2007 Apple Research Review
January 22-23
Confluence Technology Center
Wenatchee, WA
DAY ONE AGENDA

Time	Page	PI	Project Title	Funding period
8:00		McFerson	Introduction and update	
			Final Reports	
8:15	1	Hirst	Mechanisms of apple fruit growth under WA conditions (videoconference)	05-06
8:30	7	Greene	Monitoring apple fruit growth for predicting chemical thinning response (teleconference)	04-06
8:45	15	Fallahi	Water use, quality and growth as affected by irrigation and/or rootstock (teleconference)	04-06
9:00	25	Whiting	Impact of cultural practices on apple canopy physiology	04-06
9:15	34	Elfving	Effects of preharvest sprays of MCP on apple	05
9:30	38	Mattheis	Regulation of apple fruit ripening	04-06
9:45	47	Shekarriz	Ethylene measurement in post-harvest storage	05-06
			Break	
10:15	59	Dandekar	Role of sorbitol in sugar and acid accumulation in apple fruit	04-06
10:30	69	Song	Proteomic approach to study scald disorder of apples	04-06
10:45	80	Lu	Hyperspectral reflectance and fluorescence scattering	04-06
11:00	89	Bliss	Consulting for the Washington apple breeding project	05
			Continuing projects: Video and Teleconference	
11:15	92	Norelli	Trait modification through genetically engineered rootstocks	04-06
11:20	98	Hirst	Flower bud development in apple	06-07
11:25	105	Klein/Riley	Testing of a sticker for ethylene release from apples	06-07
11:30	112	Fazio	Replant disease tolerance of Geneva rootstocks	06-08
11:35	119	van Nocker	Auxin and ethylene dynamics in the abscission zone	06-08
11:40	125	McArtney	Performance of air induction nozzles under variable wind speeds	06
11:45	131	Cheng	High temperature stress on apple fruit peel: physiology and detection	06-07
Group #	**Page #**		**Continuing Projects Poster Session 1:00-3:30**	
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2	160	Barritt	Apple scion breeding	05-07
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3	174	Elfving	Growth and crop load management in apple trees with bioregulators	05-07
3	179	Schmidt	Chemical thinning of apple	internal
3	186	Castillo	WTFRC research collaboration	internal
4	192	Hanrahan	Improving apple fruit finish by suppressing sunburn and russet	internal
4	198	Hanrahan	Lenticel-based superficial skin disorders of apple	internal
4	204	Schrader	Improving fruit finish in apple	05-07

FINAL PROJECT REPORT
WTFRC Project Number: AH-05-501

Project Title: Mechanisms of apple fruit growth under Washington conditions

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Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries		3380	
Benefits		1159	
Wages	7494	9584	
Benefits	757	968	
Equipment			
Supplies	6750	8850	
Travel			
Miscellaneous			
Total	15000	23941	

SIGNIFICANT FINDINGS

- Gala fruit grew at the same rate as those of red delicious, and were only smaller at harvest due to their earlier harvest date.
- Different patterns of cell division, in terms of the number of cells dividing, were found in the 2 years of this study. In 2005 cell division occurred over a protracted period, but was much shorter in 2006.
- In 2006, fruit size was more closely related to cell size than cell number.

RESULTS AND DISCUSSION

Flower and fruit samples were collected from a Washington orchard during the 2005 and 2006 growing seasons. The treatments were cultivar (Gala and Red Delicious) and crop load (heavy crop load, no thinning, and light crop load achieved by thinning at bloom). Sample collection began soon after bloom and continued until harvest. On each sampling date, fruit were shipped immediately from Washington to Indiana where fruit size was measured followed by preparation of samples for flow cytometry and dissection.

During 2005, our thinning treatments were insufficient to affect fruit size (Fig. 1). In 2006 we imposed a more severe thinning treatment and achieved a fruit size response, both in terms of fruit diameter and fruit fresh weight (Fig. 2). It is interesting to note that in both years, the growth of Gala fruit was similar to that of Red Delicious, but the greater final size of Red Delicious fruit was due to their later harvest date.

The main components of fruit growth are cell number and cell size. Cell number increased rapidly until approximately 50 days after full bloom but from that point until the end of the growing season, there was little change in over cell numbers (Fig. 3). There was no difference between Gala and Red Delicious fruit in terms of cell number. Likewise, reducing crop load did not increase fruit size by increasing the number of cells in the fruit. When cell size was plotted against accumulated growing degree days, the pattern was very similar to that in Fig. 3 plotted against days after full bloom (data not presented).

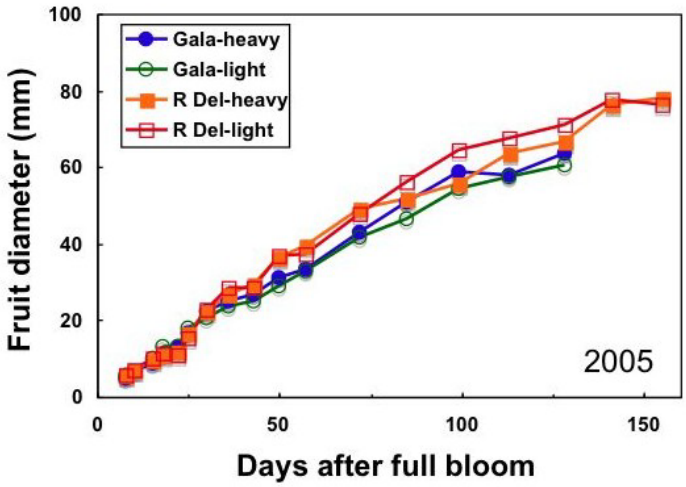

Fig. 1. Fruit diameter of Gala and Red Delicious fruit under different crop loads during the 2005 growing season.

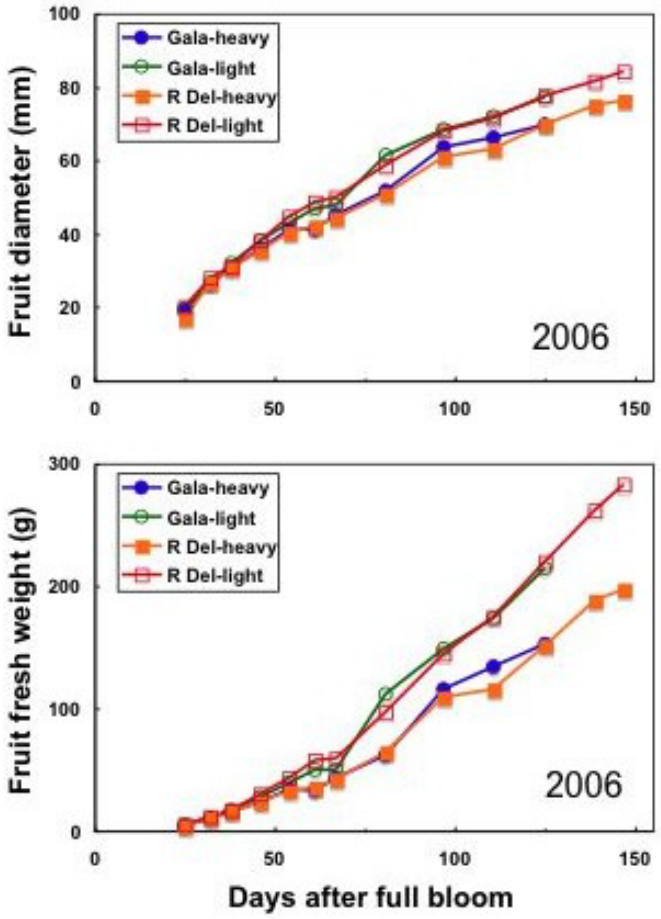

Fig. 2. Fruit diameter and final fresh weight of Gala and Red Delicious fruit under different crop loads during the 2006 growing season.

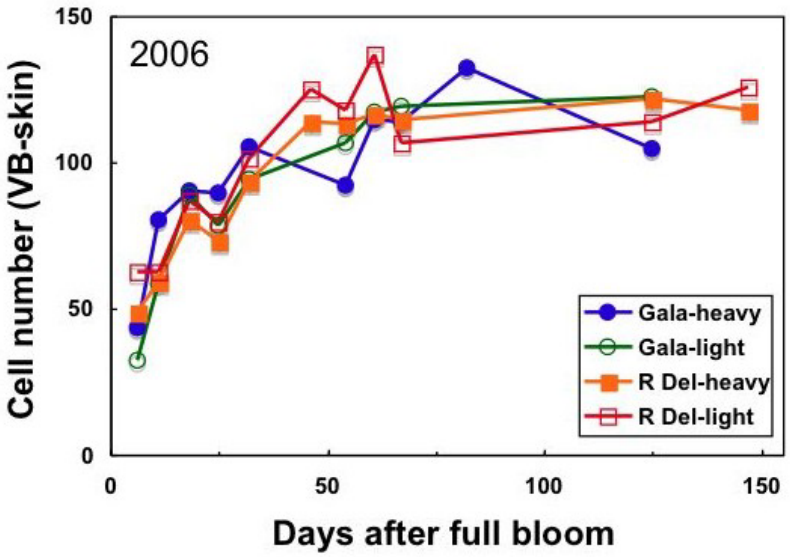

Fig. 3. Cell numbers in Gala and Red Delicious fruit under different crop loads during the 2006 growing season. Cell numbers were determined by counting a file of cells from the sepal vascular bundle to the epidermis.

An increase in the fruit cell number is brought about by cell division processes. One key component of this that has not been examined previously is the proportion of cells in the fruit actively dividing at any given point in time. Apple fruit contain a heterogenous population of cells, ie, they are not all doing the same thing at the same time. Using flow cytometry, we estimated the proportion of cells in a fruit that were actively dividing. As can be seen from Fig. 4, during the 2005 growing season, both Gala and Red Delicious reached a peak of about 15% of their cells dividing at one time. Both had a peak of cells dividing early in the season, but the proportion of Gala cells dividing declined soon after. Red Delicious on the other hand, had a second peak of cells dividing.

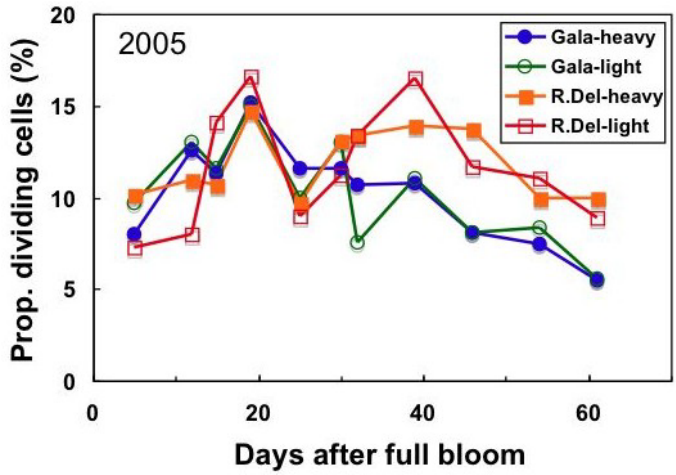

Fig. 4. The proportion of cells in Gala and Red Delicious fruit actively dividing during the 2005 growing season as estimated by flow cytometry.

In 2006 however, there was no difference between Gala and Red Delicious iin terms of the proportion of cells dividing. In addition, crop load did not affect the proportion of cells dividing. As noted earlier (Fig. 3), neither cultivar nor crop load affected fruit cell number.

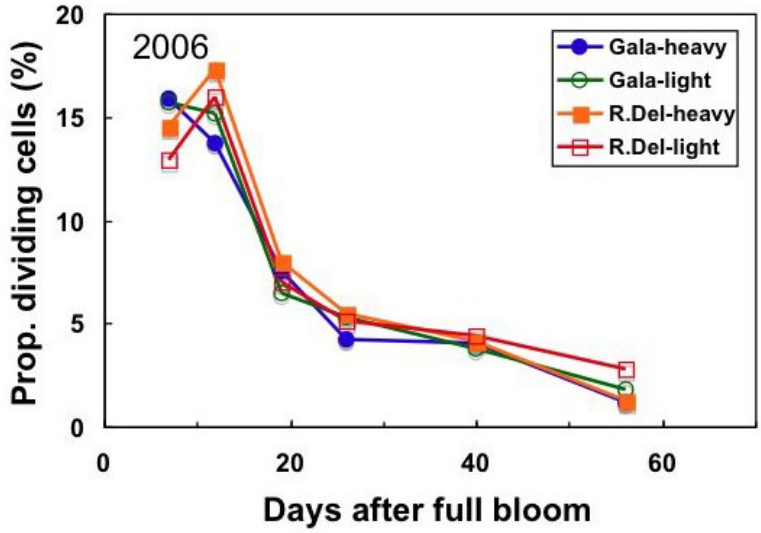

Fig. 5. The proportion of cells in Gala and Red Delicious fruit actively dividing during the 2006 growing season as estimated by flow cytometry.

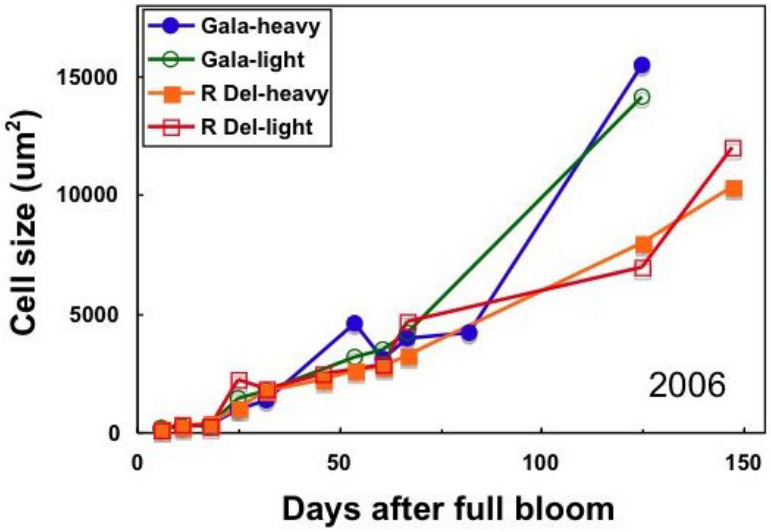

Fig. 6. Size of cortical cells in Gala and Red Delicious fruit under different crop loads during the 2006 growing season.

Crop load had no effect of fruit cortical cell size (Fig. 6). During the early part of the season, both Gala and Red Delicious had similarly sized cells. Cells in Red Delicious increased linearly over the season, whereas Gala cells appeared to increase dramatically at the end of the season. This result is puzzling but it should be borne in mind that is relies on one sample at the end of the season.

Fruit size throughout the season was much more closely related to cell size, rather than cell number Figs. 7-8). Caution should be used in concluding that cell size is the only driver of fruit size, since this analysis includes data from early in the season through to harvest. Cell size is also a product of how rapidly cells are dividing.

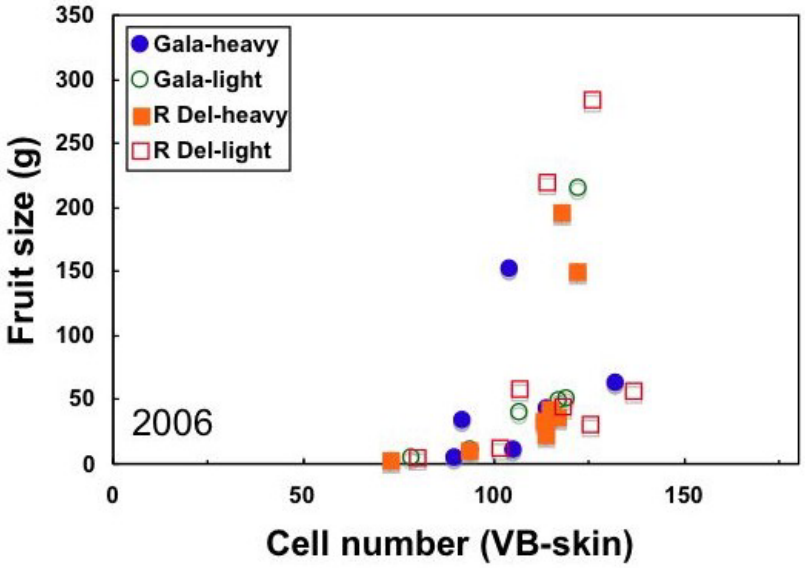

Fig. 7. The relationship between fruit size and cortical cell number in Gala and Red Delicious fruit under different crop loads during the 2006 growing season.

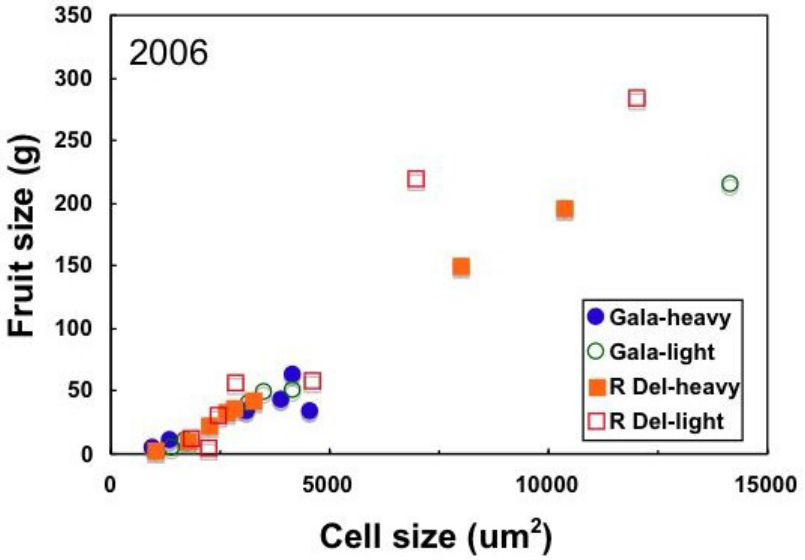

Fig. 8. The relationship between fruit size and cortical cell size in Gala and Red Delicious fruit under different crop loads during the 2006 growing season.

FINAL PROJECT REPORT
WTFRC Project Number: AH-04-419

Project Title: Monitoring apple fruit growth for predicting chemical thinning response

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Budget History:

Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries			
Benefits			
Wages	$13,100.00	$6,700.00	$15,000.00
Benefits	$4,978.00	$2,345.00	$5,719.00
Equipment			
Supplies	$1,322.00	$355.00	$1,600.00
Travel			
Crop Loss	$600.00	$600.00	$600.00
Spreadsheet			$400.00
Miscellaneous			
Total	$20,000.00	$10,000.00	$23,319.00

Objectives:
1. Follow fruit growth after thinner application to confirm that fruit growth is an appropriate and timely indicator of final indicator of final fruit set. Many things may ultimately determine whether a fruit persists or abscises, but the ultimate indicator appears to be fruit growth. The goal is to be able to determine a thinner response within 7 or 8 days of application, allowing for additional thinner follow up if deemed necessary while fruit are still susceptible to normally used thinners.
2. Develop a fruit growth model and procedure that can be used by growers to predict fruit set and thinner response.
3. Develop a procedure for selecting fruiting spurs that will assure that spurs selected and measured represent the response that is occurring on the whole tree.
4. Incorporate a heating degree component in the predictions system and confirm its usefulness in predicting thinning. Although following fruit growth has been a reliable predictor of thinner response, in some cool years it has required up to 12 days to make a judgment about thinner effectiveness. The hope is to establish a heating degree unit threshold that must be accumulated before an assessment of thinner response can be made.
5. Assemble an easy to follow and clear set of instructions that will outline spur selection, fruit numbering and measurement and data collection that will streamline the setting up and carrying out the procedure necessary for accurate thinner response prediction. The goal is to have the instructions and procedures sufficiently straight forward so that hourly laborers may carry out this activity.
6. Construct an Excel-based spreadsheet template that will simplify data input and automate the majority of required calculations. The hope is to ultimately have a system in place where data can be entered directly into the data base in the field and then download this into a computer to receive an near instantaneous result.
Utilize the Phytech® fruit growth sensor to accurately and continuously monitor and record growth information following thinner application. This activity will allow determination of the most precise information and earliest possible time that responses to a thinner can be detected.
7. Determine if the variability in tree response to the same thinner treatment applied at different times can be explained, then predicted, by accounting for tree sensitivity with a carbohydrate supply/demand estimate.

Significant Findings:

- We have confirmed that following fruit growth is an extremely accurate way to predict the fruit response to chemical thinner application and weather.
- The weather following thinner application, especially temperature, influences not only the extent of thinner response but also the time required to see a response. We now have a clear picture of the number of degree days required to see and observe a response.
- We have established a very simple and straight forward system so that workers can be trained in a minimum amount of time to collect and process the appropriate data.
- We have established a proactive chemical thinning approach where a decision at bloom is made about the crop load density that an orchardist hopes to achieve at the end of June drop and then allows that grower to monitor the progress toward that goal and allow for corrections if the goal is not predicted to be achieved.
- We have constructed an Excel-based spreadsheet that data can be downloaded into and where nearly all required calculation are done by the macros contained in the spreadsheet.
- Physiological and environmental data collected over the course of several years has allowed us to improve and refine the ability of the carbohydrate model to predict fruit abscission.

Methods:

Number of Spurs to Measure and Location of Spurs

Six 7-year-old Honeycrisp/M.9 were selected. Three lower scaffold limbs, three upper scaffold limbs and the central leaser were separately tagged and all blossom clusters counted at the pink sage of flower development. Six 4-year-old trees each of Desert Rose Fuji and Braeburn trained as super spindle trees were selected and the trees divided equally with tape into 4 sections: bottom, lower 25-50%, upper 50-75%, and the top of the tree. At bloom time every other blossoming spur on the Honeycrisp, Fuji and Braeburn were tagged. At the end of June drop all fruit on each limb or tree segment was counted and fruit set was taken on individual spurs. Fruit were identified as being located on a tagged or untagged spur.

Heating Degree Day Studies and Fruit Growth Studies

Heating degree days were calculated from temperatures taken in the orchard where the thinning studies were conducted from 2003 through 2006. Spurs were tagged and measurements taken on tagged and numbered fruit according to the method outlined in the Results. Thinners were then applied and fruit periodically measured. Based upon growth rate of measured fruit, the days after thinner application when a prediction with 90% accuracy could be made was noted.

Results and Discussion

The Basis for Predicting Thinner Effectiveness

The basis for the prediction is the slowing of growth of fruit that are destine to drop. This slowing of growth can be evident within 3 days when very warm conditions follow thinner application but may take as long as 8 days if the weather is cool. Fruit do not stop growth immediately but this reduction in growth can be detected before growth stops with careful measurements. Figure 1 illustrates the typical growth curve of a fruit that will persist to harvest and one that will abscise as the result of thinner application. A fruit is predicted to drop if the growth rate falls to 50% or less than the growth of the fastest growing fruit being measured. Although the relationship between fruit drop and fruit growth rate is a curve, based upon previous experience, we have selected 50% as our simplified cut-off level (Compact Fruit Tree 38(3)17-20, 2005).

Number of Spurs to Measure

Tagging, marking and measuring spurs is a time consuming process. Consequently, we wanted to determine the approximate number of spurs to measure to get a good prediction of response on the tree without incurring unnecessary work. The result in Table 1 show how close prediction of set on spurs was to the actual number of fruit on the tree. These are the average results from 5 trees. A 100% prediction is where the prediction and the actual number of fruit on the tree are exactly the same. A 90% or 110% represent a prediction that is either 10% too low or 10% too high, respectively. In our estimation a sampling error of +/- 10% is a reasonable compromise for precisions vs number of spurs measured. For the Honeycrisp 1 in 22 spurs measured gave a good representation whereas on Fuji 1 in 16 or 18 spurs seemed adequate. The Honeycrisp had 350 to 400 blossom cluster whereas the Fuji had 150 to 175. Based upon these data we feel that measuring 15 spurs on 6 different trees (a total of 90 spurs) would give a representative prediction for the whole tree. These results also confirm our original protocol suggestion of using a total of 100 spurs on several trees.

We evaluated fruit set on various portions of the tree to use as a guide in determining the distribution of spurs on the tree. On both Honeycrisp and Fuji in both years fruit set was not the same on all portions of the tree, even though blossom cluster density was frequently similar (data not shown). In general, fruit set was higher on the upper portion of the trees and in some instances it was more than twice the amount. This confirms that the distribution of the tagged spurs to measure must

be in all portions of the tree and that they should be distributed in direct proportion to the blossom cluster density.

Table 1. Effect of the number of spurs sampled on a tree to predict final set on the accuracy of the prediction. The percent given represents the amount above or below the actual number of fruit counted on the tree at the end of June drop.

Spurs	Honeycrisp (%)		Desert Rose Fuji (%)	
Sampled	2005	2006	2005	2006
All spurs	100	100	100	100
1 of 2	111	106	100	94
1 of 4	107	101	107	94
1 of 6	102	100	97	88
1 of 8	109	107	95	100
1 of 10	112	88	101	84
1 of 12	94	108	130	103
1 of 14	119	95	85	96
1 of 16	121	121	91	83
1 of 18	91	100	93	117
1 of 20	103	86	134	---
1 of 22	96	114	---	---
1 of 24	68	106	138	---
1 of 30	131	83	---	---

Heating Degree Day Studies and Fruit Growth Studies

While doing the research to develop a protocol for predicting thinning we measured fruit at 2 to 3 day intervals after thinner application and based upon growth rate of fruit we could make a prediction at each of the times of measurement. The accuracy of or prediction was tested by comparing actual set at the end of June drop. If this method is to be a useful tool for orchardists a call must be made soon after thinner application, but without the benefit knowing the final set. We noted in most years that an accurate prediction could be made within about 6 to 7 days from thinner application but sometimes it took as long as 12 days. In nearly every situation where the time of prediction was unduly delayed, it was associated with cold weather following thinner application. We initiated this study to see if we could use a heating degree day model to tell us when sufficient heat unit had a heating degree units following thinner application in 13 experiments over 4 years. We also determined when we could make a prediction of final set that was accurate to within 10% of the final set. Two things should be noted from this table. First, it requires 6 to 7 days after application for a thinner to cause physiological events that result in fruit abscission. If trees are exposed to 130 to 140 heating degree units (base 50) following thinner application, a thinning prediction can be made in about 7 days. However, if cold weather follows thinner application it took much longer. Our conclusion from these data is that an accurate prediction can be made 7 days after application if trees are exposed to approximately 130 heating degree units. If cool weather follows application then you must wait not only the 7 days but also until the 130 heating degree unit has been accumulated. The data for 2006 should be explained. The Fuji, Braeburn and Gala trees were all sprayed with a petal fall spray of carbaryl. This was then followed at the 7 mm stage with MaxCel. The days after application are for MaxCel and not carbaryl. Following carbaryl application weather turned warm and over 70 heating degree units was accumulated before MaxCel application. Therefore, the prediction results represent the earlier application of carbaryl.

Table 2. Growing degree days (base 50) following application of thinners in 2003-2006. Thinning and prediction of thinner response could be accurately made in 2003 and 2005 at 5-8 days after application, whereas it required 10-12 days for a similarly accurate prediction in 2004. The * indicates the time at which a thinning prediction could be made with 90% + accuracy.

Days	2003		2004		2005			2006
after	Delicious	Golden Delicious	Golden Delicious	Delicious	Braeburn	Delicious	Braeburn	Fuji
application	McIntosh			McIntosh	Fuji			Braeburn
								Gala
1	14.3	22.8	9.2	10.3	7.9	10.0	12.8	28.3
2	31.6	38.6	19.5	25.4	18.4	22.8	34.0	50.0
3	46.4	56.0	30.3	29.0	28.4	44.0	58.6	84.1
4	60.1	74.2	45.2	40.5	41.2	68.6	76.1	110.8**
5	82.9	97.9	55.9	47.8	87.0	86.1	100.1	132.0*
6	98.7	119.6	70.3	57.0	104.5	110.6	126.1	144.1
7	116.1*	145.7*	84.8	67.3	129.0*	136.1*	151.4*	156.8
8	134.3	161.7	84.8	78.1	154.5**	161.4	174.3	172.4
9	158.0*	181.8	98.7	93.0	179.8	184.3	199.6	193.8
10	179.7	207.3	110.2	103.7	202.7	209.6	223.4	206.3
11	205.8	224.3	120.4	118.1**	228.0	233.4	250.6	219.8
12	221.3	240.9	132.9*	132.6	251.8	260.6	275.7	237.3

We evaluated the basic method to predict thinning in 2006 in both New York and Massachusetts and we found it to be very accurate in predicting the final set within 7 days. Following application at both locations a warm period occurred which resulted in the exquisite accumulation of over 130 heating degree units within the 7 day period.

The Carbon Balance Model

Over the period of this grant we have used the information collected to refine the model to predict and explain thinner response under differing environmental conditions. The use of a mathematical carbohydrate supply-demand balance model to integrate the weather effects was found again to help explain a pattern of relative thinner response to a timing trial with the same NAA/Sevin treatment. When the carbohydrate balance was good, there was mild thinning. However, a very warm period occurred at the 10-14 mm stage, leading to a poor carbohydrate balance and much stronger thinning response at that time. This again shows that using such a model to integrate weather effects can help explain and better predict (within the limits of weather predictions) the thinning response related to weather

The Generalized Procedure for Predicting Thinner Response

Select and tag spurs

On 5 to 8 representative trees select 10 to 15 spurs for a total of about 100 flowering spurs. The distribution of these spurs should be in proportion to the flower distribution on the tree. If you feel that set on your trees is uniform throughout, placement of spurs may be made for convenience and ease of measurement.

Time to select spur and mark fruit

Spurs should not be selected until average fruit size has reached at least 6 mm. At this time fruit are sizing rapidly and this is the time when there is the start of intense competition among fruit and growing fruits for available carbohydrates. Each fruit in each cluster should be marked with a pen with indelible ink (eg Sharpie) to identify each fruit. The method that works well is the simple numbering system 1, 2, etc. in each cluster.

Measuring the fruit

There are two key things that must not be deviated from during the measuring and data collection process. First, fruit must be measured at the same location each time. Fruit are frequently asymmetrical and measuring the fruit at a different location can cause variability that is greater than the fruit growth over an individual measurement period. Secondly, the growth of individual fruit must be identified so that their growth rate can be calculated individually.

Determine bloom density and target fruit set

We suggest that before you put on any thinner you establish a target crop load that you would like to see at the end of June drop. This can be done by counting all blossom clusters on 2 limbs per tree on 5 to 6 trees or all blossom clusters on smaller trees at the pink stage of flower development. You then determine ideally how many fruit you would like to set and persist to harvest. For example, if you have 200 blossom clusters and you would like to see 100 fruit in total from these spurs then your goal is to have a final set of 50% or 1 fruit for each 2 flowering spur. The spurs that you have selected are representative of the spurs on the whole tree. If you have tagged 100 spurs and after measuring all fruit you have 430 developing fruit. On those spurs you would like to see 50 fruit, or an average of 1 fruit per 2 spurs. Therefore, you would like to see 50/430 x 100 = 11.9% of those developing fruit to persist to give you the ideal crop load.

Identifying fruit that will persist to harvest

To determine if fruit growth is slowing you must identify fruit that will persist. Initially it may appear that identification of the fruit that will persist to harvest may be an impossible task. In actuality, identification of these has proven to be relatively easy and reliable. The largest and fastest growing fruit are the most able to compete with smaller and slower growing fruit. Usually 99% of the fastest growing fruit will persist. To arrive at the growth rate of fruit to persist to harvest at each measurement period we take an average of the 20 fastest growing fruit. The Excel spreadsheet automatically identifies these rapidly growing fruit and makes the calculation.

When to measure fruit and when you can make a reliable prediction

During the development of this procedure for predicting thinner response we measured fruit initially and again at 2 to 3 day intervals. This was necessary for development of the model. When put into practice in a grower orchard fewer measurements probably will not be necessary. The initial measurement may not be required. Once applied it requires 3 to 4 days for a thinner to slow growth. At least one measurement will be necessary at this time. Normally, it requires about 7 days for growth reduction to be manifest, but temperature has a strong modifying effect (Table 2). Therefore, we suggest that you monitor the accumulation of heating degree units and make no final measurement or determination until at least 130 to 140 heating degree units (base 50) have accumulated. We also suggest initially that at least 3 measurements should be made between 3 and 8 days after application.

Predicting which fruit will persist or drop

A fruit is predicted to drop if the growth rate of a fruit is less than 50% of the growth rate of the average of the 20 fastest growing fruit. Conversely, a fruit is predicted to set if the growth rate of that fruit is 50% or greater of the 20 fastest growing fruit.

Taking and recording data

Attached is a portion of a fruit size data base that we used in one of our experiments (Table 3). The Excel spreadsheet is set up to accept data in this format. In this experiment we used 5 trees with 20 spurs per tree tagged. Each spur had an initial set of 2 to 5 fruit. We suggest that you use a format similar to this when organizing and collecting fruit growth data.

Table 3. Excel data template showing fruit size measurements in mm fruit diameter that may be useful in organizing and entering fruit growth data.

Tree	Spur	Fruit	28May	31May	2 June	5June	7Jun
1	1	1	8.0	10.9	12.1	13.9	15.0
1	1	2	7.3	10.2	11.1	12.0	13.6
1	1	3	7.7	8.1	8.0	8.0	8.0
1	2	1	7.7	10.4	11.7	12.9	14.2
1	2	2	6.7	9.0	9.7	11.3	11.5
1	2	3	6.8	7.0	7.0	6.9	6.8
1	3	1	6.9	9.4	10.8	12.2	13.3
1	3	2	7.1	9.5	10.9	12.3	13.2
1	3	3	6.2	7.0	7.2	7.1	7.0
1	3	4	6.4	9.9	10.0	10.7	10.8

Predicting thinning where multiple thinners are used.

Increasingly growers are using a multiple thinner approach to achieve appropriate crop loads. Frequently a bloom or petal fall spray is used. The system outlined is valid in predicting final set. No adjustments need to be made. There is a danger however, of taking measurements too early before fruit reach 6 to 7 mm. If one starts the timer too early, before the period of rapid growth begins and fruit competition is not great, it is like to take several days longer to note reductions in fruit growth and make a prediction.

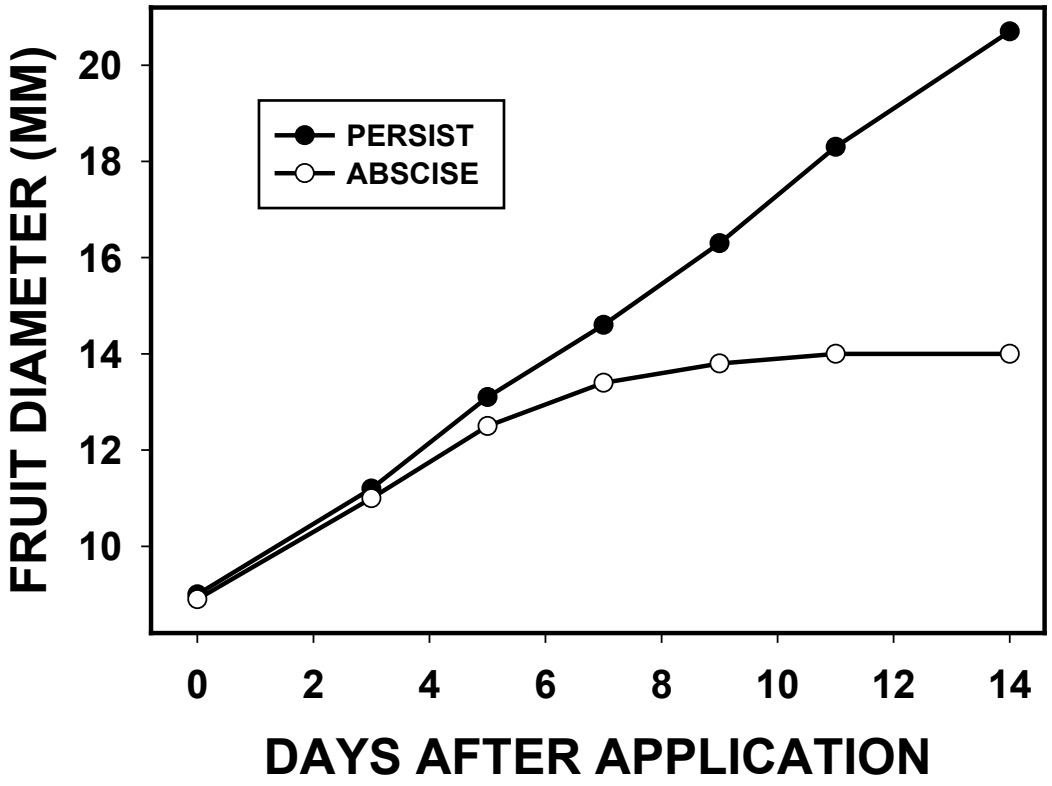
Is an untreated control necessary?

Initially we included in our experimental protocol an untreated control. The primary purpose was to use fruit on these trees to establish the growth rate of the fastest growing trees. However, we felt that under most conditions growers are unlikely to leaves trees unthinned and measuring fruit on more trees was time consuming. We have made successful predictions on thinned trees in the past couple of years. The only danger of not using an untreated control is if the thinner(s) are so aggressive that no fruit set, and this is an unlikely occurrence.

The Excel spreadsheet with automatic calculations

Macros have been written in the Excel spreadsheet to make all of the calculations automatically. The spreadsheet consists of 6 sheets: Input, Staging, Setup, Summary, Diameter and Growth Rate, and a Count sheet. The Input sheet is where the data is downloaded. The Staging sheet is where the data is reorganized for calculations. The Setup sheet shows tables of the sampling date and the target fruit number and percent set. The Summary sheet is most informative in that it shows on each sampling date the fruit diameter of the fastest growing fruit the number of fruit that grow greater and less that the 20 fastest growing fruit, a predicted % set and a predicted % drop. Finally there is a Diameter sheet and a Count sheet.

FIGURE 1. FRUIT GROWTH



FINAL PROJECT REPORT
WTFRC Project Number: AH-04-418

Project Title: Water use, quality and growth as affected by irrigation and/or rootstock
PI: Dr. Essie Fallahi, Professor and Research Director, Pomology,
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Cooperators: Dr. Jim McFerson

Budget History:

Item	Year (2004)	Year 2005	Year 2006
Salaries*	6000	6000	$10,000
Benefits (45%)	2700	2700	$4,500
Wages**	2760	2760	$4600
Benefits (25%)	690	690	$1150
Equipment	450	450	$750
Supplies (Lab & Orchard) & other charges	720	720	$1200
Travel***	720	720	$1200
Miscellaneous (Land Charge)	960	960	$1600
Total	15,000	15,000	$ 25,000

* Salary is for technical assistants and FB for that is 45%; ** Wages are for various part of this project conducted by part-time helpers

*** Travel includes mileage charges for local and regional travel related to the project. We use the university motor vehicle and they charge us 42.5 cents per mile.

SIGNIFICANT FINDINGS:

Findings in 2003 and 2004 Seasons:

1) Water use was highest in July during 2004. Water usage per tree for full drips and full sprinklers were higher in 2004 than 2003. In 2004, about 1437 gallons of water per season was used by each tree with sprinkler system while each tree with full drip used 639 gallons. Trees with full sprinkler and partial zone sprinkler received greater water than those with drip systems in both 2003 and 2004. Trees with full sprinkler system received about 28 inches in 2003 and about 33 inches in 2004 while those with partial zone drip received less than 15 inches in 2003 and about 17 inches of water in 2004. At the peak of the water use (July), each full sprinkler tree received an average of 14.15 gal water per day while each tree with full drip system received an average of 5.5 gal/tree in 2004.

2) Trees with partial zone drying drip irrigation were smaller than other treatments. Tree under full drip system had the same size as those under full sprinkler in 2004. Size of 'Desert Rose Fuji' trees with double drip irrigation was not noticeably larger than those with single drip system in 2003 or 2004.

3) Trees with sprinkler irrigation had greater leaf N than those on other treatments in 2003. Total leaf value for double drip was 2.19% dwt while that of single drip was 2.16% dwt in 2003.

4) In 'Autumn Rose Fuji', yield of trees with sprinkler system was lower than those with other irrigation treatments in 2004. Fuji trees with partial sprinklers had smaller but sweeter fruits than those on other treatments in 2004.

5) Trees on Supporter 4 were larger followed by those on BUD 118, G30, RN29, and Bud 9 in 2004. In Gala, drip irrigation resulted in higher yield than sprinkler system in 2004. Fruit size of Gala on RN 29 was larger than that on other rootstocks in 2004.

6) Following soil moisture may provide a useful guide but was not very exact indicative of water needs.

Findings in 2005 Season:

1) Each tree with full micro-sprinkler (sprinkler) treatment received 1541 gallons of water (total of 35.36 inches/acre) while each tree with full drip had 908 gallons (total of 20.80 inches/acre) in 2005.

2) 'Autumn Rose Fuji' from trees with full drip had higher yield and sugar than those of sprinkler in 2005.

3) In 'Autumn Rose Fuji', partial sprinkler had lower yield and smaller fruit than many other treatments in 2005.

4) In 'Desert Rose Fuji', fruit color and percentage of sunburn were lower in trees with Double drip irrigation system as compared to Single Drip. Other yield and quality parameters were similar in Double and Single Drip irrigation systems.

5) 'Gala' Trees on RN 29 had higher yield and larger fruits than those on all other rootstocks in 2005. 'Gala' fruits from trees on Bud 9 had higher yield efficiency, fruit sugar, color, and starch degradation but lower firmness as compared to other rootstocks in 2005.

6) 'Gala' trees with drip irrigation had significantly higher yield, fruit size, and starch degradation pattern than those with sprinkler system in 2005. However 'Gala' fruits from drip irrigation had lower color, sugar, and firmness as compared to those in sprinkler system in 2005.

Findings in 2006 Season:

1) The amount of water used in each treatment in 2006 was higher than that in 2005 but the patterns of use in within treatments were similar to those in 2005 in Fuji and Gala apples.
2) Trees on B.9 had at least 79% more bourse shoots with fruit than those on other rootstocks and at least 30% more bourse shoot without fruit than Supporter 4 and G.30.
3) Gala trees on B.9 ceased its terminal growth and formed its terminal buds about one once before the trees on other vigorous rootstocks, and thus leaves from trees on B.9 can be sampled for mineral analyses about one month before the trees on more vigorous rootstocks.

4) 'Pacific Gala' fruits from trees on G.30 and B.9 rootstocks had at least 13% higher starch degradation patter (SDP) than those on RN-29 and Supporter 4 rootstocks, whereas trees on RN-29 had higher fruit weight than those on B.9 and Supporter 4. 'Pacific Gala' on G.30 had more than double the amount of fruit crack than those on any of the other rootstocks.
5) 'Pacific Gala' on B.9 had at least 8% higher shoot leaf N, than those on Supporter 4 and G.30. Trees on B.9 had 36% less shoot leaf K than those on other rootstocks. In contrast, trees on B.9 and RN-29 had at least 8% higher shoot leaf Mg and trees on B.9 had at least 24% higher shoot leaf Ca than those on the other rootstocks.

RESULTS AND DISCUSSION

Water Usage

Water usage in all irrigation systems in 'Fuji' and 'Gala' was higher in 2005 than 2004. Trees used the highest amount of water in July and August in 2004, 2005, and 2006. Trees with FS treatment received significantly greater volume of water than those with drip systems in both 2004-2006. During the entire growing season, total of 5401.2 L and 5832.7 L of water was applied to each tree with FS system while 2403.5 L and 3436.8 L was applied to each tree with full drip system in 2004 and 2005, respectively. Each tree with PRS received more water than those with any type of drip systems in 2004 and more than DD and PRD in 2005. Trees with FS treatment received 846.6 mm and 898.1 mm while those with PRD received 248.9 mm and 350.5 mm of water during the entire 2004 and 2005 growing seasons, respectively with minor or no visible damage to the trees with PRD system. 'Pacific Gala' receiving drip irrigation used about 38% less water as compared to those receiving sprinkler system (Table 12 and Figure. 6). Each tree receiving drip used 3872 liters of water whereas each tree receiving sprinkler used 6250 liters of water during 2006 growing season.

Tree Growth, Leaf Area, and Leaf Mineral Nutrient Concentrations in 'Autumn Rose Fuji'

Trees with FS and FD had similar TCSA, but both treatments had significantly greater TCSA than those of other irrigation treatments (Table 2). Fresh weight, dry weight, and leaf area tended to be higher in trees receiving FS and FD treatments than other treatments, although differences were not always significant. This difference led to a lower leaf N, Mg, and Zn concentrations in FS and FD treatments due to a dilution effect. (Table 2). Irrigation treatments did not affect percentage of dry matter (Table 2) or N content per leaf (data not shown). Leaf K concentrations (Table 2) and leaf K content and fruit K concentration (data not shown) in FS and FD treatments were significantly higher than those in other treatments. Leaf K differences in this study must be due to the difference in the volume of irrigation applied. This finding suggests that water deficiency can reduce K status and this point should be taken into account when interpreting leaf analysis data. Trees with FS and FD irrigation systems also had lower Mg concentration than other treatments due their higher K concentrations, causing an antagonistic effect between these two elements. Leaf Cu concentration in FS was higher than those in all treatments.

Yield and Fruit Quality Attributes at Harvest in 'Autumn Rose Fuji'

Trees in all drip systems had significantly higher yield and yield efficiency than those in FS (Table 3), perhaps because drip systems induce varying levels of stress on the tree, forcing them to induce higher number of fruit spurs. Fruits from trees receiving PRS and DD treatments had significantly smaller fruits. However, fruits with PRS had higher SSC than all other treatments, perhaps either due to its smaller size or due to higher level of abscissic acid (ABA) production, resulting in higher SSC. Fruit from trees receiving PRD treatment had greater starch degradation pattern but lower firmness than most other treatments. This suggests that PRD treatment advances fruit maturity. Trees with FS and FD had relatively lower sunburn due to their larger canopy, resulting in more shading and fruit protection.

Rootstock Effects on Tree Growth, Yield, and fruit Quality of 'Pacific Gala'

Results in Gala in 2004-2005: 'Pacific Gala' trees on Bud 9 were least vigorous followed by those on RN-29, G 30 and Supporter 4 (Table 4). Yield and yield (Table 4) and yield efficiency (data not shown) were higher in B9 and RN-29. 'Pacific Gala' fruit were also larger in trees on RN-29. Fruits from trees on B9 had lower firmness but higher SSC and starch degradation pattern than those on RN-29 and Supporter 4. This finding suggests that 'Pacific Gala' on this B9 rootstock matures earlier, perhaps partially due to it's smaller and more exposed canopy. Relatively smaller fruits and higher yield in the trees on B9 mandates a more aggressive thinning program on this rootstock than those on other rootstocks. Fruits from trees on G30 had better color than those on RN-29 and Supporter 4. Supporter 4 did not show over-all satisfactory performance in our evaluation. Trees on this rootstock were too vigorous and had low yield. Considering all growth, yield, and quality attributes, both RN-29 and B9 showed excellent performance. However, we strongly suggest that a fruit protection such as Surround must be applied on these trees to protect the fruits from sunburn, as canopies of these trees, particularly those on B9, are not sufficiently protected by shading.

2006 Results in Gala:

2006 Growth Analysis - *Rootstock effects* (Table 6 and Fig 1): Rootstocks influenced the scion numbers of developing spurs (DS), side shoot with fruit (SSWF), side shoot without fruit (SSWOF), bourse shoots with fruit (BSWF), and bourse shoots without fruit (BSWOF) (Table 6). For each rootstock, the numbers of scion DS, SSWF, BSWF and BSWOF in June were similar to those in August. Therefore, only values for August are reported in this report. 'Pacific Gala' on Supporter 4 and G.30 had higher numbers of DS than those on the B.9. Trees on Supporter 4 had higher number of SSWOF than those on other rootstocks. Side shoot with fruit among different rootstocks were similar. Trees on B.9 had at least 79% more BSWF than those on other rootstocks and at least 30% more BSWOF than Supporter 4 and G.30. 'Pacific Gala' Trees on B.9 always had smaller trunk cross sectional area and their terminal buds were formed and terminal growth was ceased before trees o other rootstocks (Figures 1 and 2).

2006 Growth Analysis in Gala - *Irrigation effects*

For each irrigation system, the numbers of scion DS, SSWF, BSWF and BSWOF in June were similar to those in August. Therefore, only values for August are reported in this report. Trees receiving drip irrigation had more of SSWF, SSWOF, and BSWF than trees receiving sprinkler irrigation (Table 7). However, trees with sprinkler irrigation had more BSWOF than trees receiving drip system. The number of DS was unaffected by irrigation systems.

2006 Shoot Leaf mineral analysis in Gala- *Rootstock effects*

Leaves from trees on B.9 had at least 17% less leaf area and 22% lower fresh weight, 11% less dry weight than those on other rootstocks (Table 8). 'Pacific Gala' on B.9 had at least 8% higher shoot leaf N, than those on Supporter 4 and G.30. Trees on B.9 had 36% less shoot leaf K than those on other rootstocks. In contrast, trees on B.9 and RN-29 had at least 8% higher shoot leaf Mg and trees on B.9 had at least 24% higher shoot leaf Ca than those on the other rootstocks. Trees on B.9 and Supporter 4 had 13% more P levels in their shoot leaves than those on RN-29.

2006 Fruit quality attributes at harvest in Gala- *Rootstock effects*

Rootstocks influenced the scion fruit SDP (starch degradation pattern), fruit weight, percentage of fruit crack, yield, SSC (soluble solids concentration), and fruit color (Table 9). Fruits from trees on G.30 and B.9 rootstocks had at least 13% higher SDP than those on RN-29 and Supporter 4 rootstocks, whereas trees on RN-29 had higher fruit weight than those on B.9 and Supporter 4. 'Pacific Gala' on G.30 had more than double the amount of fruit crack than those on any of the other rootstocks. Trees on RN-29 had at least 23% higher total fruit yield than those on other rootstocks, whereas trees on Supporter 4 had lower SSC than those on B.9 and G.30. Trees on B.9 had 10% more fruit color than those on Supporter 4. Fruits from G.30 had significantly lower fruit firmness.

2006 Fruit quality attributes at harvest in Gala- *Irrigation effects*

Trees receiving sprinkler irrigation had significantly 8% more fruit color than trees receiving drip irrigation (data not shown). Other fruit attributes of 'Pacific Gala' were unaffected at harvest time by irrigation systems in 2006.

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Table 1. Cumulative ET and depth of applied water and average daily and total water applied per tree in irrigation systems in 'Autumn Rose Fuji' in 2004 and 2005.

Treatment[z]	Cumulative ET values or depth of applied water (mm) in 2004						Applied in 2004 (L/tree)	Cumulative ET values or depth of applied water (mm) in 2005						Applied in 2005 (L/tree)
	June	July	Aug.	Sep.	Oct.	Cum.		June	July	Aug.	Sep.	Oct.	Cum.	
ETr	212.1	218.9	208.0	97.5	43.2	924.8		192.3	243.6	264.7	133.1	18.5	956.3	
Etc	191.5	215.6	207.3	94.7	33.8	846.6		181.9	252.0	275.1	133.1	14.6	931.2	
FS	191.5	215.6	207.3	94.7	33.8	846.6	5401.2	163.8	249.7	274.6	129.0	10.4	898.1	5832.7
PRS	95.8	107.9	103.6	47.5	16.8	455.7	3285.4	81.8	124.7	137.4	64.5	5.2	469.6	3046.9
FD	72.9	107.9	85.1	47.4	19.6	369.3	2403.5	88.9	169.2	148.6	79.5	6.4	528.3	3436.8
DD	47.2	70.1	55.1	31.2	12.8	248.9	1620.0	57.9	110.0	96.5	51.6	4.1	350.5	2282.4
PRD	47.2	70.1	55.1	31.2	12.8	248.9	1620.0	57.9	110.0	96.5	51.6	4.1	350.5	2282.4

[z] Abbreviations for irrigation treatments: FS=Full Sprinklers; PRS=Partial Root-Zone Drying Sprinklers; FD= Full Drip; DD=Deficit Drip; PRD=Partial Root-Zone Drying Drip.

Table 2. Effect of different irrigation systems on trunk cross sectional area (TCSA), leaf area, and leaf macronutrient of 'Autumn Rose Fuji' apple[z].

Irrigation[z]	TCSA (cm^2) [Y]	Fresh wt/leaf (g)	Dry wt/leaf (g)	Percent dry wt	Leaf area (cm^2/leaf)	N (% dwt)	K (% dwt)	Ca (%dwt)	Mg (% dwt)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
FS	50.8 a	0.80 a	0.33 a	42.8 a	25.5 a	2.11 b	1.59 a	1.71 b	0.32 d	55 bc	14 b	8.3 a	55 ab
PRS	43.2 b	0.70 c	0.30 b	50.8 a	23.3 b	2.20 a	1.29 c	1.62 c	0.40 a	59 a	18 a	7.5 b	60 a
FD	51.6 a	0.77 ab	0.31 ab	40.7 a	23.7 b	2.10 b	1.41 b	1.63 bc	0.34 c	53 c	13 b	7.5 b	53 b
DD	44.8 b	0.72 bc	0.30 b	42.2 a	23.1 b	2.21 a	1.23 c	1.62 c	0.38 b	57 ab	17 a	7.3 b	59 a
PRD	45.2 b	0.70 c	0.30 b	41.3 a	23.5 b	2.21 a	1.28 c	1.81 a	0.39 ab	57 ab	16 a	7.4 b	60 a

[Z] Abbreviations for irrigation treatments: see footnote for Table 1. Each value is the average over 2004 and 2005. There were 5 blocks, each with 4 trees, with total of 20 trees per treatment per year. Mean separation within columns with LSD at 0.05.
[Y] TCSA= Trunk cross sectional area. %TCSA change = (TCSA in 2005-TCSA2004) /(TCSA 2004) x 100.

Table 3. Effect of different irrigation systems on fruit quality attributes in 'Autumn Rose Fuji' apple[z].

Irrigation[z]	Yield (T/ha)	Yield efficiency (kg/cm^2)	Fruit wt (g)	Fruit color (1-5)	Firmness (kg)	Sol. solids (°Brix)	Starch pattern	Sunburn (%)	Fruit Russet (%)	Water core (%)
FS	16.7 c	0.59 b	293.7 a	3.5 ab	8.37 ab	15.4 b	3.4 b	15 bc	44 a	47 ab
PRS	17.7 bc	0.81 b	245.7 c	4.8 a	8.40 a	16.3 a	3.4 b	23 a	38 a	49 ab
FD	23.3 a	0.77 a	291.3 a	3.4 ab	8.34 ab	15.5 b	3.6 b	10 c	38 a	53 a
DD	22.6 ab	0.98 a	272.5 b	3.3 b	8.23 bc	15.5 b	3.6 b	22 ab	26 b	41 b
PRD	23.0 a	0.97 a	286.4 a	3.4 ab	8.13 c	15.3 b	3.9 a	24 a	28 b	45 ab

[Z] Abbreviations for irrigation treatments: see footnote for Table 1. Each value is the average over 2004 and 2005. There were 5 blocks, each with 4 trees, with total of 20 trees per treatment per year. Mean separation within columns with LSD at 0.05.
[Y] Yield efficiency = yield per tree/TCSA.

Table 4. Effect of different rootstocks on yield and fruit quality of 'Pacific Gala' apple in 2004-2005[z].

Rootstock	TCSA (cm^2)	Yield (T/ha)	Fruit wt (g)	Fruit color (1-5)	Firmness (kg)	Sol. solids (°Brix)	Starch pattern
Bud 9	10.4 d	13.7 ab	204.5 b	3.4 ab	8.07 c	14.5 a	4.5 a
RN 29	19.3 c	15.8 a	223.2 a	3.2 b	8.34 ab	13.9 b	4.0 b
Supporter 4	26.4 a	7.2 c	206.0 b	3.1 b	8.57 a	13.9 b	4.0 b
G 30	22.1 b	12.3 b	205.7 b	3.5 a	8.14 bc	14.1 b	4.4 a

[Z] Each value is the average over 2004 and 2005. There were 5 blocks, each with 2 trees, with total of 10 trees per treatment per year. Mean separation within columns with LSD at 0.05.

Table 5. Effect of different irrigation systems on trunk cross sectional area (TCSA) , yield, and fruit quality in 'Pacific Gala' in 2004-2005[z].

Irrigation	TCSA (cm^2)	Yield (T/ha)	Fruit wt (g)	Fruit color (1-5)	Firmness (kg)	Sol. solids (°Brix)	Starch pattern
Drip	16.1 b	14.0 a	216.7 a	3.1 b	8.07 b	14.0 a	4.3 a
Sprinkler	17.9 a	10.4 b	202.9 b	3.5 a	8.49 a	14.2 a	4.1 b

[Z] Each value is the average over 2004 and 2005. Mean separation within columns with LSD at 0.05.

Table 6. Effects of rootstocks on the mean number of different types of shoots in 'Pacific Gala' apple in August, 2006.

Rootstocks	DS[z]	SSWF	SSWOF	BSWF	BSWOF
B.9	0.96 c[y]	0.09 a[x]	1.98 c	6.50 a	10.48 a
RN-29	1.88 bc	0.03 a	6.78 b	3.63 b	8.39 ab
Supporter 4	3.57 a	0.18 a	9.13 a	1.63 c	8.07 b
G.30	2.75 ab	0.08 a	7.08 b	1.53 c	7.12 b

[z]Abbreviations: DS = Developing spur, SSWF = Side shoot with fruit, SSWOF = Side shoot without fruit, BSWF = Bourse shoot with fruit, BSWOF = Bourse shoot without fruit. The values represent mean number of shoots on a segment of a limb that was 1.2 m in length in June, 2006.
[y]Means with different letters within a column are significantly different, by using protected LSD at 0.05 level. [x]Each value was the average of 5 blocks each with 4 trees (20 trees/rootstock).

Table 7. Effects of irrigation on the mean number of different types of shoots in 'Pacific Gala' apple in August, 2006.

Irrigation	DS[z]	SSWF	SSWOF	BSWF	BSWOF
Drip	2.70 a[y]	0.18 a[x]	0.18 a	0.18 a	7.59 b
Sprinkler	2.30 a	0.00 b	0.00 b	0.00 b	9.48 a

[z]Abbreviations: DS = Developing spur, SSWF = Side shoot with fruit, SSWOF = Side shoot without fruit, BSWF = Bourse shoot with fruit, BSWOF = Bourse shoot without fruit. The limb was 1.2 m in June. Each value was the average of two limbs on tree.
[y]Means with different letters within a column are significantly different, by using protected LSD at 0.05 level.
[x]Each value was the mean over 4 rootstocks with 5 blocks each with 4 trees (40 trees/irrigation treatment).

Table 8. Effects of rootstocks on the mean fresh weight, dry weight, % weight, leaf area and macronutrient concentrations in the shoot leaves of 'Pacific Gala' apple in 2006.

Rootstocks	Fresh wt (g/leaf)	Dry wtght (g/leaf)	Leaf % dry weight	Leaf area (cm^2/leaf)	N (% dwt)	P (% dwt)	K (% dwt)	Mg (% dwt)	Ca (% dwt)
B.9	0.9 b[z]	0.40 b[y]	43.06 a	33.00 b	1.97 a	0.15 a	1.00 c	0.40 a	2.00 a
RN-29	1.1 a	0.45 a	41.35 b	39.40 a	1.90 ab	0.13 b	1.25 b	0.39 a	1.61 b
Supporter 4	1.1 a	0.43 ab	39.36 c	39.80 a	1.80 b	0.15 a	1.60 a	0.32 c	1.61 b
G.30	1.1 a	0.43 ab	40.40 bc	38.90 a	1.81 b	0.14 ab	1.51 a	0.37 b	1.60 b

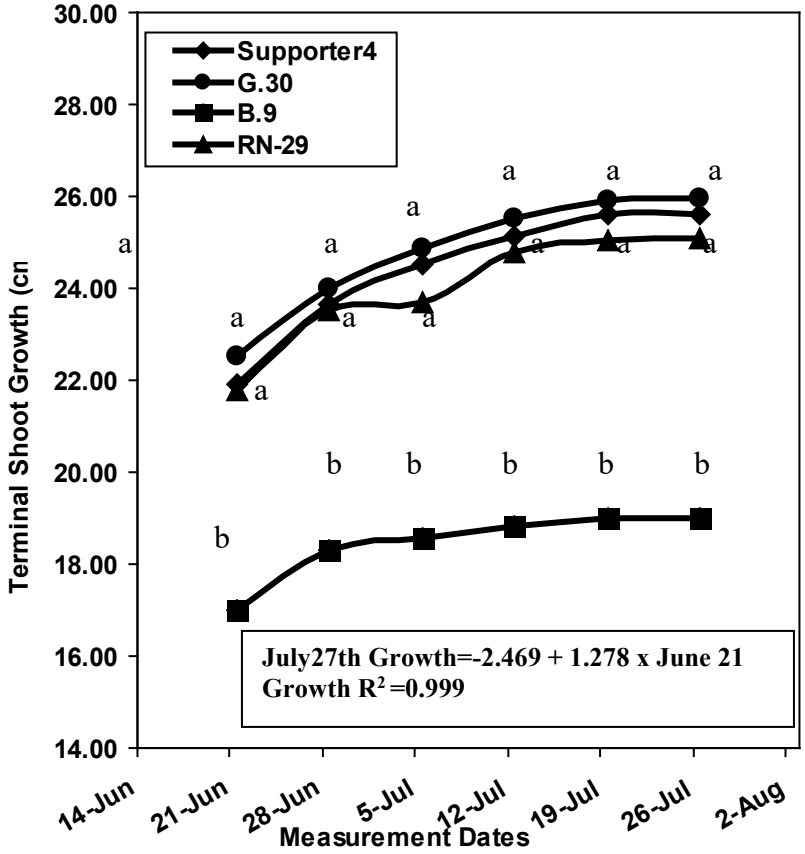
[z]Each value is the average of 5 blocks each with 4 trees (20 trees/rootstock).
[y]Means with different letters within a column are significantly different, by using protected LSD at 0.05 level.

Table 9. Effects of rootstock on the mean at-harvest fruit quality of 'Pacific Gala' apple in 2006.

Rootstocks	Avg. fruit wt (g)	Color	SSC[z] (Brix)	Firmness (Kg)	SDP	% Crack	Yield
B.9	189.2 b[y]	4.1 a[x]	14.5 a	8.8 a	4.1 a	13.4 b	19.4 b
RN-29	209.8 a	3.8 ab	14.0 ab	8.8 a	3.3 b	16.0 b	25.3 a
Supporter 4	194.0 b	3.7 b	13.8 b	9.0 a	3.2 b	20.1 b	6.1 d
G.30	201.0 ab	3.8 ab	14.4 a	8.4 b	3.8 a	45.4 a	13.2 c

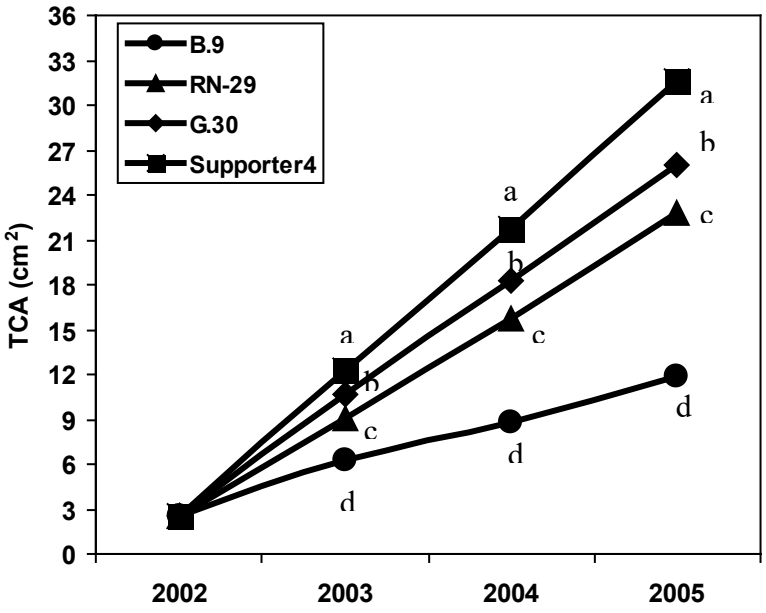
[z]SSC = Soluble solids concentration, SDP = Starch degradation pattern, Color was rated from 1 to 5 and SDP was rated from 1 to 6.
[y]Means with different letters within a column are significantly different, by using protected LSD at 0.05 level.
[x]Each value is the average of 5 blocks each with 4 trees (20 trees/rootstock).

Figure 1. Growth curves of terminal shoots on 'Pacific Gala' trees on different rootstocks during the season 2006[z].



[z]Mean separation within the sampling dates by LSD at the 5% level.

Figure 2. Mean trunk cross sectional area of 'Pacific Gala' on different rootstocks 2002-2005.[zy] .



[z]Trunk circumference was measured at 30 cm above the bud union.
[y]Mean separation within the sampling years by LSD at 5% levels.

FINAL PROJECT REPORT
WTFRC Project Number: AH-04-412

Project Title: Impact of cultural practices on apple canopy physiology

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries			
Benefits			
Wages	14,000	14,000	14,000
Benefits	2,240	2,240	2,240
Equipment	4,000	4,000	4,000
Supplies	2,500	2,500	2,500
Travel	3,000	3,000	3,000
Miscellaneous			
Total	25,740	25,740	25,740

OBJECTIVES:

- Understand the impact of cultural practices on canopy carbon acquisition and water use
- Understand the horticultural significance of altered rates of gas exchange
- Continue to refine the design and efficiency of the whole-canopy gas exchange measurement system
- Begin initial development of whole-tree carbon budgets and models

SIGNIFICANT FINDINGS (2004-2006):

Chemical thinner trials

- Fish oil + lime sulphur (FOLS) and soy oil (SO) reduced significantly whole-canopy net CO_2 exchange rate
- FOLS and SO reduced canopy NCER similarly
- mean canopy NCER over 9 measurement days and 3 applications was ca. 76% and 77% of control for FOLS- and SO-treated trees, respectively
- a 4-d spray interval appeared effective for maintaining a deficit carbon balance
- trees recovered fully from thinning treatment
- trees recovered quicker from SO treatment than from FOLS treatment

Deficit irrigation trial

- no deficit irrigation strategy (DI or PRD) affected significantly whole-canopy or leaf gas exchange or stomatal conductance
- water use efficiency did not vary among irrigation treatments
- withholding irrigation for more than 3 weeks did not cause physiological stress to 14-year-old 'Fuji'/M.26 trees
- for many orchards, water resources may be conserved without reducing canopy carbon balance
- soil water content is not a good indicator of physiological status of canopy

Calcium trials

- both Mora-leaf® and calcium chloride at 6lbs/ac reduced leaf NCER and stomatal conductance compared to water-treated control
- both calcium treatments affected leaf physiology similarly
- leaf NCER was 21-27% lower and stomatal conductance was 34-36% lower from treated trees two days after application
- Mora-leaf® alone and combined with a cover-spray reduced slightly (ca. 10%) leaf gas exchange

METHODS:

Chemical thinner trials

- The effects of multiple applications of the chemical bloom thinners fish oil + lime sulphur (FOLS) and soy oil (SO) on 'Sun Fuji' whole-canopy gas exchange were studied in relation to water-sprayed 'control' trees. Thinning treatments consisted of 2% FO + 3% LS and 4% soy oil emulsion applied by PropTec sprayer at 100 gallons per acre. Treatments were applied at a 4-d

interval beginning at 80% full bloom, three applications were made (e.g., 80% FB, 80% FB+4d, and 80%FB+8d). Leaf and canopy gas exchange measurements were collected using a CIRAS-2 and CIRAS-1 gas analyzer, respectively, prior to, and following thinner applications. Whole-canopy measurements were taken before and after the initial application (80% full bloom) and again following the applications at 80%FB+4d and 80%FB+8d. Following the 80%FB+8 application, we collected gas exchange for three days. Three replications of each treatment were measured (i.e., total of 9 trees). System flow rates were calculated from 21 velocity measurements per inlet pipe.

