old vertical leader shoots of 'Cameo'/M.26 trees at trellis wires doubled the number of new shoots forming at the wire compared to no treatment.
2. The Promalin formulation alone (no surfactant) was concentrated enough to assure a branching response in the absence of bark injury and did not cause phytotoxicity.
3. In apple, cytokinin is as important as gibberellin for inducing shoot formation. This approach can help growers developing apple canopies on trellises, such as for "sleeping-eye" systems.
4. In vigorous, grafted 'Fuji'/MM.106 trees, four Apogee (prohexadione-Calcium, P-Ca) sprays did not maintain control of shoot growth. Shoot growth on Apogee-treated trees eventually equaled that of untreated control trees.
5. When four Apogee sprays were followed by one or two ethephon applications, two abscisic acid (VBC30051) applications or one or two tank-mix applications of ethephon and VBC, shoot growth was essentially halted after treatment.
6. The various treatment combinations of four Apogee sprays with or without follow-up ethephon and/or ABA applications showed minor effects on fruit quality.
7. In 'Fuji'/M.26 trees of roughly comparable vigor, untreated shoots grew normally. Four Apogee applications were sufficient to control shoot growth. Curiously, Apogee-treated shoots receiving one or two ethephon treatments grew enough to be the same as controls. Additional treatments with ABA alone or ABA+ethephon were equivalent to four Apogees alone.
8. Pretreating 'Fuji'/M.26 apple trees later in the growing season with Apogee followed by ethephon and/or ABA resulted in no overall control of growth. Waiting until 24 June to start this program was too late. Nonetheless, shoot growth was less vigorous where ethephon and/or ABA were used.
9. A preliminary trial testing the potential vegetative growth control agent FAL 1210 was carried out on vigorous 'Fuji'/MM.106 apple trees. FAL 1210 was applied at 125 ppm three times and at 250 ppm only once in early spring, while Apogee (125 ppm) was applied four times. Apogee-treated shoots eventually grew as much as untreated control shoots. A single application of 250 ppm FAL 1210 or 3 applications of 125 ppm were both effective for controlling terminal shoot elongation in this trial. This product shows promise as a vegetative

growth control agent for apple. Three applications of the FAL1210 product appeared to reduce fruit grade-out and resulted in lower titratable acidity. These observations should be considered as preliminary.

10. 'Fuji'/M.26 trees were treated with a single late-June application of Apogee followed one week later by single applications of ethephon and/or ABA. These treatments were applied too late to significantly reduce terminal growth, although combination treatments did have somewhat shorter terminal shoots. There were no effects of any treatment on fruit quality.

Significant findings 2009:

1. Three or four applications of Apogee at 6 oz/100 gallons (125 mg/liter) to 'BC2 Fuji'/M.7 apple trees provided initial control over terminal shoot extension but later-season regrowth canceled out any differences.
2. Ethephon (900 mg/liter) applied either once or twice after three applications of Apogee had no effect on control of regrowth in response to Apogee applications.
3. ABA (500 mg/liter) applied either once or twice following three applications of Apogee provided good control of shoot regrowth. Two applications were better than one. Combining ethephon with ABA did not improve treatment effectiveness over ABA alone.
4. Neither three nor four applications of Apogee nor supplemental ABA in June had any effect on fruit set or fruit size at harvest.
5. Two applications of Apogee at 12 oz./100 gallons (250 mg/liter) reduced terminal shoot growth in 'BC2 Fuji'/M.7 trees by approximately 50%.
6. A single application of daminozide (1000 mg/liter) at petal-fall slowed initial shoot growth for about 6 weeks in treated trees but did not reduce final shoot growth at the end of the season.
7. Applications of the candidate vegetative growth control agent FAL1210 at from 0 to 1000 mg/liter applied once or three times provided no control of apple shoot growth.
8. None of the growth-control treatments had any effect on either fruit set or fruit size at harvest.
9. Five treatments, 1) Promalin (PR, 20,000 ppm, pure formulation), 2) ProVide (GA_{4+7} 5,000 ppm + Pentra-Bark 3% v/v), 3) ProVide (GA_{4+7}, 10,000 ppm + Pentra-Bark 3% v/v), 4) thidiazuron (TDZ, 20,000 ppm + Pentra-Bark 3% v/v), and 5) thidiazuron (TDZ) + ProVide (GA_{4+7}) (20,000 ppm + 10,000 ppm + Pentra-Bark 3% v/v) painted on one-year-old vertical leader shoots of 'Granny Smith'/M.9 trees at trellis wires nearly doubled or doubled the number of new shoots forming at the wire compared to no treatment.
10. PR and ProVide produced adequate to more-than-adequate lateral branching for training to trellis wires.
11. TDZ consistently caused undesirable tissue proliferation at the bases of induced lateral shoots.
12. Yield in a high-density sleeping-eye apple block ('Fuji'/'Mark') under both Promalin-treated and cyclanilide-treated tree-training regimes reached 46 bins/acre in the third leaf and 91 bins/acre in the fourth leaf.

Results and Discussion:

In apple, the Promalin formulation alone, undiluted, continues to be effective for stimulation of branch induction on one-year-old leader shoots in the early spring without resort to scoring or notching cuts. Use of strong surfactants (e.g., Pentra-Bark at 3% v/v) helps produce a branch induction response to GA alone. The capacity to place new branches adjacent to trellis wires should facilitate proper canopy development in trellised high-density plantings. This type of tree structure has much to recommend it from the physiological point of view, so facilitating this process without

the need for pruning is an important development. Although such a process is labor-intensive, our most successful treatments minimize the labor cost involved. We have now compiled positive results with two very hard-to-branch cultivars, 'Cameo' and 'Granny Smith'.

Apogee programs in commercial apple orchards continue to be plagued by the problem of later-season "regrowth" after a successful initial control response to early-season Apogee applications. The advent of commercially formulated abscisic acid opens a potential new avenue for approaching a resolution to this problem. One or two applications of ABA (500 mg a.i./liter) successfully extended the period of control over shoot growth well beyond that of Apogee alone, with the result that, on average, a single ABA application in late June resulted in terminal shoots about 2/3 the length of untreated shoots, while two applications reduced terminal shoot length by about half. These results are very promising and suggest that ABA should also be tested for growth-control properties on its own. ABA may offer a way to extend the benefits of early-season growth control with Apogee.

Trials in 2009 showed clearly that ethephon has little or no role to play in controlling shoot growth under these conditions. Whether mixed with ABA or applied alone following three previous Apogee applications, ethephon did not produce useful growth-control results. No further work with ethephon for this purpose is planned.

Single or double applications of ABA in early and late June (500 mg/liter) had no effect at all on fruit set or on fruit size at harvest. No observations were made that would suggest a problem with fruit drop. No other fruit-quality parameters were examined in this study. These results support the plan for further studies with this product for control of vegetative growth in apple.

In 2008, Fine Americas introduced a candidate vegetative growth control product (FAL 1210) for testing. After two years of tests, it is clear that this product does not provide any significant control over apple tree shoot growth. No further trials with this product are planned.

Cyclanilide (registered as Tiberon^{TM} in 2009) has proven to be as beneficial as Promalin for branching developing leader shoots at trellis wires on sleeping-eye 'Fuji'/'Mark' apple trees. In this trial, cyclanilide rates below recommended nursery levels (e.g., 50 mg a.i./liter) induced good branching with minimal inhibition of terminal shoot extension. Yields in the 3rd and 4th years in this trial have been very large (46 and 91 bins/acre on average, respectively), demonstrating the benefit of rapid, early development of a productive canopy through directed growth.

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EXECUTIVE SUMMARY

In trellised apple plantings, securing adequate and well-positioned lateral branching is critical for early production success. Lateral branching can be induced on vigorously-growing current-season's shoots in summer or on one-year-old wood in spring. Each approach has its benefits and drawbacks, but either strategy, if properly implemented, will work.

Abscisic acid, a newly-available bioregulator product, may have an important role to play in vegetative growth control. Initial trials indicate that ABA applications can nearly eliminate the undesirable second growth flush ("regrowth") that often follows multiple Apogee applications in the spring and early summer. More work is needed to explore the interactions of dose and timing of ABA treatments, and it also warrants evaluation as a growth-control agent on its own. ABA appears to have no effects on fruit set, fruit size or fruit drop when applied in June, after fruit cell division is completed. Its effects on very early fruit growth are unknown. Ethephon has been discarded as a viable approach in conjunction with Apogee and ABA to control later-season regrowth. Applied alone, or in combination with ABA, ethephon did not produce effective and extended control over vigorous apple shoot elongation in previously Apogee-treated trees.

The candidate growth-control product FAL 1220 (Fine Americas) has been shown to have no potential as a vegetative growth-control material for apple trees. Further work with this product is not planned.

FINAL PROJECT REPORT

Project Title: Mapping M. sieversii: a valuable genetic resource for apple breeding

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Other funding sources: None

Total Project Funding: $54,000

Budget History:

Item	2008	2009	
Salaries	10,000	10,000	
Benefits	3,500	3,500	
Wages			
Benefits			
Equipment			
Supplies	12,000	15,000	
Travel			
Miscellaneous			
Total	$25,500	$28,500	

Objectives

The objectives of the present proposal addressed the following high priority research area defined by the Washington Tree Fruit Research Commission: Continued I.D. of genes and improvement of gene marker tools for continued increase in the efficiency of apple breeding program as well as improved disease and pathogen resistance.

The objectives were:

1) Produce genetic marker data for a family (**GMAL 4593)** of 190 seedlings derived from a 'Royal Gala' x *M. sieversii* cross using a collection of 159 SSR markers. Use the resulting marker data to produce a basic "framework" map for *M. sieversii*.

2) Identify additional markers (SSR and SNP) to fill any gaps in the evolving map and enrich regions of interest with a high density of markers.

3) Anchor the generated map to other apple maps by using identical SSR markers. In particular, exchange markers with New Zealand Institute for Plant and Food Research who will be mapping another family (**GMAL 4591**) from a different 'Royal Gala x *M. sieversii* cross.

4) 200 SNP markers will be identified by IASMA and placed on the genetic map for the GMAL4593 population.

5) Collaborate with others to define QTLs for important traits as the population is characterized. Specifically, **GMAL 4593** will be assessed for fire blight resistance in 2008 and 2009.

Note: **The finished maps and marker data will be deposited in the Genomic Database for the Rosaceae (GDR) in order for it to be available to the entire apple breeding and research community.**

Significant Findings

Objective 1 – One hundred of the 159 SSR markers available for the study have been screened on the entire population of 190 seedlings. Of the SSR primer combinations screened, 64 SSRs were polymorphic within the family. Fifty segregated in *M. sieversii* (MS) and sixty-four segregated in 'Royal Gala' (RG). The markers were assigned to linkage groups using Joinmap® 3.0. Statistics for both the SSRs and SNPs are presented in **Table 1**.

Objective 2 – A linkage map for both *M. sieversii* and 'Royal Gala' have been constructed using Joinmap® 3.0 and presented in **Figure 1**. Some linkage groups are represented as several sub-groups. It is expected that as the mapping effort continues all subsets of a linkage group will be able to be combined into a single linkage group. To accomplish this several hundred additional SNPs specific to the missing linkage group are currently being screened at FEM-IASMA, additional SSRs are being screened, and efforts to identify *sieversii*-specific SNPs are in progress. These extended efforts are part of new proposal submitted to the WTFRC for 2010.

Objective 3 –Additional SNP primer sets were obtained from the New Zealand Institute for Plant and Food Research covering all 17 linkage groups. These SNPS were screened in our population using HRM analysis on the Roche LightCycler and added to the genetic framework map (**Figure 1**).

Objective 4 – While the genetic framework maps for *M. sieversii* and 'Royal Gala' are incomplete, a sufficient number of loci have been established to allow comparisons with existing maps. These comparative maps are presented in **Figs. 2 and 3.** Mapping of the GMAL 4591 population by the New Zealand Institute of Plant and Food Research was not conducted due to a lack of funding so this comparison could not be made.

Ojective 5 – In collaboration with FEM-IASMA, 287 SNP markers were screened in a 96-member subset of the GMAL4593 population by FEM-IASMA. One hundred and seventy-nine were informative and placed on the genetic framework map (**Figure 1)**. As mentioned, this effort is continuing and several hundred additional SNPS are being screened at FEM-IASMA focusing on the missing and incomplete linkage groups.

Objective 6 - All the individual members of the GMAL4593 population have been scored for fire blight resistance. A replicated copy of the mapping population GMAL4593 was planted in Kearneysville in the fall of 2008. Further fire blight resistance evaluation and scoring will be conducted on these individuals in the spring of 2010 in order to obtain a more comprehensive data set to use for QTL analysis. This will also provide time to enrich the *M. sieversii* genetic map so that a meaningful QTL analysis of fire blight resistance can be conducted. The framework map will also be available to those conducting other phenotypic analyses of the GMAL4593 population.

Results and Discussion

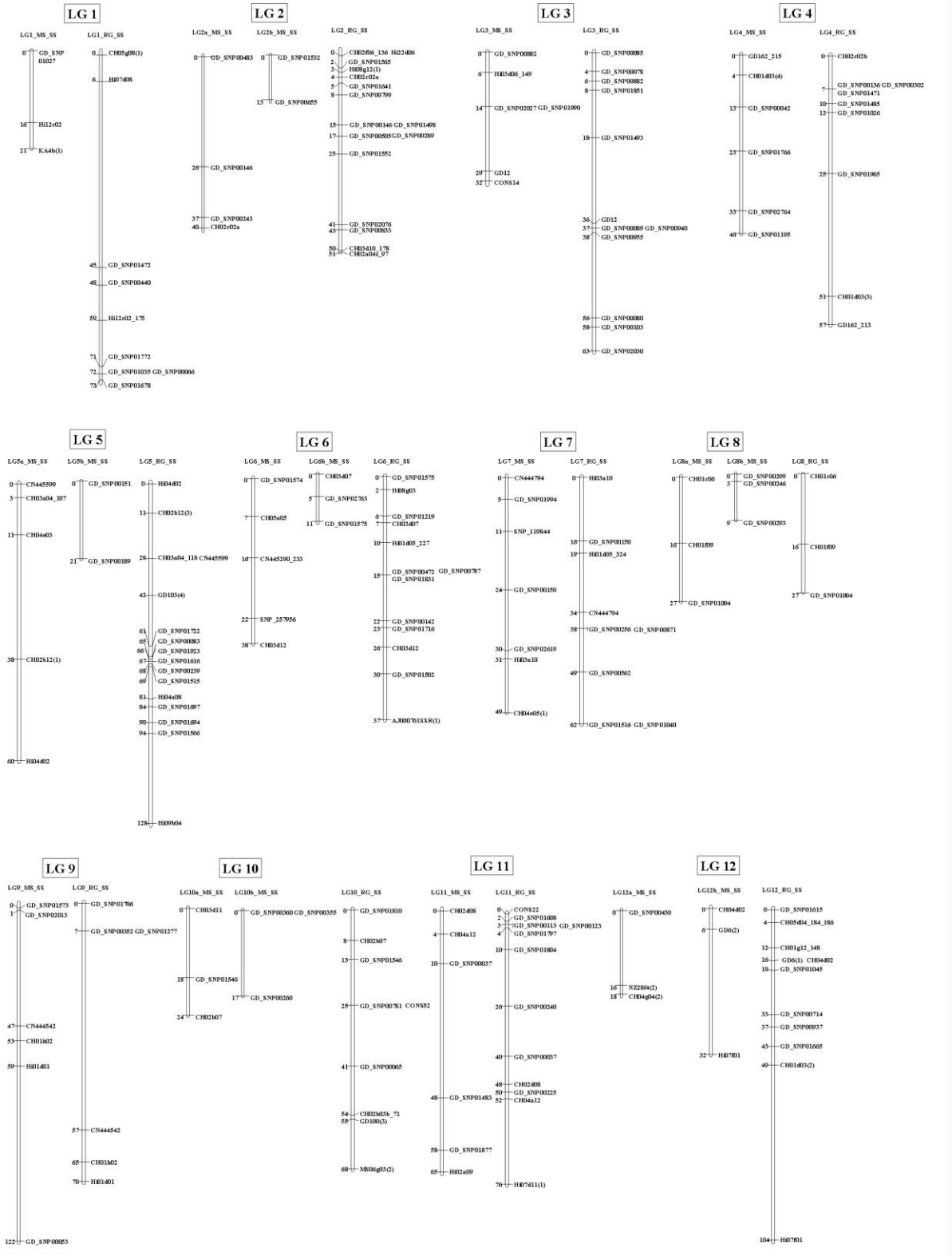
The USDA-ARS Plant Genetic Resources Unit has established a collection of *Malus* from around the world in order to preserve and develop genetic resources important to the apple industry. Among this collection is *Malus sieversii*, the main progenitor of the domestic apple, collected from Central Asia (Khazakhstan). To enable QTL analysis for important traits in *M. sieversii*, we have undertaken the construction of a genetic framework map for the family (F1) GMAL4593 ('Royal Gala' X *M. sieversii* PI 631981 [GMAL 4448]). One-hundred-ninety progeny were analyzed using Joinmap® 3.0 (Van ooijen and Voorips 2001) to establish a framework map for each individual parent of the F1 population GMAL4593 ('Royal Gala' x *Malus sieversii*). Linkage groups were established at a recombination fraction of 0.40 a LOD score $\geq$ 4.0. The Kosambi mapping function was used for the calculation of map distances.

Of 107 SSR primer combinations screened, 81 SSRs were polymorphic within the family. Fifty-three segregated in both *M. sieversii* (MS) and 'Royal Gala' (RG), 15 segregated in MS only and 21 in RG only. Additionally, 287 SNPs were screened in a subset (96) of the population. Ninety-nine SNPs were not informative for mapping in this population and 179 were mapped using Joinmap. 24 of the SNPs segregated in both MS and RG, 158 with RG only and 52 with MS only. The current 'Royal Gala' map consists of 188 molecular markers (124 SNPs and 64 SSRs) assigned to 17 linkage groups and the MS map consists of 107 molecular markers (57 SNPs and 50 SSRs) assigned to 26 linkage groups representative of all 17 linkage groups.

The construction of a framework map for *Malus sieversii* is the first step in enabling the identification of regions of the genome that control important traits such as apple scab and fire blight resistance. This map will be used to place QTLs for fire blight resistance and apple scab resistance. Identifying molecular markers in these regions will help breeders streamline the breeding process by allowing the breeders to make early selections of resistant material. The framework map presented in **Figure 1** will not only serve to identify genomic locations of important traits in *Malus sieversii*, but by using molecular markers common in other apple maps, allow the comparison of these locations in

the *Malus sieversii* genome to other apple genomes. Figure 2 illustrates the cross comparison of *Malus sieversii* linkage groups with the corresponding linkage groups from the apple consensus map recently constructed by N'Diaye et al. (2008) and the 'Fiesta' x 'Discovery' map (www.rosaceae.org and Silfverfberg-Dilworth et al. 2006).

Figure 1 – Genetic framework maps for the parents, *M.sieversii* (MS) and 'Royal Gala' (RG) of mapping population GMAL4593. LG = Linkage group. All SNP-based markers are designated as GD SNP where GD= Golden Delicious. SNPs analysis was conducted by IASMA based on the identification of SNPS from the genomic sequence of 'Golden Delicious.'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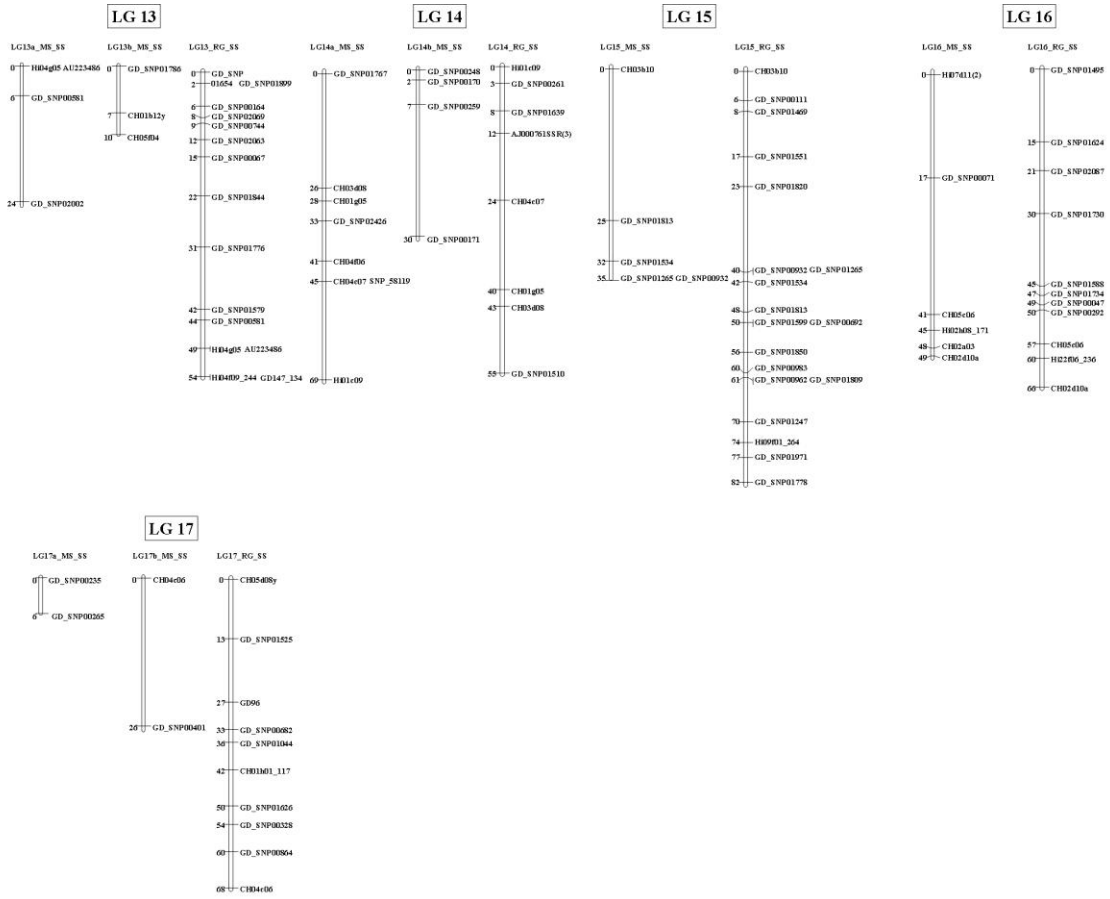
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0 GD_SNP00235
6 GD_SNP00265
LG17b_MS_$$
0 CH04c06
26 GD_SNP00401
LG17_RG_$$
0 CH05d08y
13 GD_SNP01525
27 GD96
33 GD_SNP00682
36 GD_SNP01044
42 CH01h01_117
50 GD_SNP01626
54 GD_SNP00328
60 GD_SNP00864
68 CH04c06

Figure 2 – Anchoring of *M. sieversii* map to the apple consensus map. LG1 is not included because none of the markers from *LG1-M. sieversii* were on the apple consensus map.

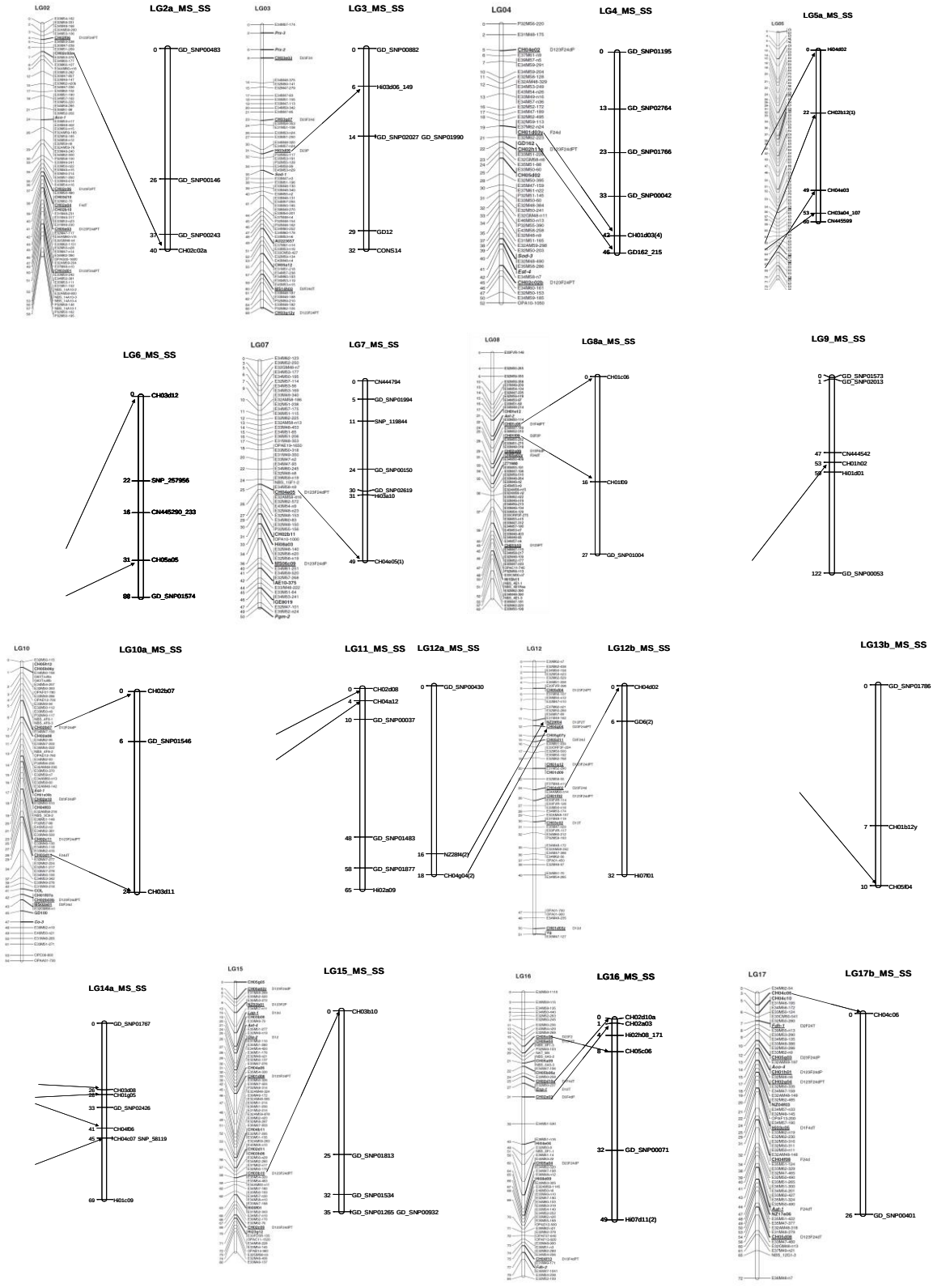

Table 1 – Mapping statistics for each parent in GMAL4593 ('Royal Gala' x *M. sieversii*).
LG = Linkage Group Number, **#loci** = total number of loci identified on the linkage group, **#SSR** = number of SSR markers mapping to the linkage group, **#SNP**s = number of SNPs mapping to the linkage group, **CM distance** = centimorgans of distance spanning the linkage group by the collective SNP and SSR markers.

Malus sieversii

LG	#loci	#SSR	#SNPs	CM distance
1	3	2	1	21.182
2a	4	1	3	39.827
2b	2	0	2	13.242
3	6	2	4	31.911
4	6	2	4	46.343
5a	5	5	0	59.873
5b	2	0	2	21.637
6a	5	3	2	37.565
6b	3	1	2	11.41
7	7	3	4	48.937
8a	3	2	1	26.922
8b	3	0	3	9.065
9	6	3	3	121.687
10a	3	2	1	24.302
10b	3	0	3	17.329
11	6	3	3	64.69
12a	3	2	1	17.692
12b	3	3	0	31.599
13a	4	2	2	23.832
13b	3	2	1	10.11
14a	8	5	3	68
14b	4	0	4	29.634
15	5	1	4	35.253
16	6	5	1	49.341
17a	2	0	2	5.773
17b	2	1	1	26.275
totals	100	47	53	832.422

Royal Gala

LG	#loci	#SSR	#SNPs	CM distance
1	9	3	6	72.594
2	16	6	10	50.712
3	12	1	11	63.342
4	9	3	6	57.299
5	16	7	9	127.644
6	13	5	8	37.495
7	9	3	6	62.442
8	3	2	1	27.139
9	6	3	3	70.111
10	9	4	5	67.596
11	12	3	9	73.066
12	11	6	5	103.555
13	15	4	11	53.873
14	8	5	3	54.535
15	19	2	17	81.892
16	11	3	8	66.413
17	10	4	6	68.245
totals	188	64	124	1137.953

Executive Summary

Malus sieversii, the main progenitor of the domestic apple, collected from Central Asia (Khazakhstan), is an important genetic resource for apple breeders developing new cultivars for the industry. In addition, to being a source of drought, pest and disease resistance, it offers the potential of many new fruit quality traits. As opposed to many more exotic apple genetic resources, elite selections of *M. sieversii* have large, palatable fruit. Breeding apple varieties for novelty and superior quality, meeting grower demands and satisfying consumer requests is a challenging task. Marker-assisted selection (MAS) is a very promising genomic approach to make the breeding process more efficient and precise. This method uses molecular markers linked to the genes of desirable traits to monitor how the desired traits of each parent have combined in individual seedlings. Although marker assisted selection does not replace the need to carefully evaluate mature trees for complex traits like flavor, texture and aroma, it provides the breeder with a tool to identify plants with unfavorable combinations of genes when they are young seedlings. By eliminating the need to grow unfavorable seedlings to maturity before identifying them as "losers", the breeder can maximize their limited resources. Marker assisted selection also allows the breeder to "pyramid" different resistance genes to enhance the durability of resistance in new varieties, a process which is extremely difficult by current apple breeding methods.

Essential to the process of marker-assisted selection is the availability of detailed genetic maps. Small, repetitive DNA sequences in the genome or DNA of organisms called 'simple sequence repeats' (SSRs, aka microsatellites) can be used as markers to create genetic maps. Genes controlling desirable traits are then mapped to a specific location between two markers on the chromosome map. The regions of a chromosome that are associated with a greater or lesser portion of a multi-gene trait are referred to as quantitative trait loci (QTLs) that can likewise be mapped or defined by specific markers (Fig. 2). **To enable QTL analysis for important traits in *M. sieversii*, we have undertaken the construction of a genetic framework map for the family (F1) GMAL4593 ('Royal Gala' X *M. sieversii* PI 631981). One-hundred-ninety progeny were analyzed using Joinmap® 3.0 (Van ooijen and Voorips 2001) to establish a framework map for each individual parent of the F1 population GMAL4593 ('Royal Gala' x *Malus sieversii*).**

Over 100 SSRs and 300 SNP markers have been screened in our mapping population and those that were informative (i.e. had segregating alleles) were mapped to either or both parents. Using this data, we have created a basic genetic framework map for both the *M. sieversii* and 'Royal Gala' parents of the GMAL4593 mapping population. Additionally, the entire population has been evaluated and scored for fire blight resistance. The GMAL4593 population has also been propagated and established as a replicated planting at the USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV for additional fire blight resistance screening. Using the genetic framework map, the collective data on fire blight resistance can be used to determine the genetic location (QTL) of the fire blight resistance in *M. sieversii*.

