2006 Apple Hort/Path - Post Harvest Research Review January 19-20
Red Lion Hotel - Pasco, Washington
DAY ONE AGENDA

Time	Page	PI	Project Title	Funding period
			Final Reports	
10:00	2	Fazio	Replant disease tolerance of Geneva rootstocks	03-05
10:15	12	Caspari	Effects of PRD on apple tree physiology, fruit quality and yield	04-05
10:30	13	Rom	Alternative thinning strategies for tree fruits	04-05
10:45	14	Schupp/Robinson	Efficacy and physiological effect of oil/ lime sulfur combinations	03-05
11:00	21	van Nocker	Developing genetic tools for regulation of flowering, thinning, & fruit drop	05
11:15	22	Wisniewski/Fuchigami	Evaluation of apple lines overexpressing the antioxidant APX	03-05
11:30	32	Schrader	Fuji stain: causes and prevention	03-05
11:45	42	Schrader/Cheng	Photoprotection of apple fruit by the xanthophyll cycle	03-05

Continuing Projects Poster Session - 2:00pm - 5:30pm

Group #	Page	PI	Project Title	Funding Period
1	51	Fallahi	Water use, quality and growth as affected by irrigation and/or rootstock	04-06
1	58	Norelli	Trait modification through genetically engineered rootstocks	04-06
1	64	Barritt	Apple scion breeding	05-07
1	67	Mazzola	Employing biological elements of orchard ecosystems	05-07
1	73	Riga	Synthetic and bio-nematicides for plant parasitic nematodes	05-07
2	79	Lakso/Greene	Monitoring apple fruit growth for predicting chemical thinning response	04-06
2	85	Hirst	Flower bud development in apple	05-06
2	91	Byers	Influence of temperature on pollen germination & tube growth	05-07
2	97	Elfving	Growth and crop load management in apple trees with bioregulators	05-07
2	102	Schmidt	Chemical thinning of apple	internal
2	108	Müller	Sprays to improve packouts and annual cropping in apple	internal
3	114	Whiting	Impact of cultural practices on apple canopy physiology	04-06
3	120	Campbell	Target-specific control of fungal pathogens by natural compounds	04-06
3	127	Schrader	Improving fruit finish in apple	05-07
3	133	Xiao	Sphaeropsis rot in apple	05-06
3	139	Xiao	Holistic approach to decay management	04-06
3	145	van Nocker	Preserving and improving the aroma of apple fruit	02-04
4	146	Dandekar	Role of sorbitol in sugar and acid accumulation in apple fruit	04-06
4	152	Lu/Beaudry	Hyperspectral reflectance and fluorescence scattering	04-06
4	158	Mattheis	Regulation of apple fruit ripening	04-06
4	163	Dandekar	Defining ethylene regulation of apple fruit quality traits	05-07
4	169	Andrews	Identifying disease prevention benefits of apple consumption	05-06
4	170	Shekarriz	Ethylene measurement in post-harvest storage	05-06

FINAL PROJECT REPORT

Project title: Replant disease tolerance of Geneva rootstocks.
PI: Dr. Gennaro Fazio
Organization: USDA-ARS / Cornell University
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Geneva NY, 14456
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Introduction

Apple Replant Disease (ARD) is one of the major problems that Washington State growers face as virgin land optimal for orchards becomes less available. Growers planting orchards on "replant" sites have few options to avoid losses due to this disease complex which include fumigation (Chloropicrin, Telone, Metam sodium, etc.), fallow, or treatment with bio-derived agents – all these options however can be expensive and give spotty results. Geneva apple rootstocks were bred using intensive disease screening methodology and diverse germplasm as the source of resistance. Preliminary studies indicate that some Geneva rootstocks show tolerance or resistance to ARD in New Zealand and NE United States. These rootstocks (including advanced selections) have not yet been tested in replant soils in Washington State. Knowledge of their performance in WA replant soils could give growers another viable option to maintain productivity in combination with available soil fumigation treatments. Preliminary results also indicate that there may be differences among rootstocks in the way they interact with beneficial organisms such as fluorescent pseudomonads which have been shown to antagonize the replant disease complex. Knowledge about the genetic components of these plant-microbe interactions may yield new methodology for selection of improved rootstocks and more viable options to combat the ARD complex.

Objectives

1. Test Geneva™ rootstocks in three or four grower cooperator replant sites where ARD has been known to occur. Compare these rootstocks to M9 standards with commercial scion varieties such as Gala, Pink Lady™ and Fuji.
2. Investigate genetic interactions between beneficial microorganisms that antagonize ARD and rootstock selections.

Significant Findings:

- **We have been able to detect tolerance to ARD among rootstocks. We have established that M.26 is very susceptible to ARD. We are still establishing long term performance of rootstocks in ARD soils.**
- **The effect of fumigation on relative tree growth sharply decreases with time.**
- **Relationships between apple rootstocks and beneficial pseudomonads are too complex to be unraveled with current resources.**

Methods: 2004 planting

Three new trials were planted in the spring of 2004. The location of these trials was picked on the basis of existing replant problems. The locations were Wapato (WA), Chelan (CH) and Naches (NA). The rootstock trials in WA and CH were planted in a split plot fashion where one half of the orchard was fumigated with Telone C-17 and the other half not fumigated. The trial in NA was also planted as a split plot however the orchard was split into three main plots treated with Telone C-17, Metam Sodium and

unfumigated. The variety used for the WA and CH locations was Brookfield Gala and the variety used for the NA location was Honeycrisp. Table 1 shows the rootstock genotypes and locations where they are being tested. During winter 2005 trees were pruned in to uniform scaffolds at the CH location whereas trees at the WA and NA locations were pruned according to individual tree needs. Trunk circumference data was taken at planting and in October 2004 and 2005and trunk cross sectional areas (TCSA) were derived. The mean amount of TCSA growth was calculated for the rootstocks using a mixed model approach adapted to a split-plot experimental design. Another method was used to measure the site variation of each location: a mixed model analysis with the rootstock genotype as the main effect was used to calculate the residual for each sampling unit. That residual was then plotted in a contour plot based on the orchard map which shows the effectiveness of fumigation as well as the effectiveness of the tolerance of certain rootstocks.

Table 1. Locations and rootstocks planted in 2004.

Rootstock	Location*	Scion Varieties
G.16	WA, CH, NA	Brookfied Gala, Honeycrisp
G.11	WA, CH	Brookfied Gala
G 3041	WA, CH	Brookfied Gala
G 5935	WA, CH, NA	Brookfied Gala, Honeycrisp
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	Brookfied Gala
4003	NA	Honeycrisp
4814	NA	Honeycrisp
4210	NA	Honeycrisp
G.30	NA	Honeycrisp
5087	NA	Honeycrisp
G 4202	NA	Honeycrisp
4013	NA	Honeycrisp
4213	NA	Honeycrisp
M.9 EMLA	NA	Honeycrisp

*** WA=Wapato, CH=Chelan, NA=Naches**

General Results

The tree growth data that was collected at all three locations showed that there was a definite positive effect of fumigation for the first year of trunk measurements. The relative effect of fumigation was less at the WA and CH locations during the second year of the experiment (Figure 1). This is may indicate that either the effect of fumigation is temporary (i.e. the disease pressure is returning to pre-fumigation levels) or that the rootstocks are generally being affected less by the soilborne diseases as they grow larger. At planting time the mean size of the trees for each rootstock genotype was different and that difference had a significant effect on the relative growth potential of that tree. We tried to account for those differences in the statistical analysis and corrected this effect where possible. There were significant rootstock by treatment interactions at every test location – this means that some rootstocks show at least a partial tolerance to the biological components of replant disease. The rootstocks that were affected the most at all locations by replant were M.26 and PiAU56-83. Overall clones of M.9 did not do as bad as M.26. The most interesting results will come in the future, where we will be able to quantify the increase in production (and profit) due to fumigation and rootstocks and make some inferences on the long term

effects of pre-plant decisions. It will be useful to have a field day in the near future at each location to discuss the findings.
The relationship between ARD microbial antagonists (such as fluorescent pseudomonads) and tolerant apple rootstocks was investigated in Geneva. We isolated fluorescent pseudomonads from stoolbeds in Geneva and collaborators (Rumberger et al.) performed tests on stool bed root samples and found no significant difference in the composition of the general bacterial community. We are continuing to investigate the possibility of strain specific relationships that would not have been detected by the methods used given current resources.

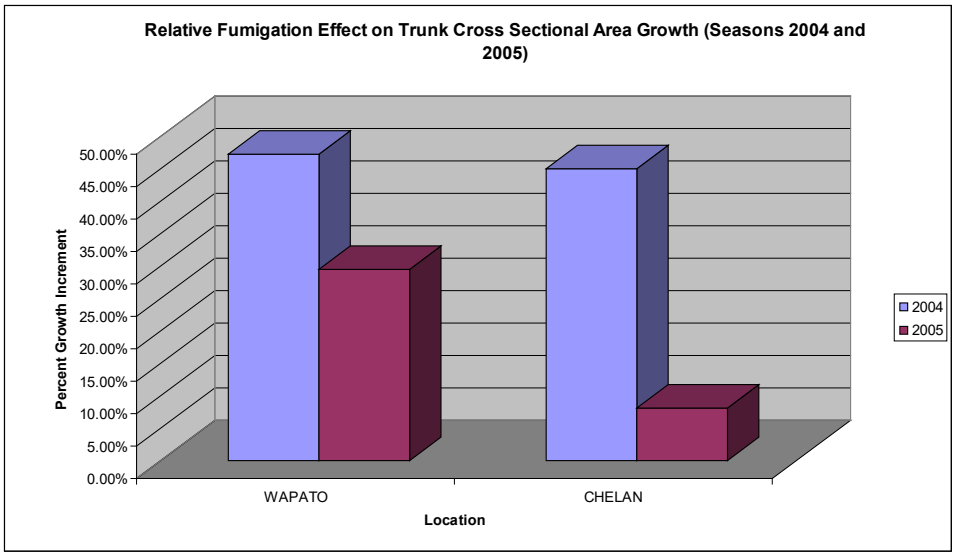

Figure 1. Relative TCSA increase of Brookfield Gala trees due to fumigation over two growing seasons. Although fumigation had the effect of increasing tree growth 45-50% in the first season of growth its effect has decreased considerably in the second season.

Wapato Location Findings

A decision was made to let all trees crop in 2005 and the results are shown in Figure 3. The next few years will tell whether each rootstock is able to overcome the effects of replant and still maintain productivity. As a general rule the trees that had a crop this year grew less – the correlation between fruit number per tree and TCSA growth was negative (-0.24, $p < 0.001$). One rootstock stands out as very vigorous and non precocious (PiAU56-83) being almost twice the size as B.9. At planting PiAU56-83 had the largest initial caliper of all trees. The vigor of this rootstock may give an opportunity to test the hypothesis that more vigorous trees perform better in weak replant sites than more dwarfing rootstocks. Although rootstocks G.11, G3041, G4214, Supporter 2 and M.9 Pajam 2 did not show a significant difference in growth during the first season between the fumigated and unfumigated treatments, during the second season the trends were not the same and only Supporter 2 showed no significant differences. Rootstocks M.26, M.9 Nic 29 and PiAU56-83 exhibited the largest differences in growth between fumigated and unfumigated treatments during both years. Trees on Bud.9 and G.11 were on average the smallest in the group. One major finding that has to be noted is that this site had been fumigated once before prior to the fumigation treatment that was performed for this experiment. The fact that we witnessed a positive effect of a second fumigation on tree growth shows how temporary the fumigation effect is and that only a proper choice of rootstock genotypes may give a long term solution to the replant problem.

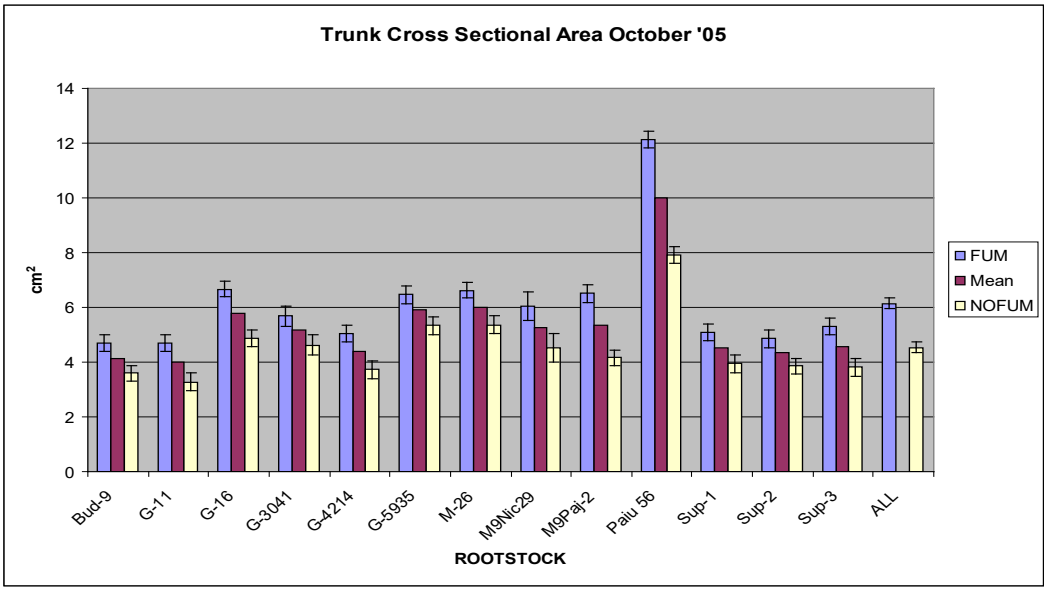

Figure 2. Mean TCSA of Brookfield Gala trees on 13 different rootstocks in fumigated and non fumigated soils at the Wapato (WA) location. Trees on fumigated land were on average larger than trees on unfumigated. For some rootstocks (Pi AU 56-83) the relative difference was greater than others (Supporter 2 and G3041).

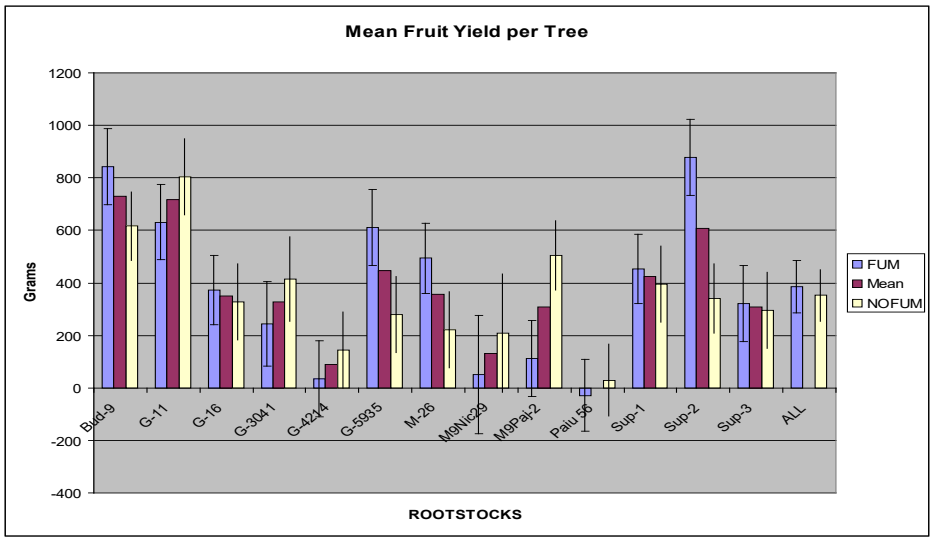

Figure 3. Mean fruit yield per tree (Brookfield Gala) in the first bearing year (2005) at the WA location. The error bars are quite large showing that there was a lot of inconsistence in fruit bearing within each plot. Yield differences among rootstocks give a measure of their precocity potential. There was a significant negative interaction between fruit yield and tree growth (trees with the most yield grew less – this may have an effect on return bloom in 06'.

Naches Location Findings

The experimental design at this location (Figures 3,4,and 5) is different than WA or CH as it was set up as single trees for each replication. There was a positive overall effect of fumigation (Telone C-17 and Metam Sodium). There were some surprising effects seen in some of the Geneva rootstocks where Metam Sodium was able to impact growth more positively than Telone C-17. Whereas M.26, Bud.9 and G.935 where impacted equally by both fumigations. This effect may be an indication that Metam Sodium was more effective at eliminating a specific component of replant disease that affects these rootstocks differentially. Although B.9 rootstock did not seem to differ significantly between the fumigated and unfumigated treatments there were significant tree losses of B.9 rootstock at this location. Geneva 4210

was the only rootstock that came close to not showing statistical differences among treatments in the first season. This site was also characterized by significant root lesion nematode populations (as indicated in the report from Mark Mazzola) for which no plant disease resistance is known. This factor may have impacted the genetic resistance potential to the other components of the replant disease complex. Another significant finding measured in a separate experiment at the same location was that organic pre-plant treatment did not have a significantly different impact on growth of Honeycrisp/M.9 sleeping eyes when compared to the no-dig treatment (Figure 6). It is possible (but unlikely) that by random chance all the no-dig treatments in this experiments landed on better plots. All treatments however grew much better than the control dig plots. We are puzzled by the fact that the control dig treatment exhibited the worst growth of all treatments.

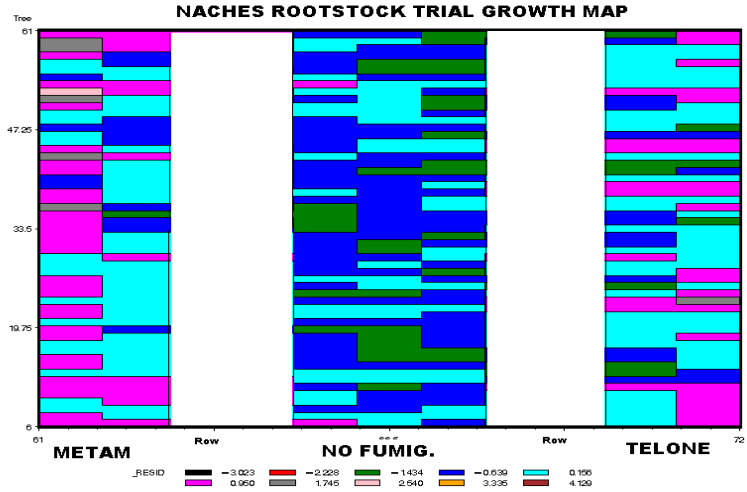

Figure 4. Contour plot of circumference growth of Honeycrisp trees at the Naches location. Unfumigated rows are sandwiched between rows treated with Telone C17 and Metam Sodium. Although it is not clearly demarcated we can see the effect of the fumigation treatment (lighter colors) on both sides of this map. Trees treated with Metam Sodium grew better on average.

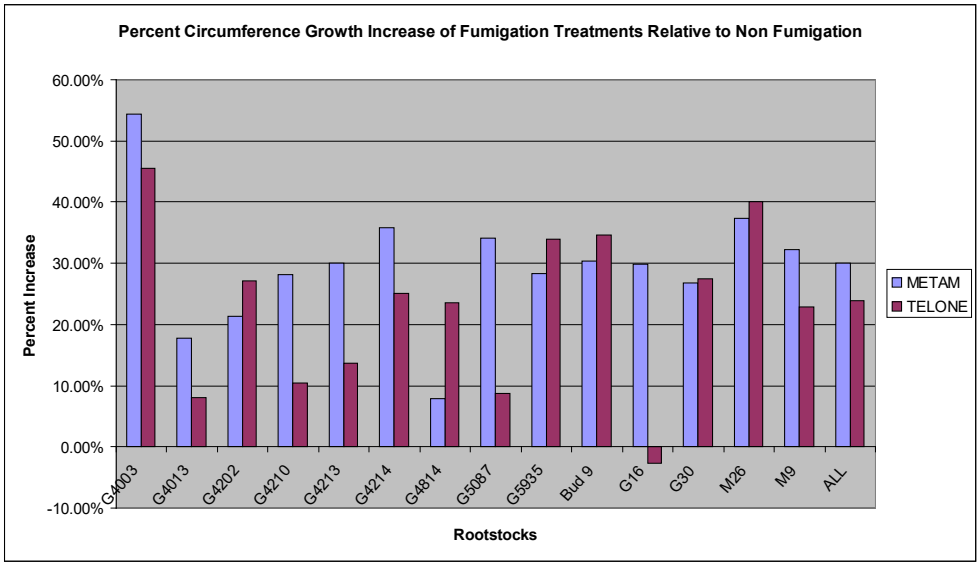

Figure 5. Percent increase of circumference growth (two seasons) of a variety of rootstocks at the Naches replant location. Overall the Metam Sodium treatment worked better than Telone C17 treatment. Some rootstocks like G4013 and G4814 seem to be more tolerant to the replant problem at this location since they did not experience the same relative growth increase of susceptible M.26 and G.4003. It is also evident that some rootstocks perform much better with the addition of Metam Sodium relative to Telone.