Deficit irrigation trials

- The effects of irrigation regime on whole-canopy gas exchange and water relations were studied on 11-year-old 'Fuji' trees in Prosser. Two novel season-long irrigation strategies have been established in these orchards (both receiving approximately 50+% evapotranspiration replacement): deficit irrigation, and partial rootzone drying (see Caspari project #AH-04-413 for complete experimental details). Using whole-canopy chambers, gas exchange was monitored at key physiological growth stages. In 2005 data were collected between 7 August and 12 August and again between the 6th and 9th of September. Single leaf gas exchange data were also collected throughout the season using a TPS-1 (PP Systems) on a weekly basis (data not shown).
- A separate trial was conducted in 2006 to evaluate canopy gas exchange in relation to soil water content. Two treatments were imposed: an irrigated control, which received irrigation by drip emitters (four/tree) beneath the chambers, and an un-irrigated treatment which did not receive irrigation throughout the trial. From 22 August until 13 September, we measured whole-canopy gas exchange of six trees. In addition, almost every day we measured at noon, single leaf gas exchange (CIRAS-2, PP-Systems) of 3 sunlit and 3 shaded leaves per tree, and stem water potential (PMS Instruments pressure bomb) from 3 leaves acclimated within foil bags for a minimum of 3 hours. Soil water content was recorded daily from tensiometers (three per tree) at three depths (6″, 12″, and 18″ below soil surface). Tensiometers were buried halfway from the trunk to the tree's dripline (ca. 2.5 feet from the trunk).

Calcium + cover spray trial

- The effects of calcium chloride, Mora-leaf®, and a prescribed codling moth cover spray on leaf gas exchange were compared in two separate trials in late summer 2005. In the first, trees were treated with water (control), Mora-leaf®, or Mora-leaf® + cover (5 lbs Imidan™, 8 fl. oz. Success™ per acre plus 1 pint Regulaid™ and 0.5 pint TriFol™ per 100 gallons) applied at 200 gallons/ac. Applications were made on 6 August (Day of year 218). In a separate trial, calcium chloride and Mora-leaf® were compared to untreated control. Applications of water, calcium chloride (6 lbs/ac), and Mora-leaf (6 lbs/ac) were made on 23 September to whole trees at 200 gallons per acre. For both trials, leaf gas exchange was measured on 3 sunlit leaves from 5 trees per treatment within 1 hr of solar noon. Data was collected the day before application and for several days following applications.

RESULTS & DISCUSSION

System for measuring canopy gas exchange

Over the course of this trial we have improved significantly our ability to monitor canopy gas exchange in remote orchard locations, and increase the number of trees (i.e., treatments or replications) we can evaluate. A 12kW continuous-duty diesel powered generator was mounted to a trailer and connected to an electrical service panel. This setup is supplied by a 40-gallon fuel tank and can provide electrical power for computers, gas analyzers, vacuum pumps, dataloggers, and

blowers in remote locations. By using vacuum pumps to continuously withdraw gas samples from every chamber to the gas analyzer, and 12V solenoid valves and a manifold, we can switch among chambers/treatments every minute. We made only subtle changes to the chamber design and still utilize mylar and hook-and-loop fastening. The plenum base allows high chamber volume exchange rates, important to minimize temperature buildup, at low air velocity. In short, we have developed and refined an efficient, reliable, and mobile system for studying fundamental canopy physiology.

Chemical thinner trials (2004 & 2005)

One likely mechanism of blossom/fruitlet thinning is via a reduction in net photosynthesis (or more specifically, carbon balance) and therefore, the supply of carbohydrate growth resources to developing fruit. Our lab has collaborated with others studying the horticultural benefits (i.e., thinning efficacy) of various blossom thinning agents (e.g., fish oil + lime sulphur (FOLS)) to better understand the tree's physiological response to this thinning agent and develop a successful organic thinning program. In 2004 and 2005 we compared the effects of several blossom thinning treatments on canopy gas exchange. In 2005, we studied the effects of applying FOLS and soy oil emulsion (SO) at a 4-day interval. Our data from research in 2004 had suggested that the reductions in net photosynthesis from a 7-day interval were insufficient to elicit an adequate thinning response (see our report in 2004 Apple Hort/Path book).

In 2005, due to poor weather conditions before 22 April, we were unable to collect gas exchange data prior to the first (e.g. 80% full bloom) thinner application. Within 24 hr of the initial application however we had recorded significant reductions in canopy NCER from both treatments (Table 1). Mean daily (0600 – 1730HR) NCER was 32% and 19% lower from SO- and FOLS-treated trees, respectively. High winds blew down our canopy chambers around 1800HR. As a result, we are missing data from late on the 23rd through to late morning on the 25th when conditions permitted chamber setup and data collection. By 3 days after the 80% FB application, trees had recovered from the initial reduction in NCER – mean NCER was only 13% lower than untreated trees, irrespective of treatment. It is not known, but unlikely that the greater initial reduction in NCER from the SO treatment is significant with respect to thinning efficacy, particularly because both treatments had similar NCERs two days later. We recorded greater reductions in NCER from the second application (e.g., 80% FB+4d) on 26 April. Both thinners reduced canopy NCER by 28% compared to the control. This reduction was recorded only over a 5-hr period in late afternoon because winds again blew down the chambers on the night of the 26th. By two days after the second application, canopy NCER was 42% and 35% lower from SO- and FOLS-treated trees, respectively. The greater reductions are not a result of a delay in thinner impact but due to differences in period of measurement of NCER between the 27th and 28th. We hypothesize that on the day after the second application (27th) we would have recorded similar or even greater reductions in NCER than those on the 28th had we recorded the full diurnal impact. Trees once again showed recovery from thinning treatments. By three days after the second treatment, SO-treated trees exhibited NCERs only 14% lower than those of untreated trees. FOLS-treated trees appear to recovery less rapidly – on the same day, canopy NCER had not appreciably recovered from the initial reduction. On 30 April, the third application was made (e.g. 80% FB+8d). We were able to record gas exchange late in the afternoon on the day of application and recorded slight reductions (15 – 18%) in NCER from both treatments. However, over the next day, both treatments again reduced canopy NCER similarly and by ca. 32%. Over the next two days SO-treated trees exhibited significant recovery – 3 days after the third application, NCERs were only 13% lower than those for the control. In contrast, on the same day, FOLS-treated trees had 22% lower NCER than control. Therefore, it appears that both FOLS and SO have similar immediate effects on canopy physiology but that FOLS is slightly more phytotoxic from characterized by a more persistent effect. It is not know whether the longer-lived reductions in NCER confer any greater thinning efficacy.

Overall, the reductions in canopy NCER we recorded match very closely those we reported previously from FOLS applications. What remains unknown is the relationship between thinner phytotoxicity and thinning efficacy. Future investigations should attempt to better define whole-tree carbon balance, taking in to account reproductive and vegetative growth rates as well as the impact of thinning agents on canopy carbon budgets. Moreover, further research must distinguish between thinner effects on canopy carbon balance and interference with pollination/fruit set, and the relative roles of these modes of action for thinning.

Calcium + cover spray trial (2005)

In 2005 we also investigated the effects of commonly-applied micronutrient and insecticide cover sprays on apple tree gas exchange. In these preliminary investigations Mora-leaf® was tested alone and in combination with a standard codling moth cover spray. We recorded only slight negative effects of both treatments on leaf gas exchange (data not shown). Leaf NCER was reduced by 10 – 18%, irrespective of treatment. There was no additional effect of the cover spray. In addition, there was no consistent effect of either treatment on leaf transpiration or stomatal conductance. We hypothesize that the slight reductions in leaf NCER from Mora-leaf® are not horticulturally relevant – there is little chance that the reductions had any negative impact on fruit yield or quality though these parameters were not determined in this trial. However, in this first year we investigated only the response to a single application. We do not know whether trees would respond similarly to multiple applications or if chronic treatment with these products would impact yield or quality via reductions in carbon assimilation.

In 2005 we also compared the phytotoxicity of Mora-leaf® and calcium chloride by studying their effects on leaf gas exchange. Interestingly, there was little effect of either treatment the day after application but significant reductions in NCER two days after application. Approximately 24 hr following treatment, leaf NCER was ca. 10% lower from treated trees. However, leaf NCER from treated trees was 23% - 27% lower by 48 hr after application. Unfortunately we did not document the trend beyond 48 hr. Reductions in NCER were likely a result of lower stomatal conductance in treated trees. Again, there were only slight reductions 24 hr after treatment but significant reductions by 48 hr – both treatments exhibited ca. 34% lower stomatal conductance than untreated control. This delayed response is difficult to reconcile and merits further investigation. However, we discovered that both products are similar in their effect and that neither is particularly phytotoxic in single applications.

Deficit irrigation trials (2004 – 2006)

In 2004 and 2005, this project also documented the effects of two season-long irrigation regimes on whole-canopy and single leaf gas exchange in Prosser. For complete trial details including fruit yield, fruit quality, and soil water content see Caspari's report in the 2005 report (AH-04-413). Our results have been consistent across several years. In 2005 we recorded no effect of irrigation regime on whole-canopy gas exchange measure over a week in August and 3 days in September (data not shown). Similarly, in 2004 and 2003, neither deficit irrigation negatively impacted canopy net CO_2 exchange rates. Consistently we discovered that any differences among treatments were subtle and not statistically significant. Interestingly, this occurred despite significant differences in soil water content among treatments. In fact, across and within year, there was no clear or close relationship between soil water content and canopy NCER within the range studied in the current study. Soil water status has been often taken as a measure of drought stress in plants on the assumption that soil water status is proportional to plant water status. Clearly, this is an over-simplification of complex physiological phenomena and our data suggest that soil water content is not a good indicator of physiological stress. However, it is possible that we did not impose a physiologically significant water stress in this trial. Moreover, it may be more appropriate to examine only the soil profile where active root function exists. For the current analyses, we are comparing

NCER to the water content of the entire 3.5 – 4′ soil depth. Future trials should include measurements of leaf/stem water potential to improve our assessment of stress.

In 2006, we conducted our most detailed assessment of the relationship between soil water content and canopy/leaf physiology. We recorded whole-canopy gas exchange for ca. three weeks. Equipment malfunction spoiled the first week of data collection. Between the 3rd and 13th of September however, we were able to collect a tremendous amount of data. Soil water potential was monitored closely by tensiometers. At no point during the trial period did we record significantly different soil water potential between the unirrigated and irrigated trees (data not shown). Similarities may have been due to the distance between the drip emitters and the tensiometers (i.e., tensiometers did not perceive the wetted soil). In addition, we did not apply large irrigation volumes to irrigated trees. Moreover, the tensiometers may not have been in proper contact with the soil and therefore, been generating false readings. If similar research were to be attempted, greater control over irrigation application and soil water content monitoring would be necessary to reconcile physiological data.

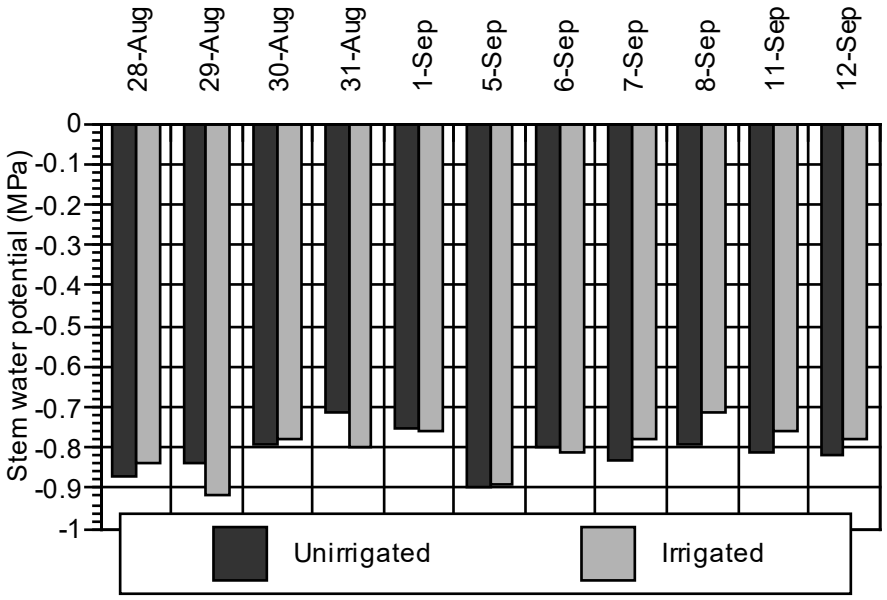

Figure 1. Midday stem water potential from leaves of irrigated and unirrigated 'Fuji'/'M.26' trees. Each bar is the mean of 9 leaves.

Our estimates of tree physiological stress support the contention that we did not impose a significant stress - we recorded no effect of irrigation treatment on stem water potential (SWP) (Fig. 1). In fact, despite not receiving any irrigation for 16 days, unirrigated trees exhibited no sign of water stress – there was no decline in midday SWP throughout the course of the trial. On 12 September, the penultimate day of this trial, SWP was not statistically different from the beginning of the trial (-0.87 vs. -0.82, respectively). This suggests that M.26 at maturity is reasonably drought resistant. This may be due to its ability to access water resources deep in the soil profile, or to access a large soil volume. Very little research has investigated apple rootstock rooting patterns and the response of apple rootstocks to water deficits. Physiological stress (i.e., stomatal closure and decreased NCER) is believed to occur in apple at leaf and stem water potentials much lower than those we recorded (i.e., less tha 2.0 MPa).

Single leaf gas exhange We recorded midday leaf gas exchange almost every day over the three week trial period. At no time did we record significant effects of irrigation treatment on any component of gas exhange (i.e., net CO_2 exchange rate, transpiration, stomatal conductance) (data not shown). However, we did record, throughout the trial, significantly lower leaf NCER and E and g_s from shaded leaves vs. sunlit leaves. Shaded leaves exhibited NCER of between 10% and 40% of sunlit leaves. Shaded leaf E was nearer to that of sunlit leaves, typically being only 15% to 30% lower. Therefore, water use efficiency of shaded leaves was much lower than that from sunlit leaves.

Whole canopy gas exchange We setup canopy chambers around 6 trees (3 irrigated, 3 unirrigated) on the 28th of August and collected data through to the 13th of September. Canopy transpiration from both treatments followed a similar trend over the course of the trial (Fig. 2). In general, unirrigated trees exhibited higher rates of transpiration – overall mean canopy transpiration was 68% higher from unirrigated trees. This may not reflect a treatment effect but have been related more to differences in canopy leaf area. Data are presented as liters of water per tree per day and do not account for potential differences in leaf area. With the exception of the 6th of September, whole-tree water use was fairy consistent over the 10 day sample period. Unirrigated trees transpired between 27 l and 43 l, irrigated trees transpired between 17 l and 36 l. In addition, there was little variability among replicates in transpiration.

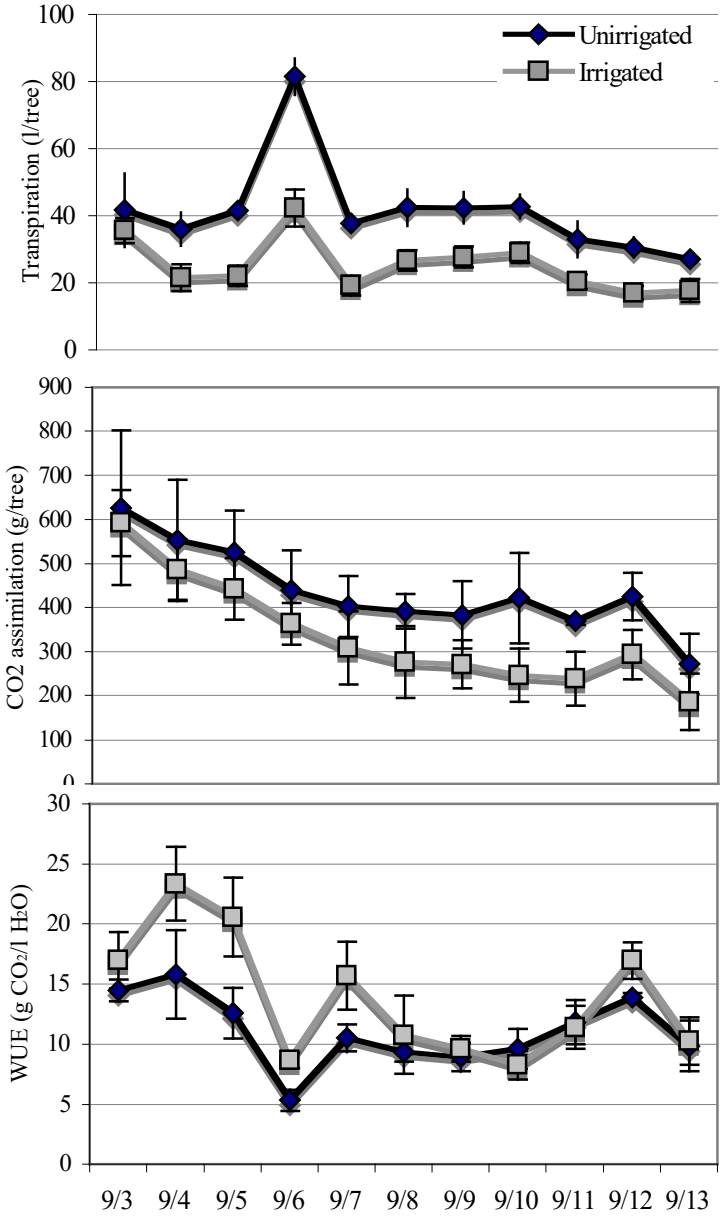

Figure 2. Whole-canopy transpiration (l/tree), CO_2 assimilation (g/tree), and water use efficiency (g CO_2/l H_2O) of irrigated and unirrigated 'Fuji'/'M.26' trees. Each data point is the mean ± standard error of 3 trees.

Canopy CO_2 assimilation for all trees declined throughout the trial period (Fig. 2). Assimilation was roughly 50% - 60% lower at the end of our trial compared to the outset. The cause of this decline is not clear but not likely related to any change in canopy/stomatal conductance because transpiration was not affected. Our treatments may have elicited a metabolic response not related to canopy/soil water status. Interestingly, we did not document a similar decline in single leaf NCER over the same period. Overall, canopy CO_2 assimilation was about 29% higher from unirrigated trees (437 g/tree vs. 337 g/tree). This again may be related more to differences in canopy area rather than a direct treatment effect. Moreover, trees exhibited significant variability in daily CO2 assimilation – we documented greater than 3-fold differences among individual trees (data not shown). Differences may be related to canopy architecture, light distribution/interception, and leaf area. Crop load (fruit number and yield) was similar among all

trees (data not shown) and therefore, presumed to be irrelevant with respect to CO_2 assimilation. In addition, key environmental conditions were similar throughout the trial (Fig. 3, Table 1).

Tree water use efficiency (WUE, g CO_2/l H_2O) was higher for irrigated trees overall (Fig. 2). Mean WUE was ca. 27% higher in irrigated trees. This was due in large part to lower rates of transpiration, rather than differences in CO_2 assimilation. However, in the latter half of the trial, WUE was similar for both treatments.

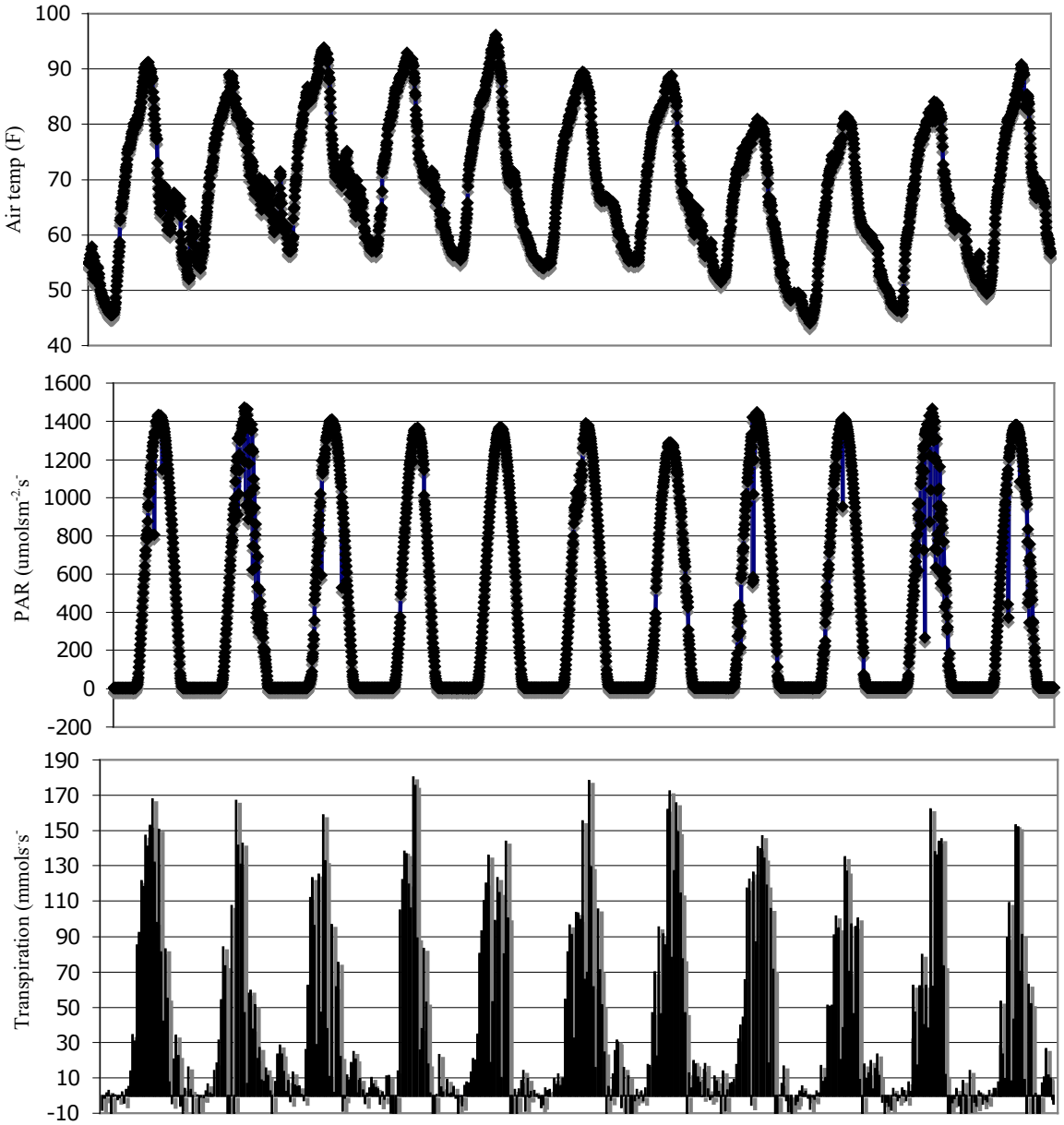

Figure 3. Trend in air temperature (F), photosynthetically active radiation ($\mu mols \cdot m^{-2} \cdot s^{-1}$), and whole-canopy transpiration ($mmols.s^{-1}$) of a representative tree throughout the 10-day period of measuring canopy gas exchange. Data are from the 3rd of September to the 13th of September.

Table 1. Environmental conditions between 3rd and 13th of September, 2006 during measurements of canopy gas exchange.

	3-Sept	4-Sept	5-Sept	6-Sept	7-Sept	8-Sept	9-Sept	10-Sept	11-Sept	12-Sept	13-Sept
Mean air temp (F)	68.9	72.9	73.5	73.7	70.5	69.9	62.7	59.8	64.2	69.0	61.0
Max air temp (F)	80.8	87.3	88.8	90.6	85.4	85.7	73.5	75.1	78.6	86.2	74.3
Min air temp (F)	56.2	58.3	59.1	60.4	57.0	56.2	49.7	44.3	47.9	54.4	47.0
Solar radiation (MJ/M2)	10.73	12.1	11.96	12.26	12.16	11.31	12.4	12.13	11.5	11.82	8.33

FINAL PROJECT REPORT
WTFRC Project Number: PH-05-512 (WSU Project 13C-3655-4297)

Project Title: Effects of preharvest sprays of MCP on apple

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Budget History:

Item	Year 1: 2006
Salaries	1,000
Benefits	340
Wages	800
Benefits	80
Equipment	0
Supplies	200
Travel	400
Miscellaneous	0
Total	$2,820

Objectives:

1. Test the new sprayable MCP formulation for efficacy on 'Scarletspur Delicious' and 'Cameo' apple fruit quality retention after storage.
2. Compare preharvest MCP treatments of the new formulation with the standard postharvest treatment approach and with AVG applied four weeks before first harvest.
3. Test preharvest MCP timing and concentration effects in relation to harvest date to identify relationships among MCP timing, concentrations and harvest date on fruit postharvest behavior.
4. Evaluate potential for AVG, ethephon, preharvest MCP or postharvest MCP as tools for adjusting fruit maturity in 'Cripps Pink' apples to permit earlier harvest with fruit quality and postharvest durability equivalent to untreated apples harvested at the normal time

Significant findings:

1. MCP applied to apple trees as a dilute spray with a handgun was much more effective in influencing 'Scarletspur Delicious' fruit behavior than a comparable spray applied with a Proptec airblast sprayer at 100 gallons per acre. Application technology is a critical factor in successful use of the current formulation of MCP.
2. Preharvest MCP applied one week before harvest (WBH) controlled fruit drop in 'Scarletspur Delicious' for one month regardless of MCP concentration, but drop in 'Cameo' was too small to show an effect.
3. Preharvest MCP delayed starch hydrolysis, reduced ethylene production and reduced red color measurably in both 'Scarletspur Delicious' and 'Cameo' apples at harvest one week after treatment. One set of applications was made one week before normal harvest (fruit sampled at normal harvest), while a second set of applications was made the week of normal harvest and sampled one week later.
4. For both cultivars, the principal differences among treatments after 60 days RA, 120 days CA or 210 days extended CA (ECA) were mainly in flesh firmness and internal ethylene concentration (IEC).
5. Patterns of flesh firmness and IEC were similar for fruit immediately out of storage and after seven days of stimulated ripening. For fruit treated only with preharvest MCP, flesh firmness was greater with higher concentrations of MCP, but the response was curvilinear as concentration increased.
6. Similarly, IEC was decreased with higher concentrations of MCP.
7. Where postharvest treatment of fruit with MCP was also carried out, the concentration-dependent responses of flesh firmness and IEC were entirely eliminated and all fruit showed the same level of control over firmness loss and IEC regardless of preharvest treatment.
8. 'Cripps Pink' apples treated six weeks before normal harvest (WBNH) with AVG (ReTain®) and 4 WBNH with ethephon showed an unusual amount of preharvest drop compared to untreated trees or trees sprayed with either AVG or ethephon alone. As much as half the crop was lost on these trees, even though starch hydrolysis and IEC were not different from other treatments receiving ethephon with or without MCP.
9. 'Cripps Pink' apples treated with either preharvest AVG or MCP showed a very marked reduction in development of red color, while preharvest ethephon stimulated red color development such that treated fruit harvested two weeks early had about the same red color as untreated fruit harvested on the normal date.
10. The optimum strategy for encouraging early fruit maturity (commercial harvest two weeks before the normal time) in 'Cripps Pink' while preserving good color development, internal condition and good storability appeared to be a preharvest application of ethephon (300 ppm) four weeks before normal harvest followed by a postharvest MCP fruit treatment.

11. Spraying MCP one week after ethephon resulted in comparable starch and IEC levels as ethephon alone but red color development was strongly inhibited, making this approach for promoting early fruit maturity unattractive.

Methods:
Trials were established in mature, cropping trees of 'Scarletspur Delicious,' 'Cameo' and 'Cripps Pink' apple to determine effects of various bioregulator products on fruit maturation, fruit quality and post-storage behavior. All trials employed single- or double-tree plots in randomized complete block designs.

Results and discussion:
Preharvest applications of MCP appear to work well in apple if appropriate application technology is employed. Preliminary results suggest that large droplet size and full coverage aid in producing effective tree and fruit responses. Because MCP is a gas when in aqueous solution at normal temperatures and pressures, the product is probably quite volatile and off-gasses quickly. This fact, combined with the limited wetting of concentrate spray applications, is consistent with observations that small droplets and limited spray volume per acre produce unfavorable results.

In 'Scarletspur Delicious' and 'Cameo' apples, preharvest dilute sprays of MCP applied one week before harvest produced results close or comparable to a standard postharvest application when MCP concentration (a.i.) in the spray solution was at least 45 ppm. The observed effects on retention of flesh firmness and control of IEC continued to be present up to 210 days in CA storage following harvest. This suggests that once ethylene receptors in apple are combined with MCP and control over ethylene effects in the fruit is established these sites are not reactivated or replaced and control is maintained. The level of control achievable with preharvest MCP represents as much as a 4-lb. benefit in flesh firmness under long-term storage, even after seven days of stimulated ripening, equal to what can be obtained with a postharvest application of MCP. This technology appears very promising and may offer other advantages as well, including the potential for extending the harvest season, which could help improve crop recovery in a limited labor situation.

'Cripps Pink' apples in Washington are subject to potentially significant crop loss from preharvest freezes, which have happened several times in the last decade. Bioregulators that can control fruit maturation might be able to play an important role in encouraging earlier fruit maturity while permitting the preservation of fruit quality. Ethephon can stimulate fruit maturation but is commonly associated with more rapid deterioration of fruit quality during storage. Ethylene production or action inhibitors (e.g., ReTain, MCP) retard fruit maturation and extend fruit storability. Various combinations of bioregulators that either stimulate or retard fruit maturation and ethylene production were tried to determine if fruit could be brought to the same maturity condition two weeks earlier than untreated fruit reach when harvested at the normal time. Because of the profound effect of maturation inhibitors (preharvest AVG or MCP) on red color development in this cultivar, the best treatment for accomplishing this goal was the combination of ethephon (300 ppm dilute) applied four weeks before normal harvest followed by postharvest treatment of fruit with MCP after harvest two weeks following the ethephon application. Further trials are warranted to confirm this result.

Summary:
MCP applied preharvest produces effects on apples that are the equivalent of postharvest treatment, but the sprayable technology allows application before harvest. One unanswered question that immediately surfaces is whether a preharvest application can control fruit ripening enough to permit a significant (1-3 week) delay in harvest without the normal loss of fruit storability and quality. If so,

spraying MCP might also help smooth out peaks in harvest labor requirements, aiding the maintenance of good fruit quality and facilitating harvest labor management in a limited labor environment. The stimulation of fruit drop observed in 'Cripps Pink' apples treated with AVG and later with ethephon is unusual since other factors thought to be associated with stimulation of drop, such as starch hydrolysis or ethylene production, were not accelerated in this treatment. A combination of preharvest ethephon plus postharvest MCP appears to offer the best opportunity for successful earlier harvest of 'Cripps Pink' apples.

Acknowledgments:
The assistance and support of the following persons and organizations are gratefully acknowledged: Lynnell Brandt, Clyde Buechler, Dean Christie, Dr. S.R. Drake, Dale Goldy, Jeff Henry, Dr. Chris Ishida, Kathy Monroe, Chris Olsen, Dr. A.N. Reed, Brenda Steady, Peter Van Well, Dwayne Visser, AgroFresh, Inc., Bayer Environmental Science, E.W. Brandt & Sons, Inc., Goldy Orchards, Valent BioSciences, Van Well Orchards, the Washington Tree Fruit Research Commission, and the WSU Agricultural Research Center.

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FINAL PROJECT REPORT
WTFRC Project Number: PH-04-0443

Project Title: Regulation of apple fruit ripening

PI: Jim Mattheis
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Cooperators: Dave Rudell, Postdoctoral Research Associate
USDA, ARS TFRL, Wenatchee, WA 98801

Budget History:

Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries	$26,800*	$13,232**	$23,282***
Benefits	$13,200	$6518	$11,468
Wages	$0	$1675	$0
Benefits	$0	$825	$0
Equipment	$0	$0	$0
Supplies	$1500	$1500	$1500
Travel	$0	$0	$0
Miscellaneous	$0	$0	$0
Total	$41,500	$23,750	$36,250

*0.5 FTE Postdoctoral Research Associate
**0.25 FTE Biological Science Technician
***0.44 FTE Biological Science Technician

Objectives:

1. Characterize apple fruit production of nitric oxide during fruit development and ripening.
2. Characterize apple fruit response to exogenous nitric oxide at harvest and during storage.
3. Characterize apple fruit response to activators/inhibitors of nitric oxide metabolism.

Summary Findings:

1. Nitric oxide (˙NO) production increases during apple maturation.
2. Whole apples exposed to ˙NO or NO_2^- can have reduced ethylene production and respiration rate.
3. Development of superficial scald, CO2-induced internal browning, and softening were inconsistently altered by whole fruit exposure to ˙NO or NO_2^- at harvest or during storage.
4. Apples convert NO_2^- to ˙NO in low O_2 or air sufficient to reach ˙NO treatment target concentrations without development of phytotoxicity.
5. Treatment of whole apples with a strobiluron fungicide (a compound reported to stimulate ˙NO production) inconsistently impacts fruit ethylene production and respiration rate.
6. Treatment of cut apple slices with ˙NO donors results in reduced ethylene production relative to non-treated control slices.

Results and Discussion:

Fruit Maturity and ˙NO production

Delicious apples harvested at weekly intervals were assessed for maturity including ethylene and ˙NO production. An increase in ˙NO production between the last two harvest dates accompanied increases in starch score and ethylene production. The increase in ˙NO production, while significant, occurred after the onset of ripening as indicated by increased ethylene production. The increase in ˙NO also is in contrast to published reports indicating an inverse relationship between ethylene and ˙NO production at the onset of fruit ripening. Based on these results with Delicious as well as results with other cultivars, the increase in ˙NO production occurs after ripening has initiated and as such may not be an early indicator of harvest maturity.

Harvest Date	starch	lbs	watercore %	ethylene ppm	NO ppb
Sep 19	1.6	16.0	0	0.0	0.07
23	1.7	17.0	0	1.5	0.00
Oct 1	1.9	17.0	0	7.1	0.23
5	2.0	16.6	0	7.2	0.17
19	3.9	15.9	50	43	0.68
$LSD_{0.05}$	0.4	ns	-	11.3	0.35

LSD: least significant difference; ns: not significant

Table 1. Progression of Delicious maturity including ethylene and ˙NO production. Fruit were evaluated the day after harvest (n=20).

Responses of fruit ethylene production and respiration to exogenous ˙NO and NO_2^-

Delicious apples held in 0.5% O_2 were exposed to 0,1,5,10, or 50 ppm ˙NO or 50 ppm NO_2^- for 2 hours the day of harvest (16.6 lbs, 2.0 starch). During holding in air for 5.5 days at 68 ºF after treatment, fruit respiration and ethylene production were monitored, and analyses of fruit quality were performed on day 7. All ˙NO and NO_2^- treatments delayed and/or reduced ethylene production with the largest impact from 10 ppm ˙NO. Respiration rate was also reduced by most ˙NO and NO_2^- treatments with the largest impact after exposure to 50 ppm NO_2^- . These results were the basis for subsequent ˙NO and NO_2^- treatments at harvest and during storage.

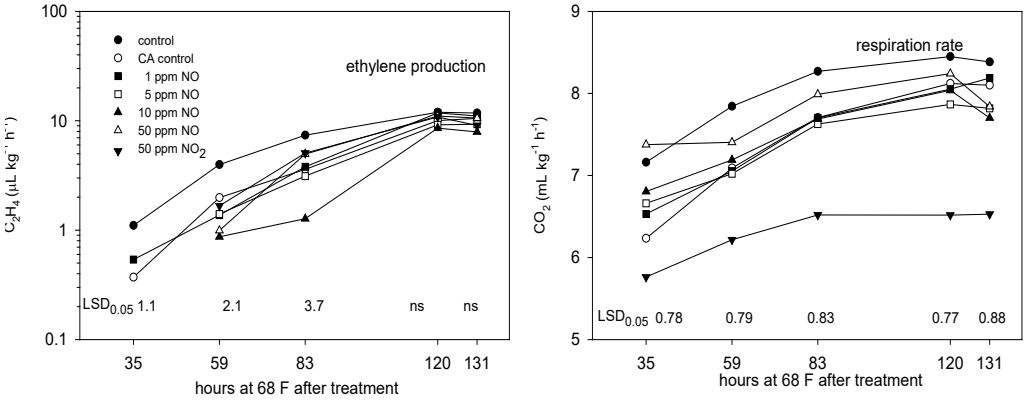

Figure 1. Delicious apple ethylene production and respiration rate. Fruit treated at harvest with ˙NO or NO_2^- then held in air at 68 ºF .

Impacts of ˙NO and NO_2^- treatments on fruit quality and physiological disorder development

Internal CO_2 injury: Braeburn apples were treated with 1 ppm 1-MCP, 2000 ppm DPA, 1-MCP + DPA, 10 ppm ˙NO , 1-MCP + ˙NO, then stored at 33 ºF in air or CA (1.5% O_2, 3% CO_2). The only fruit stored in air that developed internal disorders was treated with 1-MCP and stored for 6 months. For fruit stored in CA, controls and 1-MCP-treated apples had the highest rates of injury. DPA treatment prevented injury on control as well as 1-MCP-treated fruit. The ˙NO treatment reduced injury incidence in control and 1-MCP-treated fruit through 4 months, however, after 6 months differences between control, 1-MCP, ˙NO , and 1-MCP+˙NO were less than at 2 or 4 months.

Months CA	control	1-MCP	DPA	1-MCP/DPA	NO	1-MCP/NO
2	61%	50	0	0	11	11
4	50	44	0	0	6	17
6	28	39	0	0	17	22

Table 2. Incidence (%) of internal browning+cavities in Braeburn apples treated at harvest in 2004. Fruit were stored in CA (1.5% O_2, 3% CO_2) and evaluated 1 day after removal.

A study conducted in 2005-06 did not confirm the 2004 results. ˙NO treatments (10 ppm) at harvest or during CA storage did not effectively control disorder development during CA (1.5% O_2, 3% CO_2) storage. Fruit from two orchards were used in this study, Orchard A was harvested at an earlier date (Oct 8 vs. 18) with greener peel ground color (2.1 vs. 3.9, 1-5 scale; 1=green, 5=yellow). Orchard B was fruit source for 2004 study as well.

Months	control	˙NO at harvest	˙NO weekly	˙NO monthly
4	14	22	28	22
8	67	83	61	56

Orchard A near Royal City, WA.

Months	control	˙NO at harvest	˙NO weekly	˙NO monthly
4	56	35	44	56
8	68	49	47	55

Orchard B near Manson, WA.

Table 3. Incidence (%) of internal browning + cavities in Braeburn apples treated with 10 ppm ˙NO at harvest or during CA (1.5% O_2, 3% CO_2).

A similar lack of internal browning control in ˙NO-treated fruit was observed for Delicious apples in 2005-06. Apples were held for two hours at harvest in sealed jars in air (control), 0.5% O_2, or O.5% O_2 with 10 ppm ˙NO, then stored in RA (5 months) or CA (1% O_2 1% CO_2).

treatment	Orchard 1		Orchard 2		Orchard 3	
	RA	CA	RA	CA	RA	CA
control	0	0	22	41	28	94
0.5% O_2	0	0	33	39	44	83
0.5% O_2+˙NO	0	0	28	59	22	63

Table 4. Incidence of internal browning in Delicious apples treated with 0 or 10 ppm ˙NO at harvest. Fruit stored in air (5 months) or CA (8 months) and evaluated after 7 days at 68 ºF .

The lack of effective disorder control in 2005-06 indicates the potential for ˙NO treatments to manage internal browning is not consistent across seasons. Factors that may be influencing efficacy remain to be determined.

Superficial Scald

Delicious apples harvested at CA maturity (starch 1.9, 17.7 lbs) in 2004 were exposed to 10 ppm NO while held in 0.5% O_2 for two hours at harvest. Fruit were then stored at 33 ºF in air or CA (1% O_2, 1% CO_2). No scald developed on fruit stored in CA. For fruit stored in air, scald developed on controls and fruit held in 0.5% O_2 for 2 h at harvest, however, no scald was present on apples exposed to ˙NO. After 6 months, peel analysis for accumulation of conjugated trienes (CT), indicative of α-farnesene oxidation and putatively related to scald development, showed the amount of CT in peel from fruit with scald symptoms was greater compared to ˙NO-treated fruit. ˙NO treatment did not impact fruit ripening as measured by firmness and acidity loss.

treatment	scald %	CT	lbs	TA %	% red
control	28	74	10.1	0.162	89
0.5% O_2	61	75	10.6	0.150	81
0.5% O_2+˙NO	0	30	9.8	0.148	92

CT: conjugated trienes; TA: titratable acidity; % red: visual rating of amount of fruit surface with red color

Table 5. Poststorage quality and scald incidence of Delicious apples. Fruit treated at harvest with 10 ppm ˙NO then stored in air for 6 months. CT: conjugated trienes

Scald incidence was too low to evaluate treatment effects in a similar experiment conducted with Delicious in 2005-06, however, results with Granny Smith did not show a significant ˙NO treatment response for scald reduction.

treatment	Orchard 1		Orchard 2		Orchard 3	
	5M RA	8M CA	5M RA	8M CA	5M RA	8M CA
control	94 %	6	56	33	17	78
0.5% O_2	83	0	67	33	22	89
0.5% O_2+˙NO	89	6	50	44	17	78

Table 6. Granny Smith scald incidence 7 days after removal from storage. Fruit were held in air or 0.5% O_2 with or without 10 ppm ˙NO for 2 hours at 70 °F, then stored in air at 33 °F or CA (1.5% O_2, 1.5% CO_2) for 5 or 8 months, respectively.

NO treatment during storage

Delicious and Granny Smith apples exposed to 0 or 10 ppm NO at harvest in 2004 then stored in CA (0.5% O_2, 1% CO_2) were exposed to 10 ppm NO or NO_2 twice a week, or 10 ppm NO once or twice a month. ˙NO accumulated in CA chambers following injection of NO_2^- indicating likely conversion of NO_2^- to ˙NO by fruit. Through 6 months, effects of ˙NO or NO_2^- treatment on Delicious firmness, respiration rate and ethylene production were detected, but no other treatment effects were apparent. No impacts from any of the treatments on Granny Smith fruit quality were detected, and no phytotoxicity was observed on either cultivar. There was no incidence of superficial scald on any fruit (NO/NO_2-treated or controls) through 6 months plus 7 days at 68 °F .

Delicious: 6 months CA plus 7 days at 68 °F.

Treatment	lbs	CO_2 ppm	C_2H_4 ppm	SSC (%)	TA (%)	decay (%)
control	13.2	315	1.88	13.7	0.188	11
˙NO@harvest	13.4	263	2.01	13.0	0.214	6
˙NO monthly	14.4	267	1.62	13.2	0.211	0
˙NO 2Xmonth	15.1	217	0.76	13.2	0.207	11
˙NO 2Xweek	14.4	228	1.16	13.5	0.205	0
NO_2^- 2X week	15.2	277	1.19	13.2	0.178	0
$LSD_{0.05}$	1.8	50	0.74	ns	ns	ns

LSD: least significant difference; ns: not significant

Table 7. Impact of repeated ˙NO and NO_2^- treatments on Delicious quality. ˙NO and NO_2^- concentrations were 10 ppm for 2 hours during treatments.

Similar experiments conducted in 2005-06 using 3 lots each of Delicious and Granny Smith with treatments limited to weekly or monthly ˙NO addition did not result in observable impacts on scald development, internal disorders, or fruit quality throughout an 8 month storage period. The lack of responses in 2005-06 to either ˙NO treatments at harvest or during storage indicates minimal likelihood of development of ˙NO gas treatments as performed in these studies as an effective means to manage fruit quality in the postharvest environment.

Fruit responses to activators of ˙NO production

Studies related to Objective 3 were conducted initially using cortex disks prepared from Golden Delicious apples. Treatments were applied as aqueous solutions to disks contained in 50 mL Erlenmeyer flasks. Solutions contained either potassium nitrite (NO_2-), *S*-nitrosoglutathione (GSNO), a ˙NO donor, oxidized glutathione (GSSG), a GSNO control, sodium nitroprusside (SNP), also a ˙NO donor, sodium ferrocyanide [$NaFe(CN_6)$], a SNP control, or potassium nitrite (NO_2^-). NO_2^- can be metabolized to form ˙NO in plant tissues. Following a 30 min equilibration period, flasks were sealed for an additional 30 min then sampled to measure evolved ethylene and ˙NO.

Treatment with NO_2^- resulted in increased ˙NO production and, likewise, decreased ethylene biosynthesis. Generation of ˙NO increased linearly while ethylene generation decreased exponentially with increasing NO_2^- treatment concentration. Treatment with solutions containing the ˙NO donors GSNO and SNP reduced ethylene biosynthesis compared to treatments containing equimolar concentrations of GSSG or sodium ferrocyanide, respectively. GSSG and sodium ferrocyanide did not affect ethylene biosynthesis. These results indicate ethylene production in apple disks can be manipulated by the presence of ˙NO. These results may have commercial potential for sliced apples if the reductions in ethylene production and respiration rate are sufficient to slow quality deterioration. No studies to investigate that potential have been conducted.

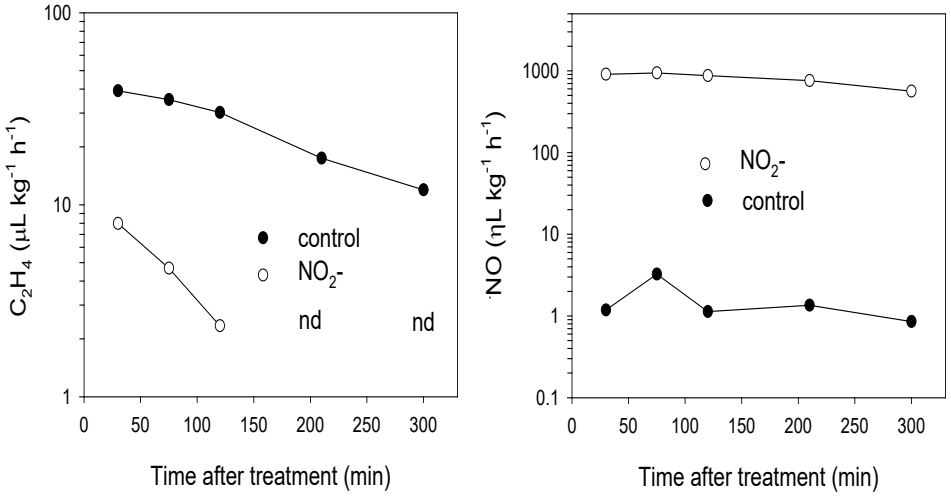

Figure 2. Ethylene and ˙NO production by Golden Delicious apple fruit disks exposed to nitrite. nd: not detected.

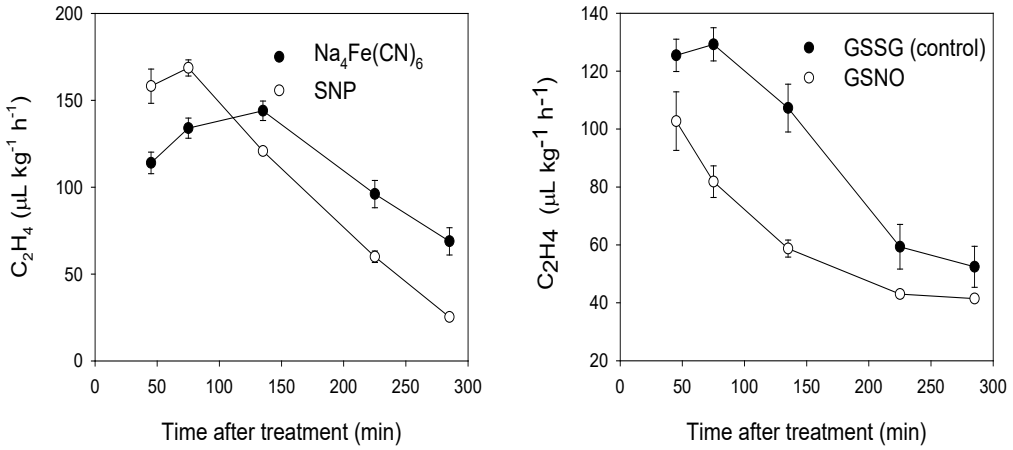

Figure 3. Ethylene (C_2H_4) production by 'Golden Delicious' apple fruit disks. Disks were exposed to solutions containing ˙NO releasers sodium nitroprusside (SNP), S-nitrosoglutathione (GSNO), or controls containing sodium ferrocyanide [$Na_4Fe(CN)_6$]or oxidized glutathione (GSSG).

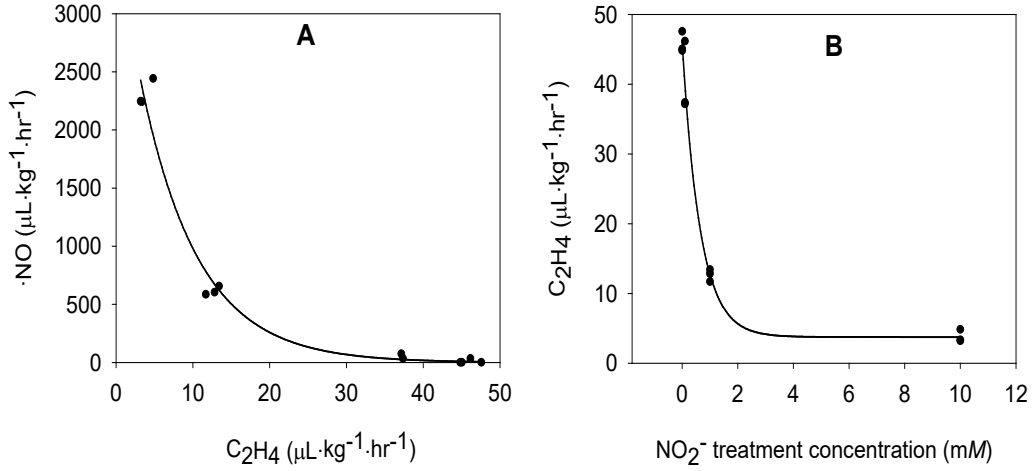

Figure 4. Relationships between ˙NO and ethylene production in response to treatment of 'Golden Delicious' apple disks with increasing concentrations of nitrite.

The reactive nature of ˙NO also prompted a study examining whether a direct reaction between ˙NO and ethylene occurs that results in reduced ethylene concentration. Both compounds were injected into sealed glass jars to a concentration of 10 ppm. One jar contained air, the other was purged with nitrogen prior to introduction of ˙NO and ethylene to reduce the rate of ˙NO oxidation by O_2 in air. Ethylene concentration did not change over a 3 hour incubation period in either jar indicating no reaction between these compounds is favored under the conditions of the experiment. In light of this result, changes in ethylene production by apple disks in response to exposure to ˙NO donors or nitrite may be the result of metabolic rather than chemical changes promoted by ˙NO.

Studies with pyraclostrobin, a strobiluron fungicide, and putative stimulant of plant ˙NO production

Strobilurons are a class of compounds with fungicidal activity that have been demonstrated to stimulate plant ˙NO production. Strobiluron fungicidal activity is thought to occur primarily via inhibition of mitochondrial electron transport and respiration rate. A commercial material (Cabrio, BASF, 20% a.i.) was used in 2006 to evaluate the potential of this water dispersible compound to impact fruit ripening via alteration of fruit ˙NO metabolism. Studies were conducted with several cultivars (Early Gold, Gala, Golden Supreme, Delicious, and Granny Smith) where fruit were treated at harvest with Cabrio at concentrations up to 4000 ppm with or without surfactant. Apples were held in air at 68 °F or in cold storage and evaluated for treatment effects on fruit quality and disorder development. Consistent positive impacts on fruit quality or decreased disorder development have not been observed with any cultivar.

In an experiment with Early Gold apples, fruit treated with up to 1000 ppm Cabrio in water had lower ˙NO production relative to untreated controls after 7 days at 70 °F.

treatment	lbs	CO_2 ppm	C_2H_4 ppm	˙NO ppb
control	5.8	938	13.9	2.7
Cabrio: 10 ppm	6.1	914	12.5	1.8
100	6.1	823	12.8	1.6
1000	5.9	935	13.0	1.5
$LSD_{0.05}$	ns	ns	Ns	0.5

Table 8. Early Gold firmness, respiration rate, and ethylene and ˙NO production after 7 days at 68 °F . Fruit were treated at harvest with water or Cabrio in water without surfactant.

Golden Supreme apples treated with 4000 ppm Cabrio had higher color ratings (yellower), higher ethylene and ˙NO production and higher respiration rate compared to controls 7 days after treatment.

treatment	green-yellow 1-5	CO_2 ppm	C_2H_4 ppm	˙NO ppb
control	2.3	215	1.8	0.27
Cabrio:4000 ppm	3.0	348	5.8	0.43
$t\text{-test}_{0.05}$	0.4	85	2.2	0.11

Table 9. Golden Supreme peel color, respiration rate and ethylene and ˙NO production after 7 days at 68 °F after treatment. Fruit were treated at harvest with water or Cabrio in water with surfactant.

Gala apples treated with up to 4000 ppm Cabrio had lower firmness, and higher respiration and ethylene production compared to controls 7 days after treatment.

treatment	lbs	CO_2 ppm	C_2H_4 ppm	˙NO ppb
control	16.5	466	1.9	0.27
Cabrio: 100 ppm	16.7	469	1.9	0.3
1000	15.4	539	2.7	0.3
4000	15.4	536	3.2	0.23
$LSD_{0.05}$	1.0	48	0.6	ns

Table 10. Gala responses to pyraclostrobin (Cabrio). Fruit treated at harvest in water plus surfactant with or without pyraclostrobin, then held in air for 7 days at 68 °F .

Cabrio treatment effects on Delicious apples were concentration dependent. Fruit respiration rate was enhanced following treatment at 100 or 1000 ppm Cabrio, but reduced by 4000 ppm treatment relative to controls. Similar effects on ethylene or ˙NO production were not observed, nor were impacts on fruit quality detected.

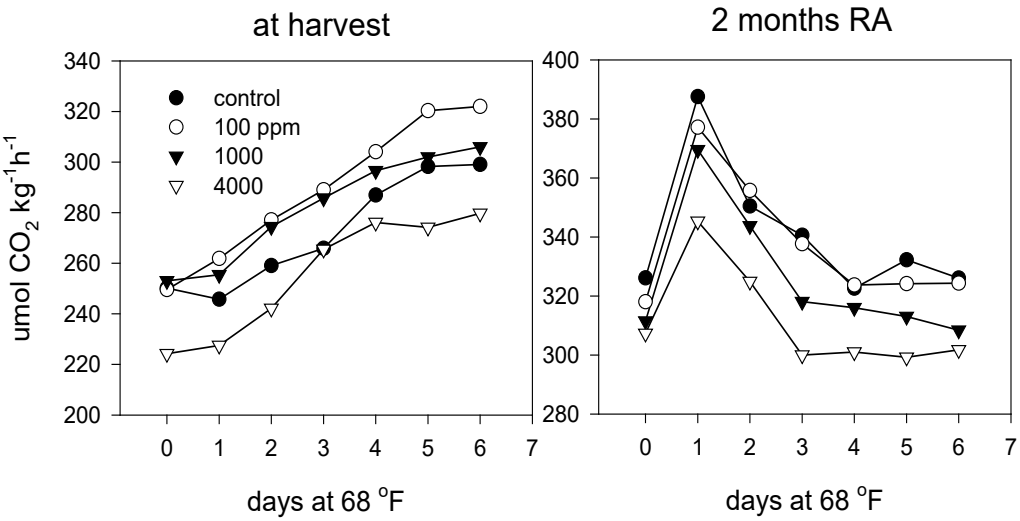

Figure 4. Delicious respiration following treatment with Cabrio with surfactant. Fruit were held at 68 °F after 0 or 2 months cold storage in air.

No Cabrio treatment have been observed to date for Granny Smith apples from two harvest dates (commercial, commercial plus 3 weeks) treated with up to 4000 ppm Cabrio then stored in RA.

The results with this strobiluron fungicide do not support an active role of the material for altering postharvest ripening of apples in a manner consistent with commercial development. Where physiological impacts on respiration or ˙NO production were observed, no accompanying quality or disorder development effects have been observed. The lack of consistent alterations in fruit ˙NO production is in contrast to published reports with vegetative plant tissues where increased ˙NO emission occurs following treatment. Whether formulation issues contributed in part to the lack of consistent treatment effects (the material used is labeled for field use) is unknown.

General Summary

Results of studies conducted over 3 crop years show apple fruit produce ˙NO during development and ripening (ripening data not presented). The pattern of endogenous ˙NO production in relation to fruit maturation is not consistent with previous reports indicating ˙NO and ethylene have an antagonistic relationship (i.e. increased ˙NO accompanied by lower ethylene production). This trend was observed in cut apple slices treated with ˙NO donors and in some experiments where whole fruit were exposed to ˙NO or NO_2^- , but consistent impacts of these treatments on fruit quality or disorder development have not been apparent. Factors that could possibly influence treatment responses (˙NO, NO_2^- gas diffusivity, maturity, seasonality, storage conditions) require further investigation. However, no continuation of this project is proposed due to a perceived high risk of success based on results to date. While roles for nitrogen radicals as effectors of developmental regulation as well as plant responses to stress are increasingly well documented in plant tissues, an obvious horticultural utility in the postharvest system for apple fruit is not apparent based on the studies conducted in this 3 year project.

FINAL PROJECT REPORT
WTFRC Project Number: # 2005-10

Project Title: Ethylene Measurement in Post-Harvest Storage

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Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	25000	16000	
Benefits	10000	6400	
Wages	8900	6120	
Benefits			
Equipment	3000	1500	
Supplies	1000	2000	
Travel	500	1500	
Miscellaneous	3738	2570	
Total	57138	39290	

I. Project Objectives

I.1. Year 1: 0.1 ppm Ethylene Sensor Development and Demonstration

1) *Design and fabricate prototypes for near real-time ethylene testing at 0.1 ppm and higher, with simple operation and maintenance.* This system will be designed in a modular fashion such that manufacturing, assembly, and component replacement for maintenance are rendered very efficient.

2) *Test and evaluate performance of prototype in laboratory and field*. The critical parameters to be evaluated are sensitivity, selectivity, accuracy, and stability. We will use the results as a basis for production engineering and commercialization of a hand-held package, which would include all the required cost and performance attributes necessary for the apple post harvest market.

I.2. Year 2: Low Cost Miniaturized Prototype Development and Demonstration

Design, fabricate, and field test a miniaturized and low cost version of the prototype for near real-time ethylene testing at 0.1 ppm levels.

II. Significant Findings

Several major accomplishments are highlighted below and further discussions of the results will follow in the upcoming sections.

1. *High sensitivity near real-time ethylene detection:* During the course of this study our electrochemical sensor demonstrated typical sensitivity of approximately 100-ppb. By careful fabrication of the sensor cell 10-ppb sensitivity could be realized for ethylene in air or pure nitrogen backgrounds, representing RA and CA rooms, respectively.

2. *Miniaturization and packaging of the system:* The entire sensor cell, the fluidic system for sampling the air, the data interpretation electronics system that comprised of two circuit boards and power module all fit inside a field-portable compact sensor unit.

3. *Alpha prototype development and field demonstration:* A battery-operated alpha prototype was completely engineered, manufactured, assembled, and tested in the laboratory and in the field. The utility of this system was demonstrated for measurement of the amount of ethylene produced by a single apple and for a variety of apples, even for those producing very little ethylene.

4. *Low cost ethylene sensor development:* Depending on the specific sensing options and built-in functionalities of the system, the projected price is below $5,000 and may be as low as $2,000. Given the capabilities of the system, this provides a very competitive product and one that offers a cost effective and higher throughput sampling and monitoring option for determining maturity of fruit in the harvest and post-harvest phases.

III. Methods: Electrochemical sensing OF ETHYLENE

As a fruit ripening hormone, ethylene gas is effective at greater than 0.1 ppm; one part of ethylene per million parts of air represents one cupful of ethylene gas in 62,000 gallons of air – not much.[1] Constant ethylene monitoring is essential because automotive emissions, plastics, smoke and fluorescent lights all increase ethylene gas levels.[2]-[3] A single propane-powered forklift can cause

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1 California Fresh Market Advisory Board, Informational Bulletin No.12, June 1, 1976.

2 Ethylene Control, Inc. "About Ethylene Gas" (taken from independent study in 1997 at the University of California Davis). Retrieved from WWW Aug. 18, 2004 www.onsolution.com.au/EthyleneControl/about.html.

3 California Fresh Market Advisory Board, Informational Bulletin No.12, June 1, 1976.

serious damage in highly ethylene gas-sensitive commodities. Ethylene will permeate through produce cardboard shipping boxes, wood, and even concrete walls.[4]

The current approach for post-harvest ethylene sensing is based on the electrochemical oxidation of ethylene as described above. The oxidation of ethylene at a gold anode in an electrochemical cell provides a cell current that can be measured and used for the determination of ethylene in air. This electrochemical process is interestingly similar to the ozone oxidation of ethylene that produces a chemiluminescence signal that is used in one of the ethylene sensors discussed below. The oxidation of ethylene by ozone is driven by the chemical energy resident in the ozone. The driving force for electrochemical oxidation of ethylene in the cell is the electrical cell potential and the pH of the electrolyte. Since oxidation of ethylene is common to both processes, the selectivity of both processes is very similar. The important difference is that the electrochemical process requires neither an ozone generator nor a photodiode for converting chemiluminescence photons to electrical current. Compared to chemiluminescence, the electrochemical process is not only simpler in terms of hardware required but it is much more amenable to miniaturization and mass production. Therefore, we believe the electrochemical process using the gold anode as the technology base can provide a new generation of ethylene sensors that have high sensitivity and are low in manufacturing cost.

Interestingly, research on the process and application of the oxidation of ethylene at a gold electrode has been reported in the scientific literature during the past decade. The chemistry of the oxidation of ethylene on gold was studied and reported by Volkmar Schmidt.[5,6] They reported that an electrolytic cell using porous surface gold with 0.05 M H_2SO_4 electrolyte produced a maximum cell current just below 1.3-V in the presence of ethylene. Products for both partial oxidation of ethylene to acetaldehyde and for full oxidation to CO_2 were detected through mass spectral analysis of cell off gases.

$$C_2H_4 + H_2O = CH_3CHO + 2H^+ + 2e^- \quad (1)$$

Ethylene was isotopically enriched with deuterium in order to determine the difference between acetaldehyde and CO_2, which both have a normal mass of 44 amu. They speculated that the first step in the process is the complexation of ethylene to the gold surface through the π-bond in ethylene. The researchers concluded "Gold in acid solution can in principle be regarded as a selective electrocatalyst for the oxidation of unsaturated hydrocarbons as well as unsaturated alcohols (Schmidt and Pastor, 1994)." In their testing, Schmidt and Pastor bubbled ethylene into the cell electrolyte directly. Shortly thereafter, Peter Hauser reported using a layer of gold plated onto a Nafion membrane as the anode in an electrochemical cell for determining ethylene and other oxidizable molecules present in air.[7,8] Professor Hauser utilized Nafion as a Solid Polymer Electrolyte (SPE) in the cell such that it provided a partition between the gas side of the cell and the liquid electrolyte. When gas was passed over the gold, ethylene in the air was catalytically oxidized. Hauser's cell bears a significant resemblance to the electrodes and SPE in a PEM fuel cell. The difference is that the SPE in a fuel cell provides the partition between two gas chambers. Hauser reported that cell response to ethylene was "linear at least up to 500 ppm." He established that 40 ppb was the detection limit based on a signal-to-noise-ratio (SNR) of 3 in his measurement system.

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4 California Fresh Market Advisory Board, Informational Bulletin No.12, June 1, 1976.

5 Schmidt, V.M., and E. Pastor, 1994, Adsorption and oxidation of acetylene and ethylene on gold electrodes." Journal of the Electrochemical Society, 376, pp. 65-72.

6 Pastor, E, and V.M. Schmidt, 1995, "Electrochemical Reactions of Ethene on Polycrystalline Au Electrodes in Acid Solution by Differential Mass Spectrometry and Isotope Labeling", Journal of Electroanalytical Chem., *383*, 175-180.

7 Jordan, L.R. and P.C. Hauser, 1997 "Amperometric Sensor for Monitoring Ethylene", Anal. Chem. *69*, 558-562.

8 Hodgson, A.W.E., P. Jacquinot, L.R. Jordan and P.C. Hauser, 1999 "Amperometric Gas Sensors with Detection Limits in the Low ppb Range." Analytica Chemica Acta, *393*, 43-48.

It is clear that this method of amperometric gas analysis presents a novel and potentially practical approach as a basis for a commercial sensor business. For this to become reality, further research into electrode configuration, cell conversion efficiency, signal analysis and conditioning, cell engineering, and water and air flow management needed to be performed to achieve the desired sensor sensitivity, SNR, and reliability.

IV. RESULTS AND DISCUSSION

The current work plan covers tasks in areas that encompass the objectives component engineering, system integration, and performance evaluation testing.