The genetic framework maps for *M. sieversii* and 'Royal Gala' are incomplete at this stage. Some linkage groups are represented by only a few markers and some linkage groups are represented as subsets. The placement of additional markers on the map in order to enrich the map and join all the subsets of a linkage group into a single linkage group is being pursued. Specifically, several hundred more SNPS are being screened by FEM-IASMA and once the results have been obtained, the additional markers will be added to the parental framework maps. **Identifying markers that segregate in the *M. sieversii* parent has been difficult and many more markers are mapping to the 'Royal Gala' parent. This was not anticipated and the identification of *M. sieversii*-specific SNPS by re-sequencing in order to enrich the *M. sieversii* genetic map and the identification of QTLs for fire blight resistance is the subject of a new proposal submitted to the WTFRC, entitled, "Identifying fire blight resistance in *M. sieversii* for scion breeding."**

FINAL PROJECT REPORT

Project Title: Apple genome project

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Other funding sources

Agency Name: USDA - NRI
Amount awarded: $ 224,000
Notes: Supplemental funding provided by USDA for scaffold sequencing complementing the objectives of this project.

Agency Name: IASMA, Italy
Amount awarded: $ 30,000
Notes: These funds were dedicated towards generating sequence from DH apple at IASMA using the BAC library constructed with funds from USDA-NRI project. The data generated will also help in generating the scaffold.

Total Project Funding: 37,750

Budget History:

Item	**2009**		
Salaries			
Benefits			
Wages	5000		
Benefits	485		
Equipment			
Supplies	30,265		
Travel	2000		
Miscellaneous			
Total	**$37,750**		

Note: Funding was approved for one year enabling us to focus on one objective.

ORIGINAL OBJECTIVE

1. Generate additional genome sequence information to increase the depth of coverage of the apple genome.

SIGNIFICANT FINDINGS

The basic purpose of this project was to ensure efficient and complete assembly of the Double Haploid Apple genome sequence. Prior to the start of this project our program along with the collaborators had acquired sequence data randomly from the DH apple genome. At the same time USDA funding allowed us to construct a library of DNA fragments representing the DH apple genome. We were granted supplemental funding from USDA-NRI program to modify our approach of sequencing the ends of the DNA fragments to establish a new method of scaffold sequencing. The funds provided by USDA and this project was utilized for acquiring sequence information using our novel method. The objective of generating the information is 80% complete. We are currently in the process of refining the computational methods to integrate random and scaffold sequencing data for building a complete assembly of the apple genome. Thereafter this dataset will be compared with the apple genome sequence completed at IASMA with whom we continue to actively collaborate. We have provided the DH Apple genome DNA library to IASMA for generating sequences from the ends of DNA fragments. All this data will aid in our final goal of assembling the DH apple genome. They have committed their own funds to generate this information as part of our ongoing collaboration.

RESULTS & DISCUSSION

Sequence information can be rapidly utilized for developing molecular markers for the apple improvement program. It can also provide complete sequence information for genes where we only have partial information. Over the last year we have utilized the preliminary assemblies for mining such information for various colleagues at WSU. Most importantly this information has been the basis of identifying the complete coordinates and sequence for the putative bitter pit-controlling gene in apple that we identified in another project funded by WTFRC. Knowledge of genes underlying important traits can also serve as targets for improving existing varieties using controlled sports induction (CSI) using non-transgenic approaches. We have a continued emphasis on refining the CSI approach in our program to improve existing varieties thereby circumventing the marketing and retail shelf space issues.

The significance of this information will far outlive the duration of this project. Each economically important trait or desirable quality in the fruit tree is controlled at some level by genes. Availability of the genomic blueprint of apple enables us to pin point what gene or group of genes are responsible for such traits. This information will guide apple improvement year after year from now on. Another testimony to this fact is that scientists have now discovered the gene underlying skin and lung cancer in humans utilizing the human genome information. As in case of humans, the potential economic benefits to the industry are apparent. With the apple genome sequence in hand, we can develop unique varieties for the PNW combining all priority traits that can create unique economic opportunities ranging from production to post-harvest stages.

BROADER IMPACTS

Presentations: The apple genome information has been highlighted at several forums over the last year including WSHA meetings. In 2009, the PI was invited to speak at the Hort Show about Enabling Economic Resilience through Genomics Research. Besides that the work has been shown as poster presentations at annual international meetings like American Society of Plant Biology and Plant and Animal Genome Meeting.

Publications: The data generated from WTFRC, WSU and USDA-supported DH apple genome has been integrated with the sequence information generated at IASMA and the seminal paper describing the results has been provisionally accepted at Nature Genetics.

Research: The apple genome sequencing project has enabled us to now sequence pear and cherry

genomes. We are also a part of the strawberry and peach genome project consortia.
Training opportunities: This project has been steered by graduate student Scott Schaeffer who is independently supported by an NIH fellowship. We have graduated a computer science student Vandhana Krishnan who utilized the apple genome data for her MS thesis. A high school senior utilized the apple genome data for her senior project and has been accepted at MIT for higher studies.

EXECUTIVE SUMMARY

Significant progress: The objective of generating additional sequence information has been accomplished. We have devised a new method of generating far more useful information using a scaffold-sequence approach. At present we continue to refine the computational methods for creating complete and efficient apple genome assembly. It is a reiterative process owing to the computational constraints that involves testing different parameters to arrive at the best possible assembly.

Outcomes and summary of finding: Preliminary DH apple genome assemblies are available that are being used by our program to identify coordinates and sequence information of important genes linked to desirable traits. In summary this is just the start of the most efficient way of connecting traits to genes, an emphasis of our genomics program.

Future directions: We have two proposals under review at NSF and others at various stages of writing to build upon this foundational information. Our programmatic approach is to connect traits with genes using function information and the future projects are aimed at doing just that.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Crop load and canopy management of apple

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State/Province/Zip WA 98801

Cooperators: Jim McFerson, Ines Hanrahan, Felipe Castillo, Tom Auvil - WTFRC

Budget 1:
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: (509) 665-8271 **Email address:** kathy@treefruitresearch.com

Item	2008	2009	**2010**
Salaries	23,230	26,220	25,000
Benefits	6,770	7,650	7,200
Wages	27,150	25,700	24,000
Benefits	12,750	12,100	11,500
Equipment	2,500	3,000	3,000
Supplies	2,500	3,000	3,000
Travel	2,000	2,000	2,000
RCA rental	1,200	4,200	4,200
USDA facilities fee	750	750	750
Total gross costs	76,850	84,620	80,650
Reimbursements	(27,600)	(25,000)	(25,000)
Total net costs	$49,250	$59,620	**$55,650**

Footnotes: RCA rental based on fiscal year billing cycle
Travel includes fuel costs for driving to trial sites
USDA facilities fee covers storage space and use of research packing line

NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

OBJECTIVES:

1) Evaluate pre-bloom, bloom, and post-bloom chemical thinning agents and mechanical thinning technologies with particular focus on complete programs to achieve three goals:
   a) Minimize costs of green fruitlet thinning
   b) Maximize fruit quality
   c) Encourage annual bearing
2) Investigate influence of important variables (drying conditions, spray technology, carrier volume) on chemical thinner efficacy and fruit finish
3) Develop practical PGR programs to manipulate floral initiation and promote annual bearing
4) Evaluate horticultural effects of reflective materials (Extenday, mylar products)
5) Profile natural tree-to-tree variation in long-term cropping patterns in a newly planted apple block
6) Expand collaborative efforts with other research programs

SIGNIFICANT FINDINGS:

Effective chemical thinning programs reduce hand-thinning, improve fruit size and quality, and increase return bloom; bloom thinners generally achieve these goals more consistently than postbloom programs (Tables 3, 5)

Oil (dormant, summer, vegetable, fish) + lime sulfur programs are the most efficacious options for bloom thinning; results with Crocker's Fish Oil are most consistent (Table 3)

Endothall (ThinRite) was as effective as Crocker's Fish Oil + lime sulfur in three 2009 trials and may provide a viable alternative for chemical bloom thinning of apple (Table 2)

Thinning efficacy and fruit finish were not clearly affected by variations in spray technology (AccuTech vs. Proptec vs. airblast), carrier volume (100 vs. 200 gal/acre), or drying conditions (dawn vs. noon vs. evening sprays) of chemical thinning programs (data not shown)

BA + carbaryl thinning programs give results equal or superior to NAA + carbaryl or ethephon + carbaryl programs; BA often shows a positive effect on fruit size (Tables 4, 5)

Crops may be effectively thinned chemically without use of carbaryl; BA + NAA programs demonstrate positive results (Tables 4, 5) with no deleterious effect on fruit quality (data not shown)

Summer applications of NAA have not increased return bloom in WTFRC trials; GA trials to inhibit return bloom show promise for mitigation of biennial bearing (Figure 1)

Factorial field trials indicate that chemical thinning (bloom and postbloom) and PGR (BA or BA+GA) programs are not affected by the presence/absence of Extenday throughout the growing season, although increased fruit set is often observed with Extenday deployment during bloom (data not shown)

Extenday products improve yields of target fruit in apple by:

1) Increasing fruit set without sacrificing fruit size (Tables 6, 7)
2) Increasing fruit size without reducing fruit set (Tables 6, 7)
3) Increasing fruit color (Tables 6-8)

Trees treated with Extenday products over multiple seasons demonstrate increasing capacity to carry high quality fruit (Tables 6, 7)

Mylar products increase apple fruit color, but not as dramatically as Extenday in WTFRC trials (Table 8)

Ongoing collaborative efforts across disciplines, institutions, and regions (Greene, McArtney, Hirst, Yoder, Elfving, Lewis, Toye) increase relevance and impact of research

BACKGROUND:
We have scaled back internal research efforts in chemical thinning to accommodate more collaborative work in other areas, but also in part because of the success of earlier work. Many programs and principles put forward by our research, especially aggressive bloom thinning with lime sulfur, are now firmly established across the Washington industry. We will continue screening new materials and programs for crop load management, but our focus is now increasingly on collaborative projects exploring mechanical thinning techniques (see Lewis/Schupp technology committee project report for more details), the genetic and physiological basis for cropping, and increasing the precision and predictability of crop load management programs through web-accessible developmental models and decision systems.

We continue to evaluate the relative success of chemical and mechanical thinning programs through three measurable targets which are directly tied to a grower's economic bottom line:

1. Reduction of green fruitlet hand-thinning
2. Improved fruit size and quality
3. Increased return bloom/annual bearing

The degrees to which our chemical thinning programs achieve each of these goals are reflected in our data labeled fruitlets/100 floral clusters, harvest fruit size, and percent return bloom, respectively.

Our protocols generally assume two applications of each bloom thinning program, at 20% and 80% full bloom. Likewise, most postbloom thinning programs are applied twice, typically at 5mm and 10mm fruitlet size. Programs in 2009 are reflected in Table 1; in those which show a range of possible rates, higher concentrations are typically reserved for cultivars known to be difficult to thin, such as Fuji and Golden Delicious. In most cases, additional chemical thinning treatments were left to the discretion of individual grower-cooperators, provided that each experimental plot receives the same programs.

Table 1. Chemical thinning programs evaluated. WTFRC 2009.

BLOOM THINNERS
2-4 gal ammonium thiosulfate (ATS)/A
2% Crocker's Fish Oil (CFO) + 2-3% LS
16-32 oz ThinRite/A
16-24 oz ThinRite + Regulaid/A
16-24 oz ThinRite + Bronc/A
0.5% GSL 90 + 1-2% Sulforix
5% NC99
POSTBLOOM THINNERS
48 oz Sevin (carbaryl) + 3 oz NAA/A
48 oz Sevin (carbaryl) + 128 oz BA/A
128 oz BA + 3 oz NAA/A

BLOOM THINNING:
Results from 2009 chemical bloom thinning trials were generally unimpressive, with very few programs showing significant treatment effects. A preliminary evaluation of Sulforix plus a non-

ionic surfactant suggested some potential for reduced fruit set, but fruit marking (Table 2) may preclude this material from providing a viable sulfur alternative to the standard lime sulfur.

We also evaluated endothall (ThinRite) at three locations at several concentrations and in combination with various surfactants; some programs were encouraging and corroborate our results with ThinRite from several years ago. We suspended our work with the material in 2003 because its development was mothballed, but its new owner, United Phosphorus, is now aggressively seeking to register ThinRite as a blossom thinner of apple. Endothall is currently registered as an aquatic herbicide for use in irrigation canals and already has established tolerances on several tree fruit species, including apple, which should facilitate its registration as a chemical thinner. We plan to further test the material in 2010, as well as a novel postbloom material which has shown promise in European trials, and a black food dye which "conceals" flowers from bees and depresses photosynthesis during bloom.

Table 2. Crop load effects of bloom thinning programs. Gala/M.9, Manson, WA. WTFRC 2009.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		%	%	g		%
4 gal ATS	60 ns	51 ns	39 ns	175 a	109	62 abc
2 gal ATS	58	52	39	161 ab	118	54 abc
2% CFO + 2% LS	52	58	34	170 ab	112	69 abc
0.5% GSL 90 + 2% Sulforix	49	59	33	166 ab	115	72 ab
0.5% GSL 90 + 1% Sulforix	42	63	30	166 ab	115	59 abc
5% NC99	49	58	35	161 ab	118	76 a
16 oz ThinRite 1x	63	49	41	154 b	124	46 bc
16 oz ThinRite 2x	56	58	31	165 ab	116	59 abc
24 oz ThinRite 1x	55	56	34	165 ab	116	59 abc
Control	56	55	35	159 ab	120	45 c

Even though we have reduced our work in bloom thinning, we continue to corroborate prior results of ATS and oil + lime sulfur programs in the context of other experiments. No thinning program we have evaluated to date outperforms oil + lime sulfur combinations. Table 3 summarizes results from all apple bloom thinning trials conducted by the WTFRC since 1999, reflecting a very conservative standard by which to assess our most frequently studied programs.

Table 3. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials WTFRC 1999-2009.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	15 / 57 (26%)	10 / 60 (17%)	4 / 51 (8%)
NC99	15 / 31 (48%)	7 / 33 (21%)	2 / 27 (7%)
Lime sulfur	25 / 54 (46%)	12 / 48 (25%)	9 / 47 (19%)
CFO + LS	59 / 103 (57%)	26 / 94 (28%)	21 / 91 (23%)
JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	14 / 27 (52%)	4 / 26 (15%)	4 / 26 (15%)
VOE	13 / 29 (45%)	4 / 28 (14%)	2 / 30 (7%)

[1]Does not include data from 2009 trials.

[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

POSTBLOOM THINNING:
Results from 2009 grower-applied trials are consistent with prior outcomes which demonstrate that 1) tank mixes of carbaryl and BA outperformed tank mixes of carbaryl and NAA (Tables 4, 5) and 2) BA + NAA programs are equal or superior to any standard postbloom thinning programs utilizing carbaryl (Tables 4, 5). Perhaps most striking about Table 5 is the overall dearth of significant effects from any postbloom chemical thinning program; when compared to the general success rates of bloom chemical thinners (Table 3), it becomes all the more clear that early, aggressive thinning is critical to effective crop load management.

Table 4. Crop load effects of postbloom thinning programs (grower applied). Gala/M.9, Grandview, WA. WTFRC 2009.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		%	%	g		%
BA + NAA	126 b	38 a	23 ns	202 ns	94	9 ns
Carbaryl + BA	104 c	45 a	23	199	96	14
Carbaryl + NAA	122 bc	39 a	21	204	93	4
Control	164 a	26 b	21	191	100	5

Table 5. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials WTFRC 2002-2009.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	2 / 18 (11%)	0 / 19 (0%)	0 / 18 (0%)
Carb + BA	28 / 75 (37%)	9 / 73 (12%)	8 / 71 (11%)
Carb + NAA	11 / 51 (22%)	7 / 51 (14%)	4 / 48 (8%)
BA + NAA	5 / 11 (45%)	2 / 11 (18%)	0 / 9 (0%)
Carb + NAA + Ethephon	0 / 5	0 / 5	2 / 5
Carb + NAA + BA	0 / 8	0 / 8	3 / 8

[1]Does not include data from 2009 trials.
[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

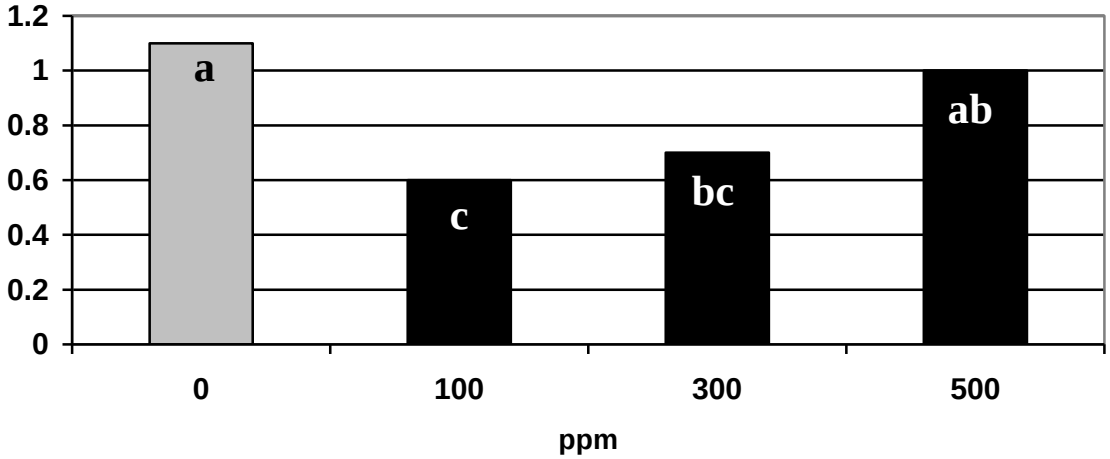
At this stage, we are confident that BA + NAA programs can provide satisfactory, if not superior, alternatives to postbloom thinning programs which rely on carbaryl. We have not observed any pygmy fruit in any of our 11 trials evaluating that combination, nor any other harmful effects to fruit quality; language on product labels warning against tank mixing of BA and NAA products is likely an artifact of historic concerns of NAA causing pygmy fruit in isolated cases and has little relevance to combining the two chemistries.

RETURN BLOOM PROGRAMS:
We have discontinued our trials with summer applications of NAA and ethephon to promote return bloom; evaluation of three 2008 trials in the spring of 2009 affirmed the poor results seen in more than 20 trials over 5 years. After testing programs successful in East Coast trials, programs employed by prominent Washington growers in their own orchards, and numerous rates, timings, and combinations of materials, we are forced to conclude that summer NAA and ethephon programs should not be relied upon to increase flowering under our conditions.

Although tree-to-tree variability continues to confound our results, we are increasingly optimistic that GA_3 has the potential to provide cost-effective relief for biennial bearing by suppressing flowering in

the "on" year of an alternate cycle. Interestingly, our results often indicate that higher concentrations do not amplify treatment effects (Figure 1); 2010 trials will explore programs utilizing multiple applications of low concentrations.

Figure 1. 2009 return bloom effects (flower clusters/cm^2 TCSA) of 10mm applications of GA_3. Fuji/M.26, Orondo, WA. WTFRC 2008.



Since 2005, we have conducted approximately 25 trials evaluating reflective materials in commercial Washington apple orchards. Products tested have included the woven plastic fabrics Extenday, Daybright, and Daywhite, all distributed by Extenday USA, as well as Brite N'Up, a Mylar-based material. The Extenday products are designed for use throughout the growing season and may be reused for 6-8 years with good maintenance, while Mylar products cannot be reused and are generally only deployed 2-3 weeks before harvest.

Each material we tested is designed to reflect sunlight striking the orchard floor back up into plant canopies. Increased light saturation as harvest approaches can increase red color development, while increased light saturation throughout the growing season is associated with enhanced carbon fixation (photosynthesis), cell division, and cell expansion. While all products tested improved apple fruit color when deployed shortly before harvest (Table 8), Extenday products have also consistently increased fruit set and/or fruit size in WTFRC apple (Tables 6, 7), pear, cherry, peach, and nectarine trials. Because these materials specifically promote the production of high yields of large, well-colored, high quality fruit, they have tremendous potential to significantly improve grower returns.

Table 6 reflects three years of results from a Red Delicious block treated with Extenday from bloom until harvest. In each year, yields were significantly higher in the treated blocks due to increased fruit set and fruit set. Note that the gap in production was maintained in the off year of a biennial cycle (2008) and widened in the on year (2009). Fruit color development was superior in treated trees in 2008 and 2009, despite carrying higher crop loads.

Table 6. Full-season reflective material effects on fruit yield and color. Red Delicious/M.26, Royal Slope, WA. WTFRC 2007-2009.

	YIELD			COLOR GRADE	
	Fruit set	**Fruit wt.**	**Yield**	**WAXF**	**WAF**
	(#/tree)	(g)	(kg/tree)	(%)	(%)
2007					
Extenday	496 ns	206 a	98 a	60 ns	37 ns
Control	469	182 b	86 b	59	40
2008					
Extenday	202 ns	219 a	39 a	79 a	20 b
Control	198	187 b	35 b	67 b	32 a
2009					
Extenday	510 a	193 a	99 a	31 a	43 ns
Control	442 b	174 b	71 b	14 b	67

Increased yield differentiations in second and third years of a trial are not unique; we have frequently observed a cumulative increase in yields over the course of multiple year studies. Table 7 summarizes the average effects of Extenday in each season of every full-season apple trial we have conducted since 2005. While modest yield gains are typical in the first year of trials, the effects are more dramatic in subsequent seasons, likely due to increased carbohydrate reserves and renewed fruiting wood, especially in lower, shaded portions of tree canopies.

Table 7. Mean cumulative yield effects relative to untreated controls of full-season multiyear use of Extenday in all WTFRC apple trials. 2005-2009.

Trial age	*n*	Fruit set (harvested fruit/tree)	Individual fruit size (g)	Total yield (kg/tree)
1st year	12	+ 9%	+ 6%	+ 15%
2nd year	7	+ 24%	+ 2%	+ 26%
3rd year	4	+ 17%	+ 8%	+ 23%

Reflective materials deployed late in the growing season have little effect on apple fruit set or size, but can improve fruit color in red or partially red cultivars. Table 8 shows effects of Extenday and Brite N'Up on Gala fruit color; both materials were deployed at the same timings using equal material widths. While the mylar product improved color, Extenday was more effective in both seasons.

Table 8. Effects of reflective materials deployed 4 weeks prior to harvest on harvest sequence and fruit color. Gala/M.9, Othello, WA. WTFRC 2007-2009.

	TOTAL YIELD HARVESTED				COMMERCIAL COLOR GRADE		
	1st pick	**2nd pick**	**3rd pick**	**4th pick**	**WAXF**	**WAF**	**US#1**
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
2007							
Extenday	39 a	40 ns	19 b	2 b	92	7	1
Brite N' Up	21 b	42	30 a	7 a	82	17	1
Control	16 b	40	35 a	8 a	78	21	1
2008							
Extenday	32 a	59 ns	9 b	na	99	1	0
Brite N' Up	19 b	63	19 b		96	4	0
Control	14 b	56	30 a		95	5	0
2009							
Extenday	68 a	26 b	6 b	na	87	12	1
Brite N' Up	38 b	40 a	22 a		65	30	6
Control	24 c	47 a	29 a		49	46	5

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-09-908

Project Title: Modeling Washington apple bloom phenology and fruit growth

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Cooperators: Tim Smith (WSU Extension, Chelan County), Gwen Hoheisel (WSU Extension, Prosser), Norman Suverly (WSU Extension, Okanogan), Tom Auvil (WTFRC), Ines Hanrahan (WTFRC), Felipe Castillo (WTFRC), Mark Bell (WTFRC), Matt Whiting (WSU-IAREC), Todd Einhorn (OSU-MCAREC), Vince Jones (WSU-TFREC)

Total project funding request: **Year 1:** $4,180 **Year 2:** $7,938 **Year 3:** $8,090

Other funding Sources: None

WTFRC Collaborative expenses:

Item	2009	2010	2011
Stemilt RCA room rental			
Crew labor[1]	5,000	5,000	5,000
Shipping			
Supplies			
Travel[2]	1,800	1,800	1,800
Miscellaneous			
Total	$6,800	**$6,800**	$6,800

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

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

Item	2009	**2010**	2011
Salaries[1]		2,941	3,059
Benefits		847	881
Wages[2]	1,000	1,000	1,000
Benefits	180	150	150
Equipment			
Supplies			
Travel[3]	3,000	3,000	3,000
Total	$4,180	**$7,938**	$8,090

[1] Salary (benefits at 28.8%) for Nairanjana Dasgupta
[2] Wages (benefits at 15%) for part-time help in Wenatchee for bloom observations.
[3]Cooperator in-state travel for bloom observations (5 persons at $600 each)

Objectives:

1. Develop functional models for apple bloom development from bud break to petal fall for three cultivars: 'Red Delicious' (standard with historic data), 'Cripps Pink' (early bloomer), and 'Gala' (mid-late bloomer).
2. Develop fruit growth models for the same three cultivars from petal fall until harvest.
3. Incorporate models into WSU DAS system.

Significant Developments:

- Co-PI Jim Olmstead left his position with WSU Extension; Karen Lewis will administer WSU budgets and Tory Schmidt will assume most other project leadership responsibilities

- Bloom phenology observations successfully recorded at 11 location nodes throughout Central Washington, including 11 Red Delicious, 11 Gala, and 9 Cripps Pink blocks (Table 1)

- Digital photographs taken at most sites reflecting phenologic development from bud break to 20mm fruitlet size

- Fruit growth measured throughout growing season at 9 Red Delicious, 9 Gala, and 7 Cripps Pink blocks; fruit diameter recorded at all sites, as well as fruit length for Red Delicious blocks (Table 1)

- Data and photographs successfully collated for analysis

- Autonomous digital cameras field tested during summer as possible alternative to routine human observations; cameras will be deployed throughout the spring bloom season at two sites to redundantly record bloom phenology and assess their viability for future use (Figures 1, 2, 3)

- WSU statistician (Dasgupta) was successfully recruited to develop models and advise project protocol

Methods:

Bloom phenology: Team members from WSU Extension and WTFRC internal program observed and evaluated flagged apple blocks around the state (Table 1) at 2-5 day intervals from bud break until mean fruitlet size reached 20mm. Twenty buds/clusters at chest level on the northwest side of trees of each cultivar were categorized by phenologic stage and digital pictures were taken of representative buds/flower/fruitlets. Data were recorded on a tally sheet by each individual and eventually submitted to the WTFRC internal program for collation. Hobo data loggers were deployed at each site to record ambient temperatures throughout the season.

Fruit growth: After June drop and hand-thinning, 100 surviving fruit were tagged in the same blocks used for the bloom phenology observations (20 fruit in each of 5 trees). All fruit were measured by WTFRC staff for diameter and Red Delicious was additionally measured for length as an indicator of fruit type at regular intervals (10-20 days) until the blocks were harvested in the fall.

Table 1. Roster of sites utilized for apple bloom phenology observations and fruit growth measurements. 2009. (RD = Red Delicious, CP = Cripps Pink, G = Gala)

LOCATION	GROWER	CVs	ELEV (ft)	STAFF	FRUIT GROWTH
Omak	Root	RD, G	1250	Suverly	Y
S Shore Chelan	Easley	CP	1120	Auvil	Y
	Sunshine	RD, G	1450	Auvil	Y
Brays Landing	Podlich	RD, CP, G	900	Auvil	Y
S Orondo	C & O Nursery	RD, CP, G	755	Bell	Y
E Wenatchee	Gausman	RD, CP	910	Esteban	Y
	Witte	G	1025	Esteban	Y
Rock Island	WSU-TFREC	RD	910	Bell	N
	WSU-TFREC	G	880	Bell	N
	Zirkle CRO	CP	775	Bell	Y
Royal Slope	Delay	CP	1095	Lewis	Y
	Delay	RD, G	1055	Lewis	Y
Naches	Rowe	RD, G	1580	Hanrahan	Y
Parker	Brandt	RD, CP, G	879	Olmstead	RD & G only
Sawyer	WTFRC Rootstock	G	870	Hanrahan	Y
	Badgely	RD	870	Hanrahan	Y
	Weippert	CP	870	Hanrahan	Y
Prosser	Ballard	RD, CP, G	681	Hoheisel	N

Results & Discussion:

Project cooperators successfully made observations at their assigned sites (Table 1) at appropriate intervals in 2009 with minimal gaps in the data. Photos taken in the field have been collated and will be used to develop an improved form for field observations in 2010 and 2011; these images will also be available for use in presentations, extension publications, etc. After June drop, WTFRC took over routine fruit growth measurements at all phenology sites except in Prosser (too far to travel), Parker Red Delicious, and WSU-Sunrise, where fruitlets thinned off all trees to prevent cropping in their 3rd leaf.

The greatest expense and ongoing challenge of this study remains the considerable time commitment required by several cooperators during a busy time of year. As such, we are interested in investigating automated technologies which can reduce the human inputs. After consulting Carnegie Mellon engineers involved in the Comprehensive Automation for Specialty Crops project, we purchased 2 autonomous game trail cameras (Figures 1, 2) used by hunters to photograph big game in

the wild. Initial field testing of the cameras produced time-stamped images of reasonable quality taken at hourly intervals (Figure 3); we plan to deploy at least two of these cameras in March for use at an established sample site to capture images which could eventually be evaluated in the lab to determine phenologic stages throughout the spring.

Hobo data loggers were deployed at all nearly all sites to record ambient temperatures in the immediate microclimate of the sampled trees; most sites were selected due to their proximity to AWN stations (usually within a mile), and models using temperatures from both systems will be evaluated for the best statistical fit. Potential discrepancies between temperatures recorded by AWN and individual data loggers could have many explanations, but may be instructive regarding broad extrapolation of readings from either system.

Efforts to attract collaborators with statistical and/or modeling expertise within the tree fruit research/extension community proved unfruitful. Amit Dhingra (WSU), recommended we contact Nairanjana Dasgupta, a professor of statistics at WSU-Pullman. Dr. Dasgupta believes the demands of the project to be beyond the skill level of most students, and has agreed to join the project as its statistician. She will provide consultation for data management, sampling design, and construction of the bloom phenology and fruit growth models for Red Delicious, Gala, and Cripps Pink for the amount originally set in the project budget ($3000/yr for 2010 and 2011).

Figure 1. Stealth Prowler DVS game camera tested for automated field observations.

Figure 2. Moultrie D 40 game camera tested for automated field observations.

Figure 3. Sample photo taken by Stealth Prowler DVS camera. Note optimal focal range is beyond 3 feet (factory set), which may be too far away to observe fine detail in bloom development.

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

Project Title: Identifying causes of variability in fruit quality

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Cooperators: Craig and Mike O'Brien, Olsen Brothers, Hansen Orchards, Qin Zhang, Yaxin Yu

Total project funding request: **Year 1**: 49,135 **Year 2**: 50,291

Other funding Sources

Agency name: Emerging Research Issues, WSU-ARC
Amt. awarded: $64,756 awarded
Notes: This funding was used to purchase laser scanning equipment, computer, and software necessary for the methods proposed in the current proposal. Additionally, the ERI grant will cover a Ph.D. student for one year.

WTFRC Collaborative expenses: None

Budget 1
Organization: WSU-TFREC **Contract Administrator:** ML Bricker
Telephone: 509-335-7667, 509-663-8181 x221 **Email:** mdesros@wsu.edu

Item	2009	2010	
Salaries	26,952	28,030	
Benefits	1,955	2,033	
Wages	7,500	7,500	
Benefits	728	728	
Equipment			
Supplies	2,000	2,000	
Travel	10,000	10,000	
Miscellaneous			
Total	$49,135	**$50,291**	

Footnotes: Salaries for Ph.D. student for two years (third year covered by ERI grant); wages for timeslip assistance; supplies for consumables in lab analyses

OBJECTIVES

1. Document role of fruit developmental history at key stages (i.e., flowering, thinning, fruit maturation) and management interventions in fruit quality
2. Map fruit quality and position within high resolution 3D digital canopies
3. Develop practical strategies for reducing variability and maximizing genetic potential.