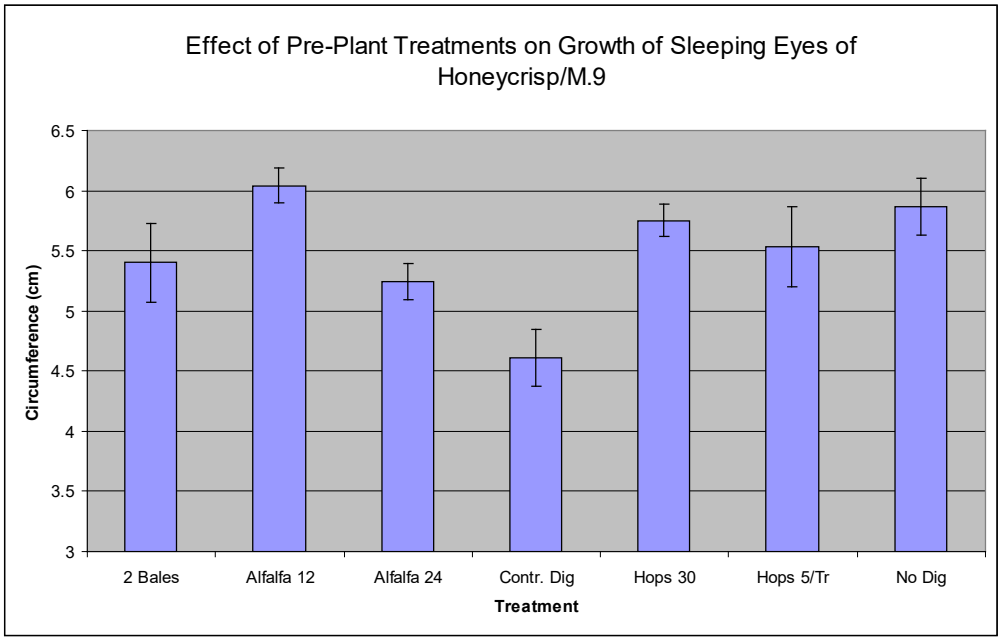

Figure 6. This experiment had the goal of evaluating alternatives to fumigation in the same replant plot as the rootstock trials in NA. The soil was amended with hops waste, alfalfa, and straw and compared to No-Dig and Control-Dig treatments. The plots were planted with sleeping eyes (Honeycrisp/M.9) and circumference measurements were taken at the end of the first leaf. The measurements show that trees in the Control-Dig plots exhibited the least growth and trees with Alfalfa 12 grew the best.

Chelan Location Findings

The positive effect of fumigation on tree growth sharply decreased to 8% in the 2005 growing season from 45% in the 2004 growing season (Figure1). The planting in CH was pruned to a uniform scaffold after the first year (2004) and therefore unlike the WA location there was no fruit production in 2005. Bud.9 was the weakest rootstock overall. G.935, Pajam 2 and Supp. 1 showed no significant differences in tree size between the fumigated and the unfumigated plots. M.9T337 and M.26 had the largest differences in tree growth between fumigation treatments. The most vigorous rootstock in this trial was PiAU56-83 (almost double the size of Bud.9). This may be a good opportunity to test whether the practice of using more vigorous rootstocks on replant sites is a viable solution to the replant problem. This site is also unique as it is under organic management. This site may provide useful information on relative rootstock performance given increased weed competition because of the absence of an herbicide strip and the somewhat different composition of nutrients applied in the form of organic fertilizers.

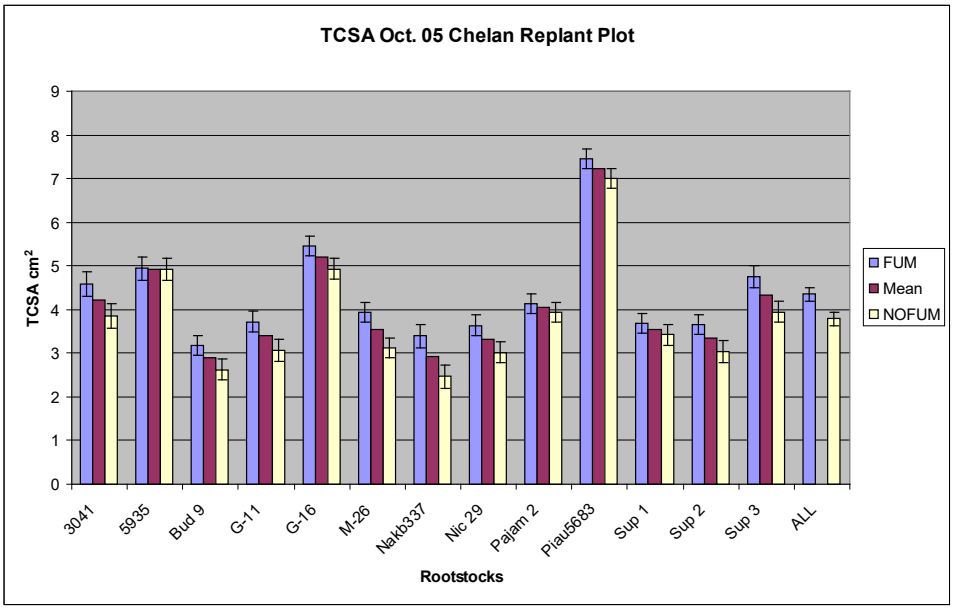

Figure 7. Trunk cross sectional area of Brookfield Gala trees on 13 different rootstocks at the CH replant site. This site is under organic management.

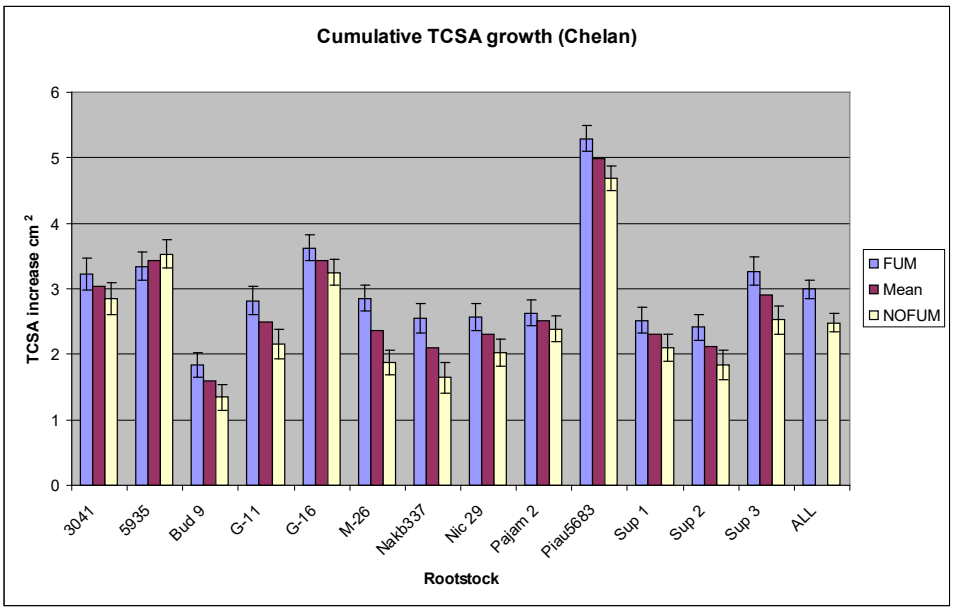

Figure 8. TCSA increase over the life of the orchard at the CH location.

Other activities supported by this grant not included in initial proposal:
Frenchmen Hill Honeycrisp rootstock trial

In the spring of 2003 24 rootstock genotypes with the scion Honeycrisp were planted in two rows at the Frenchmen Hill location. This grower-cooperator trial is managed under the auspices of the WFTRC. Over 500 trees and 25 rootstock genotypes were planted. These trees were propagated and grown in Geneva's apple rootstock nursery. Trunk circumferences have been recorded for Spring 2003, Fall 2003 and Fall 2004, Fall 2005. Bloom, yield and fruit size data was collected in the fall of 2005. Data was analyzed with the SAS 8.2 statistical package and PROC MIXED. Some of the results are summarized in figures 8 and 9. Although we detected significant yield differences, there was a definite effect of fruit

load on fruit size and some of the yields (G.16 and B.9) exhibited smaller fruit than normal (Figure 11). The trees on M.9 were not significantly different in size than M.26 – M.26 probably does not grow that well at this location. This site was plagued by two major irrigation accidents that have compromised two to four replications.

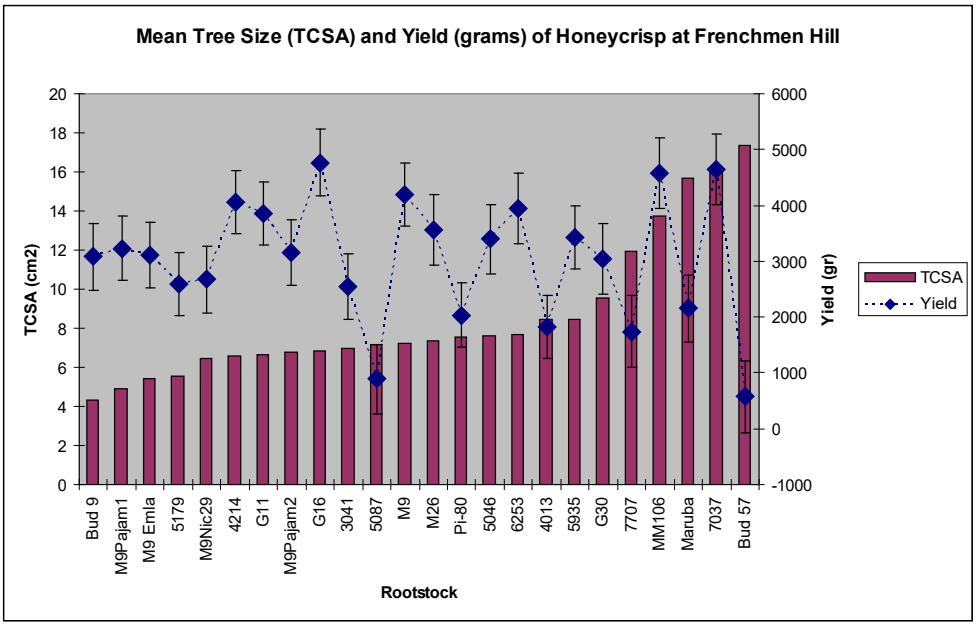

Figure 9. Mean yield per tree and mean tree size measured in the fall of 2005. Several classes of rootstock vigor are represented in this experiment (Dwarf: G.3041, G.11 B.9, M.9 clones. Semi-Dwarf: M.26, G.5935, G.6253, G.30, G.4013. Vigourous: G.7037, G.7707, MM.106 and Marubakaido).

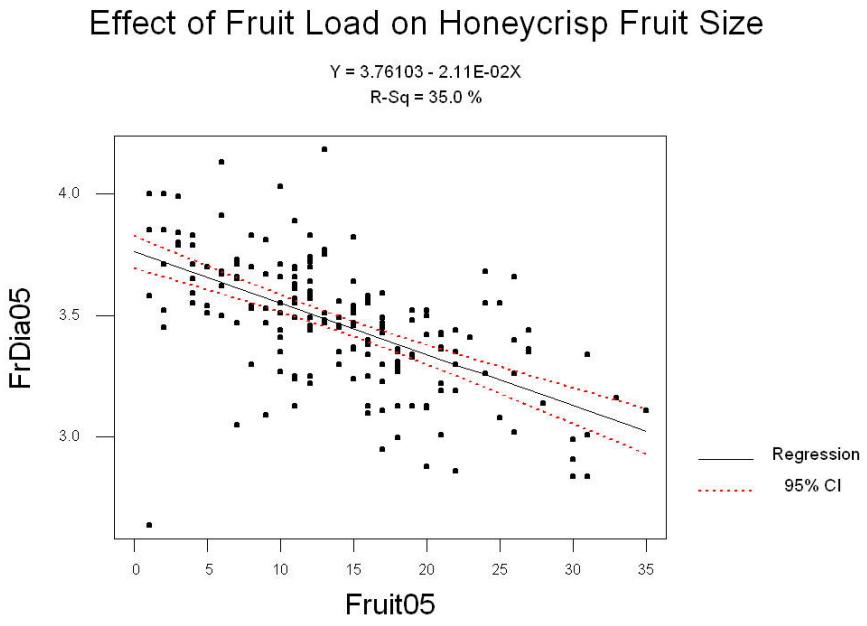

Figure 10. Fruit size was affected by the fruit number per tree at the Frenchmen Hill location. In an ideal situation where fruit are thinned to a uniform cropload the fruit should have a more uniform size.

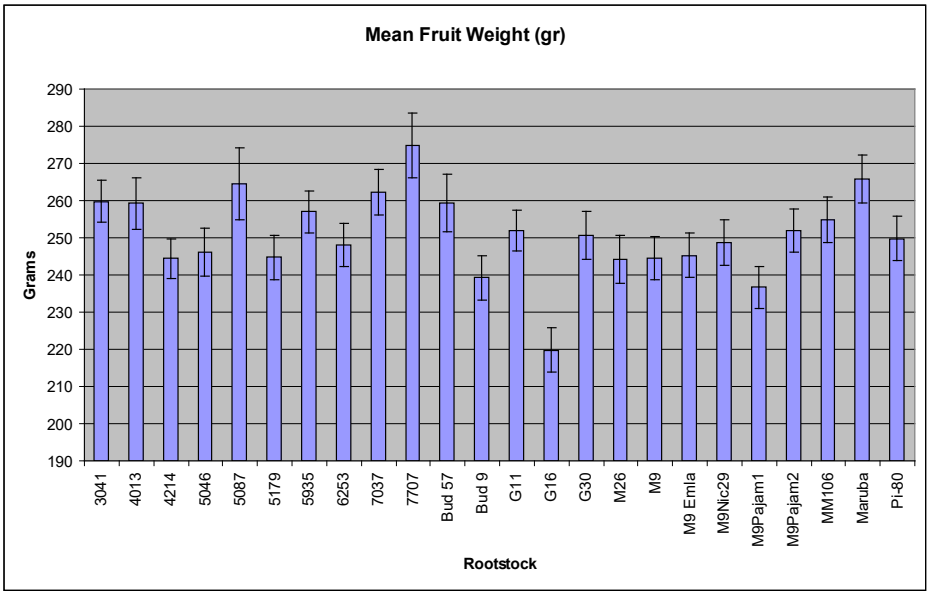

Figure 11. Average fruit weight of Honeycrisp at the Frenchmen Hill location.

Stormy Mountain (Chelan) Honeycrisp Rootstock Trial

This trial mirrors the trial on Frenchmen Hill. This trial was planted on virgin ground and is under organic management. Several trees of have been lost to rodents, however the statistical capacity of the trial is still very good. Rootstocks G.5087 and G.4214 had the highest yield followed by G.5935, G.16 and G.30. Fruit size was not significantly affected by crop load at this location. On average the trees at this location grew less than Frenchmen Hill – this is probably because of different light and season conditions. When compared to Frenchmen Hill we are surprised by the differential performance of rootstock G.5087 at the two sites. G.4214 ranked high in production at both locations.

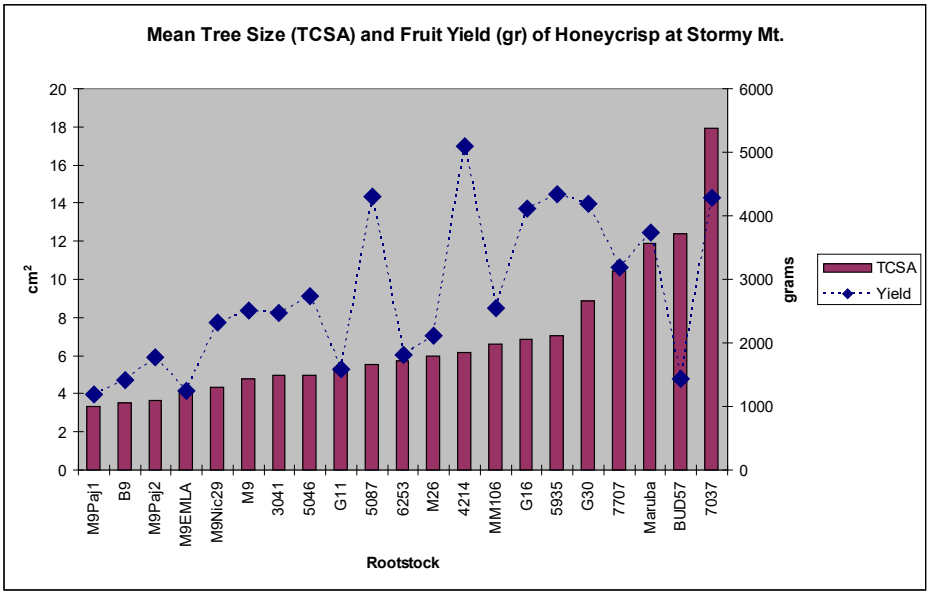

Figure 12. Rootstocks have been arranged by tree size. A surprising find in this trial when compared to Frenchmen Hill is that trees on MM.106 are smaller than semi-dwarf rootstocks

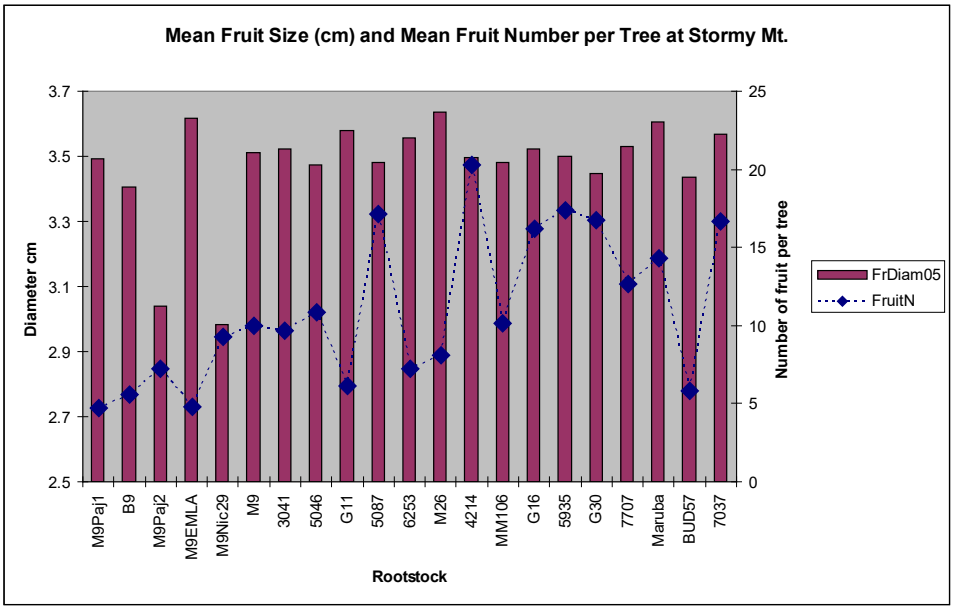

Figure 13. Mean fruit size (Diameter) and fruit number per tree. There was no detectable effect of crop load on fruit size at this location.

Budget:
Project title: Replant disease tolerance of Geneva rootstocks
PI: Dr. Gennaro Fazio
Project duration: 2003-2010 (8 years) first three years shown.
Current year: 2005
Project total (3years):

Item	Year 1 (2003)	Year 2 (2004)	**Year 3 (2005)**
Salaries[1]	3,000	3,000	1,500
Benefits (38.31%)	1,149.3	1,149.3	574.65
Wages[2]	8,000	8,000	4,000
Benefits (38.31%)	3,064.80	3,064.80	1,532.40
Equipment[3]	2,000	0	0
Supplies[4]	17,085.90	11,085.90	4092.95
Travel[5]	3,000	3,000	3,000
Miscellaneous[6]	700	700	300
Total	38,000	30,000	15,000

[1]Technician salary for part-time assistance in propagation budding and maintenance of stoolbeds.
[2]Wages for assistance in trial design and establishment and for laboratory technician part time help.
[3]Includes digital calipers for measuring TCSA etc.
[4]Includes cost for rootstock liners, trees, support system, laboratory supplies etc.
[5]Travel to and from trials.
[6]Includes shipping expenses, communication costs etc.