IV.1. Task 1: Gold Electrode Design and Development

At the heart of our device is an especially developed nanoporous gold catalyst deposited on the surface of a Nafion® membrane using two different techniques. Although gold is considered to be a noble and extremely unreactive metal, numerous reports in recent years show that atomic gold clusters ranging in size from 2 to 20 nm are highly active as heterogeneous catalysts in a number of chemical reactions.[9,10,11] Other typical catalytic metals such as platinum, palladium, nickel, cobalt and others have been shown to have interesting catalytic properties as nanoparticles, but these metals are already known heterogeneous catalysts.[12] Gold in a nanoparticulate state however, provides a new area of catalysis research, sometimes known as the "New Gold Rush."

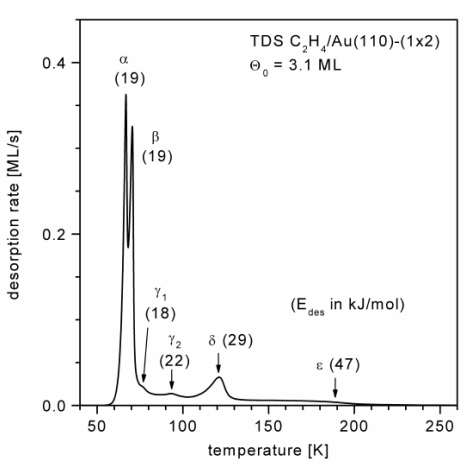

Figure 1. Thermal Desorption Spectorscopy (TDS) for adsorption of ethylene on gold (Gottfried, 2003).

Typically, a heterogeneous catalyst consists of a small amount of metal deposited on the surface of a metal oxide. The metal oxide: alumina, ceria, ferric oxide, titanium dioxide or mixtures of metal oxides provides a support for the catalytic metal, and in oxidation reactions, provides a source/sink for oxygen in the reaction. To be a catalyst, the dispersed metal must first adsorb both reactants, and not attract the products significantly. This mechanism provides the pathway for bringing reactant molecules together to facilitate the reaction. Interestingly, nanoparticle gold on a number of metal oxide substrates has attracted much attention for the catalytic oxidation of many small molecule and carbon-containing compounds. This is an important property for our detection of small molecules of ethylene adsorbed to the nanoparticle gold (Figure 1).[13] This makes them ideal targets for electrochemical oxidation using our nanoparticle gold electrode cell operating at moderate cell potentials. The successful oxidation of ethylene, propylene,

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9 Didier Astruc, Feng Lu, and Jaime Ruiz Aranzaes. Nanoparticles as recyclable catalysts: The Frontier between Homogeneous and Heterogeneous Catalysis. Angew. Chem. Int. Ed. 2005, 44, 7852-7872.

10 .A. Sanchez, et al. When gold Is Not Noble: Nanoscale Gold Catalysts. J. Phis. Cham. A 1999, 103, 9573-9578.

11 Cortie, M.B. and E. van der Lingen, 2002, "Catalytic Gold Nano-Particles," *Materials Forum*, 26, pp. 1-14.

12 Thompson, D., 1999, "New Advanced in Gold Catalysis – Part II," *Gold Bulletin*, 32(1), pp. 12-19.

13 Gottfried, J. M., 2003, "CO Oxidation over Gold Adsorption and Reaction of Oxygen, Carbon Monoxide, and Carbon Dioxide on an Au(110)-(1×2) Surface," Ph.D. Dissertation, fu Berlin, http://www.diss.fu-berlin.de/2003/133/indexe.html.

ethylene glycol, formaldehyde, formic acid and carbon monoxide with air or oxygen has been reported.[14]

The specifics of our deposition approach constitute some of our intellectual property for producing highly reliable and repeatable high surface area catalysts. Figure 1 shows a sample of our gold catalyst at different magnifications. Note that the top left image is a gold film with little to no porosity, appearing as a brilliant gold and highly reflective layer under the microscope. The right image labeled as 1X has the right appearance for a very effective gold catalyst and appears to have small lumps under a microscope with 200X magnification. The lower image is an image taken with SEM at 25,000X magnification, which shows the gold particles of smaller than 100-nm in diameter.

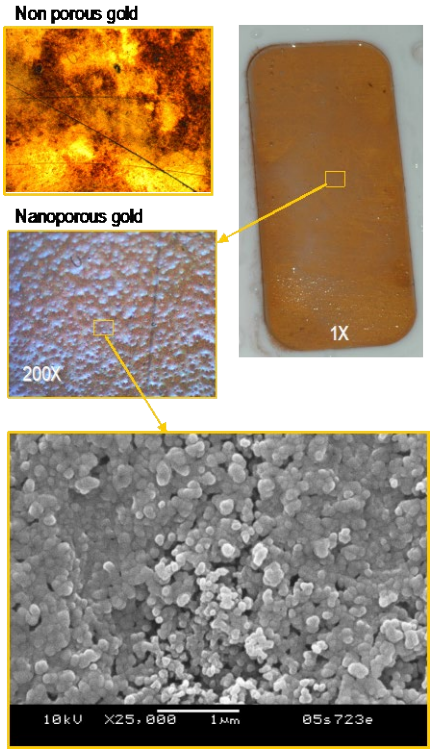

Figure 2. Nanoporous Gold Catalyst.

IV.2. Task 2: Prototype Design and Fabrication

In order to construct a handheld, easy to use prototype, a lot of design work had to occur. Factors that had to be considered in the design included ease of use, adaptability to different testing configurations and environments, chemical compatibility, size, rigid handling of plated membranes and catalyst surface area. After looking at each of these factors, the design was fabricated. A rendering of the design along with the finished product are shown below.

The test cell is small, measuring only 3.25 inches tall and approximately 2 inches wide with an additional 1.5 inches in width being due only to the mounting structure. The cell's inlet and outlet ports can be adapted to almost any pipe or compression fitting size, which will make it easy for testing different gas inlet configurations or environments. The catalyst exposed surface area is approximately 2.5 times larger than any previously tested surface area which rendered our cell response larger making smaller ethylene concentrations easier to detect. One of the biggest additions in this test cell that was not addressed in any previous version is the addition of a semi-rigid frame for the catalyst membrane. This provided a more robust construction and easier handling during assembly.

[14] Burke, L.D. and P.F. Nugent, 1998, "The Electrochemistry of Gold: II The Electrocatalytic Behaviour of the Metal in Aqueous Media," *Gold Bulletin*, 31(2), pp. 39-50.

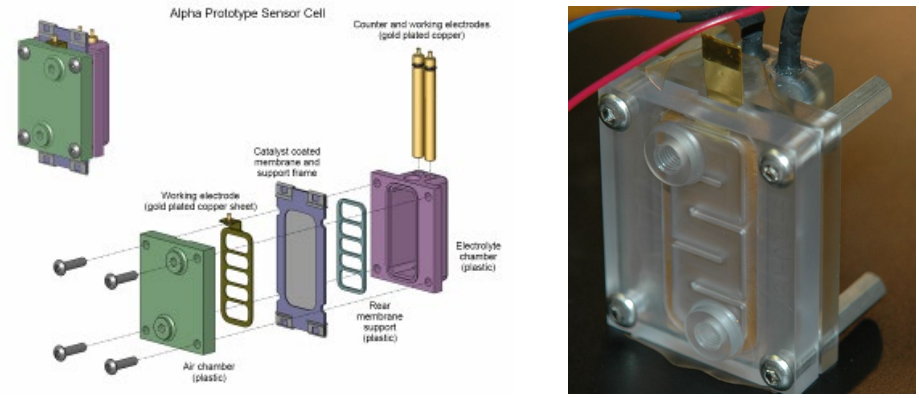

Figure 3. Electrochemical cell design for the alpha prototype .

The complete ethylene sensing instrument is packaged in an engineered sheet metal enclosure. The enclosure serves as s a rugged framework for mounting the internal components, while providing an attractive and protective shell. Figure 4 shows the rendered CAD design of the box, and a photograph of the finished enclosure.

Figure 4. Photograph of complete alpha prototype.

IV.3. Task 3: Prototype Performance Testing and Evaluation

A new test apparatus was designed and built keeping many factors in mind. Some of the main factors considered when designing the new test apparatus include: (1) ensuring that the mixing of 10 ppb of ethylene in nitrogen is achievable with a higher level of confidence than was previously achievable; (2) designing such a system that the actual cell response would not be left in question due to inability to fully characterize the test system response time; (3) allowing for ease of use; and (4) minimizing overall test setup size. After careful consideration of these factors, a design was created that satisfied the needs.

Also incorporated into the new test apparatus is a Princeton Applied Research (PAR) potentiostat, model 263A-1 as well as three tanks of cylinder gas (10-ppm ethylene in nitrogen, 1-ppm ethylene in nitrogen, and 100% nitrogen). The potentiostat gives us in house capabilities to perform all testing without the need to travel to other laboratories. Step testing performed with membranes 0009 and higher was done using the complete prototype and the potentiostat boards as opposed to the PAR 263A-1 potentiostat.

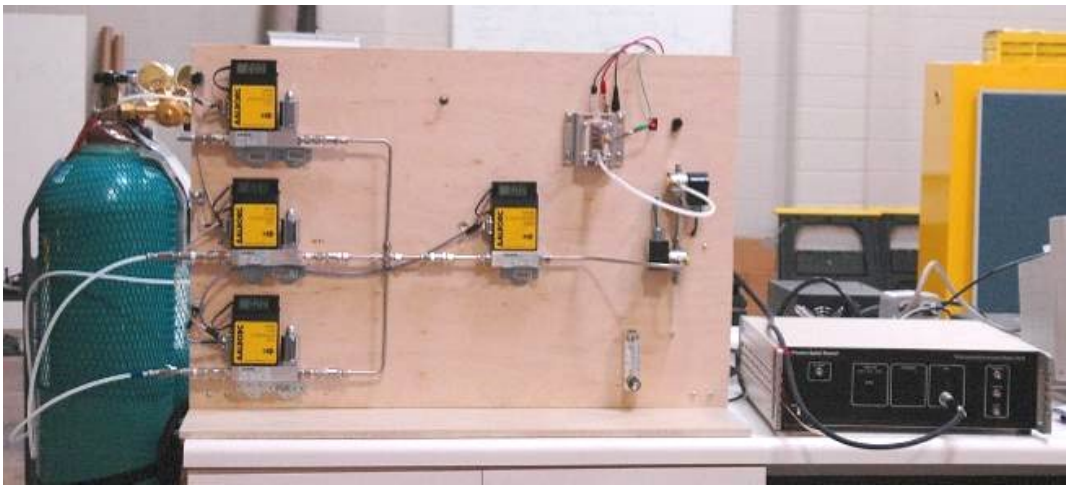

Figure 5. The final test apparatus incorporates all of the goals put forth and is currently fully functional.

Initial step tests consisted of keeping the operating voltage and flowrate constant and changing the ethylene concentration. The standard initial step test changes the concentration from 0 ppm to 5 ppm and from 0 ppm to 10 ppm. These concentration changes are easily seen and made a good quick evaluation point in order to compare performance between different membranes. Figure 6 shows step test results from membrane 0006. Membrane 0006 showed approximately 4 microamps per ppm response. As mentioned before, fabrication of the membrane has a tremendous impact on the sensitivity of the device. While membrane 0006 showed good sensitivity, we have results from electrodes that showed little to no sensitivity to ethylene because of variability in the plating process. The figure on the right shows the linear relationship between the ethylene sensitivity and ethylene concentration, showing sensitivity down to 10-ppb of ethylene.

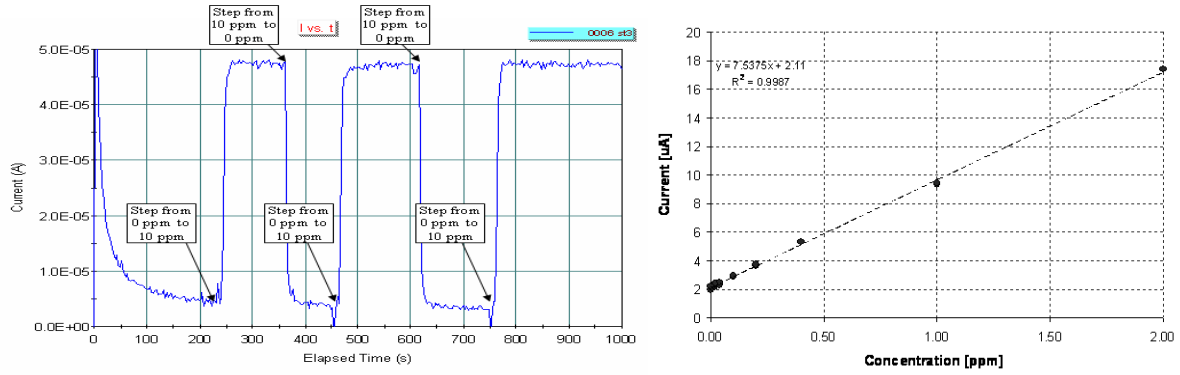

Figure 6. (a) Initial step test for membrane 0006; (b) Sensitivity versus concentration.

Because of the positive results achieved with membrane 0006, additional tests were performed on this membrane for a more inclusive characterization. The next tests performed on membrane 0006 were voltage tests. From a cyclic voltammogram we get a rough idea of the optimal operating voltage, but

it does not tell us what happens if the voltage is shifted slightly. The shape of the response versus voltage curve is useful in determining the level of accuracy needed in our electronic voltage control, as well in determining the optimal voltage for sensing a particular species, in this case ethylene molecules in air. Voltage tests consisted of first running the cell at 0.5 V (the baseline voltage for these tests), performing two sets of a 0 ppm to 10 ppm, longer than 200 s pulse, then changing voltage and repeating the concentration pulses. Flow rate was kept constant at 200 sccm for the entire test. The plot shown in Figure 7 represents the sensor response at different biased voltages maintained on the electrode. The sensor response at ~0.5V and above (within the tested range) does not show a large dependence on the operating voltage. This is very useful information that was used in selecting suitable electronics.

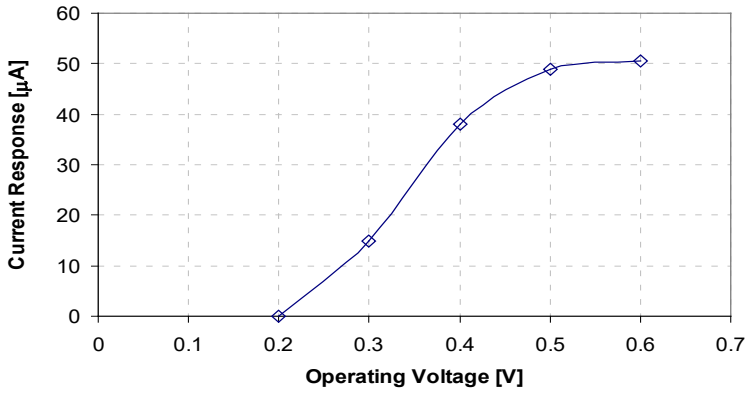

Figure 7. Operating voltage vs sensor response curve shows a plateau starting at ~0.5V

Figure 8 shows the results of pH variation vs sensitivity and offset. Sensitivity increases with decreasing pH (aka increasing acidity). In the highest pH solutions tested the system was not very stable and large fluctuations were observed. Testing is still in progress to evaluate the range of pH from 1 to 2.5. We are also planning tests to evaluate the impact of position and geometry of the counter electrode on cell electrical stability.

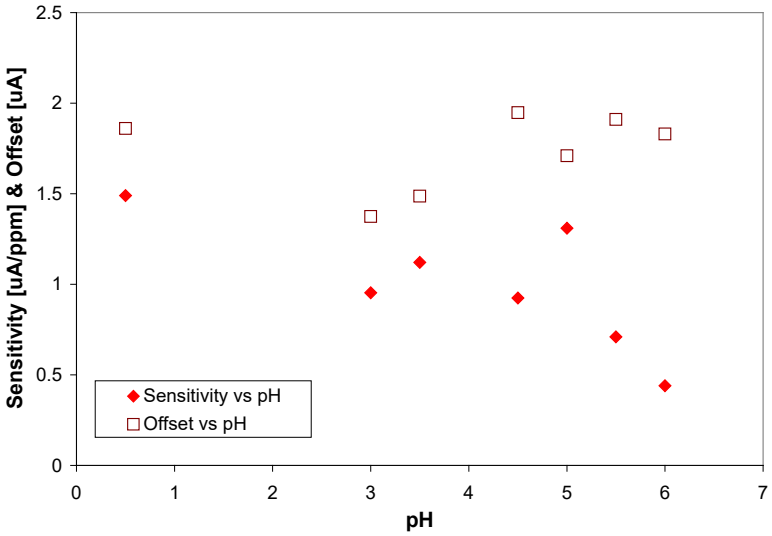

Figure 8. Sensor response vs pH.

Two factors that affect operation of the sensor under the cold storage environment are variation in the oxygen content in the air and changes in the temperature. The variation in sensor sensitivity as a function of temperature was determined using a set of tests in a controlled atmosphere chamber. The sensitivity of the sensor seems to be linearly correlated to temperature and increased with increasing temperature as shown in Figure 9. Note that the sensitivity in cold storage temperature conditions is nearly half of what we would expect at room temperature. The tests in the standard room air condition versus nitrogen only environment shows slight increase in sensitivity with exposure to air or higher oxygen levels (Figure 10).

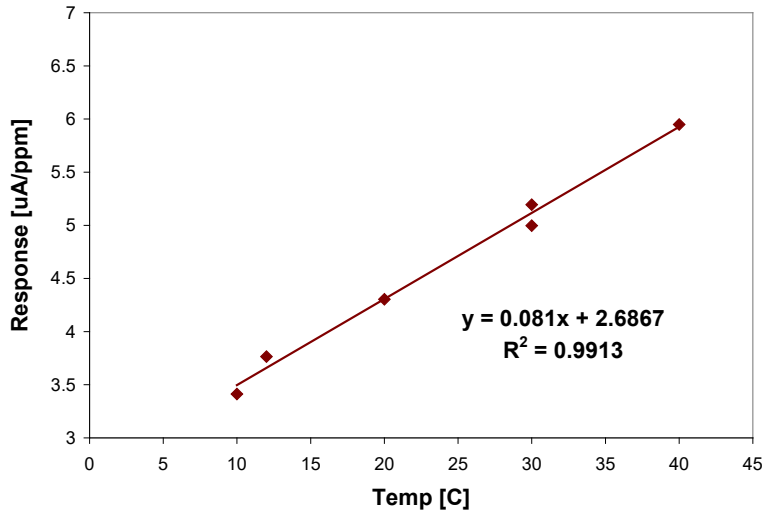

Figure 9. Sensitivity appears to increase with increasing temperature.

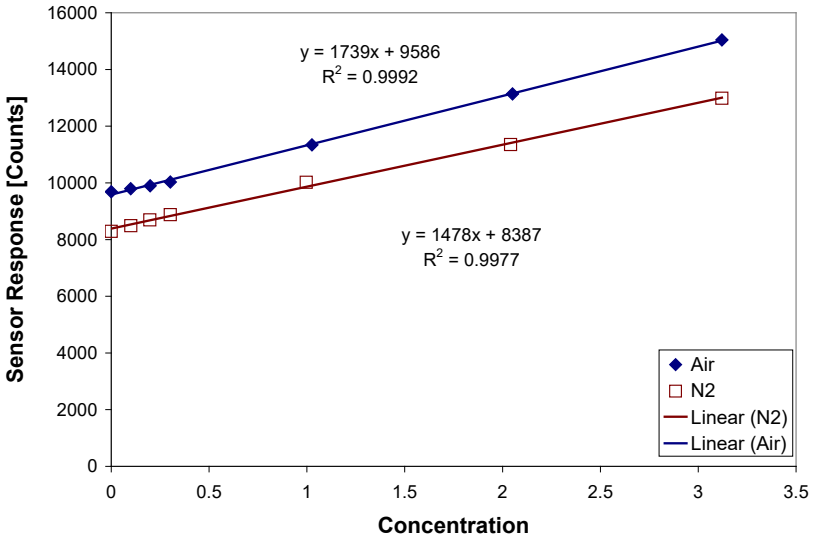

Figure 10. Sensitivity in N2 vs sensitivity in air.

In addition to ethylene there are other gases that tend to react on our catalyst potentially causing interference. The extent of interference is determined from the sensitivity of the sensor to these other gases at the operating conditions corresponding to ethylene. One gas that has been explored here is

carbon monoxide due to concern about the presence of CO in the air generated by the fork lifts and agricultural equipment. A test was performed using the mixing apparatus and an additional cylinder containing 10 ppm carbon monoxide in nitrogen. The results of these tests are shown in Figure 11. The results show that although there is some sensitivity to carbon monoxide, the sensor is more than 40 times more sensitive to ethylene than to CO. This suggests that interference from carbon monoxide is not a significant issue, unless the levels of carbon monoxide are extremely high (higher than the EPA set hazard threshold) or the ethylene measurement requirements are in the 10-ppb level.

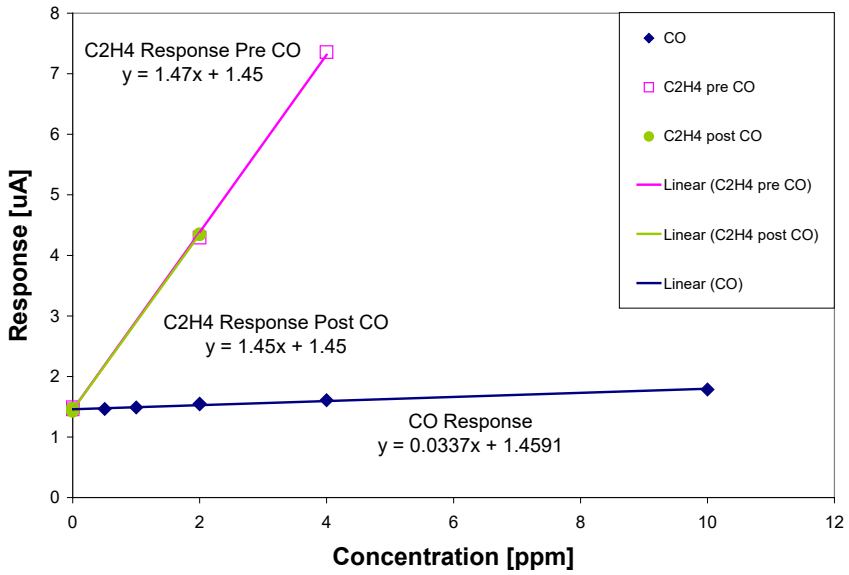

Figure 11. Sensitivity to carbon monoxide.

We also tested our sensor against a gas chromatography system, which is currently the "gold standard" in ethylene detection, for measuring the ethylene produced by a single apple (Figure 12). Single apples were placed in 0.5 liter jars and allowed to build up ethylene in the head space of the jar for several hours. Using appropriate fittings, the ethylene containing air within the jar was then recirculated through the ETH-1010 ethylene sensor. As evidenced in this figure, the total time required to get reading from a single apple is just a few minutes, which shows the built up ethylene within each jar for the different apples. Because of the high sensitivity of ETH-1010, the rate of ethylene production during each run was also measurable (Insert).

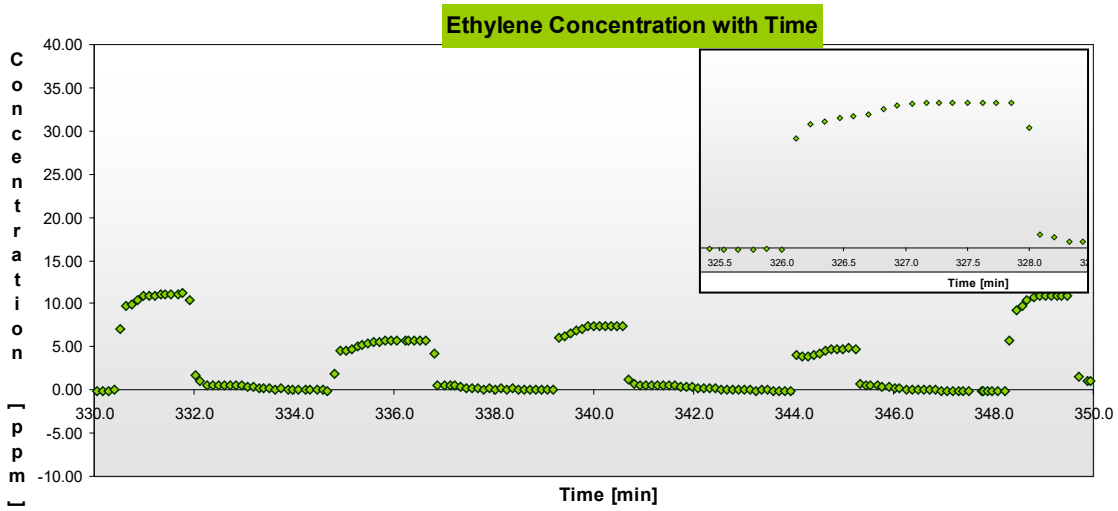

Figure 12. Time traces for single gala apple measurements performed at AgroFresh Wenatchee, October 2006 (Data provided with permission by Dr. Nate Reed).

Figure 13 shows an overlay of our sensor results and GC results for ethylene produced by a single apple. As you can clearly see, there is a correlation. One advantage to our sensor is that it can provide near real-time measurement of ethylene, which is much faster than the GC detection.

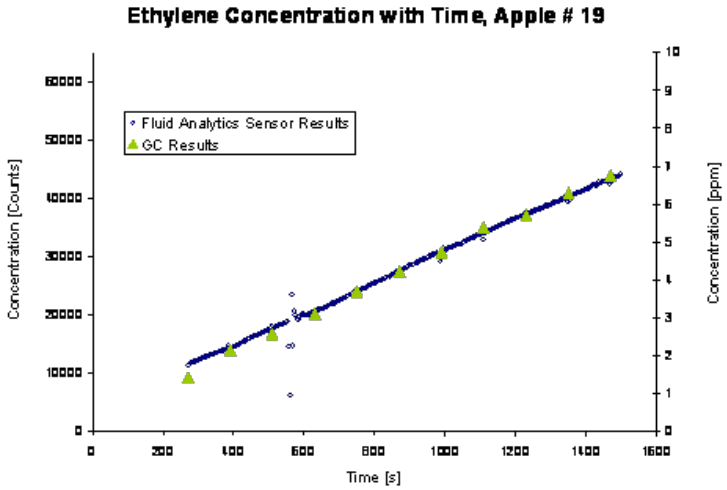

Figure 13. Single Apple Ethylene Generation Rate and Comparison with GC (experiments performed in collaboration with Dr. Dana Faubion at WSU Extension in Yakima, March 2006).

V. Summary

In this report, we presented and discussed the results of our tests performed with a new electrochemical ethylene sensor prototype developed by Fluid Analytics, Inc. We showed that this sensor has the following attributes:

1. High sensitivity of down to 10-ppb;

2. Fast response of the sensor to the measurements (within seconds) and fast recovery (within a minute or so);
3. Low sensitivity to interferent gases such as carbon monoxide;
4. Higher sensitivity to ethylene in air than in an oxygen-deprived environment (pure nitrogen);
5. More than two times higher sensitivity to ethylene at room temperature than at cold storage temperatures;
6. Sufficient sensitivity and response rate to measure single apple ethylene production rates;
7. Portable and easy to use for laboratory or field measurement of single apple ethylene generation rate or flow through measurements of ethylene concentration in cold storage facilities.

VI. Acknowledgements

Fluid Analytics would like to than the commission for providing this and other funding for advancing our sensor development and qualification testing both in the laboratory and in the field. The PI would also like to thank the technical contributions provided by Dr. Dana Faubion and Dr. Nate Reed.

FINAL PROJECT REPORT
WTFRC Project Number: AH-04-416

Project Title: Role of Sorbitol in Sugar and Acid Accumulation in Apple Fruit

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Department of Plant Science, University of California, Davis, CA 95616

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	$21,364	$22,342	$23,554
Benefits	$ 3,843	$ 4,035	$ 4,237
Wages			
Benefits			
Equipment			
Supplies	$12,493	$12,233	$13,409
Travel	$ 800	$ 800	$ 800
Miscellaneous			
Total	$38,500	$39,500	$42,000

Objectives:

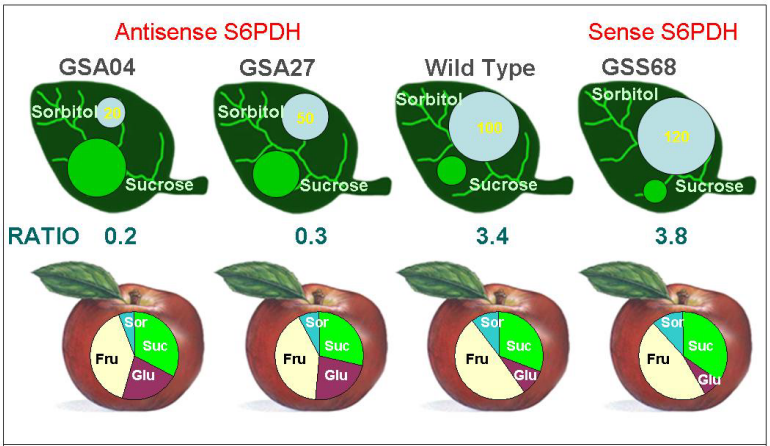

Fig. 1: Metabolic phenotypes of major transgenic genotypes with alterations in sorbitol metabolism due to antisense/sense expression of cDNA encoding S6PDH.

This project focuses on understanding biochemical mechanisms responsible for metabolism and partitioning of sorbitol in apple fruit. We have outlined the key role of sorbitol in: 1) sugar composition, 2) starch accumulation, 3) malic acid metabolism, and 4) development of the watercore disorder. For these reasons, understanding metabolism and partitioning of sorbitol in fruit tissues should remain a priority for an industry dependent on production of high quality apple fruit. Unfortunately, defining the role of sorbitol in normal apple fruit is complicated by the presence of sucrose, which is partitioned simultaneously with sorbitol. Both sorbitol and sucrose are synthesized from the same products of photosynthesis, creating two pathways of carbon flow into the fruit for conversion to common sugars and other products that determine fruit quality. For example, fructose in fruit can be derived from either sorbitol or sucrose. We have solved this problem by creating transgenic apple trees specifically altered in the ability to make and translocate sorbitol. These plants are in essence specific mutants, and they provide a unique opportunity to evaluate the role of sorbitol in fruit quality. Figure 1 lists the phenotypes of transgenic lines that are currently under study. The sorbitol to sucrose ratio (So/Su) indicates important differences among these lines. Untransformed apple trees have a ratio around 3; we have lines with a ratio more than ten times lower (Fig. 1). The ratio of sorbitol to sucrose decreased from 3.4 to 0.3 and 0.2 in leaves of antisense clones GSA27 and GSA04. In sense clone GSS68, it increased to 3.8. The ratio in fruit pedical phloem exudates is similar to the ratio found in leaves. As a result, some quality-determining factors such as fruit growth, size, and firmness do not change, but others, such as sugar composition, acid content, and starch content, do change (Fig. 1). Thus, sorbitol plays an important role in the quality of fruit flavor. This research seeks to determine key enzymes which regulate fruit quality, especially flavor, through analyses of activity and expression of encoding genes. We examined one transgenic line in detail and demonstrated that sorbitol plays a key role in the sugar composition, starch distribution and malic acid accumulation in fruit tissues. In this study we extend our analysis of sorbitol metabolism to identify critical proteins/enzymes and genes that regulate sugar and acid composition and starch accumulation (Fig. 2).

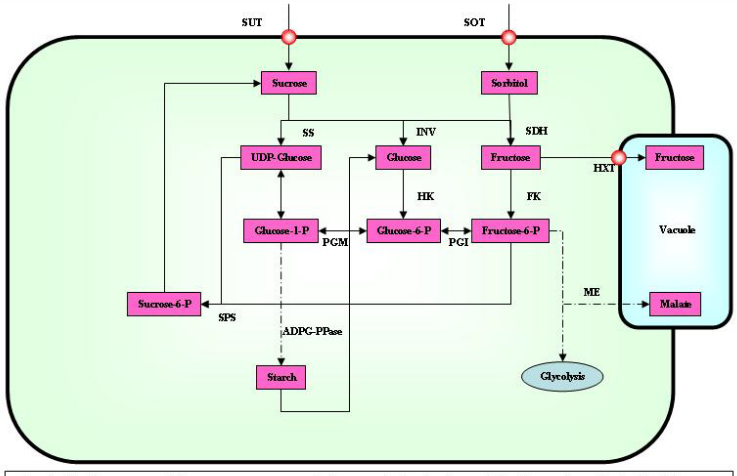

Fig. 2: Pathways and key enzymes regulating carbohydrate metabolism of apple fruit. SS – Sucrose Synthase; INV – Invertase; SDH – Sorbitol Dehydrogenase; HXT-Vacuolar Hexose Transporter; SUT – Sucrose Transporter; SOT – Sorbitol Transporter; HK – Hexokinase; FK – Fructokinase; PGM –Phosphoglucomutase; PGI-Phosphoglucoisomerase; SPS -Sucrose Phosphate Synthase; ADPG-PPase – ADPG Pyrophosphorylase; ME – NADP+-Malic Enzyme.

1 Define and validate the role of sorbitol-related enzymes in accumulation of sugar and acid in apple fruit.

2 Determine sorbitol's role in development of water core in apple fruit.

Significant Findings:

1. The Sorbitol/Sucrose ratio regulates malic acid accumulation and thus acidity levels in fruit.
2. Sorbitol partitioned into fruit regulates accumulation of nonstructural carbohydrates but does not significantly affect firmness.
3. High sorbitol concentration is responsible for water core development.
4. The sorbitol concentration supplied to fruit regulates activity and gene expression of sorbitol related enzymes and thus metabolism in fruit tissues during development and in the final product.

Methods:

Field planting of transgenic apple plants: Transgenic plant samples for this project were collected from two fields:
Field A: Source planting of all transgenic apple tree clones expressing sense/antisense S6PDH. This orchard has trees planted in a randomized design on their own roots in a 15x15 ft. spacing. The plot has 354 apple trees, of which 53 are control trees of golden delicious apple cultivars 'Greensleeves' (GS; 26) and 'Ginger Gold' (GG; 27). The other trees are transgenic, of which 106 express anti-sense S6PDH, 111 express sense-S6PDH, and the remaining 74 express other constructs not relevant to this study.
Field B: This transgenic production orchard planted in 2001 has 5 clones (all GS): 1026-8 (GSS68), 1026-27(GSA27), 0701-10, 0601-4, and 1020-4(GSA04). These five clones, including a GS control, were bud grafted onto M26 rootstock and planted in 5-tree replicates; each replicate was repeated 5 times in a randomized complete block design in a one acre block. 'Gala' grafted to M26 was planted as a pollinator in between each replicate of 5 trees. This block has micro-sprinkler irrigation and is managed with standard horticultural practices.
Fruit: Fruit samples were collected at 30, 54, 70, 91, and 118 days after flowering (DAF). Fresh samples were peeled, frozen in liquid nitrogen and kept at -80^{o}C until analyses. For the SDH and ME enzyme assays, fresh samples were used.
Enzyme Extraction and Assays: NAD-sorbitol dehydrogenase (SDH) and NADP-malic enzyme (ME) were extracted according to Yamaki and Ishikawa (1986) with slight modifications. Sucrose synthase (SS), sucrose phosphate synthase (SPS), soluble acid invertase (sAI), neutral invertase (NI), fructokinase (FK), hexokinase (HK), and ADP-glucose pyrophosphorylase (AGPase) were extracted according to Tanase and Yamaki (2000) with slight modifications.

SDH activity was assayed by modifying the method described by Yamaki and Ishikawa (1986). The reaction mixture contained 100 mM M Tris-HCl buffer (pH 9.5), 1 mM NAD^{+}, 300 mM sorbitol, and the enzyme extract. Enzyme activities were determined as changes in absorbance at 340 nm at 25^{o}C. ME activity was assayed according to Yoshioka et al. (1989). The reaction mixture contained 80 mM M Tris-HCl buffer (pH 7.5), 0.3 mM $NADP^{+}$, 1 mM $MnSO_4$, 6 mM malate, and the enzyme extract. Enzyme activities were determined as changes in absorbance at 340 nm at 25^{o}C. Soluble and cell-wall bound acid invertase activities were assayed according to Tanase and Yamaki (2000). The assay mixture contained 30 mM K acetate (pH 4.5), 200 mM sucrose, and enzyme solution. The mixture was incubated for 1 h at 30^{o}C, and the reaction was stopped by boiling before adding 0.75 M Tris-HCl buffer (pH 8.5). The activities were measured as glucose production from sucrose by the enzyme coupling method described earlier (Yamaki, 1980). For NI activity, the assay mixture was identical to that of acid invertase except that 30 mM HEPES-KOH (pH 7.0) was substituted for 30 mM K acetate (pH 4.5). The reaction was stopped by heating the mixture in boiling water for 3 min. The activities were determined as glucose produced from sucrose by the enzyme coupling method of

Yamaki (1980). SS and SPS activities were assayed according to Tanase and Yamaki (2000) with a slight modification. For SPS activity, the reaction mixture contained 15 mM HEPES-KOH (pH 8.5), 15 mM fructose-6-phosphate, 2 mM UDP-glucose, 5 mM $MgCl_2$, 50 mM NaF, 1 mM sodium orthovanadate, and the enzyme extract. For SS activity, the reaction mixture contained 15 mM HEPES-KOH buffer (pH 8.5), 15 mM fructose, 2 mM UDP-glucose, 5 mM $MgCl_2$, and the enzyme extract. The mixture was incubated for 30 min at 30ºC, and the reaction was stopped by addition of 2.5 N NaOH. Sucrose production was determined by Roe's method (1934). For the sucrose cleavage activity of SS, the reaction mixture contained 30 mM HEPES-KOH (pH 7.0), 200 mM sucrose, 5 mM UDP, and the enzyme solution. The reaction was stopped by heating the mixture in boiling water for 3 min. The production of fructose was determined by the enzyme coupling method using ATP, NAD^+, hexokinase, phosphoglucose isomerase, and NAD-glucose-6-phosphate dehydrogenase (G6PDH) (Morell and Copeland, 1985). FK and HK activities were assayed according to Kanayama et al. (1997) with a slight modification. For HK activity, the reaction mixture contained 30 mM HEPES-NaOH (pH7.5), 1 mM $MgCl_2$, 0.6 mM EDTA, 9 mM KCl, 1 mM NAD^+, 1 mM ATP, 2 units of G6PDH , 30 mM glucose, and the reaction mixture. For FK activity, 2 units of phosphoglucose isomerase and 30 mM fructose were added. Enzyme activities were determined as changes in absorbance at 340 nm at 25ºC. ADPGase activities were assayed according to Smith (1990) with a slight modification. The reaction mixture contained 100 mM Hepes-NaOH (pH 7.8), 5 mM $MgCl_2$, 10 mM NaF, 2 mM ADP-Glu, 1 mM NAD, 5 units of G6PDH, 2 units of phosphoglucomutase, and the reaction mixture. Enzyme activities were determined without (control) and with 2 mM sodium pyrophosphate as changes in absorbance at 340 nm at 25ºC.

Real time quantitative TaqMan PCR: PCR primers and compatible TaqMan® probes were designed using Primer Express (Applied Biosystems, Foster City, CA). To prevent co-amplification of contaminating genomic DNA (gDNA), TaqMan PCR primers were designed to cover exon-exon junctions where possible (Leutenegger et al., 1999).

Total cellular RNA was isolated by the hot borate method (Wan and Wilkins, 1994). gDNA contamination in the total RNA fraction was digested with RNase-free DNase I (Invitrogen, Carlsbad, CA) for 15 min at 37°C and inactivated at 95°C for 5 min followed by chilling on ice. Absence of gDNA contamination was confirmed using a universal 18S TaqMan PCR system on digested total RNA. Complementary DNA (cDNA) was synthesized using 50 units of SuperScript III reverse transcriptase, 600 ng random hexadeoxyribonucleotide (pd(N)6) primers, 10 U RNaseOut (RNase inhibitor), and 1 mM dNTPs (all Invitrogen) in a final volume of 40 ml. Reverse transcription proceeded for 120 min at 50°C. After addition of 60 ml water, the reaction was terminated by heating for 5 min to 95°C and cooling on ice.

Each PCR reaction contained 20x Assay-on-Demand primer, probes for the respective TaqMan system, and commercially available PCR mastermix (TaqMan Universal PCR Mastermix, Applied Biosystems) with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 2.5 mM deoxynucleotide triphosphates, 0.625 U AmpliTaq Gold DNA polymerase, 0.25 U AmpErase UNG, and 5 ml diluted cDNA sample in a final volume of 12 ml. The samples were placed in 96 well plates and amplified in an automated fluorometer (ABI PRISM 7700 Sequence Detection System, Applied Biosystems). AB's standard amplification conditions were used: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C and 60 s at 60°C. Fluorescent signals were collected during the annealing phase and CT values extracted with a threshold of 0.04 and baseline values of 3-10.

Housekeeping gene validation experiment: To determine the most stably transcribed housekeeping gene, a housekeeping gene validation experiment was run on representative samples from all tissue types. Three commonly used housekeeping genes were selected: a TaqMan PCR system recognizing plant 18S rRNA (ssrRNA), apple glyceraldehyde 3-phosphate dehydrogenase, and apple ribosomal protein S19. 18S rRNA was transcribed most stably, and therefore had least standard deviation across all tissues. 18S rRNA CT values were used to normalize the target gene CT values.

Relative quantitation of gene transcription: Final quantitation was done using the comparative CT method (User Bulletin #2, Applied Biosystems) and was reported as relative transcription, or the n-fold difference relative to a calibrator cDNA (i.e. lowest target gene transcription). In brief, the housekeeping gene, 18S rRNA, was used to normalize the CT values of the target genes (ΔCT). The ΔCT was calibrated against the weakest signal within each target gene. The linear concentration of target molecules relative to the calibrator was calculated by 2-ΔCt. Therefore, all gene transcription were expressed as an n-fold difference relative to the calibrator.

Results and Discussion:

Comparison of activities of key emzymes regulating sugar metabolism: The most important enzyme in apple fruit is SDH, which catabolizes sorbitol, the principal translocated sugar in fruit. SDH activity is highest late in fruit development, when sugar content in fruit drastically increases. In control trees, SDH activity was not detected in immature fruit at 30 DAF. Then, activity increased and peaked in premature fruit at 91 DAF. At harvest, activity was detected at very low levels (Fig. 3). In GSS68, SDH activity was also not detected at 30 DAF. The pattern of SDH activity was the same as in the control, but the activity tended to be higher throughout fruit development. In antisense clones GSA04 and GSA27, activities were lower than the control, especially at 91 DAF when the activity was highest in control fruit, and were not detected at harvest. Earlier work showed interruption of assimilate by girdling led to decreased SDH activity in apple fruit (Beruter and Feusi 1997). Low SDH activity of girdled apple fruit was recovered by *in vitro* sorbitol (Archbold, 1999). These reports suggest that sorbitol supply regulates SDH activity in fruit. Our results with transgenic apple fruit support this hypothesis with direct genetic evidence. In cultured celery cells, expression of the mannitol dehyrogenase (MDH) gene was repressed by hexose, and it was suggested that HK and sugar phosphorylation are involved in signaling its repression (Prata et al., 1997).
SS and invertases play are critical to sink organs since they catabolize sucrose, the other translocated sugar in apple. In general, these enzymes contribute to sequential stages of sink initiation, expansion, and storage/maturation (Koch, 2004). Girdling during active starch synthesis as mentioned above also led to decreased neutral invertase activity (Beruter and Feusi 1997), which suggests neutral invertase is important at this stage. In this study, no differences in these enzymes were detected between control and transgenic fruit, though sucrose contents in phloem exudates were different. Fructokinase and hexokinase are crucial enzymes for further metabolizing hexose. Sucrose synthase (synthesis) and sucrose phosphate synthase re-synthesize sucrose in fruit. ADP-glucose pyrophosphorylase synthesizes starch. We did not detect prominent differences of these enzymes among control and transgenic fruit (Fig. 3), though sugar and starch concentrations were different. The difference in sugar concentration may be too small to overcome homeostasis and change enzyme activities in fruit, unlike SDH, which is directly affected by phloem sap with altered sugar composition.

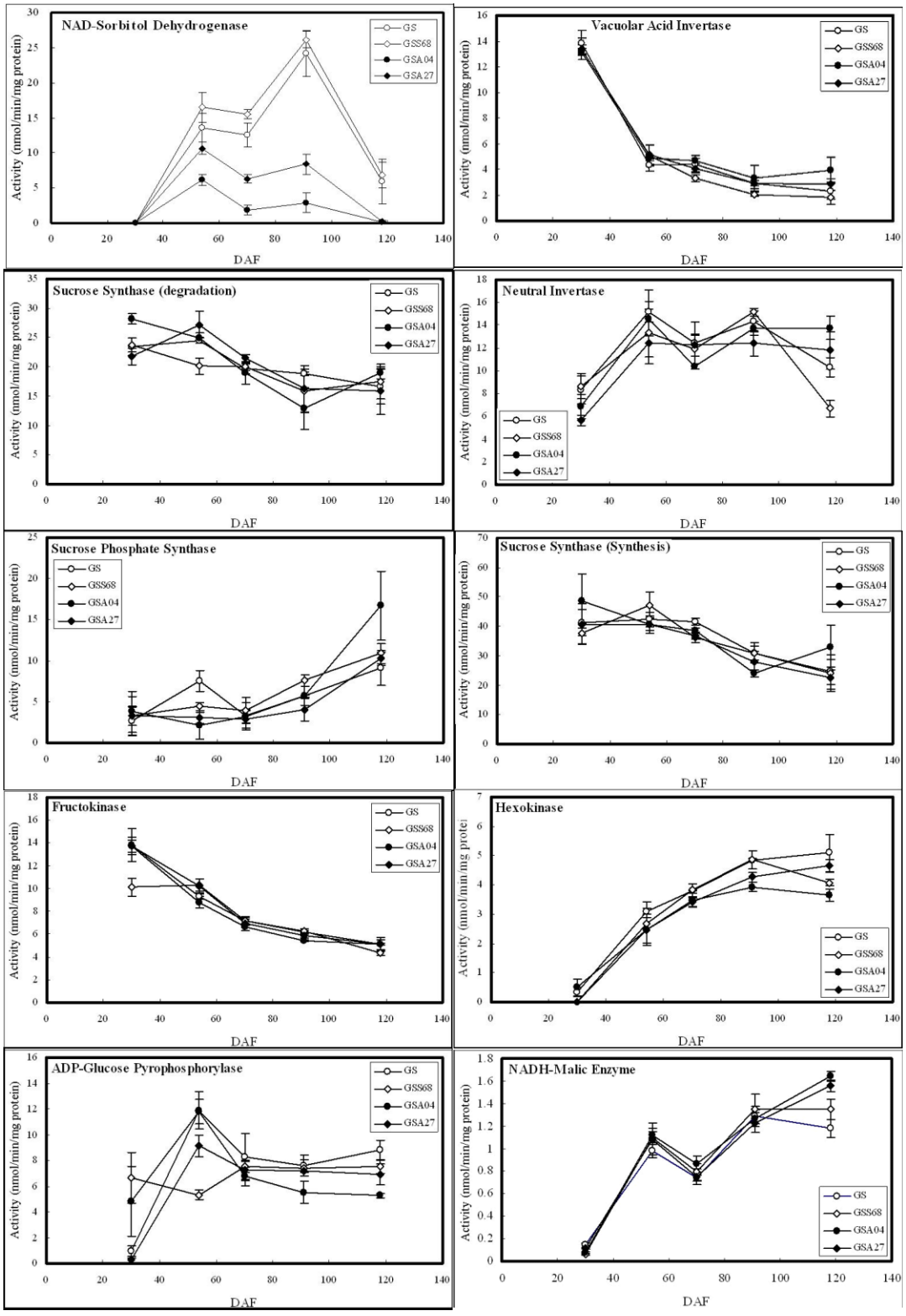

Fig. 3: Analysis of key enzymes regulating carbohydrate metabolism in normal and transgenic apple fruit at various stages of development.

It has been suggested that ME is associated with decreased malic acid in apple fruit (Yoshioka et al. 1989). ME activities increased during development (Fig. 3). They were similar in control and transgenic plants until 91 DAF, but at harvest activities in antisense clones GSA04 and GSA27 were higher than those of the control and sense clone GSS 68. There is less acid in the antisense clones than in the control and sense clone (previous report). This suggests low acid causes high ME activity.

Comparison of gene expression of key proteins regulating sugar metabolism: To characterize sorbitol transport (SOT) and metabolism (SDH) proteins, we analyzed individual alleles with real time quantitative TaqMan® PCR . For each target gene, there was more than one sequence representing allelic forms; we analyzed 21 sequences corresponding to each of the 8 alleles used in this analysis (4 *SOT*s and 4 *SDH*s). PCR primers and TaqMan® probes were designed based on information available for these sequences in the apple unigene set on the NCBI web site (http://www.ncbi.nlm.nih.gov/UniGene/UGOrg.cgi?TAXID=3750). Figure 4 shows data for 4 alleles for each of the 2 genes (*SOT*s and *SDH*s). Expression of *SDH2*, *SDH3*, and *SDH4* were higher than that of *SDH5* (Fig. 4). Expression of *SDH2* in control and GSS68 lines was generally higher than that observed for GSA04 and GSA27, except at 54 and 70 DAFB. At 30 DAFB, expression of the *SDH2* gene was detected, though enzyme activity was not detected (Fig. 3 & 4). Expression of *SDH3* in control and GSS68 was higher than in GSA04 and GSA27 except at 30 DAFB. Low expression of *SDH3* in lines GSA04 and GSA27 may result from low SDH activities through fruit development. In addition, low expression of *SDH2* in GSA04 and GSA27 after 91 DAFB may partially be a result of low SDH activities at the later stages of fruit development.

Expression of *SOT1*, *SOT3*, and *SOT4* in control and GSS68 lines at 30 DAFB was higher than that in GSA04 and GSA27, and though expression of *SOT2* in GSA04 and GSA27 was higher at 54 DAFB (Fig. 4), we did not detect prominent differences in *SOT*s expression among control/sense and antisense clones.

We also detected no prominent differences in expression of genes for *AGPase*, *HXT1*, *HXT2*, *HXT3*, *NIN1*, *NIN2*, *SS1*, and *SS2* in this experiment. No differences in expression of *AGPase* were observed in clones thoughout fruit development. Changes corresponded roughly with changes in activities. Expression of *HXT1*, *HXT2*, and *HXT3* in control and GSS68 was only higher than GSA04 and GSA27 at 30 DAFB. No other differences in expression of *HXT*s genes were observed in clones thoughout fruit development. We did not observe significant differences in expression of *NIN* genes or their activities, although *NIN1* was expressed more than *NIN2*. Expression of *SS* genes in control and GSS68 fruit was very high at 30 DAFB, then decreased drastically and kept almost constant.

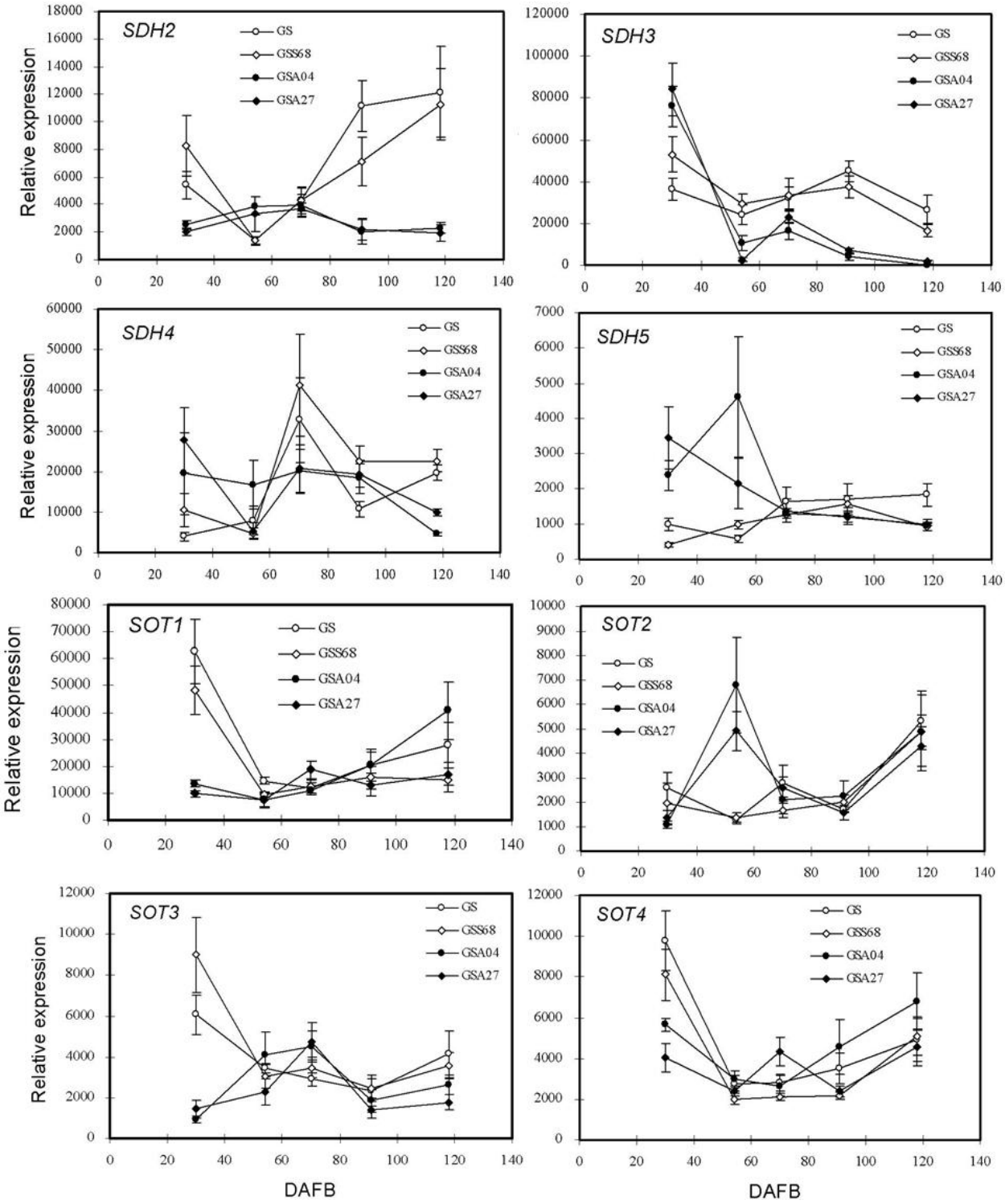

Fig.4. Gene expression of key proteins regulating sugar metabolism in control (○) and transgenic apple fruit, GSS68 (◇), GSS04 (●) and GSS27 (◆) at various stages of development as revealed by Real time PCR. Each value is the mean (±SE) of 6 replicates.

Except at 30 DAFB, expression of *SS* genes was almost the same among clones, like their enzymatic activities.

Sugars can play pivotal roles as signaling molecules (Rolland et al. 2006), and thus sorbitol may regulate gene expression. In sliced tissues of Asian pear fruit, the amount of SDH protein, activity, and mRNA increased in the presence of not only sorbitol, but also sucrose, glucose, and mannitol (Iida et al., 2004). In cultured celery cells, gene expression of mannitol dehyrogenase was repressed by hexose, and it was suggested that HK and sugar phosphorylation are involved in signaling its repression (Prata et al., 1997). In this research, greatly decreased sorbitol in phloem of antisense lines could be responsible for suppression of *SDH2* and *SDH3* expression in antisense fruit, which results in low SDH activities. This suggests sugar composition, especially sorbitol, in phloem sap affects sugar metabolism and thus fruit quality.

Sorbitol transporters were not apparently regulated by sorbitol, although they are an important component of sorbitol metabolism. No prominent differences were observed among different lines (Fig. 4). *SOT* gene expression is low in tissues of watercored fruit (Gao et al., 2005), where there are high concentrations of sorbitol. This suggests *SOT* expression may be regulated by factors other than sugars.

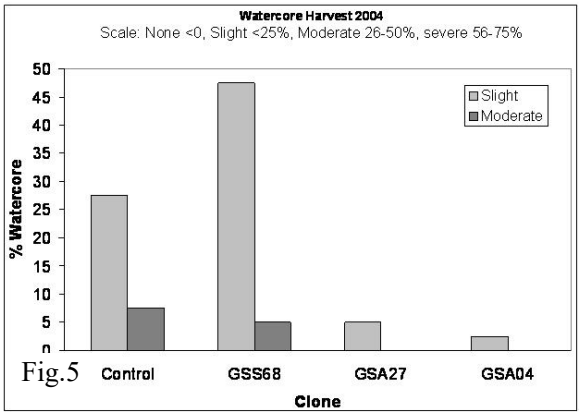

Fig.5

There is a relationship between sorbitol and watercore formation in our transgenic lines that produce different levels of sorbitol (Fig. 5 and 6). GSS68 makes more sorbitol and less sucrose, and these fruit display a very significant incidence of watercore when compared to the control. In contrast, lines with less sorbitol (GSA27 and GSA04) also have very low incidences of watercore. Watercore formation is related to sorbitol metabolism. Thus, we focused on sorbitol dehydrogenase (SDH), which catabolizes sorbitol, and sorbitol transpoter (SOT), which translocates sorbitol from apoplast into cytoplasm. We did not detect different levels of SOTs gene expression among clones. Gene expression of SDHs in control and GSS68 were higher than in GSA04 and GSA27. These results show that high sorbitol, not SOT and SDH, are responsible for watercore formation. They also suggest that watercore formation may be caused by low activity of SOT and SDH in comparison to the sorbitol supply.

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Final project report **Year 2/2**

WTFRC Project#: PH-04-0444 **Organization project:** AFHRC-53086

Project Title: Proteomic approach to study scald disorder of apples

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Objectives:

To improve our fundamental understanding of scald disorder, the objectives of this study were:

1) Using proteomic tools, characterize proteins associated with scald development in scald susceptible apple cultivars.

2) Map protein changes associated with factors that impart scald resistance including ripeness and treatment with DPA or 1-MCP.

3) Identify proteins that are responsible for scald development and resistance and propose metabolic pathways involved.

Significant findings (2004):

1) Improved the methodologies of 2-dimensional electrophoresis (2-DE) analysis and characterization of proteins in apples.
2) Significant development of scald was found in untreated "Red Delicious" apples during storage. Treatment with 1-MCP and DPA inhibited scald development in all tested apples.
3) Using proteomic tools, characterized proteins associated with scald development in the scald susceptible apple cultivar "Red Delicious".

Significant findings (2005):

1) Data analysis from experiments conducted in 2004-2005 was completed. The analysis of 2-DE gels, mapped apple proteins and identified up- and down-regulated proteins associated with control and treatments with DAP and 1-MCP were conducted. At day 0, a total of 16, 28 and 29 proteins were found to be in specially associated with the scald, DPA and 1-MCP treatment, respectively. Among them, 5, 17 and 17 proteins from the control, DPA and 1-MCP treatments, respectively were identified. Four proteins were found to be present in fruit treated with DPA or 1-MCP. At day7, 134 proteins were found to be specific to the DPA treatment, while 71 proteins were common to both the DPA and 1-MCP treatments.
2) In 2005-2006, two additional proteomic studies were conducted to analyze 2-DE gels, mapped apple proteins and identify up- and down-regulated proteins associated with control, DAP and 1-MCP treatments.
First, we characterized proteins profiles in scald and non-scald tissues. Gel analysis identified 211 proteins in non-scald tissues, while 90 proteins in scalded tissues. Among them, 69 proteins in non-scald tissues and 54 in scalded tissues were present with a normalized intensities great enough to be identified. In total, 114 protein spots were excised from gel and sent for protein identification.
3) A chemiluminescence detection based technology was developed to measure the H_2O_2 concentration in peel and flesh tissues of apples.
4) Established collaborations with Drs. Beaudry at MSU and Zhu at USDA to conduct gene expressions studies in association with scald development using micro-array and substractive hybridization expression techniques.

Materials and Methods:

Apple fruit

2004: "Cortland", "Law Rome" and "Red Delicious" were included in this year's study. Fruit were harvested before the climacteric stage, with internal ethylene concentration below 0.1-0.2 ppm (one week before commercial harvest). After harvest, fruit were divided into three groups, control, DPA treatment and 1-MCP treatment. DPA (2000 µl/L) and 1-MCP (1.0 µl/L) were applied. Fruit were stored under cold air and CA (3.0 kPa +1.0 kPa CO_2) at 0-1°C for 4 and 6 months. Scald

development was evaluated immediately after removal from storage, or after an additional 7 days at 22 °C. Scald was rated using a scale of 1 to 5: 1=no scald; 2=1-10%; 3=11-33%; 4=34-66 %; and 5=67-100% (Whitaker, et al., 2000). Two sub-samples of peel from eight apples were taken from each treatment group. Peels were quick frozen in liquid N_2, and stored at -86 °C for further analysis. 2005: Further research in 2005-2006 was concentrated on the "Red Delicious" cultivar with a new experimental design. Fruit were obtained from two orchards and two harvest maturities (early and late, one week before commercial harvest, before the climacteric stage, with internal ethylene concentration below 0.1-0.2 ppm and post-climacteric stage). After harvest, fruit were divided into three groups, control, DPA treatment and 1-MCP treatment. DPA (2000 µl/L) and 1-MCP (1.0 µl/L) were applied. Fruit were stored under cold air at 0-1°C for 5 months. Scald development was evaluated immediately after removal from storage, or after an additional 7 days at 22 °C as described above. Three sub-samples of peel from twenty apples were taken from each treatment group. Peels were quick frozen in liquid N_2, and stored at -86 °C for further analysis.

Proteomic analysis
Total protein extraction: In 2004, total protein was extracted from 2.0 g peel samples with 12 mL SDS extraction buffer containing 2% SDS, 20 % glycerol, 60 mM DTT and 40 mM Tris-base buffer (pH 8.5). In 2005, total protein was extracted from ground fruit peel tissue (2.5 g) with a protocol modified phenol-based protocol from Saravanan and Rose (1). The final extract corresponds to the total protein extract and was stored at B86 °C for further analysis.

Protein assay
Protein concentration was measured using the RC DC protein assay kit (Bio-Rad Laboratories), with BSA (bovine serum albumin) as a standard. The protein concentration was expressed as mg/g FWt.

2-D electrophoresis (2-DE)
For 2-DE analysis, proteins were first separated by iso-electrofocusing (IEF). IEF was carried out with the IPG Phor System II (Amersham Bioscience) using Immobiline DryStrip gels (18 cm) with non-linear pH gradients (pH 3-11 NL) according to the manufacturer=s instruction. 80.0 µg protein was resolublized in DeStreak Rehydration solution (Amersham Biosciences) loaded onto the gel and incubated overnight or for 10 h at room temperature using the in-gel rehydration procedure. After the first dimension separation, second dimension separation was conducted on large format 12.5% acrylamide SDS-PAGE gels (24 cm x 18 cm) on an Ettan Dalt*six* multi gel system (Amersham Biosciences) for 30,000VH at 20°C. After 2-D electrophoresis, gels were placed in a protein fixation solution in preparation for staining. Fluorescent stain (SYPRO Ruby, Bio-Rad Laboratories) was applied to visualize proteins.

Image analysis and protein identification
2-DE gel images were captured with a digital camera and analyzed using computer assisted image analysis software (PDQuest Version 7.4, Bio-Rad Laboratories). Spot detection, quantification and gel comparisons were conducted. Spots that changed in association with scald development were selected and cut from gels to send for MS identification.

Protein identification:
Excised spots were identified by NanoSpray LC/MS/MS (3200 Q Trap LC/MS/MS system. Mass spectrometry was performed on a hybrid quadrupole linear ion trap (Q-TRAP LC/MS/MS, Applied Biosystems, Foster City, CA, USA) equipped with a nanospray ion source at the DalGEN Proteomics Core Facility, Atlantic Research Centre, Dalhousie University, Nova Scotia. The raw MS/MS data

were searched against NCBI viridiplantae entries, 278115 sequences, updated Nov 7th 2006 (NIH, Bethesda, MD, USA) using the MASCOT algorithm (Matrix Science, London, UK). The MS and MS/MS mass tolerance was 0.8 and 0.5 Da respectively and one missed cleavage was allowed. Carboxamidomethyl cysteins and oxidized methionines were set as variable modifications. Proteins with significant peptide matches were selected for error tolerant searching. The data was also searched against the SwissProt database, 234112 sequences, updated Dec 11th 2006 (Sprot version 50.8) to ensure no peptides from trypsin or keratin were present. Peptide ion scores greater than 41 indicate identity or extensive homology ($p < 0.05$) and are referred to as significant hits. Peptides below the significance threshold were only reported where other significant hits to the same protein were present. The Pro ID algorithm (Applied Biosystems, Foster City, CA, USA) was used to search data against the EST database for apple, 195553 entries, updated Dec 19th 2005 and strawberry, 9213 entries, updated Dec 19th 2005 (Genome Database for Rosaceae, Washington State University, Pullman, WA, USA http://www.mainlab.clemson.edu/gdr). Search parameters for Pro ID were the same as for those used with MASCOT. Peptides with a Pro ID confidence value at least 95 were considered significant hits.

Statistical analysis.
Experimental design and data analysis were conducted using GenStat, Release 8.1, (VSN International Ltd., 2005. Lawes Agricultural Trust). ANOVA was conducted from a factorial design with three cultivars, three treatments, two storage conditions, two removals, two evaluations and two sub-samples for 2004 and with one cultivar, two locations, two harvests, three treatments, and two removals for 2005.

Results and Discussion
2004

Incidence of scald disorder. Scald development in three apple cultivars was evaluated for "Red Delicious", "Cortland" and Law Rome". Significant scald developed only on the "Red Delicious" with a scald index rating between 1-2 after 4 and 6 months storage. There was a slight increase in scald development from 4 to 6 months, while there was no significant increase of scald following 7 days at room temperature after storage. Treatment with DPA and 1-MCP inhibited scald development (Tab. 1). The low scald development on "Cortland" and "Law Rome" may be due to the relative cool season last year. Therefore, our further analyses have been focusing on "Red Delicious".

Protein quantification and SDS-PAGE: With the standardized protein procedure developed previously, proteins were successfully extracted from all samples. More than one hundred sharp bands were seen on the SDS-PAGE gels with no evidence of protein breakdown or contamination (data not shown). This procedure ensured that our protein extraction procedure was successful and reliable.