SIGNIFICANT FINDINGS:

- Variability in Gala apple fruit quality attributes are largely associated with bloom timing
- Early blooms in Gala and Fuji have greater size potential than later flowers
- In high density orchard systems, fruit position does not affect fruit quality
- We have created 3D virtual trees, mapping fruit quality attributes within canopies
- In Gala there was no difference in fruit quality potential between king and side blooms
- Crop load (fruit number per tree) is a key control of fruit quality
- Gala 'Buckeye' studied are able to grow to maturity with aborted seeds and have comparable fruit quality to fruit with seeds.
- This work has led to our playing a key role in a new SCRI proposal (ca. $760,000 requested for WSU portion)

METHODS

Approach to objectives 1 & 2. Our methodology for documenting the role of key stages in a fruit's development on its quality is straightforward yet painstaking. We propose to select three contiguous, representative trees from modern Fuji and Gala orchards trained as slender spindles or fruiting walls. At budbreak, we will tag flower clusters and individual flowers within clusters, labeling each with the day of flowering (determined as the day that pollinators can access the flower). Detailed records of the composition of each numbered cluster (i.e., number of flowers and timing of flowering of each flower) will be recorded. In addition, digital images of each cluster will be collected at regular intervals throughout anthesis and included in the cluster's database. Cluster composition will be recorded post-anthesis at weekly intervals. Standard crop load management practices will be followed in each orchard. These will include bloom and post-bloom thinning (chemical and hand). Our data will therefore inform thinning efficacy on a cluster basis. At the point of hand thinning, equatorial diameter of each remaining fruit will be recorded.

Each tree will be laser-scanned prior to budbreak to create a 3D digital structure with which we can study positional effects on bloom and fruit quality. This will be accomplished using a Topcon total station purchased with funding from a grant from WSU Emerging Research Issues program (Whiting and Kise, PIs). We have used this approach successfully in 2008 to create fruit quality maps superimposed upon actual 3D tree structure. Digital canopies are created currently using 3D CAD software, an off-the-shelf package that is not well-suited to the current application. We intend to collaborate in 2009 with research associates working in newly-hired senior automation engineer Dr. Qin Zhang to investigate the potential to develop novel 3D software.

We propose to work with our grower cooperators for coordinating commercial harvest of each tree. We will utilize the total station again the day before commercial harvest to identify the position of each fruit. Each fruit will be identified by its cluster, harvested, and evaluated for quality parameters (e.g., weight, diameter, soluble solids, firmness, and skin disorders including sunburn) in the quality lab in Prosser. In addition, at harvest the woody structure of each tree will again be laser-scanned to account for positional shifts during the growing season. Fruit quality data will be then be mapped within the virtual canopy to assess relationships between 3D canopy position and fruit quality

parameters. We will have a database for each tree on cluster composition, timing of flowering, and fruit quality. Each harvested fruit will have the following key data:

1. time of flowering
2. hierarchy of flower (i.e., king, secondary, etc.)
3. dynamic cluster composition (i.e., how many other flowers in cluster, how many fruitlets/fruit, when the cluster changed, etc.)
4. fruit diameter at hand thinning
5. 3D position in relation to other fruit and shoots
6. full fruit quality assessment (e.g., weight, diameter, soluble solids, firmness, etc.)

We will use the same trees in both years to gain two consecutive years of data.

King and Side Bloom Assessment. In a separate experiment, entire 'Buckeye' Gala trees were bloom thinned at various stages of bloom to leave one flower in each cluster: either a random accessory bloom or the king bloom. Hand thinning of flowers occurred on 17 April, 21 April, 27 April, 5 May, 13 May, or 26 May with three trees per treatment on each date. At harvest, each tree was harvested in three sections i.e. lower 0-1.2 m, middle 1.2-2.4 m and upper 2.4-3.6 m and collected in labeled lugs. The fruit were counted and weighed as total mass per tree in the field then sent to the fruit quality lab at WSU Prosser where a subsample of 15 fruit were randomly selected for the same quality checks as in the floral timing experiment. The same cultural practices were applied to the bloom assessment trial as the floral timing trial except that these trees were not hand thinned.

Approach to objective 3. Using data collected in the first season in Washington, we intend to adopt strategic thinning strategies in an effort to reduce variability and improve fruit quality. The concept is illustrated in Figure 1. By understanding better the role of a fruit's position and developmental environment from bloom we will work with growers to develop precision management strategies (which will focus on crop load management at this stage). In the second year we will impose differing strategies and compare their effects on fruit yield and quality.

RESULTS AND DISCUSSION:

From labeling every flower for its date of opening we were able to evaluate the relationship between date of anthesis and fruit quality at harvest. In the first year of this research project, we studied this relationship for Gala ('Buckeye') and Fuji ('Aztec'). In Gala, flowers opened between the 21st of April and the 16th of May. In total, ca. 2100 flowers were tagged from three contiguous trees. Forty five percent of the flowers opened in the first 9 days. Progression of bloom has been modeled with growing degree hours (data not shown). By 10 days from the first bloom, 60% of the floral buds were open and these flowers accounted for 92% of the fruit retained for harvest.

Thinning treatments with lime sulfur and carbaryl began 1 May, and by 21 June, 83% of the blooms/fruitlets had abscised (Fig. 1). Hand thinning on 25 June removed another 8% leaving only 9% of the total possible fruitlets to mature. The efficacy of the two thinning treatments is questionable since we did not record notable fruit drop in the weeks following application (Fig. 1).

Fruit quality analyses of Gala revealed the role of date of anthesis on fruit quality (if a single harvest approach is implemented). Early blooms have larger distribution in fruit quality parameters weight, diameter, and height. At harvest on 27 August, least significant difference analyses identify clear separation of the means ($P \leq 0.05$) by date in fruit weight, height, and diameter (Table 1). When a comparison is calculated as percentage, fruit weight exhibited a 42% decrease, diameter is 17% less, and height losses 22% comparing fruit quality from the earliest and latest flowers 22 April and 12 May, respectively.

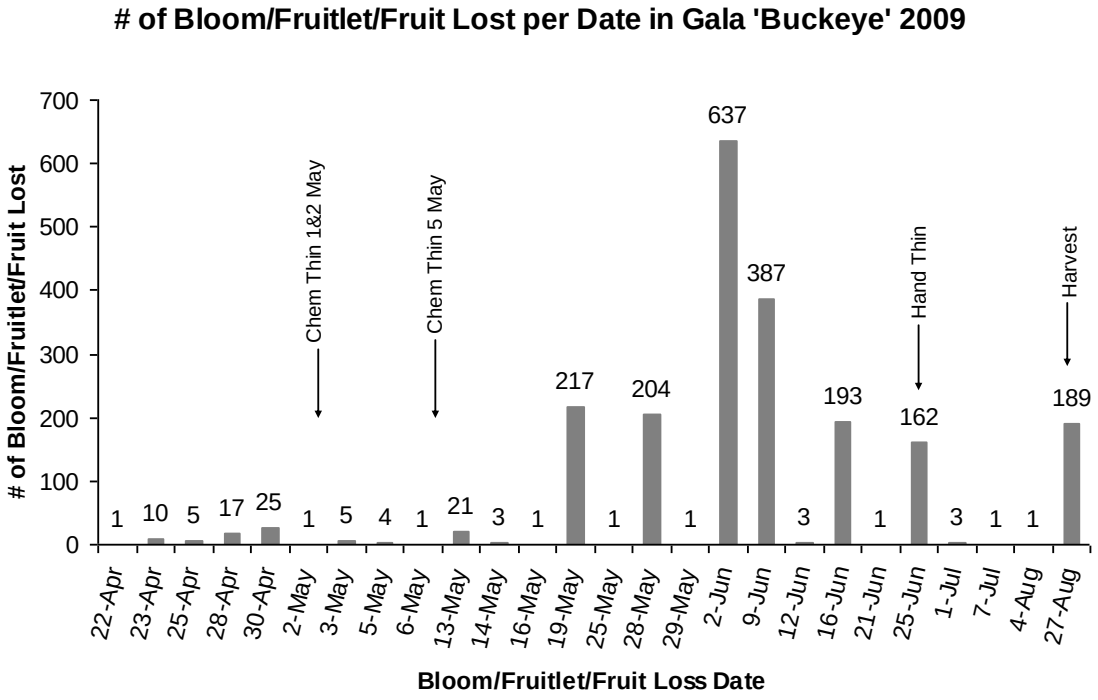

Figure. 1 Seasonal loss of flowers/fruit from 3 replicate 'Buckeye' Gala trees subjected to standard cultural practices. Chemical thinning commenced on 1 May and hand-thinning occurred on 25 June. Harvest was performed on 27 August. Note – x axis is not to scale.

Table 1. Comparisons of fruit quality by bloom date in 'Buckeye' Gala by least significant difference ($P \leq 0.05$)

Bloom Date	% Red (Closest 10%)		Weight g		Diameter mm		Height mm		Firmness lb		Starch Rating 1-6		Brix	
22-Apr	85.56	ab	245.3	a	80.14	ab	80.59	a	16.57	bc	3.8	abcd	11.68	b
23-Apr	88.89	a	242	ab	78.9	ab	79.61	a	16.11	c	5	a	12.12	ab
24-Apr	85.33	abc	234.2	ab	77.7	ab	79.98	a	16.81	bc	3.9	abc	12.17	ab
25-Apr	84.44	abc	228.3	ab	78.4	ab	79.39	a	17.4	bc	3.8	abcd	12.12	ab
28-Apr	87.18	ab	236	ab	78.8	ab	79.08	a	17.71	abc	3.8	abcd	11.86	ab
30-Apr	85.71	ab	230.4	ab	78.7	ab	77.86	ab	17.12	bc	3.4	bcde	11.87	ab
1-May	76.67	bc	242.1	ab	79.5	ab	79.7	a	17.6	abc	4.7	ab	12.2	ab
2-May	90	a	159.9	d	71	cd	67.5	c	18.6	ab	5	a	11.6	b
5-May	80	abc	177.1	cd	73.4	bc	69.4	c	18	abc	3	cde	11.5	b
6-May	85	abc	192.1	bcd	75.2	abc	69.87	bc	19.83	a	2.5	cde	12.7	ab
8-May	75	c	169.2	d	71.1	cd	69.35	c	16.95	bc	2	e	13.15	a
12-May	86	ab	142	d	66.7	d	63.16	c	18.7	ab	2.4	de	11.78	ab
lsd	10.4		52.6		6.37		8.04		2.31		1.49		1.39	

In Fuji, bloom started on 22 April and progressed until by 8 May (476 GDD). Similar to Gala, Fuji had the largest number of blooms on the 8th day from first bloom (29 April). By 8 days from first bloom 35% of the flowers had opened, and these fruit from these flowers accounted for 62% of the fruit harvested. Chemical thinning started on 8 May with a mixture of carbaryl, alkali monovalent

metal salt, and ethephon and by 7 July, 68% of the bloom/fruitlets had abscised. Hand thinning removed another 20% leaving 9% of the total blooms for harvest (Fig. 2).

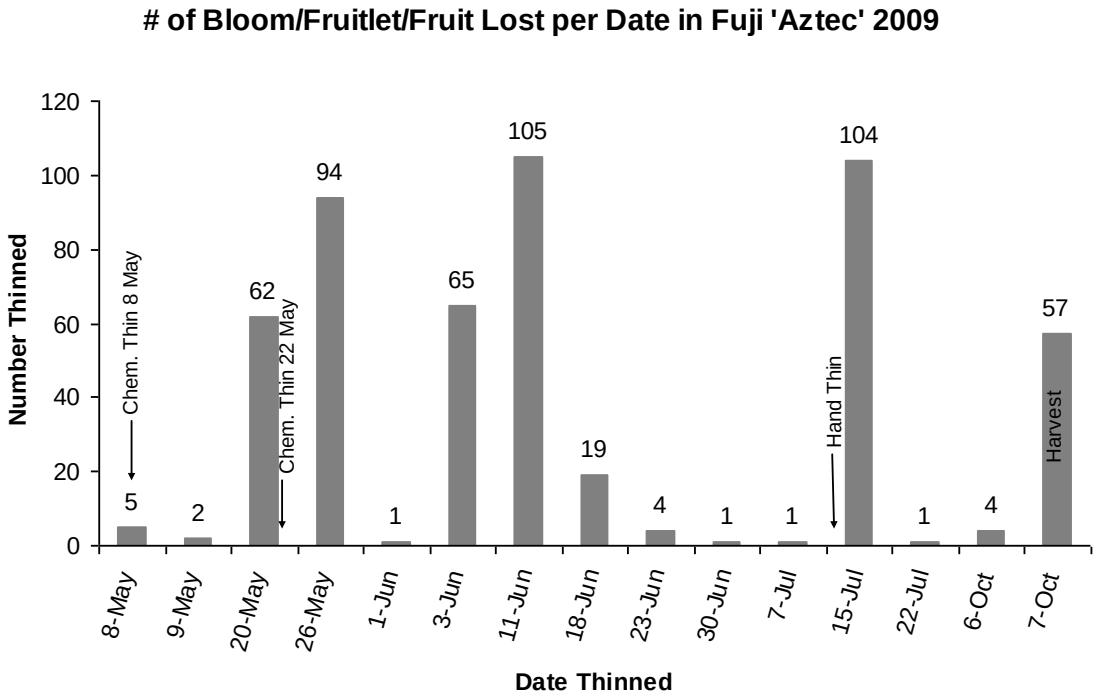

Figure 2. Mean seasonal loss of flowers/fruit from 3 replicate 'Aztec' Fuji trees subjected to standard cultural practices. Chemical thinning commenced on 8 May and hand-thinning occurred on 15 July. Harvest was performed on 7 October. Note – x axis is not to scale.

Table 2. Comparisons of fruit quality by bloom date in 'Aztec' Fuji using least significant difference ($P \leq 0.05$)

Bloom Date	% Red (Closest 10%)		Weight g		Diameter mm		Height mm		Firmness lb		Starch Rating 1-6		Brix	
25-Apr	100	a	357	ab	91	a	82	ab	13.2	a	1.7	ab	15.1	ab
29-Apr	96	a	284	abc	85	ab	75	ab	15.8	a	3.1	ab	15.2	ab
30-Apr	100	a	367	a	91	a	86	a	13.4	a	4	a	16.5	ab
1-May	100	a	283	abc	86	ab	77	ab	14.9	a	2.2	ab	17.2	a
2-May	100	a	271	abc	85	ab	77	ab	13.5	a	2	ab	16.3	ab
3-May	100	a	241	bc	80	ab	74	b	14.3	a	2.7	ab	14.4	b
7-May	100	a	251	abc	81	ab	73	bc	15.7	a	1.3	b	16.9	ab
8-May	100	a	196	c	76	b	62	c	16	a	2	ab	15.8	ab
lsd	8.7		121		14		11		3.1		2.4		2.6	

Fruit quality analyses of Fuji also showed that if a single harvest approach is implemented, fruit from early blooms are larger and heavier (Table 2). Analyses by least significant difference identify clear separation of the means ($P \leq 0.05$) by date in fruit weight, height, and diameter (Table 2). When a comparison is calculated as percentage, fruit weight declines by 45%, fruit diameter is 16% less, and height is 24% less comparing fruit from blooms open on 22 April vs. 8 May. For Fuji there was a

less obvious relationship between flowering date and fruit size, compared with Gala. Only the latest opening blooms were consistently significantly smaller than earlier blooms. Interestingly, fruit firmness, soluble solids, and maturity do not seem to be related to bloom date (Table 2). This suggests that, if left on the tree, the undersized fruit from late blooms would not achieve similar size as the fruit from early blooms.

King and Side Bloom Comparison. Gala floral buds/blooms/fruitlets were pinched back to either one king bloom or side bloom to assess fruit quality at different stages in floral development. Statistical comparisons of king, side, and control (fruit from the timing of flowering experiment above) show that quality of fruit from king blooms is not any better than fruit from side blooms considering fruit quality characteristics such as weight, diameter, and height (Table 3). Trees in this study that had similar crop loads (kg/cm^2) to the floral timing trees, fruit quality was not significantly different. However, trees with crop loads above 6 fruit/cm^2 began to show greater variability within the treatment (Table 4). Our trees in this study were not hand-thinned to adjust crop load. To truly identify fruit superiority the whole crop load adjustment regime should have been applied through the treatments. However, our data underscore the importance of crop load management for growing superior quality fruit.

Table 3. Comparisons of fruit quality of 'Buckeye' Gala trees thinned to one flower per cluster (king, side) with control (hand thinned) by least significant difference ($P \leq 0.05$)

Treatment	% Red (Closest 10%)		Weight g		Diameter mm		Height mm		Firmness lb		Water Core Rating 1-5		Starch Rating 1-6		Brix	
Control	86.057	a	229.86	a	78.13	a	78.37	a	17.47	a	1	a	3.77	a	11.96	a
King	54.701	c	203.42	b	74.13	b	74.96	c	17.28	a	1	a	2.92	c	10.68	b
Side	62.704	b	229.39	a	77.92	a	76.44	b	17.47	a	1	a	3.23	b	10.62	b
lsd	3.105		7.67		1.16		1.23		0.39		0.02		0.22		0.17	
r x r	0.35		0.08		0.08		0.04		0.002		0.002		0.07		0.28	

Table 4. Comparisons of mean fruit yield and quality from trees thinned to only king or side bloom at various timings. All comparisons are calculated as least significant difference ($P \leq 0.05$).

Treatment	# of Fruit		Yield (kg)		Fruit Weight (g)		TCSA (cm*cm)		Fruit/cm2
4/17/ King	107	ab	23.3	a	216	a-c	16.4	a-d	6.5
4/17/ Side	87	bc	18.5	b-c	211.2	bc	13.5	cd	6.4
4/21/ King	93	a-c	18.6	a-d	200.8	bc	15.5	b-d	6.0
4/21/ Side	87	bc	18.6	a-d	214.1	a-c	11.9	d	7.3
4/27/ King	97	a-c	19.7	a-c	204.13	bc	16.3	a-d	6.0
4/27/ Side	60	d	14.8	de	246.6	a	22.9	ab	2.6
5/5/ King	91	a-c	20.1	a-c	226.5	ab	21.5	a-c	4.2
5/5/ Side	52	d	12.5	e	244.6	a	18.3	a-d	2.8
5/13/ King	109	ab	21.4	a-c	196.7	bc	17.6	a-d	6.2
5/13/ Side	97	a-c	21.7	ab	223.3	ab	23.9	a	4.1
5/26/ King	110	a	20.2	a-c	183.5	c	12.3	d	8.9
5/26/ Side	84	c	16.8	c-e	199.1	bc	20.9	a-c	4.0
lsd	21.7		4.6		33.3		8.13		
r x r	0.73		0.62		0.56		0.49		

Figure 3. 3D model of 3rd leaf Fuji tree recreated from points collected with total station laser scanner. Woody structure at full canopy (left) including fruit (right). The size of the virtual fruit in the image is proportional to actual fruit size at harvest. The color of the fruit is proportional to the actual color at harvest (percent red). Note – tree is not viewed from same perspective in both pictures.

CONTINUING PROJECT REPORT **YEAR**: 1 OF 3
WTFRC Project Number: AP-09-912

Project Title: Development of pollen tube growth model for Washington State growers

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Co-PI(2): Dr. Rongcai Yuan
Organization: Va. Tech
Telephone/email: (540)-869-2560
Address: 595 Laurel Grove Rd.
Address 2: Va. Tech AHS-AREC
City: Winchester
State/Province/Zip: VA 22602

Cooperators: Leon Combs, Research Specialist, Va. Tech AHS-AREC; Winchester, VA; e-mail: lecombs@vt.edu
Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA
Dr. Vincent P. Jones, Washington State University Tree Fruit Research and Extension Center

Total Project Request: Year 1: $39,868 **Year 2:** $45,904 **Year 3:** $47,729

Other funding sources: None

WTFRC Collaborative expenses:

Item	2009	2010	2011
Travel		200	
Salary		2000	
Benefits		600	
Total		**$2800**	

Footnotes:

Budget 1

Organization Name:
Virginia Polytechnic Institute and State University (Va. Tech)
Telephone: (540)-231-2068

Contract Administrator:
Sharron McElroy, (Grant Administrator)
Email address: mcelroys@vt.edu

Item	2009	2010	2011
Salaries*	23,900	27,980	29,213
Benefits	11,467	13,424	14,016
Supplies	750	750	750
Travel (to Wash. St. orchard sites)	3000	3000	3000
Miscellaneous	0	0	0
Contractual services & repairs	750	750	750
Total	$39,867	**$45,904**	$47,729

***Note:** Salary for Research Specialist Leon Combs.

OBJECTIVES:

Our overall goal for 2009-11 is to collaborate with the Washington Tree Fruit Research Commission and Washington State University Tree Fruit Research and Extension Center in the development of a computer generated pollen tube growth modeling program.

The specific objectives are:

1) Validate our prior work on pollen tube growth and thinning by conducting field studies at selected cooperating orchard sites in Washington State. Assist, if needed, with field implementation of beta testing of the modeling program with cooperating growers.

2) Repeat Washington State "in-orchard" tests conducted in 2008 to accumulate multiple-year data on commercially important cultivars. Try to collect more "normal year" data (as compared to 2008) on Red Delicious, Golden Delicious, Fuji, Gala, Honeycrisp, Jonagold, Pink Lady, Granny Smith, and Braeburn.

3) Assimilate data into development of a functional model of pollen tube growth for growers to test on selected specific apple cultivars.

4) Continue studies of pollen germination/tube growth under natural field temperature and light conditions compared to 2005-08 laboratory and field experiments, expanding studies to additional commercially important cultivars.

5) Further develop reliable laboratory techniques for the study of a wide range of constant and variable temperatures on pollen germination and tube growth.

2010 Objectives:

- Develop model parameters for Red Delicious, Pink Lady, Honeycrisp
- Generate computer model and incorporate into DAS (working with Vince Jones and Ute Chambers, WSU)
- Provide training to recognize desirable amount of king bloom open to "start the clock".
- Beta field testing of models for Gala, Fuji, and Golden Delicious

SIGNIFICANT FINDINGS:

(Where we are after 2009)

- Better understanding of temperature effect on pollen tube growth
- Better understanding of actual time required for fertilization
- Better awareness of cultivar differences in time required for fertilization
- Recognize the need to fine-tune "triggering method" for start of bloom thinning applications
- Have completed controlled testing of Gala, Fuji, and Golden Delicious
- Have developed preliminary pollen tube growth modeling program for beta testing on Gala, Fuji, and Golden Delicious
- Have initiated testing of pollen tube growth modeling program in Washington St. sites.

METHODS:

A. Computer Modeling Program

Assist with initial implementation by helping the model programmer with interface, and dissemination of relevant data in computer generated output programs.

B. Field Studies

Controlled pollination studies will be conducted by Leon Combs in selected Washington orchards and sampled flowers will be preserved in vials, refrigerated, and transported to be examined in our laboratory at Winchester using previously developed techniques. Flowers will be harvested from cooperating orchards at designated times after pollination, petals will be removed, flower styles will be placed in a solution of sodium sulfite (5%) and stored at 40°F. Prior to microscopic examination, samples will be boiled for 15 minutes. Pistils will be dissected from the remaining flower tissue, rinsed with distilled water and squashed under a coverslip or slide and stained with 0.01% aniline blue in 0.067M K_2HPO_4. Slides will be stained for 24 hours before examination at 100x under fluorescent light using a Zeiss HBO-50 high-pressure mercury vapor light source and a Nikon Optiphot microscope. Collected data include abundance of pollen germination/ tube growth on the stigma surface (0-10 rating scale), number of tubes penetrating the stigma base, mean length of the longest pollen tube, mean style length, and number of pollen tubes reaching the base of the style.

C. Additional Field Studies

Controlled bloom thinning tests will be conducted at beta-test sites using field data model program developed from previous research project data. These studies will be used as a preliminary guide in estimating % king bloom open, pollen tube growth, and when bloom thinners should be applied according to model parameters. Data will be recorded on fruit size, yield, and quality. By actual use of the program concept, growers can evaluate the program and suggest improvements or modifications that would help refine the model into a more grower-friendly tool.

RESULTS AND DISCUSSION:

Proper bloom thinning produces the largest fruit, the best return bloom and less biennial bearing. This 3-year study applies findings from our previous research project: "Temperature Effect on Pollen Germination/Tube Growth in Apple Pistils". Our ideal for bloom thinning would be to track fertilization of the desired % of king bloom needed to make the crop, then apply a bloom thinner to prevent set of later bloom. The critical needs to accomplish this are knowing how much time is needed to fertilize king bloom after pollination and preventing set before fertilization of later bloom.

To import our data into a predictive model to be used by Washington growers, extensive field research studies using previous experimental data are required to validate and justify use of this program as an aid in bloom thinning of apples. Understanding the progression of pollen tube growth after pollination is critical for proper timing of bloom thinner applications. Crop loads not sufficiently thinned can result in trees being thrown into biennial bearing with little or no crop in the off year. Our goal is to assimilate data into development of a functional model of pollen tube growth to be used on specific apple cultivars, and to continue developing the laboratory and orchard database on additional varieties now being tested and other varieties yet to be tested.

In 2009 we launched research studies at several locations across the Washington apple growing region. With the participation and years of experience of key individuals as beta-testers for this research, we can better analyze, evaluate, and develop this tool for apple crop load management by the industry. Practical utilization of our experimental data was demonstrated on Gala at Goose Ranch, Finley, WA (Figure 1). Shown are hourly temperatures (dark line across the center) and the predicted progress of pollen tubes in Gala styles based on those temperatures. The broken line near the top indicates mean style length (9.49 mm for Buckeye Gala), at which point fertilization is assumed to occur. The right sides of each curve coincide with the timing of the first three lime sulfur sprays. The steep curve at the left shows rapid pollen tube growth with unusually warm temperatures soon after a

desired 10% of clusters had king bloom open on April 21. The first bloom thinning application was intentionally delayed slightly to permit some additional fruit set in delayed bloom in the treetops. The more gradual curves in the center and the right in Figure 1 show slower pollen tube progress in more moderate temperatures, and the timing of lime sulfur applications before the pollen tubes had reached the length of the styles. These applications prevented set of pollinated blossoms before they were fertilized, thereby reducing the need for hand thinning. Figure 2, also at Finley, shows that the rate of pollen tube growth can change in a matter of a few hours. Adding projected growth from actual temperatures near 80°F for the previous four hours (broken line, starting from the four open bars at the left) shows that significant changes in growth would have occurred, requiring earlier thinning application to achieve the desired crop load.

We are continuing to add to the varietal database for predictive models to be used by Washington apple growers. Two illustrations of this from 2009 are with Cripps Pink (Pink Lady). The progress of Snowdrift pollen tubes on Pink Lady styles appeared to be somewhat different than the norm (Figure 3), as tube growth appeared to be slower at 75°F than at 65°F. Gala and Manchurian were less effective or slower pollinizers for Pink Lady than were Snowdrift, Golden Delicious and Fuji pollen (Figure 4). This raises some questions about time of fertilization and bloom-thinning in the presence of various pollinizers.

Our field studies in 2009 helped to validate the current Excel model as an aid for growers to use in bloom thinning of apples. Our ideal for bloom thinning would be to track fertilization of the desired percent of king bloom needed to make the crop, then apply a bloom thinner to prevent set of later bloom. Critical needs to accomplish this are knowing how much time is needed to fertilize king bloom of each variety after pollination, and then preventing set before fertilization of later bloom. Varietal responses must be recognized and must be compensated for in models, and eventually we would like to see separate model for each important cultivar. Also there are questions about unfertilized blossom longevity: How long do they last if they aren't fertilized?

What's next?

Harold Ostenson (Stemilt Growers), Tom Butler (Washington Fruit & Produce Co.), Mike Robinson (Double Diamond Fruit), Dan Flick (Wilbur-Ellis), and Tory Schmidt (Washington Tree Fruit Research Commission) were part of the pollen tube growth model beta-testing group for 2009. After the season was completed, they evaluated and recommended areas that they feel need to be addressed to enhance the use of the modeling program and make it a more useful aid in bloom thinning. The following is a summary of their suggestions:

- Determining the timing of king bloom set and beginning the clock.
- A way of measuring the % of fruit set in the field.
- Streamline the process to determine fruit set, real time.
- Understand the mode of action of the bloom thinners (suppression of photosynthesis vs depression in carbon fixation.
- Pollen and pollen tube viability.
- More work needs to be done on varietal tube growth differences.
- Learn more about the stigmatic burning and pollenicide potential of Lime Sulfur and others bloom thinners.
- Is it BOTH pistil damage AND plant stress/reduced photosynthesis (and maybe more) that contribute to thinning?

These and others are questions we will continue to address as we work with beta testers and Vince Jones and Ute Chambers, WSU, to generate computer models and incorporate them into DAS.