FINAL PROJECT REPORT

Project Title: Effects of PRD on apple tree physiology, fruit quality and yield
PI: Horst Caspari

No report submitted

FINAL PROJECT REPORT

Project Title: Alternative thinning strategies for tree fruits
PI: Curt Rom

No report submitted

FINAL PROJECT REPORT
WTFRC Project # AH-03-308

Project Title:	Efficacy and physiological effect of oil/lime sulfur combinations
PI:	Jim Schupp
Organization:	Department of Horticulture, Penn State University
Address:	Fruit Research and Extension Center, P.O. Box 330, Biglerville, PA
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Terence Robinson, Department of Horticultural Science, NYSAES, Geneva, NY
Alan Lakso, Department of Horticultural Science, NYSAES, Geneva, NY
Martin Goffinet, Department of Horticultural Science, NYSAES, Geneva, NY

Cooperators:
AppleEye, Mustang and Baird Orchards in WA
Sunny Slope Orchard in PA
Prospect Hill Orchard in NY
Singer Farms in NY

Objectives:

Research has demonstrated that apple can be thinned by photosynthetic inhibition caused by shading or sub-lethal doses of herbicides (Byers, et al., 1990). In the era preceding modern pesticides the use of lime sulfur was known to reduce apple fruit set (Burrell, 1945). In Japan, where lime sulfur is a registered thinner, two applications are typically made, the first at full bloom, and the second at petal fall (Koike and Ono, 1998). Burrell (1945) noted that lime sulfur at petal fall or shortly after caused the greatest reduction in fruit set. Conversely, Byers et al. (1990) showed that applying photosynthetic inhibitors closer to June drop caused more thinning than earlier timings.

Recent research in both WA and NY has shown that a tank mix of Crocker's Fish oil and liquid lime sulfur (FOLS) is an effective thinner when applied during bloom. Research conducted in N.Y. in 2001 and 2002 demonstrated that post-bloom sprays of FOLS were even more effective than sprays applied during bloom. If, as we suspect, photosynthetic inhibition is the primary cause of thinning, then post-bloom sprays should provide greater efficacy. These studies were conducted to evaluate the effect of timing on FOLS thinning efficacy and photosynthetic responses.

A second question focuses on alternatives to fish oil. There are several concerns with fish oil when used in this thinning mixture. Phytotoxicity is one concern. Apple growers have a reluctance to utilize this thinner because of its expense and repulsive odor. Research to date has been conducted using oil from a single small source in Washington State. The purity and consistency of fish oil from other sources is unknown. Fish oil may function as a surfactant and penetrant, and it may also have a direct thinning effect. A second objective of these studies was to evaluate the efficacy of several surfactants and oils in combination with lime sulfur for thinning apples.

The objectives of these studies were:
- To evaluate the effect of timing of fish oil / liquid lime sulfur (FOLS) applications on fruit set, size and quality, photosynthesis and leaf anatomy of apple.
- To evaluate the effect of alternatives to fish oil in combination with liquid lime sulfur on thinning efficacy and physiological responses of apple trees.
- To evaluate the effect of spray volume and concentration on FOLS efficacy and safety.

Significant Findings:

- FOLS sprays reduced photosynthesis (Pn) rates shortly after treatment, with recovery evident within four to five days in WA. Recovery of Pn following FOLS sprays in eastern sites was much slower.
-Double sprays of FOLS were effective for consistent thinning in eastern orchards, while three sprays were necessary in WA.
-Post-bloom FOLS sprays were more effective in the east, while FOLS sprays during bloom were better in WA.
-Spacing three FOLS sprays at a 4 day interval thinned Washington State Gala more effectively than longer or shorter intervals (2004).
-Tank mixing LS with fish oil or soybean oil provided more thinning of Gala fruit than dormant (petroleum) oil, while LS alone was ineffective (2004).
- FOLS reduced the size of leaf epidermal cells, palisade cells, and spongy mesophyll cells in leaves of Gala trees in N.Y. State in 2004, but had no effect on leaf anatomy in either WA or N.Y. in 2005.
- Spraying FOLS at 200 gallons per acre was slightly more effective than at 100 or 50 gallons per acre. Concentrating spray materials in lower spray volumes did not give a greater response.

Methods:

Timing and Spray Interval Studies:

To evaluate the effect of post-bloom applications of FOLS, treatments were made using different timings and different intervals. In 2003 the treatments in both N.Y. and WA consisted of single and double applications of FOLS beginning at PF and extending through 21 days after petal fall. One triple spray was applied in WA starting at PF and repeated at seven day intervals. Based upon the 2003 results, the 2004 protocol was adjusted to start treatments at 80% full bloom in WA, with two repeat sprays at two, four, or eight day intervals. Similar studies were conducted in N.Y. and PA, using two applications of FOLS at the same intervals.

Fruit set was evaluated after June drop. Yield was recorded in eastern studies, and fruit size and quality were evaluated at all sites using standard methods.

Leaves were measured for Pn activity 48 hours after spraying and thereafter until treatment differences dissipated. Leaf phytotoxicity ratings were taken three days after treatment using a 1-5 scale. Data were gleaned from anatomical examination of Gala leaves harvested in Geneva, New York from trees treated with FOLS and from untreated trees in 2004, and from both N.Y. and WA studies in 2005.

Evaluation of Alternatives to Fish Oil:

The thinning efficacy of lime sulfur was evaluated by itself, and with either a non-ionic or an organosilicone surfactant, or in combination with various oils. Petroleum- and vegetable-based oils were compared to Crockers fish oil. Oils were applied at 2% (vol:vol), and LS was applied at 2.5%. Surfactants were applied according to the respective labels. All treatments were applied with an air-blast or Prop Tech sprayer.

Fruit set, yield, fruit size, fruit color and russet were evaluated. Fruit size distribution was determined in N.Y. and PA, using commercial grading equipment. Leaf damage was rated.

Spray Volume and Concentration Studies:

Studies were conducted in 2003 and 2004 to evaluate the effect of spray concentration and volume on FOLS efficacy. Spray volumes of 50, 100, and 200 gallons per acre were applied and the materials either concentrated to apply the same amount of materials per acre, or not concentrated so as to apply the same amount of materials per 100 gallons of spray mix.

Results and Discussion:

Timing and Spray Interval Studies:

Post-bloom applications of FOLS were not as effective in Washington as in the eastern states. Over all the experiments in the three years of this project, post-bloom oil and lime sulfur treatments were effective in reducing fruit set in just 14% of the treatments applied in WA. This contrasts with the outcomes in N.Y. and PA over the same period, where 70% of the post-bloom treatments reduced set. The low incidence of successful post-bloom thinning in WA also contrasts sharply with bloom thinning trials in WA, were oil and lime sulfur treatments thinned in 61% to 74% of the cases, depending on which oil was used (WTFRC data, 1999-2004).

Evaluation of photosynthesis following treatment in 2003 showed that FOLS reduced net photosynthesis for about 4-5 days following treatment in WA (Figure 1). We theorized that the effects of the single and double timings on Pn were too short in duration to impact fruit set, and the fairly rapid Pn recovery rate suggested that greater thinning efficacy may have been obtained by shortening the intervals between applications to less than seven days.

Following review of the 2003 results, we adjusted the timing study in WA to include treatments that began at 80% bloom, and increased the number of sprays to three to see if we could increase efficacy. In 2004, three sprays of FOLS at 4 day intervals starting at 80% bloom gave the greatest thinning response, and fruit size was increased by FOLS spray protocols that started at 80% bloom and were repeated at either 2- or 4-day intervals. Unfortunately, results in 2005 were not consistent enough to confirm this outcome.

Our results suggest that, if photosynthetic inhibition is important to cause thinning, an interval of about four days between sprays may prove to be the most effective in WA, because it suppresses photosynthesis over the longest time period and without allowing recovery of Pn between sprays. Based upon our results, post-bloom FOLS protocols have been less effective in WA than in eastern U.S. trials, and haven't increased fruit size as well as earlier timings.

WA orchards receive higher light intensity than do eastern orchards, which may explain why photosynthetic inhibition is less effective as a thinning mechanism here. The total amount of light received by WA orchards may raise the threshold for thinning efficacy with photosynthetic inhibitors. Reducing Pn to 50% the level of the untreated trees for four or five days under high light conditions, as our treatments did, may not reduce the carbohydrate supply below the fruit thinning threshold under such conditions. High light intensity in WA results in thicker leaf cuticle development (Table 1), which may also lessen FOLS efficacy by limiting chemical uptake.

While this research has confirmed that FOLS efficacy isn't limited to the bloom period, the bloom period appears to still be the best application window for WA, as it includes not only photosynthetic inhibition but also the effect of FOLS as a blossom thinner. While we had hoped that our research would increase the utility of FOLS to western growers by extending the effective application window beyond petal fall, as occurs in eastern U.S. orchards, this appears not to be the case.

Leaf Anatomy:

Leaf anatomy analysis in N.Y. showed no obvious external or internal phytotoxic changes in cell or organ structure between controls and FOLS leaves in 2004. Parameters measured are illustrated in Figure 2. The lower photosynthetic capacity of FOLS-treated spur leaves appeared to relate to differences in leaf anatomy. FOLS treated primary spur leaves had smaller epidermal cells, smaller cells in the palisade layers, and thinner cells in the spongy mesophyll region (Table 1). Bourse leaves were much less affected by FOLS than were the primary leaves. The influence of primary spur leaves is important for fruit set and early fruit growth, but diminishes once shoot leaves, such as bourse leaves fully emerge and begin to contribute to assimilation. Additional anatomical work in 2005 was conducted to see if primary spur leaves are consistently more impacted by FOLS sprays than are shoot leaves, which, if it were the case, would provide insight into why the earlier timings seem to have been more effective than later timings in WA.

The anatomy component of the overall project was designed to test whether anatomical differences accompany or help explain differences in fruit thinning between FOLS and non-sprayed control trees,

especially through FOLS' possible negative impact on photosynthesis or through phytotoxic effects on leaves. We did see some phyotoxiciy (stippling) of leaves treated with FOLS in both WA and NY, but it was inconsistent as to position on the leaf or to position within the canopy. We hypothesized that we would see more limited cuticle development in FOLS-treated leaves compared to controls, especially in NY State, where normally cooler weather and decreased light intensity compared to WA State could limit cuticle development. Although cuticle thickness did not vary much between spray treatments, WA spur leaves did develop a thicker cuticle than did NY leaves (Table 1). This difference may partially explain the extra thinning effectiveness of FOLS sprays seen in NY compared to WA. The lower photosynthesis readings found for FOLS-treated spur leaves in NY (Figure 1) did not appear to affect leaf anatomical features a day or two after treatment. There also did not appear to be a carry over effect of such treatments as discovered in the anatomical analysis of NY leaves 1 month post bloom. The FOLS treatment at bloom time in WA also did not appear to affect leaf structure as seen in the 1-month post bloom collection. We did not have a bloom time collection of WA spur leaves to see whether there was a short-term affect on leaf anatomy just after spraying the younger spurs. We saw little evidence for this in the NY situation. Because so much of the effect of FOLS appears to impact the biochemical/physiological mechanism operating during photosynthesis, any future study of the effect of FOLS should include more detailed study of chloroplast ultrastructure using electron microscopy.

Fish Oil Alternatives:

Applications of lime sulfur alone were not effective for thinning apples in any of our experiments. Following inconclusive results in WA in 2003, tank mixing LS with either fish oil or soybean oil provided the most thinning activity in both WA trials in 2004. Petroleum oil plus LS also provided a moderate amount of thinning. Individual fruit size was greatest with soybean oil+ LS at both locations, while petroleum oil+LS also increased fruit size at one of the locations in 2004. None of the oil+lime sulfur treatments were effective in WA in 2005. Fruit russet has not been a problem with any of the oil and lime sulfur combinations

Combining lime sulfur with surfactants was shown to be ineffective in two years in NY. The addition of petroleum-based oil with lime sulfur, such as dormant oil and JMS Stylet oil, has been consistently effective. In several of our studies the petroleum oil tank mixes have resulted in the best fruit size, despite slightly less reduction in fruit set. It would seem that the challenge in eastern orchards is to use caution not to inhibit Pn so long as to hamper fruit growth, while in WA the challenge is to get a strong enough reduction in Pn so as to achieve thinning.

Spray Volume and Concentration:

As with oil alternatives, the discussion on spray volume and concentration must be based upon results in eastern orchards due to the lack of significant results in WA. Regression analysis showed that the greater the spray volume of water the greater the thinning efficacy of FOLS sprays (Figure 3). This would suggest that dilute sprays provide better coverage of flowers and young spur leaves giving greater effect. The rate of lime sulfur and fish oil did not affect thinning performance of this combination at either 2X or 4X spray volumes.

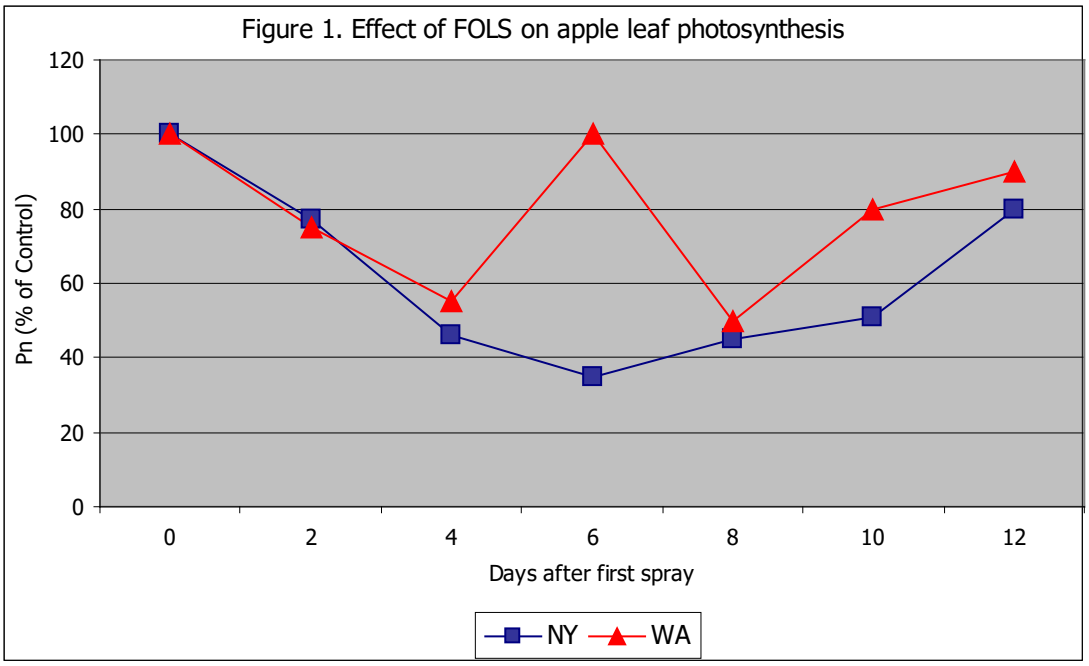

Table 1. Comparison of means for Gala primary spur leaves of control trees and trees treated with FOLS or FOLS+Shade in New York or with FOLS in Washington State in 2005.

Location and Date	Treatment	Pn: Net Photosyn-thesis[1]	Upper Cuticle Thick. (μm)[2]	Upper Epid. Cell Thick. (μm)	Upper Palisade Cell Ht. (μm)[3]	Upper Palisade Chloro-plast L. (μm)[4]	Lower Palisade Cell Ht. (μm)[5]	Lower Palisade Chloro-plast L. (μm)	Spongy Meso-phyll Thick. (μm)[6]	Lower Epid. Cell Thick. (μm)	Total Leaf Thick. (μm)
New York 19 May	Control	12.1 ± 0.7	0.70 ± 0.08	12.5 ± 0.8	36.7 ± 2.6	3.4 ± 0.1	37.6 ± 2.2	3.6 ± 0.1	118 ± 7	12.1 ± 0.7	217
	FOLS	5.6 ± 1.1	0.73 ± 0.08	12.8 ± 0.9	36.5 ± 2.1	4.2 ± 0.1	36.1 ± 2.1	4.6 ± 0.1	119 ± 5	11.8 ± 0.9	217
	FOLS+Shade	8.5 ± 2.1	0.70 ± 0.08	12.0 ± 0.7	34.9 ± 1.7	4.0 ± 0.1	34.5 ± 1.7	4.2 ± 0.1	111 ± 6	11.3 ± 0.5	204
New York 21 June	Control	*	0.58 ± 0.03	13.4 ± 0.4	48.4 ± 1.1	4.8 ± 0.1	47.6 ± 1.28	5.2 ± 0.1	133 ± 4	12.4 ± 0.4	254
	FOLS	*	0.63 ± 0.03	13.5 ± 0.4	45.5 ± 1.7	4.8 ± 0.1	43.0 ± 1.6	5.2 ± 0.1	140 ± 6	11.6 ± 0.2	254
	FOLS+Shade	*	0.57 ± 0.04	13.3 ± 0.4	45.5 ± 0.9	4.8 ± 0.1	42.9 ± 1.0	5.4 ± 0.1	120 ± 6	12.2 ± 0.6	234
Washington 1 June	Control	9.5 ± 0.4	0.85 ± 0.17	15.7 ± 0.7	36.9 ± 3.2	4.2 ± 0.1	30.2 ± 2.4	4.2 ± 0.2	90 ± 6	12.4 ± 0.7	186
	FOLS	7.6 ± 1.2	0.78 ± 0.15	15.4 ± 0.1	38.1 ± 2.0	4.4 ±0.1	34.0 ± 1.4	4.1 ± 0.2	106 ± 5	12.2 ± 0.7	205

[1]Pn measured as µmol/m2/s.
[2]Thickness of waxy covering on cells on upper leaf surface.
[3]Vertical height of photosynthetic cell layer just below the epidermal cell layer.
[4]A measure of the size and capacity of the chloroplast to produce photosynthate or to store starch.
[5]Vertical height of photosynthetic cell layer just below the upper palisade cell layer.
[6]Vertical thickness of the aerated photosynthetic tissue filling the lower half of the leaf's thickness.
*Not recorded for samples leaves in NY in the June collection.

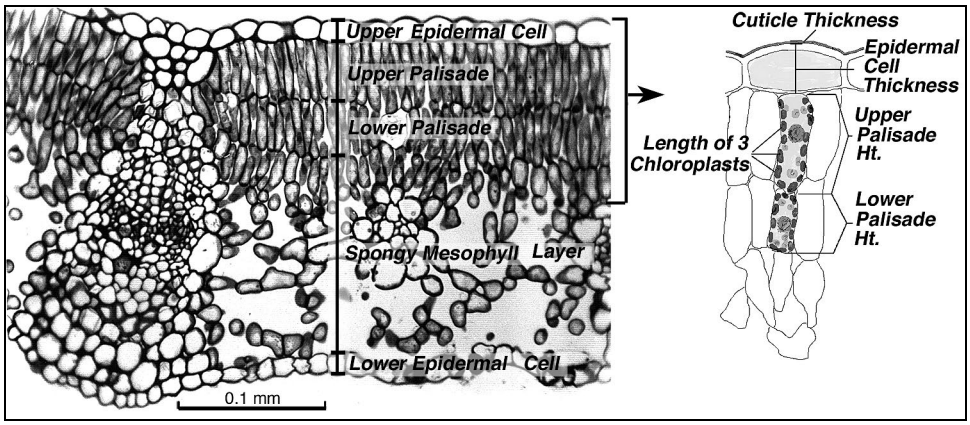

Figure 2. Anatomical parameters measured for each leaf processed for histological examination.