Improvement of methodology for 2-DE protein analysis: Several improvements have been archieved during this study, including maximizing protein extraction capacity while maintaining good protein quality, analyzing proteins on large scale SDS-PAGE gels and analyzing proteins qualitatively and quantitatively after fluorescence staining (Sypro Ruby). Similar sensitivity to silver stain was achieved by the use of a modified fluorescence stain. This protocol allows us to quantitatively detect ~1-10 ng protein. More than 900 polypeptides (spots) from apple fruit are shown on a 2-DE SDS-PAGE gel (Fig.1), some of which have been identified.

Identification of proteins from 2004 study: Gel analysis of the 2004-2005 study indicated that protein populations and profiles changed significantly among control, DPA and 1-MCP treated apples

during storage. To date, we have focused on "Red Delicious" apples under cold storage and have compared scalded fruit (control) with those treated with DPA or 1-MCP after 6 months storage. Apple peel proteins were separated by 2-DE and gels stained with SYPRO Ruby. Based on the protein extraction protocol used in 2004, protein profiles from control fruit (with scald), DPA and 1-MCP treated fruit are shown in Fig 1. (1A, 1B, and 1C). A total of 900, 851, and 954 spots have been visualized for the control, DPA and 1-MCP treated fruits, respectively. Substantial quantitative and qualitative differences can be seen. At day 0, there were 16, 28 and 29 proteins associated with the scald, DPA and 1-MCP treatments, respectively. Among them, three proteins were consistently present in fruit treated with DPA or 1-MCP. At day7, 134 proteins were found to be specific to DPA treatment, while 71 proteins were common to both the DPA and 1-MCP treatments. LC/MS identification was conducted on day 0 samples, and 5, 17 and 17 proteins from control, DPA and 1-MCP respectively, were putatively identified and annotated in Table 3. For control fruit, the identified proteins are endoplamin homolog precursor, malic enzyme, plastid 5, 10-methylene-tetrahyrofolate dehydrogenase and non-symbiotic hemoglobin class 1. In DPA treated apples, NAD binding/ glyceraldehyde-3-phosphate dehydrogenase, pectin methylesterase, malate dehydrogenase, alcohol dehydrogenase, cholorphyll a/b binding enzyme, polyphenol oxidase, heat shock proteins and ATP synthase subunits were identified, indicating that multiple pathways involved in cell wall, chloroplast function, browning and electron transport system may act as protecting mechanism against scald. The occurance of NAD binding/ glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, and alcohol dehydrogenase in DPA treated fruit may be similar to a report that all at these proteins being inhibited or brokendown by H_2O_2 treatment of *Arabidopsis*, and are related to oxidative stress responses in plants (2, 3). 1-MCP treated fruit had four proteins the same as in DPA treated fruit (Fig.1D), which were phosphopyruvate hydratase, pectin methyl esterase, malate dehydrogenase, and alcohol dehydrogenase. In addition, calmodulin binding, sucrose synthase, isopropylmalate synthase, cinnamoyl CoA reductase, glutamate decarboxylase 2 and putative 26S proteasome beta subunit were also identified. Both cinnamoyl CoA reductase and glutamate decarboxylase 2 are also stress response proteins in plants. These findings indicated that additional mechanisms are involved in tissue protection against scald. Among the scald hypotheses, some evidence suggested that oxidation products of (*E.E*)-α-farnesene could be the causal agents of apple scald (4, 5). Other evidence suggests that a general oxidative stress triggered by disruption of mitochondrial electron transport at low temperature results in the production of superoxide, which may lead to scald (6). DPA inhibits oxidation of α□-farnesene *in vivo* and *in vitro* and prevent scald development (7, 8). Application of 1-methylcyclopropene (1-MCP) can also prevent scald development in apples (9). The blocking of ethylene action seems to be critical to reduce the production of □α-farnesene and the development of scald (10, 11), while the complete prevention of scald by 1-MCP was better correlated with antioxidant capacity (12). At this stage of this study, we speculate that DPA and 1-MCP might have their own unique function against scald, but may also share a common mechanism by protecting fruit tissue from oxidative stress damage caused by H_2O_2 and/or ROS. A much clearer picture will be drawn, when protein identifications from 2005-2006 are completed.

2) Further research in 2005-2006 was concentrated on "Red Delicious" with a new design that included two blocks, two harvest maturities (early and late) and treatments with DAP and 1-MCP. 2005: *Incidence of scald disorder*. Significant scald developed only on the "Red Delicious" with a scald index rating between 4-5 after 5 months storage. There was a significant increase in scald development from 4 to 6 months, while there was no significant increase of scald following 7 days at room temperature after storage. Treatment with DPA and 1-MCP inhibited scald development (Tab.2).

Identification of proteins from 2005study:
In 2005-2006, two proteomic studies were conducted using an improved protein protocol (13). We analyzed 2-DE gels, mapped apple proteins and identified up- and down-regulated proteins associated with control and treatments with DAP and 1-MCP. On average, the number of spots in each proteins populations increased from 900 to 1400. This will allowed us to investigate even broader protein profiles.
First, we characterized proteins profiles in scald and non-scald tissues. Gel analysis was completed and 211 spots were identified to be present in non-scald tissues, while 90 spots were found to be in scald tissues. Among them, 69 spots in non-scald tissues and 54 in scald tissues were found to be present with a normalized intensity great enough to be excised from gel (Fig.2). A total of 114 protein spots were excised from gels and sent to protein identification by LC/MS in Dec. 2006.

Second, we characterized protein populations and profiles from tissues associated from control and DAP and 1-MCP treatments. Gel analysis of this study is currently being conducted. Spot identifications will be conducted in summer 2007.

Potential outcome and benefits:
1) Application of proteomic technology is a feasible tool to investigate the biochemical mechanisms of apple scald. Hundreds of proteins and enzyme system can be monitored at one time as apples respond to the conditions which cause scald and to the compounds that prevent scald.
2) Investigation on the effects of DPA and 1-MCP treatments to alter protein profiles in apple fruit may help to identify proteins responsible for scald development and/or resistance.

Identification and quantitative analysis of the proteins profiled in scald tissue, combined with the knowledge of previous findings, may help to identify the proteins and possible metabolic pathway(s) involved in the development of the disorder. Understanding the mechanism of scald formation in stored apples and the physiology/biochemistry of resistance could lead to new, effective strategies to reduce or eliminate this disorder. Using this technology on apples may lead to new insights into the long-standing mystery of scald resistance and development.

Project title: Proteomic approach to study scald disorders of apple fruits
PI: Dr. Jun Song
Proposed project duration: Two years (2004-2005)
Current year request (2005): $30,000

Item	Year 1 (2004)		**Year 2 (2005)**	
	WTFRC	AAFC[1]	**WTFRC**	**AAFC**
Salaries[2]	$15,000	$14,000	**$14,000**	**$14,000**
Benefits	$3,000	$3,000	**$3,000**	**$3,000**
Equipment[3]	$5,000	$8,000	**$2,000**	**$7,000**
Supplies[4]	$3,000	$1,000	**$7,000**	**$1,500**
MS identification[5]	$3,000	$2,000	**$4,000**	**$2,000**
Statistics[6]	$0	$0	**$0**	**$500**
Travel[7]	$0	$500	**$0**	**$1,500**
Miscellaneous	$0	$500	**$0**	**$500**
Total	$29,000	$29,000	**$30,000**	**$30,000**

[1]Matching funds in Canadian dollars from Agriculture and Agri-Food Canada's Matching Investment Initiative (Contingent on approval of WTFRC funding). [2]Salary dollars (part) for a postdoctoral research associate for two years (Dr. Zheng, QiFa, start date June 06, 2005). [3]Additional image analysis equipment and upgrades. [4]Fruit, chemicals, gases, electrophoresis, staining supplies. [5]Identification of proteins at proteomic facility. [6]Support for statistical analysis.
[7]Travel to present results of project.

References

(1). Saravanan, S.R.and Rose, J. K. C. *Proteomics*. 2004**4**, *4*, 2522-2532 (2). Hancock, J.T., et al., 2005. Plant. Physio. and Bio. 43: 828-835. (3). Sweetlove, L.J., et al., 2002. The Plant journal. 32: 891-904. (4) Huelin FE, Coggiola IM. 1970. J. Sci. Food Agric 21:584–589.(5). Rowan DD et al., 2001. J Agric Food Chem 49:2780–2787. (6) Whitaker BD. 2005. HortScience 39(5), 933-937 (7)Rao, MV., et al., 1998. J. Amer. Soc. Hort. Sci. 123:299-304. (8) Whitaker, BD., et al. 2000. Postharvest Biology and Technology 20: 231-241. (9) Fan X, et al., 1999. J Agric Food Chem 47:3063–3068. (10) Rupasinghe HPV, et al., 2000. J. Hort. Sci. Biotechnol. 75:271–276. (11) Watkins CB et al., Postharvest Biol Technol 19:17–32. (12) Shaham Z, et al., J. Amer. Soc. Hort. Sci. 128:761-766. (13) Zheng, Q., et al., 2007. J. Agri Food Chem. (in press).

Table.1 Scald disorder in Red Delicious apple fruit at day 0 and 7, after 6 month storage under air and CA in 2004.

	Red Delicious					
	Control		DPA		1-MCP	
4 m	D0	D7	D0	D7	D	D7
	2.0	3.0	1.0	1.3	1.3	1.3
6 m	D0	D7	D0	D7	D0	D7
	2.5	3.0	1.0	1.0	1.0	1.0

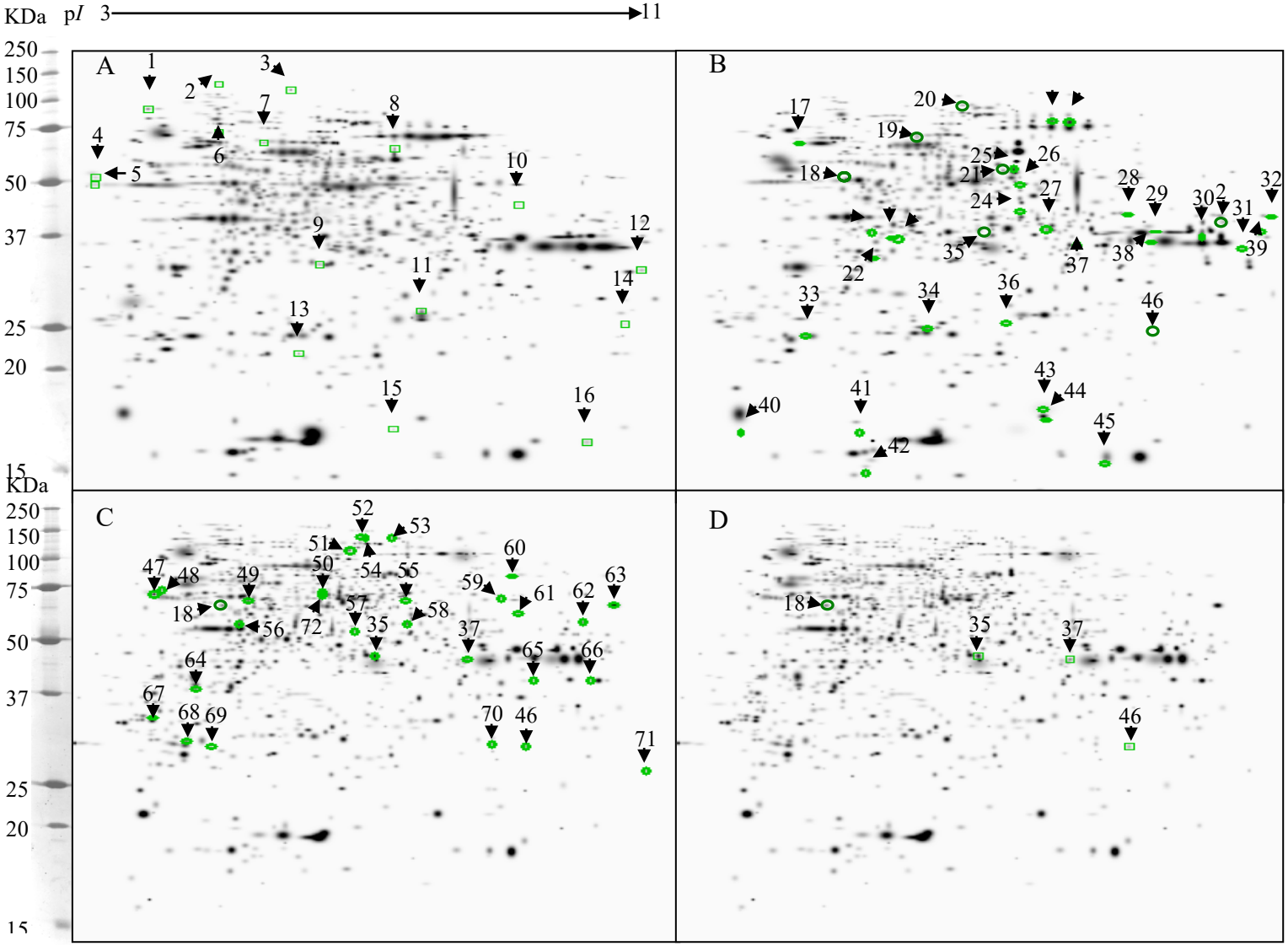
Table.2 Scald disorder in Red Delicious apple fruit at day 0 and 7, after 5 month storage under air in 2005.

	Red Delicious					
Early	Control		DPA		1-MCP	
	D0	D7	D0	D7	D0	D7
	1.9	2.0	1.0	1.1	1.8	1.9
Late	D0	D7	D0	D7	D0	D7
	2.1	3.3	1.0	1.0	1.0	1.0

Table 3. Putative identification of protein extracted from "Red Delicious" apple in control and after 1-MCP and DPA treatments. Protein spots excised from gels stained with Sypro Ruby were subjected to digestion with trypsin and identified following mass spectrometry analysis (LC/MS/MS)*. Spot numbers are also shown in the figure 2**.**

Control		DPA		1-MCP	
Spot	Protein name	Spot	Protein name	Spot	Protein name
1	Endoplasmin homolog precursor (GRP94 homolog)	17	High molecular weight heat shock protein (*Malus x domestica*)	18	Phosphopyruvate hydratase (*Arabidopsis thaliana*)
		18	Phosphopyruvate hydratase (*Arabidopsis thaliana*)	35	NAD-dependent malate dehydrogenase (*Prunus persica*)
8	Malic enzyme/ oxidoreductase, acting on NADH or NADPH, NAD or NADP as acceptor (*Arabidopsis thaliana)*	19	Polyphenol oxidase precursor (*Prunus armeniaca*)	37	Allyl alcohol dehydrogenase (*Nicotiana tabacum*)
		20	Os04g0118400 [*Oryza sativa* (japonica cultivar-group)]	46	Pectin methylesterase (*Nicotiana tabacum*)
		21	Putative protein (Arabidopsis thaliana)	49	Phosphopyruvate hydratase (*Arabidopsis thaliana*)
		22	Eukaryotic initiation factor 4A-2 (ATP-dependent RNA helicase eIF4A-2) (eIF-4A-2)	50	Calmodulin binding (*Arabidopsis thaliana*)
		23	glyceraldehyde-3-phosphate dehydrogenase (*Pinus sylvestris*)	51	Sucrose synthase 1 (*Pyrus pyrifolia*)
11	Plastid 5,10-methylene-tetrahydrofolate dehydrogenase (*Prototheca wickerhamii*)	25	ATP synthase subunit alpha, mitochondrial	55	Putative 20S proteasome beta subunit 5 (*Prunus persica*)
		29	NAD binding / glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)/ Glyceraldehyde-3-phosphate dehydrogenase (*Arabidopsis thaliana*)	56	Actin (*Pisum sativum*)
				57	Aminotransferases class-I pyridoxal-phosphate-binding site(*Medicago truncatula*)
				58	Isopropylmalate synthase (*Brassica oleracea*)
12	Prohibitin (ISS) (*Ostreococcus tauri*)	30	Glyceraldehyde-3-phosphate dehydrogenase (*Pinus sylvestris*)	63	Elongation factor 1-alpha (*Hordeum vulgare* subsp. Vulgare)
16	Non-symbiotic hemoglobin class 1 (*Malus x domestica*)	33	Light harvesting chlorophyll a /b binding protein (*Hedera helix*)	64	Unnamed protein product [*Oryza sativa* (japonica cultivar-group)]
		35	NAD-dependent malate dehydrogenase (*Prunus persica*)	67	Acidic chitinase III (*Nicotiana tabacum*)
		37	Allyl alcohol dehydrogenase (*Nicotiana tabacum*)	68	Cinnamoyl CoA reductase CCR2 (*Arabidopsis thaliana*)
		40	Trypsin inhibitor subtype A (Glycine max)	70	Putative 20S proteasome beta subunit 5 (*Prunus persica*)
		43	(Segment 5 of 10) Putative oxygen-evolving enhancer protein 1 (OEE1) (33 kDa subunit of oxygen evol	72	Glutamate decarboxylase 2 (*Brassica juncea*)
		44	ATP synthase F0 subunit b (*Pseudendoclonium akinetum*)		
		46	Pectin methylesterase (*Nicotiana tabacum*)		

*Please note that *all* the MS/MS data can be made available as supplementary material in whatever format the WTFRC prefers.


KDa
pI 3
11
250
150
100
75
50
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25
20
15
A
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D

Fig. 1. 2-DE analysis of proteins from apples stored for 6 months under air or CA storage. 80.0 µg protein was loaded onto a 12.5 % polyacylamide large format gel and visualized with SYPRO Ruby stain. The molecular weight of protein standards are indicated on the left. The image is inverted from a dark image with red spots. Arrows indicate proteins identified by LC/MS/MS with corresponding numbers listed in Table 3. A: Control; B: DPA; C: MCP; and D: Proteins present in both DPA and 1-MCP.

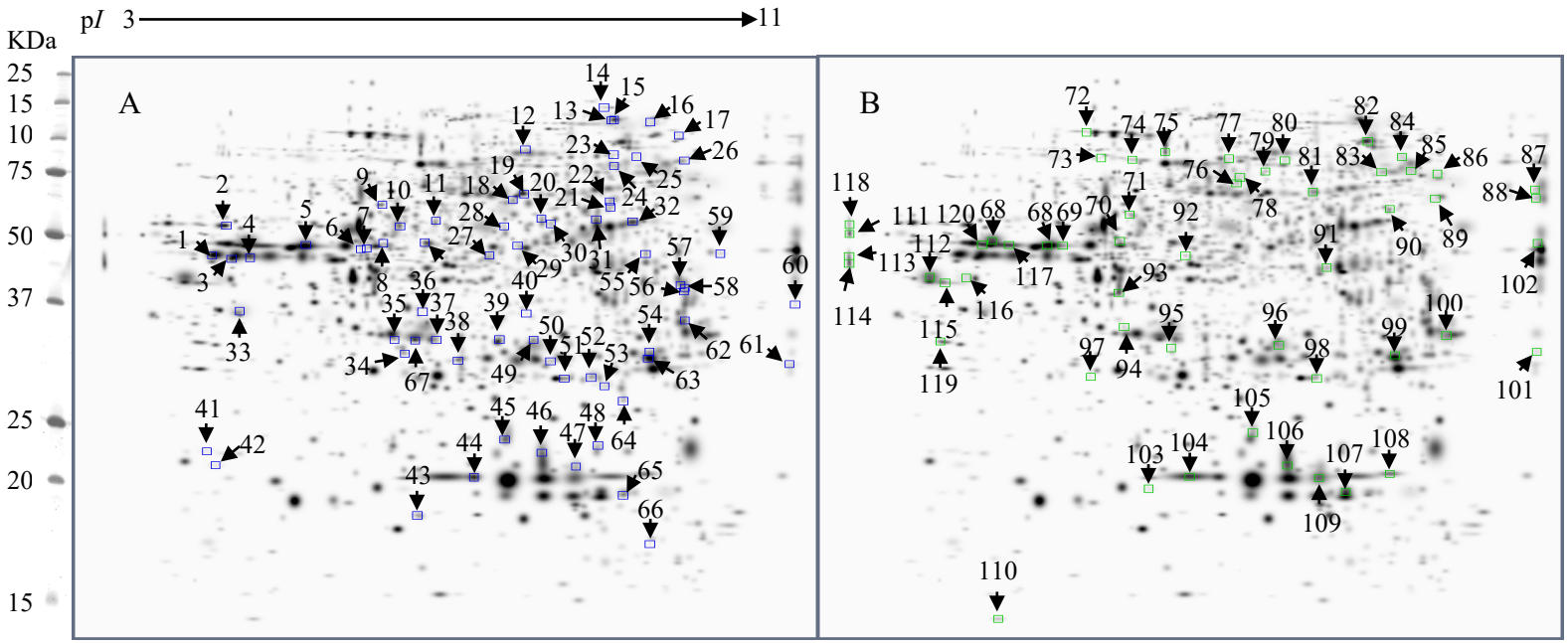

Fig. 2. 2-DE analysis of proteins from apples stored for 6 months under air or CA storage. 100.0 µg protein was loaded onto a 12.5 % polyacylamide large format gel and visualized with SYPRO Ruby stain. The molecular weight of protein standards are indicated on the left. Arrows indicate proteins which are only present in the group and will be identified by LC/MS/MS. A: Only in non-scald tissues; B: Only in scald tissues.

FINAL PROJECT REPORT
WTFRC Project Number: PH-04-0442

Project Title: Hyperspectral Reflectance and Fluorescence for Assessing Apple Maturity

PI:	Renfu Lu	**Co-PI(2):**	Randolph M. Beaudry
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City:	East Lansing	**City:**	East Lansing
State/Province/Zip	MI 48824	**State/Province/Zip:**	MI 48824

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	18,400	18,952	9,760
Benefits	6,624	6,823	3,514
Wages	4,576	4,713	2,428
Benefits			
Equipment			
Supplies	1,500	1,500	750
Travel		800	400
Miscellaneous			
Total	31,100	32,788	16,852

OBJECTIVES

The overall objective of the project is to develop a sensing technique of integrating reflectance (or interactance) and fluorescence for measuring multiple apple quality parameters including skin and flesh color, fruit firmness, soluble solids, starch, and acid. Specific objectives are to:

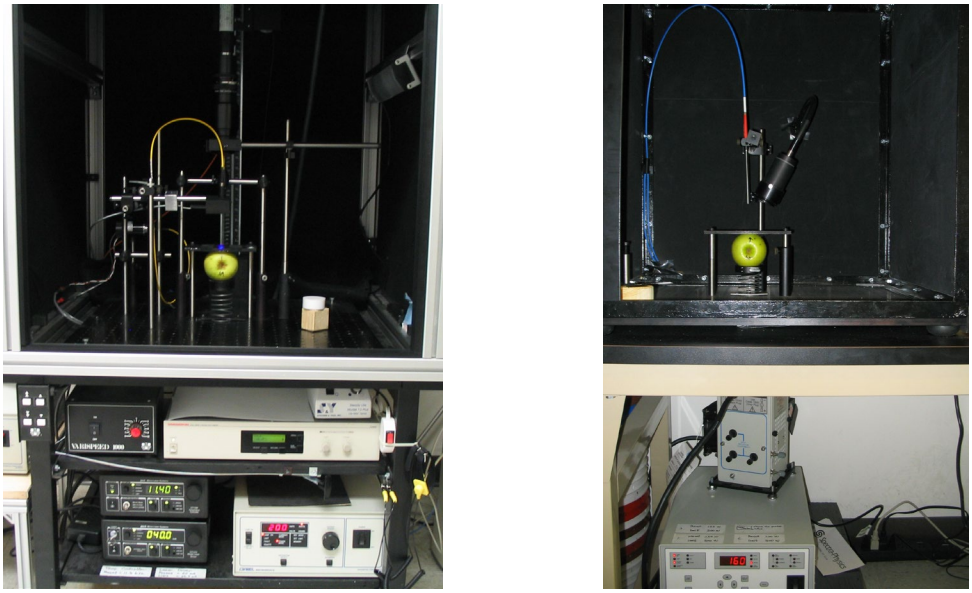
- Investigate the feasibility of using single sensing methods to measure both reflectance (or interactance) and fluorescence from apple fruit;
- Develop mathematical methods and algorithms to integrate reflectance and fluorescence data for predicting fruit maturity parameters;
- Develop a prototype for measuring reflectance and fluorescence to predict fruit maturity parameters including fruit firmness, soluble solids, starch, acid, and skin and flesh color.

SIGNIFICANT FINDINGS

- Reflectance and fluorescence are useful for measuring apple quality parameters including skin and flesh color, fruit firmness, soluble solids, starch, and acid. However, each sensing mode alone may not be sufficient for accurate measurement of all maturity parameters.
- Reflectance (or interactance) is generally better than fluorescence for the measurement of fruit maturity, which is especially evident for firmness, soluble solids and acid measurement.
- Integration of reflectance and fluorescence improves predictions of all maturity parameters; the improvements are more pronounced for firmness, starch, and acid predictions than for soluble solids and skin and flesh color. These improvements are critical since neither reflectance nor fluorescence can provide good predictions of fruit firmness and starch.
- Reflectance or fluorescence alone may be sufficient for providing good predictions of fruit skin and flesh color.
- A compact laboratory prototype was assembled and tested, which is capable to acquire both reflectance (interactance) and fluorescence spectra. The prototype provides excellent measurements of fruit skin and flesh color and soluble solids content. The prototype also gives relatively good prediction of fruit firmness.
- Reflectance and fluorescence techniques are complementary and the integration of the two sensing techniques can provide a better means for measuring multiple quality attributes of apples.

METHODS

In the first year, we assembled and tested a hyperspectral reflectance and laser-induced fluorescence (R/LIF) imaging system (Fig. 1a) and a reflectance and UV-induced fluorescence (R/UV-F) spectroscopic system (Fig. 1b). The R/LIF imaging system consisted of a hyperspectral imaging unit and two separate light sources. A blue laser was used for inducing fluorescence in apples and a broadband light source for acquiring the reflectance scattering images. The R/LIF imaging system was capable of acquiring both reflectance and fluorescence scattering images from apples over the visible and short-wave near-infrared region between 500 and 1000 nm. The R/UV-F spectroscopic



(a) (b)

Figure 1. The hyperspectral reflectance and laser- induced fluorescence (R/LIF) imaging system (a) and the reflectance and UV-induced fluorescence (R/UV-F) spectroscopic system (b).

system also had two sensing modes: reflectance and fluorescence. A xenon lamp was used as an ultra-violet (UV) light source to induce fluorescence and a broadband halogen tungsten light was used for reflectance. A low-cost spectrometer was used in the R/UV-F system for acquiring both reflectance and fluorescence spectra from each fruit.

The two systems were tested on after-storage 'Golden Delicious' and 'Red Delicious' apples in two separate experiments as a first step in developing an integrated reflectance/fluorescence sensing technique for measuring multiple fruit quality parameters. In the first experiment, the R/UV-F spectroscopic system was used to collect diffuse reflectance and fluorescence spectra from 300 'Golden Delicious' and 350 'Red Delicious' apples, which had been kept in controlled atmosphere storage for 4-5 months. In the second experiment, the R/LIF imaging system was used for collecting hyperspectral reflectance and fluorescence scattering images from 400 'Golden Delicious' apples. After reflectance/fluorescence images and spectra had been collected from individual apples, standard destructive methods were used for measuring multiple quality parameters of each fruit, including skin and flesh color, fruit firmness, soluble solids (SS), and titratable acid. Starch pattern index was not measured because these apples had been in CA storage for 4-5 months and were not suitable for starch determination.

After all data had been collected, prediction models for individual quality parameters were first developed from either reflectance or fluorescence data. Prediction models that combine reflectance and fluorescence data were then developed. Single sensing models and the combined models were compared to determine how they measured fruit quality parameters.

Based on the experimental findings from after-storage apples in the first year study, improvements to the R/LIF imaging system and the R/UV-F spectroscopic system were made before they were used to test freshly harvested apples in the 2005 harvest season. These improvements allowed better control of UV or laser illuminations for fluorescence measurements and faster acquisition of reflectance and fluorescence data. Furthermore, a calibration procedure was developed to compensate for the effect of light source fluctuation on fluorescence measurements. For the R/UV-F system, the sensing mode changed from reflectance (light illumination and detection is on the same

area of the sample) to interactance or semi-transmittance (the light detection area is separate from the illumination area). The use of the interactance mode allowed better light penetration into the fruit and thus improved the measurement of flesh properties. The two improved systems were tested on 750 'Golden Delicious' apples within 24 hours after harvest. Apples were harvested once a week and the first harvest started two weeks before the optimal commercial harvest time. Five harvests were made over a period of four weeks in the fall of 2005.

Prediction models using both the artificial neural networks and statistical methods were developed for individual quality parameters using either reflectance (or interactance) or fluorescence data. Prediction models were also developed using the combined reflectance and fluorescence data. A method that is different from the one used in the previous study was used for analyzing the combined data of reflectance and fluorescence. We first applied a mathematical method (called principal component analysis or PCA) to extract essential information (or principal components or PCs) for each set of data. The two sets of PCs from reflectance and fluorescence were input into an artificial neural network. Once the neural network was properly trained, it was then used to predict maturity parameters of other samples. Finally, single sensing models and the combined models were compared to determine their performance in predicting fruit quality parameters.

With the promising results from the first one and half year study of after-storage and freshly harvested apples, our focus in last year was to assemble a laboratory interactance and UV-induced fluorescence prototype. The prototype consisted of two miniature visible/NIR spectrometers and two light sources (one for interactance measurement and the other for fluorescence measurement) (Fig. 2). The use of two spectrometers allows more efficient measurement of interactance and fluorescence since fluorescence signals are often much weaker than the interactance signals. The prototype was able to acquire both interactance and fluorescence within a short time (<250 ms). The spectrometers used were compact (about the size of a wallet) and covered the spectral range between 400 nm and 1100 nm.

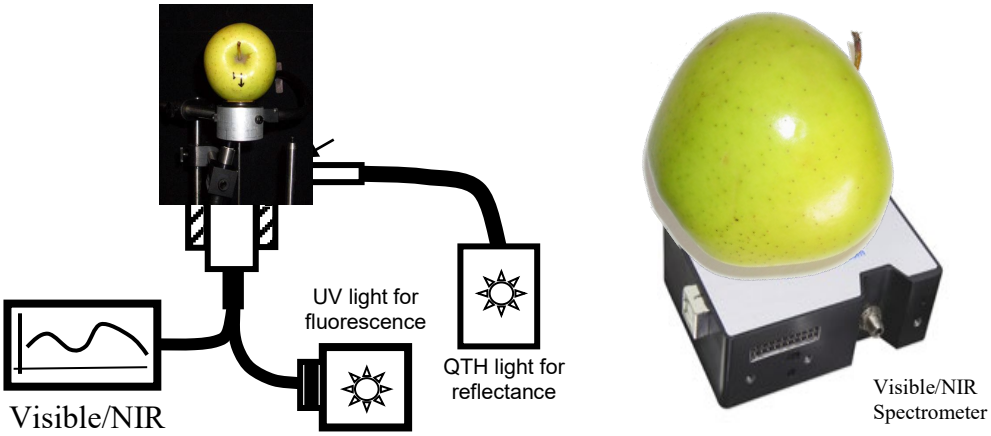


Figure 2. Schematic of a laboratory prototype for measuring interactance and fluorescence spectra from apple.

This prototype was used to collect interactance and fluorescence data from 'Golden Delicious' apples in the 2006 harvest season. Nine hundred 'Golden Delicious' apples were harvested over a period of four weeks. Interactance and fluorescence measurements were performed within 24 hours after harvest. After spectral measurements, standard destructive tests were performed on each fruit to measure individual maturity parameters (skin and flesh color, fruit firmness and soluble solids). The 2006 season was quite unusual; the starch conversion phenomenon was not observed in Golden Delicious apples during the four-week harvest period. As a result, starch measurements were not performed for the test apples.

The approaches for developing prediction models were similar to those used in the previous years. The artificial neural network models were developed relating interactance and fluorescence data to individual maturity parameters. Finally, the combined data of interactance and fluorescence were used in developing the integrated models for predicting apple fruit maturity.

RESULTS AND DISCUSSION

1. The R/LIF and R/UV-F Systems for Measuring After-Storage and Freshly Harvested Apples (Year One/Two)

Table 1 summarizes prediction results for fruit firmness, soluble solids content (SSC), titratable acid, and skin and flesh color (hue and chroma) for after-storage 'Golden Delicious' apples using the R/LIF imaging system for reflectance, fluorescence, and their combined data. Both reflectance and fluorescence correlate with fruit quality parameters. Overall, the reflectance mode is better than fluorescence for predicting apple fruit quality. When reflectance and fluorescence were combined, better predictions of fruit firmness, SSC, and titratable acid were obtained over either reflectance or fluorescence. Skin and flesh color predictions from the combined data are similar to those from the reflectance data. Good to excellent correlations were obtained for all quality parameters except for titratable acid which had low correlation.

Table 1. Prediction results (correlation coefficients) for after-storage 'Golden Delicious' apples obtained with the R/LIF imaging system for three types of data (reflectance, fluorescence, and the combined data) in 2005

Quality Parameter	Reflectance	Fluorescence	Combined Data
Firmness	0.80	0.75	0.86
Soluble Solids	0.72	0.66	0.75
Titratable Acid	0.63	0.57	0.66
Skin Hue	0.97	0.93	0.97
Skin Chroma	0.86	0.78	0.83
Flesh Hue	0.78	0.74	0.76
Flesh Chroma	0.66	0.59	0.70

When the R/UV-F spectroscopic system was used, similar prediction result trends were obtained for after-storage apples of 'Golden Delicious' and 'Red Delicious' (results are not shown here). Again, reflectance is better than fluorescence for predicting individual apple quality parameters, as measured by the coefficient of correlation. When reflectance and fluorescence were combined, prediction results for individual quality parameters in generally improved.

Table 2 summarizes the maturity predictions of freshly harvested 'Golden Delicious' apples obtained with the improved R/LIF imaging system for each sensing mode (i.e., reflectance, fluorescence, and the combined data). For the two single sensing modes, the reflectance mode performed consistently better than the fluorescence mode in predicting all maturity parameters except for flesh hue, in which the two sensing modes had the same results. Predictions for all maturity parameters from the combined data were consistently better than those from either reflectance or fluorescence. The improvements from the combined data over the reflectance data range between 1% and 12% in terms of the correlation coefficient values and up to more than 14% in terms of prediction errors (standard error of prediction). The results for freshly harvested apples in Table 2 also compare favorably to those for after-storage apples (Table 1), although freshly harvested apples are known to

Table 2. Maturity prediction results (correlation coefficients) for freshly harvested 'Golden Delicious' apples obtained with the improved hyperspectral reflectance and laser-induced fluorescence (R/LIF) imaging system for three types of data (reflectance, fluorescence, and the combined data) in 2005

Maturity Parameter	Reflectance	Fluorescence	Combined Data
Firmness	0.74	0.63	0.79
Soluble Solids	0.74	0.72	0.78
Starch Pattern Index	0.84	0.70	0.88
Titratable Acid	0.67	0.50	0.75
Skin Hue	0.95	0.94	0.96
Skin Chroma	0.83	0.73	0.87
Flesh Hue	0.90	0.90	0.93
Flesh Chroma	0.66	0.61	0.71

be more difficult to measure over after-storage apples by near-infrared technology. The relatively good results for freshly harvested apples were mainly attributed to the improvements made to the R/LIF system.

Table 3 shows the predictions of maturity parameters for freshly harvested 'Golden Delicious' apples obtained with the improved R/UV-F spectroscopic system for the interactance, fluorescence, and combined data. The prediction trends for the R/UV-F system are similar to those for the R/LIF (Table 2). Interactance had consistently better predictions for all maturity parameters than did fluorescence with the exception of skin color, for which the two sensing modes had the same results. Again, the integration of interactance and fluorescence yielded considerably better results than either sensing mode. Relatively low correlation for titratable acid was obtained and this was also observed in the previous study. This indicates the difficulty of achieving accurate predictions of

Table 3. Maturity prediction results (correlation coefficients) for freshly harvested 'Golden Delicious' apples obtained with the improved interactance and UV-induced fluorescence (R/UV-F) spectroscopic system for three types of data (interactance, fluorescence, and the combined data) in 2005

Quality Parameter	Interactance	Fluorescence	Combined Data
Firmness	0.75	0.62	0.77
Soluble Solids	0.74	0.67	0.77
Starch Pattern Index	0.82	0.81	0.89
Titratable Acid	0.60	0.53	0.62
Skin Hue	0.97	0.97	0.99
Skin Chroma	0.74	0.74	0.77
Flesh Hue	0.96	0.87	0.96
Flesh Chroma	0.79	0.47	0.77

titratable acid with either sensing mode or the combined data. Relatively poor correlation for titratable acid could also be attributed, in part, to the titration method used, which is prone to experimental error and thus could adversely affect the prediction accuracy by the nondestructive techniques.

Our study and other studies have shown that near-infrared spectroscopy generally has relatively poor predictions of apple fruit firmness and SSC for freshly harvested apples than for apples after storage. This is mainly because freshly harvested apples continue to have high physiological activities immediately after harvest, thus making it more difficult to accurately measure their quality parameters. In addition, the relatively narrow range of firmness and SSC readings for the test apples also contributed to lower correlations. Both reflectance (or interactance) and fluorescence modes had excellent predictions of skin and flesh hue with values for the correlation coefficient of 0.96 and 0.97, respectively, but had lower predictions of skin and flesh chroma (R=0.77 in both). Interactance and fluorescence had similar results for predicting the starch pattern index with values for the correlation coefficient of 0.82 and 0.81, respectively (Table 3). The integrated model had a much better correlation (R=0.89) for starch pattern index prediction.

The results for freshly harvested apples (Tables 2 and 3) are comparable to those for after-storage apples (Table 1). Apparently, improvements to the R/LIF and R/UV-F systems resulted in better maturity predictions for freshly harvested apples.

2. The Laboratory Prototype for Freshly Harvested Apples (Year Three)

The relatively low correlations for SSC and skin and flesh color obtained in the previous were also in part attributed to the narrower spectral range of the spectrometers used. In order to further improve SSC and skin/flesh color predictions, we used two different spectrometers for the prototype, which covered the entire visible range and a greater portion of the near-infrared region (400-1,100 nm). As a result, the performance of the prototype for predicting SSC and fruit skin/flesh color improved. The maturity predictions of 'Golden Delicious' apples tested within 24 hours after harvest with the laboratory prototype are shown in Table 4. Results for starch index and titratable acid are not included in the table. The starch conversion was only observed in a few test apples (which was rather unusual and we still do not know why after consulting with a

horticulturist) and thus no starch prediction was performed in the study. Titratable acid measurements have been not been completed yet.

Table 4. Maturity predictions (correlation coefficients) for freshly harvested 'Golden Delicious' apples obtained with the interactance and UV-induced fluorescence prototype from three types of data (interactance, fluorescence, and the combined data) in 2006

Maturity Parameter	Interactance	Fluorescence	Combined Data
Firmness	0.69	0.68	0.74
Soluble Solids	0.89	0.79	0.89
Skin Hue	0.95	0.93	0.97
Skin Chroma	0.80	0.73	0.84
Flesh Hue	0.95	0.90	0.96
Flesh Chroma	0.84	0.87	0.88

The prediction trends for individual maturity parameters obtained with the prototype (Table 4) are again similar to those obtained with the two systems shown in Fig. 1. The prototype had much better predictions of soluble solids and skin/flesh color than the R/LIF and R/UV-F systems (Tables 1-3). The improved results were primarily due to the use of the new miniature visible/NIR spectrometer which had a broader spectral range (400-1100 nm) versus the one (~530-1000 nm) used in the previous systems. The combined data gave better predictions of firmness, skin and flesh hue and chroma, whereas the correlation coefficient for soluble solids from the combined data is the same as that from the interactance data. Firmness predictions from the three data types (interactance, fluorescence, and the combined data) are not as high as those from the previous studies (Tables 1-3). This is mainly because the apple samples from the 2006 harvest season had a narrow range of firmness distributions, thus causing lower correlation.

This research demonstrated that integration of reflectance (or interactance) and fluorescence can lead to improved predictions of fruit maturity parameters, especially for firmness, starch and acid. The integrated technique can provide more consistent and accurate measurement of fruit maturity. The prototype was designed and assembled using two miniature spectrometers, which could facilitate further development of a portable device for field applications. Further improvements in the prototype are needed, especially in selection of appropriate light sources in order to make the prototype truly portable. We will continue our research towards the goal of developing a low cost, portable device for measuring the maturity of fruit on the tree and after harvest.

PUBLICATIONS

Noh, H. K. and R. Lu. 2005. UV/blue light-induced fluorescence for assessing apple maturity. Proceedings of SPIE 5996-19. Bellingham, WA: SPIE.

Noh, H. K. and R. Lu. 2005. Hyperspectral reflectance and fluorescence for assessing apple quality. ASAE Paper No. 053069. St. Joseph, MI: ASABE.

Noh, H. K. and R. Lu. 2006. Hyperspectral laser-induced fluorescence imaging for assessing apple quality. Postharvest Biology and Technology. (In print)

Noh, H. K., Y. Peng, and R. Lu. 2006. Integration of hyperspectral reflectance and laser-induced fluorescence imaging for assessing apple maturity. ASABE Paper No. 066182. St. Joseph, MI: ASABE.

Noh, H. K. and R. Lu. 2006. Integrating fluorescence and interactance measurements to improve apple maturity assessment. Proceedings of SPIE 6381-23. Bellingham, WA: SPIE.

Noh, H. K., Y. Peng, and R. Lu. 2006. Integrating hyperspectral reflectance and fluorescence imaging for assessing apple maturity. Transactions of the ASABE (in review).

FINAL PROJECT REPORT
WTFRC Project Number: AH-05-511

Project Title: Consulting for the Washington Apple Breeding Project
PI: Fredrick A. Bliss
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Address: 214 Inca Pl.
City: Davis
State/Province/Zip CA 95616

Cooperators: Bruce Barritt, Yanmin Zhu, Jim McFerson.

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel	$ 5,280.		
Miscellaneous	$ 7,680.		
Total	$12,960.		

Significant Activities and Findings:

Presented ideas and plans for integrating molecular marker opportunities into the apple breeding program.

- Developed a decision tree showing where molecular markers can be used effectively in an apple breeding program.
- Led assembly of a comprehensive list of apple traits for setting genetic and breeding priorities.
- Developed the document - Apple Trait Decision Tree for Implementing Marker Assisted Selection to guide decisions about potential for marker-assisted selection in apple.
- Coordinated monthly teleconferences at which important traits were discussed to determine feasibility of MAS. Traits discussed were: fruit acidity, fruit firmness, fruit crispness, fruit juiciness, fruit sweetness, mildew reaction, tree juvenility, lenticel breakdown, and skin overcolor.

Conducted literature reviews and prepared reports for the consulting work.

- Reviewed literature related to apple breeding and genetics for use by Bruce Barritt, Yanmin Zhu and Jim McFerson.

Traveled to meeting in Washington to evaluated project and participate in program activities.

- January 18 – 20, 2006. Traveled to Pasco, WA to participate in the Apple Research Meeting. Met with Bruce Barritt, Jim McFerson and industry members from Washington to discuss activities and progress in the apple breeding program. Reviewed research proposals and reports to become more familiar with activities related to apple improvement.

Submitted invoices for expenditures on a quarterly basis.

- **Quarter one (Oct.1, 2005 – Dec. 31, 2005): $ 720.00**
- **Quarter two ((Jan. 1, 2006 –Mar. 31, 2006): $3,096.70**
- **Quarter three (Apr. 1, 2006 – June 30, 2006): $ 440.00**
- **Quarter four (July 1, 2006 – Sept.30, 2006): $ 240.00**
  - **Total $4,496.70**

Results and Discussion:

A priority recommendation from the review of the WTFRC Apple Breeding Program conducted Oct. 10-12, 2004 was, “Incorporation of molecular tools – Tools for practical DNA-marker aided selection are nearly ready for routine incorporation in apple breeding programs. They may be useful for selection of certain traits. … It will be important to identify a researcher with the interest and capability to add this dimension to the program. This capability could be developed at WSU or contracted at another institution or the private sector” (From the Committee Report to the Board).

During the course of the year I initiated a series of monthly discussions among Bruce Barritt, Jim McFerson, Yanmin Zhu and myself to identify apple traits most amenable to enhanced improvement using MAS. I developed the document - Apple Trait Decision Tree for Implementing Marker Assisted Selection to guide decisions about potential for marker-assisted selection in apple. This decision tree includes scientific and economic factors that are evaluated for each trait in order to determine feasibility and commercial potential for improving these traits leading to new cultivars and whether MAS can be employed cost effectively to improve efficiency

The traits discussed were: fruit acidity, fruit firmness, fruit crispness, fruit juiciness, fruit sweetness, mildew reaction, tree juvenility, lenticel breakdown, and skin overcolor. This discussion and ranking provided guidance for Bruce and Yanmin to begin developing screens for priority traits. Also, the critique and ranking of the traits provide a basis of the newly hired scientists, Amit Dhingra and Cameron Peace to develop research proposals relevant to the needs of the apple industry.

Addition of these new scientists and their enthusiasm to work with apple improvement addresses the priority recommendation of the Committee Report of 2004.

I conducted literature reviews that identified new research findings and resources relevant to the breeding program. These were provided to the collaborators during the monthly conference calls and via email correspondence.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3
WTFRC Project Number: AH-04-421

Project Title: Trait modification through genetically engineered rootstocks

PI: Jay Norelli
Organization: USDA, ARS
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Address:
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Cooperators: Gennaro Fazio, USDA, ARS / Cornell University, Geneva, NY
LaiLiang Cheng, Cornell University, Ithaca, NY

Budget 1:
Organization Name: USDA, ARS **Contract Administrator:** Ingrid Charlton
Telephone: (215) 233-6402 **Email address:** ingrid.charlton@ars.usda.gov

Item	**Year 1: 2004**	**Year 2:** 2005	**Year 3:** 2006
Salaries	29,130	0	5,826
Benefits	8,739	0	1,748
Wages	0	6,163	9,610
Benefits	0	471	735
Equipment	0	0	0
Supplies	4,800	3,366	3,600
Travel	0	0	1,200
Miscellaneous	0	0	0
Total	**$42,669**	**$10,000**	**$22,719**

Objectives:

The goal of this project is to develop technology to modify traits in conventional scion varieties through genetically engineered rootstocks. This will mitigate many of the hurdles facing the use of genetically engineered apples in the orchard, including:

- Eliminate the risk of transgenic pollen spread since pollen would not be produced by the transgenic rootstock;
- Improved consumer acceptance since fruit will not be "GMO";
- Facilitate commercialization since a single genetically engineered rootstock could be used to enhance the value of many different commercial fruiting varieties.

This project does not aim to develop a specific rootstock to alter a specific trait. Rather, the project aims to develop technology that will allow any scion trait to be altered through a transgenic rootstock. The use of a genetically engineered rootstock will not automatically result in trait modification of the scion; specific biological mechanisms will need to be employed in the rootstock to make the trait modification graft-transmissible.

Objective: Determine if rootstocks genetically engineered to silence specific genes can be used to modify traits in apple scions. Gene silencing in genetically engineered plants has been demonstrated to be an effective method to develop plants with improved disease resistance, fruit quality, tree architecture and several other agronomic traits. In tobacco, gene silencing has been shown to be graft-transmissible from genetically engineered rootstock to conventional scions. Graft-transmissible gene silencing has not been adequately investigated in fruit trees to predict how it will function in apple. However, it has a high likelihood of being a useful mechanism to facilitate trait modification in apple scions by genetically engineered rootstocks.

Deviations from the original schedule: In 2006, most of the plants "micro-grafted" for laboratory and greenhouse testing were destroyed during dormancy storage by voles accidentally introduced into the cold-storage facility with field grown liners. Unfortunately, this prevented completion of the project on schedule. Therefore, I am requesting that the project be extended for one year without additional funding. The funds received last year to evaluate the plant material will be used to complete the project. The plant material is currently being propagated and grafted.

The goals for the next year (2007) are to:

- Produce micro-grafted plants for growth chamber and greenhouse tests.
- Evaluate trait modification through genetically engineered rootstocks in greenhouse and growth chamber tests.

Methods:

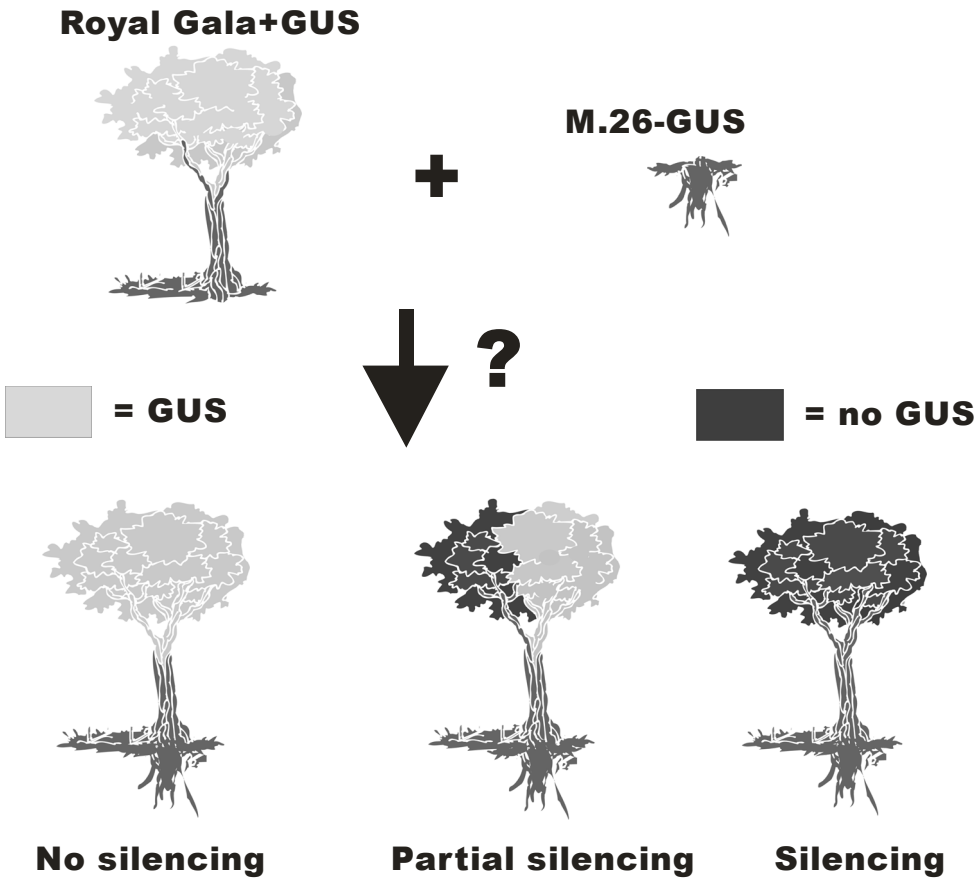
Experiments with GUS marker:

Purpose: Determine if genetically engineered rootstock can modify scion traits by gene silencing.
Description:

- 'Royal Gala' that was genetically engineered to produce GUS ('Royal Gala+GUS') will be grafted to M.26 rootstock engineered to silence GUS (M.26-GUS).
- By determining the amount of GUS expression in 'Royal Gala+GUS' trees grafted onto M.26-GUS rootstock we will determine if the engineered rootstock is capable of silencing GUS in the scion and if there is uniform silencing throughout the entire tree and over time (Figure 1).

- The advantage of using the GUS marker for these experiments is that it is relatively easy to monitor and quantify, allowing sampling throughout the tree and over time to determine uniformity of silencing in the scion.

Figure 1:



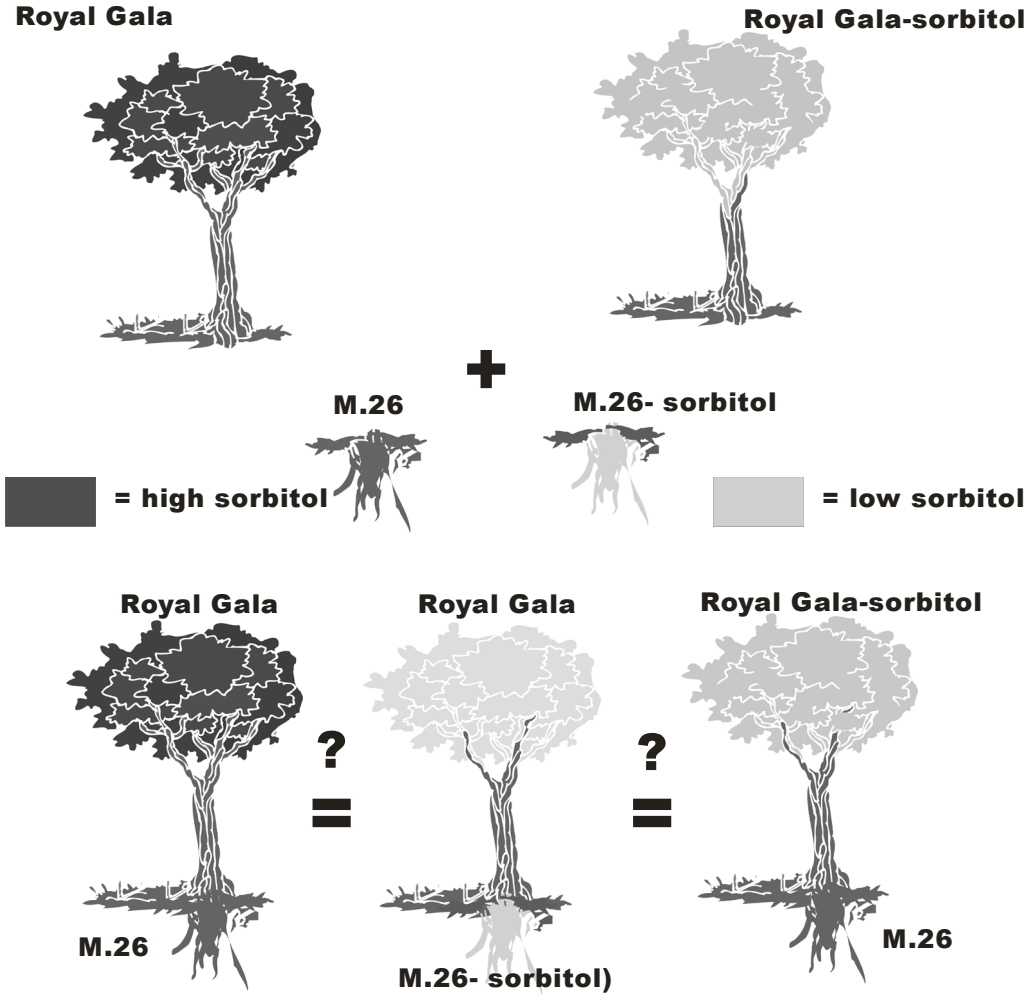
Experiments with sorbitol production:

Purpose: Determine if a genetically engineered rootstock will be as effective as a genetically engineered scion in altering a trait.

Description:

- 'Royal Gala' will be grafted to M.26 rootstock engineered to silence sorbitol production (M.26-sorbitol). In addition, 'Royal Gala' and 'Royal Gala' silenced for sorbitol production ('Royal Gala-sorbitol') will be grafted to M.26 rootstock.
- By comparing the level of sorbitol in 'Royal Gala-sorbitol' trees with the sorbitol level of 'Royal Gala' trees grafted to M.26 and M.26-sorbitol rootstocks we will determine if the silenced rootstock is as effective in reducing sorbitol production as the silenced scion.
- In addition, we will confirm that an engineered rootstock can silence a native apple gene.

Figure 2:



Results and discussion:

2004:

- made the vectors necessary to genetically engineer silencing of GUS and sorbitol in apple
- initiated work to genetically engineer both 'Royal Gala' and M.26 rootstock

2005:

- successful engineered M.26 rootstock to silence GUS (12 lines at present) and sorbitol production (5 lines), and 'Royal Gala' to produce GUS (4 lines) and silence sorbitol (1 line)
- propagated 131 genetically engineered plants for grafting
- initiated grafting of 'Royal Gala+GUS' to M.26-GUS rootstocks

2006:

- completed work to genetically engineer 'Royal Gala' and M.26 to silence GUS and sorbitol production
- due to vole damage of plant material grafted for laboratory and greenhouse experiments, project was not completed on schedule

Plans for 2007:

- continue to characterize plants genetically engineered to silence sorbitol production (are they silenced?)
- propagate transgenic plants and make appropriate grafts to conduct experiments outlined above
- conduct greenhouse trials to determine if genetically engineered rootstock can modify scion traits using GUS marker gene as outlined above
- conduct greenhouse trials to determine if a genetically engineered rootstock will be as effective as a genetically engineered scion for scion trait modification as outlined above

The goals of this project are to:

- conduct preliminary experiments to determine if rootstocks genetically engineered to silence traits in a scion show sufficient promise to justify the cost of orchard trials
- produce and select the genetically engineered plants best suited for an orchard trial

If experiments conducted in the greenhouse with micro-grafted plants show sufficient promise for this technology, orchard trials will be conducted using trees propagated by standard commercial practices. Stoolbeds will be established with the rootstocks most suitable for the orchard trials, field grown liners will be budded with the appropriate scions and test orchards planted. Using trees propagated by such means are more likely to provide a realistic evaluation of the technology in commercial orchards. However, due to the time and cost of performing orchard trials with trees propagated by standard commercial practices, these initial greenhouse experiments are required to determine if the orchard trials are justified.

<u>Why are these experiments being done with useless marker genes rather than traits of commercial interest?</u>

Although gene silencing has been used to produce apple varieties with improved horticultural traits, these traits are usually difficult to monitor. For example, suppose we silenced a gene to increase fire blight resistance. The amount of fire blight that will occur on individual trees is very variable. Therefore, it would be difficult to determine if the difference we see between the amount of fire blight on two trees is due to a difference in gene silencing or normal fire blight variation. In addition, the

tree is susceptible to fire blight primarily in spring and early summer. In contrast, the GUS marker being used in these experiments is easy to quantify and is present in all tissues at all times of the year, allowing us to accurately measure the amount of silencing, and determine the uniformity of silencing throughout the tree and over time. Sorbitol production has been successfully silenced in apple; therefore it was selected as a native gene to target.

BUDGET SUMMARY

Project Title: Trait modification through genetically engineered rootstocks
PI: Jay Norelli
Proposed project duration: 2004-2007
Current year: 2007 (year 4)
Project total (4 years): $75,388
Current year request: $0

Deviations in budget requests from the original proposal:

Year 1 (2004): No deviations.

Year 2 (2005):

- Original funding level was reduced by WTFRC from $42,731 to $10,000 resulting in termination of GS6 technician.
- Although I requested all $10,000 of the revised budget to be listed as 'Supplies', a part-time student was hired for part of the year to assist in tissue culture maintenance and propagation of transgenic plants; $6,634 was used for wages and benefits.

Year 3 (2006)

- Total request reduced from $43,994 in original proposal to $22,719.
- Salaries, wages and benefits reduced from $31,072 (technician, GS6-3) in original proposal to $17,919 to support 20% of a technician and a part-time student.
- Travel funds requested for 4 trips to Geneva, NY (3 days per visit at $100 per day).

Year 4 (2007)

- No funds requested.
- Funds received in 2006 will be used to evaluate the plant material and complete the project.

Other funding sources: There is no other current or pending external funding to support this project. However, the project was previously supported by "in-house" ARS CRIS project 1931-21220-014-00D "Management of Abiotic Stress in Fruit Crops" and is currently supported by ARS CRIS project 1931-21000-016-00D "Using Functional and Applied Genomics to Improve Stress and Disease Resistance in Fruit Trees".

CONTINUING PROJECT REPORT **YEAR**: 2 of 2
WTFRC Project Number: AP-06-601

Project Title: Flower bud development in apple

PI: Peter Hirst
Organization: Purdue University
Telephone/email: 765-494-1323/hirst@purdue.edu
Address: 625 Agricultural Mall Drive
Purdue University
West Lafayette, IN 47907-2010

Cooperators: Felipe Castillo, WTFRC

Budget 1:
Organization Name: Purdue University **Contract Administrator:** Rich Binder
Telephone: 765-494-1058 **Email address:** rbinder@purdue.edu

Item	Year 1:	Year 2:	Year 3:
Salaries	3380	3481	
Benefits	1159	1194	
Wages	4893	4893	
Benefits	495	495	
Equipment			
Supplies	200	200	
Travel	700	700	
Miscellaneous			
Total	10827	10963	

Footnotes: Travel to Washington to set up the field study and establish the treatments in the spring.

Budget for assistance requested from WTRFC

Item	Year 2
Labor	1300
Shipping costs	300
Supplies	200
Mileage	80
Total	1880

Objectives:

The original objective of this 2-year project was to further understand the root causes of biennial bearing by tracking the development of potential flower buds from early in the season through to dormancy. Bourse buds were selected with different histories:

- vegetative
- flowering but not fruiting
- fruiting

We described the transition of buds from a vegetative to a floral state. As well as determining how the above fruiting patterns affect flower formation, we also measured the degree of floral differentiation in these buds. More highly differentiated buds might be expected to give rise to more developed flowers the following spring. Fruit growth on these spurs will be monitored in the second year of this study.

We encountered a slight problem with bud collection this last year, in that the buds collected during the first few sample dates were not suitable, but this was corrected in the later sample dates. Although this resulted in data being collected from fewer sample dates, we still managed to track the pattern of floral development well. In addition, the Gala trees used in this study flowered profusely, such that no representative vegetative spurs could be selected and sampled.

In the second year of this study we intend to collect and analyze another set of buds from the same bud history categories (see above). Data from the past year will enable us to fine tune our schedule of sample collection in the second year of this study. We also intend to measure the pattern of fruit growth from flowers arising from these buds.

Significant findings:

- flower formation in Fuji starts earlier in the season in Fuji than it does in Gala.
- Gala buds have the capacity to become floral over the entire season. The ability of Fuji buds to do this is questionable.
- While fruiting did not affect the proportion of Fuji buds forming flowers for the following season, it did appear to compromise the complexity of those buds, and perhaps also fruit growth the following year.

Methods:

On 10 mature trees each of Gala (regular bearing, small fruit size) and Fuji (biennial, larger fruit size), 20 buds from each of the following categories will be selected at flowering.

- vegetative
- flowering but not fruiting (flowers removed at full bloom)
- fruiting (thinned to the king flower at full bloom)

These buds will be selected and tagged at full bloom and sampled on 10 sampling dates throughout the season, from 60 DAFB until leaf fall. On each sampling date, 2 buds per tree will be sampled and stored in a preservative solution until later dissection.

Buds will be dissected and the number of bud scales, transition leaves, true leaves and bracts will be counted. The degree of floral differentiation will be measured using a rating scale we have previously developed (Hirst and Cashmore 1997). In addition we will measure the size of the king flower, both within the bud at the time of leaf fall, and at flowering time the following spring. Fruit size on buds of each of these classes will be measured in year 2 of the study (all spurs thinned to king flower only).

From these data, we aim to determine:

- the degree to which the presence of a flower or a fruit on a spur inhibits floral bud formation for the following year's crop.
- the degree to which the presence of a flower or a fruit on a spur affects the complexity of flower buds
- the relationship between the degree of flower bud development and fruit size potential the following year.

Therefore on these 2 cultivars differing in their tendency for biennial bearing, we will examine not only the number of flower buds that develop, but the quality of those flower buds.

Due to different environmental conditions each year, and inherent year-to-year variation, I propose to repeat this study in year 2 of the project (2007), which will also incorporate fruit size monitoring on spurs of each of the classes.

Results and Discussion

As buds develop, they form (in order from the outside) bud scales, transition leaves (appearing as a cross between bud scales and true leaves), true leaves, then bracts. The true leaves represent the very small leaves that will first emerge from the buds the following spring. If the meristem of the bud appears flattened, this indicates that there is no visual sign that the bud has formed a flower therefore such buds are classified as vegetative. In floral buds however, the meristen becomes domed, then forms first the king flower then lateral flowers.

As buds develop over the course of the season, the number of appendages increases, as can be seen in Fig.1. In Gala there was no difference in the development of appendages between those buds bearing a fruit and those that were defruited at full bloom. In Fuji however, there was a slight tendency for vegetative (non-flowering) buds to have more appendages than flowering buds, which in turn had more than fruiting buds. It should be pointed out that the differences were small. The decrease in the number of appendages in vegetative Fuji buds towards the end of the season is a reflection of a slightly lower proportion of floral buds in that sample.

Because temperature plays such a central role in tree development, we looked at the course of growing degree day (GDD) accumulation over the season. During the course of our sampling, the accumulation of GDD was essentially linear. The linear nature of GDD accumulation was borne out by comparing the increase in appendage number plotted against days after full bloom with the same data plotted against accumulated GDD. In the interests of space these figures are not presented here but the curves were almost identical.

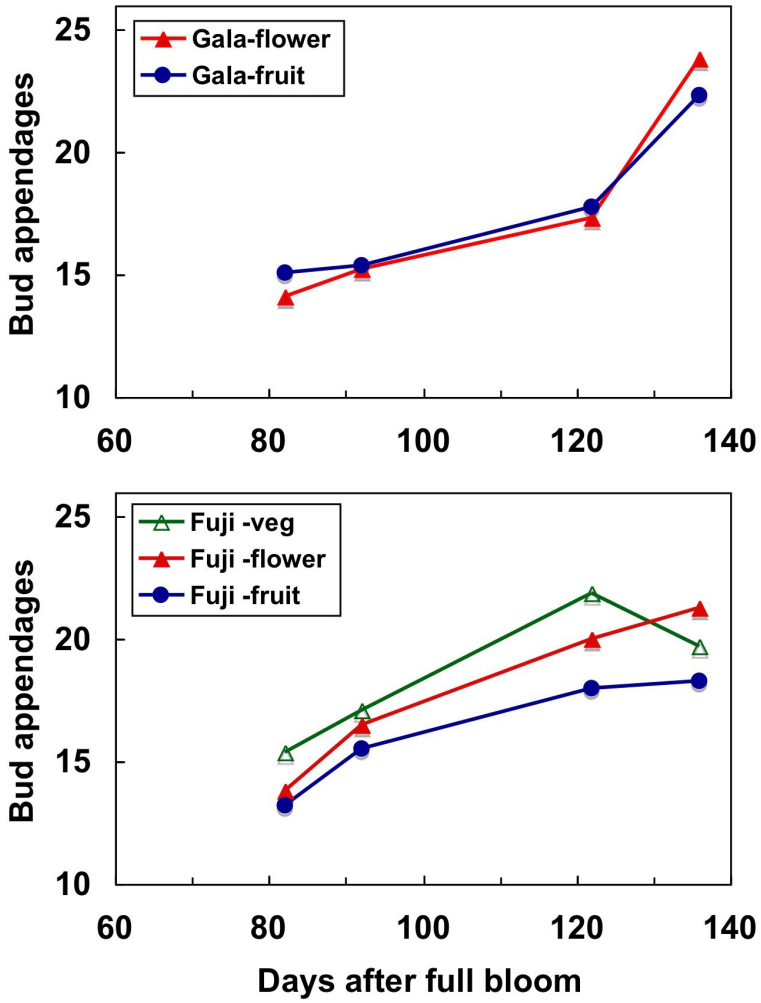


Fig. 1. Total number of appendages (bud scales + transition leaves + true leaves + bracts) in buds of Gala and Fuji. Buds were either vegetative (Fuji only) (non flowering), flowering (carried a flower that was manually removed at full bloom) or carried fruit (thinned to a single fruit at full bloom). Full bloom was April 30, 2006.