FIGURE 1

GOOSE RANCH - FINLEY, WASHINGTON
POLLEN TUBE GROWTH MODEL THINNING TEST
BUCKEYE GALA (2009)

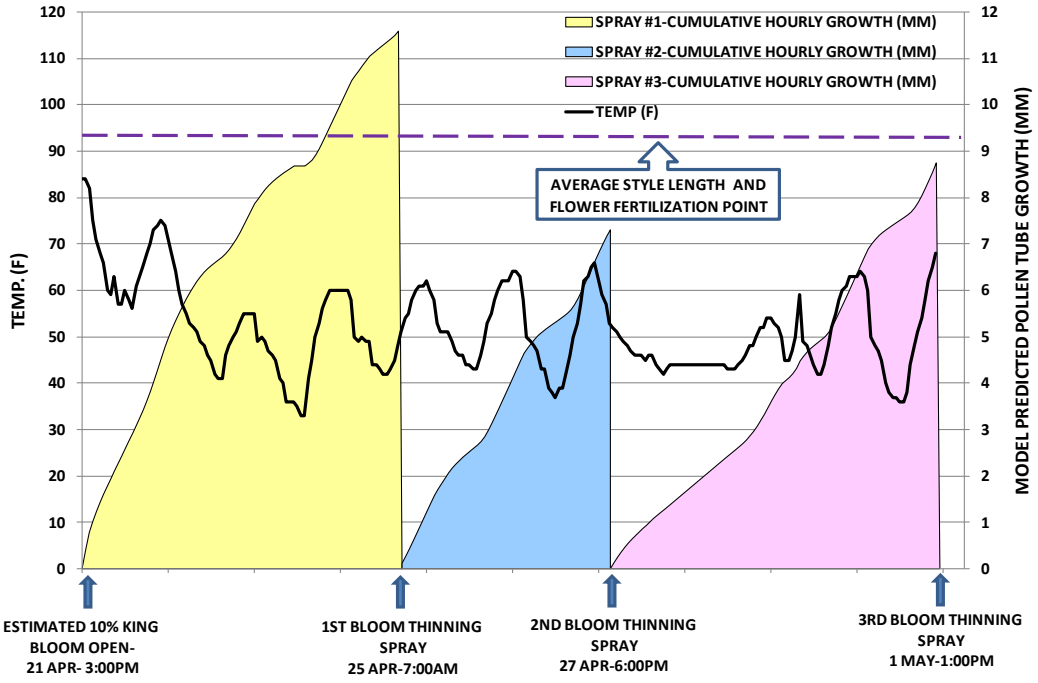


FIGURE 2

GOOSE RANCH - FINLEY, WASHINGTON
POLLEN TUBE GROWTH MODEL THINNING TEST
BUCKEYE GALA (2009)

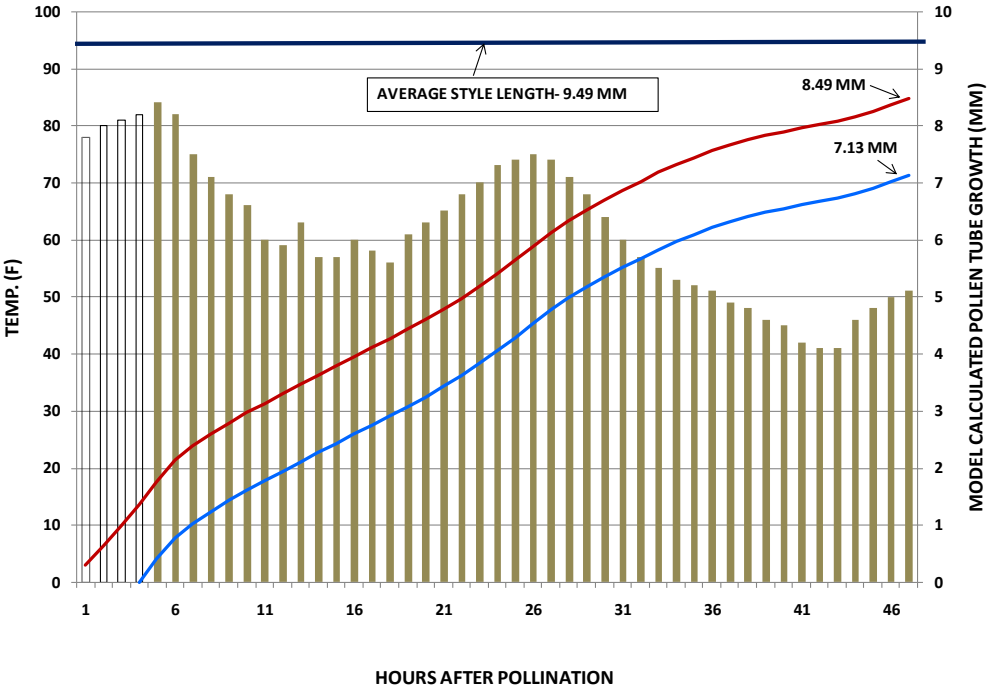


FIGURE 3

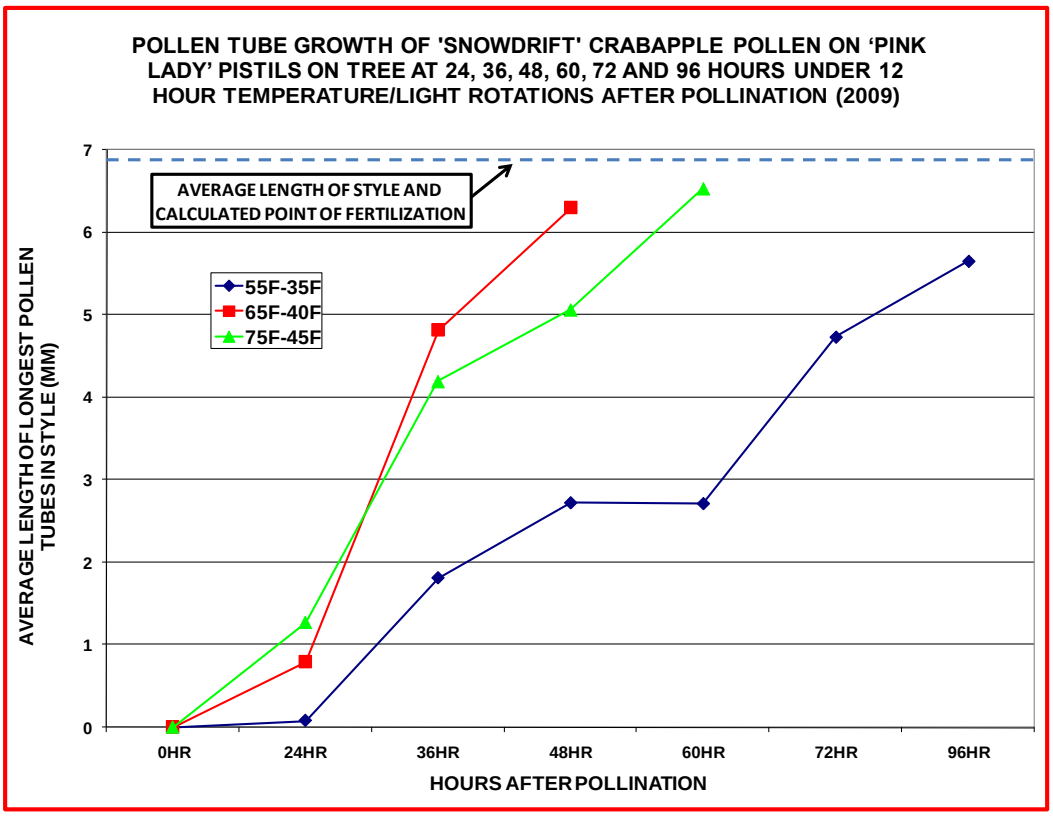
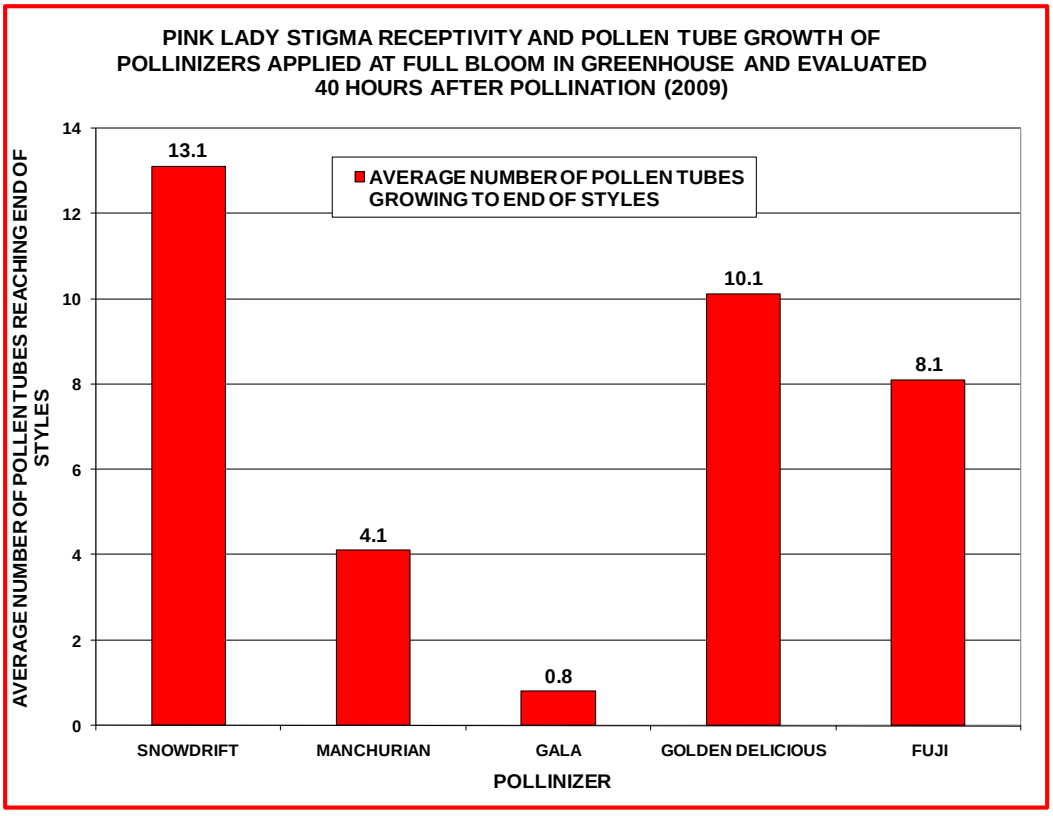


FIGURE 4



CONTINUING PROJECT REPORT **YEAR**: 2009
WTFRC Project Number:

Project Title: Apple rootstock and scion evaluation

PI: Tom Auvil
Organization: WTFRC
Telephone/email: 509-669-3060 auvil@treefruitresearch.com
Address: 1719 Springwater Ave.
City: Wenatchee, WA

WTFRC Staff cooperators: Felipe Castillo, Tory Schmidt, Jim McFerson, Wenatchee, WA

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

Cooperators: Dave Allan, Bob Brammer, Ray Fuller, Del Feigal, Ron Wilcox, Dale Goldy, Tim Welsh, Jose Ramirez

Total project funding request: Year 1: $83,054 **Year 2**: $87,102 **Year 3**: $100,725

Other funding sources: None

WTFRC Internal expenses:

Item	2009	2010	2011
Salaries[2,3]	34,925	29,500	30,500
Benefits[2,3]	11,176	9,440	9,760
Crew Wages[3]	21,025	25,880	26,655
Crew Benefits[3]	6728	8281.6	8529.6
Stemilt RCA room rental	4,200	8400	8400
Shipping			
Supplies[4]			10880
Travel[1]	5,000	5600	6000
Miscellaneous			
Total	83,054	**87,102**	100,725

Footnotes:
[1]Fuel and maintenance
[2]Salaries and benefits for Auvil, Schmidt, Castillo, Hanrahan and Stone apportioned to this project.
[3]Rootstock salary and wages are down 66% due to ending trials, WTFRC is now doing harvest and storage on Phase 3 trials which will increase significantly in 2010.
[4]Phase 3 trees for WABP

NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

OBJECTIVES:

1. Evaluate apple rootstocks, particularly disease resistant rootstocks, in commercial settings in Washington State with known replant conditions.
2. Integrate the processes of evaluation and industry adaptation.
3. Extend procedures for rootstock evaluation into scion breeding program.
4. Establish protocol for scion evaluation program.

Table 1: Rootstock and scion trials in the Northwest

Location	Term	Tree # / Acreage	Apple Scion	Apple Root	Pear Root	Cherry Scion	Cherry Root
Brewster	5 - 8 years	436 / 0.3	**x**				
Mattawa	5 - 8 years	477 / 0.32	**x**				
Quincy/Babcock	5 - 8 years	437 / 0.3	**x**				
Prosser	5 - 8 years	471 / 0.32	**x**				
Brewster	2006-2012	837 / 1		**x**			
Chelan[2]	2004-2010	1147 / 0.83		**x**			
Chelan[2]	2003-2009	231 / 0.18		**x**			
Frenchman Hills[2]	2003-2009	275 / 0.25		**x**			
Naches[2]	2004-2010	728 / 0.41		**x**			
Vantage	2006-2012	731 / 0.31		**x**			
Wapato[2]	2004-2010	970 / 0.86		**x**			
Wapato	2006-2012	768 / 0.7		**x**			
Wapato[2]	2006-2012	768 / 0.7		**x**			
Royal City	2008-2016	300/.5		**x**			
Cashmere[1]	2002-2012	na			**x**		
Hood River[1]	2002-2012	400 / 1.5			**x**		
Hood River[1]	2004-2010	300 / 0.33			**x**		
Hood River[1]	2006-2014	1000 / 2.2			**x**		
Tonasket[1]	2002-2012	Na			**x**		
Hood River [1]	5 years	Na				**x**	
Manson	5 years	Na				**x**	
Mattawa	5 - 8 years	Na				**x**	
Prosser[1]	5 years	Na				**x**	
Manson	8 years	700 / 1.75					**x**
Mattawa[3]	5 - 8 years	700 / 1.75					**x**
Mosier[1]	8 years	700 / 1.75					**x**
Prosser[1]	8 years	700 / 1.75					**x**

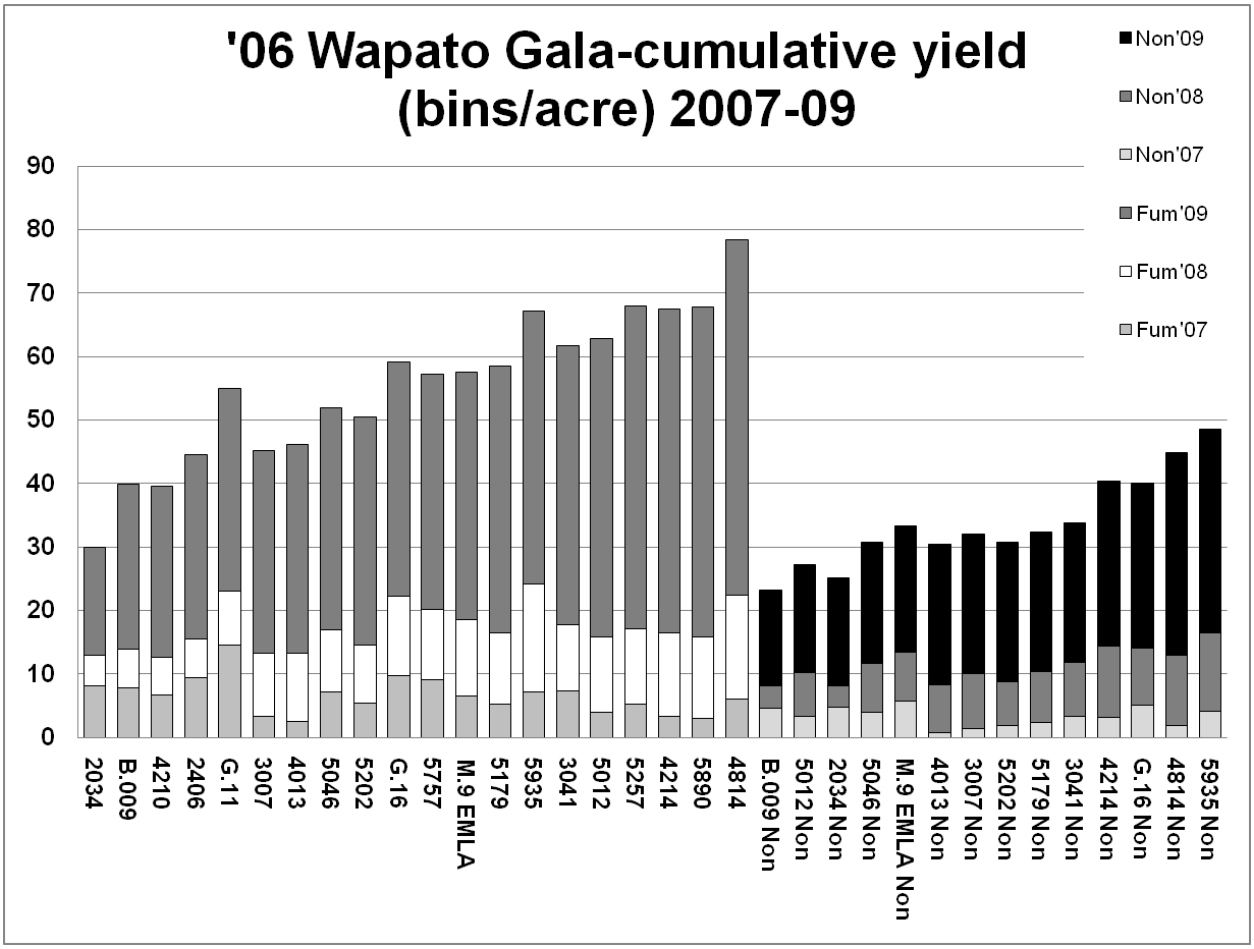
[1]Trials conducted by WSU / OSU faculty

[2] Apple rootstock trials ended fall of 2009.

[3]Trial cancelled due to a lack of trees

Scion evaluation accomplishments

- 2009 was the first significant crop for Washington Apple Breeding Program Phase 3 plantings: There are a range of flavors / sugar-acidity ratios in the advanced selections.
- It is anticipated to release a new genotype annually for evaluation by Washington State growers.
- The low ethylene gene in WA 2 really works! Texture of fruit is unchanged after two months of regular storage. Fruit retains 'starchy' harvest flavor two months of Regular Storage.
- Protocols for post harvest handling tests are being established.
- The strip pick harvest regimen offered insight to the color/maturity tradeoff.
- Need 75+ trees per site to have fruit volume for storage and handling trials.
- Crisp to hard textured fruit may be prone to stem and calyx splitting.



2006 Wapato Gala rootstock trial also shows impact of marginal irrigation system. Cumulative yields through the end of fourth growing season is about two-thirds of expectation.

Significant Rootstock Findings

- Geneva rootstocks continue to show genetic mitigation of replant disorders. Elite selections including CG4214, G.41 and G.935 continue to perform well in fumigated and non-fumigated sites. CG.5890, CG.4011 and CG. 4814 are performing well in non-fumigated sites and warrant more evaluation.
- Availability of Geneva rootstocks is increasing, though slowly there are signs of hope. A significant increase in G.41 and G.11 is expected for spring 2011 liner planting, spring of 2013 planting of finished trees.
- G.41 and G.11 are smaller than M.9 337. Some older eastern US literature has G.11 similar to M.26.
- CG.4214 is larger than M.9 337
- G.935 is similar in size to Pajam 2 or M.9 emla. Eastern trials utilizing data from young trees report G.935 is at least M.26 size. In Washington, G.935 is M.9 class in tree size. G.935 can be vigorous as a newly planted non-bearing tree.
- CG.4214 is scheduled to be released in early 2010. It is the best replant site performer.
- G.41, G.11, CG.4214, G.935 are all fireblight resistant.
- In tough replant sites, G.41, CG.4214, and G.935 may improve consistency of performance.

2010 Activities

- Monitor and discuss cultural practices with scion and rootstock cooperators.
- Closely monitor and adjust cropload of scion trials.
- Efficiently harvest, transport and place into storage trials 80 bins of fruit.
- Collaborate with Dr. Hanrahan on storage and post harvest handling experiments on scion trial fruit.
- Collect rootstock data including trunk circumferences and per tree yields.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-09-903 **(WSU Project 13C-3655-3260)**

Project Title: Apple scion breeding

PI:	Kate Evans	**Co-PI (1):**	Cameron Peace
Organization:	WSU Tree Fruit Research and Extension Center	**Organization:**	WSU-Horticulture and Landscape Architecture
Telephone:	509-663-8181 x245	**Telephone:**	509-335-6899
Email:	kate_evans@wsu.edu	**Email:**	cpeace@wsu.edu
Address:	1100 N. Western Ave	**Address:**	P O Box 616414
City:	Wenatchee	**City:**	Pullman
State/Zip	WA-98801	**State/Zip:**	WA-99164-6141
Co-PI(2):	Carolyn Ross	**Co-PI(3):**	Yanmin Zhu
Organization:	WSU School of Food Science	**Organization:**	USDA-ARS Tree Fruit Research Lab
Telephone:	509-335-2438	**Telephone:**	509-664-2280
email:	cfross@wsu.edu	**email:**	zhu@tfrl.ars.usda.gov
Address:	P O Box 646376	**Address:**	1101 N. Western Ave
City:	Pullman	**City:**	Wenatchee
State/Zip	WA 99164-6376	**State/Zip:**	WA 98801

Cooperators: Bruce H. Barritt, Professor Emeritus, WSU; Amit Dhingra, WSU Pullman; Doreen S. Main, WSU Pullman; Tom Auvil, WTFRC; Roger Adams, Willow Drive Nursery, Ephrata.

Total project funding request: Year 1 :$169,910 **Year 2: $239,628** **Year 3**: $225,663

Other funding sources: None

WTFRC Collaborative expenses:

Item	2009	2010	2011
Stemilt RCA room rental	4,200	**8,400**	8,400
Crew labor	3,500	**18,840**	19,405
Crew benefits	0	**6,028.8**	6,209.8
Supplies	0	**0**	10,880
Travel – to plots	5,000	**5,600**	6,000
WTFRC staff salary+benefits	15,790	**23,984**	24,856
Total	$28,490	**$62,853**	$75,751

Budget 1
Organization: WSU-TFREC **Contract Administrator:** ML Bricker and Kevin Larson
Telephone: 509-335-7667, 509-663-8181 x221 **Email:** mdesros@wsu.edu, kevin_larson@wsu.edu

Item	2009	2010	2011
Salaries[1]	60,540	**50,352**	52,366
Benefits	23,005	**16,616**	17,281
Wages[2]	15,500	**20,000**	20,800
Benefits	2,790	**2,960**	3,078
Equipment[3]	50,000	**0**	0
Supplies[4]	0	**19,700**	17,500
Travel	14,200	**15,500**	16,938
Total	166,035	**$125,128**	127,963

Footnotes:
[1]Salaries for Agricultural Research Technologist.
[2]Wages for time-slip labor for orchard establishment and trait phenotyping.
[3]For equipping new fruit phenotyping lab (instrumentation for measuring soluble solids, acidity and texture).
[4]For orchard establishment supplies.

Budget 2
Organization: Willow Drive **Contract Administrator:**

Item	2009	2010	2011
Trees[1]	700	**6,000**	3,700
Seedling propagation[2]	3,175	**108,500**	94,000
Total	3,875	**$114,500**	97,700

Footnotes:
[1]Trees for four sites at 100 trees per grower site + 25 extra for WSU plots, three scion per year. Five trees of two commercial varieties will be planted as standards for two years at each grower site.
[2]Propagation of seedling populations on M.9 rootstock for WSU by Willow Drive Nursery.

OBJECTIVES

1. Produce, through traditional breeding methods, promising selections and subsequently elite selections with outstanding eating quality and commercial potential.
2. Use extensive trait phenotyping in combination with genomic tools (phenotype/genotype associations) to develop Marker-Assisted Breeding for key fruit and tree traits.
3. Use both objective (instrumental) and subjective (sensory) evaluation techniques to identify selections with outstanding eating quality.

SIGNIFICANT FINDINGS

1. Fourteen new crosses were made and approximately 15,000 seeds produced in the WSU Apple Breeding Program (WABP). Seedlings from 16,000 seeds from 2008 crosses were grown in the greenhouse and transplanted to the nursery.
2. Over 15,000 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards at TFREC in 2010.
3. Fifteen promising selections made in 2008 were propagated in 2009 for planting in 2011 Phase 2 trials at three diverse sites in Central Washington.
4. Promising selections in Phase 2 trials (planted in 2004, 2005, 2006, 2007 and 2008) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
5. Twenty-three new promising selections were planted at three evaluation sites in Phase 2 trials in 2009.
6. Trees of one further elite selection were planted in 2009 in Phase 3 grower trials.
7. Leaf samples were collected from all Phase 2 and 3 mother trees ready for DNA extraction and fingerprinting.
8. Fruit from seven elite selections were profiled by the sensory panel at WSU-School of Food Science under the supervision of Dr. Ross in February 2009. Eight elite selections were tested in November.
9. An industry advisory council (IAC) was established. IAC visits included the Phase 1 seedling evaluation orchards as well as the Phase 3 grower trials in 2009. The IAC actively participated in drawing up evaluation and commercialization guidelines for WSU apple releases with WSU and the WTFRC.
10. Trees of 'WA 2', the first release from the WABP, were offered to growers for evaluation at the WA Hort Show in December 2009.
11. Fruit from several different seedling progenies were phenotyped and the trees genotyped for projects improving molecular markers for skin color and texture lead by Dr. Zhu and Dr. Peace.
12. The WABP was selected as one of the core breeding programs in the newly funded SCRI RosBREED project. As this project will develop and implement marker-assisted breeding, the WABP will benefit from being at the forefront of this technology.

METHODS

1. Breeding
    a. Use traditional hybridization of parents with desirable traits to produce seed (10,000 to 20,000 per year). Germinate seed and grow seedlings in the nursery and bud each seedling onto M.9 rootstock to produce large trees for the fruit evaluation orchard. From promising seedlings, fruit are evaluated for quality after two months in regular cold storage. Selection is based on appearance (primarily color, uniformity, freedom from defects) and desirable eating quality (primarily firmness, crispness, juiciness, sugar/acid balance).
    b. Promising selections are propagated on M.9 and placed in replicated second stage trials (five trees/selection) at three diverse sites in central Washington. Data on tree health, productivity and fruit quality are collected and outstanding selections are given elite status.

c. Elite selections are propagated on M.9 and planted in multi-tree (25 to 100 trees/selection) grower third stage trials in commercial orchards at four diverse sites in central Washington. Trials are conducted in cooperation with the WTFRC, managed by Tom Auvil. Certified, virus tested, bud wood is produced for elite selections.
d. Outstanding selections are proposed for commercialization.

2. Genomics, Marker-Assisted Breeding (MAB) and Pedigree-Based Genotyping
    a. In conjunction with the supporting scientists in genomics and genetics (Drs. Peace, Dhingra, Main and Zhu) take advantage of new information and tools to improve breeding efficiency. Develop phenotype/genotype associations for important fruit quality traits including firmness, crispness, juiciness, acidity, soluble solids, fruit color and tree characteristics including precocity, vigor, disease and insect resistance. Use developed markers in MAB. Specifically for the ethylene genes *Md-ACS1* and *Md-ACO1*, which influence fruit softening and storage life, genotype seedlings in populations resulting from crosses between parents with different textural characteristics (Honeycrisp, Crimson Crisp and Cripps Pink) and use these genes as selection tools to eliminate undesirable seedlings. Use performance data of parent cultivars, selections and breeding populations with the statistical approach of Pedigree-Based Analysis to determine the predictive power of markers in each potential cross.

3. Sensory/Consumer Evaluation
    a. In cooperation with Dr. Carolyn Ross, establish a trained sensory evaluation team. Using established sensory evaluation methods, characterize promising selections for consumer acceptability in terms of fruit texture, flavor and appearance.

RESULTS & DISCUSSION

The transition between Dr. Barritt and Dr. Evans in management of the WABP has gone smoothly with useful input from Dr. Barritt throughout the year. Crossing has continued with several new progenies being derived from the best selections from earlier WABP breeding generations. No new Phase 1 seedling orchards were planted in 2009 following a lack of budwood in the nursery, however, two years worth of seedlings have been propagated for a large planting for 2010. The 23 new second stage selections, planted in 2009, were the start of a new Phase 2 planting at the WSU Sunrise Orchard which will replace the previous WSU Phase 2 planting at Columbia View over time. An overview of the WABP was presented to visitors to the Sunrise Orchard Open Day in July. Leaf material of all the mother trees from the advanced and elite selections from the WABP was collected ready for fingerprinting in Pullman for future reference should the need arise for patent applications and to confirm identity of propagated material.

Following the move of the breeder's fruit phenotyping laboratory from the USDA building into the Overley building, the necessary equipment was installed prior to the start of the fruit season. Equipment purchased included an autotitrator for measuring acidity, a refractometer for measuring soluble solids/sweetness and a DigiTest which measures firmness, crispness and mealiness. Systems are under development to link data collected to the individual tree and harvest date using bar codes.

The breeding team is working with Dr. Main's group to develop the breeders toolbox database to house the data from the breeding program in a searchable format that will readily provide objective information to support breeding decisions and background data on elite selections prior

to release. 2009 data from the WABP is ready for the database as soon as the structure is finalized.

The newly established Industry Advisory Council (IAC) met several times in 2009 following an initial meeting at the WTFRC Apple Review in January. The group visited the Phase 1 seedling evaluation orchards in September and the Phase 3 grower plantings in May, September and October to view and discuss the elite selections and met again in November for a further look at the fruit. The group was impressed by the quality of the elite selections and interested by the variation in performance of the selections at the different sites which may reflect local adaptation that can be exploited in future cultivar deployment. These plantings were assessed and harvested weekly by the WTFRC team led by Tom Auvil who has also played a large part in promoting these elite selections with visits to groups such as the Fieldman's Association as well as organizing the variety showcase function at the Washington State Horticultural Association Show in Wenatchee, on 6th December.

The IAC was also involved with the WTFRC and WSU Research Foundation in the discussion of new evaluation and commercialization guidelines for releases from the WABP. The patent application was submitted in May for the first release 'WA 2' from the WABP and the commercialization process was presented in the October issue of the Good Fruit Grower magazine as well as being presented on several occasions at the Hort Show. Three poster stands were present at the Hort Show: one focusing on 'WA 2', one on the phase 3 grower trials and one on the WABP itself. All three stands had fruit available for tasting.

Samples of seven second and third stage selections were tested for sensory attributes and consumer preference by Dr. Ross and her team in February. The sensory panel was provided with fruit exhibiting a wide range of quality attributes for training prior to assessing the selections and consumers compared the samples to a reference variety. A further set of eight samples were tested in November to see how these selections were rated earlier in the season.

WABP breeding parents and seedling populations served as a valuable germplasm resource for conducting ongoing molecular genetic studies that are aimed at developing decision support tools for this program. Seedling progenies of crosses among Honeycrisp, Cripps Pink, and Crimson Crisp, totaling thousands of seedlings, continued to be used as the "Trial Use" material to demonstrate the application of genetic markers for high-throughput seedling selection. A set of 96 WABP parent cultivars and advanced selections continued to be tested with genetic markers of potential value reported in the literature. However, the WABP will be represented more thoroughly in future studies with the Pedigree Set of 475 seedling trees and the Parent Set of 37 cultivars that were planted last year at the Sunrise Research Orchard. As this material was not yet adequately fruiting in the 2009 season, older breeding progenies at the Columbia View orchard were used for the 2009-2010 NRI- and WTFRC-funded project focusing on apple texture genetics. In collaboration with the WTFRC research team, fruit of 230 WABP seedlings, selections and cultivars were harvested and evaluated for fruit quality. Texture-related DNA marker analysis was conducted on this material in the NRI project. Fruit from further breeding progeny were phenotyped for skin color and the trees genotyped by Dr. Zhu's team to evaluate the utility for the WABP of a skin color marker (based on the *MdMYB1* gene).

The WABP was selected as one of the 12 core breeding programs in the newly-funded, large-scale, SCRI RosBREED project, lead by Dr. Amy Iezzoni (Michigan State University). As this project will develop and implement marker-assisted breeding to optimize selection, the WABP will benefit from being at the forefront of this technology using the infrastructure already developed at WSU following support from the WTFRC.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-09-907

Project Title: Developing an online toolbox for tree fruit breeding

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Cooperators: Amy Iezzoni (MSU), Jim Olmstead (UF), Gennaro Fazio (USDA-ARS), Gayle Volk (USDA-ARS)

Total project funding request: **Year 1**: 38,808 **Year 2**: 38,808

Other funding sources: None

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

Item	2009	2010	
Salaries	26,000	26,000	
Benefits	8,808	8,808	
Wages	3,000	3,000	
Benefits	0	0	
Equipment	0	0	
Supplies	0	0	
Travel	1,000	1,000	
Miscellaneous	0	0	
Total	38,808	**$38,808**	

PROJECT OB JECTIVES

1. Integrate publicly available gene, trait, QTL and breeding data for apple and cherry with the current genomics and genetics data and tools in the Genome Database for Rosaceae.
2. Develop web interfaces for molecular geneticists and breeders to upload new data.
3. Develop web interfaces and online tools suitable for accessing and mining all breeder relevant data.

Year One Goals and Activities:

- Design data schema for new features
- Collect gene, trait, QTL and breeding data for apple and cherry and upload to database.
- Design queries for data retrieval
- Start web interface/tool design process

Year Two Goals and Activities:

- Continue to collect to gene, trait, QTL and breeding data for apple and cherry and upload to database. Integrate the resistant gene into a susceptible variety to confirm resistance function
- Complete web/interface tool design process

Anticipated Accomplishments:
After this two year project is complete the apple and cherry breeding programs will have a robust, and secure data management and data querying database that will link directly to the integrated genomics and genetics data housed in the Genome Database for Rosaceae (GDR). GDR will have a publicly available breeding toolbox that is integrated with existing genomics and genetics data. This breeders database and toolbox will be part of a larger open-source genomics, genetics and breeding database for Rosaceae, that can also be utilized by other plant communities.