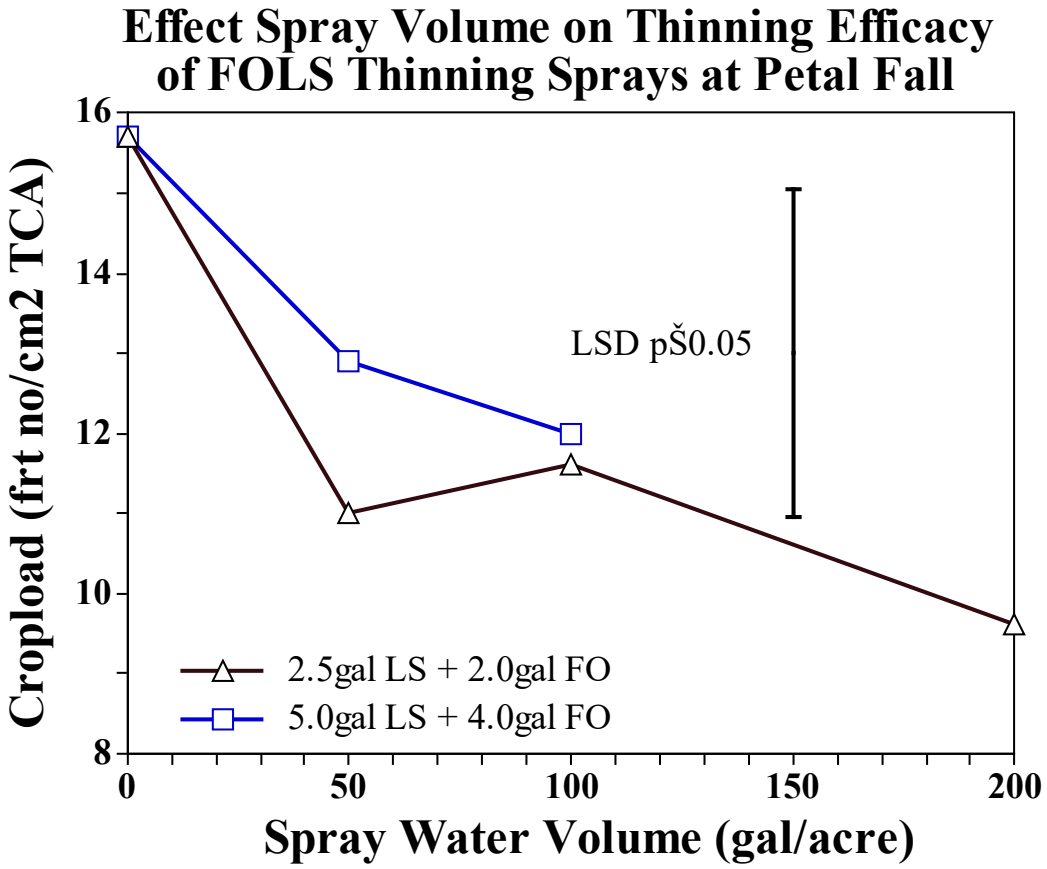

Figure 3. Effect of FOLS spray volume on cropload of Gala/M.9 apple trees from sprays applied at bloom.

Literature Cited:

Burrell, A. B. 1945. Practical use of our newer knowledge of apple scab control. Proc. 90th NY State Hort. Soc. pp. 9-16.

Byers, R.E., J.A. Barden, R.F. Polomski, R.W. Young and D.H. Carbaugh. 1990. Apple thinning by photosynthetic reduction. J. Amer. Soc. Hort. Sci. 115: 14-19.

Koike, H., and T. Ono. 1998. Optimum crop load for apples in Japan. Compact Fruit Tree 31:13-16.

Budget:

Project Title: Efficacy and Physiology of Oil / Lime Sulfur Apple Thinners.
PI: Jim Schupp
Project duration: 2003-2005
Current year: 2005
Project total (3 years): $54,000

Item	Year 1(2003)	Year 2 (2004)	Year 3 (2005)
Salaries	6165	0	0
Benefits	2362	0	0
Wages	4680	10,000	11,206
Benefits	1793	4100	4594
Equipment	0	0	0
Supplies	3000	2200	2200
Travel	0	1700	0
Miscellaneous	0	0	0
Total:	18,000	18,000	18,000

FINAL PROJECT REPORT

Project Title: Developing genetic tools for regulation of flowering, thinning, and fruit drop

PI: Steven van Nocker
Michigan State University-Horticulture
390 Plant and Soil Sciences. East Lansing, MI 48824
Email: vannocke@msu.edu
517-355-5191 ext 394

No report submitted

FINAL PROJECT REPORT
WTFRC project number: AH-03-317A

Project Title: Evaluation of apple lines overexpressing the Antioxidant APX

PI: Michael Wisniewski
Plant Physiologist
USDA-ARS
Appalachian Fruit Research Station Kearneysville, WV

Co-Principal Investigator: Les Fuchigami
Department of Horticulture, ALS 4017
Oregon State University
Corvallis, OR 97331

Cooperator: Lailiang Cheng
Cornell University
Ithaca, NY 14853

Objectives:

- Evaluation of three transgenic apple lines overexpressing APX. Evaluations will be conducted in both West Virginia (Kearneysville) and Oregon (Corvallis). Evaluation of material will be as self-rooted plants and as scions grafted on a commercial rootstock.
- Evaluation will consist of monitoring growth parameters, photosynthetic and antioxidant parameters on lines grafted on commercial rootstocks, especially under conditions of environmental stress.
- Continued characterization of the resistance of potted plants to environmental stresses such as drought, and high and low temperatures, and high light.
- Construction of transgenic lines of apple overexpressing other antioxidant enzyme genes such as SOD (superoxide dismutase).
- Characterization of seasonal expression of the APX gene, and enzyme activity in different tissues (leaves, stem, and bud).
- Determining how overexpression of APX affects other parameters of the antioxidant system (SOD, glutathione reductase, catalase, etc.).

Significant Findings and Accomplishments over the Life of the Project:

Ten to Fifteen Lines of Transgenic Apple Were Created and Several of the Lines Were Shown to Have Increased Resistance to Environmental Stress. We were the first to demonstrate that Overexpression of the Antioxidant Enzyme Gene for Ascorbate Peroxidase (APX) Could Enhance the Resistance of Apple to Several Different Types of Environmental Stress (Low and High Temperature, Drought, and UV-B).

- APX levels were higher in transgenic leaves than in wild-type leaves and had significantly higher total antioxidant status.
- The overexpression of APX produced plants that were more resistant to freezing stress, high temperature stress, and drought stress. (See details in previous annual reports and final report).
- Plants were also resistant to UV-B-induced oxidative stress.
- Transgenic APX plants exposed to drought maintained higher levels of photosynthesis.
- Higher levels of APX in transgenic leaves were consistent throughout the growing season.
- Higher levels of APX levels in transgenic leaves resulted in lower levels of hydrogen peroxide compared to wild-type leaves when leaves were exposed to UV stress.

- No significant differences were observed in growth rates between the wild-type and transgenic lines indicating that the overexpression of APX did not have a negative impact on general metabolism. This is an important finding when assessing the cost of increasing resistance to environmental stress.
- Several genetically-enhanced line of apple were established that overexpress a cytosolic SOD (superoxide dismutase) gene. This line is now being propagated to assess its resistance to environmental stress.

Methods Used in Final Year of Study:

Drought Stress and Enzyme Activities

MATERIALS AND METHODS

Plant materials

Wild type and APX transgenic *royal gala* apple plants were cultured and selected with selective medium. Plants were planted in 8 inch pots and grown under controlled environment conditions (PPFD 200 µmol m^{-2} s^{-1} during a 16 h photoperiod at 25 °C) in a greenhouse.

Drought stress

Leaves were freshly detached and weighed. Then leaves were exposed to the air on a bench in the laboratory (25°C) for various periods and the weight loss were recorded. Parallel samples were dried at 80 °C to determine the water content of the leaves.

Enzyme activity assay

Abbreviations: dehydroascorbate reductase (DHAR); monodehydro-ascorbate reductase (MDAR); glutathione reductase (GR); ascorbate peroxidase (APX);

Four enzymes involved in the ascorbate-glutathione cycle were studied. Antioxidant enzymes were extracted according to Grace and logan (1996). Leaf discs were weighed, ground with a pre-cooled mortar and pestle in 2.5 ml extraction buffer containing 50 mM KH_2PO_4-KOH (pH 7.5), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.3% (w/v) Triton X-100, and 4% (w/v) insoluble polyvinylpolypyrrolidone (PVPP). The extract was then centrifuged at 13, 000 *g* for 10 min in an Eppendorf microcentrifuge, and the supernatant was used immediately for enzyme activity assay.The ascorbate consumption was monitored by the reduction of absorbance at 290 nm taking 2.8 $(mmol/\ L)^{-1}$ cm^{-1} as the absorption coefficient (Nakano and Asada, 1981)

For the MDAR assay, a reaction mixture containing 0.9 ml of 2 mmol/L ASA in phosphate buffer (pH 7.0), 0.04 ml of ASA oxidase (2 units) in phosphate buffer (pH 5.6), 0.03 mL of 2 mmol/L NADPH in buffer (pH 7.6) and 0.03 mL crude enzyme was used. The consumption of NADPH was monitored by the reduction of absorbance at 340 nm taking 6.2 $(mmol\ /L)^{-1}cm^{-1}$ as the absorbance coefficient (Krivosheeva *et al*, 1996).

For the DHAR assay, a reaction mixture containing 0.7 ml buffer (pH 7.0) , reduced glutathione (GSH) [20 mmol/L 0.1 ml in buffer (pH 7.0)], 2 mmol/L DHA 0.1 mL and crude enzyme 0.1 ml was used. DHA was freshly prepared and kept on ice until addition of the reaction mixture in the cuvette to prevent oxidation at room temperature. The reduction of DHA to ASA was monitored by the increase in absorbance at 265 nm using 14 $(mmol/\ L)^{-1}\ cm^{-1}$ as the absorbance coefficient (Krivosheeva *et al*, 1996).

Glutathione reductase (GR) activity was determined at 340 nm by following the change in absorbance occurring as NADPH is oxidized to NADP (Glutathione reductase assay kit, Calbiochem).

All the enzyme assays were repeated three times.

Results:

In the drought stress experiment the APX enzyme activity of the wildtype (control) leaves increased rapidly after only 10% loss of water and then quickly decreased after 20% and 30% water loss. In contrast the APX activity of the transgenic leaves gradually increased after 10% and 20% loss in water followed by a gradual decrease at 30% water loss.

The rapid increase in APX activity after 10% water loss suggests that wild type were able to acclimate quickly to the level of the unstressed transgenic leaves but further stress caused a rapid loss of APX activity. It is interesting to note that APX enzyme activity of the APX transgenic plants responded slower than the wild type suggesting that perhaps the higher initial APX activity of the transgenic plants was sufficient to withstand the gradual loss of water followed by a gradual increase in activity to acclimate to the continued loss of water.

The higher initial APX enzyme activity in the APX transgenic plant leaves may enable the stressed cells to better scavenge the hydrogen peroxide produced in response to the stress thus protecting the cells from being oxidized. The initial higher APX activity of the transgenic plants may be important for enabling the transgenic plants to have a buffer against rapid and greater exposure to stresses. The higher maintenance of APX activity of the transgenic leaves with increasing water stress up to 30% water loss suggests that the transgenics were able to acclimate to water stress than the wild type plants.

The initial and sustained higher APX activity of transgenic shows better protection from drought stress.

APX activity was sensitive to the rapid changes in water content of leaves. In contrast the MDAR and DHAR activities seemed much lower, and responded slower to drought stress than APX.

The activity of MDAR and DHAR is higher in the APX transgenic than the wild type plants. These enzymes are important for regenerating ascorbate and the results are consistent with the higher APX activities found in the transgenic plants. This implies that the APX transgenic plants have faster ascorbate-glutathione cycle.

The GR activity in the transgenic plants was lower initially and responded slower to 10% water loss than the wild type. At 20% water loss the GR of the transgenic plants increased rapidly and was greater than the GR activity of the wild type. At 30% water loss GR activity of both plant types decreased however, the activity was greater in the transgenic plants.

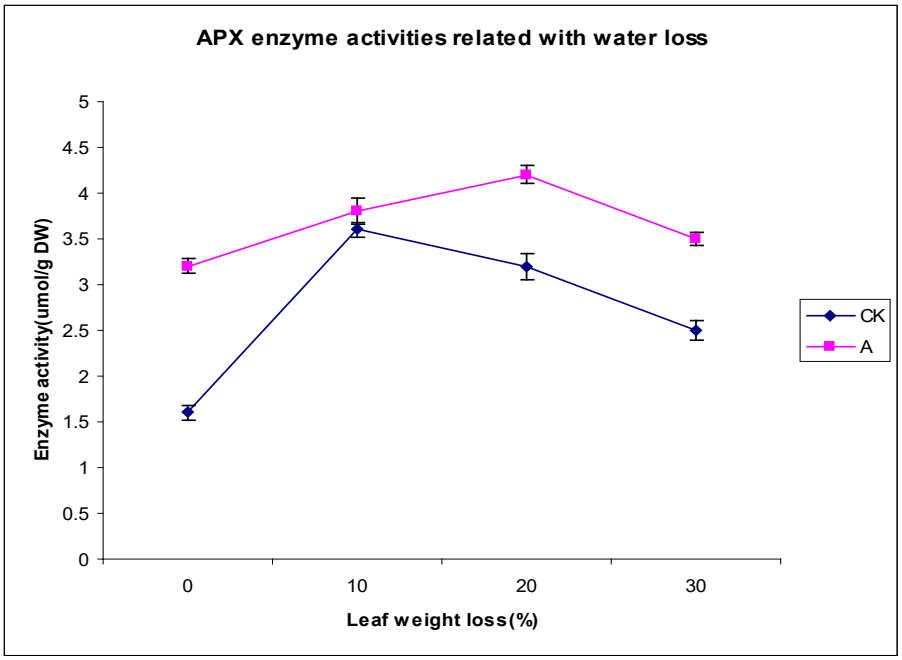

Fig1. APX enzyme activities change with leaf water loss

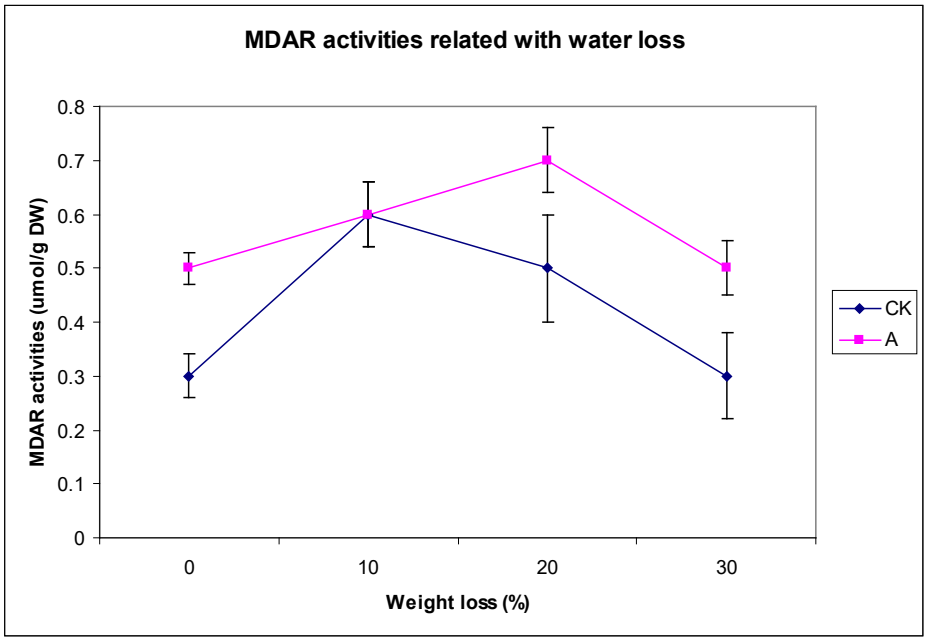

Fig2. MDAR activities with leaf water loss

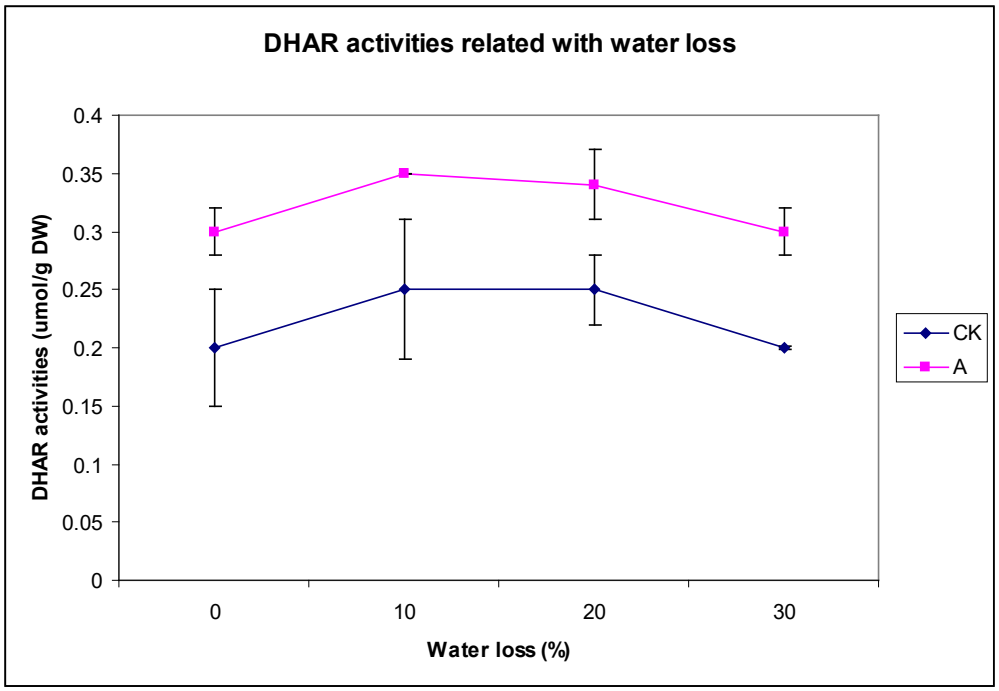

Fig3.DHAR activities with leaf water loss

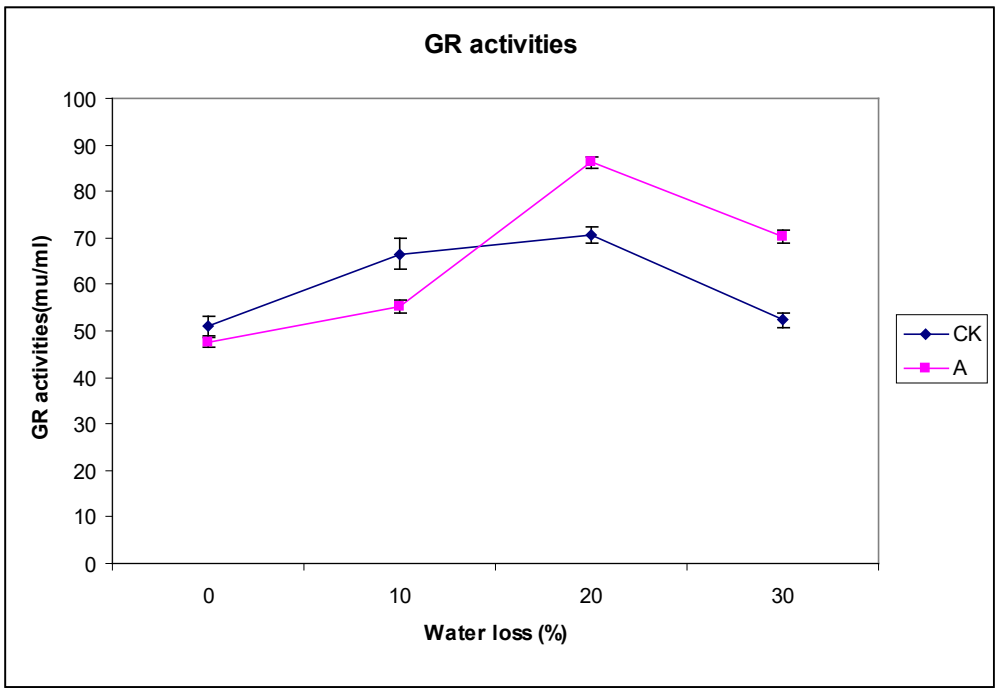

Fig 4. Glutathione Reductase (GR) activity in relations to changes in water loss (%) of leaves from wild-type and APX-trangenic plants

Seasonal change of APX enzyme activity and shoot growth

Material:

Two leaf discs from first fully expanded leaves from wild type and APX transgenic 'Gala' apples growing under natural conditions at Corvallis, OR were collected monthly from April to October. The samples were put into 1.5ml centrifuge tube and placed into liquid nitrogen immediately. All samples were stored in a freezer at -80^{o}C until analysis.

Shoot growth

Shoots from different lines and wild type plants growing in containers at Corvallis, OR as described previously were labeled, and during the beginning of each month, the shoot length was determined. Six replications per each line and wild type were determined.

Extraction and assay of APX

The method for APX assay was the same as described above. One sample from wild type was used as a reference protein. It was divided into 0.5ml centrifuge and 25 µl/ tubes. All tubes were put into the freezer (-80^{o}C) immediately. The enzyme assays by native gels for all the plant types.

Results:

1.
The APX enzyme activity of the wild type and APX transgenic plants showed two peaks during the 2005 season (Fig5). In general the APX activities of the transgenic liens were greater throughout the sampling period than the wild type plants. In both the wild type and transgenic lines the activity increased slowly to a peak at June followed by a decrease in July in the transgenic line and a decrease in July and further decrease in August by the wild type. The activity increased again to a new peak in August for the transgenic line and in September for the wild type plants. This trend was similar as the results of 2004.