The first visible signs of flower formation in Gala buds appeared just after 90 DAFB (Fig. 2). This coincides with the timing of floral differentiation in Red Delicious buds in Ohio (Hirst and Ferree, 1995) and Gala buds in New Zealand (Hirst and Cashmore, 1997). Buds of Fuji however showed a different pattern to Gala. Fuji buds formed flowers earlier, and there appeared to be a window of opportunity early in the season for flower formation. With buds that flowered (flower and fruit treatments) there may be a second flush of flower formation, but it is hard to determine this from our limited data. From our earlier work with Red Delicious in Ohio, flowers formed during the period 90-120 days after full bloom, and after this period essentially no more flowers formed. This was consistent over 3 growing seasons. Washington may be more like New Zealand in terms of having a long growing season, and certainly Gala in Washington showed similarities to NZ in that it was able to form flowers over an extending period. With more intensive sampling in the upcoming season, we should to be able to determine whether Fuji forms flowers early with only limited increases thereafter, if they truly exhibit 2 flushes, or whether they are able to form flowers throughout the season.

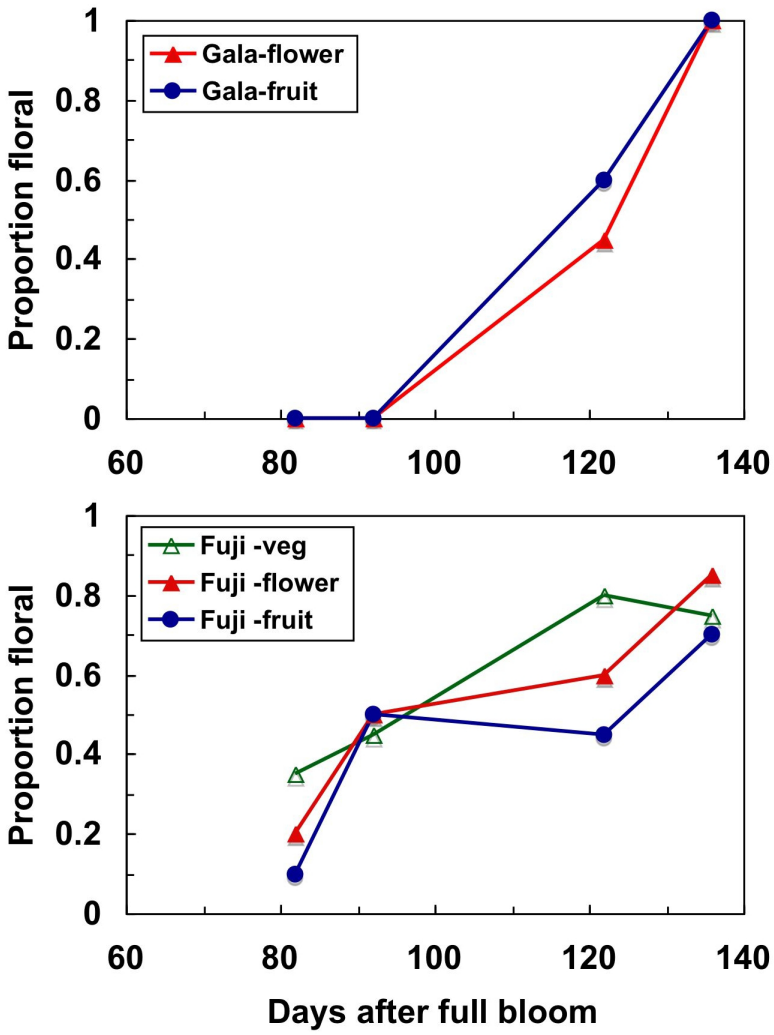


Fig. 2. The proportion of buds in which floral formation was visible.

With both Gala and Fuji, there appeared to be little difference in terms of floral formation whether a bud flowered and had the flower removed, or whether a fruit was carried. Unfortunately vegetative buds were only present on Fuji trees, and the data were too variable to allow meaningful comparison of vegetative with flowering buds.

The level of complexity a bud attains before flowers are formed can be determined by linear discriminant analysis and is called the critical appendage number. Basically what this analysis does is to determine the threshold level in terms of number of bud appendages that must be reached before a flower is formed. This analysis predicts the critical appendage number, then gives a measure of what proportion of all buds would have had their floral status predicted correctly using this model.

The models used to predict the floral status of buds performed well, in all cases classifying over 80% of buds correctly (Table 1). With Gala, carrying a fruit compared with flowering then being defruited had little effect on the flowering threshold. With Fuji however, the results showed marked differences based on the history of buds. Vegetative Fuji buds were the most complex when the switch to a floral status was made. Carrying a fruit however, had a large effect in that buds formed flowers at a lower level of complexity. This may compromise such flowers the following season in terms of fruit

growth. Fruit growth monitoring to be carried out in the second year of this study should help answer this question.

Table 1. The critical appendage number of proportion of buds correctly classified as vegetative or floral using linear discriminant models.

Cultivar/bud type	Critical app. No.	% correct
Gala		
Flowering	18.5	88.8
Fruiting	18.2	87.5
All Gala	18.3	88.1
Fuji		
Vegetative	18.0	81.3
Flowering	17.7	81.3
Fruiting	16.6	88.8
All Fuji	17.4	83.3

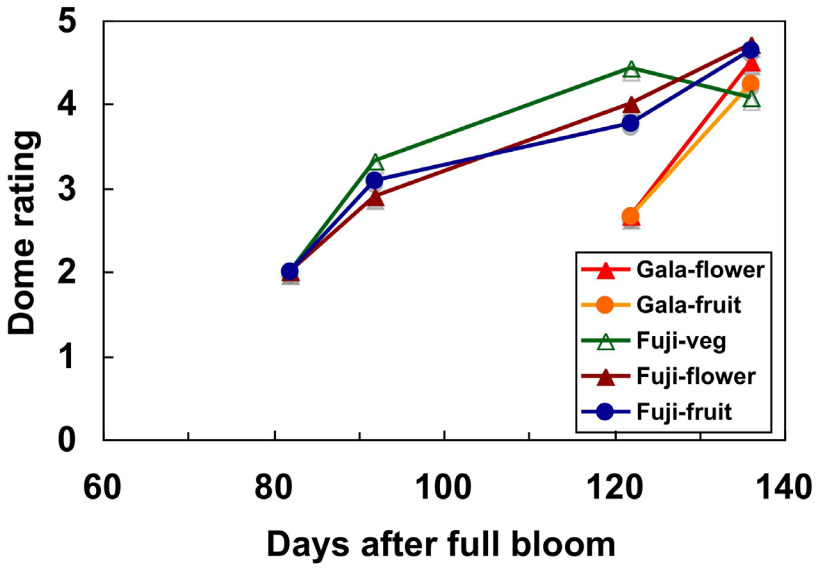


Fig. 3. The degree of differentiation of floral buds of Gala and Fuji. Only floral buds were considered for this analysis.

Growers are obviously concerned not only with the number or proportion of buds that flower, but with the "quality" of those flowers. We measured the degree of floral differentiation according to a scale we developed in our previous work:
1. Apical meristem flat (vegetative)
2. Apical meristem domed
3. Bracts differentiating on the king flower
4. Sepals differentiating on the king flower and bracts forming on lateral flowers
5. Sepals differentiating on the lateral flowers

As also seen in Fig. 2, Fuji formed flowers earlier than Gala (Fig. 3). Although Gala flowers started differentiating later, they developed rapidly and well highly differentiated by the end of the season. Fuji buds on the other hand, showed a much more gradual development. There was no difference in

the course of development as a result of bearing fruit compared with flowering only. Once again, we did not have sufficient data to make meaningful conclusions regarding vegetative buds, although there was a slight indication they may develop more quickly.

As can be seen from much of the above data, flowering can be quite variable. In year 2 of this study we will intensify the sampling regime in an attempt to iron out some of the variation observed here.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-06-605B

Project Title: Testing of a sticker for ethylene release from apples

PI:	Riley	**Co-PI(2):**	Klein
Organization:	U of AZ	**Organization:**	RediRipe™
Telephone/email:	520-626-9120	**Telephone/email:**	RKlein@rediripe.com
Address:	1177 E. 4th St	**Address:**	
Address 2:	Shantz 403	**Address 2:**	
City:	Tucson	**City:**	Albuquerque
State/Province/Zip	AZ 85721	**State/Province/Zip:**	NM

Cooperators: Dr. James Mattheis, USDA

Total Budget Request: $23,905
Budget 1:
Organization Name: Univ of Arizona **Contract Administrator:** Brenda Lee
Telephone: 520-621-9208 **Email address:** bvlee@email.arizona.edu

Item	Year 1: 2006	Year 2: 2007	Year 3:
Salaries	11283	11283	
Benefits	372	372	
Wages			
Benefits			
Equipment			
Supplies	9000	6500	
Travel	1000	1000	
Miscellaneous			
Total	23905	19155	

Footnotes:

Budget 2: (*Complete only if funding is split between organizations***)**
Organization Name: RediRipe©, LLC **Contract Administrator:** Robert Klein
Telephone: 505-247-4665 **Email address:** RKlein@rediripe.com

Item	Year 1	Year 2 (fill in year here – optional)	Year 3 (fill in year here - optional)
Salaries	2250	4750	
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Total	2250	4750	

Objectives: The goal of this research program is to develop a simple and inexpensive device to provide a noninvasive means to determine ethylene release from apples. This objective will be met through design of a sticker based device that presents a gradual color change indicative of the amount of ethylene released by an individual apple. Specifically, the proposed device is a flat, inexpensive, thin permeable membrane sandwich in the form of a patch or "sticker" that self-adheres to the surface of the apple. The sticker detects the emissions of ethylene from an individual apple (rather than the atmosphere around many apples) and consequently displays a color change indicating ripeness on the external surface of the detector. Ethylene is a demonstrated fruit ripeness indicator and its release correlates with ripening. The stated objective would be met by addressing the following aims.

Specific aim 1: Develop a stable and reproducible device using ethylene sensitive reagents integrated into membranes.

Specific aim 2: Tune the responsiveness of the device for fast (hours) to slow (days to weeks) response to ethylene.

Specific aim 3: Test the devices in the laboratory, orchard, and packinghouse.

Timeline:

Year 1: 1/06 – 12/06

Improve stability of device based on results of small field trials from Fall 2005.
Begin development of slow responding device by modifying reagents and supports.
Field trials in the orchard and packinghouse

Year 2: 1/07 – 12/07

Improve stability of device based on results of small field trials from Fall 2006.
Improve time of response of devices.
Moderate scale field trials in the orchard and packinghouse

Significant findings: Over the past year, we have made substantial progress in the development of this device. To summarize accomplishments of 2006:

1) Improved stability of the membrane device
    a. Addressed the non-specific color change which was due to sunlight and elevated humidity during construction of the device.
        i. Sunlight is kept away from the device by using a small cover
        ii. Elevated humidity during manufacture is addressed by drying devices with a heat gun, curing them in a dessicator with humidity set by incorporating water with hygroscopic salts
        iii. Shipping and storage related color changes diminished by cold storage.
        iv. Identified difficulties with adhesives.
2) Begin development of a slow responding device.
    a. Through improved stability of the reagents and membrane, stickers can maintain color for weeks to months.
    b. We have begun to modify reagents to make multiple devices with high, moderate, and low sensitivity.
    c. We have begun to make an easier to read formulation in which patterns appear based on utilizing multiple sensitivity spots.
3) We have run three separate sets of trials
    a. Laboratory studies on multiple cultivars of apples
    b. Orchard studies on multiple cultivars of apples (Mattheis)
    c. Packinghouse trials on pears which had been stored for 4 months (Mattheis)

These trials are summarized below.

Plans for the 2007 activities:
Our goals for 2007 activities are to continue the progress made in the previous year in improving device stability and sensitivity, to develop a prototype with separate components with variable response to ethylene, to run additional trials in the orchard and packing house, and to explore opportunities for manufacture of the device.

A likely major component of each of these tasks for 2007 incorporates a device which does not require a membrane, but rather uses a white powder loaded with the ethylene responsive reagents. At this time the paste is akin to "White out" or liquid correction fluid. This powder is highly sensitive to ethylene (yielding a strong color change from white to blue), is less sensitive to humidity effects, can be removed with a gentle wash, is less expensive than the membrane device, and is in general easier to use.

We will focus activities of the first half of 2007 on formulating this powder, making a useable device, and comparing performance to the membrane system. We will, of course, continue to improve the membrane system, in particular to reduce the water content effects by incorporating hygroscopic salts. Dr. James Mattheis will assist on trials in the packinghouse this spring and in the orchard in the fall.

Research progress and methods:
Device stability

The primary focus on this issue has been the impact of sunlight and humidity. Sunlight has shown consistently to cause a non-specific color change. Elevated humidity during manufacture of the devices has been shown to cause inconsistent, but usually poor results.

We have investigated different preparations of our reagents and support materials to directly address the sunlight caused color change. Fluorescent lighting as well as UV light has no measured effect for any length of time at normal intensity, but sunlight may have the effect of reducing the chemistry, turning it blue or grey/black. Approaches used have been to increase the cross-linking of reagents to supports, varying the chemical and physical environment of the reagents, changing the support materials, and altering the processing and construction methods. UV protective films were not effective. At this time, the most effective means to reduce the sunlight effect is to place an opaque, reflective material on the surface of the device. The device is highly stable in the laboratory or in storage and so eventual use in conditions less harsh than in the field are not likely to need such a cover. Aluminum foil has proven to be very successful as a cover and eliminates all of the sunlight / temperature induced color changes.

The second stability issue we have addressed is related to elevated humidity. The final design of our device has low response to varying humidity (the materials allow very little water penetration and interaction with the reagents). The concern with humidity is an issue predominantly with the manufacture and shipping of the device. This challenge was not immediately clear as the ambient humidity in Tucson, AZ, is typically very low. Most of the year our humidity is around 10-20%, but during our rainy season of July – Aug reached 60-70% for extended periods of time in the summer of 2006 which was one of the wettest summers in many years.

Elevated ambient humidity during the production of devices can lead to inconsistent results often yielding very low response to ethylene. We took several steps to maintain a consistent and low humidity by placing wet stickers (membranes to which had been added our reagents) in vacuum dessicators which were evacuated of air. By varying the amount and type of hygroscopic salts added to the dessicators we could modify the equilibrium humidity levels that were reached as water

evaporated off of the membranes. A humidity of 20% ± 2% during the production process yielded the most stable stickers. A heat gun was used gently to dry the bulk water away from the membranes. The rate of this drying was found to impact the stability and response of the sticker. The type of drying agent also impacted sticker performance. Drierite, the standard laboratory drying agent, causes a non-specific color change; however, CaSO4, the primary component of Drierite, does not cause a color change. We continue use a combination of the heat gun and pure salts to maintain humidity. We have begun to evaluate using these salts as a component of the device to ensure that humidity levels are maintained.

Stickers were designed to have a coated membrane remain in direct contact with the fruit covered by a clear viewing material. A light-reflecting material, aluminum foil, was placed on top of the coated material to protect from exposure to sunlight. This cover was able to be lifted up so the coated material could be inspected and then reattached to the apple using bright orange labels (merely to simplify location of the apples in the field).

Field trials

The goals of these initial orchard trials were to assess the feasibility and utility of the device in the field, to evaluate stability in response to environmental exposure, and to provide feedback on device design and ease of application. These trials were conducted from August 2006 – October 2006. The device was stable with minimal to no non-specific color change in the field. Stickers were moderately easy to apply, but in some cases did not adhere well to the fruit surface. The degree of color change was small but consistent across trials and consistent with expectations based on device response to 1-5 ppm ethylene in the laboratory.

Figure 1: Stickers prepared with an aluminum cover for field trials.

Dr. James Mattheis performed these field trials in Wenatchee, WA, on our reagents placed on Durapore membranes. The procedure for the trials has been the following. Stickers are manufactured at the University of Arizona, in Tucson, and shipped overnight to Wenatchee. Stickers are placed on apples on the tree in an orchard at the TFRL and maintained on the apples for 24 to 48 hours. Two stickers are used per apple. At that time the degree of color change is assessed compared to a graded color scale (1-4, with 1 being white, 4 being dark blue). Typically three individuals grade each sticker independently and their visual evaluations are averaged. Apples are harvested and their internal ethylene concentration (IEC) is quantified by gas chromatography. Additional measures of apple firmness, color, and sugar content are also quantified.

Sunlight was found to be a major factor in laboratory studies. For membrane stickers placed in the field under an aluminum cover, sunlight and temperature had no significant impact. Nearly all of the control stickers (placed in the field on a tree, but not on an apple) showed no to minimal color change.

There is a color change observed for stickers placed on apples and this change has a relationship to the IEC, but the correlation is less strong than desired to provide an easily readable measure in bright sunlight.

Based on the results of these orchard trials, multiple modifications were made to the device. We identified ways to increase stability of the sticker before, during, and after shipping (maintaining 4ºC (household refrigerator)) temperatures improved stability. Formulations of the device cover were improved. Methods of drying the reagents prior to encapsulation were improved and standardized. Moderately fast drying with a heat gun improves stability and decreases "splotchiness" of the color development. Drying too fast causes a non-specific color change.

Following the apple orchard trials, we performed a number of laboratory studies and Dr. Mattheis' group performed one study on pears removed from controlled environment storage after 4 months in storage. The impact of these improvements are best described using Figure 2 below which displays the results of a laboratory trial on 5 types of pears and one Granny Smith apple. Color in the membranes developed over 24 – 48 hours (negative controls of membranes without reagents remain white). Note that the Asian pear in the upper right is a non-climacteric fruit and so does not release ethylene. Reagent loaded Durapore membranes and loaded PVDF powder showed a strong color development that reached and remained at a peak by 24 hours.

Figure 2: Performance of Durapore membrane containing device on pears and apple. Trials performed at The University of Arizona. Clockwise from upper left are: red pear, bosc pear, Asian pear, Granny Smith apple, Bartlett pear, D'Anjou pear. Each fruit was tested for up to 7 days. The color became darker for up to approximately 48 hours and remained for at least 7 days. White membranes with a small blue dot are an alternate formulation in which the reagents do not fully wet the membrane.

Color development on commercial apples in the laboratory were assessed with these device improvements. Color development was significantly greater than that observed in the field (Figure 3).

Figure 3: Color development on 10 apple varieties evaluated in the laboratory in Tucson, AZ, in the second stage of this project (during field trials and after some improvements had been implemented).

Dr. Mattheis' group performed trials on the improved devices on pears which had been stored for 4 months in a controlled atmosphere (cold storage). Bartlett pears (some treated with 1-MCP; control pears had no MCP), were removed from storage, and labeled with Durapore membranes. A summary of the results is presented below. Stickers were evaluated using a color scale ranging from 1-8 (white to blue). A larger color number is expected once the pears have awoken from storage and have begun to ripen and hence to release ethylene. A good correlation was observed between ethylene release and degree of color change for 3 out of 4 conditions. The 1-MCP, day 5 measurement shows a darker color than anticipated based on ethylene release. Note that the color scale ranges from 1-8 in degree of color development but has not yet been calibrated directly to ethylene release.

Treatment	Day	# of Obs	Variable	Mean	Std Dev
Control	1	18	C2H4	7.32	0.359
			Stickers	7.9	0.3
	5	18	C2H4	3.04	1.02
			Stickers	4.1	0.3
1-MCP	1	18	C2H4	2.84	1.83
			Stickers	2.4	0.7
	5	18	C2H4	0.93	0.99
			Stickers	4	0

Key: C2H4: ppm in headspace of closed jar w/pear 20 for min; stickers rating 1-8 (white to blue).

Discussion
In the past year we have improved the device formulation with much of the progress occurring during and after orchard trials. These improved devices were utilized in the laboratory studies and pear trials described above. The results indicate that the Durapore stickers have good potential for indicating fruit maturation out of cold storage and could be particularly useful for management decisions. We cannot yet draw final conclusions on the potential for orchard use due to the changes and improvements made based on the results of the trials. We aim to continue to improve performance and will spend much time on testing on fruits from the packinghouse and in the orchard.

We have received additional funding for this work in a phase I SBIR (small business initiative for research) project from the USDA to RediRipe™, LLC. These funds have been used to hire additional personnel to broaden our development activities (testing more conditions and device constructions, purchasing equipment, supporting field trials, etc.). RediRipe™ plans to pursue additional funding for a phase II SBIR to support larger trials in the fall of 2007 with the goal of having a commercial device available for use by the Washington apple and pear industry in late summer / early fall 2008. Supporting this industry remains our primary goal. We encourage commission members to contact us to discuss how we can best improve the quality and economics of Washington apples and pears. Future development will target other fruits and vegetables.

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

Project Title: Replant disease tolerance of Geneva rootstocks.

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Address:
City: Wenatchee
State/Province/Zip WA
Cooperators: Tom Auvil, Tim Smith and others at WTFRC

Budget 1:
Organization Name: USDA ARS PGRU **Contract Administrator:** Dianne Emerson
Telephone: (315)787-2329 **Email address:** dianne.emerson@ars.usda.gov

Item	**Year 1:** 2006	**Year 2: 2007**	**Year 3:** 2008
Salaries	24,000	**33,000**	33,000
Benefits		**9,470**	9,470
Supplies	4,000	**5,000**	6,000
Travel	1,000	**1,530**	1,530
Micropropagation		**10,000**	
Miscellaneous	1,000	**1,000**	1,000
Total	30,000	**60,000**	51,000

Footnotes: [1]Technician salary for assistance in laboratory, propagation, budding and maintenance of stoolbeds.
[2]Includes cost for rootstock liners, trees, support system, lab supplies etc.
[3]Travel to and from trials.
[4]Includes shipping expenses (trees, rootstocks), communication costs etc.

Reasons for Budgetary increases for Year 2 of operations (2007)
Increases in technician salary costs are due to the lack of base funding and budgetary shortfalls in the USDA ARS and Cornell systems. The micropropagation entry represents the removal of a major roadblock to get major quantities of plant material for testing and release to the WA industry – this will be accomplished by sending new selections to a tissue culture lab for micropropagation and the establishment of large stoolbeds.

Note: This project is in collaboration with the WTFRC. Internal research budgetary projections can be found in Tom Auvil's report

Progress Report
Objectives

1. To study the relative performance of Geneva dwarfing apple rootstocks compared to commercial controls in replant soils and the study of genetic mechanisms related to tolerance to ARD.
2. In the most recent visits we have come to appreciate the need by a certain segment of the industry to plant liners in place in the orchard either as sleeping eyes or as bench grafts. We would like to modify our existing protocol to discover "nursery in place" properties of rootstocks and how they interact with replant disease when the plants are so young. The question we are trying to answer is: how well do ARD tolerant sleeping eyes and bench grafts do in a replant situation?
3. To set up an early evaluation protocol for newly developed genotypes that screens for components of apple replant disease resistance in the early stages of breeding.

SIGNIFICANT FINDINGS AND DEVELOPMENTS

- **A graft in place replant experiment with the widest selection of Geneva rootstocks ever tested in WA has been planted in Vantage, WA and a new replant experiment with a wider selection of Geneva rootstocks has been planted in Brewster, WA.**
- **With regards to tree deaths due to fire blight and other causes in the Wapato replant trial M.9 Pajam 2 came in first with 25 trees and Supporter 1 and 2 with 15 trees each. Geneva 41 and Supporter 3 only suffered 2 tree deaths each. We can still detect an effect on tree growth from the fumigation treatment and we can also detect rootstocks that show tolerance to the replant components in Wapato. The rootstock PI AU 56-83 had to be removed from the experiment because it was unproductive and large.**
- **The rootstock B.9 has been one of the weakest and least productive rootstocks in the replant experiments that have been planted so far. G.41 has performed well in the replant settings of Wapato (conventional) and Chelan (organic).**
- **The positive effect on season's tree growth of fumigation treatment has virtually disappeared in the third season while the cumulative effect is still detectable.**

OBJECTIVE 1. There are three phases within this objective: 1) propagation of rootstocks and tree design, 2) Establishment of replant trials, and 3) Data collection at the sites and analysis.
At the onset of this period we had already established 3 ARD (Table 1) and two non ARD field trials. In the spring of 2006 we established three additional ARD field trials (Wapato, Vantage and Brewster) the Vantage field trial is unique because even though it has been placed in a fumigated replant setting, it utilized grafted liners instead of finished trees thus fitting elements of proposed Objective 2. The new Wapato trial is part of the NC-140 replant trials being established in the U.S. All trials have generally fit the major requirement that the trials be placed into a commercial setting (i.e. large enough to be managed by growers and of economical value to growers). The establishment more of such trials will depend on the availability of rootstock liners in large enough quantities to be tested. A new series of rootstocks that have passed fire blight resistance and productivity trials in Geneva have been selected. These rootstocks are all of a dwarfing category between 20-35% of seedling (M.27-M.26) and upon receipt of funding they will be sent to a tissue culture facility to be propagated in large enough numbers to provide large scale testing in 2008.

Table 1. Locations and rootstocks planted in ARD trials 2003-2006.

Rootstock	Location*	Scion Varieties
G.16	WA, CH, NA	Brookfied Gala, Honeycrisp
G.11	WA, CH, BR, VA	Brookfied Gala, Torres Fuji, Aztec Fuji
G 3041	WA, CH, VA	Brookfied Gala, Aztec Fuji
G 5935	WA, CH, NA, VA	Brookfied Gala, Honeycrisp, Aztec Fuji
PiAU-56-83	WA, CH	Brookfied Gala
Pajam 2	WA, CH	Brookfied Gala
M.26 EMLA	WA, CH, NA	Brookfied Gala
Bud 9	WA, CH, NA	Brookfied Gala
Supporter 1	WA, CH	Brookfied Gala
Supporter 2	WA, CH	Brookfied Gala
Supporter 3	WA, CH	Brookfied Gala
4214	WA, NA, BR, VA	Brookfied Gala, Torres Fuji
4003	NA	Honeycrisp
4814	NA, BR, VA	Honeycrisp, Torres Fuji, Aztec Fuji
4210	NA, BR, VA	Honeycrisp, Torres Fuji, Aztec Fuji
G.30	NA, VA	Honeycrisp, Aztec Fuji
5087	NA, VA	Honeycrisp, Aztec Fuji
G 4202	NA	Honeycrisp
4013	NA	Honeycrisp
4213	NA	Honeycrisp
M.9 EMLA	NA, BR	Honeycrisp, Torres Fuji
5757	BR	Torres Fuji
G.202	BR, VA	Torres Fuji, Aztec Fuji
6879	BR	Torres Fuji
MM.106	BR	Torres Fuji
6006	BR	Torres Fuji
7707	BR	Torres Fuji
5257	BR, VA	Torres Fuji, Aztec Fuji
3007	BR, VA	Torres Fuji, Aztec Fuji
4011	BR, VA	Torres Fuji, Aztec Fuji
5937	BR	Torres Fuji
5463	BR, VA	Torres Fuji, Aztec Fuji
4003	BR	Torres Fuji
6001	BR	Torres Fuji
6210	WA	
M.7	BR, WA	Torres Fuji
JTE-B	BR	Torres Fuji
Ottawa 3	BR	Torres Fuji
JTE-C	BR	Torres Fuji
5890	BR	Torres Fuji
2034	VA	Aztec Fuji
2406	VA	Aztec Fuji
3001	VA	Aztec Fuji
4002	VA	Aztec Fuji
4004	VA	Aztec Fuji
4013	VA	Aztec Fuji
4172	VA	Aztec Fuji
4288	VA	Aztec Fuji
5046	VA	Aztec Fuji
5179	VA	Aztec Fuji
5202	VA	Aztec Fuji
4019	VA	Aztec Fuji
Mark	VA	Aztec Fuji
Supporter 4	VA	Aztec Fuji

*** WA=Wapato, CH=Chelan, NA=Naches, VA=Vantage, BR=Brewster**

FINDINGS BY LOCATION (Please refer to poster for more graphs and data):

CHELAN AND WAPATO REPLANT TRIALS

A fumigation effect on cumulative tree growth and productivity is evident; however this year (2006) when we compared girth growth between fumigated and non fumigated treatments we could not detect a significant difference (Figure 1) signifying that any effect that the fumigation treatment has on growth is restricted to the first two growing seasons – it remains to be seen how long the head start conferred by fumigation will last. The differences in yields and efficiencies between the Wapato (WA) and Chelan (CH) sites may be explained by the cultural practices (Conventional vs. Organic) and by the fact that the CH location received a heading back treatment that was meant to equalize the trees, thus setting the production of some trees back one year. The relative rankings though are similar in the two plantings. In general B.9 had the lowest yields whether it was in fumigated or non fumigated treatment. G.41 (3041) performed well in both trials showing strong tolerance to replant and resistance to fire blight.

Table 2. Summary table of productivity data of replant experiments in Wapato and Chelan.

WAPATO 2006 Means

Treatment	FUMIGATED				NON FUMIGATED			
Rootstock	TOTAL FRUIT	NET WT Kg	TCA Cm^2	YEFF Kg/Cm^2	TOTAL FRUIT	NET WT Kg	TCA	YEFF Kg/Cm^2
Bud-9	43.4	6.53	7.87	0.83	30.61	4.84	5.89	0.82
G-11	68.39	11.16	6.92	1.69	49.08	7.49	4.77	1.46
G-16	71.92	10.27	9.66	1.04	66.75	9.04	7.12	1.3
G-3041	58.63	9.17	9.82	0.98	64.06	9.97	7.45	1.43
G-4214	63.04	9.79	8.25	1.22	40	6.26	6.68	0.95
G-5935	80	11.8	9.83	1.22	71.45	11.31	8.49	1.37
M-26	55.43	8.74	10.56	0.83	45.04	6.55	8.28	0.8
M9Nic29	59.5	9.86	9.97	1.04	38.13	5.96	6.57	0.92
M9Paj2	72.5	11.58	9.65	1.27	47.03	7.66	7.51	1.02
Sup 1	45.27	7.66	8.75	0.89	50.42	8.07	6.11	1.48
Sup 2	51.63	8.38	7.85	1.37	39.89	6.18	6.08	1.06
Sup 3	59.65	9.36	8.42	1.18	51.36	7.79	6.22	1.29

CHELAN 2006 Means

Treatment	FUMIGATED				NON FUMIGATED			
Rootstock	TOTAL FRUIT	NET WT Kg	TCA Cm^2	YEFF Kg/Cm^2	TOTAL FRUIT	NET WT Kg	TCA	YEFF Kg/Cm^2
Bud 9	6.21	1.09	3.88	0.27	4.48	0.8	3.72	0.19
G-11	15.64	3.05	5.01	0.62	12.93	2.28	4.21	0.57
G-16	23.08	4.16	7.47	0.56	14.91	2.48	7.05	0.35
G-3041	18.8	3.99	6.79	0.61	14.58	2.82	6.01	0.48
G-5935	19.04	3.15	6.65	0.46	25.38	4.36	6.4	0.67
M-26	13.34	2.43	5.26	0.45	6.4	1.11	3.97	0.26
M9T337	7.23	1.35	4.08	0.34	8	1.43	3.37	0.38
M9Nic29	7.82	1.59	4.69	0.32	4.88	0.82	3.7	0.26
M9Paj2	6.7	1.17	5.44	0.21	5.78	0.99	5.05	0.17
Piau5683	2.97	0.51	12.81	0.04	3.97	0.66	13.08	0.05
Sup 1	4.76	0.85	9.48	0.2	11.24	1.95	4.29	0.48
Sup 2	4.91	0.99	4.96	0.22	4	0.71	3.73	0.18
Sup 3	8.92	1.61	6.28	0.27	12.88	2.24	5.2	0.42

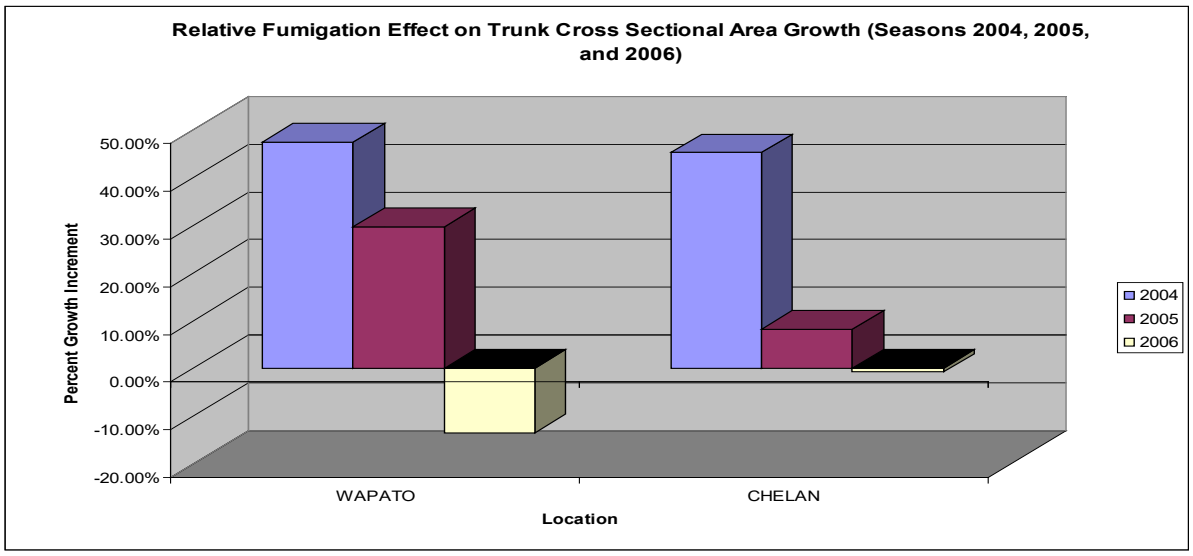


Figure 1. Percent increase in growth (TCA) that can be explained by the fumigation treatment for the Wapato and Chelan replant trials.

NACHES REPLANT TRIAL

This was the first good cropping year and the effect of fumigation detected on tree growth in previous years was translated into fruit. This year we were able to see how fruit quality (Bitter Pit) is affected by rootstocks and fumigation treatment – overall the non fumigated trees had more Bitter Pit than other treatments and some rootstocks had lower incidence no matter the treatment (Figure 3). Evidence of fumigation treatment could still be detected in some rootstocks (M.9, G.202, 4213 and 4814) but some showed very little or no difference in the amount of growth (TCA) that they experienced (Figure 4) and may now be considered tolerant to the replant conditions that affect the Naches location.

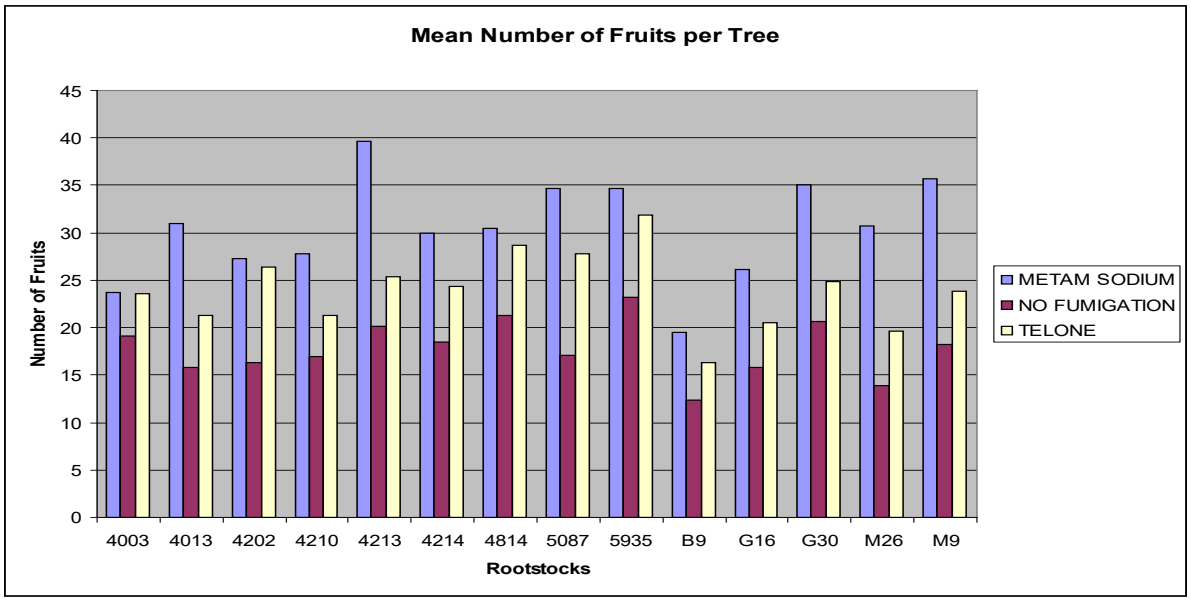


Figure 2. Mean number of fruits per tree (Honeycrisp). Overall the Metam Sodium treatment seems to have performed better than other treatments. Productivity of the rootstocks was correlated with their dwarfing category. One rootstock (4003) did not have a significant difference in productivity due to treatment (e.g. the fumigated trees were not very different from unfumigated).

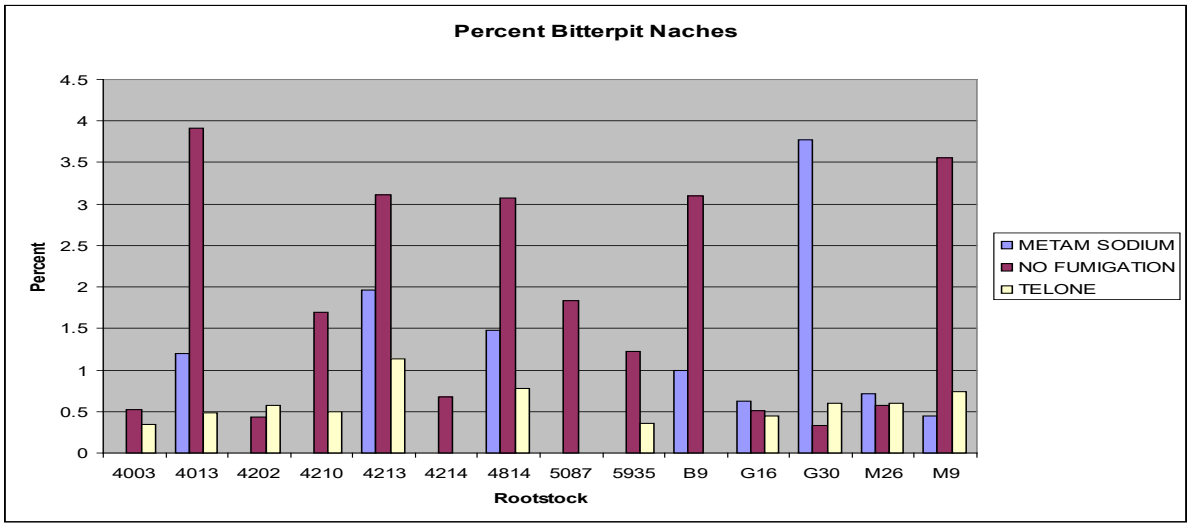


Figure 3. Percent Bitter Pit of Honeycrisp fruit according to genotype and treatment (Naches replant trial).

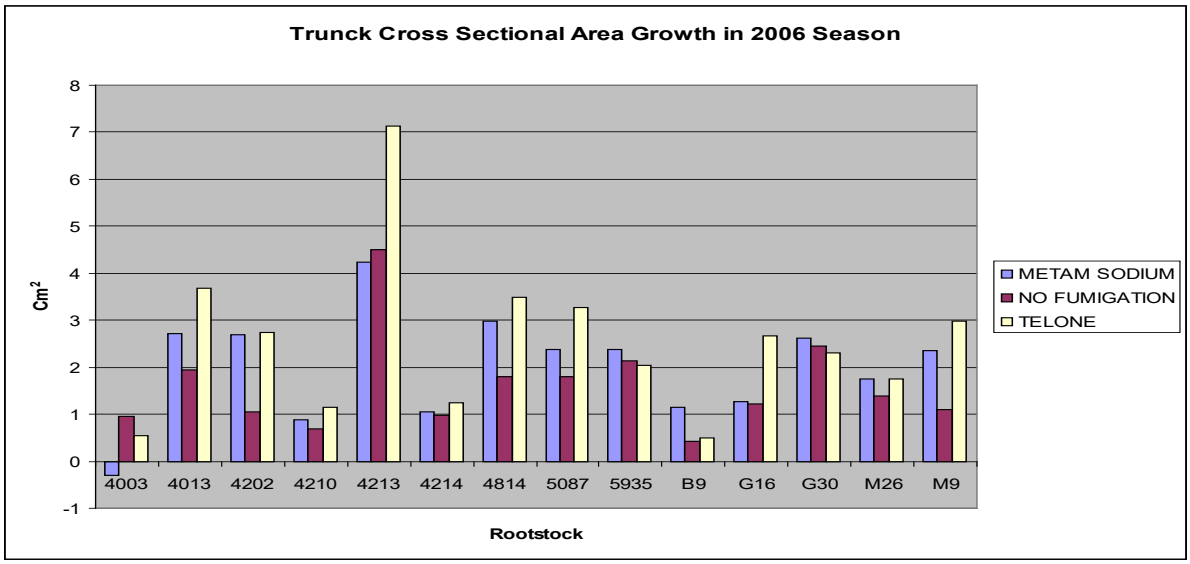


Figure 4. Growth in TCA according to genotype and treatment (Naches replant trial).

OBJECTIVE 2

Many growers are purchasing rootstock liners and contracting nurseries to make sleeping eyes (SE) or bench grafts (BG) instead of planting finished trees. This year we planted a bench graft experiment in a fumigated replant trial location at the Auvil Fruit Tree Farm (Vantage, WA) questions we are trying to answer in this experiment are:

1. Are certain rootstocks better adapted at producing healthy trees from BG in ARD fumigated soil?
2. What are the long term effects on fruit production (yield, quality) from growing trees in place according to different rootstocks?

The planting in Vantage is on a V trellis and has the widest array of Geneva rootstocks planted in WA. Bench grafts are allowed to push growth for two season to build a scaffold that closely matches the wires in the trellis. Trees are expected to reach optimum height prior to fruiting. Data on central leader growth and number of trained branches has been taken this year (Figure 5). A few rootstock genotypes (G.16, 4002, 4019) showed latent virus susceptibility and have been eliminated from the

trial. Liner quality (roots) may have had a strong effect on graft and tree establishment on a few select rootstocks but in general it was not a major factor for success given the TLC that the grower gave to the trial.

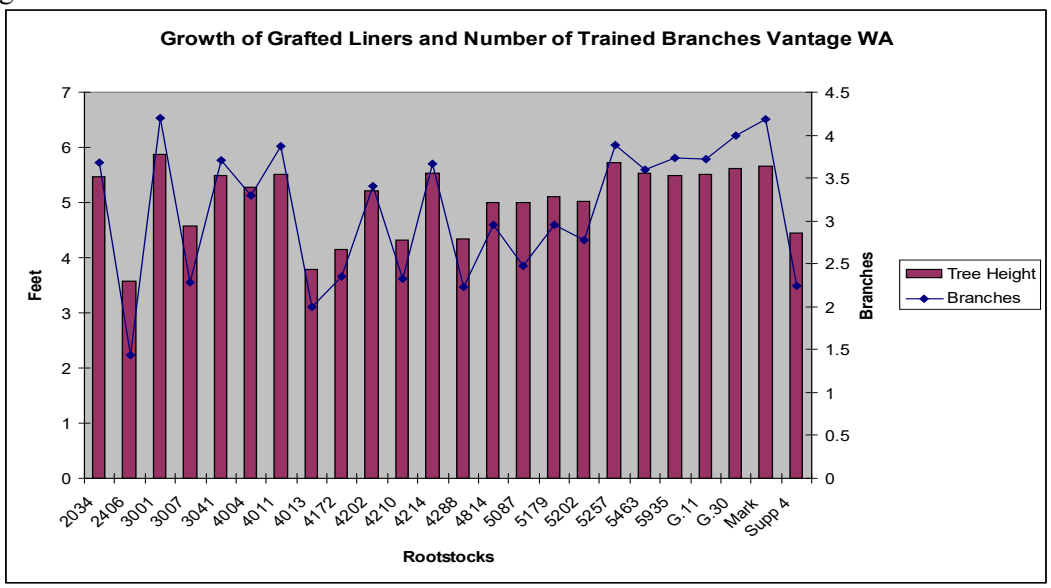
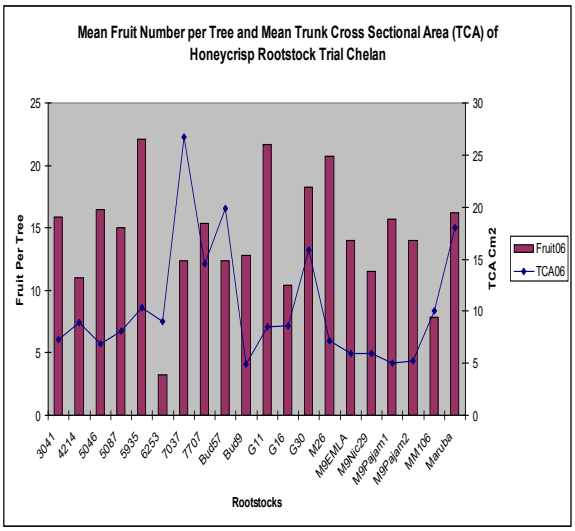
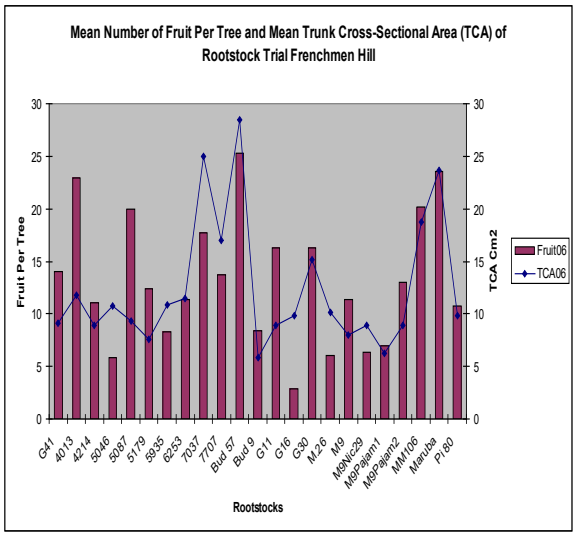


Figure 5. Rootstock effect on growth of Aztec Fuji grafts at the Auvil Fruit Tree Farm in Vantage.

OTHER TRIALS: Below are two graphics representing the TCA and yield (mean number of fruit per tree number) of Honeycrisp rootstock trials in Chelan and Frenchmen Hill. G.11 and G.935 had the highest yields in Chelan (Organic) and Bud 57, 4013 and Marubakaido in Frenchmen Hill.





OBJECTIVE 3

We have made progress on Objective 3 (acquiring additional screening methods for replant) but it has not been as quick as I wished due to lack of base funding.

AKNOWLEDGEMENTS

We would like to express heartfelt gratitude to the growers that are cooperating in this effort as well as the staff members at the WTFRC that have worked very hard to obtain this data. THANK YOU!!!

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-06-602

Project Title: Auxin and ethylene dynamics in the abscission zone

PI: Steve van Nocker
Organization: Michigan State University
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Address: 390 Plant and Soil Science Bldg
City: East Lansing
State/Province/Zip MI 48824

Cooperators: M John Bukovac, MSU Horticulture

Budget 1:
Organization Name: Michigan State Univ **Contract Administrator:** Lorri Busick
Telephone: 517-355-5191 x363 **Email address:** busick@msu.edu

Item	Year 1:	Year 2:	Year 3:
Salaries	8,676[1]	8,936	9,204
Benefits	819[2]	693	956
Wages	4,200	3,846	4,456
Benefits	0	0	
Equipment	0	0	
Supplies	1,800	1,600	4,200[3]
Travel	150	150	150
Miscellaneous	3,200[4]	3,200	
Total	18,845	18,425[5]	18,966

Footnotes: [1]We have obtained matching funds from the Michigan State Agriculture Experiment Station. [2]Will support $^1/_2$ effort by a graduate student (stipend). [3]Costs include production and screening of microarrays. [4]Costs for DNA sequencing in Year 1 and Year 2. [5]Does not includes funding for collaboration with WTFRC; see Budget 2.

Budget 2: (*Complete only if funding is split between organizations***)**
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: 509-665-8271 x2 **Email address:** Kathy@treefruitresearch.com

Item	Year 1	Year 2 (fill in year here – optional)	Year 3 (fill in year here - optional)
Salaries			
Benefits			
Wages		480	
Benefits		192	
Equipment			
Supplies		200	
Travel			
Miscellaneous			
Total		872[6]	

Footnotes: [6]Projected costs for supplies and technical help for experiment in the Wenatchee area in spring '07. These amounts are not included in the total for Budget 1.

Objectives:
The goal of this three-year project is a methodical characterization of auxin and ethylene signaling components in the flower and fruit abscission layers, and analysis of the effects of cultural practices (including application of bloom and postbloom thinners) and environment on the interactions between these components. We will identify apple counterparts of known components of auxin and ethylene signaling (enzymes involved in biosynthesis, degradation, receptors, transporters, signaling intermediates, and regulatory proteins). We will engineer new tools to study the activity of all of these genes in the flower/fruit abscission layers during natural progression of abscission (pollination/fruit set, fruitlet abscission, wound- or pathogen-induced abscission, and ripening-associated abscission), and in response to exogenous manipulations (bloom thinners, reduction in photosynthate flow to the fruit, postbloom thinning compounds, and biochemical inhibitors of auxin transport and ethylene biosynthesis and perception). In addition, we will compare the activity of these genes between varieties that are difficult to thin (e.g., Fuji) and those that are prone to overthinning (Delicious) in order to understand the biochemical basis for this difference.

Objectives for Year 1 (past year): Identification of auxin- and ethylene-related genes, construction of a *DNA microarray*[1], optimization of field work design and manipulations.

Objectives for Year 2 (coming year): Continued field work in the Wenatchee area and at MSU, gene expression analysis of various abscission-related processes using the completed microarray.

Schedule of activities and anticipated accomplishments:

Feb '07 Microarray construction completed.
Mar '07 Initial tests of microarray using samples collected in spring and fall '06.
Apr '07 Replication of '06 tissue sampling during thinning trials in Wenatchee area.
May '07 Field work and tissue sampling - pollination/fruit set, fruitlet competition within a cluster, fruit abscission promoted by wounding, fruit drop in response to reduced photosynthate, postbloom thinning response to NAA, BA, and ethylene.
Jul '07 Field work and tissue sampling - pedicel abscission in response to fruit removal.
Sep '07 Replication of '06 field work looking at natural abscission and manipulation of fruit drop by ethrel, NAA, and ReTain.
May-Nov '07 Microarray analysis and data processing.

Significant findings for Year 1:
<u>Identification of auxin- and ethylene-related genes.</u>

- We completed analyses of the *expressed sequence tag (EST)*[2] information currently available in public sequence databanks and updated our web-accessible database, Tree Fruit Technology (www.genomics.msu.edu/fruitdb). This work was published in the top-rated plant physiology journal, *Plant Physiology*[3].
- We performed extensive literature searches to identify and catalog all suspected auxin-related and ethylene-related genes characterized in other plants.

[1] **DNA microarray** is a collection of microscopic DNA spots attached to a solid surface, such as glass, plastic or silicon chip forming an array. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously.
[2] **EST (expressed sequence tag)** is a short DNA sequence of a randomly selected gene active in a given tissue. ESTs are a useful resource for gene discovery and for designing probes for DNA microarrays used to determine patterns of gene expression.
[3]Park S, Sugimoto N, Larson MD, Beaudry R, van Nocker S. 2006. Identification of genes with potential roles in apple fruit development and biochemistry through large-scale statistical analysis of expressed sequence tags. Plant Physiol 141(3), 811-824.

- We used bioinformatics techniques to identify apple counterparts of known components of auxin and ethylene signaling, and identified a total of ~414 apple genes potentially involved in auxin signaling, and ~190 apple genes potentially involved in ethylene signaling.

Microarray construction.

- Construction is underway of a microarray containing all DNA sequences of the auxin or ethylene signaling genes that we identified. This will be completed before the end of the first year of the project. This microarray was designed as a resource to be used among the apple research community, and also represents genes with known or suspected roles in flowering, fruit ripening, color and aroma production, and other developmental processes important for production and storage.

Optimization of field work design and manipulations.

- We sampled flower and abscission zone tissues from bloom thinning trials on Gala in Orondo, WA. This was in collaboration with the more extensive thinning trials done by WTFRC staff. The Orondo study compared CFO/LS, Ethrel, Regulaid, and CFO/LS in combination with Retain+Regulaid. Only CFO/LS had a significant effect on final fruitlet number (data will be presented by WTFRC staff). Tissues were saved for upcoming analysis of gene activity using microarrays.
- In experiments at MSU, we sampled flower and abscission zone tissues from trees where flowers were treated to LS or jasmonic acid (a mediator of plant defense responses), or subjected to physical wounding. We also measured ethylene production resulting from these treatments. LS resulted in a rapid and sustained increase in ethylene production by floral tissues, and this treatment reduced final fruitlet number. However, other treatments did not result in ethylene production or thin effectively! Tissues were stored for upcoming analysis of gene activity.
- We collected abscission zone tissues from trees during the course of natural fruit drop. We were also able to accelerate drop using ethrel and delay drop using NAA or ReTain, and we collected tissues from these trees as well for the upcoming analyses.

Methods:

Public sequence databanks now contain ~300,000 apple EST entries. We have organized these data into a web-accessible database, Tree Fruit Technology (www.genomics.msu.edu/fruitdb). We performed extensive literature searches to identify and catalog all suspected auxin-related and ethylene-related genes characterized in other plants, and used our web software to scan the organized EST information for apple genes related to these. We anticipate updating this resource once again before the end of this project.

Our microarrays are being commercially produced by a microarray company, CombiMatrix. Each microarray allows the analysis of all of the identified genes under four different conditions, and these microarrays are re-usable. Outside investigators will be able to order these custom apple microarrays for their own research, directly from CombiMatrix.

This past year, field work was done at Orondo, WA and at the MSU Research Center in E. Lansing, MI. Our experiments so far have concentrated on Gala, because this cultivar is being developed as a genomics model. Bloom thinning treatments were carried out at ~50% bloom. For study of natural abscission, samples were taken at 2-d intervals, commencing before the first sound fruit dropped. Ethrel or NAA (Fruitone N) was applied when the first sound fruit began to drop in response to branch shaking, and samples were taken every two days until peak drop. ReTain treatments commenced 3 weeks prior to anticipated peak drop, and sampling dates corresponded to natural abscission (no treatment). This coming year, we will replicate the '06 studies and carry out five

additional studies to evaluate gene activity patterns through several distinct circumstances of abscission, as originally proposed:
1) Flower abscission or retention associated with pollination/fruit set. We will analyze the progression to abscission in a set of flowers that are prevented from being pollinated. A matched set of hand-pollinated flowers will be used as a control, utilizing the king flower (most likely to be retained) within each cluster. Three samples will be collected during the time between anthesis and flower abscission.
2) Fruitlet abscission associated with competition within a cluster. We will analyze fruitlets most likely to abscise within a cluster (lateral fruitlets) in comparison with those most likely to be retained (the king fruitlet). Samples will be taken throughout the period of early fruitlet drop.
3) Fruit abscission promoted by wounding. Fruits will be subjected to artificial wounding following the period of early fruitlet drop. Non-wounded fruit will be used as a comparison. Samples will be taken at 1d, 2d, and at one additional time point before abscission.
4) Fruit abscission promoted by reduced photosynthate. Cool, cloudy conditions early in the season promote excessive fruitlet drop associated with reduced photosynthate availability. We will attempt to identify the biochemical pathway leading to abscission layer activation under these conditions. We will attempt to induce abscission using two approaches:

i. shading to 50% canopy with shade cloth
ii. treatment with a photosynthesis inhibitor, Terbacil

We will take samples 1d, 2d and at one additional time point before enhanced drop of fruitlets is observed. Non-treated trees will be used as controls.
5) Fruit removal. After June drop, if fruit is removed from the tree by severing the pedicel, the segment of pedicel remaining attached to the tree will abscise in a very reproducible manner. This provides a time course of the induction and progression of abscission. We will take samples at daily intervals, until day 4, when about half of pedicels have abscised.

We intend to perform many of these manipulations with cultivars that exhibit the extremes of thinning/drop responses seen in the field. For example, for postbloom thinning assays we will also evaluate both Red Delicious (sensitive) and Fuji (relatively insensitive). For evaluations of preharvest drop we will also evaluate Red Delicious (prone).

Discussion:
We have developed a model for fruit abscission involving the interaction between two endogenous PGRs, auxin and ethylene. Specifically, at a very early stage in abscission we have found changes in the activity of a number of genes that participate in mediating auxin signal transduction, and this preceded observed changes in the activity of several genes that function in ethylene signaling. Taken together with a variety of studies of the effects of bloom and postbloom thinners, and with very recent findings in basic plant biology, our results allow us to propose a model for the initiation of flower and fruit abscission. In this model, loss of directional auxin transport through the abscission layers triggers enhanced ethylene signaling in abscission layer cells, culminating with activation of genes that promote cell separation.

Auxin is produced by actively growing tissues of the flower, fruit, leaf, and apices and is transported towards the base of the plant. Reduction in auxin flow from the flower or fruit, through the abscission layers, could result naturally from several circumstances, including (1) lack of adequate pollination and fertilization of the flower, (2) limited fruitlet growth after fruit set due to competition for photosynthates within a cluster, (3) limited fruit growth due to reduced photosynthate availability in periods of cool and cloudy weather, (4) cessation of fruit growth associated with maturity, or (5) organ senescence in response to damage or pathogen infection. Loss of auxin flow through the abscission cell layers is expected to result in auxin accumulation in these cells, which is hypothesized to enhance the ability of these cells to sense ethylene; where ethylene is present at appreciable

concentrations, its perception will promote autocatalytic ethylene production in these cells. Ethylene is known to directly activate various genes involved in cell separation such as pectate lyase, an enzyme that degrades the middle lamella (the 'glue' that holds cells together). Because the ability of auxin to trigger abscission is limited to circumstances where the concentration of ethylene reaches or exceeds the ethylene sensitivity of the abscission layer cells, the dynamics of auxin and ethylene signaling are tightly linked.

This model is adaptable to explain the activity of many thinning chemicals. For example, as verified in our '06 study with LS, damaged floral tissues resulting from caustic bloom thinners should induce production of ethylene, which promotes flower senescence (thus terminating auxin transport from the flower), and also diffuses through the pedicel to directly promote autocatalytic ethylene production and cell separation in the flower abscission zone. As another example, NAA applied to the entire tree as a postbloom thinner may weaken the apical-basal auxin gradient, enhancing abscission of those fruitlets transporting the least auxin (i.e., the smallest fruitlets in a cluster).

Many of the ~600 genes that we identified are expected to function in auxin- or ethylene- mediated processes unrelated to abscission, but a subset of these will be important intermediaries in the abscission process. Without evaluating the activities of all of these genes during abscission-related processes, it is impossible to know which have important roles in abscission, and which have unrelated functions. Work during the first year of this project was mainly preparatory, as planned, and this information will be generated in the second and third years of the study.

FINAL PROJECT REPORT
WTFRC Project Number: AP-06-603

Project Title: Performance of air induction nozzles under variable wind speeds

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Budget History:

Item	**Year 1:** 2006	**Year 2:**	**Year 3:**
Salaries	0		
Benefits	0		
Wages	1000		
Benefits	0		
Equipment	950		
Supplies	500		
Travel	0		
Miscellaneous	2500		
Total	4950		

Significant Findings

A series of field experiments were undertaken on 'Pink Lady'/M.7 during 2006 to compare the performance of an axial fan air blast sprayer with either a conventional hollow cone (HC) nozzle configuration or a modified air induction/HC nozzle configuration. Spray drift, spray coverage, and spray efficacy were quantified in order to compare each nozzle configuration. Digital analysis of water-sensitive cards was used to quantify spray drift and spray coverage. The efficacy of a standard post-bloom chemical thinning spray and a "high-risk" cover spray program applied with each nozzle configuration was compared. Spray coverage and chemical thinning efficacy were assessed at three different across-row wind speeds. The major findings were…

- Spray drift was greater from a standard HC nozzle configuration compared to a modified AI/HC nozzle configuration.
- A modified AI/HC nozzle configuration produced very high spray fallout at distances of 20-30 feet from the sprayer. This effect could result in significant carry-over and deposition of spray onto trees two rows over from the sprayer at a between-row spacing of 16-20 feet.
- Spray coverage was greater with a modified AI/HC nozzle configuration compared to a conventional HC nozzle configuration under "high" across-row wind speeds, equivalent to 11.3 mph at a height of 6 feet in the middle of the tree canopy.
- The incidence of fruit with sooty blotch and flyspeck was higher with the AI/HC nozzle configuration under a "high-risk" fungicide program compared to the conventional HC nozzle configuration,
- The effects of each nozzle configuration on efficacy of a post-bloom chemical thinning spray could not be compared because of variability in the fruit set data resulting from a severe fire blight outbreak in the experimental orchard.

Objectives

Most common orchard sprayers are configured with disc/core type hollow cone (HC) spray nozzles producing small droplets that under even moderate wind speeds can result in poor coverage[1] and off-target drift[2]. Spray drift becomes more of an issue as wind speed increases, resulting in greater movement of pesticides away from the intended target. Air induction (AI) nozzles generate large, air-filled droplets that behave in a ballistic way as they travel, quickly falling to the ground once the air support drops below a critical value[3]. Spray droplets generated by AI nozzles are less prone to drift than droplets produced by HC nozzles due to their increased size, and tend to break apart into many smaller droplets once they hit a solid surface. AI nozzles have been shown to considerably reduce airborne drift in field studies in Europe using a cross-flow sprayer producing a planar air jet applied to trees 10-13 feet in height[4]. The performance of AI nozzles has not been evaluated on an axial fan sprayer of the type most frequently used to apply pesticides to the larger tree canopies typical of apple orchards in the US. Output from the upper nozzles on an axial fan sprayer can potentially provide the major contribution to off-target drift under moderate wind speeds. It seems logical to assume that switching the nozzle type in these upper positions from HC to AI may provide the greatest benefits in terms of increased spray deposition and reduced drift under low to moderate wind speeds.

The objectives of the present studies were to compare the performance of an axial fan sprayer equipped with a standard HC nozzle configuration or AI nozzles in various configurations. Sprayer performance included measurements of (i) spray drift, (ii) spray coverage throughout the tree canopy, and (iii) efficacy of thinning and cover sprays. In addition, the effects of each nozzle configuration on spray coverage and thinning efficacy were determined under different across-row wind speeds.

Methods

Sprayer configuration and wind speed control. An axial fan sprayer was configured with either conventional HC nozzles or a modified AI/HC configuration where the upper four nozzles were replaced by AI nozzles as described in Table 1. The sprayer was operated at a pressure of 100 psi and the tractor speed was 2 mph for each configuration. The test trees were mature 'Pink Lady'/M.7 planted in 1998 at the Mountain Horticultural Crops Research and Extension Center in Fletcher, NC at a spacing of 20 feet by 10 feet. These parameters equate to a spray output of 177 and 178 gallons per acre for HC and AI/HC configurations respectively, estimated to be 80% of the calculated full canopy TRV of the orchard. Across-row wind speeds were artificially generated by controlling the fan speed on two additional tractor mounted air blast sprayers parked adjacent to each treatment tree as described in Figure 1. Three levels of across-row wind speed (zero moderate, high) were generated using this approach. Actual wind speed values within the tree canopy were quantified with a digital anemometer at 2 feet intervals in height along the tree trunk.

Table 1. Description of HC and modified AI/HC nozzle configurations.

Nozzle Position	Configuration			
	Hollow cone		Air induction/Hollow cone	
	size	gal/min@100 psi	size	gal/min@100 psi
1 Upper	off		off	
2	45/7	1.11	AI1106VS	0.95
3	45/7	1.11	AI1108VS	1.26
4	45/7	1.11	AI1108VS	1.26
5	45/8	1.35	AI1108VS	1.26
6	45/8	1.35	45/8	1.35
7	45/7	1.11	45/7	1.11
8	off		off	
9	off		off	
10 Lower	off		off	
total gal/side/min		**7.14**		**7.19**
gal/acre @ 2 mph		**177**		**178**

Spray drift and spray coverage. **The effects of HC and AI/HC nozzle configurations on spray drift was measured by placing water sensitive cards on the ground at 5 feet intervals along a 70 foot transect perpendicular to the direction of the sprayer in an area of open ground adjacent to the test orchard. The cards were removed after they had dried and drift was quantified by measuring the percent total area covered by droplets in an area representing approx. 5 cm^2 on each card, using the UTHSCSA ImageTool software program (*ftp://maxrad6.uthscsa.edu*). Spray coverage was assessed by analyzing percent spray coverage on water-sensitive cards that had been stapled to the upper surface of a leaf adjacent to the trunk every 2 feet from the soil line using ImageTool software.**

Spray efficacy. Two replicated field experiments were undertaken to compare the efficacy of HC and modified AI/HC nozzle configurations. In the first experiment a post-bloom chemical thinning spray (100 ppm MaxCel plus 1 lb/100 gal. Sevin) was applied with each nozzle configuration at the same three across-row wind speeds generated in the *spray coverage* study. Fruit set was calculated from counts of flower cluster and fruit number on representative sample limbs in the lower (two limbs) and upper (three limbs) canopy. The test orchard suffered a severe fire blight outbreak during 2006 with many clusters on the sample limbs becoming infected so that fruit set data were too variable for meaningful statistical analysis. To try to quantify the treatment effects on fruit set the total number and weight of fruit per tree was recorded at harvest. In addition to the nozzle configuration and wind speed treatments there was an unsprayed control treatment. Treatments were applied to fully guarded single trees with four replications arranged in a split-plot design experiment with nozzle configuration as the main plot and wind speed as the sub plot.