SIGNIFICANT FINDINGS

1. Through face-to-face project team meetings at Pullman, Prosser and Wenatchee we identified data management and analysis tool needs for the apple (Dr. Evans) and cherry breeding (Dr. Oraguzie) programs at Washington State University. This included identifying shorter term and longer term needs.

2. We researched existing breeding data management software packages and found the most useful to be the commercially based AgroBASE. This software is rather limited at the current time for perennial species such as tree crops and does not provide integration with genomics and genetics data housed in the Genome Database for Rosaceae (GDR).

3. Modified data structure of open-source “Chado” genomics database schema to accommodate breeding data requirements including pedigree breeding module. We did this in consultation with Chado developers so these modifications can be useful to other plant communities who use this open-source resource. Meeting with Chado collaborators at the Plant and Animal Genome Conference in San Diego in January to finalize common design.

4. Developed computational scripts to extract WSU apple breeding program data from access database and reformat for upload to Chado database.

5. Designing queries for data retrieval from Chado using Drupal web interface tools.

6. Met with Dr. Pankaj Jaiswal at Oregon State University, Corvallis, principal investigator of the NSF funded Plant Ontology project, to discuss methods to standardize ontology association of the Rosaceae traits.

METHODS

1. In collaboration with the SCRI funded RosBREED project we will continue to collect and upload to the database available phenotypic data including simple trait, and QTLs from the literature and organize the Rosaceae community to help curate this data using Plant, Trait and Environment Ontology terms.

2. We will develop web interfaces suing Drupal for WSU breeders to access their private data and available public data in GDR.

RESULTS AND DISCUSSION

We are on track to meet all our year 1 goals (end of March 2010) and complete the project within the two year framework outlined above. The major findings to date are that we have successfully modeled a database system that will accommodate not just the data and analysis needs for the apple and cherry breeding programs in Washington State but also other Rosaceae and tree fruit species. The provision of breeder focused datasets and analysis tools integrated within GDR will significantly accelerate identification and application of the genes and markers underlying important economic traits such as pre and post harvest fruit quality, and pest and disease resistance. Improvement of metric traits through the application of bio-informational methods will give a more predictable outcome to plant breeding than is currently the case with conventional one-gene-at-a-time genetics or phenotypic selection approaches. This database will allow the collection, storage and analysis of appropriate genotype, phenotype and germplasm datasets which can then be linked to traits that are of interest to breeders and industry stakeholders. This database resource will aid marker-assisted tree fruit breeding and facilitate the creation of new cultivars which meet consumers' needs, and sustainable agricultural practices , helping to ensure economic competitiveness of the Pacific North West tree fruit industry.

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

Project Title: Genetic marker assistance for the Washington apple breeding program

PI:	Cameron Peace	**Co-PI(2):**	Kate Evans
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Co-PI(3):	Jim Olmstead	**Co-PI(4):**	Jim Mattheis
Organization:	(no longer involved)	**Organization:**	USDA-ARS TFRL Wenatchee
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City:		**City**:	Wenatchee
State/Zip:		**State/Zip**:	WA 98801

Cooperators: Jim McFerson (WTFRC), Fred Bliss (Davis, California), Bruce Barritt (WSU), Yanmin Zhu and Dave Rudell (USDA-ARS Wenatchee), Deven See (USDA-ARS Pullman), Jim Luby (U Minnesota), Eric van de Weg and Marco Bink (Plant Research International), Fabrizio Costa and Riccardo Velasco (IASMA), Sue Gardiner, Stuart Tustin, and David Chagne (HortResearch), Susan Brown (Cornell), Schuyler Korban (U Illinois), Phil Forsline and G. Fazio (USDA-ARS Geneva), Walter Guerra (Laimburg), Francois Laurens (INRA), Rozemarijn Dreesen and Mark Davey (KULeuven), Amy Iezzoni (MSU), and others.

Total project funding request: **Year 1**: $68,500 **Year 2**: $53,000

Other funding sources

Awarded

Agency Name: USDA-CSREES National Research Initiative
Amount awarded: $400,000 (2009-2010)
Notes: "Functional gene markers for Rosaceae tree fruit texture" PI: Cameron Peace. Co-PIs: Costa, van de Weg, Luby, McFerson, Gardiner, Hamblin, and Oraguzie. Closely coordinated with activities 2-4 of this WTFRC apple project.

Agency Name: WTFRC Technology Review
Amount awarded: $50,000 (2009)
Notes: "ABI 3730 DNA Analyzer to augment tree fruit breeding and research" PI: Cameron Peace. See also next source.

Agency Name: Washington Wheat Commission
Amount awarded: $50,000 (2009)
Notes: PI: Deven See. Matches WTFRC funding (see above) to obtain a refurbished ABI 3730 DNA Analyzer ($100,000 total) for high-throughput genotyping of tree fruit and cereals, based at Pullman.

Agency Name: WSU Agricultural Research Center
Amount awarded: $100,000 (2009)
Notes: Additional support to C. Peace for high-throughput DNA extraction and genotyping equipment, complementing the ABI 3730 and removing technical bottlenecks for routine tree fruit genotyping. Part of this support was used to leverage a further $50,000 from the Washington Wheat Commission and $8,000 from D. See's USDA base funding to obtain a BioMek Laboratory Automation Station – a "robot" for high-throughput DNA extraction and genotyping sample preparation – which was operational from September 2009.

Agency Name: WTFRC Cherry Review
Amount requested: $45,000 (2009)
Notes: "Establishing the Marker-Assisted Breeding Pipeline for sweet cherry" PI: Cameron Peace. Co-PIs: Olmstead, Iezzoni, and Oraguzie. Synergistic project for establishing equivalent marker-assisted breeding infrastructure for the PNW sweet cherry breeding program.

Agency Name: WTFRC Apple Review
Amount requested: $635,201 (2009-2011)
Notes: "Apple Scion Breeding" PI: Kate Evans. Co-PIs: Peace, Ross, Zhu. The foundation program on which the current WTFRC project builds.

Agency Name: WTFRC Apple Review
Amount requested: $77,616 (2009-2010)
Notes: "Developing an online toolbox for tree fruit breeding" PI: Dorrie Main. Co-PIs: Evans, Oraguzie, Peace, Jung. Establishment of bioinformatics and databasing support to facilitate the translation of genomics information into application in WSU tree fruit breeding programs. Synergistic with activity 5 of the current WTFRC project and SCRI project "tfGDR" below.

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

Agency Name: USDA-CSREES, Specialty Crops Research Initiative
Amount requested: $7,200,000 plus equal amount matching from universities and industry (Sep 2009 – Aug 2013)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Amy Iezzoni. Co-PIs include Peace, Olmstead, and Evans. A synergistic project proposal to establish sustainable marker-assisted breeding infrastructure for U.S. Rosaceae crops, based on the Marker-Assisted Breeding Pipeline concept that involves Pedigree-Based Analysis.

WTFRC Collaborative expenses:

Item	2009		
Stemilt RCA room rental	$ 6,000		
Crew labor	$13,000*		
Total	$19,000	undetermined	

Footnotes: * $8000 for fruit quality data collection, $3000 for tree architecture and phenology data collection, $2000 for data handling and analysis

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

Item	Year 1 (2009)	Year 2 (2010)	
Salaries			
Benefits			
Wages[1]	$21,186	$22,034	
Benefits	$ 3,814	$ 3,966	
Supplies[2]	$10,000	$10,000	
Travel (in-state)	$ 2,000	$ 2,000	
Travel (other)	$13,500		
Outreach[3]	$ 5,000	$ 5,000	
Miscellaneous[4]	$13,000	$10,000	
Total	**$68,500**	**$53,000**	

Footnotes:

[1] Casual technical assistance for activities 6 and 7. Benefits are calculated at 18%. The total is designated 40% ($10000 wages + benefits in Year 1, 4% increase in Year 2) for activities 5 and 6, 60% ($15,000 in Year 1, 4% increase in Year 2) for activity 7.

[2] Genotyping lab supplies for activities 6 and 7.

[3] Activity 4 development, hosting, and publicizing Extension coordinated workshops and website development. In 2009 we will purchase a portable media projector ($2000).

[4] Activity 1 nursery propagation and Sunrise planting/maintenance costs. In 2009, $10000 is for propagation costs of the Diversity Set, any additional genotypes identified during the 2009 growing season, and any necessary re-propagation, and $3000 is for plot fees at the WSU Sunrise orchard ($1000/acre). In 2010, $6000 is for propagation of the Genetic Stock set and any necessary re-propagation and $4000 is for plot fees.

OBJECTIVES

The overall goal is to provide comprehensive molecular genetics support for the WABP utilization of marker-assisted breeding. Specific objectives are to:

1) Establish a world-class long-term reference apple germplasm planting in Washington.
2) Obtain comprehensive fruit quality phenotypic data on representative industry and breeding stock of Washington and the nation.
3) Ensure Washington fruit quality phenotyping is contemporary and coordinated with national and international collaborators.
4) Enhance outreach efforts to demonstrate local impacts of genetic marker use for apple.
5) Create a high-throughput seedling genotyping database for the WABP.
6) Develop a DNA fingerprinting system for new cultivar releases from the WABP.
7) Continue to pipeline new markers for high priority traits into the WABP.

Activities to address these objectives are similar between 2009 and 2010. The next year will therefore continue work in each area. *Deviations*: Substantial travel within Obj. 3 (coordination) was planned for late summer-fall 2009, but has been rescheduled for summer 2010. The departure of co-PI J. Olmstead greatly reduced progress in 2009 toward Obj. 4 (outreach). We are seeking alternative personnel to further such activities in 2010; if necessary, unspent funds allocated to this component will be returned.

SIGNIFICANT FINDINGS

- The Apple Germplasm Library was established, maintained, added to, presented, reported on, and some harvesting begun.

- Fruit quality evaluations on a comprehensive set of germplasm (~1000 trees) across the U.S. and world are underway, for use in elucidating and validating genetic control.

- We strengthened coordination with an international network of apple fruit quality experts.

- Outreach efforts on genetic marker assistance for local apple breeding were made at the WSHA.

- Funding for developing a streamlined seedling genotyping database was multiplied with new collaborative partnerships.

- The foundation was developed for a DNA fingerprinting system that determines uniqueness and parentage of WSU selections to better characterize and protect selections and new cultivar releases.

- Several promising genetic tests are in the MAB Pipeline for the highest priority breeding trait categories of texture, flavour, and appearance. Routine use of DNA information could be used in 2010 to support breeding parent and seedling selection decisions for all three of these trait categories.

All areas of effort described above will be addressed further in 2010.

METHODS

Adding to key infrastructure already in place for routine marker-assisted breeding (MAB) in the Washington Apple Breeding Program (WABP), we will take the MAB Pipeline approach. This Pipeline has been adopted for the multi-institutional project "RosBREED" (www.rosbreed.org) and involves eight stages to translate research findings into routine breeding application. Methodologies specific to each objective are evident in descriptions below of 2009 progress.

RESULTS & DISCUSSION

1) Reference germplasm sets

The reference germplasm sets are now called the Apple Germplasm Library. About 1.5 acres is already planted, with more than 2 acres to be added next spring (Figure 1).The concept, progress, and plans for this Library were presented on three occasions in 2009:

May: During planting of a portion of the Mapping Set, we were interviewed by Geraldine Warner which resulted in a Good Fruit Grower article ("In search of superior apples", pp14-15, July 2009; http://www.goodfruit.com/issues.php?article=2519&issue=97)

July: at the 1st Annual Sunrise Orchard Field Day. A color 2-page diagram-filled description was included in the booklet provided to attendees. Figures 1-2 below are from that 2-pager.

October: at the 2009 annual Apple Crop Germplasm Committee (CGC) meeting. After a visit to the WABP at Columbia View, attendees (U.S. apple germplasm users and curators) were brought to the Sunrise Research Orchard. The 2-page flyer was provided.

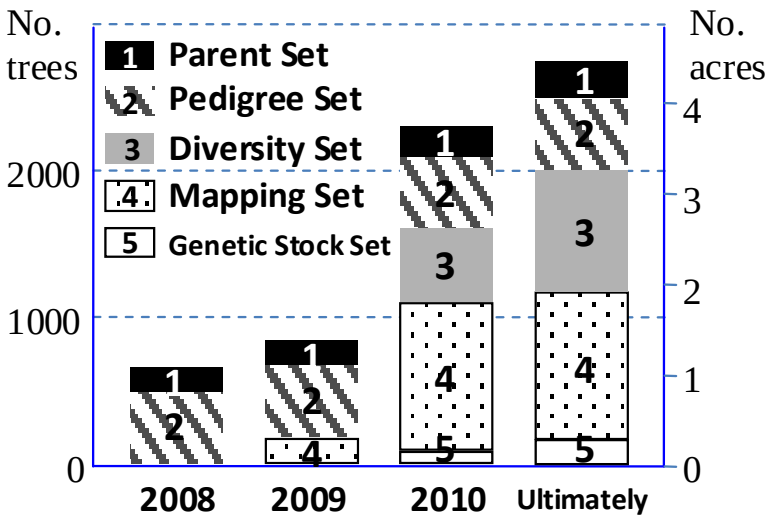

Figure 1. The Apple Germplasm Library consists of about 2700 trees (with more than 2000 unique individuals) grouped into five Sets. The first planting was in Spring 2008, and more trees were added in 2009. The largest additions are planned for 2010.

The intent and composition of each Set are described below.

Parent Set: Fifty elite cultivars and selections, 5 trees of each, used as parents in the Washington apple breeding program. First 37 parents planted 2008. Pedigrees can be traced back to 32 known founder ancestors. Golden Delicious, Red Delicious, Splendour, and McIntosh are the most common

founders of Washington breeding material, together accounting for 59% of the genetic background of the current Parent Set.

Pedigree Set: 475 trees, a snapshot of the Washington apple breeding program. Generated from crosses among 10 parents, with each parent crossed to 1-7 of the others. Planted 2008. Seedlings will be scrutinized for performance and DNA profiles to determine the breeding value of each parent (and their ancestors) and identify exceptional genetic combinations. New DNA tests to support the Washington breeding program will be refined by screening on the Pedigree Set.

Diversity Set: 400 unique apple types with diverse and novel traits, 2 trees of each, covering about 40 apple species. First 250 (x2) to be planted in 2010, propagated from trees of the USDA-ARS Plant Genetic Resources Unit core collection in Geneva, NY. Sources include wild forests of Kazakhstan and China, heirloom varieties, and distinct material representing popular cultivars of the world. Performance under WA conditions will be recorded, with attention to traits of value for both industry and consumers. All individuals are genetically compatible, so the Diversity Set will serve as a repository of diverse and novel traits for a truly diverse portfolio of new cultivars by breeding.

Mapping Set: Up to 1000 seedlings from crosses among interesting apple types, for genetic mapping of traits and development of diagnostic DNA tests. Used to genetically dissect those traits of greatest value for the WA industry (especially consumer traits of taste, texture, and appearance), to understand, monitor, and improve those traits. Molecular genetics tools will be used to genetically map the DNA controlling these traits, so we can devise DNA tests to inform breeding decisions for efficient production of superior cultivars. Includes 750 seedlings of a three-way cross of Honeycrisp, Cripps Pink (Pink Lady), and Co-op 39 (Crimson Crisp), contrasting for taste, texture, and appearance, to be planted 2010 (Figure 2). Also includes 180 seedlings of Gala crossed with *Malus sieversii*, the wild ancestor of domesticated apple, to be used to obtain and apply genomic information to accelerate the process of breeding with undomesticated types. The Gala x *M. sieversii* population (the same population as that being genetically mapped and fire blight-characterized by the USDA-ARS West Virginia group with WTFRC funding support) was provided by G. Fazio under serendipitous circumstances, allowing our future WA-based studies to be synergistically linked with those of USDA researchers.

Genetic Stock Set: Up to 200 miscellaneous trees of interest that do not fit the other sets, such as cross-species hybrid breeding lines and new sports/mutants.

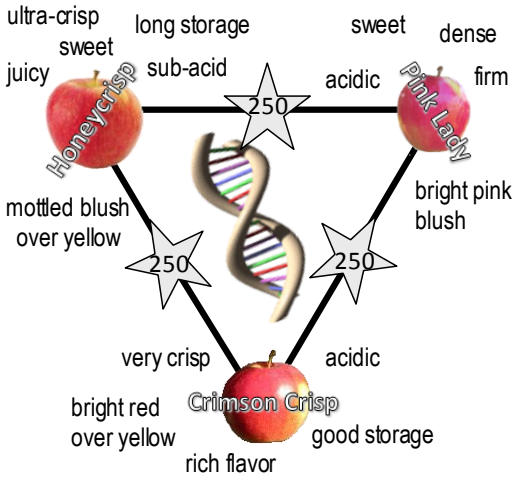

Figure 2. Three major seedling populations of the Mapping Set of WSU's Apple Germplasm Library. Fruit quality traits of each of the three parent cultivars, Honeycrisp, Pink Lady, and Crimson Crisp, are shown. Performance variation in texture, flavor, and appearance is expected to occur in the seedling populations, allowing their fine genetic dissection under WA conditions.

The Pedigree Set and Parent Set planted in 2008 were only just beginning to fruit in the 2009 season, which was a year too late for the NRI project, "Functional gene markers for Rosaceae tree fruit texture" – only 7 cultivars from Sunrise were used in that project in 2009 (Activity 2 below). However, the efforts in the last few years to choose, create, obtain, plant, and maintain these trees is well timed for the SCRI "RosBREED" project. In an unprecedented undertaking, major apple breeders and germplasm curators of the U.S. are currently working together to define a U.S. apple Crop Reference (CR) Set of approximately 500 trees. The CR Set will then be genetically characterized and performance-evaluated (especially for fruit quality) using state-of-the-art technologies and approaches from mid 2010 to 2013, coordinated across programs. Adding to this, each the three major U.S. apple breeding programs (WA, MN, and NY) are developing their own Breeding Pedigree Sets to complement the CR Sets and ensure that specific germplasm of interest to their individual breeding programs is represented in RosBREED genetic evaluations. The Pedigree and Parent Sets of WSU's Apple Germplasm Library will feature prominently in that research, created as they were to represent the WABP in anticipation of such Pedigree-Based Analysis studies.
Personnel involved: *Plant material choice*: C. Peace and B. Barritt, and J. Olmstead. *Provision of trees*: B. Barritt, K. Evans, P. Forsline, G. Fazio, Y. Zhu. *Planting*: Sunrise Research Orchard field crew, B. Konishi, J. Olmstead, K. Evans, C. Peace, CP's Pullman crew of T. Rowland, D. Edge-Garza, S. Haldar. *Plant maintenance*: Sunrise Research Orchard field crew, B. Konishi. *Flyers*: C. Peace, J. McFerson.

2) Description of fruit quality traits

This activity is coordinated closely with the NRI project, "Functional gene markers for Rosaceae tree fruit texture", where WTFRC funds (collaborative expenses) are for fruit quality phenotyping in Washington, and NRI funds of $400K and various in-kind contributions from co-PIs and collaborators are for phenotyping at other locations, genotyping, statistical analyses, and knowledge dissemination for practical application.

In the first half of 2009, standardized phenotyping protocols were hammered out with an international consortium of apple fruit quality and genetics experts. The collaborative approach to developing these protocols, and the protocols themselves, will be useful for similar future efforts such as in the "RosBREED" SCRI project. A presentation on these protocols was made at the ASHS annual conference in June by NRI project co-PI J. Luby (http://www.ashs.org/db/horttalks/detail.lasso?id=609). Eight guiding principles for conducting phenotyping (for genetics studies) included within the ASHS talk were also presented at the Apple CGC meeting in October 2009.

During the 2009 fruiting season, fruit were harvested from ~1000 trees in WA (n=225), MN (n=195), NY (n=155), New Zealand (n=100), Italy (n=200), and Belgium (n=100). Data collected in previous seasons is being provided in-kind by Plant Research International (Netherlands) and East Malling Research (U.K.). WTFRC support is providing funding for fruit quality evaluations currently underway in Wenatchee (n=380 from WA and NY-PGRU trees). Estimated costs for phenotyping in Washington were well below actual costs, and the discrepancy was largely made up by reallocating phenotyping "slots" from two other programs in the U.S., who were unable to obtain fruit this season, to WA. At harvest, fruit were evaluated for maturity, weight, crispness, firmness, juiciness, SSC, and TA. Internal ethylene concentration was also measured for fruit from WA trees. After 10 weeks of cold storage and 1 week of room temperature ripening, fruit were evaluated for crispness, firmness, juiciness, SSC, TA, and storage disorders. In 2010, a third set of measurements will be taken after 20+1 weeks of storage to identify those cultivars, and ultimately those specific DNA types, that are able to withstand long storage. Data will be used in NRI project in 2010 to understand role of major genes in apple texture and put knowledge to industry and breeding application.
Personnel involved: *Standardized phenotyping protocol development*: C. Peace, J. Luby, S. Brown, F. Costa, R. Dreesen, K. Evans, T. Schmidt, I. Hanrahan, P. Forsline, W. Guerra, J. Johnston, F.

Laurens, J. Mattheis, J. McFerson, S. McKay, N. Oraguzie, J. Palmer, S. Tustin, E. van de Weg. *WA germplasm choice*: C. Peace, K. Evans, B. Konishi. *Harvest of WA fruit*: B. Konishi, K. Evans, WTFRC crew. *Harvest of NY fruit for WA evaluation*: P. Forsline, N. Gutkin. *WA fruit quality evaluations*: T. Schmidt, I. Hanrahan, M. Bell, F. Castillo, B. Konishi, K. Evans, J. Mattheis.

3) National and international collaboration

In February, C. Peace attended and presented at a plant breeding education workshop in Christchurch, New Zealand, as one of four invited international guests. WTFRC funds enhanced this trip with visits to Plant and Food Research colleagues in Auckland (hosts: Andy Allan), Palmerston North (host: Sue Gardiner), and Hawke's Bay (host: Stuart Tustin), including discussions with Drs. Jason Johnston, R. Schaeffer, R. Atkinson, D. Chagne, and A. White. New opportunities were forged for phenotyping technologies and standardization, molecular genetics, and functional genomics of apple fruit quality. The standardized phenotyping protocols described in Activity 2 above were subsequently developed with international communication via email and telephone. C. Peace and K. Evans will visit apple breeding programs and germplasm collections in the U.S., Canada, and Europe in Summer 2010 to discuss and coordinate phenotyping efforts and MAB opportunities and challenges.

4) Outreach

Primary outreach aims are establishing cultivar performance groups based on fruit texture genes, demonstration of practical application of MAB, and soliciting stakeholder feedback. These efforts were coordinated where appropriate with the WTFRC-funded project, "Establishing the Marker-Assisted Breeding Pipeline for sweet cherry", and will be coordinated in later 2010 with outreach activities in the NRI project, "Functional gene markers for Rosaceae tree fruit texture". However, the departure from WSU of J. Olmstead leaves us without a leader for this part of the project.

In 2009, we participated in the 2009 WSHA Annual Meeting (Wenatchee, WA). A talk was presented by K. Evans, "Tree Fruit Breeding in Washington: Technologies, Tools and Teams", which included a description of marker assistance to the WABP. Also presented were a poster with genotyping props and a laptop-delivered 3-minute looping slideshow on "DNA-informed breeding: Efficient improvement of Washington tree fruit", attended by Pullman-based crew of WSU's Tree Fruit Molecular Genetics Program.

Personnel involved: *WSHA presentations*: K. Evans, C. Peace, and CP's Pullman crew of D. Edge-Garza, S. Haendiges, T. Rowland, S. Haldar, G. Lightbourn, S. Verma, and C. Starr.

5) Genotyping database

We continued efforts to develop a marker-processing database schema to manage the data inputs and outputs of routine high-throughput genotyping. We are teaming up with D. See and D. Main to hire a postdoc programmer for one year (2010) to develop the database and software tools. WTFRC funds will provide $10K, while D. See and D. Main will cover the other ~$45K. The WTFRC-funded project, "Developing an online toolbox for tree fruit breeding", will enhance the scope and functionality of this database. In addition, substantial funding obtained for new SCRI projects in 2009 will ensure that WTFRC seed-funding in this critical area is amplified many times over. The Breeding Information Management System (in the "RosBREED" project) will be developed from 2010 and the Breeders Gateway (in the "tfGDR" project) is under development in 2009-2010. In the meantime, a spreadsheet was developed for automatic summarizing of data for the ACS and ACO genotyping of several thousand WABP seedlings.

The Seedling Selection Efficiency Tool (as it is being called in the "RosBREED" SCRI project), which was developed in previous years and is in Excel spreadsheet format, was generalized to model selection schemes in other breeding programs and was applied to the PNW sweet cherry breeding program. Besides planned expansion and refinement of this decision-support Tool within "RosBREED", a postdoc funded by the blueberry breeding program of J. Olmstead (University of Florida) will develop the Tool further in 2010, including a visit of the postdoc to Pullman.

Personnel involved: *Databasing and software needs*: D. Main. D. See, K. Evans, C. Peace. *Coordination with other projects*: C. Peace, D. Main. *Interim spreadsheet*: C. Peace, D. Edge-Garza.

6) New cultivar DNA identification.

This task is benefitting from the NRI project described above that requires choice and application of 17 SSR markers for a "genome scan" of apple cultivars and their ancestors. SSR markers are well suited to the task of establishing uniqueness of individuals resulting from outcrossing species. We researched potential SSR markers from more than a decade of worldwide marker development, and chose one excellent SSR for each of the 17 chromosomes of apple. Based on reported information, we chose markers that were popular (averaging 3.9 papers for the chosen set of 17), had a high allele number in cultivars (averaging 8.1 alleles), had relatively high heterozygosity scores (averaging 0.82), and were preferably part of the recommended set from HiDRAS (16 of 17). Not all 17 of these SSRs will be required for the needs of this objective – in fact, just several highly informative SSR markers may be sufficient. We tested the 17 SSRs, and local germplasm genotyping is underway.

Marker data previously collected for functional markers such as the *Md-ACS1* and *Md-ACO1* genes and SSRs nearby other genes of interest can also be used for establishing uniqueness of WABP selections. We are developing a spreadsheet tool that uses all available DNA marker information to establish identity of WABP selections by calculating their probability of uniqueness and that can confirm or deduce parentage. As more marker data is obtained and added to the spreadsheet, uniqueness probabilities and parentage verification/deduction power will increase.

Personnel involved: *SSR choice*: C. Starr, C. Peace, E. van de Weg. *Uniqueness and parentage spreadsheet*: C. Starr, C. Peace.

7) Marker-assisted breeding for the WABP

As new promising DNA information for apple surfaces, we are taking them through the 8-stage MAB Pipeline, so that those with WABP utility and cost-efficiency are applied in breeding. Currently, for routine marker-assisted seedling selection (MASS) in 2010, one genetic test is available (*Md-ACS1* for long storability), and another genetic test will probably be ready (*Md-ACO1* for long storability). Five more tests could be ready with concerted effort in early 2010 (*Md-Exp7* for firmness; markers for the *Ma* locus simultaneously influencing acidity, crispness, and juiciness; markers for a second major genomic region influencing acidity; markers for the *2MBAc* locus for "ripe apple flavor"; and *Md-MYB1* for degree of skin blush). Crossing decisions can be and have been supported (i.e., marker-assisted parent selection, MAPS) with three genetic tests (*Md-ACS1*, *Md-ACO1*, and *Ma*), and could be supported further in 2010 with two other genetic tests (*Md-Exp7* and *Md-MYB1*) and with more precise functional information on the *Ma* locus.

Three talks in this area were given in 2009 to diverse audiences, particularly drawing on experiences in MAB development for the WABP. In February, C. Peace presented "Tree fruit marker-assisted breeding" at the Trends in Plant Breeding workshop in Christchurch, New Zealand. At the United Fresh annual meeting in April, C. Peace presented "Marker-assisted breeding for tree fruit" in Las Vegas, NV. A presentation on MAB pipelining of *Md-ACS1* ("Successful application of marker-assisted seedling selection in the Washington apple breeding program") was the basis of a scientific talk by D. Edge-Garza in July at the ISHS Symposium on Molecular Markers in Horticulture (http://oregonstate.edu/conferences/molecularmarkers2009/Symposium-Presentations/Session2/8-Garza.pdf). C. Peace also described MAB pipelining within the new "RosBREED" project at the ISHS Symposium in July and at the Apple CGC meeting in October.

Personnel involved: *Prioritization*: K. Evans, F. Bliss, C. Peace. *Genotyping efficiency*: D. Edge-Garza, T. Rowland, D. See. *Marker improvement*: D. Edge-Garza, S. Haendiges, D. See, C. Peace. *Validation and Utility*: C. Peace, E. van de Weg, M. Bink, Y. Zhu, K. Evans. *MAPS*: K. Evans. *MASS cost efficiency*: C. Peace, D. Edge-Garza, J. Olmstead, K. Evans, F. Bliss. *MASS trial use*: D. Edge-Garza, T. Rowland, B. Konishi, C. Peace, K. Evans.

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

Project Title: Apple ACS3 genotypes and fruit ripening

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Cameron Peace DHLA, WSU, 509-335-6899 cpeace@wsu.edu

Other funding sources

Agency Name: AgroFresh
Amt. awarded: $35,000 for the second year study
Notes: Funds cover 0.5 FTE GS9 Postdoctoral Research Associate

Budget:

Organization: USDA, ARS		**Contract Administrator:** Charles Myers, Extramural Agreements Specialist
Telephone:(510) 559-6019		**Email:**cwmyers@pw.ars.usda.gov
Item	**2009**	**2010**
Salaries*	25,000	26,000
Benefits	9,000	9,000
Wages		
Benefits		
Equipment		
Supplies	11,000	11,000
Travel	1,500	1,500
Miscellaneous	1,500	1,500
Total	$48,000	**$49,000**

*0.5 FTE GS9 Postdoctoral Research Associate

Supplies include common molecular biology reagents and fruit from commercial orchards.

OBJECTIVES

1. Characterize apple fruit ripening characteristics including ethylene evolution for 6-10 cultivars at defined developmental stages.
2. Investigate cultivar-specific fruit softening rate and ethylene regeneration in fruits treated with 1-MCP.
3. Examine expression patterns of ACS3 and other ethylene biosynthesis and perception related genes during fruit ripening and in response to 1-MCP treatments (including Harvista on-tree spraying).
4. Explore the potential polymorphism at the ACS3 locus for potential functional molecular marker generation, based on gene expression results.

SIGNIFICANT FINDINGS

1. During the 8-week period of apple fruit maturation (from -6 to +2 weeks with commercial maturity as 0 stage), considerable variation in the expression levels of MdACS3 were observed among 14 elite apple cultivars and breeding parents.

2. Two distinguishable expression patterns of MdACS3 in apple fruit peel tissues were identified. Pattern a: higher expression level with progressively-increased patterns, and Pattern b: lower expression level with a transient peak.

3. Higher expression of MdACS3 usually correlated with early ripening cultivars; and lower expression of MdACS3 usually correlated with late ripening cultivars.

4. The two MdACS3 expression patterns were also correlated with "on-tree fruit firmness retention" during last 6 weeks before harvest, i.e. the early-ripening cultivars showed larger decease and late-ripening cultivars had smaller decease in fruit firmness.

5. Postharvest 1-MCP treatment did not suppress MdACS3 expression level, instead a slight increase was observed for most cultivars; the suppression on MdACS1 expression was apparent for most cultivars as late as three months after 1-MCP treatment.

6. Correlation between cultivar-specific MdACS3 expression patterns and 1-MCP treatment efficacy was weak. However, early-ripening cultivars showed a greater average "fruit firmness retention" (difference between firmness values from 1-MCP treated fruit and control) than that of late-ripening group.