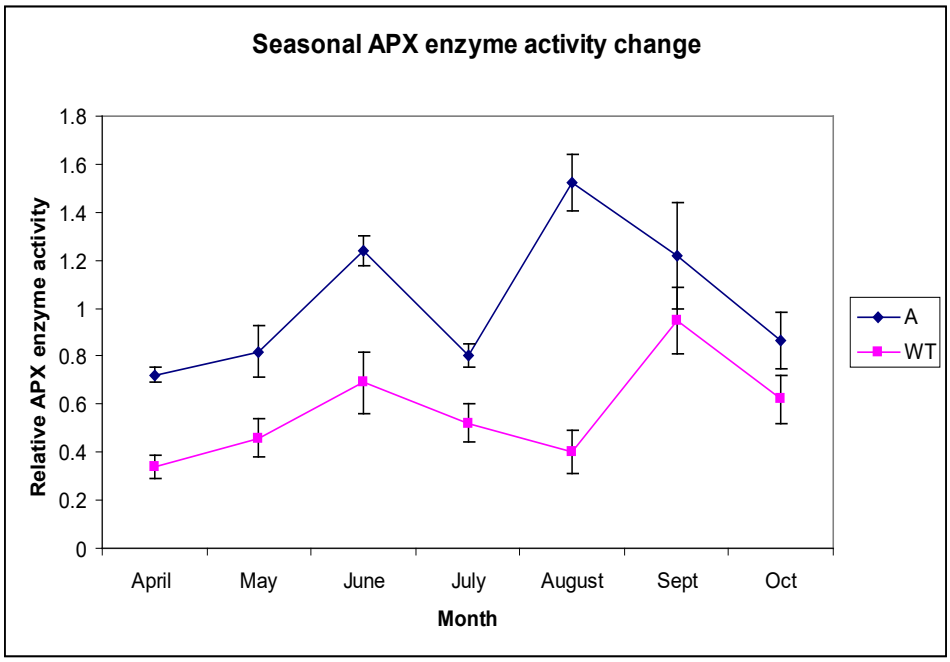

Fig 5. Seasonal APX enzyme activity change

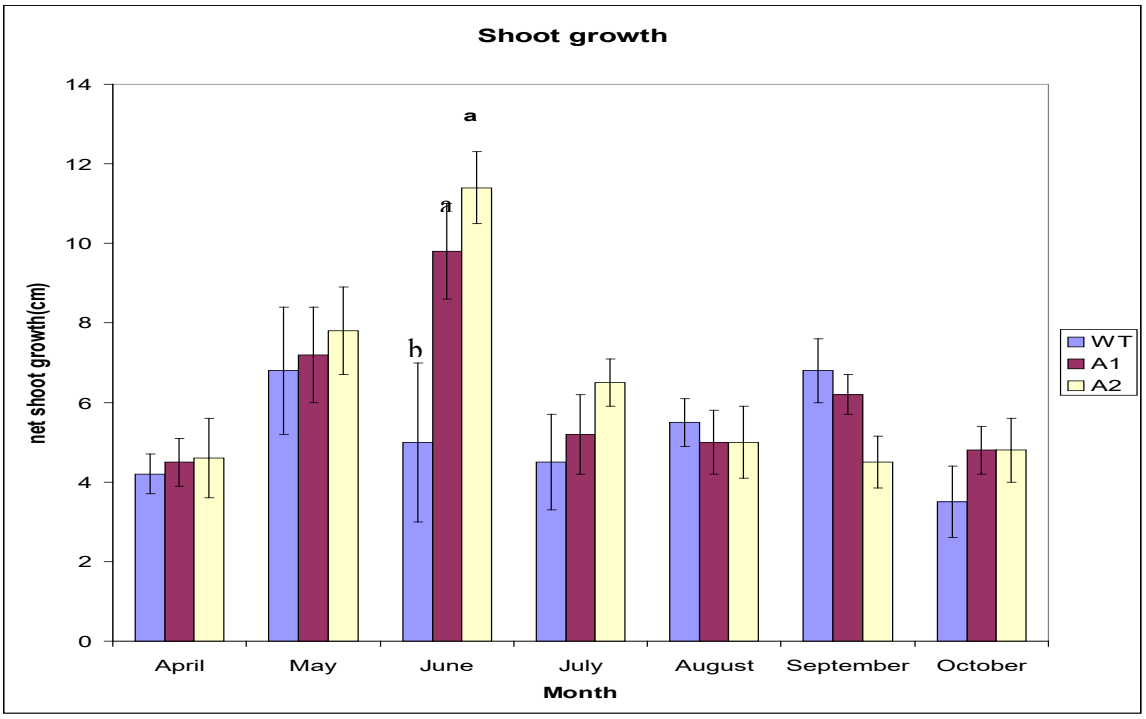

Fig 6. Seasonal shoot growth of APX transgenic plants and wild type

2.
The only significant difference found in the monthly growth between the wild type and two transgenic lines were found in June when both transgenic lines grew significantly more than the wild type plants. At all other sampling periods from April to October no differences in growth were found in general between the plant types. The only other exception was found in September when the growth of the wild type was greater than one of the transgenic line.

The Relationship of Chlorophyll Fluorescence under drought stress

Material
Three year old potted apple trees were used. Six replications of transgenic plants and wild type plants growing in 8 inch pots containers were completely randomized at Oregon State University, Corvallis, OR. Plants were treated without watering for 3 days. Chlorophyll [variable fluorescence/maximal fluorescence (Fv/Fm)] ratios were taken on the same leaf at day 0,1,2,3 in the morning.

Measurements of chlorophyll fluorescence

Modulated Chlorophyll fluorescence measurements were made in attached leaves of apple plants at midday with a Hansatech portable FMSII fluorometer (Hansatech, UK)

The plant was dark adapted overnight prior to the measurement by put the plants into the dark room at night. Upon the exposure to a saturating flash (8000 □mol m^{-2} s^{-1} for 1 s) of light, fluorescence increases from the ground state value (Fo) to its maximum value, Fm. In this condition, QA, the first electron acceptor of PSII, is fully reduced. This enables one to determine the maximum quantum efficiency of photosystem II (PSII) primary photochemistry, given by Fv/Fm = (Fm-Fo)/Fm.

Results:

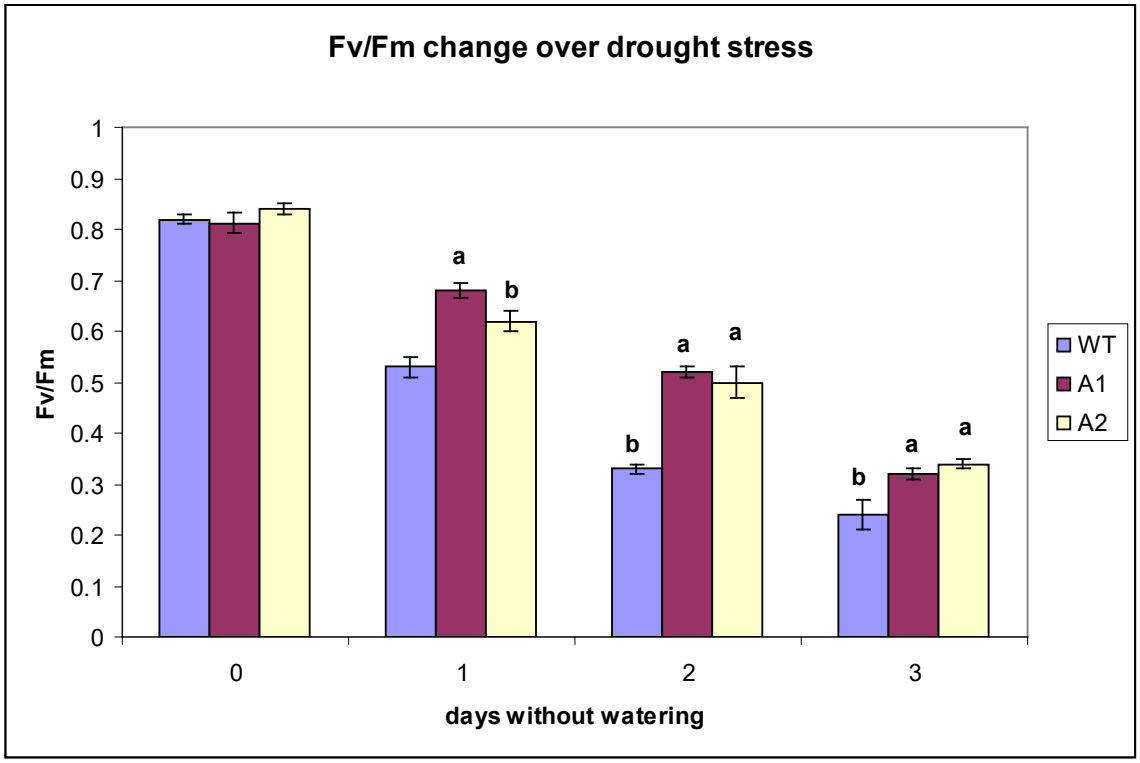

Fig 7: PSII efficiency of wild type and transgenic plants under drought stress

Efficiency of PSII photochemistry (F_v/F_m) is an indicator of photoinhibition.. After one, two and three days of water stress the APX transgenic plants maintained significantly higher PSII efficiency(Fig.7) than the wild type plants, This suggests that a proportion of PSII reaction centers in the wild type plants suffered greater damage due to exposure of plants to water stress conditions. This study suggests that higher APX enzyme activity in the APX transgenic lines can protect apple leaves from photoinhibition.

Budget

Project Title: Evaluation of apple lines overexpressing the antioxidant APX

Principal Investigator: Michael Wisniewski
Co-Principal Investigator: Les Fuchigami
Project Duration: 2003-2005
Current Year: 2005
Project Total (3 years):

$45,000 – Represents Awards at Levels Significantly Less than Requested. Although the project was approved, it was funded during the first two years at half the level requested and funding was drastically reduced in the third year.

Budget: USDA-ARS

Item	**Year 1 (2003)**	**Year 2 (2004)**	Year 3 (2005)
Supplies	**5,000**	**5,000**	0
Total	5,000	5,000	0

Budget: Oregon State University

Item	**Year 1 (2003)**	**Year 2 (2004)**	Year 3 (2005)
Wages	**15,000**	**15,000**	5,000
Benefits (3%)	**0**	**0**	0
Supplies	**0**	**0**	0
Total	15,000	15,000	5,000

FINAL PROJECT REPORT
WTFRC Project #AH-03-321 **WSU Project #13C-3655-7326**

Project title:	Fuji Stain: Causes and Prevention
PI:	Larry Schrader, Horticulturist
Organization:	WSU Tree Fruit Research and Extension Center, Wenatchee
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Overall objective: To understand the factors involved in the development of stain in 'Fuji' apples and search for ways to reduce it. Specific objectives are outlined below:

1. Investigate the effects of preharvest environmental stress on development of 'Fuji' stain.
2. Investigate the effects of MCP treatment on development of 'Fuji' stain.
3. Study the effect of various crop protectant formulations on development of 'Fuji' stain.
4. Determine whether blocking UV-B irradiation decreases development of 'Fuji' stain in cold storage.
5. Investigate whether nutrient imbalances enhance stain development.
6. Study the effects of drenching on development of 'Fuji' stain.
7. Investigate the effects of fumigation with methyl bromide.
8. Investigate the effects of grading (packingline) operations, such as waxing fruit, on development of 'Fuji' stain.
9. Search for ways to reduce 'Fuji' stain through use of additional techniques such as UV-B filters, orchard cooling, postharvest drenches and new grading practices.

Significant findings:

1. 'Fuji' stain, a skin disorder, appears only after cold storage (Fig. 1). Stain develops on the side of fruit previously exposed to sun.
2. Stain disorder affected only the epidermal layer (peel tissue) and was not found to extend into the flesh (Fig. 2).
3. Heat- and/or light-induced stain is directly related to the degree (grade) of sunburn on fruit as apples entered cold storage. No stain appeared in apples that had no sunburn, but stain incidence increased sharply as sunburn damage increased (Fig. 3).
4. Choice of rootstock (M.9 vs. M.26) had no significant effect on development of stain in 'Fuji' apples stored over four months (Table 2).
5. Postharvest treatment of 'Fuji' apples with MCP decreased stain during regular atmosphere cold storage (Fig. 4).
6. Preharvest applications of either ReTain or MCP reduced incidence of stain significantly (Fig. 5).
7. RAYNOX® applied three times during the 2003 season significantly reduced the appearance of 'Fuji' stain in treated apples that were held in cold storage over a period of four months (Fig. 6).
8. Apples sprayed four times during 2002 with RAYNOX® for sunburn protection developed less stain than control apples or apples sprayed four times with VaporGard® or Surround® (Fig.7).
9. Several mineral nutrients were higher in peel from stained fruit as compared to normal tissue (Table 3), but the significance of these changes is not known.
10. Brown in Tasmania studied effects of prefumigation drenching on the development of 'Fuji' stain. MCP and 2% ascorbic acid were the most effective treatments against stain (Fig. 8).

11. Evaporative cooling systems significantly reduced 'Fuji' stain when compared to controls. This suggests that high fruit surface temperature is a cause of 'Fuji' stain and that stain can be decreased in apples protected from extreme heat stress (Fig. 9).

Methods:

Objective 1: Apples were harvested from an orchard in the Yakima Valley with a history of high incidence of stain in trees grown on M.9 and M.26 rootstock. These apples were stored at 33°F and evaluated monthly for the appearance of stain over a four-month period of storage.

Objective 2: 'Fuji' apples were harvested from an orchard in the Yakima Valley that has a history of high incidence of stain. The apples were treated with 1 ppm MCP for 12 hours at 68°F, and following MCP treatment they were stored in a room at Stemilt at 33°F. The fruit were evaluated at monthly intervals to follow the appearance of stain over four months. During 2005, Gordon Brown in Tasmania applied MCP to 'Fuji' apples collected from four orchards. He then evaluated stain after cold storage. In 2004, several chemicals were sprayed on September 9 and/or 29. The details of treatments are shown in Table 1. Fruit were harvested on October 12, 2004, and put into cold storage the following day. Thirty apples were collected from each replicate. For replicate I, all the fruits were labeled and investigated at two-week intervals until mid-May, 2005. For replicates II to V, evaluation was made once a month and the stained fruit was marked once stain was observed. Duncan's multiple range test was used for statistical analysis.

Objective 3: In 2003, apples treated with RAYNOX® and untreated controls were harvested from the same orchard in the Yakima Valley. The degree of sunburn present at harvest was determined, and apples were separated into lots of thirty for each class of sunburn (according to the Schrader-McFerson classification of sunburn). These apples were placed in a regular atmosphere (RA) cold room at Stemilt and maintained at 33°F for four months. The fruit were evaluated at monthly intervals to follow the appearance of stain in these different lots of apples.

Objective 4: 'Fuji' apples to which RAYNOX®, Surround WP® and VaporGard® had been applied four times during 2002 were harvested, stored, and evaluated at one-month intervals for stain. Schrader and Sun applied RAYNOX®, a UV-B blocking material, to 'Fuji' apples during mid-September 2003 to see if decreased UV-B irradiation during later stages of development would decrease stain. Treated fruit were harvested along with untreated controls. All fruit were placed in cold storage at Stemilt and evaluated periodically to see if stain appearance was affected.

Objective 5: Nitrogen status of orchards was estimated by using a Minolta SPAD meter to estimate chlorophyll in leaf tissue to compare N status vs. stain incidence. Normal 'Fuji' apples were harvested from three orchards and put into cold storage. In January 2005, 26 stained and 15 normal fruit were selected and labeled for mineral analysis. Three types of peel samples were prepared: (1) stain peel (SP): stain area on fruit surface; (2) healthy peel (HP): healthy area near the stain patch; (3) normal peel (NP): healthy area of normal fruits. Three replicates were prepared for each group of peel samples. The typical area on each fruit of distinctive groups was peeled accordingly with a thickness of about 0.5 mm before the skin was put into a refrigerator at -7ºC for one day. The peel samples were then freeze-dried. When completely dry, the peel was ground to a fine powder in a clean mortar. The concentrations of P, K, Ca, Mg, S, Fe, B, Mn, Cu, Zn, Na, Mo and Al were assessed by inductively coupled plasma emission spectroscopy (ICP-OES), and total nitrogen was assessed by Ion analysis by flow injection analysis (LACHAT QuikChem 8000) at the Soil & Plant Analysis Lab, University of Wisconsin-Madison.

Objective 6: Brown in Australia compared several prefumigation drenches (DPA, Stopit, and ascorbic acid) with 1-MCP to limit the subsequent appearance of stain.

Objective 7: Brown in Tasmania compared additional prefumigation drenches in 2004 (Ascorbyl palmatate and an experimental formulation) with studies done in 2003 to determine effect on occurrence of fumigation scald.

Objective 8: Brown in Tasmania did additional work during 2005 to study effects of packingline operations on 'Fuji' stain. He compared three finishing waxes and treated half the fruit with methyl bromide after fruit cleared the packingline. He also placed fruit in a controlled atmosphere chamber (heater, high CO_2, and low O_2 environment) to compare heating to methyl bromide fumigation as a temperature disinfestation of fruit bound for export to Japan.

Objective 9: Orchard cooling with overhead sprinklers was studied to determine its effect on reducing 'Fuji' stain. An artificial sensor developed in our lab controlled an evaporative cooling (EC) system that was activated in a 'Fuji' apple orchard before temperatures were high enough to cause sunburn damage. These apples were harvested at maturity, stored at 33°F for four months, monitored for the appearance of stain, and compared to untreated (no EC) apples.

Table 1. Preharvest treatments tested for stain reduction.

Treatment	Concentration	Application date	
ReTain2 (powder)	125 ppm	Sep 9	Sep 29
MCP2 (powder)	250 ppm	Sep 9	Sep 29
DPA (liquid)	1000 ppm	Sep 9	Sep 29
ReTain1 (powder)	125 ppm		Sep 29
MCP1 (powder)	250 ppm		Sep 29
Ethrel (liquid)	300 ppm		Sep 29
CK	Water		Sep 29

Results and discussion:

1. ***Fuji stain appearance during cold storage***. Fuji stain does not appear at harvest but only after a few months in cold storage (Fig. 1). The symptoms of stain are diversified, and a great variation in color changes exists among individual fruit. The apple on the left was photographed in October as it was placed into cold storage. It was photographed periodically thereafter until it was removed from cold storage in April (photo on right).

OCTOBER → **APRIL**

Fig. 1. Appearance of stain as the period of cold storage was extended from October (left) to April (photo on right).

2. ***Comparisons of skin and flesh in controls vs. 'Fuji' apples with stain***. In mid-March of 2005, typical stained and normal fruit were cut in half to compare the textures of peel and flesh. The stain disorder was limited to the epidermal layer (peel) and did not extend into the flesh (Fig. 2).

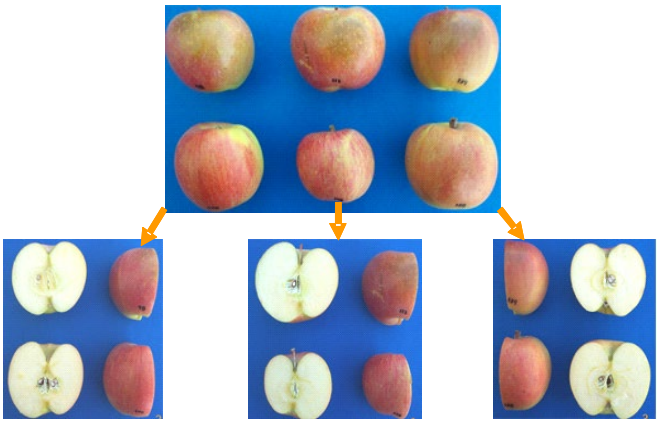
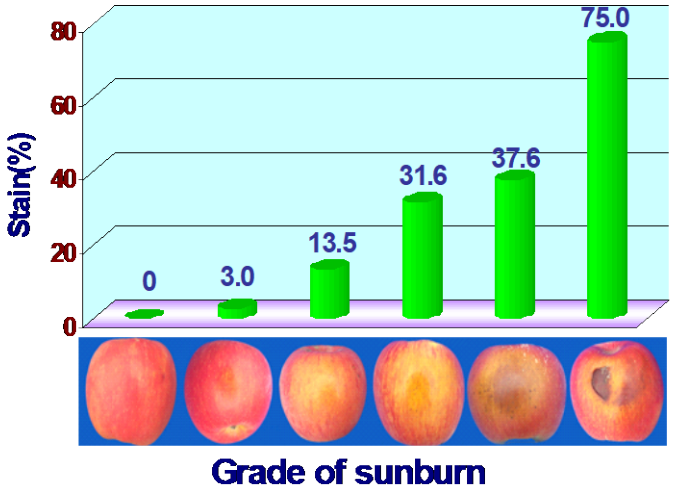


Fig. 2. Observations of flesh in apples with 'Fuji' stain.