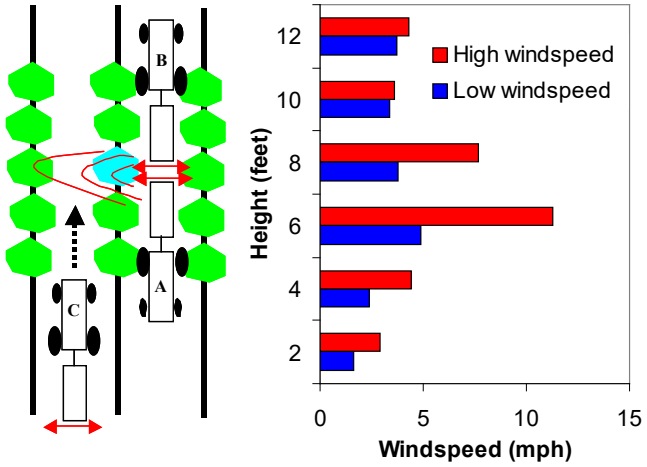
In a second *spray efficacy* experiment the cover sprays were applied with each nozzle configuration in a "high-risk" program where Topsin M was excluded order to increase disease pressure. Each plot was three adjacent rows wide to account for overspray effects and five trees along the row. There were four replications of each nozzle configuration. Two samples of 20 fruit were removed from one tree in the center row of each plot at harvest, representing the upper and lower canopy. The incidence of sooty blotch (a disease complex caused by *Peltaster fructicola*, Johnson, *Geastrumia polystigmatis* Batista and M.L. Farr, *Leptodontium elatius* (G. Mangenot) De Hoog and other fungi) and flyspeck (Zygophiala jamaicensis E. Mason) was measured on each fruit sample.

Data analysis. Data were analyzed using the generalized linear model procedure of the SAS statistical program.

Results and discussion.

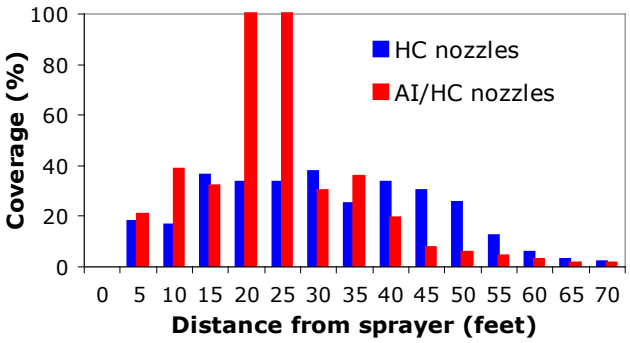
Wind speed control. Preliminary investigations revealed that two sprayers were necessary to achieve a (relatively) constant lateral wind speed across the tree canopy at any given height. However, this type of fan generated considerable variability in wind speeds at different heights within the canopy (Figure 1). Regulating the tractor rpm to generate wind speeds at the fan manifold of either 40 mph or 60 mph resulted in the "low" and "high" wind speed profiles described in Figure 1, respectively. The measured wind speeds reached a maximum at a height of 6 feet above the soil line of 4.9 and 11.3 mph for the "low" and "high" wind speeds respectively. Wind speeds at a height of 2 feet above the soil line were less that one third of the maximum value for each level, whereas at 12 feet they were 76% and 38% of the maximum value for "low" and "high" levels respectively. Although a uniform wind speed could not be generated over the entire canopy using axial fans, the efficacy of HC and AI/HC sprayer configurations was evaluated at three distinctly different levels of across-row wind speed ("zero", "low", and "high") using this approach.

Fig. 1. Schematic of arrangement for establishing variable across-row wind speeds and actual wind speeds obtained at different heights along the trunk of mature 'Pink Lady'/M.7 apple trees.



Spray drift. The two sprayer configurations had different drift profiles under still conditions in an open field, as described in Figure 2. The standard HC nozzle configuration tended to produce more drift than the AI/HC nozzle configuration, with greater spray coverage on water sensitive cards at distances of 40 feet or more from the sprayer. The AI/HC configuration resulted in 100% coverage of water sensitive cards that were placed on the ground at distances of 20 and 25 feet from the sprayer whereas coverage from HC nozzles never exceeded 40%. The

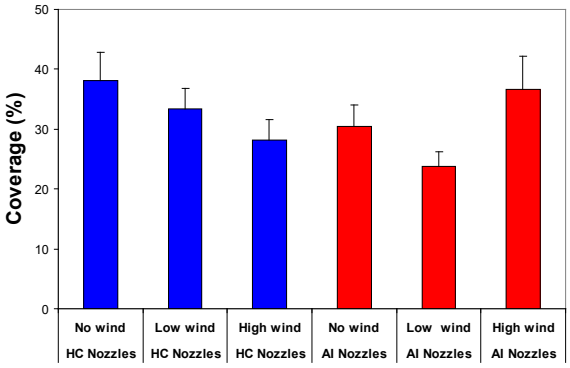
Fig. 2. Effects of sprayer nozzle configuration on drift under still conditions in an open field. Drift assessed as % spray coverage on water-sensitive cards.



dramatic increase in spray fallout from AI/HC nozzle configuration at a distance of 20-25 feet from the sprayer reflects the behavior of droplets formed by the AI nozzles. The larger droplets formed by AI nozzles appeared to exit the air support provided by the sprayer fan more quickly as described in earlier studies (3), falling to the ground much sooner than smaller droplets generated by HC nozzles.

The different aerodynamic behaviors of droplets produced by the two nozzle types may have important implications for spray efficacy in an orchard environment quite apart from any effects of droplet size. An orchard sprayer equipped with HC nozzles will produce a relatively constant and diffuse level of drift over a distance of 50 ft from the sprayer that will result in minimal carry-over of a fine spray that is unlikely to penetrate very far into the canopy of trees that are two or more rows over from the row adjacent to the sprayer. AI nozzles on the other hand produce a more intense peak of spray drift that may result in significant overspray of the canopy of trees in the row(s) adjacent to the sprayed row. The extent of this overspray will depend on the row spacing: at 20 feet between rows it will be minimal, but there may be significant overspray when the distance between rows is in the range from 12-16 feet. The extent of this overspray will depend not only on the nozzle type but also on attenuation of spray by the tree canopy in the row immediately adjacent to the sprayer.

Fig. 3. Effects of nozzle configuration and across-row wind speed on spray coverage. Spray coverage was measured on water-sensitive cards stapled to the upper side of leaves at different heights in the canopy.



Spray coverage. There was a significant main effect of above-ground height on spray coverage (P<0.0001), with coverage increasing from approx. 15% 2 feet above the soil line to approx. 50% 12 feet above the soil line. The effect of wind speed on spray coverage was not statistically significant (P=0.15), but there was a significant interaction between nozzle configuration and wind speed (P=0.02). There was a trend for decreasing coverage with increasing across-row wind speeds from HC nozzles, whereas spray coverage from the AI/HC nozzle configuration was greatest at the "high" wind speed level (Figure 3). Spray coverage was poorer with the AI/HC nozzle configuration compared to the HC nozzle configuration when there was no wind, but was better with the AI/HC nozzle configuration under high across-row wind speeds (data not shown).

Spray efficacy. Data from the thinning experiment were not conclusive. Due to severe fire blight infection in the test trees fruit set data could not be analyzed. The incidence of fruit with sooty blotch or flyspeck at harvest was 25% and 40% higher respectively when the cover sprays were applied with the AI/HC nozzle configuration compared to the standard HC configuration, however this difference was only statistically significant for flyspeck (P=0.02). An increased incidence of sooty blotch and flyspeck on fruit from trees sprayed with the AI/HC nozzle configuration may reflect the slight decrease in total spray coverage at no and low across-row wind speeds compared to a standard HC nozzle configuration (Figure 3). Alternatively, the reduction in fungicide efficacy may result from differences in droplet size and the pattern of chemical distribution on the fruit surface between the two nozzle configurations.

Literature Cited

[1] Fox et al., 1990. Downwind residue from air spraying of a dwarf apple orchard. Transactions of the ASAE 33(4): 1104-1108.

[2] Fox et al., 1985. A model study of the effect of wind on air sprayer jets. Transaction of the ASAE 28(1): 83-88.

[3] http://www.teejet.com/ms/teejet/newsStory.asp?ID=96

[4] Jaeken et al., 2003. Nozzle choice and its effect on spray deposit and distribution, uptake, drift and biological efficacy in standard apple orchards (Malus sylvestris, cv Jonagold). Pflanzenschutz-Nachrichten Bayer 56(2): 326-353.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: AP-06-604

Project Title: High temperature stress to apple fruit peel: physiology and detection

PI:	Lailiang Cheng	**Co-PI(2):**	Larry Schrader
Organization:	Cornell University	**Organization:**	WSU-TFREC
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City:	Ithaca	**City:**	Wenatchee
State/Province/Zip	NY 14853	**State/Province/Zip:**	WA 98801

Total project amount (2007): $42,488

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

Item	Year 1:	Year 2: (2007)	Year 3:
Salaries	15,000	**16,188**	
Benefits	7,479	**8,300**	
Wages			
Benefits			
Equipment	0	**0**	
Supplies	8,000	**8,000**	
Travel	0	**0**	
Miscellaneous	0	**0**	
Total	30,479	**32,488**	

Footnotes:

Salaries budgeted are for a 0.5FTE postdoc working on this project. There is a slight increase in the budget request for year 2 because effective January 1, 2007 the minimum salary for postdocs at Cornell is $32,375 per annum and the fringe benefit rate for 2007 has increased to 51.27%.

Supplies include chemicals for enzyme and metabolite analyses, oxygen evolution measurements, analytical columns, guard columns, pigment standards, solvents, vials and service for the HPLC separation and quantification of xanthophylls and other carotenoids.

Budget 2: (Budget for year 2 is highlighted)

Organization Name: WSU **Contract Administrator:** M.L. Bricker and Sally Ray
Tel: 509-335-7667; 509-663-8181 **Email address:** mdesros@wsu.edu ; saray@wsu.edu

Item	Year 1	Year 2 (2007)	Year 3 (fill in year here - optional)
Salaries	7,000	**7,000**	
Benefits	770	**770**	
Wages			
Benefits			
Equipment	0	**0**	
Supplies	2,230	**2,230**	
Travel	0	**0**	
Miscellaneous	0	**0**	
Total	10,000	**10,000**	

Footnotes:

WSU-TFREC salaries budgeted are timeslip (hourly labor) for setting up the system for monitoring fruit surface temperatures, taking fruit samples, harvesting fruit and measuring chlorophyll fluorescence. The fringe benefit rate is 11%.

WSU-TFREC supplies include temperature sensors and incidental supplies and shipping peel samples from WA to Ithaca.

Objectives

The overall objective is to better understand the underlying physiology of high temperature stress to apple fruit peel with an ultimate goal of detecting and reducing high peel temperature-induced fruit disorders both preharvest and postharvest. The specific objectives are:

1) To determine how high temperature affects the balance of photooxidation and photoprotection of apple fruit peel, leading to sunburn browning;
2) To determine if chlorophyll fluorescence reflects the damage of high temperature on fruit peel and varietal differences during the growing season;
3) To explore the possibility of using chlorophyll fluorescence to detect high temperature-induced disorders prior to the development of symptoms during postharvest storage.

Significant Findings (2006)

1) Maximum photosystem II (PSII) quantum efficiency (Fv/Fm) of the sun-exposed peel of well-exposed fruit in the southwest canopy decreased during the day in response to high peel temperatures, and very little recovery was made during overnight dark relaxation, indicating that the high peel temperature has damaged the PSII centers of the peel.

2) After a couple days of high temperature exposure, more fruit in the west side of the canopy had very low Fv/Fm value than those in the east side. This difference corresponds to the different profiles of peel temperatures and sunburn occurrence between the two sides of the canopy. This along with the diurnal Fv/Fm data indicates that Fv/Fm is a very sensitive indicator of high temperature stress in apple peel.

3) Compared with the non-sunburned peel, the sunburned peel had lower chlorophyll content, lower Fv/Fm, lower net oxygen evolution rate, and lower activities of key photosynthetic enzymes, but higher activities of antioxidant enzymes and higher content of antioxidant metabolites and higher xanthophyll cycle activity on a chlorophyll basis, and higher hydrogen peroxide and malondialdehyde content. This indicates that high peel temperature most likely has increased the photooxidation potential, rather than decreased the photoprotective capacity of fruit peel.

4) Controlled temperature treatments of fruit peel samples in the dark showed that high peel temperature led to decreases in Fv/Fm and net O_2 evolution, and appearance of "K" step in chlorophyll a fluorescence transient. This indicates that high temperature has damaged the oxygen evolution complex of the PSII, leading to oxidative stress.

Methods

1. Determine diurnal changes of Fv/Fm in relation to peel temperature: This experiment was carried out on mature Gala/M.9 trees (spacing:15 X 6.5 feet) at WSU TFREC on July 21. Fifty well-exposed fruit on the southwest part of the canopy were selected the day before and the temperature of the sun-exposed side of each fruit was monitored with a thermocouple connected to a data logger. In addition, the temperature of the shaded side of 3 fruit was also monitored along with ambient temperature. Every 4 hours starting from pre-dawn (5:00), ten fruit were dark-adapted for 30 min and then measured for Fv/Fm. The pre-dawn values of Fv/Fm were also measured the next day.

2. Determine the distribution of fruit peel Fv/Fm and sunburn occurrence on the east side and west side of the canopy after exposure to high temperatures. Ten well-exposed fruit from each side (east and west) of the canopy were selected and their peel temperatures were monitored as above from July 23 to 25. All the fruit from the east side and west side of the canopy were harvested separately and Fv/Fm of the sun-exposed peel was measured at pre-dawn on July 25. The percentage of fruit with

sunburn was counted on separate trees with similar canopy size and structure in the morning on July 26.

3. Compare the sunburned and non-sunburned fruit in terms of photosynthetic capacity, chlorophyll fluorescence, and antioxidant system. The sun-exposed peel of non-sunburned and sunburned fruit (80 fruit each) was taken from the east and west side of the canopy from 9:15 to 10:00AM and from 4:00 to 4:45PM on July 25, respectively. The samples were immediately frozen in liquid nitrogen and stored until analysis.

4. Determine chlorophyll fluorescence and oxygen evolution of the sun-exposed peel of 'Fuji' fruit in response to controlled high temperature treatments. At approximately 100 days after full bloom (mid-August), well-exposed fruit on the west side of the canopy of Fuji/M.9 trees were taken right after sunset and the sun-exposed side of each fruit was marked. All the fruit were dark-adapted overnight at 22C and fruit peel samples (0.5 mm thick, 1 cm^2) were taken from the sun-exposed side. The peel samples were placed between two layers of wet paper towel and the assembly was put onto the bottom of a small aluminum foil vessel with the top covered with aluminum foil. Then, the vessel was directly floated on water in a water bath, the temperature of which was controlled by a refrigerated water bath and the temperature equilibrium between the fruit peel and water was reached within 1 to 2 min. The peel samples were exposed to 25, 35, 40, 42, 44, 46 or 48°C in the dark for 30 min. Chlorophyll a fluorescence transient and photosynthetic O_2 evolution were measured after the peel samples had been kept in the dark at room temperature for 30 min after each temperature treatment.

Results and Discussion

1. Diurnal changes of PSII quantum yield (Fv/Fm) of the sun-exposed peel and the shaded peel in relation to peel temperature on a hot day in central WA

On July 21, the temperature of the sun-exposed peel of well-exposed fruit in the southwest part of the canopy increased almost linearly from 5:00 to 15:20, reaching 50C at 15:20, and then stayed above 45.9C till 17:30. In contrast, the shaded peel only reached a maximum of 40.7C between 17:00 and 17:30 when the highest ambient temperature was 35.5C.

Fv/Fm of the sun-exposed peel at pre-dawn was around 0.53, and remained essentially unchanged till 9:00. However, the Fv/Fm decreased linearly from 9:00 to 17:00, and then increased slowly from 17:00 to 5:00 the next day. The rapid decrease in Fv/Fm from 9:00 to 17:00 corresponds well with the period of high fruit peel temperature. Even at pre-dawn the next day Fv/Fm has not fully recovered to the previous pre-dawn level, indicating severe oxidative damage has occurred in the peel. In contrast, Fv/Fm of the shaded peel was higher than in the sun-exposed peel and remained unchanged throughout the entire day. The large error bar in the Fv/Fm data for the sun-exposed peel is due to the fact that there were large variations in Fv/Fm value among individual fruits.

2. Distribution of fruit peel Fv/Fm and sunburn occurrence in the east side and west side of the canopy after exposure to high temperatures.

We compared the distribution of fruit Fv/Fm between east and west sides of the canopy after a couple days of high temperature exposure (July 23 and 24). As shown in Fig 2, the % fruit with an Fv/Fm value less than 0.1 was much higher in the west side (34%) than in the east side (9%) of the tree canopy whereas the % fruit with an Fv/Fm value between 0.7 and 0.8 was much lower in the west side (13%) than in the east side (34%). These numbers correspond well with the difference in temperature profiles between the two sides. Interestingly, both east and west sides had 16 to 18% of the fruit with an Fv/Fm value higher than 0.8, which indicates that both sides have equal number of shaded fruit. Counting the number of sunburned fruit on each side showed that the west side had 21.9% with sunburn whereas the east side had only 6.1%.

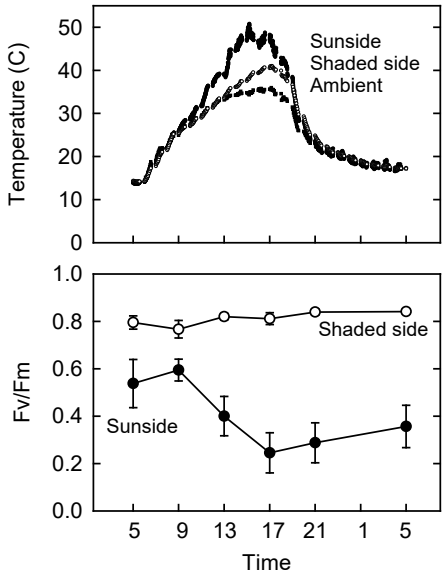


Fig 1

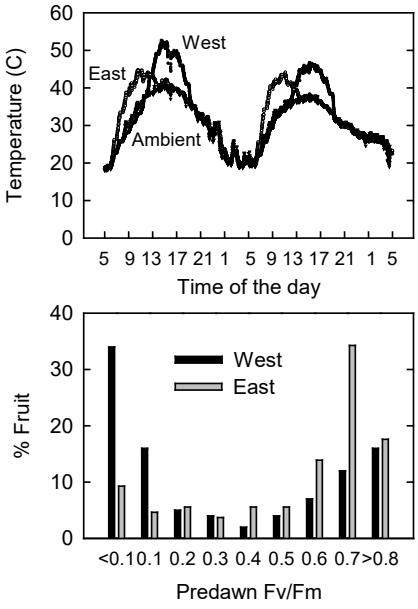


Fig 2

Fig 1. Diurnal changes of peel temperature and Fv/Fm in the sun-exposed peel and the shaded peel of well-exposed fruit in the southwest part of the canopy on July 21, 2006.

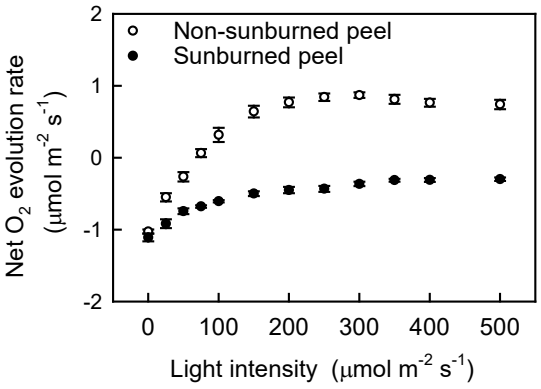
Fig 2. Diurnal changes of the temperature of the sun-exposed peel in the east and west sides of the canopy on July 23 and 24 and fruit distribution in terms of Fv/Fm measured at pre-dawn on July 25.

3. Comparison of sunburned and non-sunburned fruit peel

Photosynthetic oxygen evolution: As light level increased, net O_2 evolution rates for both non-sunburned and sunburned peels increased almost linearly first, then reached a saturation point, beyond which O_2 evolution showed little response to increasing light level (Fig 3). At each given light level, photosynthetic O_2 evolution rate was significantly lower in the sunburned peel than in the non-sunburned peel (Fig. 3). The quantum yield for O_2 evolution (the initial slope of each curve) was much lower in the sunburned peel than in the non-sunburned peel, whereas the light saturation point was higher in the sunburned peel than in the non-sunburned peel.

Hydrogen peroxide and malondialdehyde: The sunburned peel had higher concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA, an indicator of oxidative lipid metabolism) compared with the non-sunburned peel (Fig. 4), which clearly indicates that oxidative damage has occurred.

Activities of key photosynthetic enzymes: Compared with the non-sunburned peel, the sunburned peel had lower activities of key photosynthetic enzymes, including ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), NADP-glyceraldehyde-3-phosphate dehydrogenase, phosphoribulokinase, and stromal fructose-1,6-bisphosphatase (Data not shown). However, the activities of these enzymes decreased to a lesser extent than the net O_2 evolution rate.



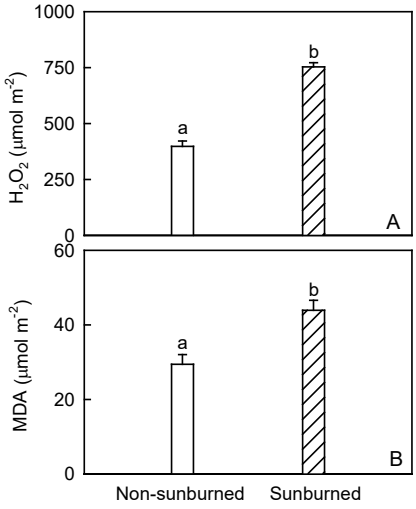


Fig 3 Fig 4

Fig 3. Light response of net oxygen evolution of sunburned and non-sunburned peels.

Fig 4. Hydrogen peroxide and malondialdehyde (MDA) content in sunburned and non-sunburned peels.

Reflectance and pigments: The sunburned peel had higher reflectance averaged over 400 – 700 nm. Reflectance spectra showed that the sunburned peel reflected more light in the range between 420 and 700 nm than the non-sunburned peel (Data not shown).

Chlorophyll, xanthophyll cycle pool size and lutein contents expressed on a peel area basis, and β-carotene and neoxanthin contents expressed on a peel area or Chl basis were lower in the sunburned peel than in the non-sunburned peel, whereas the contents of xanthophyll cycle pool size, zeaxanthin and antheraxanthin, and lutein expressed on a chlorophyll basis were higher in the sunburned peel than in the non-sunburned peel. This indicates that more chlorophylls were degraded relative to xanthophylls and other carotenoids. Almost all the xanthophyll cycle pool was converted to zeaxanthin and antheraxanthin in both sunburned and non-sunburned peels.

Antioxidant enzymes and metabolites: Activities of superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase were higher in the sunburned peel than in the non-burned peel, whereas there was no significant difference in catalase activity between the two peel types (Data not shown).

The content of total ascorbate (reduced + oxidized), total glutathione (reduced + oxidized) and reduced glutathione was higher in the sunburned peel than in non-sunburned peel, but the ratio of both reduced ascorbate to total ascorbate and reduced glutathione to total glutathione were lower in the sunburned peel than in the non-sunburned one. No significant difference was observed in reduced ascorbate content between the sunburned and the non-sunburned peels (Data not shown).

Chlorophyll a fluorescence transients: When overnight dark-adapted fruit was exposed to a saturating pulse of light, chlorophyll a fluorescence showed a characteristic rise from minimal fluorescence (Fo) to maximum fluorescence (Fm) in the non-sunburned peel (Fig 5). However, the fluorescence signal of the sunburned peel was much lower and reached Fm at a much earlier stage

followed by little change in fluorescence intensities. Chlorophyll fluorescence turns out to be the most sensitive of all the responses we have measured on the sunburned peel.

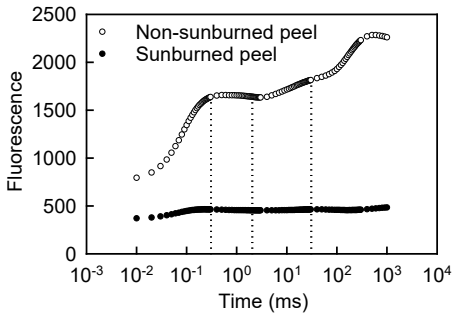


Fig 5. Chlorophyll fluorescence induction curves of sunburned and non-sunburned fruit.

4. Chlorophyll a fluorescence transients, Fv/Fm and photosynthetic oxygen evolution of the sun-exposed peel in response to temperature treatments

When the peel temperature increased from 25 to 35C, neither Fo nor Fm showed any significant change (Fig 6A). However, as peel temperature increased further, Fm decreased whereas Fo increased. In addition, the shape of the chlorophyll fluorescence induction curve changed. When the peel temperature reached 44 - 48°C, a very clear peak (called "K" step) at 300 μs appeared, followed by a pronounced dip. After exposure to 46 and 48°C, maximal fluorescence was already reached at "K" step followed by a rapid decrease to a level close to or below Fo (Fig. 6A). Fv/Fm changed very little from 25 to 40°C, and then dropped rapidly with further increase in temperature (Fig. 6B).

Net photosynthetic O_2 evolution rates remained unchanged as temperature increased from 25°C to 40°C, then dropped rapidly with any further increase in temperature (Fig. 6C). After exposure to 46 and 48C, the net O_2 evolution rate became negative. However, heat stress showed no effects on dark respiration.

Decreases in Fv/Fm and net O_2 evolution, coupled with appearance of "K" step in chlorophyll a fluorescence transient indicate that high temperature has damaged the oxygen evolution complex of the PSII, leading to oxidative stress. However, the lack of a clear K step in the sunburned peel (Fig 6) suggests that there is interaction between high peel temperature and high light, which needs to be investigated before getting a complete understanding of the mechanisms involved.

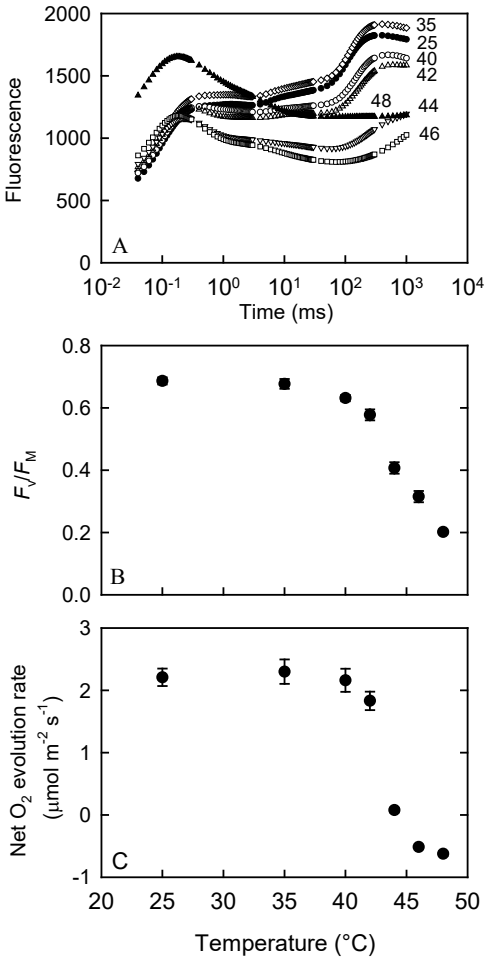


Fig 6. Chlorophyll fluorescence transient (A), maximum PSII efficiency, Fv/Fm (B), and net oxygen evolution of the sun-exposed peel of Fuji fruit in response to temperature treatments.

CONTINUING PROJECT REPORT **YEAR**: 2 of 2
WTFRC Project Number: 3055-7938

Project Title: Identifying disease prevention benefits of apple consumption

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Cooperators: Dr. Ines Hanrahan, Washington Tree Fruit Research Commission
Stemilt Growers, Inc., Wenatchee

NB: No additional funding is requested this year. We will complete our analyses of stored fruit from the 2006 crop during 2007. Our final report will be submitted in 2008 during the next proposal cycle.

Budget 1: (*Required information – please complete all information***)**
Organization Name: WSU **Contract Administrator:** Ralph Cavalieri
Telephone: 509-335-4563 **Email address:** AgResearch@wsu.edu

Item	**Year 1:** 2005-06	**Year 2:** 2006-07	**Year 3:**
Salaries	19,320	20,286	
Benefits	1,789	1,862	
Wages	8,000	8,000	
Benefits	880	880	
Equipment	0	0	
Supplies	16,000	16,000	
Travel	800	800	
Miscellaneous	0	0	
Total	46,789	47,828	

Footnotes: [1]PhD student in Pharmaceutical Sciences (Jaime A. Yáñez), without tuition.
[2]Benefits in 2005-06 ($1,499 + $290 Health) & 2006-07 ($1,558 + $304 Health).
[3]Non-student timeslip for sample collection, preparation, and data entry.
[4]Field, laboratory, and office supplies, chemicals, reagents, solvents, enzymes, instrument maintenance, and animals.
[5]Travel to orchard sites and storage facilities in Wenatchee area.

Objectives and Schedule of Accomplishments:

The goals of this study are to identify the phytochemical constituents and disease prevention capacity of different varieties of Washington apple fruit at commercial harvest and after commercial storage and shelf-life conditions. To achieve these goals, the following specific objectives will be accomplished:

1) Quantify several antioxidant, phytochemical constituents (quercetin, kaempferol, naringenin/naringin, phoretin/phloridzin) and vitamin C (ascorbic acid) in the fruit skin and flesh of different Washington State apple varieties (Gala, Golden Delicious, Red Delicious, Granny Smith, and Fuji) at commercial harvest, after commercial post-harvest treatment and storage, and after an appropriate shelf-life interval.
2) Assess the potential health benefits of apple extracts from Objective 1 by utilizing *in vitro* experimental models for inhibition of cancer cell profileration and inflammation of chondrocytes (cartilage cells), and an *in vivo* animal model for hyperlipidemia (cholesterol-lowering capacity). Selected samples that demonstrate significant differences in constituent concentrations (measured in Objective 1) will be tested.

Our proposed schedule of accomplishments include analyzing the second year of duplicate Gala, Golden Delicious, Red Delicious, Granny Smith, and Fuji samples that were harvested by Ines Hanrahan and are currently stored under commercial conditions at Stemilt Growers in Wenatchee. These analyses include fruit maturity, phytochemical constituents, and anti-oxidant, anti-cancer, anti-inflammatory, and anti-hyperlipidemia activities of the extracts. Vitamin C analysis is currently in progress. Anti-hyperlipidemia and additional anti-cancer and anti-inflammation studies will be completed after the fruit that are currently in storage are analyzed this spring for phytochemical constitutents. Our results will be reported at the annual meeting of the Washington State Horticultural Association in December 2007, at next year's WTFRC research review, and then published in scientific journals at the conclusion of the study.

Significant Findings:

- We developed a new HPLC method for analysis of phytochemicals, which include quercetin, kaempferol, phoretin/phloridzin, naringenin/naringin enantiomers, and ellagic acid.
- We successfully applied this HPLC method to quantify these phytochemicals in the flesh and peel of Gala, Golden Delicious, Red Delicious, Granny Smith, and Fuji apples, both at harvest and after a two-week, non-refrigerated shelf-life interval.
- We determined that both flesh and peel tissues of each variety contain all of the detectable compounds. However, there were differences in the amounts of these compounds among varieties. In general, peel tissue contained higher concentrations of these compounds than flesh tissue. CA storage often resulted in reduced levels of these phytochemicals, whereas the non-refrigerated shelf life did not.
- We determined that peel tissue consistently had greater anti-oxidant activity than flesh tissue, but neither CA storage nor the non-refrigerated shelf life affected anti-oxidant activity.
- We established *in vitro* cells lines for melanoma, prostate, breast, liver, and colon cancers and determined anti-cancer activity of several apple extracts in these cancer lines. Apple extracts demonstrated dose-dependent anti-cancer activity and differences inhibiting cancer cell growth.
- We developed an *in vitro* anti-inflammatory assay in chondrocytes and validated anti-inflammatory activity using biomarkers for NO, sGAG, and PGE_2.
- We also developed an *in vitro* inflammatory bowel disease (IBD) model in HT-29 (colorectal adenocarcinoma) cells and validated the potential of apple extracts in reducing IBD by a marked reduction in PGE2 levels.

Methods:

Sampling methods, HPLC phytochemical and spectrophotmetric anti-oxidant analyses, the *in vitro* anti-cancer and anti-inflammation models, and the *in vivo* hyperlipidemia model are as described in our original proposal and previous progress report.

Vitamin C analysis. Because of the apparent low concentration of Vitamin C (ascorbic acid) in apple flesh tissue, we have modified our method for measuring ascorbic acid (AsA). We are using a slightly modified version of Ma and Cheng (2003), reducing oxidized AsA with glutathione and then measuring total AsA spectrophotometrically as it reacts with the enzyme ascorbate oxidase.

Results and discussion:

During 2005 and 2006 we received shipments of Gala, Golden Delicious, Red Delicious, Granny Smith, and Fuji apples from orchards in north-central Washington. Another set was stored under commercial storage conditions. Upon receipt, we immediately measured fruit maturity (weight, firmness, soluble solids, and starch index), and collected and froze peel and flesh tissues for later analysis. We also exposed another set of fruit from each variety to one- and two-week (only two-week in 2006) non-refrigerated "shelf-life" intervals, after which we measured fruit maturity and froze peel and flesh tissues for analysis.

Soluble solids increased after the shelf-life period only at harvest (Table 1), but firmness changed little (not shown). Starch was absent from at-harvest shelf life through storage. Weight loss after a 2-week shelf life was less for Red Delicious and Granny Smith (2.6-3%) than the other varieties (4.9-5.7%).

Table 1. Soluble solids & starch index at harvest & after commercial CA storage, both before & after a 2-week, non-refrigerated shelf life.

	At harvest		After storage	
	0 week	**2 week**	**0 week**	**2 week**
Soluble solids (%)				
Gala	10.9	12.4	12.6	12.9
Golden Delicious	13.6	15.3	15.6	15.5
Red Delicious	12.4	14.5	14.8	14.4
Granny Smith	10.4	11.4	11.9	11.8
Fuji	13.8	15.5	14.6	15.1
Starch index				
Gala	2.8	6.0	6.0	6.0
Golden Delicious	3.6	5.0	5.0	5.0
Red Delicious	2.5	4.9	5.0	5.0
Granny Smith	1.9	5.8	6.0	6.0
Fuji	2.6	5.9	6.0	6.0

The most commonly reported phytochemical constituents found in apples are quercetin, phloretin/phloridzin, procyanidins, catechin, epicatechin, and chlorogenic acid (Escarpa & Gonzalez 1998). While our study is not meant to be an exhaustive analysis of all apple phytochemicals, the HPLC method we developed allowed us to simultaneously measure both the free aglycone and glycoside forms of quercetin, phloridzin/phloretin, ellagic acid, kaempferol, the R- and S-enantiomers of naringenin/naringin, and other novel polyphenolic compounds (Fig. 1).

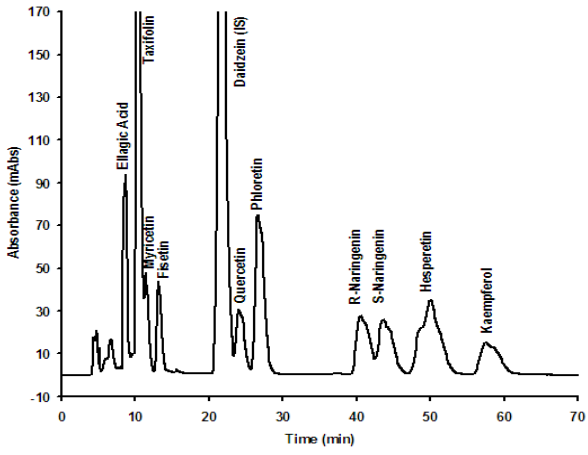

Figure 1. HPLC chromatogram of detectable aglycone phytochemicals in apple extract (IS=internal standard.)

Quercetin was the main polyphenol measured. Peel tissue had significantly higher concentrations of quercetin than did flesh tissue, with Gala peel having the highest at harvest (Fig. 2). CA storage reduced quercetin levels, especially in flesh tissue. Most quercetin was in the glycoside form, except in flesh tissue after storage. In addition, quercetin appeared to be relatively stable in just harvested apples after the two-week, non-refrigerated shelf-life interval (not shown). Although there were few differences in phloridzin/phloretin concentrations between peel and flesh tissues and among varieties, Red Delicious peel had significantly higher concentrations, both at harvest and after the two-week shelf-life, than did other varieties (not shown). CA storage, however, resulted in greatly reduced concentrations of phloridzin/phloretin, especially in flesh tissue. Quercetin has been reported to have protective effects against both cancer (Lamson & Grignall 2000) and cardiovascular disease (Peng & Kuo 2003, Kamada *et al*. 2005). Quercetin has also been shown to reduce intestinal inflammation in laboratory animals (Galvez 1996, Sanchez *et al*. 2002). Recently, strawberry extracts rich in phloretin and phloridzin have recently being reported to diminish the proliferation of colon cancer cells HT29 and breast cancer cells MCF-7 in an *in vitro* study (Olson *et al.* 2006)

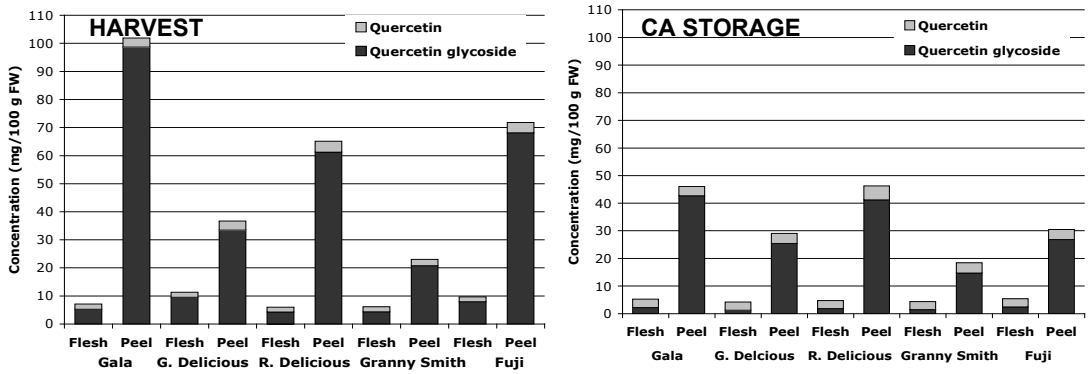

Figure 2. Total quercetin (aglycone plus glycoside forms) concentrations in flesh and peel tissues at harvest and after several months of refrigerated CA storage of apple varieties harvested in 2005.

We measured both R- and S-naringenin/naringin in peel and flesh tissues of all apple varieties (not shown). S-naringenin/naringin varied among varieties at harvest and appeared to be highest in Red Delicious tissues. However, concentrations of the S enantiomer decreased during the two-week, non-refrigerated shelf-life interval due to a significant decrease in the glycoside form, S-naringin. In contrast, for all varieties except Gala, R-naringenin (free aglycone form) increased during the two-week shelf-life. This increase in the aglycone form would likely affect bioavailability and retention of this flavonone in the human body. The concentrations of both R- and S- naringenin/naringin were lower after CA storage, but a 2-week, non-refrigerated shelf-life interval did not affect their concentrations in stored apples (not shown). There were also no differences in concentrations in peel and flesh tissues of stored apples. Both naringenin and naringin have been shown to protect against mutagenesis (Bear & Teel 2000), and to inhibit cancer cell growth (Kanno *et al*. 2005) and hyperlipidemia (Borradaile *et al*. 2003). Because naringenin is a chiral compound, one or both of its enantiomers (R and/or S) could contribute to its health benefits.

Although we did not originally intend to measure ellagic acid, our HPLC method allowed us to quantify this phenolic compound in apples for the first time. Ellagic acid concentrations were high in both flesh and peel tissues, and remained high even after the 2-week, non-refrigerated shelf-life period and CA storage (not shown). Little is known about ellagic acid's health benefits in humans, as most studies have been conducted only in animals and cell cultures. Nevertheless, ellagic acid has antiviral and antibacterial properties (Akiyama *et al.* 2001, Atta-Ur-Rahman *et al.* 2001). In addition, ellagic acid may have anti-cancer effects against liver, esophageal, prostate, and colorectal cancers (Tanaka *et al.* 1988, Stoner *et al.* 1999, Narayanan & Re 2001, Narayanan *et al.* 2002), and it also has been reported to be a potent anti-oxidant (Atta-Ur-Rahman *et al.* 2001, Festa *et al.* 2001). Ellagic acid is found at the highest concentrations in raspberries, strawberries, and pomegranates, but its

presence at significant concentrations in apples could contribute to the health benefits of apple consumption.

We also quantified the flavonoid kaempferol, which is closely related and readily converts to quercetin. Kaempferol concentrations were similar in peel and flesh tissues in all varieties, and neither increased nor decreased during the two-week, non-refrigerated shelf-life interval (not shown). There appeared to be a decrease in kaempferol concentrations, especially the glycoside form, after CA storage.

We tested the peel and flesh tissues of all varieties for anti-oxidant activity. Peel had significantly higher activity than flesh, and Red Delicious peel had the highest activity (Fig. 3). There were no significant losses in activity during storage or after a 2-week, non-refrigerated shelf life (not shown).

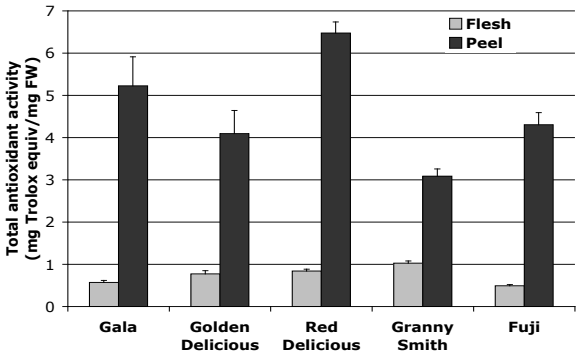

Figure 3. Total anti-oxidant activity of flesh and peel tissues of combined at harvest, after storage, and non-refrigerated shelf-life periods of apple varieties harvested in 2005. Error bars are standard error of the means.

We have only tested anti-cancer activity so far in Red Delicious peel and flesh extracts, which exhibited dose-dependent anti-cancer activity in all cell lines (Fig. 4). The lower IC_{50} (concentration at 50% cancer cell inhibition) of the peel extract indicates that it was more active against breast (MDA-MB-231) and melanoma (A-375) cancer cells than was the flesh extract, while the low IC_{50} in both peel and flesh extracts indicates equal activity against liver (Hep-G2) cancer cells by these apple tissues (Table 2).

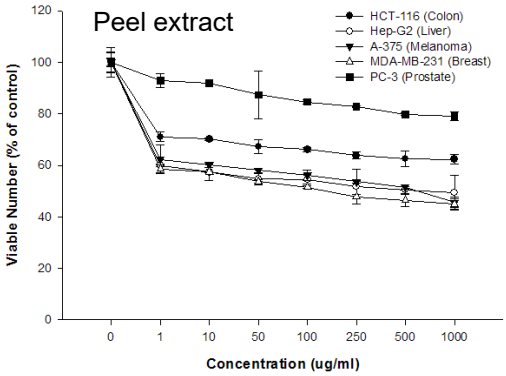

Figure 4. Anti-cancer activity of Red Delicious peel extract (flesh extract not shown).

Table 2. Concentration at 50% cancer cell inhibition (IC_{50}) of Red Delicious peel and flesh extracts.

	IC_{50} (µg/ml)	
Cancer cell line	Peel	Flesh
Colon (HCT-116)	4,890	5,890
Liver (Hep-G2)	320	326
Melanoma (A-375)	318	1,456
Breast (MDA-MB-231)	100	1,730
Prostate (PC-3)	11,672	12,125

The anti-inflammatory activity of the different peel and flesh extracts of the different apple varieties was assessed and it was observed that Red Delicious extract demonstrated the highest anti-inflammatory activity in all the measured biological markers. Peel had significantly higher anti-inflammatory activity than flesh. All the extracts reported a dose-dependant anti-inflammatory activity. The observed reduction in pro-inflammatory molecules such as tumor necrosis factor-α (TNF-α) of Red Delicious peel and flesh extracts (Fig. 5) indicate a potent anti-inflammatory effect.

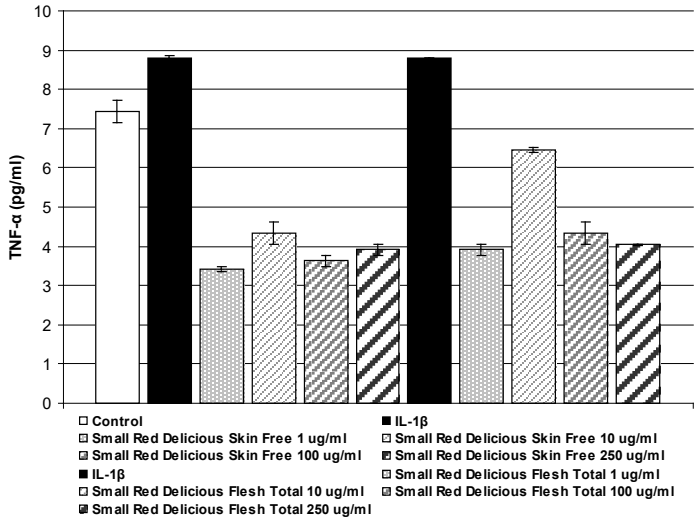

Figure 5: Anti-inflammatory activity (tumor necrosis factor-α [TNF-α]) of Red Delicious peel and flesh extracts

The presence of both known and novel phytochemicals in apple fruit produced in Washington State, and of their anti-oxidant and anti-cancer properties, provides a strong basis for claims regarding the health benefits of consuming Washington apples. More studies should be conducted on the effects of reduced phytochemical concentrations in apples following CA storage, especially in regards to the disease prevention capacity of consuming CA-stored Washington apples.

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CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: 439934

Project Title: Temperature effect on pollen germination/tube growth

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Cooperators: Leon Combs, Va Tech AHS-AREC
Tory Schmidt, Washington Tree Fruit Research Commission

Budget 1:
Organization Name: Virginia Polytechnic Institute and State University
Contract Administrator: Sharron D. McElroy (Contracts and Grants Administrator)
Telephone: (540)-231-2068 Email address: mcelroys@vt.edu

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	20,000	20,600	21,218
Benefits	7,350	8,845	9,110
Wages			
Benefits			
Equipment			
Supplies	1,000	1,316	1,000
Travel			
Contractual services and repairs	1,000	1,000	1,000
Miscellaneous			
Total	29,350	31,761	32,328

***Note:** Submitted in 2006 an amended budget for 3% salary increase for Research Specialist, Leon Combs, and revisions for fringe benefits rates, 40.75% for '06 and 44.5% for '07. Also requested a $316 increase in supplies for cost of Fuji trees for research in '06.

Long-term goal: Better understanding of pollen germination, pollen tube growth, and the potential ability of bloom thinners under a range of environmental conditions.

Specific objectives:

- Determine the effect of temperature on pollen germination and growth in styles under temperatures ranging from 35ºF to 95ºF.
- Develop a model for pollen tube growth during a typical diurnal flowering period.
- Attempt to develop a test that can simulate pollen tube growth in fluctuating temperatures and correlate it with pollen tube growth in flower styles in the orchard.
- Conduct some field studies to further evaluate selected bloom thinning compounds; to determine effective application rates and phytotoxic potential.

2006 objectives:

- Study the effects of alternating temperatures (35ºF to 75ºF) and light on pollen germination and tube growth (Table 1).

- Determine pollen germination and tube growth of 'Manchurian' and 'Snowdrift' pollen in "on-tree" 'Golden Delicious' and 'Gala' pistils and 'Fuji' pistils on spurs using alternating light and temperature regimes in regulated growth control chambers.

- Determine the minimum time required for pollen tubes to grow to the stylar base of 'Golden Delicious' and 'Gala' pistils at optimal and sub-optimal temperatures.

- Assimilate data into development of a functional model of pollen tube growth in selected apple varieties for growers to use in conjunction with thinning programs to reduce chemical/labor costs.

- Conduct limited field studies involving specific bloom thinners and timing of applications after pollination at full bloom.

- Effect of timing of application of pollination inhibitors at full bloom on hand-pollinated 'Fuji'/M.9 flowers on shoots basing timing of thinning treatments on predicted pollination times.

Significant findings:

- Application of Liquid Lime Sulfur + Crocker Fish Oil (LLS+CFO) at 4 hours (72°F) after hand pollination of 'Golden Delicious' pistils "on-tree" stopped all growth of pollen tubes into styles from stigmas. Average temperature for 24 hour period after application was 63°F.
- Application of LLS+CFO at 24 hours (72°F) after hand pollination stopped all growth of pollen tubes to base of styles from stigmas. Average temperature for 24 hour period after application was 67°F. It was predicted that ovule fertilization had not yet occurred under 2006 field conditions.
- LLS+CFO applied at 48 hours (68°F) after hand pollination of 'Golden Delicious' pistils in orchard had little or no effect on growth of pollen tubes into styles from stigmas. Average temperature for 24 hour period after application was 57°F. Ovule fertilization was predicted at 48 hours under field conditions.
- Average number of pollen tubes reaching base of styles of 'Golden Delicious' pistils "on-tree" sprayed 48 hours after hand pollination were similar to styles that received no spray treatments.
- LLS+CFO applied 4 hours after hand pollination of 'Fuji'/M.9 pistils on detached spurs and placed in 75°F/45°F (Light/Dark) rotation for 48 hours stopped all growth of pollen tubes into styles from stigmas.

- Growth progress of pollen tubes in styles affects the success/failure of application of bloom thinner (LLS+CFO).
- Controlled temperature/light tests on 'Gala' and 'Golden Delicious' hand pollinated pistils "on-tree" reinforce data findings from previous year's experiments.
- Model predictions using 2006 full bloom (FB) and temperature data from three growing locations in Washington State show a range of 30 hours (full bloom April 28) at Wenatchee to 102 hours at Omak/Pogue Flats (full bloom April 30) required for ovule fertilization after pollination.

METHODS

Pollen collection: Golden Delicious/M.27 trees grown in root bags were removed from the orchard in early March and placed in cold rooms to delay flowering, then placed in a greenhouse to induce flowering for harvesting pollen. Branches of 'Manchurian' and 'Snow Drift' crabapples were collected from the field and forced to produce pollen in the greenhouse. Some pollen was also collected from trees in the field. Balloon stage flowers with anthers that had not yet dehisced were harvested for pollen. Anthers were removed from stamens of harvested flowers and allowed to dry overnight at room temperature then pollen was screened, placed in glass vials and stored at 0°C in a larger jar containing Drierite. Pollen viability was checked on an agar/sucrose/boric acid mixture by incubating it at room temperature for 1 or 2 hours before scoring for germination under the microscope.

General procedures, growth chamber pollination studies: Gala/M.9 or Golden Delicious/M.27 trees grown in root bags were removed from the orchard row early in March and held in a cold room to delay onset of bloom, then forced in a greenhouse to induce bloom. At late balloon stage 12 flowers /treatment was selected for the pollination experiment. One day before hand pollination of test flowers, all anthers were removed from test flowers to prevent self-pollination. All other flowers on test trees were removed to prevent cross-pollination and to balance the test blossom distribution. Selected flowers were tagged and hand pollinated with '05 pollen. Pollen was applied to stigmas with #2 brush. Trees were then placed in temperature controlled rooms under HPS 1000 watt lamp (approx. 600 $\mu mols \cdot m^{-2}s^{-1}$ at the tree upper canopy) for indicated lengths of time, temperature, and lighting. Flowers were removed from trees at indicated times, placed in labeled glass containers in a solution of 5% sodium sulfite, boiled for 15 min., then refrigerated until microscopic examination. Five styles from each of three flowers were detached from the ovary, dipped in fluorescence solution, squashed between microscopic slides, and allowed to incubate 24 hrs before examination with epi-UV light using a Zeiss HBO-50 high pressure mercury vapor light source at 100X. Collected data included abundance of pollen germination/tube growth (0-10) on the stigma surface (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.

General procedures, field pollination studies: Flowers on orchard trees were selected at late balloon stage for field pollination test conducted to examine the effect of a bloom thinning treatments using Liquid Lime Sulfur + Crocker's Fish Oil (LLS+CFO), applied at selected intervals after pollination, on pollen germination and tube growth. 'Golden Delicious'/M.27 root-bagged trees in the field were used for this experiment. Trees were selected for uniformity and divided into 4 groups. Flowers at full bloom were hand-pollinated with 'Snow Drift' crabapple pollen and any unused flowers were removed. Trees were covered with white insect netting until spray applications to prevent any additional pollination from natural sources. Treatments were applied at 4, 24, and 48 hours after pollination. Only one application of LLS+CFO was applied per treatment. Flower samples were collected 48 hours after the treatment was applied. Procedures for storing and evaluation were as indicated or same for all field experiments. Hourly temperature for period from application to harvesting of samples was also taken.

'Fuji'/M.9 flowers on shoots: Flowers were hand-pollinated with 'Snow Drift' crabapple pollen and placed in under light at 75°F for 4 hours prior to application of materials used for to prevent pollen germination and tube growth. Treatments were applied 4 hours after pollination and the placed in 75°F/45°F (Light/Dark) rotation for 48 hours. Flower samples were taken at 24 and 48 hours after treatments were applied.

Model generation: Models projecting fertilization Figures B-D are generated from data such as shown in Table 1 and Figure A, using the length of time for pollen tubes to reach style bases.

Results and Discussion

In 2004, we found that pollen tubes in "on-tree" flowers under continuous light in growth chambers grew more rapidly to the base of the style than with in vitro flowers on artificial media in either the dark or light (2003). Based on these results, we believe that reserves mobilized to the flowers by the tree and additional ongoing photosynthesis are important influences in determining the rate of pollen tube growth at various temperatures. These findings led us to believe that tests should be conducted "on-tree" for more conclusive results.

Our experiments in 2005, conducted on trees under controlled light/dark temperature regimes continued to build on findings in 2004, yielding significant information on the effects of temperature and light on pollen germination, fertilization, and pollen tube growth on Golden Delicious and Gala. Data from these experiments were used to develop a model to predict pollen tube growth. Our tests involving several pollinizers and fruiting cultivars show that we cannot generalize pollen germination/tube growth rates to all pollinizer/cultivar combinations. Additional tests under in-orchard field conditions are needed to develop a modeling program that growers can use in practice. Any modeling program must be cultivar/pollinizer specific, not an all-encompassing model of one size fits all program.

2006 experiments conducted in temperature and light-controlled growth chambers and in orchard have yielded information that can be used by growers. Temperature effect or growth rate of pollen tubes after pollination has a significant impact on optimal timing of bloom thinner application. Knowledge of growth rate of tubes into styles after pollination is critical for successful thinning practices. Our tests have shown that delaying applications by one day can result in the bloom thinner LLS+CFO having little or no effect on flower fertilization. By understanding pollen tubes growth rate after fertilization in relation to temperatures, growers may save time and money by reducing sprays or by applications of sprays at optimum thinning times. By developing temperature-based pollen tube growth models, we can follow the development of pollen tubes from pollination of stigmas to fertilization of ovules. These models can be developed in consideration of the mode of action of the type of thinners used.

Table 1. Effect of temperature and light on pollen tube growth of 'Manchurian' pollen in 'Golden Delicious' and 'Gala' pistils on tree at 12, 24, 36, 48, 72, and 96 hours under indicated 12-hour temperature and light/dark (LT/DK) rotations after pollination (2006).

Time Temperature (ºF) Light exposure	Average length of longest pollen tubes in styles (mm)						Apparent number of pollen tubes past end of style (per style)					
Gala pistils[z]	12hr	24hr	36hr	48hr	72hr	96hr	12hr	24hr	36hr	48hr	72hr	96hr
55º (LT)/35º (DK)	1.3 c[y]	2.4 b	2.0 c	2.5 b	4.5 b	6.3 b	0 a	0 a	0.0 b	0.0 b	0.3 b	3.7 b
65º (LT)/40º (DK)	2.1 b	2.6 b	3.1 b	3.1 b	5.1 b	7.8 a	0 a	0 a	0.0 b	0.0 b	0.3 b	1.9 b
75º (LT)/45º (DK)	2.9 a	3.8 a	7.4 a	7.6 a	7.3 a	8.2 a	0 a	0 a	19.0 a	22.1 a	21.1 a	16.3 a
G. Del. pistils[z]	12hr	24hr	36hr	48hr	72hr	96hr	12hr	24hr	36hr	48hr	72hr	96hr
55º (LT)/35º (DK)	0.7 c	0.8 c	2.3 c	3.1 c	4.7 b	5.0 b	0 a	0 a	0.0 b	0.0 b	0.3 b	0.5 b
65º (LT)/40º (DK)	1.9 b	2.4 b	4.9 b	5.4 b	6.4 a	7.5 a	0 a	0 a	0.9 b	0.8 b	8.8 a	14.4 a
75º (LT)/45º (DK)	3.1 a	4.2 a	6.6 a	7.3 a	--[x]	--[x]	0 a	0 a	11.0 a	26.8 a	--[x]	--[x]

[z]Pollen viability test (2 hr at room temperature-70ºF) = Manchurian-55% (visual estimate).
Media= Agarose= 10g/L; sucrose= 100g/L; boric acid= 10mg/L.
[y]Mean separation within columns by Duncan's New Multiple Range Test ($P \leq 0.05$).
[x]All styles had pollen tubes reaching base of styles after 36 hours. No further evaluations were conducted after 48 hours.

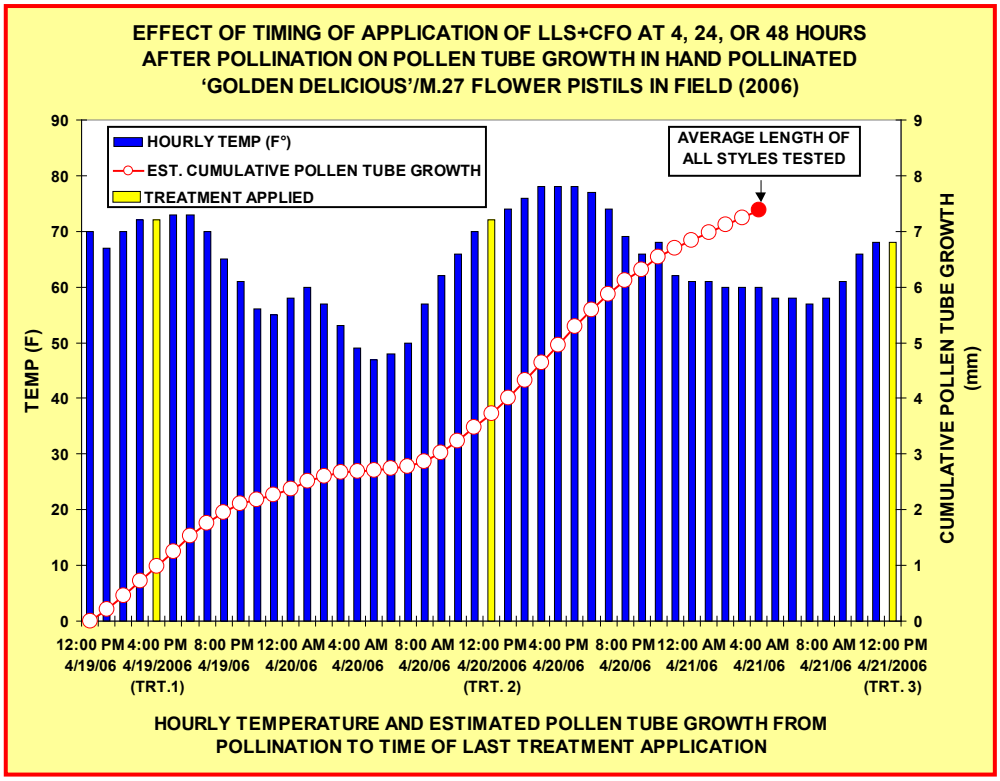


Figure A. **Generated model (above) showing predicted progress of pollen tube growth at time of application of LLS+CFO at Va Tech AHS-AREC, Winchester, VA.**

Figures B-D (below) are data-generated models of pollen tube growth for selected apple growing locations of Washington State in 2006. Temperature data acquired from the WSU Public Weather System (PAWS) website and full bloom dates were acquired from the WSU Tree Fruit Research and Extension (T. Smith) website. Graphics show predicted growth of pollen tubes after full bloom and temperatures during post full bloom period at the selected locations in 2006. Growth estimates are generated from our research conducted 2004 to present. **Figure E** emphasizes the differences in time required for fertilization due to temperature differences following full bloom at the three locations.

FIG. B

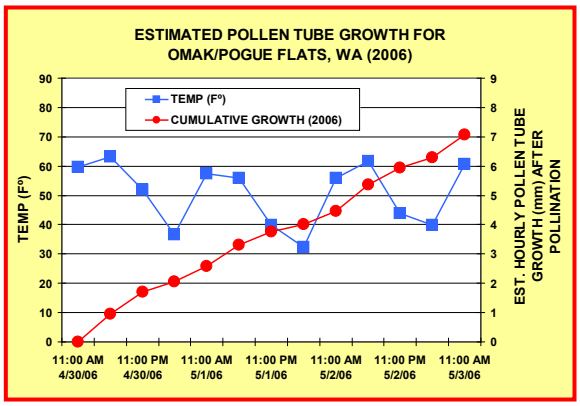


FIG. C

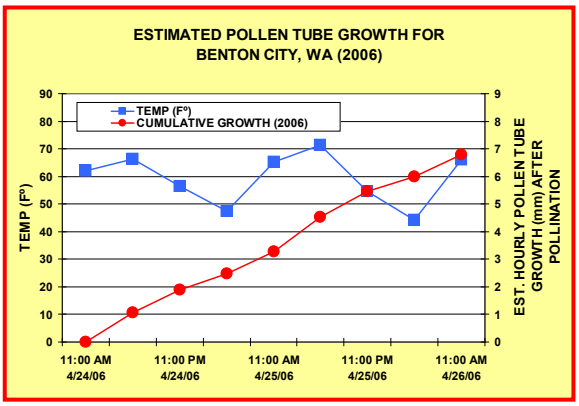
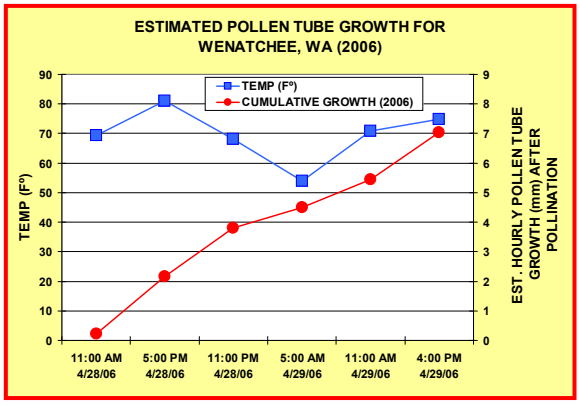
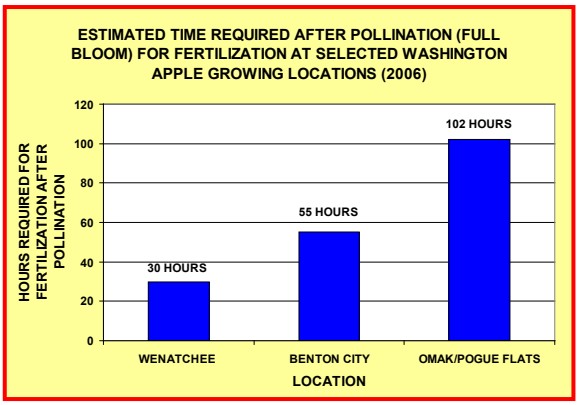


FIG. D





2007 objectives:

- Continue studies on the effects of alternating temperatures (35ºF to 75ºF) and light on pollen germination and tube growth focusing primarily on Fuji and Pink Lady.

- Assimilate data into development of a functional model of pollen tube growth in selected apple varieties for growers to use in conjunction with thinning programs to reduce chemical/labor costs, incorporating Washington bloom dates and temperatures.

- Conducts limited field studies involving specific bloom thinners and timing of applications after pollination at full bloom and pursue possible field studies at cooperating orchard sites in Washington State.

- Effect of timing of application of pollination inhibitors at full bloom on hand-pollinated flowers on fruiting spurs/shoots vs. “on-tree” application, timing of thinning treatments based on predicted pollination times.

CONTINUING PROJECT REPORT **YEAR**: 3rd Funding Period
WTFRC Project Number: 2005-09 and AP-06-606

Project Title: Estimating Apple firmness using Tensile Mechanical Properties

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State/Zip WA 98801

Budget 1: No Funds are being requested for the 3rd funding period.
Organization Name: WSU **Contract Administrator:** ML. Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	**Year 1:** July 05 – June 06	**Year 2:** July 06 – Jan 07	**Year 3:** NA
Salaries	6,478	11,622	
Benefits	2,656	4,251	
Wages	2,720	0	
Benefits	272	0	
Equipment	800	0	
Supplies	573	400	
Travel	1,500	800	
Sensory Panel	15,000	13,000	
Miscellaneous			
Total	30,000	30,073	0

Footnotes:

Introduction and Summary

This report covers the activities performed in Fall 2005 and Spring and Fall of 2006 comparing the tensile and compressive mechanical properties of apples and pears to human sensory intensity ratings of texture, and the Guss Penetrometer. The project is funded by WSTFRC grants awarded in August 2005 and February 2006. Data was collected in Fall 2005, Spring 2006 and Fall 2006. As we are currently analyzing the data from Nov 2006 tests, we are submitting this as a continuing project report and will submit a final report in January 2008.

Following Spring 2005 (year 1), we refined existing protocols for future data collections. These revisions were based on results from the Spring 2005 tests which indicated a low correlations between the mechanical properties and sensory evaluations. Revisions for Year 2 of the study (Fall 2006) included selection of apple and pear varieties, sample size and loading tests to measure the mechanical properties. Specifically, in Fall 2006, we compared the mechanical properties of apples and pears to sensory evaluations of texture, Sinclair (nondestructive) and the Guss (destructive) Penetrometer. Preliminary analysis of the Fall 2006 data indicate a relatively strong correlation between at least one sensory evaluation measurement (crispness) and the tensile material properties of apples and pears.