METHODS

1. Physiological characterization of apple fruit maturation/ripening:

Fruit from 14 apple (*Malus* × *domestica* Borkh.) cultivars, from commercial orchards or experimental orchards in central Washington State, will be subjected to systematic characterization of fruit maturation and ripening processes from 2 months before projected commercial harvest date. Commercial maturity (stage 0) for each cultivar will be retrospectively assigned based on the sample with starch staining index close to 3.5 according to Cornell composite standard. Fruits harvested at stage 0 will be treated with 1-MCP then stored in air at 33^{o} F.

2. Gene expression analysis for MdACS3 and other genes encoding ethylene receptor and signaling pathway:

Peel tissue will be collected and used for total RNA isolation, followed by DNase I digestion, RNA cleanup and cDNA Synthesis. Quantitative Real-Time PCR using SYBR Green I dye will be used for gene expression analysis, including –RT (no reverse transcriptase), no template control (no cDNA) and an actin gene as internal reference genes. Quantitative PCR reaction will be repeated twice with two independent cDNAs. Target gene expression will be normalized to that of the reference actin gene and analyzed by $2^{-\Delta\Delta CT}$ method.

RESULTS AND DISCUSSION

1. Among 14 cultivars investigated, ripening dates for these cultivars span from early August to early November (Figure 1). This germplasm collection provided a useful genetic background for studying the MdACS3 expression patterns. Among 14 cultivars, considerable variations in the expression levels or the abundance of MdACS3 transcripts were observed (Figures 2A and 2B). From the early cultivar 'Sunrise' to 'Pink Lady', the gene expression level varies up to 4 orders of magnitude.

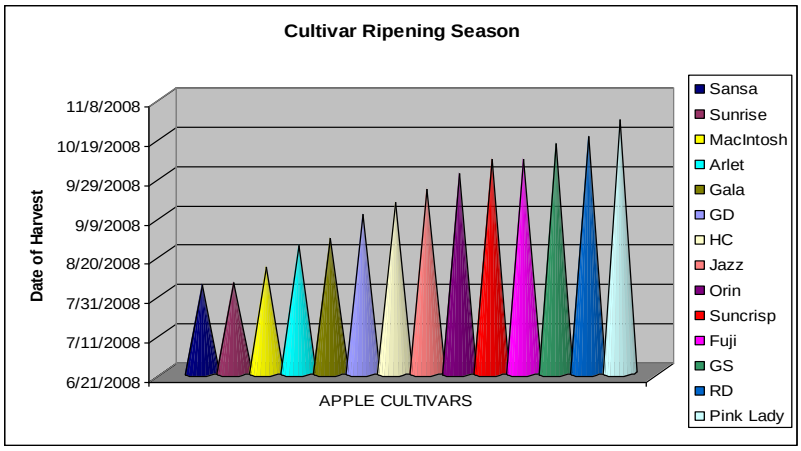

Figure 1. Ripening dates for 14 cultivars, from left to right, 'Sansa', 'Sunrise', 'McIntosh', 'Arlet', 'Gala', 'Golden Delicious', 'Honeycrisp', 'Jazz', 'Orin', 'Suncrisp', 'Fuji', 'Granny Smith', 'Delicious' and 'Pink Lady'

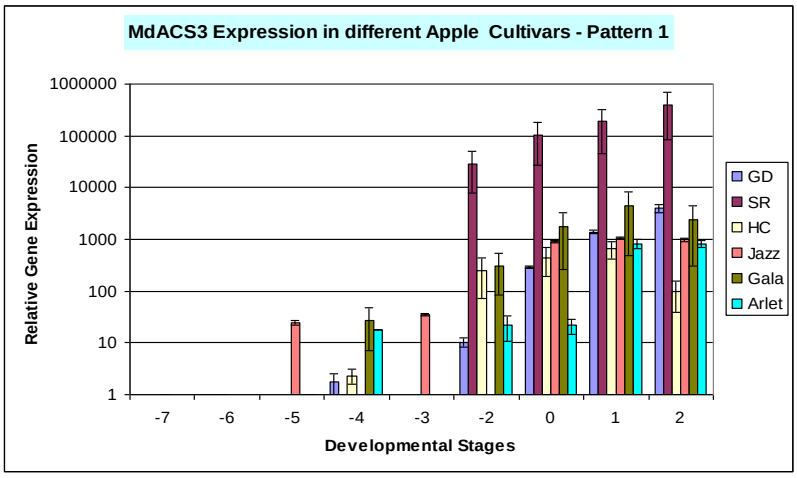

Figure 2A. Cultivars with high expression level with progressively-increased pattern, including 'Golden Delicious', 'Sunrise' 'Honeycrisp', 'Jazz', 'Gala', and 'Arlet'.

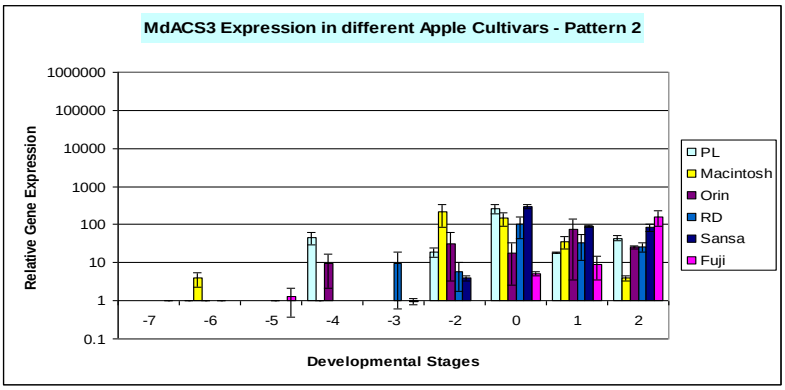

Figure 2B. Cultivars with low expression level with transient peak. These cultivar include: 'Fuji', 'Pink Lady', 'Orin', 'Sansa', 'Delicious' and 'McIntosh'

3. In general, the expression level of MdACS3 was attenuated in fruit 4 weeks after harvest. 1-MCP treatment did not suppress but in fact stimulated MdACS3 expression (Figure 3A). Treatment with 1-MCP suppressed on MdACS1 expression for all cultivars (Figure 3B).

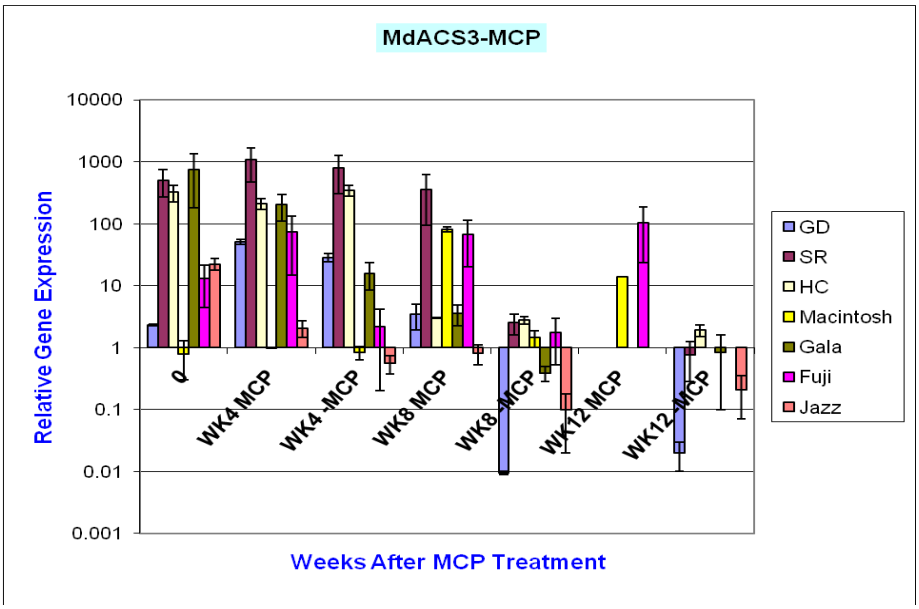

Figure 3A. Effect of 1-MCP treatment on MdACS3 expression level.

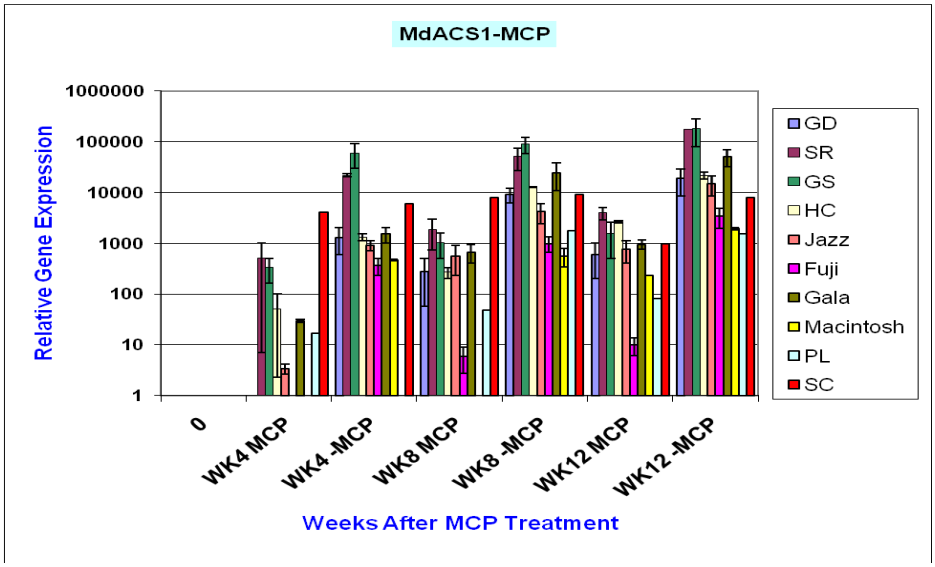

Figure 3B. Effect of 1-MCP treatment on MdACS1 expression level.

4. The cultivar-specific on-tree firmness decrease in the six weeks before commercial maturity was correlated with fruit ripening season and MdACS3 expression level in most cases (Figure 4A). Firmness loss was greater for the early ripening cultivars 'Golden Delicious', 'Sunrise' 'Honeycrisp', 'Gala', and smaller decreases were observed for later ripening cultivars such as 'Fuji', 'Pink Lady', , 'Granny Smith', and 'Suncrisp'.

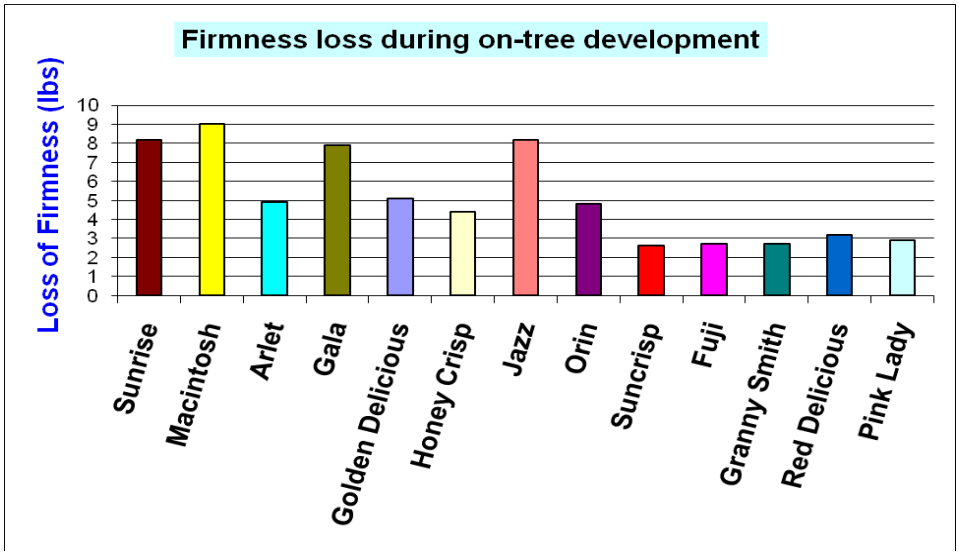

Figure 4A. The values of fruit firmness loss during last 6 weeks of "on-tree ripening" are correlated with fruit ripening season.

5. A clear relationship between 1-MCP treatment efficacy and MdACS3 expression pattern were not observed (Figure 4B) suggesting efficacy is dependent on factors other than MdACS3 expression alone. However, early-ripening cultivars had higher average firmness maintenance (3.1 pounds) than the late-ripening group (1.5 pounds).

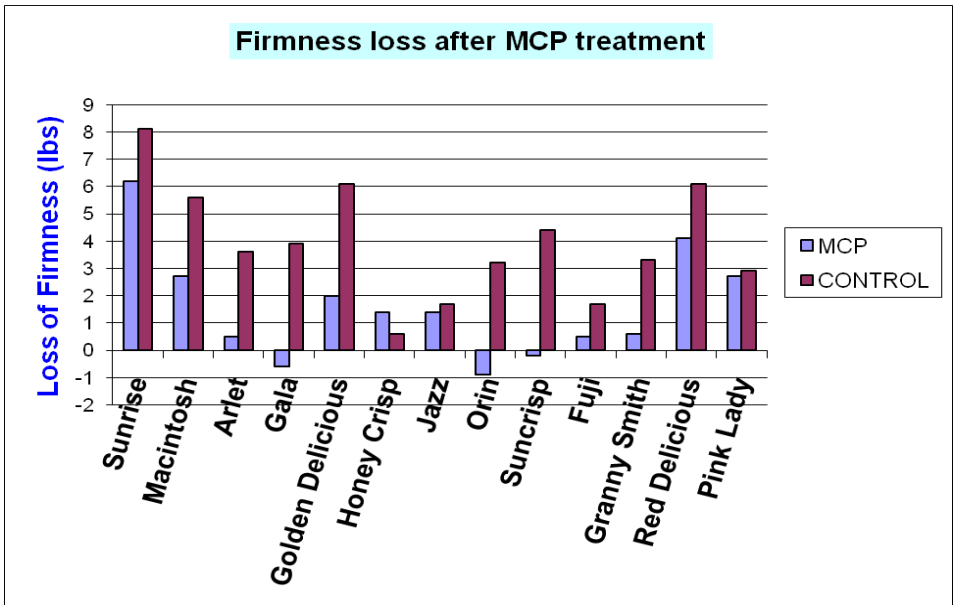

Figure 4B. Fruit firmness loss between 1-MCP treatment and non-treatment control among cultivars studied.

The results suggest high MdACS3 expression level would not be beneficial if the firm fruit is the breeding target; at the same time, allelotypes with low MdACS3 expression could be the indicator for late ripening cultivars.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-08-806

Project Title: Integration of storage technologies for fruit quality management

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Cooperators: Yanmin Zhu, USDA, ARS, TFRL, Wenatchee
Chris Watkins, Dept. Horticulture, Cornell University, Ithaca, NY

Total Project Request: Year 1: $27,075 **Year 2:** $27,863 **Year 3: $28,675**

Other funding sources

1. Agency Name: USDA-NIFA (competitive grant)
Amount requested (Federal + non-Federal): $2.4 million (total) over 4 years.
Notes: D. Rudell is Project Director, J. Mattheis is a Co-PI. The Standard Research and Extension Project, "A diagnostic toolbox for integrated management of postharvest apple necrotic disorders" was submitted (01/12/10) for the current funding cycle. WTFRC and AgroFresh, Inc. are co-sponsors.

Agency Name: AgroFresh, Inc.
Amount awarded: $19,000
Notes: Funding supports 'Honeycrisp' storage research.

Budget 1:

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

Item	2008	2009	2010
Salaries	19,605	20,198	20,808
Benefits	$6,470	6,665	6,867
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	1000	1000	1000
Travel	0	0	0
Miscellaneous	0	0	0
Total	$27,075	$27,863	**$28,675**

Objectives:
1. Identify postharvest protocols to maximize storage life of 'Honeycrisp' and other softscald-sensitive cultivars. This information will contribute to development of postharvest protocols to successfully handle and store the increasing volume of 'Honeycrisp' produced in Washington.
2. Characterize physiological events that result in softscald symptom development. The goal is to identify metabolic and/or molecular markers that could be used for cultivar improvement and/or identify in-season susceptibility to softscald development.
3. Identify minimum storage duration required to control superficial scald by use of oxygen setpoints below 1%. This information would provide warehouses an additional means to operate CA rooms to optimize quality of conventional and organic fruit.
4. Identify what if any limits for CO_2 exist during apple storage in oxygen below 1%. CA disorders related to CO_2 may be manageable using lower O_2 due to less CO_2 accumulation in the low O_2 environment.

Significant Findings:
1. 'Honeycrisp' storage regimes that included 7 days at 50 ^oF in air after harvest largely controlled development of soft scald and soggy breakdown.
2. Late harvest 'Honeycrisp' apples (starch 6.0, yellow ground color, slight greasiness) were at high risk of development of internal radial browning and peel dimpling 2 months after harvest.
3. Risk of 'Honeycrisp' radial browning: increased with decreased O_2 concentration in CA; and decreased with use of SmartFresh.
4. Harvista-treated 'Honeycrisp' apples had less softscald and soggy breakdown compared to non-treated or SmartFresh-treated fruit.
5. CA (1.5% O_2/1% CO_2) storage of 'Honeycrisp' reduced greasiness and acid loss regardless of temperature (33, 36, 39 ^oF) compared to fruit stored in air at 39 ^oF.
6. Differences in 'Honeycrisp' volatile production were evident between controls and SmartFresh-treated fruit stored in air or CA.
7. Changes in 'Honeycrisp' metabolism relateable to softscald were detected prior to symptom development.
8. 'Granny Smith' apples stored at 0.2% O_2 above the low O_2 fluorescence deflection point + 1% CO_2 did not develop superficial scald through 7 months in CA +21 days RA + 21 days at 68 ^oF, or 7 months CA +10 days RA + 28 days at 68 ^oF.

Methods

Fruit from commercial orchards were used for all experiments. 'Honeycrisp' apples from multiple lots were used for softscald and storage studies, 'Granny Smith' for low O_2 superficial scald studies. All fruit were stored utilizing existing cold storage and controlled atmosphere facilities in our laboratory. Fruit quality analyses (color, firmness, texture, soluble solids content, titratable acidity, volatile production) were conducted using established methods and existing equipment. Fruit firmness/texture assessment was conducted using a recording penetrometer. Ethylene and CO_2 production were measured using gas chromatography with flame ionization detection, and other volatiles by GC-MS. Monitoring of both volatile (aldehydes, alcohols, esters, others) and non-volatile compounds was used to characterize physiological events during the onset of softscald symptom development. Non-volatile compounds will be analyzed using GC-MS with a focus on respiratory pathway components in the outer cortex and peel. Genetic analysis of the same tissues will utilize subtractive hybridization to identify uniquely expressed genes prior to symptom development in fruit stored under conditions known to induce the disorder. Peel fluorescence of fruit stored in low (less than 1%) O_2 has been monitored using existing HarvestWatch sensors.

Results and Discussion
'Honeycrisp' storage experiments.

Experiment 1. Influence of CA and/or SmartFresh on 'Honeycrisp' quality. Fruit were stored at 50 °F for 7 days then were transferred to 33°F. SmartFresh was applied the day of harvest while fruit were held at 50°F. Two days after transfer to 33°F, controls and SmartFresh treated fruit were placed into CA chambers and an atmosphere containing 1.5% O_2 and 1% CO_2 was established. Softscald/soggy breakdown did not develop in any fruit, and incidence of bitter pit, decay, cortex and core browning were very low. Titratable acidity loss was slowed by treatment with SmartFresh and/or storage in CA. Peel yellowing was also slowed by CA as well as SmartFresh (5 months only). Radial internal browning developed between 5 and 7 months in CA-stored fruit. Incidence of radial browning was decreased if fruit had been treated with SmartFresh. Treatment effects for firmness were evident at 5 but not 7 months.

months	Treatment	Titratable Acidity %	Ground color 1-5	Radial Browning %	lbs
Harvest		0.500	3.8	--	15.7
5	Control	0.329b	5.0a	0	14.3b
	SmartFresh	0.367ab	4.6b	0	15.3a
	Control-CA	0.371ab	3.8c	0	15.0a
	SmartFresh-CA	0.395a	3.7c	0	15.3a
7	Control	0.258c	5.0a	0b	14.6
	SmartFresh	0.287bc	5.0a	0b	13.9
	Control-CA	0.320ab	4.2b	28a	14.1
	SmartFresh-CA	0.336a	4.3b	6b	14.7

Table 1. Experiment 1 results for 'Honeycrisp'.

Experiment 2. 'Honeycrisp' storage temperature in air and CA. All fruit were held at 50 °F for 7 days after harvest followed by transfer to 33, 35, or 37°F. After two additional days, CA (1.5% O_2, 1.0% CO_2) was imposed on some fruit at each of the 3 temperatures. Fruit was evaluated monthly for external disorders, and quality was assessed after 4 and 6 months plus 7 days at 70°F. No softscald/soggy breakdown or radial browning developed regardless of storage temperature and bitter pit incidence was also very low. Titratable acidity loss decreased with decreased storage temperature and CA storage. Ground color change from green to yellow was slowed by CA and 33 °F only at 4 months. No treatment effects on firmness were detected.

Months	Treatment	Titratable acidity %	Ground color 1-5	Greasiness %	Decay %	lbs
4	Air: 33 F	0.339cd	5.0a	6c	11a	13.9
	36	0.358bc	5.0a	14b	17a	14.9
	39	0.313d	5.0a	83a	3b	13.9
	CA:33	0.414a	4.3b	0c	3b	14.0
	36	0.365bc	5.0a	3c	0b	13.8
	39	0.383b	5.0a	0c	3b	14.4
6	Air: 33 F	0.290bc	5.0	4b	15b	13.8
	36	0.276c	5.0	13b	15b	13.8
	39	0.276c	5.0	40a	11b	14.2
	CA:33	0.372a	5.0	4b	0a	13.3
	36	0.381a	4.9	4b	11b	13.9
	39	0.321b	4.9	0b	7ab	13.4

Table 2. Experiment 2 results for 'Honeycrisp.

Experiment 3. Oxygen concentration during 'Honeycrisp' storage. Fruit were stored in air or in 1% CO_2 with 0.5, 1.5, or 2.5% O_2 at 33°F. All fruit was held at 50°F for 7 days prior to transfer to 33°F. No fruit developed softscald/soggybreakdown, bitter pit, or other internal disorders. All CA regimes reduced titratable acidity loss between 4 and 6 months, and some CA effects were evident for ground color. Greasiness and firmness were not influenced by CA regime.

Months	Treatment	Titratable acidity %	Ground color 1-5	Greasiness %	lbs
4	Air	0.381	5a	3	15.0
	O_2: 0.5	0.386	5a	0	14.3
	1.5	0.387	4b	0	14.9
	2.5	0.386	4b	0	14.1
6	Air	0.298b	5a	3	14.2
	O_2: 0.5	0.362a	5a	0	13.9
	1.5	0.391a	5a	0	14.2
	2.5	0.379a	4b	0	14.4

Table 3. Experiment 3 results for 'Honeycrisp'.

Experiment 4. 'Honeycrisp' CA with chlorophyll fluorescence evaluation. Fruit held at 50°F for 7 days after harvest were transferred to 33°F. After two additional days, a CA chamber was sealed and a change in chlorophyll fluorescence was detected at 0.3% O_2. CA O_2 concentrations of 0.3, 0.5, 0.8, and 1.5% were established (each with 0.5% CO_2). Titratable acidity loss decreased and radial browning increased with decreased O_2 for fruit stored 6 months. CA-stored fruit degreened at a slower rate compared to fruit stored in air after 6 months. Softscald incidence was low and no soggy breakdown was observed. Firmness in the cortex region from 0.25" inside the peel to the core line was higher in CA-stored fruit compared to air after 6 months.

Months	Treatment	Titratable acidity %	Ground color 1-5	Soft scald %	Radial browning %	Lbs 0-0.25"	Lbs 0.25-core
4	Air	0.314c	5a	0	0	15.0	22.6
	O_2: 0.3	0.346bc	5a	0	3	14.1	22.0
	0.5	0.346bc	4b	3	3	14.7	22.8
	0.8	0.413a	5a	0	11	15.5	23.4
	1.5	0.358b	5a	3	6	14.9	22.9
6	Air	0.249d	4.9a	0	0b	13.8	20.6b
	O_2: 0.3	0.387a	4.3b	6	31a	14.9	23.2a
	0.5	0.380ab	4.4ab	8	33a	14.8	23.3a
	0.8	0.350bc	4.3b	0	19a	14.4	23.0a
	1.5	0.325c	4.2b	0	6b	14.9	22.2ab

Table 4. Experiment 4 results for 'Honeycrisp'.

Experiment 5. 'Honeycrisp' softscald metabolism. Fruit obtained at commercial maturity were stored continuously in air at 33°F, or at 50°F for 7 days followed by 33°F. Apples were rated for softscald development after 1, 2, 3, 4, and 8 weeks. Softscald symptoms were evident beginning at 2 weeks after harvest in fruit stored continuously at 33°F indicating changes in fruit physiology that lead to disorder development are rapidly induced by cold temperature. No fruit held at 50° prior to 33°F developed softscald. Chemical analysis of peel tissues identified 20-30 possible markers for softscald that were detected prior to symptom development.

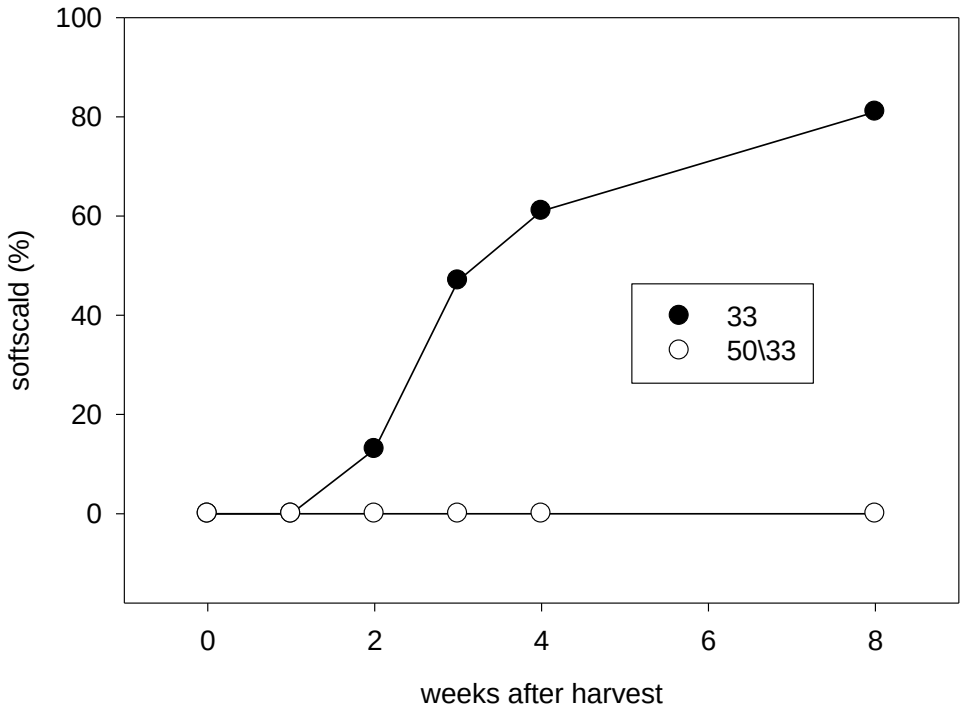

Figure 1. Incidence of 'Honeycrisp' softscald. Fruit were stored in air at 33°F continuously or at 50°F for 7 days then 33°F.

Low oxygen storage experiments

Experiment 1. 'Granny Smith' chlorophyll fluorescence during CA. Fruit harvested at commercial maturity were cooled to 33°F and held for 2 days. CA conditions were established (CO_2: 1% 'Granny Smith') at 1.2% or 0.5% O_2 determined by the O_2% at which a change in peel fluorescence was observed (0.3%) plus 0.2%. Fruit evaluated at 3, 5, 7, and 9 months were held at 33 °F in air for 10 or 21 days prior to holding at 68 °F for up to 28 days. Air-stored fruit developed scald at 3 months plus 7 days and thereafter. Fruit stored in CA (1.2% O_2) developed scald at 5 months plus 10 days RA plus 7 days at 68 °F. Fruit stored in 0.5% O_2 developed scald after 7 months plus 21 days RA plus 28 days at 68 °F, a point at which fruit was well past its market life due to other quality (firmness, color, shrivel) characteristics. At 9 months fruit held 10 or 21 days in RA developed scald during the 7 day period at 68 °F.

Month	Treatment	Days RA after CA	Days at 68 °F to superficial scald symptom expression	Scald Incidence %
7	Air	10	0	67
	O_2: 1.5%	10	7	100
	0.5	21	28	22
9	Air	10	0	72
	O_2: 1.5%	10	7	100
	0.5	10	7	25

Table 5. Experiment 1 scald results for 'Granny Smith'.

Experiment 2. 'Delicious' chlorophyll fluorescence during CA. Fruit harvested at commercial maturity were cooled to 33 ^{o}F over 2 days. CA conditions were established (CO_2: 2%) with O_2 at 0.2, 0.4, 0.7, 1.2, and 2.0% based on a change in fluorescence at 0.2%. Scald did not develop on any stored fruit. At 4 or 6 months plus 7 days at 68 ^{o}F, all fruit stored in CA had higher firmness and acidity compared to fruit stored in air, but a clear difference within CA treatments was observed only for fruit stored below 2% O_2. Some cortex and core browning developed in fruit stored 6 months in air or at 0.2% O_2.

Months	Storage	Soluble solids %	Titratable acidity %	Firmness: outer cortex lbs	Cortex browning %	Core browning %
0		11.4	0.297	15.7	0	0
4	Air	13.7ns	0.174c	10.3c	0	0
	O_2: 0.2	14.1	0.236ab	13.7a	0	0
	0.4	13.5	0.228b	12.7ab	0	0
	0.7	13.1	0.251a	13.3a	0	0
	1.2	13.0	0.251a	13.6a	0	0
	2.0	14.3	0.221b	11.3bc	0	0
8	Air	12.7ns	0.131c	10.6cd	17a	6b
	O_2: 0.2	13.4	0.201a	10.8bc	11a	15a
	0.4	13.0	0.226a	12.5a	4b	4b
	0.7	13.1	0.230a	11.7abc	0b	0b
	1.2	12.8	0.238a	12.2ab	0b	0b
	2.0	14.2	0.196b	9.1d	6b	0b

Experiment 2 results for 'Delicious'. Values followed by different letters are significantly different. ns: not significant

CONTINUING PROJECT REPORT **YEAR**: 2009

Project Title: Programs to increase packouts of apples

PI: Ines Hanrahan
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Telephone/email: 1 509 669 0267
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Address: 104 N 1st St., Suite 204
City: Yakima
State/Province/Zip WA, 98901

Cooperators: Tom Auvil, Felipe Castillo, Tory Schmidt, WTFRC, Wenatchee, WA

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

Item	2008	2009	2010
Salaries	16,847	19,860	16,000
Benefits (32%)	7,929	9,346	5,120
Wages	30,464	6,740	7,000
Benefits (16%)	14,336	1,170	1,200
Equipment + supplies	5,000	2,190	2,200
RCA rental	9,600	18,270	18,270
USDA rental	750	750	750
Travel	2,000	4,000	4,000
Reimbursements	9,400	29,000	20,000
Total	$77,526	$33,326	**$34,540**

Salaries: include proportional time spent on outlined projects for Hanrahan, Castillo, Schmidt, Auvil
Wages: covers timeslip expenses
RCA rental: numbers based on fiscal year (2.9 rooms @ $6,300)
USDA rental: access to packingline and storage space for equipment
Travel: fuel costs to travel to and from trial sites and vehicle maintenance
Reimbursements: monetary contributions by chemical suppliers
Other: all chemicals were donated by industry suppliers

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

OBJECTIVES:

1. Compare sunburn protectant efficacy in apple and evaluate ease of cleanup in the warehouse.
2. Facilitate field testing of promising approaches to mitigate lenticel breakdown in apples.
3. Can Honeycrisp apples be stored until February?
4. Evaluate horticultural effects of kelp-based products.