3. ***Effects of preharvest environmental stress on later development of 'Fuji' stain***. Apples that had been heat stressed to the extent that they were sunburned had a much higher incidence of stain during cold storage as compared to non-sunburned apples. 'Fuji' apples from an orchard with a history of high incidence of stain were sorted and stored based on grade of sunburn and then evaluated monthly during cold storage for stain development. Stain increased markedly after four months of cold storage in those apples that had more severe sunburn (Fig. 3).



4. ***Effect of rootstock on incidence of 'Fuji' stain***. 'Fuji' apples grown on M.9 or M.26 rootstocks showed no significant differences in appearance of stain during cold storage (Table 2).

Table 2. Effect of rootstock and sunburn incidence on 'Fuji' staining during cold storage.

Rootstock	Staining with sunburn	Staining without sunburn
'Fuji'/M.9	43.2	4
'Fuji'/M.26	56.9	5.2

5. ***Effects of MCP and other treatments on 'Fuji' staining during cold storage***. MCP had no significant effect on the appearance of stain in treated vs. untreated fruit after one month in storage. However, after 2, 3, and 4 months of storage, the untreated controls had significantly more ($P < 0.05$, 0.01 and 0.01, respectively) stain than MCP-treated fruit. The incidence of stain increased over the period of storage in both treated and untreated fruit. Stain in untreated fruit after four months of cold storage increased to 32.6% vs. 17.2% in fruit treated with MCP (Fig. 4).

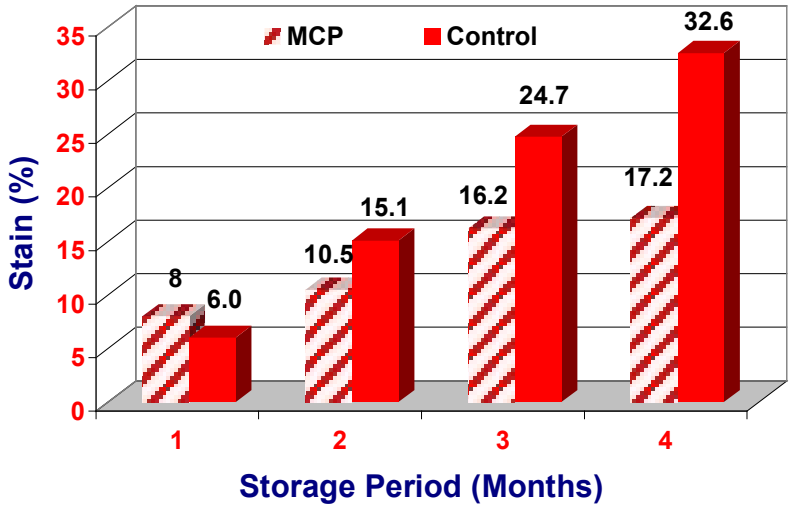


Fig. 4. Effect of postharvest application of MCP on 'Fuji' staining during cold storage.

During 2005, Gordon Brown in Tasmania applied MCP to apples from four orchards and evaluated stain after cold storage. No stain developed in any of the treatments (including the untreated controls) even after fruit were also fumigated with methyl bromide to encourage 'Fuji' stain development (data not shown). Preliminary experiments during 2005 with controlled atmosphere and temperature (CATTS) disinfestation of fruit bound for export to Japan indicated that this CATTS treatment enhances the appearance of stain to a greater extent than methyl bromide.

During 2004, we applied sprayable MCP, DPA, Ethrel and ReTain on September 9 and/or Sept. 29 (see Table 1 for details). The final stain rates of either ReTain2 or MCP2 were significantly lower than for Ethrel and the control, with no significant difference between any additional treatments and the control, suggesting that spraying with 125 ppm ReTain or 250 ppm MCP twice before harvest had a beneficial effect on prevention of stain incidence (Fig. 5).

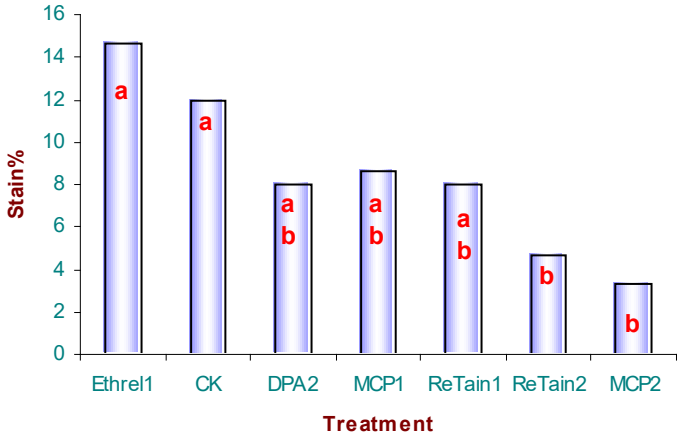


Fig. 5. Effect of preharvest treatments of Ethrel, DPA, MCP, or Retain on 'Fuji' stain.

6. ***Effects of RAYNOX® on 'Fuji' staining during storage***. RAYNOX®-treated 'Fuji' apples had a lower incidence of stain than untreated apples after four months of storage at 33°F in a regular atmosphere (RA) cold room. The appearance of stain increased over the period of storage, and untreated controls developed stain at an increasing rate compared to rate of stain development in RAYNOX®-treated fruit (Fig. 6).

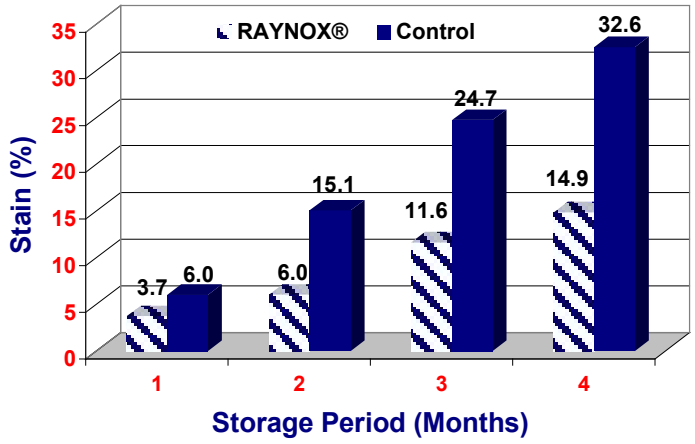


Fig. 6. Effect of RAYNOX® on 'Fuji' staining during cold storage (2003 apples).

7. ***Effects of preharvest applications of different formulations on 'Fuji' staining during cold storage***. Fuji apples sprayed during the 2002 growing season with four applications of RAYNOX®, Surround WP® or VaporGard® were placed in cold storage and evaluated periodically for stain. Stain increased with time in all treatments, but the increase was slower in apples that had been sprayed with RAYNOX® for sunburn protection (Fig. 7). This suggests that blocking some of the damaging UV-B radiation with RAYNOX® during the growing season is beneficial in decreasing stain.

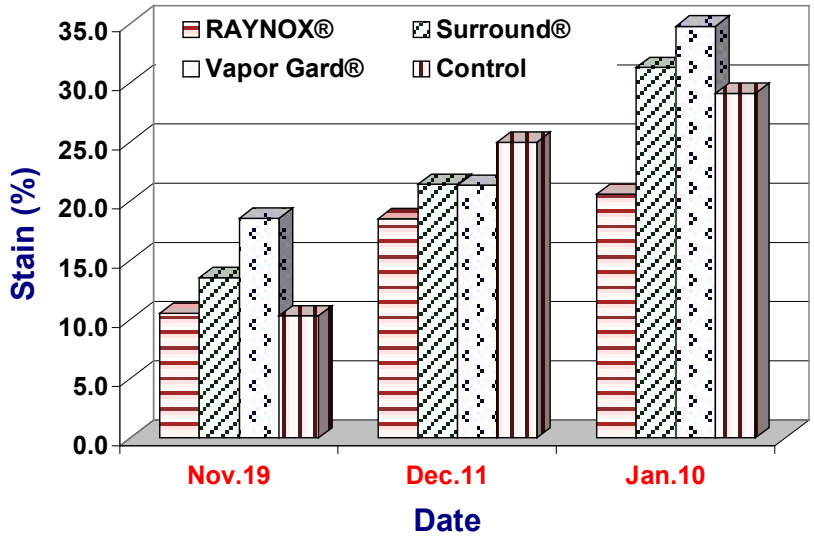


Fig. 7. Effect of different formulations on 'Fuji' stain during cold storage.

8. '***Fuji' stain in relation to mineral nutrient imbalance***. The concentrations of N, P, Mg, S, B, and Mn in stained peel (SP) were significantly higher than those in normal peel (NP) from other apples with no stain and were often higher in SP than in healthy tissue (HP) outside the stained area of the same fruit (Table 3). These assays were done in mid-January after nearly 3 months of cold storage. The relevance of this increase in certain minerals is not known at this time, but it appears to be a result of staining rather than a cause of staining.

Table 3. Mineral analyses of the peel from 'Fuji' apples with and without stain.

Type	*N*	*P*	*S*	*Mg*	*B*	*Mn*	*Na*
	%				ppm		
SP*	0.43 a	0.06 a	0.05 a	0.13 a	37.56 a	7.76 a	36.45 b
HP	0.40 a	0.05 b	0.04 b	0.08 b	31.49 ab	5.57 b	35.72 b
NP	0.33 b	0.04 c	0.03 c	0.07 b	26.87 b	5.16 b	61.29 a

*SP is apple peel from a stained area on the fruit.
HP is healthy peel from outside the stain area but from the same fruit.
NP is healthy peel from another apple that showed no stain.

9. ***Effect of drenching on development of stain during storage (work from Tasmania).*** 'Fuji' stain was not as severe in the 2003 season, with less than 10% of the fruit displaying symptoms. In contrast, up to 40% of fruit were affected in 200 so it is important to develop methods of reducing this disorder to guarantee reliable supplies of fruit with excellent skin finish. There was no significant grower and treatment interaction in the analysis of stain during 2002. Therefore, the data were averaged across all the growers. MCP and 2% ascorbic acid were the most effective treatments against stain, while none of the other treatments reduced this disorder compared to the untreated control (Fig. 8).

***Legend for Figure 8**

UTC = untreated control
DPA = Diphenylamine
1% SI = 1% Stopit®
2% SI = 2% Stopit®
1% AA = 1% ascorbic acid
2% AA = 2% ascorbic acid
MCP = 1-MCP (SmartFresh™)

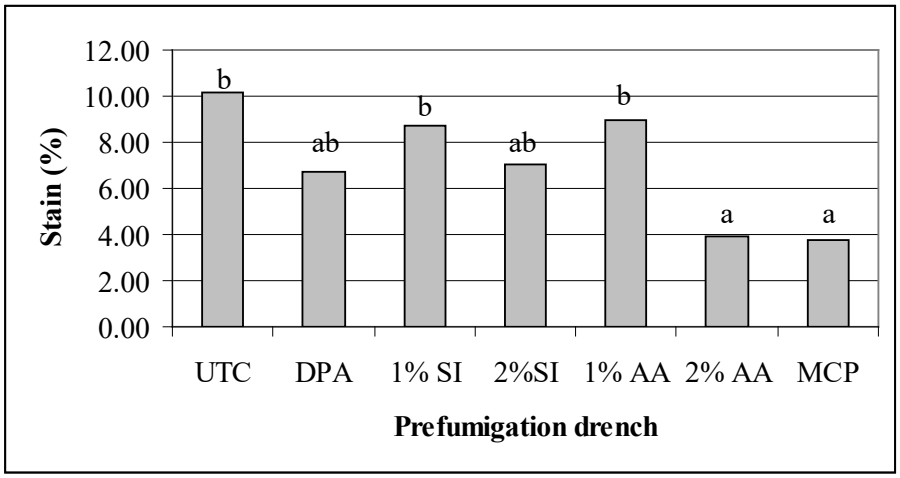


Fig. 8. Effect of drenching on development of stain during storage.

10. ***Effect of evaporative cooling on 'Fuji' staining during cold storage***. Evaporative cooling was studied to determine its effect on the reduction of 'Fuji' stain during storage. A new fruit surface temperature sensor developed in our lab was used to control an evaporative cooling system that was activated before the fruit surface temperatures were high enough to cause sunburn damage. After four months of storage at 34°F, fruit with EC during the growing season had no staining as compared to control (without EC) (Fig. 5). This suggests that stain is a heat-induced disorder that can be decreased by protecting fruit from high fruit surface temperatures.

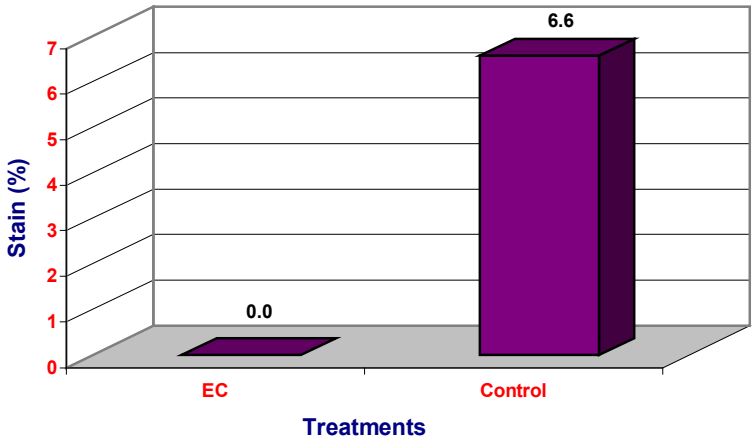


Fig. 9. Effect of evaporative cooling (EC) on 'Fuji' stain.

Budget:
Project title: Fuji Stain: Causes and Prevention

PI: Larry Schrader
Project duration: 3 years (2003-2005)
Current year: 2005
Project total (3 years): $142,818

Year	**Year 1 (2003)**	**Year 2 (2004)**	**Year 3 (2005)**
Total	$42,120	$48,112	$52,586

Current year breakdown

Item	**Year 1 (2003)**	**Year 2 (2004)**	**Year 3 (2005)**
Salaries[1]	$25,000	$28,980	$30,139
Benefits (40% - yr 1; 38% - yr 2; 42% - yr 3)	10,000	11,012	12,537
Wages[2]	2,000	2,000	2,000
Benefits (16% - yrs 1 and 2; 10% yr 3)	320	320	200
Equipment			
Supplies[3]	2,000	2,000	3,000
Travel[4]	800	800	1,000
Miscellaneous[5]	2,000	3,000	4,000
Total	$42,120	$48,112	$52,586

[1] Salary for Research Associate to work with Schrader (Dr. J. Zhang returned in 2004 to work on 'Fuji' stain).
[2] Hourly help to assist with setting up experimental apparatus, collection and analysis of data.
[3] Supplies include chemicals, materials for fabrication and maintenance of equipment, laboratory supplies, crop destruct payments, and cell phone charges.
[4] Travel to experimental plots to evaluate and harvest fruit.
[5] Collaboration continued with Dr. Gordon Brown in Tasmania, Australia. Schrader and Brown had many common interests in fruit finish (including stain and color development) and collaborated on the proposed stain experiments. $4,000 was requested for Dr. Brown's efforts in year 3. He sought most of his support and gets matching funds from the Australian apple growers through Horticulture Australia.

FINAL PROJECT REPORT
WTFRC Project # AH-03-306

Project Title:	Photoprotection of apple fruit by xanthophyll cycle
PI:	Lailiang Cheng, Department of Horticulture, Cornell University Ithaca, NY 14853. Email: LC89@Cornell.edu
Co-PI	Larry Schrader, WSU-TFREC, Wenatchee, WA.
Cooperators	Dr. Horst Caspari (CSU-WCRC), Dr. Mike Glenn (USDA-ARS), Dr. Lisong Chen (Cornell), Dr. Guohai Xia (Cornell), and David Felicetti (WSU-TFREC)

Objectives

The overall objective is to better understand the xanthophyll cycle pool size and composition in the peel of apple fruit and the role the xanthophyll cycle plays in protecting fruit from excessive light damage with an ultimate goal to reduce sunburn occurrence on apple fruit. Specific objectives are: 1) To compare xanthophyll cycle pool size and composition between sun-exposed and shaded fruit of selected apple varieties; 2) To characterize the seasonal patterns of xanthophyll cycle pool size and composition under WA conditions; 3) To determine xanthophyll cycle pool size and composition of 'Gala' fruit peel in response to nitrogen supply; 4) To determine the effects of evaporative cooling, partial root-zone drying, and deficit irrigation on xanthophyll cycle pool size and conversion of 'Fuji' apple peel under Washington conditions; 5) To compare xanthophyll cycle pool size and composition between sunburn susceptible and sunburn tolerant apple cultivars; and 6) To determine UV-screening and reflective coating on xanthophyll cycle size and composition.

Significant Findings

- The sun-exposed side of both Gala and Smoothee fruit has a larger xanthophyll cycle pool size and higher activities of antioxidant enzymes than the shaded side, indicating the sun-exposed side has a higher photoprotective capacity than the shaded side.
- Under high light at noon, the xanthophyll cycle in the sun-exposed side operates at its full capacity to dissipate excess absorbed light and the xanthophyll cycle pool size may become limiting.
- Under Washington conditions, xanthophyll cycle pool size decreases as fruit develops while activities of some of the antioxidant enzymes, especially ascorbate peroxidase, show a compensatory increase.
- Xanthophyll cycle pool size in the sun-exposed peel of 'Gala' fruit increased as nitrogen supply increased, indicating that fruit with a good nitrogen status has higher photoprotective capacity than fruit with a low nitrogen status.
- Evaporative cooling increased whereas deficit irrigation and partial root zone drying decreased xanthophyll cycle pool size.
- There were differences between cultivars in xanthophyll cycle pool size and lutein content associated with their susceptibility to sunburn.
- Pre-dawn maximum quantum efficiency (Fv/Fm) appears to reflect the varietal susceptibility to natural event of high fruit peel temperature and high light.
- Coating fruit with Surround didn't affect xanthophyll cycle pool size whereas UV-screening decreased xanthophyll cycle pool size.

Methods

Experiment 1. *Comparison of the sun-exposed side with the shaded side of apple fruit:* Both Gala/M.9 and Smoothee/M.9 trees were grown at a spacing of 1.83 × 4.88m in the field at Cornell Orchards in Ithaca, NY. They were mature trees trained as a central leader system, 3.5 m tall. At about 3 months after bloom, fruit from two canopy positions were selected: those on the exterior part of the canopy that were fully exposed to sunlight and those in the interior part of the canopy close to the central leader that were heavily shaded (receiving only approximately 5 to 8% of the full sunlight at noon). At each canopy position, the sun-exposed side was carefully distinguished from the shaded side for each fruit. Chlorophyll fluorescence measurements were made on both sides of the attached fruit under natural light exposure at noon and at predawn. Fruit peel discs (1 cm^2 each, 1 mm thick) were taken quickly at noon and/or predawn from both the sun-exposed side and the shaded side of the attached fruit, frozen in liquid nitrogen, and stored at -80 °C until analysis of pigments and antioxidant enzymes. For the noon sampling, the incident PFD and surface temperature for the sun-exposed side of exterior fruit, the shaded side of exterior fruit, the sun-exposed side of interior fruit, and the shaded-side of interior fruit was 1800 ±15, 87 ± 5, 121 ± 7, and 40 ± 3 μmol m^{-2} s^{-1}, and 34.4 ± 0.4, 27.3 ± 0.2, 27.5 ± 0.3, and 26.9 ± 0.1 °C, respectively.

Experiment 2. Seasonal changes of xanthophyll cycle pool size and composition of Gala fruit was monitored from 59 days after bloom to early August at about 2-week intervals in an 10th leaf Scarlet Gala/M.26 orchard in East Wenatchee, WA for a total of 4 times. At each sampling date, fruit peel discs (1 cm^2 each, 1 mm thick, 2 discs/fruit) of sun-exposed fruits from southwest of the tree canopy were taken at both midday (1:00 to 2:30 PM) and predawn, frozen in liquid nitrogen, and stored at -80 °C until analysis. Chlorophyll fluorescence was measured on the sun-exposed side of fruit after overnight dark adaptation. Fruit size, photon flux density (PFD), air temperature and fruit temperature for each sampling date were listed in the following Table.

Sampling date	*Fruit size (mm)*	*PFD μmol m^{-2} s^{-1}*	*Air Temp (℃)*	*Fruit Temp (℃)*
June 19	31.2 ± 0.32	947	22.0	24.6
July 8	41.8 ± 0.89	1125	28.2	35.1
July 22	47.9 ± 0.63	1905	34.7	45.8
August 4	53.8 ± 0.61	1985	27.1	36.7

Experiment 3. *Xanthophyll cycle pool size and composition in apple fruit peel in response to nitrogen supply:* Fourth leaf 'Gala'/M.26 trees grown in sand culture were used in this experiment. The cropload of these trees was adjusted by hand thinning to 5 fruit per cm^2 trunk cross-sectional area at 10 mm king fruit. They were supplied with 2 liters of 2.5, 12.5, or 25 mM nitrogen in a Hoagland's solution twice a week from petal fall to 4 weeks before harvest. Fruit growth was monitored in each N treatment. There were 6 replicates per treatment in a completely randomized design. At 70 days, 100 days, and 120 days after bloom, chlorophyll fluorescence was measured at noon to determine thermal dissipation capacity of the fruit and fruit peel samples (1 cm^2 each, 1 mm thick, 2 discs/fruit) were taken to measure xanthophyll cycle pool size and composition.