During 2007, we will complete the analysis of the Fall 2006 data, finish the finite element computer models to aid in the design of a firmness sensor for apples and pears, and submit a grant proposal to USDA for continuation of this research topic.

Objectives:

2005

1. Determine if human perceived apple firmness is related to the tensile material properties (elastic modulus, failure stress) of tissues from cultivars of apples and pears commercially grown in Washington State.
2. Determine if there is a relationship between the tensile material properties of the apple and pear varieties and the firmness pressure test originally developed by Magness and Taylor and refined over the years.

2006

1. Confirm 2005 results.
2. Develop design tools for use in designing and evaluating destructive and nondestructive firmness sensors.

Significant Findings:

Fall 2005

- Developed procedure (sample size, loading rate, photographic settings) to measure tensile forces in apple and pear tissue
- Weak correlations between tensile mechanical properties (elastic modulus, failure stress and failure strain) and compressive material properties (elastic modulus, failure stress and failure strain) in both apples and pears.
- Poor correlations between tensile mechanical properties and Guss Penetrometer measurement of firmness in both apples and pears
- Good correlations between compressive mechanical properties and Guss Penetrometer
- As the apple or pear matures, the tensile elastic modulus decreased more rapidly the compressive elastic modulus

Spring 2006

- Good correlations between compressive material properties and sensory evaluations
- Good correlations between Guss Penetrometer and sensory evaluations
- Good sensory correlations between Guss Penetrometer and compressive material properties
- Poor correlations between tensile material properties (measured in an orientation perpendicular to the core line) in apples and sensory evaluations (crispness, hardness, juiciness , chewiness and fracturability) lead to a redesign of the experimental techniques used in Fall 2006

Fall 2006 (Preliminary)

- Good correlations between tensile material properties (measured in an orientation parallel to the core line) in apples and pears and at least one sensory evaluation (crispness)
- Good correlations between compressive material properties and some sensory evaluations (hardness, fracturability).
- Good correlations between compressive material properties and Guss Penetrometer
- Good correlations between Guss Penetrometer and sensory texture attributes (hardness and fracturability)

Methods

The three testing sessions (Fall 2005, Spring 2006 and Fall 2006) used similar methods to select fruit, conduct the sensory evaluation, and measure the mechanical properties. This common methodology is described below.

Fruit Selection

Spring 2006

Gala, Granny Smith, Braeburn, Red Delicious, Golden Delicious apples were removed from air storage in late February 2006 and transported to WSU Pullman. the apples selected had a range of firmness values when evaluated by the sensory panel – a difficult prediction task. To increase the likelihood of having apples with a range of firmness values, we identified apples from historically strong and weak lots of apples. Fruit was screened twice, once nondestructively with Sinclair and samples tested destructively in Wenatchee. In February 2006, apples from these lots were pressure tested with the Slinclair nondestructive firmness to ensure a wide range of firmness.

Fall 2006

Apples were selected in Wenatchee as follows:

Golden Delicious - soft overmature fruit provided from an orchard, stored in air storage. Selected as being less than 13.5 lbf and less than 34 on the Sinclair.

Gala - soft fruit provided from an orchard stored in air. Less than 14.0 lbf and less than 37 on the Sinclair.

Red Delicious - medium firmness provided from an orchard stored in air. 15-16 lbf, not correlated with the Sinclair.

Braeburn - firm fruit again from an orchard stored in air. 19-20 lbf and above 49 on the Sinclair

Granny Smith - firm fruit from a commercial packer. Stored in air and commercially sorted. 18-20 lbf and above 38 on Sinclair.

Pears were selected in Wenatchee as follows:

Anjou pears - from an orchard stored in air.

Bosc and Bartlett pears purchased from a commercial packer stored in air.

Classification of apples for sensory evaluation
Apples from regular cold storage (1-3°C) were brought up to room temperature 24 hours before analysis. Prior to evaluation by the sensory panels, the fruit were characterized using instrumental measures of hardness using the Guss Penetrometer and the Sinclair iQ. These measurements were performed by the Kupferman group in Wenatchee and apples arrived in Pullman, characterized by their hardness level. On the day of the sensory evaluation panels, the measurements using the Guss Penetrometer and the Sinclair iQ were verified as some time had elapsed between the original measurements.

Trained sensory evaluation panel
A sensory panel of 10 panelists (2005) and 17 panelists (2006) was recruited using advertising in the WSU/Pullman community. Panelists were screened for any known allergies and anosmias. Panelists will be trained to recognize the apple texture attributes of hardness, juiciness, crispness and fracturability as defined in Table 1. In 2005, the panelists were also trained to recognize chewiness; however, this attribute was excluded from evaluations in 2006 as it was not found to yield significant results. The texture attributes were selected based upon previous literature. For training, published texture scales were used for the different texture attributes and panelists were trained to both recognize the attribute and assign it an intensity rating. Fruit of varying texture intensities and different varietals were used for the training process.

Table 1: Texture attributes of apples that were evaluated during the 2005 and 2006 trained sensory panels.

Texture:	
Hardness	Force required to bite completely through sample placed between molars
Crispiness	Amount of pitch of sound generated when the sample is first bittern with the front teeth
Juiciness	Amount of juice released on mastication in the first three chews
Fracturability	Force with which sample ruptures when placing sample between molars and biting down completely at a fast rate

During apple evaluation, panelists were presented with 6 sections of apple per evaluation session and these sessions were replicated. Following apple classification by hardness level, apples from the low, medium and hardness groupings were split in half. Half of the sample was used for tensile property measurement and half of the apple was used for sensory testing. The apple was labeled such that the sensory data and the tensile data for that apple could be compiled. The half that was used in the sensory testing was split in half. Thus, each panelist was presented with ¼ of a washed apple for evaluation and a knife to peel his/her own fruit.

Evaluations took place in individual sensory booths equipped with lap top computers for recording data. The apple sections were randomly presented to the panelists at room temperature. Apple selections were identified using three-digit codes and presented one at a time to panelists. Each panelist will be provided with water to rinse between samples as well as a cuspidor for sample expectoration. The samples were scored for intensity of each texture attribute using a 15-cm unstructured line scale, with the left end of the scale corresponding to the lowest intensity (0 mm=absent) and the right end corresponding to the highest intensity (150 mm=extreme). Results

were collected and analyzed using Compusense 6.0 software (Guelph, ON) and sensory data will be quantified by measuring the distance of the mark along the line.

Mechanical Properties

Compressive elastic modulus, failure stress and failure strain

Cylindrical tissue samples 15 mm in length and 9.22 mm in diameter were excised from the fruit. In the Fall 2005 and Spring 2006 tests, these the centerline of these samples was perpendicular to the core line of the fruit. Based on the redesign of the experimental techniques following the Spring 2006 test, in the Fall 2006 test the samples' center line was parallel to the core line of the fruit. The cylinders were compressed to failure between the parallel plates of a universal testing machine (Fall 2005, Instron Model 1350, Spring and Fall 2006, Texture Analyzer TAXT2 by SMS). Force and deformation data was collected at intervals of 10 milliseconds. Stress values were computed from the recorded force data and sample diameter; strain values were computed from recorded deformation data and the original length of the sample. From the stress and strain data the compressive elastic modulus (slope of the stress vs. strain data), failure stress and failure strain values were computed.

Tensile elastic modulus

Measuring the tensile material properties of fruit tissue is problematic due to the difficulty of forming and gripping a suitable test specimen. In our tests, the failure mechanical properties were computed using a bending apparatus and image analysis. Central to this analysis is the determination of the location of the neutral axis – the plane about which the sample deforms in response to a bending load. In this project, the neutral axis of the fruit tissue samples were determined using digital image analysis.

A rectangular block of tissue, 8.16 mm wide, 26.76 mm in length, and 8.16 mm in height was removed from the fruit. The sample was excised from the fruit so that the length dimension of the sample was parallel to the core line, and the height dimension of the sample was perpendicular to the core line.

The sample was placed in a 3 point bending jig (Image 1) and slowly deformed to failure. A digital video record was made of the deformation. Two digital images were extracted from the video; one prior to deformation of the sample (Image 1), and a second image at a point where the sample had been deformed to a point near failure (Image 2). Image 2 was then subtracted (on a pixel-by-pixel basis) from Image 1, resulting in a difference image (Image 3).

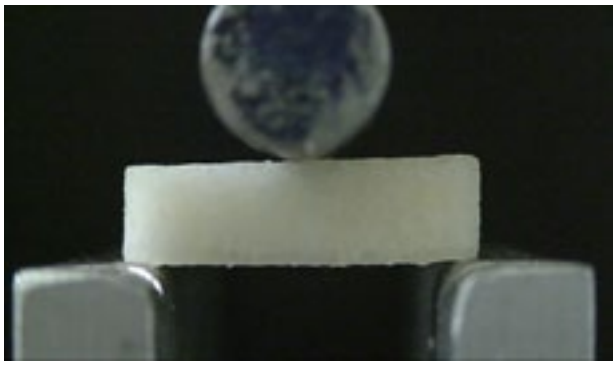

Image 1.Undeformed pear tissue sample placed in the bending jig.

Of particular interest are the dark and light triangular regions on the sides of Image 3. The dark region is where the side of the sample rotated toward the center of the sample due to the bending load. The light region is where the side rotated away from the center of the sample due to the bending load. The point where the two regions meet was the pivot of the side's rotation. This pivot point is on the neutral axis of the sample. The square of the ratio of the distance between the bottom of the sample and the neutral axis to the distance between the neural axis and the top of the sample is equal to the ratio of the compressive elastic modulus to the tensile elastic modulus. Using the

compressive elastic modulus computed from the compressive test and the square of the ration of distances to the neutral axis, the tensile elastic modulus was computed.

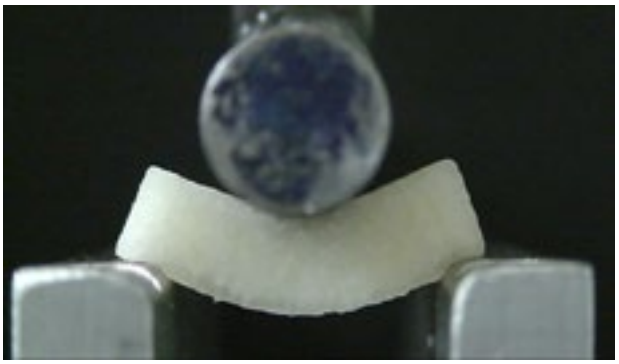

Image 2. Deformed pear sample in the bending jig

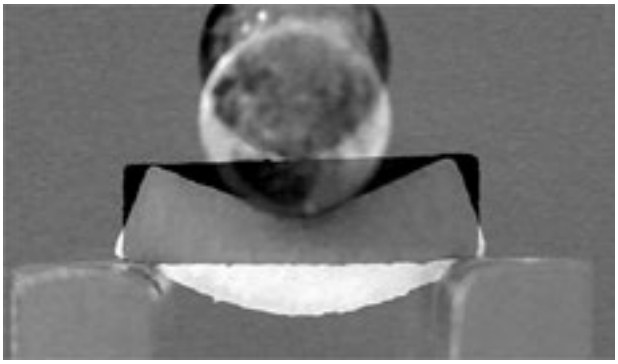

Image 3. Pixel-by-pixel difference between deformed and undeformed sample images, showing the pivot point on the sides of the sample

Results and Discussion

The data from all three testing periods consistently indicate that there is a high (R^2 greater than 0.75) correlation between compressive material properties and the Guss Penetrometer. Penetrometer devices both compress and shear tissue to failure, so a high correlation would be expected. The data from the Spring and Fall 2006 tests indicated a high correlation (R^2 greater than 0.75) between compressive material properties and the sensory evaluations made by chewing on the molars (hardness and fracturability). This correlation is also expected because the sensory evaluation is done by compressing the tissue sample between the molars. Finally, the correlations between the Guss Penetrometer and hardness and fracturability are also high.

Tensile properties measured parallel to the core line correlate well (R^2 greater than 0.85) with crispness – the only sensory evaluation done with the front teeth. Tensile material properties did not correlate well (R^2 less than 0.7) with compression material properties, the Guss Penetrometer nor with sensory evaluations that compressed the sample between the molars. Clearly the tensile material properties due not change in step with the compressive material properties as the fruit matures. The analysis on the Fall 2005 fruit indicate that the tensile material properties decline at a faster rate than compressive material properties as the fruit matures. One explanation of this observation could be that the tensile material properties are highly dependent on the strength of the pectin bonds between cells and the cell wall strength, while the compressive material properties are highly dependent on the tugor pressure in the cell, and to a lesser extent on the pectin bonds and cell wall strength. Under

certain storage environments, the fruit could mature without noticeably changing cellular tugor pressure.

The tensile material properties are highly orthotropic (the properties change with orientation of the tissue sample with respect to the core line of the fruit. The sensory evaluations and tensile properties measured in the Spring 2006 test were orientated perpendicular to each other, and showed little correlation, while in the Fall 2006 test the tensile material properties and sensory evaluations were both taken parallel to the core line, and there was a high correlation between the sensory and tensile measurements. Although we did not measure tensile material properties and sensory evaluations perpendicular to fruit core lines, we suspect that the correlations between sensory and tensile measurements would also be high in this orientation.

One very clear outcome from this project is that the orientation of the load applied by a firmness sensor must be specified. The correlation between material properties parallel and perpendicular to the core line is low, and comparing firmness measurements taken without specifying the orientation will vary widely.

Remaining activities

During the no cost extension of project through 2007 we will;

- Complete the analysis of the data from Fall 2006.
- Finish developing and testing a finite element model of an apple and a pear for use in the design and evaluation of firmness sensors, and
- Submit a proposal to USDA to continue to investigate the compressive and tensile material properties of apples, pears and other tree fruit.

CONTINUING PROJECT REPORT **YEAR 2 of 3**
WTFRC Project Number: AH-05-502A (WSU Project No. 13C-3655-5260)

Project Title: Apple scion breeding

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Cooperators: Larry Pusey, USDA-ARS, Wenatchee; Yanmin Zhu, USDA-ARS, Wenatchee; Cameron Peace, Amit Dhingra, Dorrie Main, WSU, Pullman

Budget:

Organization Name: Washington State University
Contract Administrators: Mary Lou Bricker and Sally Ray
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Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries[1]	42,766	44,477	**56,055**
Benefits	14,540	15,122	**19,059**
Supplies[2]	34,500	35,946	**43,500**
Travel[3]	6,250	6,500	**8,050**
Total	98,056	102,045	**126,664** [4]

Footnotes:
[1] Salaries of Agricultural Research Technologists Bonnie Konishi (100%) and Marc Dilley (25%).
[2] Includes trees, irrigation materials, trellis materials, spray materials. Cell phone charges are allowed.
[3] Travel to TFREC apple breeding orchards and second test trials at commercial sites in central Washington.
[4] This amount is an increase from the $106,233 requested in the original proposal. Budgets were developed in 2004 for years 2005, 2006 and 2007. Most budget items have increased significantly since 2004, particularly salaries and benefits, orchard establishment and maintenance, and gasoline. Tree propagation costs have increased by over 50%. In addition, a greater number of seedlings and second test selections than anticipated will be planted in 2007.

Objectives:

1. Select superior cultivars for the Washington apple industry from hybrid seedling populations. The primary objective is outstanding eating quality, including crisp and juicy texture, flavor based on a balance of sugar and acid, with aromatic apple flavor. Additional fruit quality objectives include long shelf and storage life, minimal sunburn and bitterpit, attractive appearance and medium fruit size. High productivity, precocity, harvest maturity from the mid-August to mid-October period, and resistance to mildew and fire blight are also objectives.

2. Develop an efficient scheme for producing and managing seedling populations through enhanced precocity and an efficient scheme for early seedling selection with marker-assisted selection.

3. Evaluate parents for their contribution to the inheritance of fruit quality traits, particularly enhanced fruit firmness resulting from low ethylene production genes.

4. Assist in the development of markers and apply these in marker assisted selection for important fruit quality traits.

Significant findings:

- Twenty-six new crosses were made and 35,000 hybrid seeds produced. Parents included Cripps Pink, HoneyCrisp, CrimsonCrisp, GoldRush and Braeburn.
- Approximately 15,000 fruiting seedlings were evaluated and over 500 selected as promising.
- Over 7,000 seedling/M.9 trees (from 2003 crosses) were propagated for 2007 planting in evaluation orchards.
- New selections were added to the three-site second test trials, which now have 40 selections. Twelve new selections were propagated for 2007 second test plantings.
- Fruit of promising selections in second test trials has been evaluated for fruit quality and productivity. Several outstanding selections will be propagated in 2007 for grower trials in 2008.
- Four elite selections were propagated (2006) for grower trials in 2007.
- Genotyping data generated by Dr. Yanmin Zhu for ethylene genes ACS1 and ACO1 were associated with phenotypic data (fruit firmness out of storage) indicating that these genes can be used in Marker Assisted Selection.

Methods:

1. Using classical fruit breeding methods, annually hybridize parents with desirable traits and produce hybrid seedling populations of 20,000 to 30,000 seedlings per year from 10 to 30 crosses. Propagate the strongest and healthiest seedlings (approximately 10,000 seedlings/year) on M.9 rootstock to increase precocity. Establish seedling/M.9 orchards at TFREC by producing trees in the nursery or by topworking existing seedling/M.9 trees in TFREC orchards. From these populations, maintained for four years, select approximately one-half of 1%, termed “selections,” based on fruit quality objectives. Only seedlings that have fruit with acceptable appearance (size, shape, color, finish) and outstanding eating quality (crisp, juicy, flavorful) are selected.

2. Propagate promising selections and establish replicated second test trials at three sites, TFREC and two commercial sites in central Washington. Trials are maintained for a minimum of six years during which selections are evaluated for tree health, productivity, fruit quality and storability.

3. Inoculate seedling populations (5,000 to 10,000 seedlings per year) with fire blight bacteria in the greenhouse. Evaluate parents for their contribution to seedling resistance. Resistant seedlings are budded on M.9 and planted in the evaluation orchard.
4. In the seedling nursery, second year seedlings are selected for resistance to natural mildew infection. Parents will be evaluated for their contribution to the inheritance of mildew resistance. Only seedlings with low susceptibility are propagated on M.9 and planted in evaluation orchards at TFREC.

5. With Dr. Yanmin Zhu, genotype advanced selections and cultivars for the ethylene production genes Md-ACS1 and Md-ACO1 (genes which contribute to fruit firmness out of storage). Use these genes as markers for selecting the firm-fruited phenotype in juvenile seedlings of the cross Cripps Pink X HoneyCrisp. Seven-eighth of the seedlings will lack the alleles for low ethylene production (have soft fruit) and can therefore be discarded.

6. Collaborate with Drs. Cameron Peace, Amit Dhingra and Yanmin Zhu in the development of gene-trait associations, gene discovery and marker assisted selection for important fruit traits including firmness, crispness, juiciness, aroma and color.

Results and discussion:

Apple breeding is a long-term activity made so by a long juvenile period and long propagation times. Promising selections are in trials at multiple sites in Washington. Conclusive data for the first selections from these trials are two years off and will be a prerequisite for deciding to commercialize a selection. Several selections appear to be excellent candidates for introduction as new varieties. Four elite selections have been propagated for grower trials to evaluate horticultural and storage characteristics.

The primary benefit will be the introduction of unique apple cultivars adapted to the central Washington environment. The aim is to provide cultivars that will be profitable for Washington growers, handlers and marketers. New cultivars will have excellent fruit quality to meet the requirements of highly competitive national and international markets and ultimately to provide consumers with a pleasurable eating experience.

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

Project Title: Apple rootstock evaluation

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Cooperators: Dave Allan, Bob Brammer, Ray Fuller, John Verbrugge
Del Feigal, Ron Wilcox

Budget 1: WTFRC Collaboration Costs
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: 509-665-8271 x2 **Email address:**

Item	Year 1:	Year 2:	Year 3:
Salaries			
Benefits			
Wages	22,000	19820	37012[2]
Benefits	3518	3171	5922[2]
Equipment			
Supplies	300	300	300
Travel (mileage)[1]	2431	1600	2601
Miscellaneous			
Total	28,249	24,891	45,835[2]

Footnotes:
[1] mileage is based on 120 miles round trip at 45 cents/mile, 8 trial sites up to 6 trips per site per season for data or plot maintenance.
[2] Time slip increase due to all trials with bloom and harvest data collection

OBJECTIVES:

1. Evaluate apple rootstocks in commercial settings in Washington State, especially those with known replant conditions.
2. Integrate the processes of evaluation and industry adaptation.
3. Extend porcedures for rootstock evaluation into scion breeding program.

Table 1 indicates the current general knowledge of rootstock performance. Information which may impact planning decisions for plant material used in replant sites include:

- M-26 and Bud-9 are sensitive to replant disorders.
- Bud-9 is significantly smaller than M.9-337 in all sites and is similar in tree size to Mark.
- M.9 clones are classified into small (<30%), medium (30 to 35%) and large tree (>35%) size categories.
- Many of the M.9 clones will have more vigor in replant sites than M.26.
- Wooly apple aphid resistance is available in G.41 and G.202.
- G.11 or Bud-9 should be considered for locations with regular fireblight outbreaks.

Table 1. Traits of available apple rootstocks grown in replant sites[1]

Rootstock	Percent Tree size	Fireblight Resistant	Crown Rot Resistance[2]	Woolly Resistant	Cold hardy[4]	Ease of propagation Liner / Finished tree
Mark	25	Susept	Tolerant	No	Tolerant	Very Good / Excellent
Bud 9	20 to 30	Field resist	Resistant	No	Tolerant	Very Good/Very Good
M.9-Fl.56	30	Susept	Resistant	No	Fair	Good / Good
G.41	30	Immune	Resistant	Resistant	Hardy	Fair / Good
M.9-337	30	Susept	Resistant	No	Fair	Good / Good
G.11	30	Very tolerant	Resistant	Tolerant	Good	Good / Good
M.26	25 to 40	Susept	Susept	No	Good	Very Good/Very Good
M.9-Emla	35	Susept	Resistant	No	Fair	Good / Good
M.9-Pajam 2	35	Susept	Resistant	No	Fair	Good / Good
M.9-Nic 29	40	Susept	Resistant	No	Fair	Good / Good
G.935	35 to 45[3]	Resistant	Resistant	No	Hardy	Good / Very Good

[1]Compiled from NC 140 trials, rootstock nursery catalogs, WTFRC rootstock trials and should be considered 'general' comparisons and not conclusions from trial data.
[2]Crown rot = Phytophthora species
[3] G.935's mature tree size may be smaller than indicated here. It is rated by Cornell as similar to M.26 in new ground. In Washington State, it appears equivalent to M.9.
[4] Relative term: Hardy>Good>Tolerant>Fair. M.9 has been known to sustain damage at -10^0 F.

Significant Findings:

- Fumigation improves performance of newly planted trees on all rootstocks.
- There is no indication that replant disease can be completely mitigated solely by a rootstock.
- Fumigation's positive affect on growth lasts only a season or two.
- Fumigation's positive affect on yield and tree health is for life of the planting.
- Vigor or tree size are a very relative terms when applied to rootstocks: performance will vary by site and management.
- Mark is the standard for plant-in-place orchard establishment. Mark grafts and buds very successfully and grows vigorously as a non bearing tree.
- Four Geneva rootstocks are unsuitable for plant-in-place, three rootstocks show promise.

Objective 1: Evaluate apple rootstocks in commercial settings in Washington State, especially those with known replant conditions. There are 9 trials with 8,000 trees being evaluated.

Year Planted	Location	Cultivar	Replant Severity	# of Rootstocks
2003	Frenchman Hills	Honeycrisp	New ground	18 Dwarf / 6 Semi-dwarf
2003	Chelan	Honeycrisp	Moderate	19 Dwarf / 6 Semi-dwarf
2004	Naches	Honeycrisp	Moderate	16 Dwarf
2004	Wapato	Gala	Moderate	12 Dwarf
2004	Chelan	Gala	Severe	12 Dwarf
2006	Wapato	Gala	Severe	12 Semi-dwarf
2006	Wapato	Gala	Severe	22 Dwarf
2006	Brewster	Fuji	Severe	16 Dwarf / 8 Semi-dwarf
2006	Vanatage	Fuji (bench grafts)	Moderate	28 Dwarf

Vantage Aztec Fuji-- Plant-in-place

Rootstock	Alive (%)	Dead (%)	Small (%)	Height (ft)	# Branches	Circ (cm)
2034	96 a	4 d	0 d	5.5 abcde	3.7 abcd	4.0 abcd
2406	82 ab	18 cd	50 a	3.6 gh	1.4 ef	3.2 ef
3001	100 a	0 d	0 d	5.9 a	4.2 ab	4.4 a
3007	96 a	4 d	35 abc	4.6 abcdefg	2.3 bcde	3.4 cdef
3041	96 a	4 d	4 cd	5.5 abc	3.7 abcd	4.0 abcd
4002	25 cd	75 ab	25 abcd	1.9 i	0.0 f	2.2 g
4004	96 a	4 d	15 abcd	5.3 abcde	3.3 abcde	3.7 abcdef
4011	96 a	4 d	16 abcd	5.5 abc	3.9 abcd	4.1 abc
4013	55 bc	45 bc	35 abcd	3.8 fgh	2.0 de	3.6 bcdef
4019	6 d	94 a	6 cd	4.1 efgh	2.0 de	3.8 abcdef
4172	96 a	4 d	33 abcd	4.1 defgh	2.3 bcde	3.1 ef
4202	100 a	0 d	4 cd	5.2 abcde	3.4 abcd	3.9 abcde
4210	100 a	0 d	37 abc	4.3 cdefgh	2.3 bcde	3.2 ef
4214	96 a	4 d	4 cd	5.5 abc	3.7 abcd	3.7 abcdef
4288	92 a	8 d	48 ab	4.3 cdefgh	2.2 cde	3.4 cdef
4814	100 a	0 d	13 cd	5.0 abcdef	3.0 abcde	3.6 abcdef
5046	8 d	92 a	8 cd	3.0 hi	0.0 f	3.0 fg
5087	91 a	9 d	28 abcd	5.0 abcdef	2.5 abcde	3.6 bcdef
5179	100 a	0 d	15 bcd	5.1 abcdef	3.0 abcde	3.3 def
5202	88 ab	12 cd	15 abcd	5.0 abcdef	2.8 abcde	3.8 abcde
5257	100 a	0 d	0 d	5.7 ab	3.9 abcd	4.1 abc
5463	91 a	9 d	6 cd	5.5 abc	3.6 abcd	4.1 abc
5935	92 a	8 d	8 cd	5.5 abcd	3.7 abcd	4.0 abcd
G.11	100 a	0 d	0 d	5.5 abc	3.7 abcd	3.8 abcdef
G.30	100 a	0 d	7 cd	5.6 abc	4.0 abc	4.3 ab
Mark	100 a	0 d	4 cd	5.8 ab	4.3 a	4.1 abc
Sup.4	94 a	6 d	27 abcd	4.5 bcdefg	2.3 cde	3.4 cdef
G16	0 d	100 a	0 d	*	*	*

2006 plant in place: The Fuji trial at Vantage has Mark as the standard. The high percentage of grafting success and first season growth combine to make Mark the standard of performance. Some Geneva stocks look promising, with good survival rates and good vigor.

Methods: The intent of this trial is to screen All the rootstocks in the trial were benched grafted by Auvil Fruit Company staff and callused at the Vantage storage facility. The rootstocks were placed outside in the day and taken inside the storage at night. When the scions were nearing ½ inch green stage the trees were planted. Four to nine trees per plot were established in four replicates in the center of a new block. Trials started on the eleventh tree from the row end. Auvil Fruit Company provided their standard management to all trees in the block. Measurements for trunk circumference, tree height and limb number were taken in late October. Mark rootstock trees grew an average 5.8 feet or 70 inches.

Wapato 04 Gala Fumigated

				GROWTH	GROWTH	TRUNK	TRUNK	TRUNK
			BINS	(cm)	(cm)	CSA	CSA	CSA
ROOTSTOCK	BLOOM	FRUIT #	ACRE	2005	2006	2004	2005	2006
Bud 9	108 bc	43 e	21 d	2.5 d	2.2 ns	2.2 abc	4.8 cde	7.9 cde
G 3041	133 ab	59 cde	30 abc	3.5 abc	2.6	1.9 cdef	5.7 abcd	9.8 ab
G 4214	108 bc	63 abcd	32 abc	3.4 abc	2.2	1.7 efg	5.0 cde	8.2 bcde
G 5935	181 ab	79 a	38 a	3.5 abc	2.1	2.4 ab	6.5 ab	9.7 ab
G-11	147 ab	68 abc	37 a	3.2 bcd	1.6	1.6 fg	4.7 de	6.9 e
G-16	185 a	78 ab	36 a	3.5 abc	1.7	2.6 a	6.7 a	9.6 abc
M 26	142 ab	57 cde	30 abc	3.8 ab	2.4	2.3 abc	6.7 a	10.5 a
Nic 29	146 ab	60 bcde	33 abc	4.0 a	2.3	1.7 def	5.9 abc	9.7 ab
Pajam 2	144 ab	69 abc	36 ab	4.0 a	1.8	2.0 bcde	6.5 ab	9.3 abcd
Supporter 1	127 ab	47 de	26 cd	2.7 cd	2.1	1.7 def	5.1 cde	8.8 bcd
Supporter 2	108 bc	52 cde	27 bcd	3.1 bcd	2.1	2.0 cdef	4.8 cde	7.8 de
Supporter 3	122 ab	58 cde	30 abcd	3.0 cd	2.2	2.1 bcd	5.4 bcd	8.5 bcde

Wapato NON fumigated

				GROWTH	GROWTH	TRUNK	TRUNK	TRUNK
			BINS	(cm)	(cm)	CSA	CSA	CSA
ROOTSTOCK	BLOOM	FRUIT #	ACRE	2005	2006	2004	2005	2006
Bud 9	64 e	34 d	18 e	2.2 cd	1.9 bc	1.6 bcdef	3.6 fg	5.9 de
G 3041	103 abc	64 abc	33 ab	2.9 b	2.3 b	1.7 abcde	4.6 abcde	7.8 bc
G 4214	69 de	40 d	20 de	2.8 bc	2.3 b	1.3 f	3.7 efg	6.6 bcd
G 5935	125 ab	71 a	36 a	3.2 b	1.9 bc	2.0 a	5.4 a	8.0 b
G-11	105 abc	49 cd	25 bcde	2.0 d	1.6 bc	1.5 def	3.3 g	4.8 e
G-16	135 a	67 ab	30 abc	2.8 bc	1.7 bc	2.0 ab	4.9 abcd	7.1 bcd
M 26	97 bcd	45 d	21 cde	3.3 b	2.0 bc	1.9 abc	5.4 ab	8.3 b
Nic 29	103 bc	38 d	20 de	2.9 b	1.1 c	1.6 cdef	4.3 cdef	5.9 de
Pajam 2	93 cde	51 bcd	27 bcd	2.7 bc	2.3 b	1.7 abcde	4.4 bcdef	7.5 bcd
Supporter 1	93 bcde	46 d	24 cde	2.8 bc	1.6 bc	1.5 def	4.0 defg	6.0 de
Supporter 2	112 abc	42 d	22 cde	2.2 cd	1.9 bc	1.4 def	3.9 defg	6.0 de
Supporter 3	96 bcd	51 bcd	26 bcde	4.0 a	5.4 a	1.7 abcd	3.8 efg	6.2 cde

Methods: Rootstock trials are randomized and replicated four times. Trunk circumferences are taken at planting in the spring and every fall. Trees are headed and all branches removed. The trees are encouraged to grow vigorously through the first two growing seasons. Competing shoots or large branches are removed as horticulturally necessary. Commencing with third leaf, bloom counts, fruit set and yield are taken tree by tree, giving three sets of crop potential data. Yield data is the total weight and fruit count on each tree to give fruit size and weight per tree data. Ten apples per rootstock plot are taken for fruit quality analysis in the laboratory.

Yields for the Wapato trial are based on 1451 trees per acre (10' x 3'). The yield difference between fumigated and non-fumigated plots follows a relationship with tree size: fumigated plots have larger trees than non-fumigated plots. G.935 and G.41 both performed well in the non-fumigated plots, both in Wapato and Chelan (data not shown). In the three 2004 replant trials, all non-fumigated plots show replant stress.

Objective 2: The trials are placed in commercial settings in different growing regions of Washington State. Interaction with trial hosts/cooperators often clarifies what is useful to growers and is logistically possible. All of the trial sites have had well attended field days arranged by WTFRC and WSU extension. The plot design with five or more trees per plot of the same rootstock is helpful for grower evaluation. Placement of trials in various geographical regions increases operating costs but has increased grower observation of the trials.

Objective 3: Starting in spring of 2007, the apple breeding program will initiate larger scale trials of elite selections. Apples from these trials will provide some indication of handling traits of these new products and give various marketing organizations some opportunity for fruit evaluation.

Acknowledgements: Felipe Castillo has managed the logistics of data collection. Ines Hanrahan has assisted in the oversight of the southern trials and assisted in harvest of the trials. Sandy Stone has patiently entered mountains of semi-legible data. Tory Schmidt has run the statistics.

Cooperators: These large rootstock trials would not be as successful without the cooperation of growers: Ray Fuller, Ron Wilcox, John Verbrugge, Dave Allan, Bob Brammer, and Del Feigal are integral to the success of these trials.

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

WTFRC Project Number: PH-05-504

Project Title: Defining ethylene regulation of apple fruit quality traits

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Budget 1:
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Item	**Year 1: 2005**	**Year 2:** 2006	**Year 3:** 2007
Salaries	$19,326	$20,292	$21,307
Benefits	$10,109	$10,651	$11,146
Wages			
Benefits			
Equipment			
Supplies	$ 9,500	$12,000	$11,000
Travel	$ 1,000	$ 1,000	$ 1,000
Miscellaneous			
Total	$39,935	$43,907	$44,453

Footnotes:

Objectives:
Our overall goal is to define the role of ethylene in the functional regulation of apple fruit quality by defining key metabolites, their precursors, biochemical pathways involved in their biosynthesis, and genes that direct their activity. We will create a database using the information available in GeneBank and develop data mining tools to discover genes that regulate flavor metabolites, especially aroma compounds, and texture in apple fruit. Transgenic apple fruit with modified capacity to synthesize endogenous ethylene and 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, will be used to validate gene discovery, along with other metabolomic and biochemical experimental approaches.

Objective 1. Identify specific transcripts that are differentially regulated in transgenic apple fruit silenced for ethylene synthesis or perception, and correlate them with flavor and texture development - The primary aim of this objective is to compile and annotate the most highly regulated transcripts expressed during apple fruit development. The transcriptome of developing apple fruit has been sampled over time, with particular emphasis on transcripts expressed in flesh and peel tissues. Our approach has been on two fronts one is to develop or deploy bioinformatic tools to do a digital analysis of expressed genes available in GenBank (NCBI) and to develop and deploy microarray technologies to investigate ethylene dependent pattern of expression in apple fruit. Currently the digital analysis and the microarray analysis is underway. This year we expect to complete this and begin validation of the expression data using real time PCR (RT-PCR). We have also developed the resource to visualize expression data as pathways to better understand the relationships between the expressed patterns of genes and the regulation by ethylene.

Objective 2. Functional validation of pathways *via* analysis of key metabolites and enzymes regulated by ethylene – We have focused on 2 of the 14 transgenic lines that make very low ethylene these are 68G expressing antisense ACC oxidase (ACO) and 103Y a line expressing a sense version of the apple ACC synthase gene (ACS). We have refined our analysis by focusing on peel and flesh tissues obtained from samples harvested last year and this year (2005 and 2006). We have carried out phenotypic, biochemical, enzymatic and metabolic analysis focusing on the postharvest behavior of gene activity. We would like to discover the subset of genes that are regulated by 'system 2' ethylene regulation, i.e., those genes/traits that are specifically regulated by autocatylytic ethylene biosynthesis. The phenotypic, metabolic and biochemical data had been collected and analysis, interpretation and validation is underway.

Significant findings/accomplishments

1. Successful deployment of digital, microarray resources and analysis tools to dissect the transcriptome of apple fruit.

2. Ethylene is positively correlated with color, starch and weight and negatively correlated with firmness and acidity. Ethylene is not correlated with soluble solids.

Methods:

Plant material: The proposed experiments use transgenic apple fruits suppressed in ethylene biosynthesis obtained from different lines grown in an experimental orchard.

Fruit collecton and handling: Apples (Golden Delicious cv. 'Greensleeves') were harvested from the research orchard when GS fruit was in a pre-climacteric stage (internal ethylene concentration lower than 0.3 $\mu L\ L^{-1}$). Apples were transported to the Postharvest Pomology Research Laboratory at UC Davis and sorted to select those that were free from defects. Matched samples of 1 to 5 apples per replicate were prepared, with 3 to 5 biological replicates per treatment.

Treatments: Fruit from selected 'Greensleeves' apples lines, including transgenic 68G (ACO-antisense), 103Y (ACS-sense), and untransformed fruit (GS), were sampled at harvest and after 14 days of storage at 20ºC in air (ethylene-free atmosphere). Relative humidity was maintained close to 90-95%. After storage, fruits from lines 68G and 103Y were treated with ethylene. Half of the fruit was kept at 20ºC in an ethylene-free atmosphere, and the other half was stored at 20ºC under a flow of air containing 80 μL L^{-1} ethylene. Because of the differences in enzymatic activities observed among fruit tissues, peel and cortical tissues were carefully separated and frozen in liquid N_2 and kept at -80 ºC until analysis. For all biochemical analysis, three replicates of five fruit each was used.

Ethylene and respiration rate measurements: Within each experiment, ethylene production and respiration rates were determined at 1 and 14 days after storage for individual fruits using a static system. Exit air samples were collected from each jar and analyzed for CO_2 concentration (by an infrared gas analyzer) and ethylene concentration (by a flame ionization gas chromatograph) (Defillipi et al., 2004).

Maturity and quality parameters: An initial sample from each harvest was evaluated for skin color (by a Minolta Chromameter), starch pattern (by IKI staining), soluble solids, (by a refractometer), titratable acidity (by an automatic titration system), and flesh firmness (by a Guss fruit texture analyzer and an Aweta Acoustic Firmness Sensor). All of these characteristics were used to defining a mature apple fruit phenotype and provide a "quality" reference for samples tested for comparison with ethylene-silenced fruit.

Microarray analysis of the transcriptome: We have started to design an oligo microarray platform made by CombiMatrix, using their bioinformatics pipeline and design criteria from the apple unigene set at GeneBank. They will synthesize the desired oligonucleotides on the chip surface and then send us the chips. We will label RNA, hybridize it to the chips, and then scan the chips at the Microarray Core Facility at UC Davis under the direction of Michael Schulz, Director of the facility.

Determination of sugars, acids, and related enzyme activities: Fruit cortical and skin tissues obtained from the various transgenic lines were analyzed for soluble sugars (sucrose, fructose, glucose, sorbitol, and inositol) and malic acid using high resolution GC/MS. Enzymes that regulate sugar-acid balance will be assayed using methods described by Dey and Harborne (1990).

Additional metabolic analysis: Metabolic analysis was carried out at the Metabolomic facility of the Davis campus (http://fiehnlab.ucdavis.edu/) under the direction of Professor Oliver Fiehn and the manager of the facility, Dr. Valdimir Tolstikov. Cortical and skin tissue samples were harvested in the field, and sample preparation was carried out at their facility. Using high resolution GC/MS equipment they resolved 400 compounds for each of our samples. We will use their existing software to resolve this data into pathways to verify the pathway data obtained from microarray analysis.

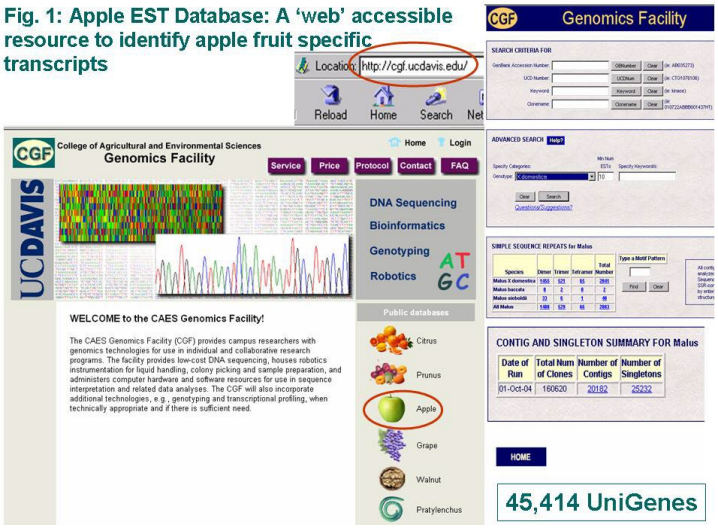
Results and Discussion

1. Successful deployment of digital, microarray resources and analysis tools to dissect the transcriptome of apple fruit.

In order to investigate the expressed genes in apple we have assembled a cDNA anlaysis pipeline at the Collage of Agriculture and Environmental Science Core Genomics Facility (CA&ES CGF) at Davis (Fig. 1). This pipeline is a series of programs that help examine all information stored in the GenBank public database, and it allows us to access raw sequence information for apple ESTs, which are terminal DNA sequences representing either the 5' of 3' end of an apple mRNA. These sequences

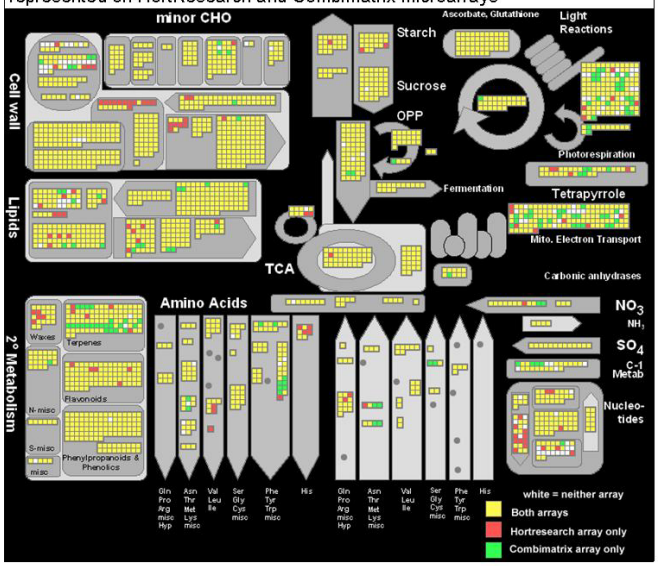
are downloaded and stored in an Oracle database at Davis, then sorted to remove extraneous sequences that represent *E. coli*, chloroplast, or mitochondria DNA sequences. Only high quality sequence information is retained for further analysis. The sequences are compared among themselves and with other sequences present in GenBank, to sort and cluster them based upon similarity and possible gene function. We have examined 160,620 of the current 256,249 entries in the public database for Apple (GenBank, NCBI). Other available resources are the GDR which is the Rosaceae community wide resource and is more current and upto date in the analysis of all available apple EST sequences. Our resource and analysis is web-accessible through the Core Genomics facility (CGF) website (http://cgf.ucdavis.edu/), available by clicking on the apple icon. Of the analyzed 160,620 ESTs, 45,414 (28.3% discovery rate) of the genes correspond to a unigene set, with a majority (25,232; 15.7%) being singletons (represented once in our database) and 20,182 (12.6%) being 'contigs' (represented more than once in our online database). This provides an estimated 45,414 unigenes. The current 'unigene' (NCBI) assembly for apple is 14,626 and is accessible: (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene. We have utilized this particular resource to design our oligonucleotide microarrays. We also use this resource and the availability of other sequences present in GenBank to design primers for RT-PCR analysis.

Fig. 1: Apple EST Database: A 'web' accessible resource to identify apple fruit specific transcripts



We currently have two options of apple microarrays available to us, one is an oligonucleotide-based spotted array developed by HortResearch (New Zealand) and the other is a set of Combimatrix arrays designed by us where the oligo nucleotides have been synthesized on the surface of the chip. The HortResearch microarray contains 15,726 gene targets whereas the CombiMatrix contains 23,181 gene targets divided among two 12K arrays, one containing all of the fruit specific unigenes and the other containing unique gene targets from vegetative tissues. The CombiMatrix arrays are being processed by our microarray facility on the Davis Campus using a one color system much like Affimatrix, whereas the HortResearch arrays will be processed by them in NewZealand. We will provide to HortResearch the RNA to be hybridized by their two-color system. We are currently using one of the CombiMatrix arrays that contains all of the fruit specific genes. This analysis is currently underway. As shown in Fig 2 we can transform microarray data and visualize the changes in expression levels in entire pathways and categories of genes in apple, using the MapMan tool developed by Mark

Fig 2: MapMan based digital analysis of biochemical and metabolic pathways represented on HortResearch and Combimatrix microarrays



Stitt et al. at the German Resource Center for Genome Research (http://gabi.rzpd.de/projects/MapMan/). MapMan project collaborators have developed an ontology which classifies Arabidopsis genes into 35 broad categories, and nearly 2000 sub-categories that correspond to all known functions in Arabidopsis. The Image Annotator, MapMan's visualization data tool, includes several pathway diagram figures where expression levels of large numbers of individual genes, and their functional classifications, can be visualized simultaneously creating an ethylene responsive knowledgebase (ER Knowledgebase) for us to sift through gene expression data. The software tool allows us to customize the ontology to the conditions that match more closely with our particular study, and add our own visualization diagrams. To date, the standard ontology and pathway figures of the MapMan package have enabled us to visualize expression data in experiments on tomato and citrus based on the orthologs in Arabidopsis corresponding to genes in each of these species. We have determined these Arabidopsis orthologs by Blast analysis for each of the HortResearch and CombiMatrix target sequences. From these we have made comparisons between the targets represented on these two arrays (Fig 2). A majority of about 16980 genes in MapMan ontology have significant hits to targets of both arrays. About 1663 of the ontology genes had significant hits to targets of Combimatrix array only and 2129 of the ontology genes showed significant hits to targets of HortResearch array only. Only 4538 of the ontology genes had no significant hits to either array targets. We expect many ethylene-related genes to be differentially regulated between the wild type, 1-MCP treated or ethylene-silenced. But our primary interest is to define pathways that are upregulated or downregulated specifically in response to ethylene or with 1-MCP.

2. Ethylene is positively correlated with color, starch and weight and negatively correlated with firmness and acidity. Ethylene is not correlated with soluble solids

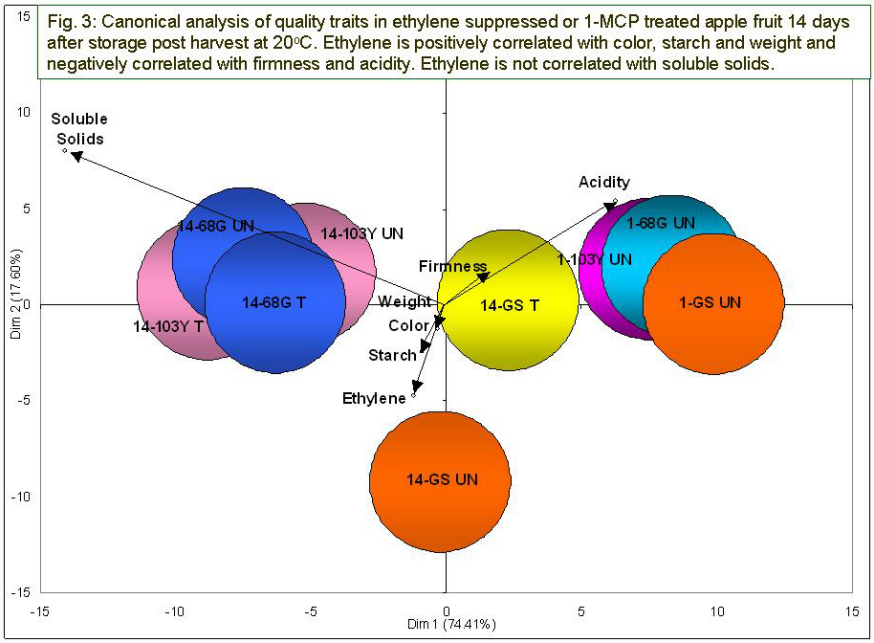


Fig. 3: Canonical analysis of quality traits in ethylene suppressed or 1-MCP treated apple fruit 14 days after storage post harvest at 20°C. Ethylene is positively correlated with color, starch and weight and negatively correlated with firmness and acidity. Ethylene is not correlated with soluble solids.

Application of 1-MCP to GS (control) apple fruit completely suppressed ethylene production. Application of exogenous ethylene to the 68G and 103Y transgenic lines did not produce increased ethylene biosynthesis. Weight, external color, firmness, starch index, and total acidity were regulated by ethylene in both 2005 and 2006, but soluble solids were ethylene-independent (Fig 3). The apple fruit from the 2006 crop was affected by unseasonal and sustained heat: maturity and quality indicators showed ethylene dependence, but not color. Firmness, a primary measure of maturity and quality, is regulated by ethylene biosynthesis. Firmness was most affected in the 1-MCP treated fruit; the effect of ethylene suppression on firmness in transgenic lines was less obvious. These results are preliminary and the final conclusions can be made after 2007 field data that we will obtain later this year. Firmness after 1 day storage at 20 ºC was measured using a Guss fruit texture analyzer

(destructive measurement method) and after 14 days firmness was measured first by an Aweta Acoustic Firmness sensor (AFS, non-destructive method) and then with the same fruits by the Guss Fruit Texture Analyzer. Sugars like fructose, glucose, sucrose, and sorbitol are the major metabolites that determine apple fruit sweetness. In lines suppressed for ethylene synthesis, fructose and sucrose do not accumulate as much as in the GS controls. However, when suppressed lines were exposed to exogenous ethylene, they reached control levels, except for glucose and fructose in the ethylene-treated line 103Y. 1-MCP did not reduce sugar accumulation in flesh, but did reduce sucrose and glucose in peel tissue. Individual sugars and malic acid were determined in flesh and peel tissues. The average number of metabolites detected 1 day and 14 days after storage at 20ºC were 155 and 136, respectively, in flesh and 132 and 125, respectively, in peel. In both cases the metabolites observed included sucrose, glucose, fructose, sorbitol, and malic acid. We are currently carrying out the complex statistical analysis of this metabolomic data that we hope to use to evaluate differences among genotypes and treatments. Metabolite profiles will be used later to resolve these into pathways and verify pathway data obtained from the microarray analysis.

Significance to the industry and potential economic benefits

Understanding the metabolic network and biosynthetic pathways active in apple fruit could facilitate extending postharvest life of flavor, aroma, and texture to match appearance. This, in turn, would promote consumption of fresh apple fruits and reduce losses during postharvest storage, stimulating demand while reducing costs to handlers and consumers. Identifying genes that determine and regulate fruit quality phenotypes can provide a new set of tools to improve management, breeding, and selection of existing and new cultivars.

Literature cited:

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CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AH-05-507 (WSU Project #13C-3655-5299)

Project Title: Growth and crop load management in apple trees with bioregulators

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Cooperators: Thomas D. Auvil, WTFRC; Eric A. Curry, USDA/ARS/TFRL
James R. McFerson, WTFRC; Dwayne Visser, WSU-TFREC

Budget 1:
Organization Name: WSU-TFREC **Contract Administrators:** Mary Lou Bricker; Sally Ray
Telephone: 509-335-7667; 509-663-8181 x221 **Email address:** mdesros@wsu.edu; saray@wsu.edu

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries[1]	8,000	8,400	**8,820**
Benefits	2,720	2,856	**2,999**
Wages[1]	2,000	2,000	**2,000**
Benefits	200	200	**230**
Supplies[2]	1,000	1,000	**1,000**
Travel[3]	3,000	3,000	**3,500**
Miscellaneous	500	500	**500**
Total	17,420	17,956	**19,049**

[1]Technical and time-slip help to set up trials, apply treatments and collect data as needed.
[2]This category includes a variety of miscellaneous supplies, non-capital equipment, consumables, etc. that are needed to carry out the research project. Cell phone charges are allowable under this grant.
[3]Travel to distant research sites is expensive. These funds will be used to defray costs of vehicular operation and maintenance, and personnel travel costs for travel for Dr. Elfving, Mr. Visser and their employees to research plots in grower-cooperator orchards through the south-central and north-central Washington fruit production areas.

Budget 2:
Organization Name: WTFRC **Contract Administrators:** Kathy Schmidt
Telephone: 509-665-8271 **Email address:**Kathy@treefruitresearch.com

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Wages and benefits[1]			**1,728**
Travel[3]			**160**
Total			**1,888**

[1]Calculated at $12/hour including benefits (4 total visits x 6 hours/visit/person = 24 man-hours/person total x 6 people = 144 total man-hours).
[3]Travel costs calculated at $0.445 per mile (360 miles).

Original objectives of the project:

1. Evaluate GA effects on return bloom in several alternating apple cultivars, e.g., 'Fuji,' 'Cameo,' and 'Braeburn.'
2. Examine single vs. multiple timings of various GA concentrations for efficacy in control of return bloom in apple to reduce amount of GA used, if possible.
3. Continue to test post-thinning ethephon as a method for stimulation of return bloom in cropping trees of several important apple cultivars, particularly those with alternate bearing problems.
4. Combine ethephon and GA programs in alternating cultivars as a possible strategy to help overcome alternate bearing.
5. Re-examine the integration of chemical thinning programs with GA/ethephon programs for beneficial effects on reduction of alternate cropping.
6. Test the use of cyclanilide and cytokinin bioregulators in high vigor sleeping-eye plantings for utility in programming lateral branch development into a specific canopy architecture as desired by the grower.

Additional objectives:

1. Evaluate various bioregulators for potential efficacy in stimulating bud activity and shoot growth on "blind wood."

Significant findings:

1. Only the strongest bloom-suppressing GA treatment applied in 2003 (GA_7) continued to influence the flowering and yield of 'Fuji'/M.9 trees in 2006. Results from this trial and others have shown that an alternating cycle can be induced by a practice intended to reduce alternate bearing. New trials exploring the potential for yearly interventions to control flowering are now underway.
2. When flowering is reduced due to GA treatment the previous year, the potential exists for an increase in fruit set to take place. This phenomenon was observed in one trial in 2006. An increase in fruit set, if significant, might offset the benefit of reduced flowering on subsequent cropping.
3. New GA/alternate bearing trials established in 2005 are testing the continued use of GA, or the application of BA/GA or ethephon to more closely control the flowering cycle as a strategy for developing better control methods for alternate cropping in difficult cultivars such as 'Fuji.' GA_{4+7} at 100 mg/liter, Promalin at 100 mg/liter or ethephon at 600 mg/liter were applied in 2006 to manage flowering for 2007 with the objective of evening out the bloom and yield.
4. Cyclanilide works well for inducing branching in sleeping-eye trees, thus saving much labor cost, but it can reduce height growth to some degree. A large comparison trial between Promalin and cyclanilide for branch induction in sleeping-eye 'Fuji'/M.9 trees was undertaken in 2006. Results are not available at this time.

Methods:

Trials were established in both cropping and non-cropping apple trees to determine effects of various bioregulator products on both growth and fruiting behavior. All trials employed single- or multiple-tree plots in randomized complete block designs. One trial was carried over from 2003 to evaluate the effects of GA applied in 2003 on flowering and crop load of 'Fuji' apple trees in 2006. Two trials from 2005 were continued with second-year applications of gibberellic acid, 6-BA plus GA or ethephon to further regulate return bloom and seek to even out production from otherwise alternating 'Fuji' and 'Braeburn' trees. New trials were established in 2006 to 1) examine effects of cytokinins on stimulation of latent bud development from feathers of 1-year-old 'Scifresh' (Jazz) trees; 2) examine similar effects on newly planted Jazz trees; 3) examine interactions between cytokinins and bark nicking for stimulation of latent bud activity in 'Cameo,' 'Granny Smith' and 'Honeycrisp' apples; and 4) compare cytokinin vs. cyclanilide for inducing branching at sequential trellis-wire levels in sleeping-eye 'Fuji'/M.9 trees.

Results and discussion:

A. Control of flowering with GA in alternating apple cultivars (Objectives 1,2)

1. In the fourth season following GA treatment in spring to control return bloom, young 'Fuji'/M.9 trees treated with GA_7 in 2003 still showed evidence of continued influence of previous crop fluctuations on 2006 production. GA_7 produced the strongest effect in 2003 on return bloom in 2004, establishing an alternating pattern still evident in 2006.

B. Induction of flowering with ethephon (Objective 3)

1. Ethephon was applied in June 2006 to trees of 'Fuji'/M.26 and 'Braeburn'/M.9 that either received GA_{4+7} in 2005 or were not treated in 2005. Supplementing GA-treated trees with ethephon may provide a way to balance return bloom and cropping so as to reduce alternation.

C. Combination GA programs for control of alternate bearing (Objectives 4,5)

1. Two trials were established in 2005 in "off"-year 'Fuji' and 'Braeburn' trees to initiate a new program of treatments designed to exploit the capability of GA to suppress flowering in apple. In these trials, GA was applied in 2005 to initiate the process of mitigating the 2005 low-crop effect on bloom in 2006. However, in these trials, intervention will continue annually with either 1) no follow-up, to observe the natural response to the initial GA treatment; 2) GA at modest concentrations to mitigate the observed tendency for GA-treated trees to develop an excess bloom the next year in response to the GA effect in the first year; 3) a BA/GA combination to provide both a thinning effect in the next year plus some suppression of bloom; or 4) some combination of NAA and/or ethephon that has shown benefit for stimulation of return bloom formation in cropping apple trees. The expectation is that one or more of these additional interventions may permit the reduction of the repeating cycle of a bloom/crop spike followed by scarcity that characterizes alternate bearing. Since flowering itself appears to be the key to the maintenance of an alternating cycle, interventions that control flowering should have significant potential for mitigating this cycle.
2. Flowering in 2006 in both trials was reduced by approximately 15% due to treatment with GA_{4+7} at 100 ppm in 2005. Yield in 2006 was comparably reduced in 'Fuji' trees but not in 'Braeburn' trees. Applications of additional GA, Promalin or ethephon were made in 2006 to investigate the potential for controlling the GA-induced reverse alternation we have observed in earlier trials.

D. Programming the induction of lateral branching in sleeping-eye apple trees with bioregulators (Objective 6)

1. Cyclanilide (CYC) has shown promise for aiding in the programmed development of lateral branches in the first year of growth of sleeping-eye apple trees.
2. In 2006, a test was established in newly planted 'Fuji'/M.9 apple trees to compare the effects of Promalin vs. cyclanilide in relation to stimulation of lateral branching at sequential trellis wires as the central leader developed vertically.
3. Cyclanilide and Promalin sprays were applied to the shoot tips and nearby leaves whenever the shoot tip reached a wire.
4. Final results are not available at this time.

E. Stimulation of bud activity on "blind wood" in apple (New Objective 1)

1. Three cytokinins were tested for efficacy in stimulating growth activity from latent buds in five trials with 'Scifresh' (Jazz), 'Cameo' and 'Granny Smith' apple trees.
2. Thidiazuron painted onto the basal halves of feathers at green-tip in second leaf and newly planted Jazz trees produced a strong budbreak response when applied at 2,500 mg/liter a.i. Chlorophenylurea (CPPU) was also effective, but 6-BA (Maxcel) produced only a marginal effect, even at 5,000 mg a.i./liter. Although budbreak was induced, very few of the activated buds formed any sort of shoot.
3. Combining nicking cuts with thidiazuron application to lateral shoots of 'Cameo' trees at green-tip produced the strongest budbreak response, but nicking only improved the response by about 10%.
4. High concentrations of thidiazuron, CPPU and 6-BA were applied to 2-year-old wood and the base of 1-year-old leader shoots on 3-year-old 'Honeycrisp'/M.9 trees. The older wood produced almost no response, while the leader shoots did show some evidence of increased budbreak, but the overall response was poor. Higher concentrations might have helped, but the low vigor of the trees may have been the principal factor limiting the growth response.

Summary:
GA applied shortly after bloom can effectively reduce return bloom in "off"-year 'Fuji' trees that otherwise would initiate a "snowball" bloom that favors continued alternation of production. So far, results have shown that a single such application of GA effectively shifts alternation to the opposite cycle but has not moderated the tendency for alternation. Results in 2005 pointed to the flowering process itself as a key factor in maintaining the alternating cycle once it becomes established. These results clearly indicate that other interventions that address flowering are required in seriously alternating cultivars if the alternating behavior is to be controlled. Thinning may not be a satisfactory approach because it does not intervene at the flowering stage.

Ethephon is registered for use on apple trees in early summer for stimulation of flower initiation. This approach could have benefit if used in the "on" year in conjunction with GA in the "off" year to better manage flowering. However, the reliability of ethephon for return bloom stimulation in cropping trees when applied well after bloom has not been demonstrated so far in Washington trials.

Cyclanilide is a very effective inducer of lateral branching in apple trees. Cyclanilide has a valuable role in managing branch development in the growth of sleeping-eye trees or other young trees where desired branch location is known and treatments can be applied at an appropriate timing to induce lateral branches in the proper locations while substantially reducing labor costs. Cyclanilide only works effectively in trees of the highest vigor. Poor-vigor trees will show NO branching response to cyclanilide.

Overcoming "blind wood," a problem with some apple cultivars, is difficult because the latent buds that populate such wood are extremely difficult to induce to grow. New methods and products are under test to try to overcome this problem. After one year of trials, thidiazuron has emerged as the most effective product for inducing bud activity on apple, with CPPU also producing useful responses. More trials are needed to better relate product concentration, cultivar and tree vigor in terms of bud development response. Also, the rather poor response in terms of shoot growth needs to be studied further.

Acknowledgments:
The assistance and support of the following persons and organizations are gratefully acknowledged: Bruce Allen, Tom Auvil, Humberto Camacho, Felipe Castillo, Phil Dormaier, Mark Gores, Jeff Henry, Steve Hull, Dr. Chris Ishida, Rick Kamphaus, Dr. Jim McFerson, Chris Olsen, Major Tory Schmidt, Dwayne Visser, Apple-Eye Orchards, BASF Corp., Bayer Environmental Science, Columbia Reach Pack, Inc., Dormaier Orchards, Firewood Orchards, Gores Orchards, Valent BioSciences, the Washington Tree Fruit Research Commission, and the WSU Agricultural Research Center.

Publications 2006:

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Smith, D., J. Turner and D. Elfving. 2006. Effects of chemically-induced branching on performance of several pear cultivar/rootstock combinations in a high-density planting. Poster, Wash. State Hort. Assoc. annual meeting, Yakima, WA.

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

Project Title: Chemical thinning of apple

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Organization: WTFRC
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City: Wenatchee
State/Province/Zip WA 98801

Cooperators: Tory Schmidt, 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	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries			
Benefits			
Wages	30,000	30,000	30,000
Benefits (16%)	4,800	4,800	4,800
Equipment			
Supplies	3,000[1]	3,000[1]	3,000[1]
Travel	500	500	500
Miscellaneous			
Total	38,300	38,300	38,300

Footnotes: [1] Chemicals, fruit purchase
NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

OBJECTIVES:

- Evaluate pre-bloom, bloom, and post-bloom chemical thinning agents with particular focus on complete programs to achieve three goals:
    1. Minimize costs of green fruitlet thinning
    2. Maximize fruit quality
    3. Encourage annual bearing
- Investigate influence of important variables (drying conditions, spray technology, carrier volume) on chemical thinner efficacy and fruit finish
- Expand collaborative efforts with other research programs

SIGNIFICANT FINDINGS:

2006 bloom thinning programs had poor results across all trials; only CFO + LS (4 sites) and NC99 (1 site) significantly reduced fruit set; no bloom thinners improved fruit size (Table 2)

Preliminary results with novel bloom thinning programs including pollenicides (from Rom), Raynox, vinegar + oil, urea, and Pacific Natural fish emulsion + lime sulfur were inconclusive

2006 return bloom was routinely improved by bloom and/or postbloom thinners in 2005 trials, often when no significant reduction in crop load was observed (Table 3)

Oil + lime sulfur programs are the most efficacious options for bloom thinning (Table 3)

Application and drying conditions relative to time of day did not significantly affect fruit set, size, or finish in 2 trials; experiments should be repeated (Table 4)

Spray technology (AccuTech vs. Proptec vs. airblast) and carrier volume (100 gal/acre vs. 200 gal/acre) utilized for bloom and postbloom chemical thinning programs did not significantly affect fruit set, size, or finish of Fuji; trial should be repeated (Table 5)

In a majority of trials, BA + carbaryl postbloom thinning programs gave results equal or superior to NAA + carbaryl programs; ethephon did not increase thinning (Table 6)

Combined bloom + postbloom thinning programs reduced fruit set and increased fruit size; carbaryl + BA outperformed other postbloom programs (Table 7)

Return bloom trials utilizing summer applications of NAA have thus far been unsuccessful

BACKGROUND:

The internal research program of the WTFRC conducted 18 apple chemical thinning trials in 2006 at thirteen commercial orchard sites around the state of Washington. As in 2005, we deployed fewer trials at fewer sites than in the past to facilitate labor efficiencies and allow intensified data collection throughout the season. Results from these trials add to our already sizable body of chemical thinning data, drawing from approximately 200 field trials since 1998 on eleven cultivars and ten rootstocks representing all important growing districts in the state. In 2006, all trials but one were applied by WTFRC staff with our Proptec research sprayer; historically, roughly half of our trials have been applied by grower-cooperators with their own equipment.

We have identified 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 call for 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 investigated in 2006 are reflected in Table 1; in programs 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 programs were left to the discretion of individual grower-cooperators as long as all experimental plots received the same treatments.

Table 1. Typical chemical thinning programs evaluated. WTFRC 2006.

BLOOM THINNERS
3.4 – 4 gal Ammonium thiosulfate (ATS)/A
5 gal NC99/A
6-8% Lime sulfur (LS)
2% Crocker's Fish Oil (CFO) + 2-4% LS
2% Pacific Natural Fish Emulsion + 2.5% LS
1% Wilbur-Ellis Supreme Oil (WES) + 3% LS
5% Canola, Corn, or Soybean Oil Emulsion
2% Canola, Corn, or Soybean Oil Emulsion + 2% LS
10% Vegetable Oil Emulsion (VOE)
17% VOE + 17% Vinegar
15% GS Long thinner (unnamed)
40 lbs Urea/A
2 pts Tergitol/A
20% Raynox
3 pts Ethrel (ethephon)/A
POSTBLOOM THINNERS
1.5-3 qts Sevin (carbaryl)/A
3 qts MaxCel, Exilis, Genesis BA (BA)/A
1-3 pts Ethrel (ethephon)/A
3 oz NAA/A
2 oz Amid Thin (NAD)/A

BLOOM THINNING:

Bloom thinning trials provided disappointing results at most sites in 2006, in part because of increased use of unproven chemistries for blossom thinning (NAA, Raynox, Tergitol, Urea, etc.). No blossom thinner significantly increased harvest fruit size in any trial, and only Crocker's Fish Oil (4 sites) and NC99 (1 site) provided significant reductions of fruit set. Evaluation of these programs will not be complete, however, until this coming spring; we are often surprised by strong increases in return bloom from otherwise unimpressive thinning treatments. Table 2 shows some encouraging

results regarding use of vegetable oil-based emulsions alone or in combination with lime sulfur. These types of programs may become critical if petroleum- or fish-based oils become less available and more expensive.