SIGNIFICANT FINDINGS:

Sunburn: Materials tested in 2009 did not increase the percentage of sunburn-free fruit. Most materials cleaned easily off fruit flanks. Residues of particle films remained visible in the stem bowl after drying.

LB: No treatment effect was noted after preharvest application of hydrophobic spray emulsions in the 2008/09 season. EpiShield applications reduced weight loss in storage.

Honeycrisp: Fruit from different locations stored well until March (approx. 6 months) when placed in CA or DCA storage conditions. RA stored fruit became greasy in January.

Kelp-products: After two years of evaluation we have not seen any measurable effect on horticultural performance of apples.

METHODS

Sunburn suppression: Three trials were established near Manson, WA, (Granny Smith/M.106, Golden Delicious/M.26 Manson, Braeburn/M.26) testing a variety of commercially available sunburn protectants (Eclipse, FruitShield, Raynox Plus, Pace Experimental, Surround WP, GM903005, SunShade). All materials were applied starting on June 24 four times according to each product's respective labeled rate except for Eclipse and SunShade which were applied five times starting June 10. At harvest, individual fruit was graded for sunburn according to the Schrader/McFerson system (0 = clean, 6 = necrosis). The ease of cleanup was evaluated by running fruit over the USDA-ARS packing line in Wenatchee while adjusting the dump tank pH to either 5.5 or 7.6. No wax was applied. Fruit was allowed to dry for 24 hours before evaluation. Trials were additionally set up in the Manson Granny block to test a) the onset of delayed sunburn in storage as influenced by Raynox Plus and b) the influence of late season applications of Raynox Plus on the development of Granny Smith blush.

Lenticel breakdown: In 2008 we conducted 2 trials sprayed with a PropTec (100gal/acre unless otherwise specified) using a randomized complete block design with 4 replications and 20 trees/treatment/rep. We tested the following materials: EpiShield as 2.5% or 1.5% solution with one or two weekly applications, Safe-T-Side at 32 oz/acre, BlueStim at 4 lbs/acre and 8 oz/acre Monterey Suoer 7 surfactant and Platina at 0.11 gal/acre. Materials were applied at 2 weekly timings starting at 2 weeks before anticipated harvest. We also conducted 4 grower-applied trials (2 Gala, 1 Fuji, 1 Golden Delicious) utilizing whole rows. Trial layout was a randomized complete block design with 4 replications. EpiShield was applied once or twice as 2.5% solution, starting either two or one weeks prior to anticipated harvest. Samples were stored under CA conditions and evaluated for LB incidence after 6 months utilizing the ARS packing line to induce lenticel breakdown.

Honeycrisp storage: We selected 3 orchards in 2008 (Prescott, Brewster, Manson) based on the following criteria: even crop load with minimal alternate bearing, trees being at least 4 years old. Harvest timings were a) one week prior to anticipated first pick, and b) first pick (or best-storing pick). Fruit was transported to Stemilt RCA facility and held for 1-3 weeks at 50F before being stored at 38F in RA, CA (0.5% CO_2, 1.5% O_2) or DCA (0.5% CO_2, 0.7% O_2) until the end of March 2009. Monthly pulls were carried out to evaluate storage performance. Trials from the 2009 growing season are currently in storage.

RESULTS AND DISCUSSION

Sunburn suppression: Sunburn is the primary physiological cause of cullage, sometimes damaging up to 50% of the fruit in a given orchard. Previously, WTFRC trials have shown calcium-based products (Eclipse, FruitShield) to perform as well as industry standards (Raynox, Surround WP). We revisited the question of sunburn protection product efficacy in 2007 and repeated the trials in 2008 and 2009 as several new products were introduced to the market (Table 1).

Table 1. Sunburn protectants used in field trials. WTFRC 2007-2009.

Active Ingredient	Commercial product(s)
Plant Wax	Raynox Plus
Kaolin	Surround WP, Cocoon
Talc	Invelop
Calcium carbonate	SunGuard, Eclipse, SunShade
Calcium carboxylic acids	Fruit Shield

Table 2. Sunburn severity readings at harvest in Braeburn, Granny Smith and Golden Delicious apples. WTFRC 2009.

	FIELD SUNBURN INCIDENCE[a]					
TREATMENT	Clean (%)	Y1 (%)	Y2 (%)	Y3 (%)	Tan (%)	Black (%)
Braeburn / M.26 - Manson						
Eclipse	86 ns	11 ns	2 ns	0 ns	0 ns	1 ns
FruitShield	81	13	3	2	0	2
GM903005	85	7	3	0	0	2
Raynox Plus	88	9	1	1	0	2
Pace experimental	88	7	1	1	0	1
SunShade	90	9	1	0	0	0
Surround WP	89	9	1	1	0	0
Control	87	9	2	1	1	1
Golden Delicious / M.26 - Manson						
Eclipse	97 ns	2 ns	0 ns	0 ns	0 ns	1 ns
FruitShield	95	3	1	0	1	1
GM903005	94	3	1	1	1	1
Raynox Plus	94	3	1	1	0	1
Pace experimental	96	3	1	0	0	0
SunShade	93	4	2	0	0	1
Surround WP	97	2	0	0	0	1
Control	93	4	2	1	0	1
Granny Smith / MM.106 - Manson						
Eclipse	86 ab	5 ns	3 ns	5 ab	1 ns	0 ns
FruitShield	80 b	6	4	7 a	3	0
GM903005	84 ab	8	5	3 ab	1	0
Raynox Plus	83 ab	9	3	3 ab	3	0
Pace experimental	85 ab	6	3	4 ab	3	0
SunShade	88 ab	4	3	4 ab	2	0
Surround WP	90 a	5	2	3 ab	2	0
Control	87 ab	6	4	2 b	2	0

[a] based on 'Schrader-McFerson' scale

Table 3. Residues of sunburn protectants following packing line treatment at two pH levels. WTFRC 2009.

	SPRAY RESIDUE pH 5.5 WASH				SPRAY RESIDUE pH 7.6 WASH			
TREATMENT	Clean (%)	Bowl (%)	Side (%)	Calyx (%)	Clean (%)	Bowl (%)	Side (%)	Calyx (%)
Braeburn / M.26 - Manson								
Eclipse	96 ab	4 b	0 ns	0 ns	95 ab	3 cd	0 b	2 ns
FruitShield	83 b	15 b	0	2	91 b	8 bc	0 b	2
GM903005	98 ab	3 b	0	0	93 b	6 bcd	0 b	1
Raynox Plus	97 ab	3 b	0	0	94 ab	6 bcd	0 b	0
Pace experimental	96 ab	4 b	0	0	87 b	12 b	0 b	2
SunShade	87 b	13 b	0	0	88 b	7 bcd	2 a	3
Surround WP	53 c	45 a	0	2	60 c	36 a	0 b	4
Control	100 a	0 b	0	0	100 a	0 d	0 b	0
Golden Delicious / M.26 - Manson								
Eclipse	99 a	1 b	0 ns	0 b	99 a	1 b	0 ns	0 ns
FruitShield	100 a	0 b	0	0 b	100 a	0 b	0	0
GM903005	100 a	0 b	0	0 b	100 a	0 b	0	0
Raynox Plus	100 a	0 b	0	0 b	99 a	1 b	0	0
Pace experimental	100 a	0 b	0	0 b	100 a	0 b	0	0
SunShade	99 a	1 b	0	0 b	99 a	1 b	0	0
Surround WP	74 b	23 a	0	3 a	88 b	12 a	0	0
Control	100 a	0 b	0	0 b	100 a	0 b	0	0
Granny Smith / MM.106 - Manson								
Eclipse	98 a	2 b	0 ns	1 b	98 a	2 b	0 ns	0 b
FruitShield	100 a	0 b	0	0 b	97 a	3 b	0	1 b
GM903005	98 a	3 b	0	0 b	97 a	3 b	0	0 b
Raynox Plus	100 a	0 b	0	0 b	100 a	0 b	0	0 b
Pace experimental	100 a	0 b	0	0 b	100 a	0 b	0	0 b
SunShade	98 a	3 b	0	0 b	98 a	3 b	0	0 b
Surround WP	65 b	30 a	0	5 a	65 b	32 a	0	3 a
Control	100 a	0 b	0	0 b	100 a	0 b	0	0 b

Although we typically have found in previous years that all materials increase the percentage of sunburn-free fruit, results of the 2009 growing season did not follow that pattern (Table 2). We can not explain the results but believe that this points out that season to season variability is an important factor when evaluating efficacy of any type of spray applied to orchards.

A common concern with sunburn protectants is the ease of cleanup in the warehouse. Ideally, fruit emerges free of residue after a standard washing and rinsing. We simulated this process by running fruit over the USDA-ARS packing line in Wenatchee while adjusting the dump tank pH to either 5.5 or 7.6. Visible residues were observed after running fruit over the line and following a 24 hour drying period. The dump tank pH did not influence the ease of clean-up in our experiments. All materials cleaned easily off fruit flanks. Residues remained in the stem bowls and the calyx at significantly higher levels for Surround WP (Table 3).

The market expects Granny Smith apples to be free of any blush. Blush develops in the last month before harvest, especially when cold nights (below 36F) and clear days (high rates of UV) coincide. We frequently observe such conditions in Washington State. Since both, low temperatures and high levels of UV radiation are necessary for the development of the blush, it might be possible to

minimize this condition by applying known UV blockers such as Raynox Plus. At our experimental Granny Smith site we observed 90+ % blush in fruit at harvest. Neither a standard Raynox Plus program nor targeted applications when nighttime temperatures dipped below 36F in the fall changed the frequency of blush occurrence (data not shown). Samples for delayed sunburn are currently in storage.

Table 4. Effects of preharvest application of hydrophobic materials on LB development and weight loss of apples after 6 months of CA storage. WTFRC 2008-09.

	WT LOSS	LENTICEL READINGS			
		CLEAN	SLIGHT	SEVERE	TOTAL LB
TREATMENT	(%)	(%)	(%)	(%)	(%)
Gala / M.26 - Manson					
Bluestim	3.1 a	66 ns	22 ab	13 ns	34 a
EpiShield 1.5 once	2.6 b	67	18 ab	7	24 abc
EpiShield 1.5 twice	3.1 a	74	18 ab	8	26 abc
EpiShield 2.5 once	2.3 b	78	16 ab	6	22 bc
EpiShield 2.5 twice	3.0 a	68	25 a	6	31 ab
Platina	3.1 a	71	20 ab	8	29 abc
Safe-T-Side	3.3 a	82	13 b	6	18 c
Control	3.2 a	73	19 ab	8	27 abc
Gala / M.9 - Selah					
Bluestim	1.8 a	90 ns	9 ns	1 ns	10 ns
EpiShield 1.5 once	0.8 c	92	8	0	8
EpiShield 1.5 twice	1.8 a	85	14	1	15
EpiShield 2.5 once	1.9 a	85	14	1	15
EpiShield 2.5 twice	2.0 a	85	14	1	15
Platina	1.4 b	83	17	0	17
Safe-T-Side	1.8 a	89	11	0	11
Control	1.8 a	87	12	1	13
Fuji / Harrah					
EpiShield once	2.2 ab	97 ns	3 ns	0 ns	3 ns
EpiShield twice	2.2 b	93	7	0	7
Control	2.4 a	96	4	0	4
Gala / Harrah					
EpiShield once	2.6 a	85 ns	11 ns	4 ns	15 ns
EpiShield twice	2.1 b	81	15	4	19
Control	2.7 a	85	13	3	15
Gala / M.7 - Othello					
EpiShield once	3.4 ns	93 ns	7 ns	0 ns	7 ns
EpiShield twice	3.3	91	9	0	9
Control	3.5	91	9	0	9
Golden Delicious / Seedling - Zillah					
EpiShield	2.0 b	na	na	na	na
Control	2.1 a	na	na	na	na

Lenticel breakdown: Lenticel-related disorders in apple have been a concern for most growers and packers in Washington since the late 1990s. In a survey conducted in the 2005/06 storage season, we found that half of the packers contacted reported lenticel-related problems such as lenticel breakdown and lenticel blotch pit. The post harvest manifestation of these disorders has resulted in significant losses – up to 30% of a storage room/orchard block/year. Cultivars most affected include Gala and Fuji. Since 2006, the Washington Tree Fruit Research Commission has facilitated testing of promising approaches utilizing hydrophobic spray emulsions applied before harvest to mitigate lenticel breakdown.

In 2008 we set up 6 trials to determine if application of hydrophobic materials within 2 weeks of harvest would alleviate LB development after storage or affect any other fruit quality parameter. All fruit was harvested at commercial maturity suitable for long-term CA storage. We found no differences in common maturity parameters at harvest between control and treated fruit (data not shown). Fruit from all orchards expressed LB symptoms after 6 months of CA storage; incidence in Gala was slightly higher than in Fuji (Table 4). No significant treatment effect was seen regarding material concentration or spray frequency (Table 4). EpiShield applications within 2 weeks of harvest did reduce overall weight loss in storage most of the time (Table 4).

After three years of field testing we have not found a preharvest material that can alleviate lenticel breakdown symptom expression after storage. However, since 2006, lenticel breakdown incidence has decreased industry wide, in part due to increased production of redder Gala strains and more careful postharvest handling of individual lots. Older Gala strains were frequently harvested beyond optimum harvest maturity in an effort to improve fruit color; recent Gala plantings have used more highly colored strains. In the warehouse, susceptible lots are typically packed early in the season, when LB incidence is low. Further, industry has increasingly adopted gentler packing line soaps and brushes, as well as abandoned pre-sizing of Gala to minimize the fruit's exposure to conditions known to aggravate lenticel breakdown.

Honeycrisp storage: High demand and premium pricing has led to rapid increases in Honeycrisp acreage in Washington. Most fruit is packed by December and sold by January. However, with increasing volumes of fruit available, the marketing window for this variety needs to be extended. Successful fruit storage of this variety is complicated by several problems, namely bitter pit and sensitivity to chilling.

In 2008 we set up an experiment to test if Honeycrisp can be stored for extended periods without compromising fruit quality. Ranges of several fruit quality parameters at harvest are shown in Table 5.

Honeycrisp is known to retain firmness well; trial fruit stored in RA lost approximately one pound during a five month storage period (February) while fruit stored under CA and DCA retained firmness levels close to harvest values (Table 6). Expression of bitterpit symptoms and development of greasiness was hastened by RA storage (Tables 7 and 8). Soluble solids and titratable acidity did not significantly change over time (data not shown) and none of the lots expressed significant levels of soft scald (data not shown).

In general, all fruit kept well until December regardless of storage regimen, harvest maturity, or orchard location. Overall we could not distinguish between CA and DCA stored fruit at any point. These results suggest that Honeycrisp apples may be stored for longer periods of time without compromising fruit quality when utilizing controlled atmosphere conditions. In 2009 we repeated the experiments with particular focus on harvest timing and the influence of crop load on overall storage performance; fruit evaluation is ongoing.

Table 5. Harvest maturity of Honeycrisp apples from three locations in 2008.

	Orchard A		Orchard B		Orchard C	
	early	standard	early	standard	early	standard
Firmness (lbs.)	-	14.4	-	16.3	17.2	16.9
Soluble solids (Brix)	-	12.1	13.4	12.0	13.0	12.9
Titratable acidity (% ma)	-	.400	.543	.384	.587	.508
Background colour (1-3)	-	2.0	2.7	2.2	2.4	2.1
Bitterpit (%)	-	9.3	0	8.0	0	0

Table 6: Storage firmness for two picks of Honeycrisp. WTFRC 2008.

	At Harvest	November	December	January	February	March
Early pick						
RA	17.2	17.8 ns	17.2 ns	17.1 ns	16.3 b	-
CA	17.2	17.6	17.4	17.0	16.9 ab	-
DCA	17.2	17.7	16.9	16.8	17.3 a	-
Standard pick						
RA	16.9	16.8 ns	16.1 ns	15.7 ns	16.4 ns	16.0 b
CA	16.9	16.6	16.0	16.3	16.4	16.8 a
DCA	16.9	16.3	16.1	16.2	16.4	16.5 ab

Table 7: Expression of bitterpit symptoms in Honeycrisp for two orchard locations (A = Prescott, C = Manson). WTFRC 2008.

	At Harvest	November	December	January	February	March
Orchard A						
RA	9.3	23 bc	13 ns	29 a	36 a	13 ns
CA	9.3	19 c	13	14 ab	12 b	15
DCA	9.3	19 c	7	10 b	17 b	13
Orchard C						
RA	0	0 ns	1 ns	0 ns	4 ns	1 ns
CA	0	0	0	0	1	0
DCA	0	3	0	0	1	1

Table 8: Honeycrisp peel greasiness (% of fruit affected) during storage . WTFRC 2008.

	January		February		March	
	Early pick	Standard pick	Early pick	Standard pick	Early pick	Standard pick
RA	1 ns	47a	27 a	24 a	-	41 a
CA	0	1 b	8 b	4 b	-	0 b
DCA	1	3 b	11 b	7 b	-	11 b

Kelp products: We have tested several commercially available kelp products (Stimplex on Gala, Kelpak on Honeycrisp) and found no effect on horticultural performance of apples (data not shown).

CONTINUING PROJECT REPORT **YEAR:** 1 of 2
WTFRC Project number: AP-09-910

Project Title: Finding scald control tools using apple peel chemistry

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Cooperators: Dr. Bruce Whitaker, Dr. Maarten Hertog, Dr. Renfu Lu, Dr. Mike McCarthy, Dr. Doreen Main

Other Funding Sources

Agency Name: USDA-NIFA (competitive grant)
Amount requested (Federal + non-Federal): $2.4 million (total) over 4 years.
Notes: D. Rudell is Project Director, J. Mattheis is a Co-PI. The Standard Research and Extension Project, "A diagnostic toolbox for integrated management of postharvest apple necrotic disorders" was submitted (01/12/10) for the current funding cycle. WTFRC and AgroFresh, Inc. are co-sponsors.

Agency Name: AgroFresh, Inc.
Amount funded: $68,700 (2009-2010)
Notes: D. Rudell is PI, J. Mattheis is Co-PI, WSU. "Defining metabolism associated with apple CO_2 injury development".

Total Project Request: **Year 1:** $53,305 **Year 2:** $55,784

Budget

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

Item	**2009**	**2010**	
Salaries[1]	37,927	39,065	
Benefits	11,378	11,719	
Wages			
Benefits			
Equipment			
Supplies[2]	4000	5000	
Travel			
Miscellaneous			
Total	$53,305	**$55,784**	

[1] 0.80 FTE Post-doctoral research associate
[2] Consumables for mRNA extraction, gene expression analysis, fruit purchase

Objectives:

1. Find peel apple peel chemicals that link scald to cultivar, harvest maturity, and other factors.
2. Identify additional apple peel chemicals that are important to fruit quality, ripening, and scald.
3. Identify apple peel chemicals or genes useful as early scald prediction or breeding selection tools.

Significant Findings:

1. More than 60% of candidate scald biomarkers selected in the first year indicated pre-scald storage stress in fruit from at a second site and season.
2. Monitoring specific biomarkers during post-storage ripening may allow earlier indication of storage stress or scald.
3. Delayed warming treatments of 1 week (68 ^{o}F) following 1-4 weeks of storage significantly reduced scald development.
4. A related group of peel chemicals associated with both superficial scald and soft-scald ('Honeycrisp') and may be a link between apple fruit chilling tolerance.

Methods:

Equipment and Cooperative Summary: Comprehensive peel chemistry analytical instrumentation (gas and liquid chromatography-mass spectrometry) and mRNA extraction and evaluation instrumentation are available at the TFRL, ARS Wenatchee. Additional chemical identification and molecular characterization will be performed in cooperation with Dr. Bruce Whitaker (BARC, USDA-ARS, Beltsville, MD). Molecular data will be processed in Wenatchee in cooperation with Dr. Doreen Main (Department of Horticulture and Landscape Architecture, Pullman, WA). Data modeling and statistical analysis will be conducted in collaboration with Dr. Maarten Hertog (Department of Biosystems, Katholieke Universiteit, Leuven, Heverlee, Belgium). New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

Year 1 Goals:

1. Determine relationships between peel chemistry, superficial scald incidence, and post-storage ripening after different storage durations.
2. Determine how peel chemistry and scald is impacted by intermittent warming treatment, which can significantly reduce scald.
3. Continue to expand the scope of our analysis procedures to find new apple peel chemicals and genes that are associated with scald.

Results and Discussion

Categorizing candidate biomarkers. Candidate biomarkers were categorized in collaboration with Dr. Hertog into groups that may predict scald development or are associated with scald symptom development. An in-depth evaluation of the data presented last year provided a more focused picture of the role(s) different chemical biomarkers may play in a biomarker-based superficial scald storage management system. Peel chemicals in control and DPA-treated fruit during the pre-symptomatic, predictive period were segregated from compounds reflecting scald symptom presence or absence. Candidate biomarker quality was ranked and overlap between candidates that predict and those associated with superficial scald symptoms were revealed (Fig. 1). Numbers of candidates associated with DPA- treated or control fruit during each period were categorized (Table 1). Results indicate candidate biomarkers may have uses beyond predicting scald for storage management. Other uses

include accurate diagnosis and/or distinguishing superficial scald from other peel defects with similar visual symptoms to troubleshooting storage, packing-line, and other supply chain issues.

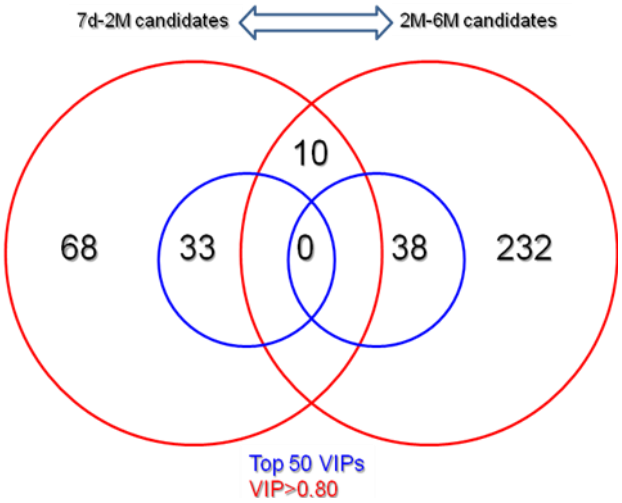


Fig. 1. Diagram showing that candidate biomarkers can be categorized into distinct groups that predict or potentially distinguish superficial scald from similar looking peel imperfections. No candidates overlapped in the "Top 50" category and only 10 in the >0.80 category (the second highest grouping). This analysis may indicate the higher the rank of a candidate biomarker, the higher the predicted quality of the biomarker.

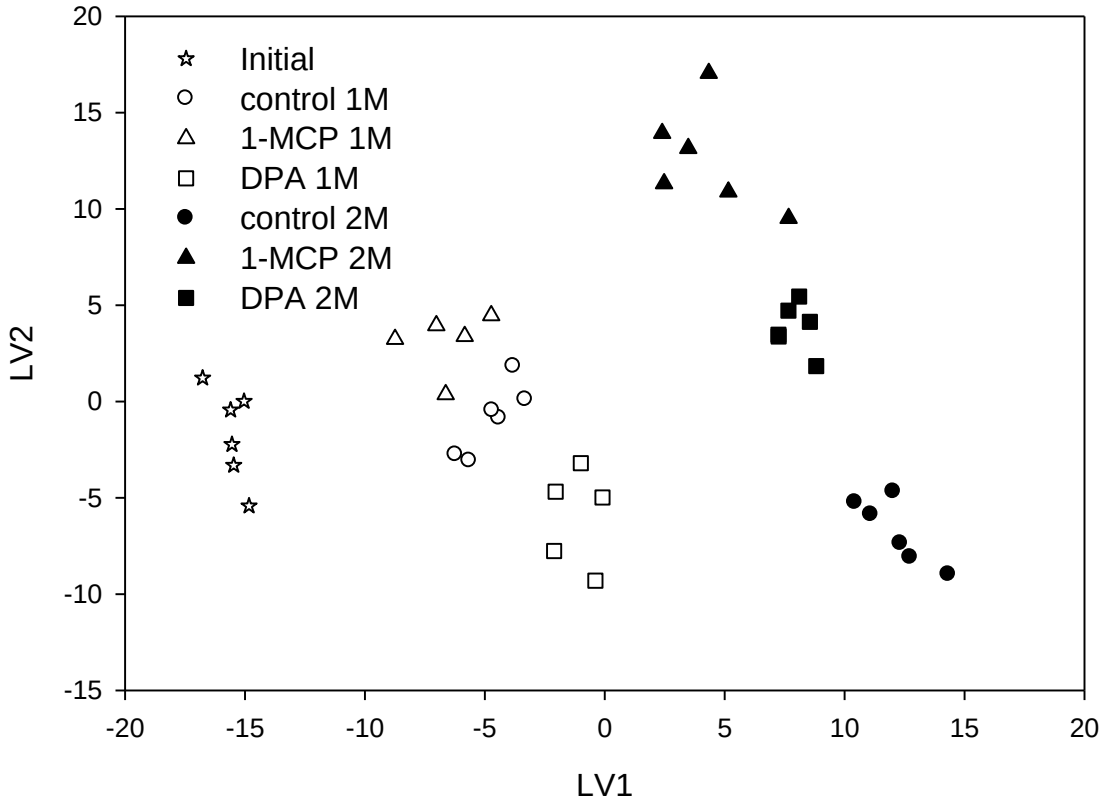
	High 50	VIP>0.8
Pre-scald (7d-2m model)		
total candidates	33	68
Greater in control	23	32
Greater in DPA	10	36
Scalding (2m-6m model)		
total candidates	38	232
Greater in control	14	82
Greater in DPA	24	150

Table 1. Ranked candidate biomarkers categorized superficial scald development period and treatment association. This demonstrates peel chemicals associated with unstressed, "healthy" peel can also be useful biomarkers indicative of storage stress levels.

Relationships between peel chemistry, superficial scald incidence, and post-storage ripening after different storage durations (Year 1, Goal 1). 'Granny Smith' apples were picked 1 month prior to commercial harvest and left untreated or treated with 2000 ppm DPA or 1 ppm 1-MCP then stored for 0, 1, 2, 4, or 6 months in 33 ^{o}F air. Scald incidence and peel chemistry was evaluated 0, 7, and 14 days after removal from storage. The storage stress leading to scald, as in previous years, principally transpired within the first month of storage, or 3 months before symptoms were apparent in control fruit. Peel chemical levels following 0, 7, and 14 days of ripening were statistically analyzed (PLS-DA) separately for the first 2 months of storage to indicate if and when peel chemistry was able to separate each treatment during storage. This demonstrated that peel chemistry was different in control and DPA-treated fruit as early as 1 month after harvest confirming our results from the previous year (Fig.2). Overall, these treatment differences were accentuated by 7 and 14 days of post-storage ripening (data not shown). Re-examination of predictive candidate markers found in the previous year indicated over 60% of the candidates distinguished fruit with and without scald during symptom development. Validation of candidate biomarkers across growing locations and seasons, and planned experiments employing other storage conditions and treatments that reduce scald such as delayed warming (see below), harvest maturity, and CA storage are expected to provide additional validation and benchmarks by which to categorize biomarkers. Results also show that levels of many candidate biomarkers change over the 2 week post-storage ripening period, either accentuating or reducing differences between control and DPA-treated fruit. Levels of a specific group of aroma volatiles associated with scald during the previous year's experiments only after scald symptoms appeared, the methanol based esters, were elevated in control peel after only 1 month

storage plus 7 or 14 days. This indicates that post-storage ripening after shorter storage periods could provide earlier information related to scald status and storage stress.

Impacts of intermittent warming treatment on scald incidence and peel chemistry (Year 1, Goal 2). 'Granny Smith' apples were harvested 1 month prior to commercial maturity. Subsets of fruit were placed in 33 ^{o}F air storage immediately or following 1 week at 68 ^{o}F. Of the fruit placed in storage immediately, additional subsets were removed after 1, 2, 4, and 8 weeks for a 1 week warming treatment at 68 ^{o}F after which they were placed back into cold air storage. Apple peel chemistry was evaluated at harvest, before and after warming treatment, and after 6 months storage. Scald severity was evaluated following 3, 4, 5, and 6 months. No scald developed after 6 months of storage on intermittent warming treatments beginning at 1 and 2 weeks and significantly reduced scald developed after treatments beginning at 4 and 8 weeks. Peel chemical evaluation is complete and data analysis is in progress. It is expected that this experiment will provide additional conditions for candidate biomarker categorization and validation.



Expanded apple peel chemical library and identification of key scald-associated chemicals (Year 1, Goal 3). The peel chemistry analysis was expanded to include over 100 additional chemicals in 'Granny Smith' and 'Honeycrisp' experiments. In collaboration with Dr. Bruce Whitaker, ARS-Beltsville, we identified a key "family" of peel chemicals called acylated steryl glycosides (ASGs) and their chemical building blocks, the steryl glycosides (SGs) in 'Granny Smith' peel. ASGs are associated with modifying important plant cell components in response to low temperatures. While there is no change in concentrations of the "building blocks" (SG and phytosterols), ASG levels are elevated in control peel both prior to and during scald symptom development. ASG levels decrease in

peel from both control and DPA-treated fruit after removal from storage. Elevated ASG levels are also associated with soft-scald in 'Honeycrisp'. Elevated ASG levels may indicate chilling stress in apples leading to many of these disorders and may also serve as potential genetic targets to modify susceptibility to chilling related injuries.

Adapting peel chemical measurement platforms for biomarker based scald-management tools. Some preliminary work has been conducted towards development of non-laboratory based platforms to estimate changes in apple peel chemistry that indicate storage stress and the potential for superficial scald development. Collaborations have been initiated with Dr. Renfu Lu (ARS, East Lansing) and Dr. Mike McCarthy (UC-Davis) for preliminary evaluation of two different on-line devices for superficial scald prediction. Experiments correspond with our apple peel chemistry evaluations and are based on our results demonstrating changes in peel chemistry prior to scald development. Dr. Lu is studying the possibility of using near infra-red spectroscopy and fluorescent scattered light imaging to differentiate apples at 0, 1, 2, and 4 months. Dr. McCarthy is studying the possibility of using a small magnetic resonance imaging platform to differentiate apples at 2 months. Both collaborators are generously performing this work using our apples without direct funding.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-09-901AB

Project Title: A sensitive indicator of Honeycrisp fruit N status for maximal quality

PI:	Lailiang Cheng	**Co-PI:**	Yanmin Zhu
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Address:	134A Plant Science Ithaca, NY 14853	**Address:**	1104 Western Ave, Wenatchee, WA

Cooperators: Ines Hanrahan and Tory Schmidt at WTFRC

Total Project Funding Request: **Year 1:** $34,595 **Year 2:** $35,391

Other funding sources: None

WTFRC Collaborative expenses:

Item	**2009**	**2010**	
Salaries & benefits	2,000	1,500	
Crew labor & benefits	2,300	2,400	
Travel	1,000	1,000	
Miscellaneous	200	200	
Total	$5,500	**$5,100**	

Budget 1
Organization Name: Cornell University **Contract Administrator:** Christine Ashdown
Telephone: 607-255-3843 **Email address:** cma20@cornell.edu

Item	2009	**2010**	
Salaries	11,500	11,960	
Benefits	6,095	5,856	
Supplies	6,100	6,675	
Travel	900	900	
Total	$24,595	**$25,391**	

Footnotes: Salaries budgeted are for a 4-month postdoc working on this project at $34,500 per year in the first year and at $35,880 in the second year. The fringe benefit rate for the second year is 48.96%. Supplies include cost of leaf and fruit nitrogen analysis, analytical columns, guard columns, standards, solvents, vials and service for the HPLC separation and quantification of amino acids, and cloning kit for the two genes.Travel expense is budgeted for one trip per year to the experimental site in WA to set up the trial and collect samples.