Experiment 4. *Effects of evaporative cooling, partial root zone drying, and deficit irrigation on xanthophyll cycle and composition of 'Fuji' fruit peel:* Eleven-year-old 'Fuji'/M.9 trees at Quincy, WA received one of the following 4 treatments: well-watered control, overhead EC on well-watered trees,

partial root-zone drying or deficit irrigation. EC treatment started on July 16 and ended about 7 days before the last sampling. The timer settings for the EC treatment were 20 minutes on then 10 minutes off from 11:30 AM to 5:00 PM every day. The EC treatment, although started on the 16th, did not provide adequate cooling until July 22. Trees in partial root-zone drying and deficit irrigation treatments were supposed to receive approximately half of the irrigation of the well-watered control. However, this year the trees received less water, resulting in water stress development. Fruit peel samples were taken on July 15, August 9, and September 8 during the 2004 growing season. At each sampling date, fruit peel discs (1 cm^2 each, 1 mm thick, 2 discs/fruit) of sun-exposed fruits from southwest of the tree canopy were taken at both midday (1:00 to 2:30 PM) and predawn, frozen in liquid nitrogen, and stored at -80 °C until analysis. Chlorophyll fluorescence was measured on the sun-exposed side of fruit after overnight dark adaptation. Fruit size, photon flux density (PFD), air temperature and fruit temperature at each sampling date were also measured.

Experiment 5. Compare xanthophyll cycle pool size and composition between sunburn susceptible and sunburn tolerant apple cultivars. Mature Cameo, Golden Delicious and Red Delicious trees that were in adjacent blocks in an orchard in Wenatchee, WA were used. Fruit peel samples (10 fruit per cultivar) of sun-exposed side were taken from the exterior canopy of the trees from 1PM to 3:00PM on July 19, August 25 and September 13 to determine xanthophyll cycle pool size and conversion. Maximum quantum efficiency (Fv/Fm) of apple peel (10 fruit per cultivar) was measured after overnight dark-adaptation to indicate the damage of high light coupled with high temperature. Fruit size, photon flux density (PFD), air temperature and fruit temperature at each sampling date were also measured.

Experiment 6. Determine UV-screening and reflective coating on xanthophyll cycle size and composition. Mature 'Gala' trees received one of the following three treatments from 2 weeks after petal fall to 2 weeks before harvest: 1) covered with a polycarbonate which transmits 97% of the photosynthetically active radiation but blocks 98% of the entire UV spectrum (2) sprayed with Surround at 25 lbs per acre every 2 weeks; or 3) untreated control. Each treatment was replicated 4 times in a completely randomized design. Sun-exposed fruits were sampled on June 21, August 9, and August 26 to determine xanthophyll cycle pool size and composition.

Chlorophyll and xanthophyll pigments were extracted and analyzed by using an HPLC procedure (Cheng, 2003). Antioxidant enzymes were measured as described by Ma and Cheng (2003).

Results and discussion

1. Comparison of the sun-exposed side with the shaded side of apple fruit

At noon, efficiency of excitation transfer (F_v'/F_m') was lower in the sun-exposed side than the shaded side for both exterior and interior fruit with the sun-exposed peel of the exterior fruit having the lowest value (Fig. 1A). This indicates that the sun-exposed side has a higher thermal dissipation capacity than the shaded side. Maximum quantum efficiency (F_v/F_m) at pre-dawn was only slightly lower in the sun-exposed side than the shaded side for both exterior and interior fruit (Data not shown), which indicates that the sun-exposed side is fairly well protected from high light.

Xanthophyll cycle pool size was larger in the sun-exposed side than the shaded side (Fig. 1B). At noon, the sun-exposed peel had a higher conversion of violaxanthin (V) to zeaxanthin (Z) and antheraxanthin (A) than the shaded peel for both exterior and interior fruit, with A+Z accounting for over 90% of the xanthophyll cycle pool in the sun-exposed side of the exterior fruit (Fig. 1C). Both the xanthophyll cycle pool size and the conversion of violaxanthin to zeaxanthin and antheraxanthin correspond well with the thermal dissipation capacity of peel type as indicated by efficiency of excitation transfer (F_v'/F_m'). This reflects the dependence of thermal dissipation on the operation of the xanthophyll cycle.

Among the antioxidant enzymes, the activity of superoxide dismutase was similar between sun-exposed and shaded peels with slightly lower values in the interior fruit (Fig. 2A). Activities of the enzymes in ascorbate-glutathione cycle: ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase all showed a similar trend, i.e. the sun-exposed peel had a higher activity than the shaded peel for both exterior and interior fruit although the difference between the sun-exposed and the shaded peels of the interior fruit was not as big as that in the exterior fruit (Fig. 2B, C, D, E). Catalase activity was higher in the shaded side than the sun-exposed side for both exterior and interior fruit, with the sun-exposed side of the exterior fruit having the lowest activity (Fig. 2F). Considering that apple fruit peel had 4 to 5 times higher ascorbate peroxidase activity, but much lower catalase activity than leaves (Cheng and Ma, 2003), the reaction catalyzed by ascorbate peroxidase may serve as the main pathway for detoxifying hydrogen peroxide in apple fruit peel.

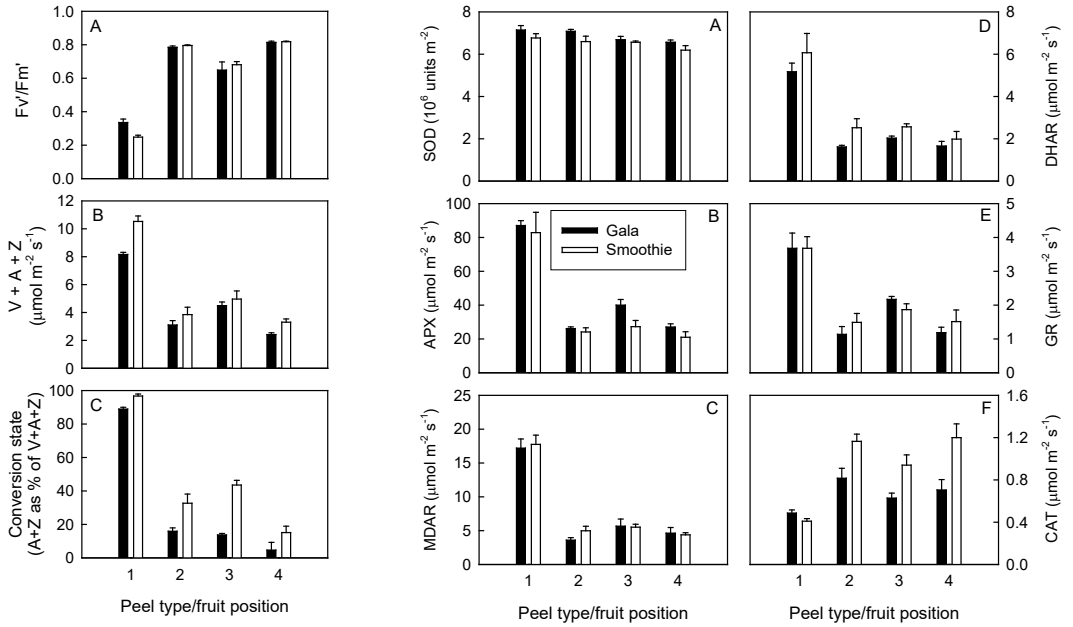


Fig. 1. Efficiency of excitation transfer, Fv'/Fm' (A), xanthophyll cycle pool size (B), and conversion state (C) in the peel of apple fruit acclimated to light exposure within the tree canopy. Peel type/fruit position: 1: the sun-exposed peel of exterior fruit; 2: the shaded peel of exterior fruit; 3: the sun-exposed side of interior fruit; 4: the shaded side of interior fruit. Solid bar represents Gala whereas empty bar is Smoothee. Each bar is the mean of 4 replicates with standard error. All the measurements were made from samples taken at noon.

Fig. 2. Superoxide dismutase, SOD (A), ascorbate peroxidase, APX (B), monodehydroascorbate reductase, MDAR (C), dehydroascorbate reductase, DHAR (D), and glutathione reductase, GR (E), and catalase, CAT (F) in the peel of apple fruit acclimated to light exposure in the tree canopy. Peel type/fruit position was the same as in Fig. 1. Solid bar represents Gala whereas empty bar is Smoothee. Each bar is the mean of 4 replicates with standard error. All the measurements were made from samples taken at noon.

2. Seasonal changes of xanthophyll cycle pool size and activities of antioxidant enzymes

The maximum quantum efficiency (*Fv/Fm*) was significantly lower on the third sampling date than the other three (Fig. 3A). This decrease reflects the photooxidative damage caused by high fruit temperature under high light.

As the season progresses, xanthophyll cycle pool size decreased (Fig 3B). In early August, xanthophyll cycle pool size decreased to about 1/3 of that at 2 months after bloom. Most of the xanthophyll cycle pool was present as violaxanthin and zeaxanthin at midday (Fig. 3C). Interestingly, even after overnight dark adaptation, violaxanthin and zeaxanthin still accounted for about 25 to 30% of the xanthophyll cycle pool size (Fig. 3C). This suggests that apple fruit retain significant amount of zeaxanthin and violaxanthin overnight so that they are ready to dissipate excess absorbed light when sunlight shines on the fruit the next day.

Both superoxide dismutase and dehydroascorbate reductase activities decreased over the sampling period (Fig. 4A, D) whereas ascorbate peroxidase activity increased (Fig. 4B). Monodehydroascorbate reductase activity increased initially, and then leveled off (Fig. 4C). Glutathione reductase activity did not show any significant change (Fig. 4E). Catalase activity remained stable except for a drop on the third sampling date (Fig. 4F). The significant decrease of catalase activity on the third sampling date is very likely caused by the high fruit temperature as catalase is very sensitive to oxidative damage.

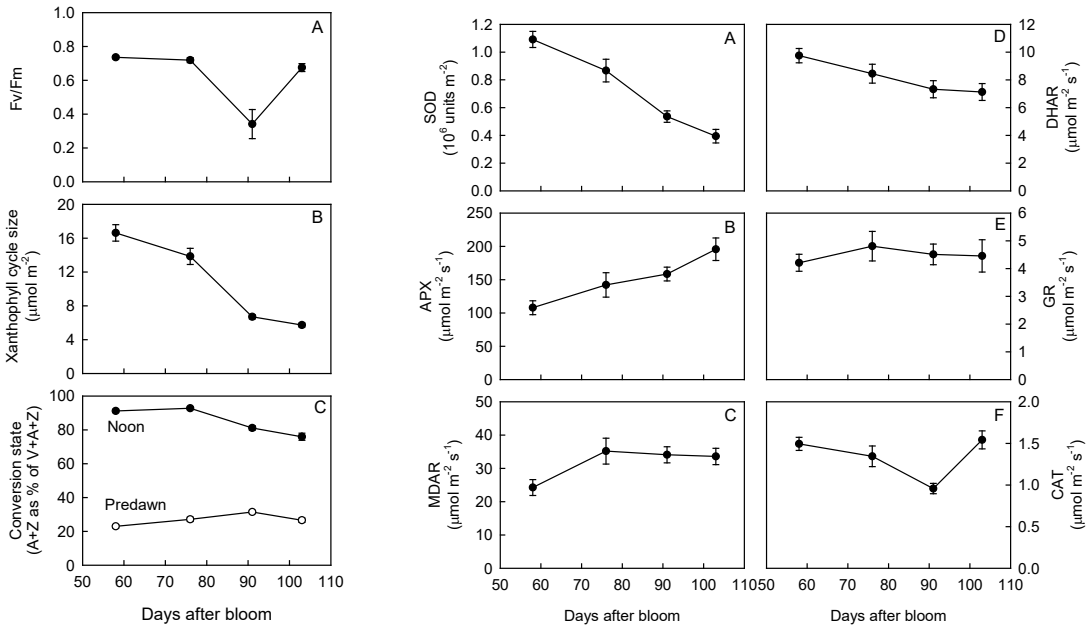


Fig. 3 (Left). Seasonal changes of maximum photosystem II efficiency, Fv/Fm (A), xanthophyll cycle pool size (B), and conversion of violaxanthin (V) to zeaxanthin (Z) and antheraxanthin (A) in the sun-exposed peel of Gala fruit in a Washington orchard. Fv/Fm was measured after overnight dark adaptation. Xanthophyll cycle pool size and conversion were measured at both midday and after overnight dark adaptation. Each point is mean with standard error of 10 fruit for Fv/Fm or 6 fruit for xanthophyll cycle pool size and conversion state.

Fig. 4 (Right). Seasonal changes of superoxide dismutase, SOD (A), ascorbate peroxidase, APX (B), monodehydroascorbate reductase, MDAR (C), dehydroascorbate reductase, DHAR (D), and glutathione reductase, GR (E), and catalase, CAT (F) in the sun-exposed peel of Gala fruit in a Washington orchard. Samples were taken at midday (1:00 to 2:30PM). Each point is mean with standard error of 6 fruit.

3. Xanthophyll cycle pool size and composition in the peel of 'Gala' fruit peel in response to nitrogen supply

At noon, efficiency of excitation transfer (F_v'/F_m') of the sun-exposed peel was higher in the low N treatment than in the medium or high N treatments (Fig. 5A). This indicates that fruit in the low N treatment has a lower thermal dissipation capacity than the medium or high N fruit. Photochemical quenching coefficient did not differ between fruits in different N treatments (Data not shown). The photosystem II operating efficiency, which represents the proportion of absorbed light used in photochemistry, was higher in the peel of low N fruit compared with medium-N or high N fruit (Fig. 5B). Maximum quantum efficiency (F_v/F_m) of fruit peel after overnight dark adaptation was similar across the N treatments (Data not shown).

On any given sampling date, xanthophyll cycle pool size was larger in the high N fruit than in the low N fruit (Fig. 5C). This corresponds well with the thermal dissipation capacity as indicated by efficiency of excitation transfer (Fig. 5A). As the season progressed, xanthophyll cycle pool size in all N treatments decreased, which is also correlated well with the seasonal change of thermal dissipation capacity of the fruit. At noon, over 95% of the xanthophyll cycle pool in the sun-exposed side was present in the form of zeaxanthin (Z) and antheraxanthin (A) regardless of N treatments (Fig. 5C). This indicates that xanthophyll cycle operates at its full capacity and the xanthophyll cycle pool size may become limiting. Chlorophyll concentration was also higher in the high N treatment than in the medium or low N treatments and decreased as fruit developed (Data not shown).

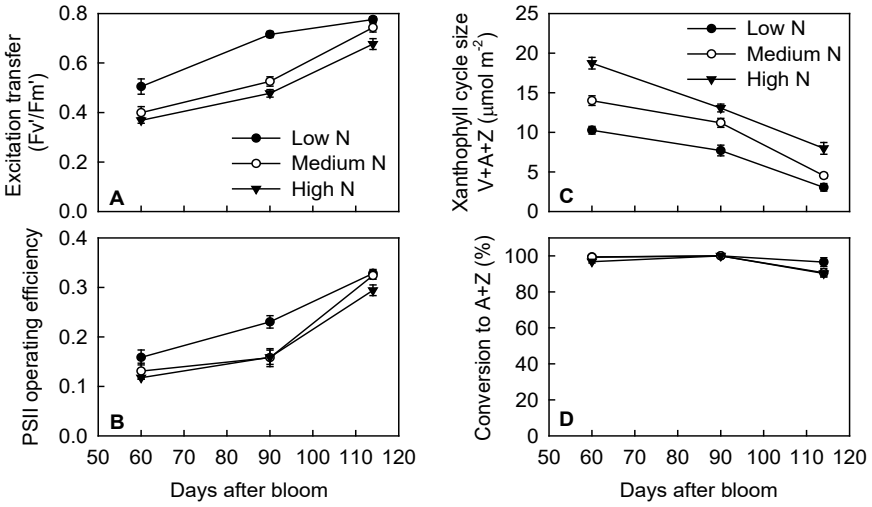


Fig. 5. Efficiency of excitation transfer, Fv'/Fm' (A), photosystem II operating efficiency (B), xanthophyll cycle pool size, Violaxanthin + Antheraxanthin + Zeaxanthin (C), and conversion of xanthophyll cycle to antheraxanthin and zeaxanthin (D) in the peel of 'Gala' fruit in response to nitrogen supply.

We have also measured antioxidant enzymes and metabolites in response to nitrogen treatments. Activities of all the enzymes in the Mehler peroxidase reaction, including superoxide dismutase and enzymes in ascorbate-glutathione cycle were higher in the high N fruit than in low N fruit (Data not shown). This indicates that high N fruit have a high capacity for detoxifying reactive oxygen species generated via direct electron transfer to oxygen under high light conditions.

4. Effects of evaporative cooling, partial root zone drying and deficit irrigation on xanthophyll cycle pool size and composition of 'Fuji' fruit peel.

Across all the treatments, both chlorophyll concentration and xanthophyll cycle pool size decreased as the season progressed (Fig.6), which is similar to what we found on 'Gala' fruit. The difference is that the decrease is less pronounced perhaps because 'Fuji' is a late season variety. Evaporative cooling tended to increase whereas both partial root-zone drying and deficit irrigation tended to decrease both chlorophyll concentration and xanthophyll cycle pool size (Fig. 6). Conversion of xanthophyll cycle to antheraxanthin and zeaxanthin was over 90% at noon with no difference between the treatments. No significant difference was found in maximum quantum efficiency (F_v/F_m) among control, evaporative cooling, partial root-zone drying and deficit irrigation treatments (Data not shown).

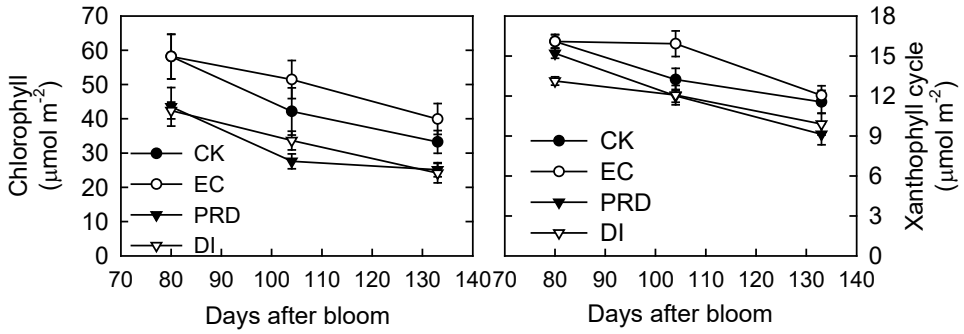


Fig. 6. Effects of evaporative cooling (EC), partial root-zone drying (PRD) and deficit irrigation (DI) on chlorophyll concentration and xanthophyll cycle pool size in the sun-exposed peel of 'Fuji' fruit.

5. Compare xanthophyll cycle pool size and composition between sunburn susceptible and sunburn tolerant apple cultivars.

Red Delicious is less susceptible to sunburn than Cameo and Golden Delicious. The xanthophyll cycle pool size was higher in Red Delicious than in Golden Delicious, but Cameo had a similar size of xanthophyll cycle pool size with Red Delicious (Fig 7C). In contrast, fruit peel lutein content was

highest in Red Delicious and lowest in Golden Delicious with Cameo in the middle (Fig 7D). Red Delicious maintained the highest maximum quantum efficiency (Fv/Fm) whereas Golden Delicious had the lowest Fv/Fm with Cameo in between throughout the growing season (Fig 7A), which appears to correlate well with the susceptibility of these three cultivars to sunburn. The lower Fv/Fm measured on July 19 was associated with a fruit peel temperature of 43°C. Chlorophyll content showed variety-specific changes as the season progressed: sharp and linear decrease in Golden Delicious, relatively constant in cameo, and moderate decrease in Red Delicious (Fig 7B).

6. UV-screening and reflective coating on xanthophyll cycle size and composition.

Throughout the growing season, coating 'Gala' fruit with Surround didn't affect the xanthophyll cycle pool size whereas screening UV light with a polycarbonate decreased xanthophyll cycle pool size.