We tried to evaluate several novel blossom thinners in 2006, including several pollenicides and essential plant oils based on recommendations from Curt Rom and Jason McAfee (U of Arkansas), as well as vegetable oil + vinegar, Urea, Raynox, and a new fish emulsion (Pacific Natural) + lime sulfur. Unfortunately, these trials showed poor results for all treatments, including our gold standard of CFO + LS, and these new treatments will need to be repeated in 2007 to fairly evaluate their efficacy.

Table 2. Crop load effects of bloom thinning programs. WTFRC 2006.

Trial	Thinning program	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
Gala / Bud.9	Lime sulfur	75 ab	56 ns	22 ns	182 ns	105
- Chelan	CFO + LS	69 b	60	20	185	103
	Canola oil emulsion	85 ab	53	20	179	107
	Canola oil emulsion + LS	90 ab	51	22	179	107
	Corn oil emulsion	72 ab	58	20	187	102
	Corn oil emulsion + LS	80 ab	56	20	178	107
	Soybean oil emulsion	85 ab	54	19	183	104
	Soybean oil emulsion + LS	80 ab	55	21	176	108
	NC99	68 b	62	18	189	101
	Control	97 a	51	19	176	108

Although 2006 was a tough season for blossom thinning, we remain confident that oil + lime sulfur programs offer consistently positive results in achieving our stated chemical thinning goals. 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. It should be noted that the success rates of ATS and CFO + LS may be undervalued relative to other thinning materials in Table 3 because these programs have been disproportionately tested in the last three years when bloom thinning results have been less spectacular than in previous seasons.

Table 3. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials WTFRC 1999-2006.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit diam	Return bloom[1,2]
Ammonium thiosulfate	14 / 51 (27%)	9 / 54 (17%)	3 / 39 (8%)
NC99 (Mg^{++}/Ca^{++} Cl^{-} brine)	15 / 29 (52%)	7 / 31 (23%)	2 / 24 (8%)
Lime sulfur	25 / 52 (48%)	12 / 46 (26%)	9 / 43 (21%)
Crocker's Fish Oil + lime sulfur	55 / 87 (63%)	24 / 80 (30%)	18 / 67 (27%)
JMS Stylet Oil + lime sulfur	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
Wilbur-Ellis Supreme Oil + lime sulfur	14 / 27 (52%)	4 / 26 (15%)	4 / 25 (16%)
Vegetable Oil Emulsion	13 / 26 (50%)	4 / 25 (16%)	2 / 25 (8%)

[1]Does not include data from 2006 trials.

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

VARIABLES AFFECTING THINNING EFFICACY AND FRUIT FINISH:
While our results have clearly demonstrated the efficacy of several chemical thinning programs, we seek to improve their consistency and predictability. Conventional wisdom holds that many extraneous factors strongly influence fruit set, including temperature, light conditions, pollination, tree nutrition, etc. In 2006, we conducted two trials to investigate the effects of drying conditions, and a third trial to examine spray technology and carrier volume effects, on efficacy of proven chemical bloom and postbloom thinning programs and their impact on fruit finish.

Table 4. Chemical thinner drying condition effects on crop load and fruit finish. WTFRC 2006.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
			%	%	g		%
Golden	Bloom+PB – 6 AM	37 b	67 abc	29 abc	159 ns	120	59 ns
Delicious/	Bloom+PB – Noon	29 b	74 ab	23 bc	186	103	72
M.26	Bloom+PB – 6 PM	39 b	65 bc	32 ab	181	105	64
- Manson	PB only – 6 AM	40 b	66 abc	29 abc	167	114	79
	PB only – Noon	25 b	78 a	18 c	173	110	66
	PB only – 6 PM	33 b	70 ab	27 abc	179	107	64
	Control	59 a	55 c	35 a	162	118	71
Fuji/M.26	Bloom+PB – 6 AM	100 ns	39 ns	32 ns	192 ns	99	39 ns
- Manson	Bloom+PB – Noon	107	32	38	187	102	30
	Bloom+PB – 6 PM	108	36	33	197	97	51
	PB only – 6 AM	141	26	28	202	94	37
	PB only – Noon	138	26	30	187	102	47
	PB only – 6 PM	129	28	31	192	99	49
	Control	135	33	25	191	100	39

Table 4 represents two trials in which identical chemical thinning programs were applied at different times of the same days. In treatments receiving bloom thinners, 2% CFO + 3% LS were applied at 20% and 80% bloom early in the morning, at midday, or in the evening. Treatments receiving postbloom thinners were sprayed with 3 pts carbaryl + 3 qts BA/acre at 5mm and 10mm fruitlet size at the same time intervals employed for bloom thinning. Morning applications were typically during cool (53-58ºF) and damp, but warming conditions; midday conditions featured temperatures continuing to rise from 60-65ºF; evening sprays occurred in relatively dry conditions cooling from 62-67ºF. All treatments on Golden Delicious significantly reduced fruit set, but, consistent with poor bloom thinning results across all of our trials, inclusion of CFO + LS did not increase thinning beyond levels achieved by postbloom thinners alone. No significant thinning was observed by any treatment in Fuji, and no treatment in either trial showed any effect on fruit finish. Unfortunately, the overall lack of treatment effects in these trials preclude analysis of the role of drying conditions in chemical thinning and fruit finish; we plan to repeat these trials in 2007.

Table 5. Chemical thinner spray technology and carrier volume effects on crop load and fruit finish. WTFRC 2006.

Trial	Sprayer	Carrier volume	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
		gal/acre		%	%	g		%
Fuji/M.26	AccuTech	100	82 bc	41 ab	40 ab	224 ns	85	100 ns
- Quincy	AccuTech	200	86 bc	38 ab	44 ab	225	85	97
	Proptec	100	106 ab	28 b	44 ab	216	88	99
	Proptec	200	82 bc	39 ab	43 ab	214	89	97
	Turbo-mist	100	87 bc	34 b	49 a	233	82	97
	Turbo-mist	200	60 c	50 a	40 ab	224	85	98
	Control	na	130 a	28 b	33 b	219	87	96

At another site, we applied identical chemical thinning programs at consistent timings using different sprayers and carrier volumes (Table 5). All treated plots received 2% CFO + 4% LS at 80% bloom, as well as 3 qts carbaryl + 3 qts BA/acre at 5mm and 10mm fruitlet size. All treatments reduced crop load, but differences relative to sprayer technology and carrier volume were not apparent. This trial block was selected in part due to a history of russet problems, and in 2006, russet pressure was sufficiently extreme to mask any treatment effects (note that 96% of unsprayed control fruit had russet). The predominant form of blemishes on fruit was Fuji flecking or "sparkle."

POSTBLOOM THINNING:

Postbloom chemical thinning trials from 2006 corroborated results from previous seasons. Combinations of carbaryl + benzyladenine (BA) often outperformed carbaryl + NAA in terms of thinning. As with bloom thinning trials, however, significant increases in fruit size were rare across all trials. Following up on our 2005 trials, we again found that earlier applications of postbloom thinners, i.e. 5mm and 10mm fruitlet size, generally provided results superior to those of later timings, i.e. 10mm and 15mm (data not shown).

Table 6. Crop load effects of postbloom thinning programs. WTFRC 2006.

Trial	Thinning program	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
Gala / M.26	NAA + Exilis	48 ab	59 ab	35 ab	186 ns	103
- Orondo	Sevin + Exilis	31 b	71 a	27 b	186	103
	Sevin + Exilis + Ethrel	51 ab	57 ab	35 ab	186	103
	Sevin + NAA	56 a	57 ab	32 ab	195	98
	Control	60 a	50 b	40 a	182	105
Gala / M.26	NAA + MaxCel	42 b	71 a	19 b	196 ab	97
- Brewster	Sevin + MaxCel	70 ab	55 b	26 ab	184 ab	104
	Sevin + MaxCel + Ethrel	73 a	51 b	31 a	185 ab	103
	Sevin + NAA	67 ab	56 ab	28 ab	206 a	93
	Sevin + NAA + Ethrel	60 ab	60 ab	25 ab	198 ab	96
	Control	72 a	56 ab	23 ab	176 b	108

We are encouraged by the effective reduction of fruit set from NAA + BA programs (Table 6), which present carbaryl-free alternatives for postbloom chemical thinning. While most treatments evaluated

provided better results than controls, the addition of ethephon did little to increase thinning of either carbaryl + BA or carbaryl + NAA tank mixes.

COMBINED BLOOM AND POSTBLOOM PROGRAMS:

Even though many of the chemical thinners we have evaluated through the years have been consistently effective, they rarely reduce cropping to levels considered satisfactory by commercial growers. In 2006, we conducted three trials evaluating various combinations of popular bloom thinning programs (ATS; oil + LS) and postbloom thinning programs (carbaryl + NAA; carbaryl + BA). Table 7 shows a trial with typical results; namely, no bloom or postbloom thinner was adequate as a stand-alone program and that a two-pronged approach provided superior results. As in 2005 trials, postbloom thinning programs featuring BA products (MaxCel or Exilis) combined with carbaryl more consistently increased fruitlet thinning and harvest fruit size than carbaryl + NAA programs.

Table 7. Crop load effects of bloom + postbloom thinning programs. WTFRC 2006.

Trial	Bloom thinner	Postbloom thinner	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
				%	%	g	
Golden Delicious/	ATS		47 a	65 bc	25 a	198 bcd	96
M.7	ATS	Sevin + NAA	41 ab	68 bc	24 a	220 ab	87
Othello	ATS	Sevin + MaxCel	15 c	88 a	11 b	209 abcd	91
	CFO + LS		35 ab	71 bc	22 ab	187 cd	102
	CFO + LS	Sevin + NAA	35 ab	70 bc	26 a	210 abc	91
	CFO + LS	Sevin + MaxCel	25 bc	78 ab	18 ab	219 ab	87
		Sevin + NAA	40 ab	68 bc	25 a	227 a	81
		Sevin + MaxCel	31 abc	75 abc	20 ab	211 abc	90
		MaxCel	33 ab	72 bc	23 a	199 bcd	96
	Control		46 a	63 c	29 a	182 d	105

SUMMER RETURN BLOOM PROGRAMS:

We are intrigued by reports from other researchers (North Carolina) and many Washington growers regarding programs which increase return bloom with serial application of NAA during summer months. The notion of influencing flowering of apple 3-4 months after bloom is particularly interesting in that conventional horticultural dogma has held that flower bud initiation is completed within 6-8 weeks of full bloom.

While we do not doubt the credibility of these reports, we have so far been unable to reproduce their successes in our trials. These previous failures have been largely attributable to site selection and/or inadequate NAA concentrations. In 2006, we initiated a new trial in a well-maintained Golden Delicious block utilizing NAA programs as recommended by the manufacturer and look forward to assessing bloom effects this spring. Even though our results with NAA have been disappointing thus far, we have observed increases in flowering due to summer applications of Ethrel, and are confident growers can reliably increase bloom with summer ethephon programs.

CONTINUING PROJECT REPORT

Project Title: Collaborative WTFRC research programs

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

OBJECTIVES:

1. Conduct field trials on crop load management, use of reflective covers, sunburn suppression, russet management, rootstock evaluation, and lenticel-based skin disorders in grower cooperator orchards.

2. Assist WTFRC funded research programs with trial setup, maintenance, and sampling.

3. Manage soil sample collection regarding Penbotec and Scholar registration.

RESULTS AND DISCUSSION

WTFRC field trials

In 2006, the Washington Tree Fruit Research Commission (WTFRC) conducted 65 trials on apple, pear, cherry, peach, and nectarine within its internal research program, covering topics such as crop load management, reflective groundcovers, sunburn suppression, fruit finish, rootstock evaluation, and lenticel-based skin disorders (Table 1). All trials were conducted in grower-cooperator orchards. Funding was used to hire seasonal labor (10 people/year), to repair and maintain equipment, to purchase supplies, and to cover crop loss. Most products evaluated were donated by industry suppliers (Table 2). A number of trails were conducted with financial support from private companies (Table 3). Detailed project reports are included elsewhere in this document.

We have developed strong ties with various organizations specializing in international student exchange (i.e. Experience International, Ohio State University International Agriculture Exchange Program). In recent years, we have hosted interns from Germany, Mexico, and Austria. We encourage students to actively participate in industry events and to educate WA growers about practices in their respective home countries.

Table 1: WTFRC internal program field trials in 2006.

	Apple	Pear	Cherry	Peach	Nectarine
Crop load management	22	3	3	1	1
Reflective fabric*	7	2	5	1	
Sunburn suppression*	1				
Fruit finish*	6				
Lenticel breakdown	4				
Rootstock	9				
				Total:	**65**

* Products donated by industry suppliers

Table 2: Companies and Institutions that contributed materials and services to the WTFRC internal program in 2006.

Contribution	Company
Chemicals	Amvac
	BASF
	Cascade Distributing Co.
	D & M Chemical
	Fine Agrochemicals
	GS Long
	JMS Flower Farms
	Nufarm
	Orcal Inc.
	Pace Intl.
	RainGard
	Rohm and Haas
	Valent
Other supplies	Extenday
	Willow Drive Nursery
	Wilbur-Ellis, Wenatchee
Labor	Crane and Crane
	Fleming's Valley View Orchards
	Stormy Mountain Ranch
	Valley Fruit
	Willow Drive Nursery
Lab space/equipment	USDA-ARS TFRL, Wenatchee
	WSU-TFREC, Wenatchee
Packing line time	Valley Fruit
	McDougall & Sons
Fruit donation	Auvil Fruit Company
	Crane and Crane
	Ron Wilcox

Collaborative Projects

The WTFRC internal program provided technical support with trial set-up, maintenance, and sampling for several WFTRC-funded research programs (Table 3). A growing number of scientists have taken advantage of the opportunity to utilize the internal program's extensive network of industry cooperators when conducting field trials. By using in-state locations in commercial orchards,

increasingly relevant data has been generated for Washington growers by research programs around the world.

Betsy Beers: The focus of this project was to evaluate collateral effects of chemical thinners (namely lime sulfur and carbaryl) on populations of phytophagous and predatory mites at WTFRC trial sites. Leaf samples were collected every other week from 5 treatments each at three trials and delivered to the Beers lab for evaluation. (sample timing: late April to late July)

Curt Rom: WTFRC performed field evaluations of novel organic chemical bloom and postbloom thinners developed by Rom and his graduate student, Jason McAfee. Materials included essential plant oils, organic acids, and organic bases with potential to kill or damage pollen. Trial location, setup, spray applications, harvest, and quality analyses were performed by the WTFRC.

Don Elfving: The internal program continued its ongoing support of data collection for Elfving's PGR work, including whole tree bloom counts in April for three sites and harvest yields in the fall.

Peter Hirst: *a*) Understanding apple flower bud development: *WTFRC* selected 10 trees from both a Gala and a Fuji block, attached tags to 100 buds per tree, and divided buds into 3 categories. Two samples of each type were collected every 10 days until August, when we switched to every 20 days until trial completion in mid October. The samples were fixed in FAA solution and shipped to Purdue for analyses.

b) Mechanisms of apple fruit growth: WTFRC selected 10 trees from both a mature Red Delicious and mature Gala block and hand thinned all trees at full bloom to reduce crop load. Samples were collected weekly starting in May, switching to bi-weekly in July, until trial completion at harvest. Samples consisted of 2 fruit from each tree, which were labeled and measured before being shipped to Purdue.

Steve van Nocker: WTFRC established a simple replicated thinning trial in Gala. Chemical thinners were applied with the Proptec sprayer, followed by 3 intensive sampling events of fruitlet parts. Hundreds of fruitlets were dissected and frozen in the field at each sampling, and eventually shipped to MSU for molecular analyses.

Gennaro Fazio: The main focus of the rootstock plantings is to evaluate new Geneva rootstocks in soils with replant problems (see Fazio report). WTFRC conducts trial layout, planting establishment, data collection, and some horticultural management on an ongoing basis.

Karen Lewis/Tom Auvil: WTFRC moves mobile platforms between locations for industry demonstration and testing, occacionally providing training in platform operation.

FruitGard: WTFRC located rain-vulnerable cherry sites, set up trials, and applied formulations designed to reduce rain cracking. Internal staff also conducted field evaluations, and collected harvest fruit samples.

Ciba-Geigy: Trial location and setup were performed by the WTFRC for 2 trials (Ambrosia and Pink Lady). Apples free of defects were selected and subsequently bagged with two component color enhancement bags about two months before harvest. Roughly a month before harvest the outer bag was removed, ten days later the inner liner was removed and the stencil was applied. At harvest, fruit was transported to the WTFRC lab for stencil removal, followed by treatment with SmartFresh, tray packed, and shipped to the company.

Whiting/Elfving: WTFRC staff helped design the trial, located an appropriate site, laid out the trial, collected field data, harvested sample fruit, and delivered it to the Whiting lab for quality analyses.

Whiting: WTFRC worked jointly with Extenday and Whiting to develop reflective groundcover trials in cherry. Internal staff maintained the trial, collected harvest fruit samples, and delivered them to the Whiting lab for quality analyses.

Fallahi: WTFRC conducted all aspects of trial design, setup, application, data collection, and analysis for peach and nectarine chemical thinning trials. Fallahi advised internal staff regarding treatments and provided Tergitol for use in soft fruit and apple.

Table 3: WTFRC internal program: collaborative support for WTFRC-funded projects.

			WTFRC technical support			
Researcher	**Topic**	**Number of trials**	Site selection	Trial set-up	Data collection	Misc.
Betsy Beers WSU - Wenatchee, WA	Chemical thinner effects on arthropod populations	3	x	x	x	
Curt Rom UA, Fayetteville, AR	Novel chemistries and pollenicides for chemical thinning	2	x	x	x	x
Don Elfving WSU - Wenatchee, WA	Use of gibberellic acid to inhibit flowering in apple	3			x	
Peter Hirst Purdue, West Lafayette, IN	Molecular basis for fruit cell division and expansion	4	x	x	x	x
Steve van Nocker MSU, East Lansing, MI	Molecular control of fruitlet abscission	1	x	x	x	x
Gennaro Fazio Cornell, Geneva, NY	Next generation rootstocks for apple	9	x	x	x	x
Lewis/Auvil WSU - Ephrata, WA / WTFRC	Mechanized assistance of orchard labor	variable	x			x
FruitGard LLC* Wenatchee, WA	Cherry cracking prevention	3	x	x	x	
Ciba-Geigy* Basel, Switzerland	Logo imprinting on apple skin	2	x	x		x
Whiting/Elfving WSU - Prosser/Wenatchee, WA	Use of gibberellic acid to inhibit flowering in cherry	1	x	x	x	
Whiting WSU - Prosser, WA	Reflective fabric to improve cherry quality	4	x	x	x	x
Fallahi UI - Parma, ID	Cropload management in softfuit	1	x	x	x	
	Total:	**33**				

* received financial support from company

Soil sample collection

In collaboration with the Northwest Horticultural Council and EPA, the internal program is managing soil sample collection supporting the registration of two new postharvest fungicides (Penbotec and Scholar). We are currently maintaining 24 sampling sites (Table 3). Soil samples are taken prior to application, directly after application, and post-season. WTFRC is responsible for collecting, shipping, and correct documentation of all soil samples. We anticipate to complete soil sampling in spring of 2007.

Table 3: Wastewater sampling: cost-effective data collection to support registration of postharvest fungicides needed by industry.

	Number of sites in WA	North central Washington	Yakima Valley
Sites established in 2005	11	5	6
New sites added in 2006	13	2	11
Total in 2006	**24**	**7**	**17**

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: Internal program

Project Title: Improving apple fruit finish by suppressing sunburn and russet

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Larry Schrader, WSU-TFREC, Wenatchee, WA

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

Item	Year 1: 2006	Year 2: 2007	Year 3: 2008
Salaries	6,000	6,000	6,000
Benefits	960	960	960
Wages			
Benefits			
Equipment			
Supplies	500	0	0
Travel	500	500	500
Miscellaneous			
Total	7,960	7,460	7,460

Footnotes: [1] All chemicals and harvest supplies were provided by industry vendors. Funds are used to rent Stemilt quality lab space.

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

OBJECTIVES:

1. Investigate chemical programs (GA, Raynox, Apogee) to improve fruit finish

2. Compare sunburn protectant (Raynox and Eclipse) efficacy in apple

SIGNIFICANT FINDINGS:

Most spray programs failed to reduce russet in Golden Delicious, Fuji, and Pacific Rose apples in 2006

Early ProVide timings reduced net and shoulder russet in one Golden Delicious trial

Application of different GAs alone or in combination with Raynox did not reduce Fuji flecking

Novel GA formulations were not effective for russet suppression

Golden Delicious and Fuji trials had less than 25% clean fruit, while Pacific Rose apples were 42-91% clean

2006 return bloom was not affected by 2005 russet treatments

Eclipse and Raynox did not suppress sunburn in 2006 trials

METHODS

Russet suppression: In 2006, we conducted 6 fruit finish trials (2 each of Golden Delicious, Fuji, Pacific Rose). All trials were sprayed with a Proptec sprayer at 100 gal/acre using a randomized complete block design with 4 replications and 5-20 trees/treatment/rep. We tested the following materials alone or in combination: ProVide (GA_{4+7}) at 15 or 19 ppm, Novagib (GA_4) at 23 ppm, Falgro 20 SP (GA_3) at 42 ppm, FAL 900 (GA_7) at 50 ppm, Apogee (prohexadione-Ca) at 12 oz/acre, and Raynox at 2.5 gal/acre. Spray programs included the following timings: 4 applications at petal fall (PF), 10, 20, 30 days after petal fall (DAPF); PF, 20, 40, 60 DAPF; 30, 40, 50, 60 DAPF. At one Golden Delicious orchard (seedling, 20 years old, Grandview) we sprayed ProVide 3 times with a hand-held sprayer at either PF, 10, 20 DAPF; 20, 30, 40 DAPF; 40, 50, 60 DAPF; PF, 30, 60 DAPF to individual branches.

Sunburn suppression: A 16 acre trial designed for commercial packout of segregated treatments was conducted on Granny Smith (M9, 8 years old, Royal City). The following treatments were applied by the grower with a powerblast sprayer diluted in 80 gallons of water/acre: Raynox at 2.5 gal/acre and Eclipse at 2.5 gal/acre. A randomized complete block design with 4 replications was used (1.55 acres/rep/trt for Raynox or Eclipse, 0.9 acres/rep for untreated control). Spray timing followed respective label recommendations (Raynox: 4 times, Eclipse: 5 times). At harvest, bins were segregated and transported to the Valley Fruit presize line for packout analysis. In addition, 4 cull bins/trt were randomly selected and each individual fruit was graded for sunburn according to the Schrader/McFerson system (0 = clean, 6 = necrosis).

RESULTS AND DISCUSSION

Russet suppression: Fruit russet is typically induced early in the growing season and is likely aggravated by a combination of weather conditions, spray chemicals, and/or topical biotic pests. Washington typically has fewer problems with severe russet than other apple production regions, since springs are mostly dry. However, the 2006 season featured extensive russet pressure, mainly caused by a wet, cool period in early spring followed by extreme heat. Few practical options are available to orchardists to suppress russet. Results from 2005 WTFRC trials confirmed the efficacy of gibberellic acid (GA) products (ProVide and Novagib) at various rates and spray regimes for improving fruit finish. In 2006, we tested additional GA products and narrowed down optimal timings and concentrations.

Golden Delicious: Within both Golden Delicious trials the amount of clean fruit was extremely low (0-29%). While none of the materials and timings tested significantly reduced russet in the Wapato trial (Table 1), more clean fruit was obtained from some of the early applications of ProVide in Grandview (Table 2). ProVide mainly reduced net russet on the cheek of the fruit, as well as shoulder russet. Whether applied by itself or tank-mixed with Raynox, GA did not reduce fruit russet in Wapato.

Table 1. Fruit finish effects of GA formulations and Raynox on Golden Delicious apples. WTFRC 2006.

				TYPE OF RUSSET					
Material	Timing	Concentration	CLEAN (%)	BOWL (%)	SHOULDER (%)	TOTAL NET (%)	NET 5% (%)	NET 10% (%)	NET 25% (%)
Golden/M.111, Wapato									
Falgro 20 SP	PF, 10, 20, 30	42 ppm	24 ns	32 ab	20 ns	44 ns	38 ns	4 ns	1 ns
Novagib	PF, 10, 20, 30	23 ppm	20	35 ab	17	44	39	4	1
Provide	PF, 10, 20, 30	19 ppm	24	35 ab	22	40	38	2	0
Provide	30, 40, 50, 60	19 ppm	29	24 b	22	43	41	2	1
Provide + Raynox	PF, 10, 20, 30	19 ppm+2.5 gal	22	41 a	20	49	44	4	1
FAL 900	PF	50 ppm	22	36 ab	22	39	35	4	1
Raynox	PF, 10, 20, 30	2.5 gal	26	33 ab	25	42	35	6	2
Control			18	34 ab	18	46	41	2	1

Table 2. Fruit finish effects of GA_{4+7} at different concentrations and timings on Golden Delicious apples. WTFRC 2006.

				TYPE OF RUSSET						
Material	Timing	Concentration	CLEAN (%)	BOWL (%)	SHOULDER (%)	TOTAL NET (%)	NET 5% (%)	NET 10% (%)	NET 25% (%)	OVER 25% (%)
Golden/ Seedling, Grandview										
Provide	20, 30, 40	15 ppm	2 bc	44 ns	28 ns	94 a	8 ns	27 ns	36 ns	24 ab
Provide	40, 50, 60	15 ppm	1 c	42	36	95 a	6	28	39	22 ab
Provide	PF, 10, 20	15 ppm	11 a	48	16	76 b	13	28	24	11 b
Provide	PF, 30, 60	15 ppm	6 abc	50	13	91 ab	23	28	24	16 b
Provide	20, 30, 40	19 ppm	11 a	50	18	82 ab	16	27	28	11 b
Provide	40, 50, 60	19 ppm	2 bc	38	40	84 ab	12	29	28	22 ab
Provide	PF, 10, 20	19 ppm	10 ab	46	15	80 ab	22	28	23	6 b
Provide	PF, 30, 60	19 ppm	4 abc	37	34	92 ab	14	23	35	20 ab
Control			0 c	31	37	97 a	3	14	34	44 a

Fuji: Both trials were severely damaged by hail and not harvested by the grower. Only 6-16% of sampled fruit was completely free of any blemishes (Table 3). Most russet was confined to the stem bowl. Severe fruit flecking was observed at both sites. Flecking was unaffected by any treatment.

Table 3. Fruit finish effects of commercial GA formulations and Raynox on Fuji apples. WTFRC 2006.

				TYPE OF RUSSET			
Material	Timing	Concentration	CLEAN (%)	BOWL (%)	SHOULDER (%)	TOTAL NET (%)	FLECKING (%)
Fuji/M7, Royal City							
Novagib	PF, 10, 20, 30	23 ppm	8 ns	12 ns	5 ns	1 ns	82 ns
Novagib	30, 40, 50, 60	23 ppm	7	11	2	1	89
Provide	PF, 10, 20, 30	19 ppm	8	9	3	0	83
Provide	PF, 20, 40, 60	19 ppm	6	11	1	1	89
Provide	30, 40, 50, 60	19 ppm	6	11	3	2	86
Control			8	12	5	1	84
Fuji/M26, Royal City							
Novagib	PF, 10, 20, 30	23 ppm	8 ns	14 ns	8 ns	1 ns	82 ns
Novagib	30, 40, 50, 60	23 ppm	11	13	9	1	83
Provide	PF, 10, 20, 30	19 ppm	9	8	5	2	85
Provide	30, 40, 50, 60	19 ppm	9	14	7	2	83
Provide + Raynox	PF, 10, 20, 30	19 ppm, 2.5 gal	16	10	7	1	81
Raynox	PF, 10, 20, 30	2.5 gal	9	15	9	0	87
Falgro 20 SP	PF, 10, 20, 30	42 ppm	11	14	5	1	90
Control			12	12	7	0	83

Pacific Rose: Despite high russet incidence in other cultivars, Pacific Rose apples across the state were comparatively clean. We observed 42-54% clean fruit in the Prosser trial and 81-91% clean fruit in the Brewster trial (Table 4). Most russet was confined to the stem bowl, within limits of premium packing grades. None of the treatments were effective in improving fruit finish.

Table 4. Fruit finish effects of commercial GA formulations, Raynox, and Apogee on Pacific Rose apples. WTFRC 2006.

				RUSSET INCIDENCE					
Material	Timing	Concentration	CLEAN (%)	BOWL (%)	SHOULDER (%)	TOTAL NET (%)	NET 5% (%)	NET 10% (%)	NET 25% (%)
Pacific Rose/ M 26, Brewster									
Apogee	PF-2	12 oz	91 ns	4 ns	0 ns	2 ns	2 ns	0 ns	0 ns
Apogee; Novagib	PF-2; PF,10, 20, 30	12 oz; 23 ppm	81	6	1	3	2	1	0
Apogee; Provide	PF-2; PF,10, 20, 30	12 oz; 19 ppm	84	7	1	2	2	0	0
Novagib	PF, 10, 20, 30	23 ppm	81	7	1	2	2	0	0
Novagib	30, 40, 50, 60	23 ppm	86	9	1	3	2	1	0
Provide	PF, 10, 20, 30	19 ppm	88	6	0	0	0	0	0
Provide	30, 40, 50, 60	19 ppm	86	8	2	1	1	0	0
Control			86	6	0	0	0	0	0
Pacific Rose/M 9, Prosser									
Falgro 20 SP	PF, 10, 20, 30	42 ppm	42 ns	38 a	16 ns	17 ns	13 ns	4 ns	0 ns
Novagib	PF, 10, 20, 30	23 ppm	47	33 ab	14	17	16	1	0
Provide	PF, 10, 20, 30	19 ppm	48	30 ab	15	17	14	3	0
Provide	30, 40, 50, 60	19 ppm	48	34 ab	12	12	11	1	0
Provide	PF, 20, 40, 60	19 ppm	45	28 ab	16	19	16	3	1
Provide + Raynox	PF, 10, 20, 30	19 ppm, 2.5 gal	45	29 ab	17	20	17	3	0
Raynox	PF, 10, 20, 30	2.5 gal	54	24 b	12	21	17	4	0
Control			46	28 ab	17	20	16	4	0

Return bloom effects: Gibberellins are known to be inhibitors of floral initiation in apple, especially materials containing GA_7, such as ProVide. We did not observe any effects on return bloom (Table 5); because most trial orchards were entering the off-year of an alternate bearing cycle, treatment effects may have been masked.

Conclusion: We can not fully explain why most treatments failed to significantly reduce russet in 2006. The lack of differentiation in the data is likely due to extremely high baseline russet pressure in most trials (Golden Delicious, Fuji), which could have masked treatment effects. Further, application timing and frequency should be revisited in 2007. Brewster was the only trial location with a weather pattern permitting fruitlets to remain dry within the first 2 weeks after full bloom; not coincidentally, Pacific Rose apples from that site had markedly cleaner finish than other 2006 trial sites.

Sunburn suppression: Sunburn is the primary physiological cause of cullage, sometimes damaging up to 50% of the fruit in a given orchard. In 2005 WTFRC trials, new calcium-based products (Eclipse and Fruit Shield) performed as well as industry standards (Raynox, Surround WP). In 2006, we compared Eclipse and Raynox in a large-scale trial to be packed in a commercial warehouse. Neither of the treatments improved the amount of marketable fruit (Table 5), nor influenced orchard performance parameters such as total yield, or fruit size. There were no significant treatment effects in individual sunburn grades (Table 6), except in the black (necrotic) category, where treated Granny Smith showed less damage than control fruit. Hail damage might have confounded results, since most of the weather-exposed fruit (roughly 20% of the crop) was not harvested.

Table 5. Commercial packout analysis of sunburn suppressants on Granny Smith and Gala apples. WTFRC 2006.

GRANNY SMITH									
TREATMENT	WXF 1+ (%)	WXF 1- (%)	blush (%)	slicer/hail (%)	culls (%)	# of bins	bins/acre	fruit weight g	box count (40 lbs box)
Eclipse	22.9	4.8	14.2	19.4	38.7	172	27.7	257	69.0
Raynox	23.3	4.5	12.7	16.3	43.1	171	27.6	244	72.8
Control	24.3	5.0	13.2	13.6	43.9	108	30.0	246	72.1
GALA / Pollenizer									
TREATMENT	WXF 1S[a] (%)	WXF 1[b] (%)	WXF 2[c] (%)	WF (%)	culls (%)	# of bins	bins/acre	fruit weight in g	box count (40 lbs box)
Eclipse	11.7	48.5	17.7	2.7	19.4	20	3.2	208	87.4
Raynox	7.8	45.4	23.4	2.9	20.6	19	3.1	200	90.7
Control	8.8	44.4	19.4	2.5	25.0	5	1.4	200	91.1

[a] Brix >14, [b] colour >65%, [c] colour > 20%

Table 6. Sunburn analysis of Granny Smith and Gala culls treated with sunburn suppressants. WTFRC 2006.

		SUNBURN INCIDENCE				
TREATMENT	Clean (%)	Y1 (%)	Y2 (%)	Y3 (%)	Tan (%)	Black (%)
GRANNY SMITH						
Eclipse	7 ns	46 ns	26 ns	9 ns	8 ns	5 b
Raynox	4	45	28	10	10	3 b
Control	9	43	17	13	7	11a
GALA						
Eclipse	19 ns	36 ns	24 ns	13 ns	5 ns	4 ns
Raynox	28	34	22	9	4	3
Control	21	35	23	11	6	4

Acknowledgement: We would like to thank Pace Intl., BASF, Fine Agrochemicals, Valent, and D & M Chemicals for graciously donating chemicals, as well as Valley Fruit for valuable time on their packing line.

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

Project Title: Lenticel-based superficial skin disorders of apple

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GROWER-COOPERATORS: Brent Milne, McDougall & Sons, Wenatchee
Jason Matson, Matson Fruit, Selah
Rick Kamphaus, AppleEye Orchards, Manson

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

Item	Year 1: 2006	Year 2: 2007	Year 3: 2008
Salaries	4,000	4,000	4,000
Benefits	640	640	640
Wages			
Benefits			
Equipment			
Supplies	500	500	500
Travel	500	500	500
Miscellaneous			
Total	5,640	5,640	5,640

Footnotes: [1] Chemicals

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

OBJECTIVES:

1. Develop a survey instrument and strategy to assess the incidence and extent of superficial skin disorders (SSDs) in apple.
2. Develop short, medium, and long term research objectives and timelines addressing SSDs.
3. Facilitate field testing of promising approaches to mitigate SSDs.

SIGNIFICANT FINDINGS:

1. **Traditional survey tools were insufficient to gauge complexity of SSDs.**

2. **Weather data, spray records, horticultural performance, and SSD incidence could be correlated in a relational database.**

3. **Data collection (incl. horticultural practices, LB severity, spray records, weather data) within sample orchards to build relational database was started in 2006.**

4. **Lack of standardized nomenclature prevents correct assessment of SSD incidence.**

5. **Identification matrix is being developed.**

6. **Pilot study found that rootstocks might influence susceptibility to SSDs.**

7. **No significant treatment effect was noted after late-season application of hydrophobic materials (summer supreme oil, soybean oil, Raynox).**

METHODS

Objective 1: To generate baseline data to be incorporated into a relational database, Gala fruit (60 fruit/orchard block) were harvested within the first or second pick (CA quality fruit), and stored at the Stemilt RCA rooms for 3-4 months under RA conditions. To keep the costs low, fruit was harvested from orchards with existing trials (i.e. crop load management), or blocks within the vicinity. Fruit was evaluated with the waxing test developed by Dr. Eric Curry, using the WSU packing line. Standard maturity parameters were taken at harvest and after storage.

Objective 3: a) To evaluate rootstock effects on lenticel breakdown (LB) development, additional fruit samples were collected at harvest from the 04 Wapato rootstock trial (3rd leaf, Brookfield Gala, 12 rootstocks). Samples were stored under RA conditions at Stemilt RCA rooms and evaluated for LB incidence after 3 months with the waxing test.

b) In 2006, we conducted 4 trials (Selah, Manson, Royal City, Desert Aire). All trials were sprayed with a Proptec sprayer at 80 gal/acre using a randomized complete block design with 4 replications and 10-20 trees/treatment/rep. We tested the following materials: Summer Supreme Oil (1 or 2%), soybean oil (1 or 2%), and Raynox at 2.5 gal/acre. Spray programs included the following timings: single applications 1, 2, or 3 weeks before anticipated harvest. Samples were stored under RA conditions at the Stemilt RCA rooms and evaluated for LB incidence after 3-4 and 5-6 months with the waxing test. Standard maturity parameters were taken at harvest and after storage.

RESULTS AND DISCUSSION

This project brings new focus from the WTFRC internal program to postharvest aspects of tree fruit production, with particular emphasis on superficial skin disorders (SSD). These include, but are not limited to, lenticel breakdown (LB) and lenticel blotch pit (LBP).

Objective 1 - Survey: In a preliminary survey during the 2005/06 storage season, more than half of the packers contacted reported lenticel-related problems (60 contacted, 45 responded, 23 reported frequent problems). Varieties most affected included Gala and Fuji.

We will utilize relational databases (Microsoft Access) to connect weather data with horticultural practices and spray records. For that purpose, approximately 30 lots of Gala have been sampled and stored at the Stemilt RCA rooms to determine LB susceptibility after storage. Results will be presented during the Apple Research Review poster session.

Objective 2 – Research directions: We have formed a SSD working group, consisting mostly of WSU and USDA-ARS researchers, to develop short, medium, and long term research objectives and timelines addressing superficial skin disorders. One of the problems when assessing SSDs is the lack of standardized nomenclature. We are in the process of developing a matrix which can be used by researchers and industry personnel as a guide to properly identify SSDs.

Objective 3 – Field testing: We will regularly test promising hypotheses about preharvest factors that make fruit more susceptible to these maladies. For the 2006 growing season, the group decided to initiate preliminary evaluations to determine if cultivar strain, rootstock, and growing region have any influence on LB development. Depending on the outcome, further collaborative studies will be initiated within the 2007 growing season.
We will use the insight gained in all these evaluations to prepare more directed proposals for the coming years. Final results will not be available until the end of the storage season.

a) Utilizing the existing rootstock evaluation trial in Wapato, we assessed fruit susceptibility to LB in relation to the rootstock used (Table 1). Compared to our orchard trials we observed higher LB symptom expression after 3 months of RA storage, possibly due to advanced maturity at harvest. Most rootstocks had no effect on LB development when scored after 2 or 7 days at room temperature. After 2 days, G.3041 exhibited more than twice the amount of LB than G.16. Keeping fruit at room temperature for 5 more days did not change the ratio between those two rootstocks. Some rootstocks (G.4214, G.11) developed significantly more LB after 7 days, while others did not change (Supporter 2, Pajam2, G.16). G.16 had higher yields and smaller fruit compared to G.3041. Differences in LB susceptibility among rootstocks may be due to secondary effects caused by different crop load and overall tree structure. Industry standards like M.26 and Bud.9 showed moderate expression of LB symptoms, indicating that these common rootstock selections might not contribute significantly to LB development after storage.

Table 1: Effect of rootstock on LB development of 3 year old 'Brookfield' Gala apples after 3 months of RA storage. WTFRC 2006.

Rootstock	LB - 2d* (%)	LB - 7d* (%)	WT/TREE (kg)	FRUIT WT (g)
Bud. 9	25 ab	32.5 ab	6.5 d	155 b
G. 3041	40 a	42.5 a	9.2 abc	160 ab
G. 4214	38 ab	43.9 a	9.8 abc	159 ab
G. 5935	27 ab	35.8 ab	11.6 a	149 b
G. 11	31 ab	40.6 a	11.2 a	168 ab
G. 16	18 b	20.0 b	11.0 a	146 b
M. 26	25 ab	27.5 ab	9.2 abc	165 ab
Nic. 29	21 ab	30.0 ab	9.9 abc	167 ab
Pajam 2	25 ab	25.0 ab	11.0 ab	157 ab
Supporter 1	28 ab	32.5 ab	8.0 cd	186 a
Supporter 2	31 ab	30.8 ab	8.3 bcd	165 ab
Supporter 3	20 ab	25.0 ab	9.1 abcd	165 ab

* days at 72F

b) Another focal point in 2006 has been the time period one month before harvest. Preliminary research by Eric Curry indicated that one of the factors contributing to development of LB in storage might be desiccation pressure during this time period or while fruit is transported to the warehouse. We set up 4 trials to determine if the application of hydrophobic materials within 3 weeks of harvest would alleviate LB development after storage.

One problem encountered when scheduling appropriate applications was the uncertainty of harvest. When working with grower cooperators, harvest decisions are adjusted on an ongoing basis, making it difficult to harvest fruit at the ideal experimental timing. Thus, we ended up with different preharvest spray intervals for all our trials (Table 2).

Table 2: Effects of preharvest application of hydrophobic materials on LB development of Gala apples after 3 months of RA storage. WTFRC 2006.

TREATMENT	App. date	Harvest date	Actual # days before harvest	LB - 2d[2] (%)	LB - 7d[2] (%)
Royal Gala / M.9 Manson					
Soy 21[1]	6-Sep	8-Sep	2	16 b	20 b
Soy 3x	6-Sep	8-Sep	2	33 a	34 a
Supreme 21[1]	6-Sep	8-Sep	2	26 ab	21 b
Supreme 3x	6-Sep	8-Sep	2	29 ab	33 a
Control				23 ab	25 ab
Brookfield Gala / M.9 Selah					
Raynox	16-Aug	31-Aug	15	19 ns	21 ns
Soybean 2%	16-Aug	31-Aug	15	18	20
Soybean 1%	16-Aug	31-Aug	15	16	19
Supreme 2%	16-Aug	31-Aug	15	18	22
Supreme 1%	16-Aug	31-Aug	15	20	23
Control				16	19
Galaxy Gala / M.9 Royal Slope					
Raynox	3-Aug	23-Aug	20	19 ns	19 ns
Soybean 2%	3-Aug	23-Aug	20	15	18
Soybean 1%	3-Aug	23-Aug	20	9	13
Supreme 2%	3-Aug	23-Aug	20	13	16
Supreme 1%	3-Aug	23-Aug	20	16	19
Control				14	17
Imperial Gala / M.26 Desert Aire					
Soy 21[1]	26-Jul	12-Aug	17	11 ns	15 ns
Soy 14 [1]	2-Aug	12-Aug	10	15	16
Soy 7 [1]	9-Aug	12-Aug	3	15	19
Soy 3x	all above	12-Aug	all above	13	17
Supreme 21 [1]	26-Jul	12-Aug	17	13	13
Supreme 14 [1]	2-Aug	12-Aug	10	21	23
Supreme 7 [1]	9-Aug	12-Aug	3	13	16
Supreme 3x	all above	12-Aug	all above	13	16
Control				18	20

[1] refers to planned number of days prior to harvest

[2] days at 72F

All fruit was harvested at commercial maturity suitable for long term CA storage. We found no differences for common maturity parameters at harvest between control fruit and treated fruit (data not shown). Symptom expression was strongest after 7 days at room temperature. Fruit from all orchards expressed symptoms of LB after 3-4 months of RA storage (low: 9%, high: 33%). We observed slight orchard-to-orchard variations, with fruit from Manson having the highest LB incidence, possibly due to advanced maturity; other factors might have included Gala strain, rootstock, harvest date, orchard elevation, and tree age. No significant treatment effect was seen regarding oil type or concentration, spray frequency or Raynox application. Earlier treatments (2-3 weeks before harvest) provided greater reduction of LB incidence after storage.

Manson apples treated with Soy 21 showed significantly fewer LB symptoms than fruit treated Soy 3x, indicating highly variable LB susceptibility within the same lot. The 5-6 month storage data and a second growing season will be necessary to determine true treatment effects.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AH-05-506A (WSU Project #13C-3655-6325)

Project Title: Improving fruit finish and fruit quality in apples

PI: Larry Schrader
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Cooperators: Jim Mattheis, USDA-ARS Tree Fruit Research Lab, Wenatchee
Jizhong Xu, Cindy Kahn, David Felicetti, Jianguang Zhang, Jianshe Sun, and Jun Tian, WSU Tree Fruit Research and Extension Center, Wenatchee
Jim McFerson and Tom Auvil, Tree Fruit Research Commission, Wenatchee
John Fellman, Professor, WSU-Pullman
Gordon Brown, Scientific Horticulture P/L, Tasmania, Australia

Total Budget Request: $116,753

Budget 1:

Organization Name:	Tree Fruit Research and Extension Center	**Contract Administrators:**	ML. Bricker / Sally Ray
Telephone:	509-335-7667; 509-663-8181	**Email address:**	mdesros@wsu.edu; saray@wsu.edu

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries[1]	74,336	76,378	**78,280**
Benefits[2]	22,586	23,859	**23,273**
Supplies[3]	8,500	8,500	**8,500**
Travel[4]	3,400	3,500	**3,500**
Miscellaneous	3,000	3,100	
Total	$111,822	$115,337	**$113,553**

[1] Salaries: for Ag Project Assistant-$26,788; for Research Associate-$32,000; for Associates in Research-$19,400. David Felicetti is the Ag Project Assistant and is a Ph.D. candidate who has completed course work and is working full time on pigment research in Wenatchee. Dr. Xu (Agricultural Univ. of Hebei), Research Associate, works primarily on the Fuji flecking disorder, russet in Goldens, and other fruit finish issues. Cindy Kahn, Associate in Research (25% appointment on this project) works on fruit quality and fruit finish. Another Associate in Research will be employed part-time during peak season (33% time = $10,000).

[2] Benefits: for Ag Project Assistant include`es health insurance and 1.5% medical aid-$1,947; Research Associate at 42%-$13,411; Associate in Research at 39%-$3,615; and the part-time Associate in Research at 43%-$4,300.

[3] Supplies will include chemicals and laboratory supplies for color analyses and for electron microscopy; general lab supplies, rental fees for use of fruit quality analyses equipment; "crop destruct"; and cell phone charges; $2,500 for Jim Mattheis for pigment analyses. Columns will need replacement periodically on the HPLC used for pigment analyses in Mattheis' lab.

[4] Travel to experimental plots for sample collection and evaluations.

Salary for Principal Investigator and 75% of salary for an Associate in Research will be provided by Washington State University (WSU). No other funding is available for this project. This is provided to the Commission for informational purposes only and does not constitute a cost-share obligation on the part of WSU. Moreover, there is no requirement for WSU to document this information as part of any cost-share or matching obligation.

Budget 2:
Organization Name: Scientific Horticulture, Tasmania, Australia
Contract Administrator: Dr. Gordon Brown
Telephone: n/a **Email address:** gordon@scientifichorticulture.com.au

Item	**Year 1** (2005)	**Year 2 (**2006**)**	**Year 3 (2007)**
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous	3,000	3,100	**3,200**
Total	3,000	3,100	**3,200**

Footnotes:
A productive collaboration has been established with Dr. Gordon Brown in Tasmania, Australia. Dr. Brown is a research scientist with Scientific Horticulture and works closely with the apple industry in Tasmania. He has interests in Fuji stain, color development of apples, and russet (flecking) of Fuji. This cooperative effort provides the advantage of two crops per year and fosters collaboration among scientists with common interests. Only $3,200 is requested for Dr. Brown's efforts. Dr. Brown will seek most of his support from the Australian apple growers through Horticulture Australia and will share his research results with us.

Overall objective: To understand the factors that influence fruit finish of apples and develop and implement management practices that will lead to better fruit finish for growers; to investigate the impact heat-induced disorders have on fruit quality during cold storage. Specific objectives related to improving fruit finish and fruit quality are outlined below.

Specific objectives:

I. Investigate the causes of the disorders called "flecking" in Fuji apples and russeting in Golden Delicious apples and study ways to prevent the incidence of both disorders.
II. Characterize pigment changes as they relate to color development and to several skin disorders (e.g. sunburn and Fuji stain) that detract from good fruit finish.
III. Examine postharvest internal fruit quality as affected by preharvest skin disorders such as sunburn, stain, flecking and other stress-induced disorders.

Significant findings:

- Fuji flecking and Golden Delicious russet were induced before seven weeks after full bloom (WAFB) but usually did not become visible until after 7 WAFB. Similarities between the two disorders suggest that Fuji flecking is a type of russet and appears to be associated with lenticels. The percentage of fruit with Fuji flecking increased between 18 to 20 WAFB.
- Various factors stimulated cell division of the phellogen to form phellem. Phellem accumulated and penetrated through the epidermal cells to form flecking.
- The frequency of stomata per fruit in Fuji was significantly higher than in Gala. Most stomata became non-functional by 7 WAFB and lenticels appeared.
- Pubescence weight decreased with fruit development in Fuji, Gala, and Golden Delicious from full bloom until 7 WAFB with most pubescence disappearing by 7 WAFB. Pubescence weight in Gala was higher than that in Fuji and Golden Delicious.
- Epicuticular wax weight/fruit and cuticle thickness were significantly higher for Gala vs. Fuji.
- Augmenting epicuticular wax of the cuticles with three or four weekly applications of RAYNOX® after 2 WAFB significantly reduced the percentage of fruit with flecking.
- Induction of flecking and russet was enhanced by chemical thinners (Sevin + NAA, ATS, and lime sulfur). ATS applied at full bloom and Ethephon or Sevin + NAA applied at 1 WAFB significantly reduced wax weight at 10, 11, and 12 WAFB.
- Chlorophyll a and b concentrations of sunburned apples were significantly lower than in non-sunburned apples for Fuji, Gala, and Golden Delicious (with and without blush).
- Anthocyanin concentrations in Fuji and Gala were significantly lower in sunburned apples as compared to non-sunburned apples. No anthocyanin was detected in non-sunburned Golden Delicious without blush.
- Fuji, Gala, and Golden Delicious had significant increases in total quercetin glycosides in sunburned apples as compared to non-sunburned apples.
- When sunburned apples were compared to non-sunburned apples, Beta-carotene and total xanthophylls increased significantly in sunburned Fuji, decreased in sunburned Golden Delicious with or without blush, and remained similar in sunburned Gala.

Methods:

Objective I on Fuji flecking and Golden Delicious russeting:

- The occurrence and development of Fuji flecking was monitored weekly with a digital camera. Ten to 15 fruitlets were monitored every week from 3 to 7 WAFB. The changes in Fuji flecking over time were also investigated weekly or biweekly from 14 WAFB until harvest. To observe the earliest symptoms of flecking, five apples were collected weekly from 7 WAFB until harvest. Cubes were cut from the apples, fixed in FAA solution, and embedded in paraffin. Paraffin sections were cut with a microtome after the process of dehydration, waxing and embedding, and staining.

- Ten to 15 fruitlets were collected weekly from full bloom to 8 WAFB. Epicuticular wax (EW) was carefully scraped from each fruit's surface with a scalpel and weighed.
- Field trials were conducted to test the effects of chemical thinners on flecking. Two percent ATS (ammonium thiosulfate) and a mixture of 2% lime sulfur and 2% fish oil were applied at full bloom; a mixture of 200 ppm Sevin and 5 ppm NAA, and 300 ppm Ethrel was applied at 1 WAFB. The treatments consisted of three replicates with one tree per replicate. Fifty fruit were collected from each tree at the time of harvest.
- Three formulations (5% RAYNOX®, 20 ppm NOVAGIB™, and a mixture of both) were applied weekly one to four times from 2 to 5 WAFB. The treatments consisted of three replicates of one tree per replicate. Fifty fruit were collected from each tree at harvest time.

Objective II on Pigment Changes of Fruit with Stress-Induced Disorders:

- Pigments were extracted from apple peel disks that were frozen and crushed in liquid nitrogen. Chlorophylls and carotenoids were extracted in 80% acetone/20% 0.1M HEPES buffer (v/v), and phenolics (anthocyanins and quercetin glycosides) were extracted with acidified methanol (1% HCl). Reverse-phase high performance liquid chromatography (HPLC) was used to analyze pigment compositions. Pigments were identified and quantified based on pure standards obtained from Sigma Chemical Company.

Objective III on Impact of Preharvest Stress on Fruit Quality of Fruit with Sunburn, Stain etc:

- Studies are in progress to examine internal quality of heat-stressed fruit and control fruit over time in regular atmosphere cold storage. Firmness, soluble solids, and titratable acidity are being determined using commonly adopted methods. Gala, Golden Delicious, Jonagold, Granny Smith, and Fuji were sorted into five grades of sunburn (0 to 4 where 0 = no sunburn and 4 = sunburn necrosis) and are being analyzed for internal quality at intervals from time of harvest. Golden Delicious with russet grades 0 to 3 and Fuji with flecking grades 0 to 4 are also being examined at monthly intervals from time of harvest.

Results and discussion:

Objective I. Fuji Flecking and Golden Delicious Russet:

1. The earliest visible symptoms of Fuji flecking occurred on the sun-exposed side of fruit at seven weeks after full bloom (WAFB). It appears that the occurrence and development of 'Fuji flecking are associated with the lenticels (Fig. 1). Fuji flecking lenticels became slightly larger and yellow-green in color. Fuji flecking increased most from 18 to 20 WAFB, with a 36.7% increase.

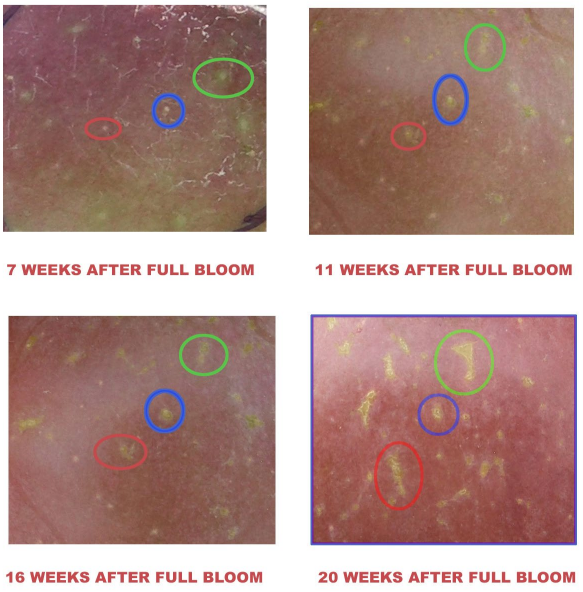

Fig. 1. Fuji flecking symptoms

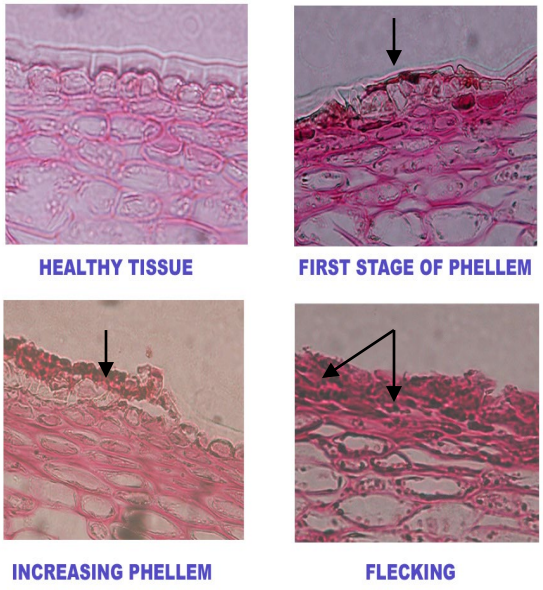

Fig. 2. Development of phellem

2. Microscopic observations showed formation of phellem as early as 9 WAFB. It appeared that epidermal cells became phellogen. It was also observed that phellem was located around or near the lenticels. Various factors stimulated cell division of phellogen to form phellem. Phellem accumulated on the surface of the apple to form flecking. (Fig. 2).
3. Epicuticular wax (EW) can absorb ultraviolet light and has some protective effects on fruit. It was found that EW weight per fruit increased from 10 to 13 WAFB as the fruit surface area increased. EW weight per fruit in Gala was significantly higher than in Fuji and Golden Delicious apples (Fig. 3). More EW on Gala may help protect this cultivar from russet.
4. To augment the natural EW on Fuji, three or four weekly applications of RAYNOX® after 2 WAFB significantly reduced flecking from 63.4% in control to 47.8%, and 46.4%, respectively (Fig. 4). Weekly applications of GA_3 two or three times after 2 WAFB significantly reduced flecking to 44.6% and 46.3%, respectively. The application of a mixture of GA_3 and RAYNOX® three or four times after 2 WAFB significantly reduced flecking from 63.4% in control to 51.3% and 46.9%, respectively. Thus, it appears that either RAYNOX® or GA_3 can reduce the amount of Fuji flecking.

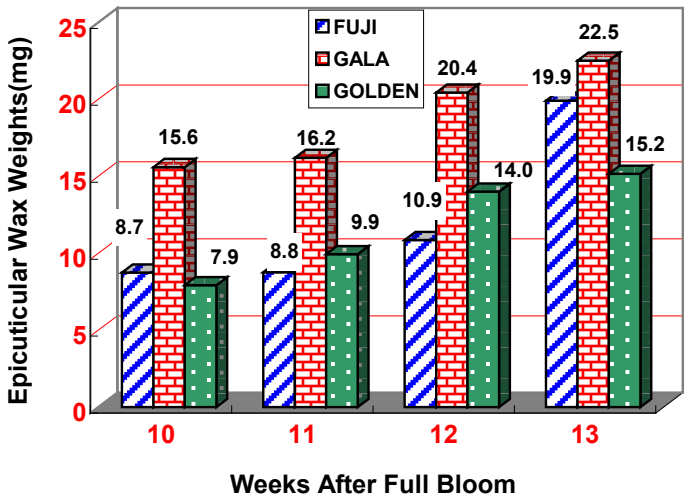

Fig. 3. Comparison of epicuticular wax weights in three cultivars.

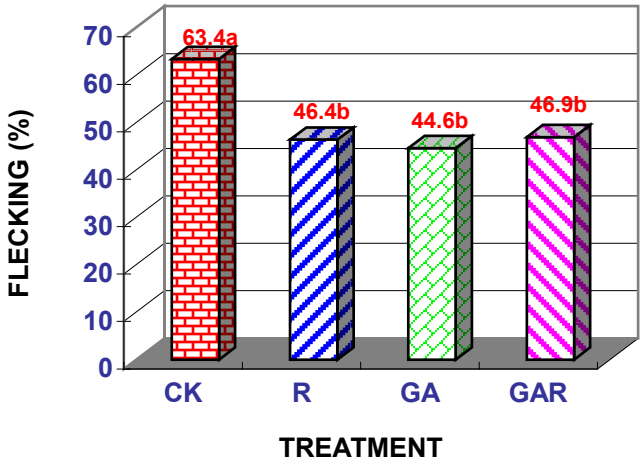

Fig. 4. Effects of RAYNOX®, GA, and a combination of RAYNOX® plus GA on flecking. (CK: Control (Water); R: 5% RAYNOX® applied four times at 2 to 5 WAFB; GA: 20 ppm NOVAGIB™ applied two times at 2 to 3 WAFB; GAR: 5% RAYNOX® + 20 ppm NOVAGIB™ applied four times at 2 to 5 WAFB).

Objective II. HPLC analyses of Pigment Changes:
These analyses of pigment changes associated with sunburn are not yet complete. Analysis of Red Delicious, Granny Smith, and all degrees of sunburn severity in Fuji will be completed in the final year of the project.

1. Changes of certain pigments resulting from sunburn damage appear to be cultivar specific.
2. Chlorophyll a and b concentrations of sunburned apples were significantly lower than non-sunburned apples for Fuji, Gala, and Golden Delicious (with and without blush) (Fig. 5).
3. Sunburned Fuji had significant increases in both ß-Carotene and total xanthophylls as compared to non-sunburned Fuji (Fig. 6). Sunburned Gala showed no significant changes in the ß-Carotene and total xanthophylls as compared to non-sunburned Gala. Sunburned Golden Delicious had significant decreases in both ß-Carotene and total xanthophylls when compared to both non-sunburned Golden Delicious with and without blush.

4. Anthocyanin concentrations in Fuji and Gala were significantly lower in sunburned apples as compared to non-sunburned apples (Fig. 7).
5. Fuji, Gala, and Golden Delicious had significant increases in total quercetin glycosides in sunburned apples as compared to non-sunburned apples (Fig. 7). Non-sunburned Golden Delicious apples with blush had significantly higher quercetin glycosides than non-sunburned Golden Delicious with no blush but had significantly lower concentrations as compared to sunburned Golden Delicious apples.

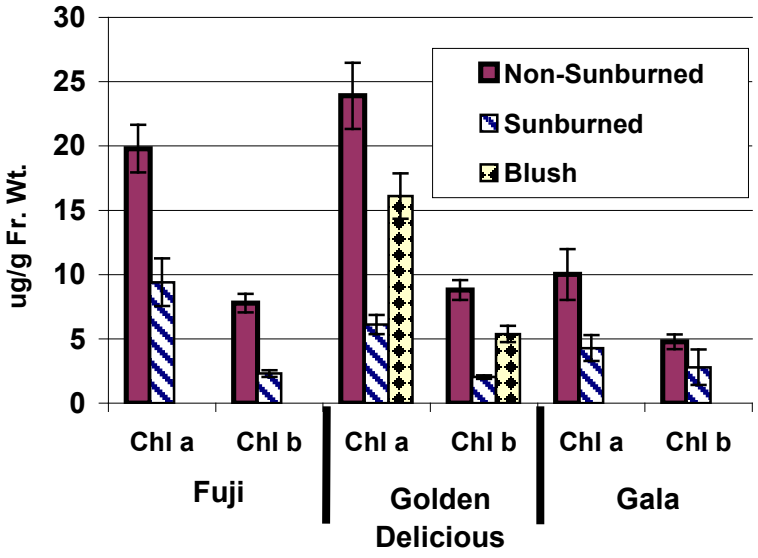

Fig. 5. Chlorophyll a and b concentrations in three cultivars.

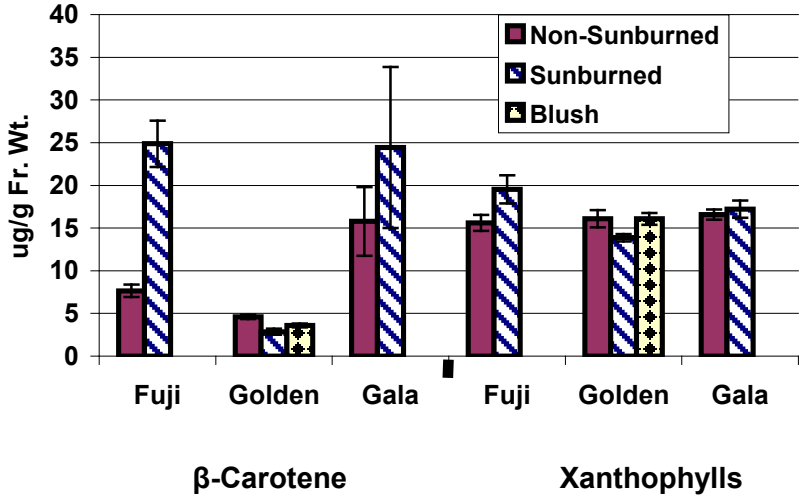

Fig. 6. ß-Carotene and xanthophylls for three cultivars.

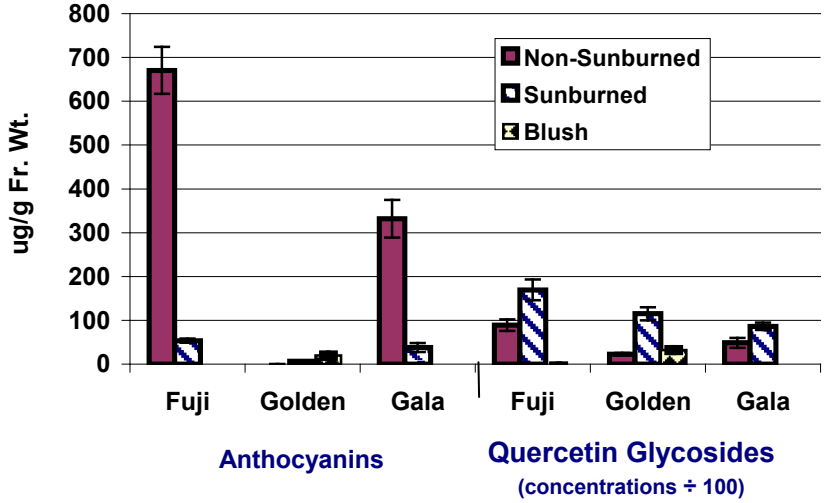

Fig.7. Phenolics in three cultivars.

Objective III. Fruit Quality Analyses of Fruit with Heat and Light-Induced Disorders:
Analyses of fruit harvested during 2006 are still in progress and will be presented next year when the data sets are complete.

Significance to Industry and Potential Economic Impact:

Fuji flecking is a serious problem in many orchards, and is having a serious economic impact on some growers. This disorder appears as a skin disorder on as many as 70% of the Fuji apples in some orchards. We have identified some of the factors that induce flecking and russet in Goldens, and also factors that enhance the expression of these disorders. We are searching for treatments that will reduce the amount of flecking and russet.

The second project involves elucidating the pigment changes that occur when apples of various cultivars become sunburned. Identifying these pigment changes will help us to understand whether beneficial pigments (e.g. antioxidants) change with sunburn. This knowledge also may allow for genetic manipulation or selection to improve the ability of apples to withstand stress conditions that cause sunburn or Fuji stain.

The third aspect combines preharvest and postharvest physiology. Research to determine the effects of preharvest-induced disorders (e.g. sunburn and stain) on internal fruit quality will provide a better understanding of how fruit quality and storability are impacted by sunburn and stain. This research should provide packers with important science-based guidelines about the effects of these preharvest stresses and disorders. Does sunburn affect internal fruit quality during the cold storage period, or is sunburn mostly an effect on appearance of fruit? Our preliminary results indicate that firmness, soluble solids, and titratable acidity are all affected by these disorders. An industry-wide system for relating internal fruit quality to these skin disorders could lead to more equitable pricing for growers who sell fruit with these skin disorders.