Budget 2

Organization Name: Cornell University **Contract Administrator:** Christine Ashdown

Telephone: 607-255-3843 **Email address:** cma20@cornell.edu

Item	2009	**2010**	
Fruit loss compensation*	10,000	10,000	
Total	$10,000	**$10,000**	

Footnotes:

***** This is a contingency plan for the worst case scenario where the high N treatments (120lbs and foliar N treatments) might make some of the fruit unmarketable so that the co-operative grower gets compensated for the loss: 20 bins of unmarketable fruit x $500/bin = $10,000. This estimate is based on 400 trees/acre after discussion with WTFRC collaborators. We'll make every effort to find a high density block to minimize the cost, and it's likely that only a proportion of the budget may be actually used. If the proposal is approved by WTFRC, the budgeted amount (Budget 2) will not be sent to Cornell upfront. Instead, WTFRC will keep the funds until the actual amount of reimbursement to the cooperative grower is determined, and will then send only that amount to Cornell for payment to the cooperative grower.

Objectives

The objectives of this project are 1) to determine how asparagine and other free amino acids in Honeycrisp fruit respond to N supply and relate the levels of free amino acids to fruit N status and fruit quality and 2) establish the optimal range of fruit N status expressed as asparagine level, with the goal of developing a sensitive indicator of Honeycrisp fruit N status for maximal quality.

Significant Findings

- Over the range of N fertilization rate 0 to 120 lbs per acre, N fertilization had only limited effect on fruit yield, fruit size, quality and physiological disorders during the first year of the trial. Although trees in the highest N treatment tended to have higher yield and bigger fruit, N fertilization beyond 30 lbs/acre didn't bring any significant benefit.

- Both leaf N and fruit N increased only slightly in response to increasing rate of N fertilization. However, fruit asparagine concentration increased significantly when N fertilization rate increased from 0 to 30 lbs N per acre. Any further increase in N fertilization rate didn't result in significant increase in fruit asparagine concentration.

- Asparagine accounted for about half of the total free amino acids in Honeycrisp fruit. As a result of the increase in asparagine concentration, the concentration of total free amino acids increased in the same way as asparagine in response to N fertilization.

- Both fruit asparagine concentration and total free amino acid concentration appear to be more sensitive than leaf N or fruit N in response to N fertilization.

Methods

Two field trials were set up in commercial Honeycrisp orchards in Washington, one at Brewster Flats and the other at Naches Heights. The trees at Brewster Flats were 6-yr-old trees on M.26 at 8 x 14 foot spacing (389 tree/acre). The trees at Naches Heights were 5-yr-old over-grafts on Delicious/MM106 at 6 x 16 foot spacing (454 trees/acre). At both sites, trees were fertilized with 0, 30, 60, 90, or 120 lbs actual N per acre per year. Each treatment was replicated 5 or 6 times, with 5 to 6 trees per replicate, in a randomized complete block design. For each nitrogen treatment, urea fertilizer was used and was applied at tight cluster and 3 weeks later in an equal split. The fertilizer was spread under the tree canopy and was watered into the soil within a few days of application. The cropload of the trees at Brewster site was 5 to 7 fruit per cm^2 trunk cross-sectional area (TCA) and that at Naches site was 9 to 10 fruit per cm^2 TCA, after chemical thinning followed by hand-thinning.

The effects of N fertilization on leaf and fruit N status, levels of free amino acids, yield and fruit quality were monitored. Leaf samples were taken in mid-August for nitrogen analysis. Fruit samples were taken at the end of shoot growth (approximately 50-g size), and at harvest for analysis of N and individual free amino acids. Fruit yield and size were measured at harvest and one bushel of fruit per replicate was stored in a regular cold room. Fruit quality (color, firmness, soluble solids, and occurrence of bitterpit and other disorders) were assessed at fruit harvest and after 4 months of cold storage. Asparagine and all other free amino acids were separated and quantified with an Agilent 1100 high performance liquid chromatography using the AccQ-Tag method (Waters Corporation). Return bloom will be assessed to determine the degree of biennial bearing in response to N supply.

Results and Discussions

1. Effects of N fertilization on fruit yield, fruit size and quality

At Brewster site, all the trees had relatively low cropload. Fruit number and fruit yield per tree were higher in the highest N treatment (120 lbs/acre) than in the control (Table 1). No significant difference in fruit size was detected between any N treatments and the control, but the trees fertilized with nitrogen tended to have slightly bigger fruit. N fertilization did not significantly affect fruit firmness, soluble solids, acids, or bitterpit incidence. Percent of clean fruit (free of russet) was significantly higher in the 120 lbs N treatment than in the control.

At the Naches site, all the trees had relatively high croload. N fertilization did not significantly affect fruit number or fruit yield per tree (Table 2). This may be partly due to the fact that the grower had put on about 30 lbs of nitrogen on all the trees before the trial was set up. Fruit size was the largest in the highest N treatment (120 lbs/acre), but for some reason, trees in the 30 lbs N /acre treatment produced the smallest fruit. Fruit firmness was slightly affected by N fertilization. No difference in fruit soluble solids or acids was detected between any N treatments and the control. Bitterpit was highest in the control, which we don't have a clear explanation for. Perhaps the trees were relatively vigorous and fruit size was relatively big. Sunburn incidence at this site was higher than that at Brewster site, and N fertilization slightly affected sunburn incidence. Similar to that found at Brewster site, percent of clean fruit was significantly higher in the highest N treatment (120 lbs N/acre) than in the control.

Fruit samples stored in regular cold storage are being evaluated, and the results will be presented at the research review.

We expect that the effects of N fertilization on tree growth and fruit quality will be bigger due to the cumulative effects of N treatments via tree reserve nitrogen levels.

2. Effects of N fertilization on leaf N, fruit N, fruit asparagine and total free amino acids

At Brewster site, both leaf N and fruit N increased only slightly from 1.9% to 2.2% and from 0.34% to 0.38%, respectively, as N fertilization rate increased from 0 to 120 lbs/acre (Fig 1). Neither increase was statistically significant. However, both fruit asparagine concentration and total free amino acid concentration increased significantly (from 4.4 to 7.2 mg/g and from 10.4 to 13.9 mg/g, respectively) when N fertilization rate increased from 0 to 30 lbs/acre. Any further increase in N fertilization rate (beyond 30 lbs/acre) did not result in any significant increase in either fruit asparagine concentration or total free amino acid concentration.

At Naches site, leaf N was higher than at Brewster site, but it increased only slightly at the two highest N fertilization rates (Fig 1). Surprisingly, fruit N levels were slightly lower than those at Brewster site, but it increased from 0.30% to 0.37% as N fertilization rate increased from 0 to 120 lbs/acre. Both fruit asparagine concentration and total free amino acid concentration showed the same trend in response to N fertilization: they increased significantly (from 6.8 to 9.3 mg/g and from 13.2 to 16.8 mg/g) when N fertilization rate increased from 0 to 30 lbs/acre, but no additional significant increase was detected with further increase in N fertilization rate.

Compared with leaf N and fruit N, fruit asparagine concentration and total free amino acid concentration were more sensitive to N fertilization, and therefore should be better indicators of fruit N status. Since the range of tree N status and fruit N status generated at both sites was fairly narrow, it is difficult to establish an optimal range of fruit asparagine concentration and total free amino concentration for maximal fruit quality based on one year's field data. Further testing is needed.

Research Plan for Second Year

During the second year of the project, we will continue the treatments initiated during the first year. Considering that the range of tree N status and fruit N status generated by soil N fertilization was fairly narrow during the first year, we will also plan to use foliar urea applications during fruit development to generate a wider range of fruit N status to determine how fruit quality is

related to fruit N, asparagine and other free amino acids in WA. Briefly, 5 to 7 year-old Honeycrisp trees grown on sandy soils will receive 0, 1, 3, 5, or 7 foliar sprays of 5 lbs urea/100 gal water at weekly intervals centered around 6 weeks before expected harvest. Each treatment will be replicated 5 times in a randomized complete block design with 4 trees per replicate. Fruit yield and size will be measured at harvest and one bushel of fruit per replicate will be stored in a regular cold room. Fruit quality (color, firmness, soluble solids, and occurrence of bitterpit and other disorders) will be assessed at fruit harvest and after 4 months of cold storage. Fruit N, asparagine and all other free amino acids will be measured to relate back to fruit quality parameters to determine the optimal fruit N status in terms of fruit N content and asparagine level.

Table 1. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Brewster site)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Bitter pit (%)*	*Sunburn (%)*	*Russet % clean*
0	11.1b	61b	180	18.1ab	12.3	0.529	1	7ab	39b
30	18.1 ab	95 ab	187	18.3 a	11.8	0.485	1	8 ab	39 b
60	18.5 ab	98 ab	186	17.9 b	12.7	0.474	1	9 a	43 ab
90	14.7 ab	73 ab	191	18.2 ab	12.3	0.520	2	5 ab	49 ab
120	22.5 a	119 a	192	18.0 ab	12.4	0.532	1	1 b	68 a

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

Table 2. Effects of N fertilization on Honeycrisp yield, fruit size and quality (Naches site)

N rate (lbs/a)	*Yield (kg/tree)*	*Fruit No*	*Fruit size (g)*	*Firmness (lbs)*	*Brix (%)*	*Acids (%)*	*Bitter pit (%)*	*Sunburn (%)*	*Russet % clean*
0	43.0	216	217ab	16.9bc	12.1	0.507	11a	16ab	57b
30	43.1	237	187 b	17.2 ab	12.5	0.499	4 b	21 a	72 ab
60	42.6	229	201 ab	17.5 a	12.6	0.537	3 b	20 a	68 b
90	49.4	274	191 ab	16.8 c	12.3	0.497	2 b	10 b	59 b
120	48.2	231	222 a	16.7 c	12.6	0.540	3 b	13 ab	85 a

Means separation were performed using Tukey's test and different letters indicate significant difference at P =0.10. Significance of percentages was based on arcsine data transformations.

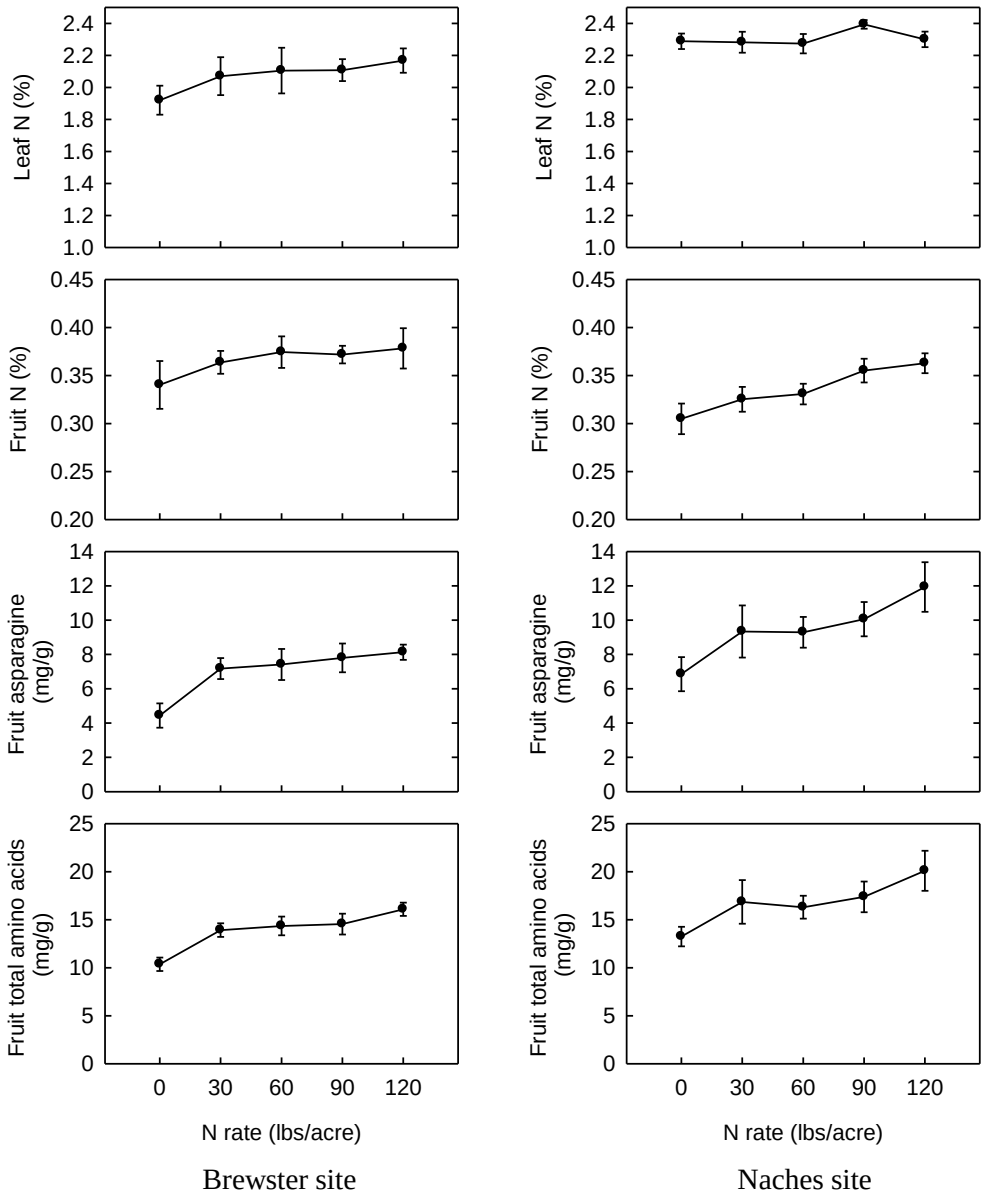

Fig 1. Nitrogen content of leaf samples taken in mid-August, and concentrations of nitrogen, asparagine and total free amino acids in fruit at harvest in response to nitrogen fertilization at both Brewster site and Naches site.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-09-906

Project Title: Validation of fresh apple packing food safety interventions

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Cooperators:

Deborah Carter, Technical Issues Manager of the Northwest Horticultural Council. Contact information: (509) 453-3193, carter@nwhort.org

Several Washington apple packers as well as chemical suppliers have been consulted to discuss aspects of this study to ensure relevancy to the apple packing industry.

Total Project Request: **Year 1:** $50,990 **Year 2: $53,030** **Year 3:** $55,152

Other funding Sources

Agency Name: Washington State USDA Specialty Crop Block Grant Program
Amt. awarded: $55,868
Notes: This project supported a literature review, initial laboratory experiments to select methodology for apple inoculation and an educational meeting with the tree fruit industry. The literature review provided information on the state of knowledge regarding antimicrobial interventions for whole, fresh apples, which was found to be relatively limited. Additionally, the review of literature indicated that methodology used in evaluating antimicrobial interventions for apples varied significantly between studies. Therefore, microbial studies were conducted to assist in selection of methods for apple inoculation, such as preparation of apples prior to inoculation, inoculation methods and media and drying time. These experiments developed a foundation for methodology that was utilized in Year 1 and the proposed work of Years 2 and 3 in order to provide the industry with scientific information using standardized methods that will allow for comparison of results. An educational food safety meeting, "Safety of Northwest Produce" was conducted with 100 participants, primarily from the Washington tree fruit industry, and provided important opportunities to discuss research needs with industry representatives.

WTFRC Collaborative expenses: None

Budget 1

Organization Name: WSU **Contract Administrator: Mary Lou Bricker**

Telephone: (509) 335-7667 **Email address: mdesros@wsu.edu**

Item	2009 (funded)	2010	2011
Salaries	22,014	**22,895**	23,811
Benefits	1,560	**1,622**	1,688
Wages	5,100	**5,304**	5,516
Benefits	816	**849**	883
Equipment	0	**0**	0
Supplies	16,500	**17,160**	17,846
Travel	5,000	**5,200**	5,408
	0	**0**	0
Miscellaneous			
Total	50,990	**53,030**	55,152

Footnotes:

[1] Graduate student and technical support in Pullman and Wenatchee

[2] Time slip assistance in Pullman and Wenatchee

[3] Fruit, chemicals, measurement devices and microbial supplies

[4] Travel to Wenatchee for apples and pilot line studies

Objectives:

1) Perform laboratory validation studies to examine foodborne pathogen and indicator organism reduction by antimicrobial treatments currently used in the apple packing industry

2) Validate antimicrobial interventions under pilot or industrial packing line conditions using indicator organisms

3) Conduct appropriate food safety extension and outreach with the apple packing industry

Significant Findings:

- Experiments in Year 1 focused on peroxyacetic acid (PAA). Project team discussions with industry representatives and chemical suppliers along with examination of currently available scientific literature indicated that industry-relevant validation data for PAA was needed. To meet industry needs, the original study design was altered to a design capable of predicting microbial reductions within a range of concentrations (between 40-75 ppm PAA) and exposure durations (between 30-120 seconds) rather than only three specific concentrations and exposure durations.

    - Our Year 1 study represents the most robust examination of PAA on whole, fresh apples using industry-relevant concentrations and exposure durations as indicated by currently available scientific literature.

- Laboratory results in Year 1 indicate that peroxyacetic acid at concentrations and exposure durations currently used in the industry resulted in at least a 1.5 – 2 $\log_{10}$ reduction of *E. coli* O157:H7. Additional replications are necessary before statistically significant conclusions can be made. Therefore, the results reported should be considered preliminary.

    - At 40 ppm PAA, a 1.7 $\log_{10}$ reduction in *E. coli* O157:H7 was observed and a 1.3 $\log_{10}$ reduction in generic *E. coli* was observed. At this concentration, duration of exposure (30, 60 or 120 seconds) did not appear to affect microbial reduction.

    - At both 60 and 75 ppm PAA, a 2.0 $\log_{10}$ reduction was observed in *E. coli* O157:H7 and a 1.6 $\log_{10}$ reduction in generic *E. coli* was observed. Statistical analysis of the complete data set is necessary to determine the effect of duration of exposure at these concentrations.

    - Current data indicate that, for some treatments, duration of exposure (30, 60 or 120 seconds) did not appear to affect microbial reduction. Statistical analysis of the completed data set is necessary to confirm this preliminary observation. Furthermore, use of brushes in an industry setting during PAA application may result in differences between laboratory and packing line data, emphasizing the need to include experiments conducted under conditions that reflect typical packing conditions.

- Preliminary data was collected for PAA application under pilot plant packing conditions. Three replications were performed. It was determined additional equipment engineering or alternative treatment delivery systems would be necessary to achieve accurate antimicrobial delivery on the packing line, so experiments were postponed until Year 2 or 3 of the project.

Methods:

Year 2

Laboratory Validation Studies.

In Year 1, discussions with several industry representatives and chemical suppliers indicated that the range of PAA concentrations commonly utilized in apple packing was quite broad. To maximize industry-relevant results and project resources, a statistician was consulted and identified a study design to predict microbial reductions at concentrations and exposure durations throughout the range of those specifically tested (40-75 ppm and 30-120 seconds). Three PAA concentrations (40, 60 and 75 ppm) and three exposure durations (30, 60 and 120 seconds) were examined. In each experiment, 12 treatment combinations were examined with 5 apples in each of the 12 treatment combinations. Both *E. coli* O157:H7 and generic *E. coli* were examined using this design. Based on current data (3 replications), optimal statistical power to provide accurate results requires 2-3 more replications. Peroxyacetic acid replications will be completed early in Year 2 followed by complete statistical analysis.

In Year 2, laboratory validation studies for chlorine and chlorine dioxide will be initiated. A similar model will be followed as in Year 1 with significant input from industry representatives and chemical suppliers to identify appropriate and relevant concentrations to include in the study design. Levels of chlorine and chlorine dioxide will be investigated using two experimental strategies to align with recommended manufacturer concentrations, standard industry practices and published literature (Rodgers et al., 2004; Suslow, 2004). Currently, in a series of experiments focusing on oxidation reduction potential (ORP), both chlorine and chlorine dioxide will be examined by adding sufficient quantities to adjust the ORP levels of the aqueous solution to 665, 750 and 850. In a second series of experiments focusing on specific chemical concentrations, chlorine will be examined at 25, 50, and 75 ppm free residual chlorine with efforts to maintain pH levels at 6.8, and chlorine dioxide will be examined at concentrations of 3, 5, and 7 ppm with efforts to maintain a pH of 6.8.

Studies will utilize pathogenic and generic *E. coli* using a similar experimental design as described above with twelve treatment combinations being examined. Inoculated apples will be exposed to three selected concentrations of the antimicrobial treatment and three selected exposure durations. After antimicrobial treatment, apples will be rinsed and serial dilutions will be performed. Appropriate dilutions will be plated on sorbitol MacConkey (SMAC) agar for enumeration of E. *coli* O157:H7 and violet red bile agar (VRBA) for enumeration of generic *E. coli*. Plates will be incubated at 95˚F and enumerated manually or by an automated Q-Count system.

Packing Line Studies:

It is critical to validate the effectiveness of antimicrobial treatments using conditions that reflect industrial packing systems. Wang (2007) demonstrated that flow velocity and agitation are factors that can contribute to microbial reduction, and these conditions are difficult to accurately simulate in the lab. In Year 2, the project team will communicate with chemical suppliers and potential industry partners to determine an appropriate study design for packing line studies. It is anticipated that pilot line or industrial scale work for PAA could be initiated in Year 2 followed by work with chlorine and chlorine dioxide in Year 3. The pilot apple packing line at the Tree Fruit Research and Extension Center could be utilized for validation of spray bar antimicrobial applications working with chemical suppliers to develop a delivery system appropriate for the pilot line. Alternatively, apple packers willing to participate in industrial scale line studies using generic *E. coli* to validate individual intervention steps or an entire packing system, including washing, rinsing, antimicrobial applications and drying could also be pursued.

Food Safety Outreach and Education:

The project team will work together to coordinate a food safety educational meeting for the apple and tree fruit industry in Year 2. All current project investigators have significant extension responsibilities. Based on participation and input at food safety educational meetings by tree fruit industry representatives in the previous year, a preliminary agenda will be developed and discussed by project leaders and industry representatives to finalize an industry-relevant workshop agenda. Potential topics include overviews of specific antimicrobial intervention effectiveness, discussion of specific foodborne pathogens, pre-requisite food safety programs and customer perspectives.

Year 3:

Protocols will be revised and optimized as scientifically appropriate based on the results produced in Years 1 and 2. Laboratory studies examining the effect of treatment combinations, such as a chlorine treatment followed by a PAA treatment, on *E. coli* O157:H7 and generic *E. coli* would provide valuable data regarding overall pathogen reduction during packing. Furthermore, validation of the ability of PAA, chlorine and chlorine dioxide to reduce *Salmonella* would be warranted.

Results from Years 1 and 2 of laboratory validation studies will demonstrate which treatment concentrations and application times are optimal for microbial reduction. In Year 3, studies conducted at the WSU Tree Fruit Research and Extension Center and/or with industry partners to validate antimicrobial interventions individually and in combination under conditions reflecting typical apple packing will be performed. It is anticipated that spray bar applications (chlorine, chlorine dioxide and PAA) as well as dump tank applications (chlorine) will be examined alone and in combination.

Furthermore, few studies have investigated the potential antimicrobial effects of other packing steps. In Year 3, the effect of waxing and drying under packing conditions to reduce microbial levels will be considered. A laboratory-based study has shown that 2 waxes when applied at 131˚F produced 0.5 – 1 $\log_{10}$ reductions in *E. coli* O157 and *Salmonella* (Kenney and Beuchat, 2000). It would be useful to examine the ability of waxing and drying to further reduce indicator organisms under packing conditions. Although the observed $\log_{10}$ reductions from wax application and drying are small, this process step has the potential to contribute to an overall antimicrobial treatment system throughout a packing line that could enhance overall system microbial reductions.

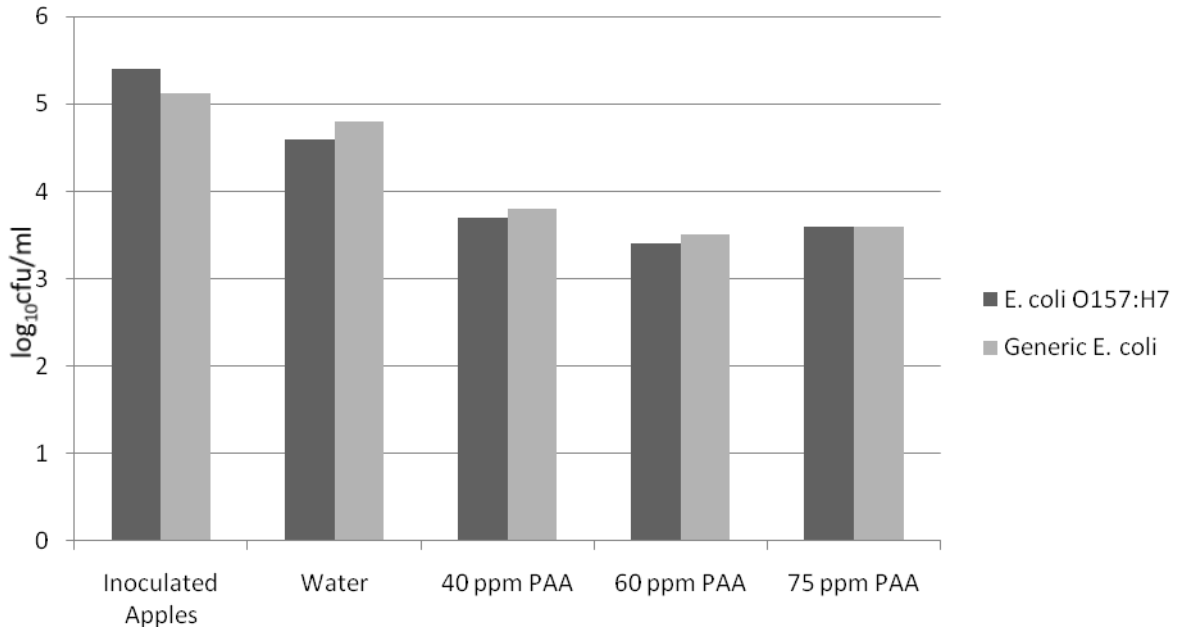
Results and Discussion:

Validation Methodology: Initial microbial studies on appropriate methodology for antimicrobial validation indicated that apples should be unwaxed and rinsed in water prior to inoculation. The most effective method of microbial inoculation was determined to be immersing and massaging the apples in an inoculation solution for 10 minutes rather than a spot inoculation at the stem end. Optimal drying time for bacterial attachment was determined to be 1 hour. The observed inoculum level on the apple of 100,000 cells is sufficient to demonstrate up to a 4 $\log_{10}$ reduction achieved by antimicrobial treatments without exceeding the detection limit for the method used for microbial enumeration.

Peroxyacetic Acid (PAA) Validation Laboratory Study: To maximize industry-relevant results and project resources, a statistician was consulted and identified a study design to predict microbial reductions at concentrations and exposure durations throughout the range of those specifically tested (40-75 ppm and 30-120 seconds). Therefore, packers who utilize 50 ppm or 70 ppm PAA or a 45 second exposure time will have access to relevant data for microbial reductions achieved under their operating systems. Three replications have been performed and at least two more replications are needed to complete the study for statistical validity.

The laboratory results of Year 1 indicate that peroxyacetic acid at concentrations and exposure durations currently used in the industry can result in at least a 1.5 – 2 $\log_{10}$ reduction of *E. coli* O157:H7 (Figure 1). However, further replications are necessary before statistically significant conclusions can be made. The validation study examined both pathogenic *E. coli* O157:H7 and generic *E. coli*. Comparison of pathogenic and generic *E. coli* response allows for the opportunity to examine microbial reductions under packing line conditions where only generic *E. coli* can be utilized.

Figure 1. Average *E. coli* O157:H7 and generic *E. coli* levels ($\log_{10}$ colony forming units/ml, cfu/ml) on apples after microbial inoculation and treatment with 40, 60 and 75 ppm peroxyacetic acid (PAA). Values are averaged over application times of 30, 60 and 120 seconds. Values are reported in $\log_{10}$ scale (5=100,000 cfu/ml, 3=1000 cfu/ml).



Overall, *E. coli* O157:H7 and generic *E. coli* appeared to have similar responses to treatments. Current data indicate that for some treatments duration of exposure (30, 60 or 120 seconds) did not appear to affect microbial reduction. Discussions with industry representatives indicated that time of PAA exposure varies based on line design and line speed. If time of exposure beyond 30 seconds is not critical for microbial reduction, scientific evidence of this phenomenon would be important information for packers who need to demonstrate the effectiveness of antimicrobial intervention strategies in their operations. Statistical analysis of the completed data set is necessary to confirm this preliminary observation. Furthermore, use of brushes in an industry setting during PAA application may result in differences between laboratory and packing line data. Therefore, pilot line studies to examine the effect of duration of exposure for PAA is an important component of work proposed in Year 2.

For the water control treatment, a 0.8 $\log_{10}$ reduction was observed for *E. coli* O157:H7 and a 0.4 $\log_{10}$ reduction was observed for generic *E. coli*. The PAA treatments achieved a 1.5 – 2 $\log_{10}$ reduction in *E. coli* O157:H7 levels. At 40 ppm PAA, a 1.7 $\log_{10}$ reduction in *E. coli* O157 was observed and a 1.3 $\log_{10}$ reduction in generic *E. coli* was observed. At both 60 and 75 ppm PAA, a 2.0 $\log_{10}$ reduction was observed in *E. coli* O157 and a 1.6 $\log_{10}$ reduction in generic *E. coli* was observed. Due to the logarithmic scale of the data, the magnitude of a 1 $\log_{10}$ or greater difference between the water treatment and the PAA treatments is an important difference (equivalent to a 90% reduction). Therefore, the PAA treatments appear to be more effective than water at reducing microbial levels.

These results provide valuable data to packers who are asked to demonstrate the effectiveness of food safety interventions through validation studies to third-party auditors, customers and regulators. The ability to document the effectiveness of PAA as a food safety intervention for apple packing is limited due to a lack of scientifically reviewed data for use as supporting documentation. Most data currently available for PAA has not been performed at concentrations or durations of exposure relevant to the fresh, whole apple packing industry (Wang, 2007; Rodgers, 2004; Wisnieskey, 2000) or has been performed on other produce items. Economic benefits include the ability to demonstrate to potential customers that food safety interventions address potential food safety risks. Furthermore, trade associations can utilize the data to demonstrate control of food safety hazards and promote whole, fresh apples as a low risk produce commodity. Finally, the economic benefit to preventing an outbreak in whole, fresh apples is tremendous as this type of event would result in devastating economic losses for the entire industry.

In year 1, a network was developed between research and extension faculty as well as research commission and trade association representatives. Moreover, several apple packing industry representatives as well as chemical suppliers were involved. This network proved instrumental to determining study design, chemical application levels and relevant industry practices. Having an established network will facilitate conducting proposed work in the coming years. Laboratory validation data to demonstrate the effectiveness of PAA as an antimicrobial treatment for apple packing was collected and will be completed and available for industry use early in Year 2, followed by similar studies for chlorine and chlorine dioxide as well as packing line studies.

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