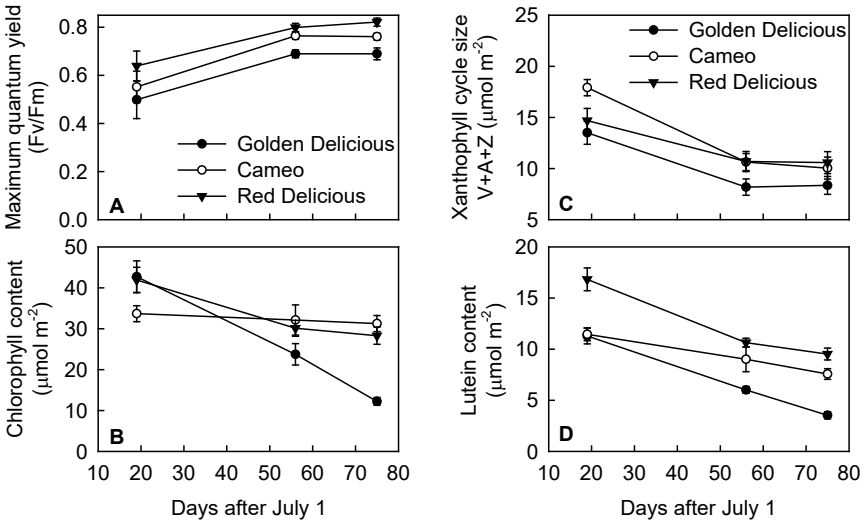


Fig 7. Seasonal changes of maximum quantum efficiency (A), chlorophyll content (B), xanthophyll cycle pool size (C), and lutein content (D) of sun-exposed fruit peel in Golden Delicious, Cameo and Red Delicious in WA.

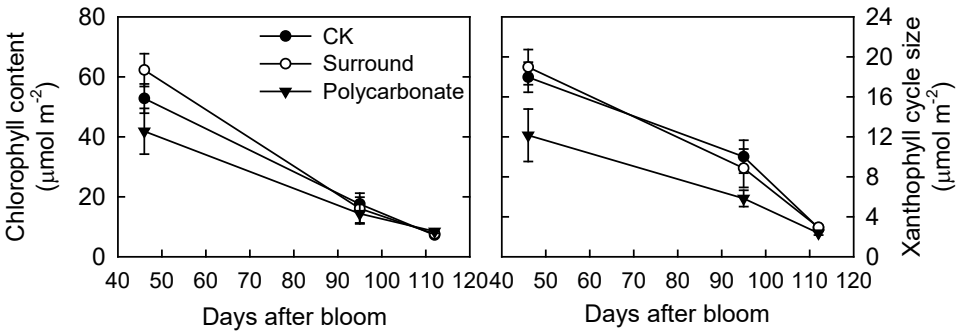


Fig 8. Seasonal changes of chlorophyll content and xanthophyll cycle pool size of sun-exposed peel of 'Gala' fruit in response to Surround treatment and UV-screening treatment via polycarbonate.

Acknowledgement: We thank Mr. Richard Raba and Mr. Leo Jedlow for technical support.

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CONTINUING PROJECT REPORT **YEAR 2/3**

Title: Water use, quality and growth as affected by irrigation and/or rootstock

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Objectives: The primary hypothesis of this research is that apple tree performance, fruit quality, and yield are affected by method of irrigation and by rootstocks. Therefore, in this comprehensive project, we have three main objectives as follows:

1. To investigate the effects of five irrigation systems, including partial root-zone drying drip and sprinkler systems on fruit quality (fruit color, soluble solids concentrations, starch index, fruit size, ethylene and respiration), tree growth and development, precocity, yield, and mineral partitioning in 'Autumn Rose Fuji'.
2. To study the influence of two irrigation systems on tree growth, yield, precocity, fruit quality, and mineral partitioning in 'Desert Rose Fuji'.
3. To study tree growth, yield, precocity, fruit quality, and nutrition of 'Pacific Gala' apple as influenced by drip and sprinkler irrigation systems, with five rootstocks, Bud 9, M.9 RN 29, Supporter 4, G 30, and Bud 118.

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 Seasons:

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.

Methods:

Irrigation Techniques for 'Autumn Rose Fuji' Apple (Objective 1):

The experimental orchard was established at the University of Idaho Pomology Orchards, at the Parma Research and Extension Center during spring and summer of 2002. 'Autumn Rose Fuji' trees on RN 29 (NIC 9) rootstocks were planted at 1.52 x 4.27 m (5 x 14 ft) spacing. The experimental design is a randomized complete block design, with five methods of drip or sprinkler irrigation systems with five blocks of 10-tree plots per irrigation treatment. Irrigation systems will be as follows:

1. **Under Ground Full-Drip:** In this system, one drip line is buried at 3-inch (7.5 cm) deep, 1 ft away from the tree row at each of the north and south sides of the tree row. Each of these lines is connected to a pressure regulator to keep the water pressure constant at 20 PSI. Emitters are located at 0.45 m (1.5 ft) spacing on each line and each emitter delivers 0.6 gal water per hour. The drip line on the north side of the tree is "off-center" with the line in the south side to have a better water coverage.
2. **Under Ground Deficit -Drip:** With the exception of the amount of water application, this system is identical to treatment 1. Trees in this treatment receive 65% of water as compared to the full drip irrigation.
3. **Under Ground Alternate-Drip (Partial Root Zone Drying Using Drip):** With exception to the frequency and amount of irrigation, this system is identical to treatment 1. At each bi-weekly irrigation cycle, trees are only irrigated by one of these drip lines and in the next bi-weekly cycle they are watered by the other line. The amount of water is same as deficit drip.
4. **Full-Sprinklers:** In this system, a 30-cm (1 ft) micro-sprinkler (Olson Ultra-jet) is connected to the lateral polyethylene line. These micro-jet sprinklers are installed mid-way between the two adjacent trees. Each replication of the full- sprinkler treatment is regulated at 15 PSI with Rain Bird regulators (low flow to provide uniform water delivery) in each row and each sprinkler head covers a diameter of approximately 4.11 m (13.97 ft). This treatment is considered as the "Control" irrigation and was watered at the rate of ET c in 2004 and 2005. ET c= ET r x Kc. Kc is crop coefficient and is determined based on canopy growth and "base Kc" (described later).
5. **Alternate-Sprinklers (Partial Root Zone Drying, Using Sprinklers):** This system is similar to the full-sprinkler except that two 30-cm (1 ft) micro-sprinklers are fastened to two lateral polyethylene lines, each located either on the south (180^{o}) or north side (180^{o})

of the tree row. At each bi-weekly irrigation cycle, trees are irrigated only with sprinklers on one side and in the next bi-weekly cycle, they will be watered by sprinklers on the other line (the number of irrigations on each side before switching to the other side of the tree row can be adjusted). At each irrigation time, trees in this treatment receive 50% of the "Full-Sprinkler" treatment (each side of the tree in both treatments receive the same amount of water at each irrigation).

Irrigation Techniques for 'Desert Rose Fuji' Apple (Objective 2):

'Desert Rose Fuji' trees on RN 29 (NIC 9) rootstock were planted at 1.52 x 4.27 m (5 x 14 ft) spacing in spring of 2002. The experimental design is a randomized complete block design, with five 25-tree blocks per treatment. Two methods of drip irrigation (two treatments) are used in this study as follows:

1. Under Ground Double-Line Drip: In this system, one drip line is buried at 3-inch (7.5 cm) deep, 1 ft away from the tree row at each of the north and south sides of the tree row.

2. Under Ground Single-Line Drip:

In this system only one drip line is buried at 3 inch deep right along the tree rows. This line is connected to a pressure regulator to keep the water pressure constant at 20 PSI. In 2003, 2004, and 2005 100% of the amount applied to trees with Under ground Double-Line Full-Drip system.

Performance of 'Gala' on Different Rootstocks and Drip and Sprinkler Irrigation Systems (Obj. 3):

'Pacific Gala' trees on five rootstocks were planted under drip and micro-jet sprinkler systems at 1.52 x 4.27 m (5 x14 ft) spacing at the University of Idaho Parma Research and Extension Center. Therefore, in this part of our comprehensive project, we are studying effects of drip and micro-jet sprinkler irrigation systems and four rootstocks on tree growth, precocity, fruit quality attributes, and other physiological measurements under conditions of southwest Idaho which are very similar to those of Yakima and some other location in Washington. The experimental design in this portion of our projects will be a randomized complete block, split plot design with two irrigation regimes (drip and sprinkler) as the main plots, and five rootstocks (Bud 9, RN 29, Support 4, Bud 118, and G 30) as sub-plots, with five blocks containing nine trees per treatment. The Sprinkler and Drip systems are similar to those described for full sprinkler and full drip treatments of Autumn Rose Fuji.

Fertigation: In 2004 , 60 $kg.ha^{-1}$, and in 2005, 89 $kg.ha^{-1}$ of N was applied to all irrigation treatments. One 3.65-m (12 ft) supporting post is placed next to each tree [24 cm (2 ft) in the ground and 3.05 m (12 ft) above the ground]. The main trunks are tied to the posts, and central leaders are trained in a central leader system. Although the flow rights are very consistent, water delivery in each irrigation station and each treatment are regularly checked to assure correct water calculation.

Water usage, Tree Growth and Development, Mineral Element Measurements, Yield, and Fruit Quality and Maturity for Objectives 1, 2, and 3:

In 2002, amounts of needed water was determined by monitoring weekly evapo-transpiration (ET) and using ETc (crop evapo-transpiration), where ETc = K_c x ET. In this calculation, the crop water use coefficient (K_c) = mature tree K_c + %M or K_c = K_c base + % M x (mature K_c – K_c base), as described by Ley (1994). %M is a measurement of canopy size. Water status of soil will be determined Aqua-Pro and TDR water sensors and a portable moisture meter. During 2002 season, K_c = 0.4 was used for all irrigation calculations. In 2003, 2004, and 2005 ET r (alfalfa-based evapo-transpiration) from Agri-Met for Parma area was used and Kc was

calculated, using a proper ground shading and canopy maturity. At the end of 2004, canopy ground shading was 50%. During 2003, 2004, and 2005, ET c was used to calculate irrigation rate for each treatment. Trees in Full sprinkler system received 100% of Etc. Full drip received 100%ET c but adjusted for the ground shading area (in drip: gal needed per tree=0.623 x ET c x 5 x 14 spacing x efficiency factor). By August 1, 2005, tree canopies were completely mature (100% maturity).

Extensive soil samples were taken before and after several irrigation periods and all physical and chemical characteristics of the soil, such as Field capacity, Bulk Density, Sand, clay, and silt content, and water penetration were determined in our lab. Regression equations were developed. Several access tubes were installed at different replications of each irrigation system and water deficit before each irrigation was determined, using the regression equations. These deficit were compared with the ET c values determined for each irrigation system at each irrigation time. Drip systems were irrigated twice a week but sprinklers were irrigated once a week.

Tree growth (growth in trunk cross sectional area), yield, time of terminal bud formation, and leaf and fruit mineral concentrations and content are measured annually. For leaf mineral analysis, thirty leaves per tree are sampled randomly from the middle of the current-season's shoot in mid-August each year. Leaves are washed in a mild Liqui-nox detergent solution, rinsed with distilled water, and dried in a forced-air oven at 65°C. Leaves are weighed before and after drying, and percent dry weight is calculated. Dried leaves are ground to pass a 40-mesh screen. Five fruits per tree are washed with a mild detergent solution and rinsed in de-ionized water. Each fruit is peeled and cut longitudinally to collect flesh and peel tissues and dried at 65ºC. Nitrogen concentration and content of leaf and fruit tissues will be determined by combusting dry tissues using a LECO Protein/Nitrogen Analyzer (Model FP-528, LECO Corp., St. Joseph, Mich.). Tissues are being analyzed for potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) by dry ashing at 500°C, digestion, and atomic absorption spectrophotometery (Perkin-Elmer B1100, Norwalk, CT, USA) as described by Chaplin and Dixon (1974) and Jones (1977). Thirty-four fruits from each tree will be picked randomly at commercial harvest time (between 17 to 20 Oct.) every year. Fruits are weighed, and placed in perforated polyethylene bags and are tested for various quality attributes at harvest.

Fruit color is rated visually on a scale of 1 = 20% pinkish-red progressively to 5=100% pinkish-red. Fruit firmness will be measured at harvest and after storage on three peeled sides of each fruit using a computerized Fruit Texture Analyzer (Guss, Strand, Western Cape, South Africa). These fruits then will be cut equatorially. One wedge from the calyx-end half of every fruit will be juiced, and the soluble solids concentration (SSC) will be measured by placing three to four drops of juice on a hand held, temperature-compensated refractometer (Atago N1, Tokyo, Japan) both at harvest and after storage. The stem-end half of the fruit at harvest will be dipped in KI solution, and the starch degradation pattern (SDP) for each fruit will be recorded by comparison with the SDP standard chart developed for apples.

We are going to continue this experiment in 2006 and will measure the same parameters that we measured in 2005 as described in this report. This study will clarify the relationship between leaf and fruit nutrient status and fruit quality of "Fuji' and 'Gala' trees under different irrigation regimes and 'Gala' trees grown on different rootstocks. Analyses of variance will be conducted by using SAS (SAS Institute, Cary, NC, USA), and means will be compared by least significant difference (LSD) at $P \leq 0.05$. Categorical data such as fruit color will be analyzed by categorical modeling, using either PROC CATMOD or PROC GENMOD in SAS.

Brief Results and Discussion:

Important findings for 2004 are presented earlier in this document and in Table 1 and 2.
2004 Season (Figures and Tables for 2004 not shown due to space limitation): In

general, partial zone sprinklers produced smaller but sweeter fruits while used more water than a full drip system. If all parameters are calculated correctly, a drip system can save water by at least 50% while producing excellent quality Fuji apples. In Gala, fruit yield was greater with drip than sprinkler systems. Rootstock significantly affected a number of quality attributes. For Gala, RN 29 seemed to perform better than other rootstocks but we need to look at the performance when trees are older.

2005 Season 'Autumn Rose Fuji' and 'Desert Rose' Fuji (Tables 1 and 2): 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 (Table 1). Fruits from trees with full drip had higher yield and sugar than those of sprinkler. Overall, fruit from trees with full drip system had excellent yield and quality while using considerably less water than those with sprinkler system. Fruits from deficit drip were significantly smaller with lower starch index as compared to those from partial drip, although the amount of water used in both systems were the same, perhaps because water in partial drip has penetrated deeper in the soil and becoming more readily available to the roots. Fruits from partial drip treatment had more starch degradation pattern and lower firmness than those of full drip, perhaps because fruits in partial drip received stress signal, leading to more advanced maturity but lower Ca. Other yield and fruit quality parameters in full drip and partial drips were similar (Table 1). Partial sprinkler had lower yield and smaller fruit than many other treatments. Fruit color and percentage of sunburn were lower in 'Desert Rose' Fuji with Double drip irrigation system as compared to single drip. Other yield and quality parameters were similar in these two irrigation systems (Table 2). In general, 2005 results are very promising and details will be presented at the Research meeting in Washington.

2005 Season Rootstock Effects on 'Pacific Gala' (Tables 3): Trees on RN 29 had higher yield and larger fruits than those on all other rootstocks in 2005 (Table 3). 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 (Table 3). Thus, fruits may have matured on Bud 9 earlier than those on other rootstocks. Trees on Bud 118 and Supporter 4 did not have satisfactory yield or fruit quality.

2005 Season Drip vs. Sprinkler Comparison for 'Pacific Gala' (Table 4): 'Gala' trees with drip irrigation had significantly larger fruit with higher starch degradation pattern and higher yield than those with sprinkler system. However fruits from drip irrigation had lower color, sugar, and firmness as compared to those in sprinkler system (Table 4). Gala trees with drip used 20.80 inches of water per acre while those with micro-sprinkler sprinkler used 35.36 inches per acre in 2005 (Table 4).

Table 1. Effects of various irrigation systems on fruit quality, yield and water consumption in 'Autumn Rose Fuji' at harvest, 2005.

	Fruit Weight (g)	Fruit Color (1-5)	Sugar	Starch Index	Firmness (kg)	Yield (kg/tree)	Sunburn (%)	ET r 2005	Etc 2005	Applied Water 2005 (")	Gal/tree 2005
Full Sprinkler	301.06 a	3.46 abp	15.47 b	3.50 bc	8.12 abc	16.52 bc	10.15 a	37.65	36.66	35.36	1541.3
Partial Sprinkler	259.64 c	3.86 a	15.95 a	3.26 b	8.24 ab	15.53 c	4.26 b	37.65	36.66	18.49	805.3
Full Drip	295.31 a	3.43 ab	15.95 a	3.60 b	8.28 a	22.06 a	3.28 b	37.65	36.66	20.80	908.3
Deficit Drip	274.94 b	3.49 ab	15.74 ab	3.56 b	8.00 c	20.71 ab	7.16 ab	37.65	36.66	13.80	602.8
Partial Drip	295.43 a	3.21 b	15.57 ab	3.94 a	8.04 bc	19.82 abc	6.22 ab	37.65	36.66	13.80	602.8

Table 2. Effects of various irrigation systems on fruit quality, yield, and water consumption in 'Desert Rose Fuji' at harvest, 2005.

Treatment	Fruit Weight (g)	Fruit Color (1-5)	Sugar (Brix)	Starch Index	Firmness (kg)	Yield (kg/tree)	Sunburn (%)	ETr 2005	Etc 2005	Applied Water 2005 (Inches)	Gal/tree 2005
Single Drip	288.91 a	4.31 a	15.89 a	3.82 a	8.35 a	15.99 a	3.17 a	37.65	36.66	20.80	908.3
								37.65	36.66		
Double Drip	281.51 a	4.01 b	16.22 a	3.85 a	8.20 a	14.83 a	1.63 b	37.65	36.66	20.80	908.3

Table 3. Effects of various rootstocks on fruit quality, yield and water consumption in 'Pacific Gala' at harvest, 2005.

	Fruit Weight (g)	Fruit Color (1-5)	Sugar	Starch Index	Firm	Yield (kg/tree)
RN 29	222.39 a	2.75 bc	13.83 b	3.71 bc	8.46 c	11.96 a
Bud 9	206.86 b	3.13 a	14.98 a	4.23 a	8.40 c	9.61 ab
GC 30	198.30 b	3.00 ab	14.17 b	3.74 b	8.66 bc	7.97 b
Supporter 4	199.84 b	2.69 bc	14.00 b	3.40 c	9.04 a	2.81 c
Bud 118	186.01 c	2.46 c	14.11 b	3.62 bc	9.00 ab	2.08 c

Table 4. Effects of various irrigation systems on fruit quality, yield, and water consumption in 'Pacific Gala' at harvest, 2005.

Treatment	Fruit Weight (g)	Fruit Color (1-5)	Sugar (Brix)	Starch Index	Firmness (kg)	Yield (kg/tree)	ETr 2005	Etc 2005	Applied Water 2005 (Inches)	Gal/tree 2005
Drip	210.74 a	2.67 b	13.95 b	3.94 a	8.36 b	8.63 a	37.65	36.66	20.80	908.3
Sprinkler	196.67 b	3.0 a	14.51 a	3.54 b	9.04 a	5.66 b	37.65	36.66	35.36	1541.3

Mean separation within columns in each Table (Table 1-4) by LSD at 5%. Fruit color: 1= green, progressively to 5=red .

Budget Information

Title:

Water Use, Fruit Quality, Tree Growth and Development, and Nutritional Physiology as Influenced by Irrigation Systems in 'Fuji' and 'Gala' Apples and by Rootstock in 'Gala'

Principal Investigator:

Dr. Esmaeil "Essie" Fallahi, Professor and Tree Fruit Physiologist

Project Duration: March 2004-December 2006

Note: This project was funded for only $15000/year in 2004 and 2005 as a 3-year project, but we are requesting $25000 for 2006 as expenses are high and we are conducting several analyses and measurements and 15000 is not sufficient. In 2006, we will evaluate fruit quality, yield and other factors as described here.

Current Year: 2006

Project Total : $55000

The following table shows how 2004 and 2005 budgets were spent:

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

CONTINUING PROJECT PROPOSAL **YEAR 2/3**
WTFRC Project # AH-04-421 **ARS Project # 1931-21220-012-10T**

Project Title: Trait modification through genetically engineered rootstocks
PI: Jay Norelli / jnorelli@afrs.ars.usda.gov / 304-724-8340 ext.2142

Organization: USDA, ARS, Appalachian Fruit Research Station
Kearneysville, WV

Cooperators: Gennaro Fazio, USDA, ARS / Cornell University, Geneva, NY
LaiLiang Cheng, Cornell University, Ithaca, NY

Contract Administrator: Ingrid Charlton / ingrid.charlton@ars.usda.gov /
(215) 233-6402

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 1: 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.

Objective 2: Determine if tissue specific expression of transgenes in the vascular system of the rootstock will result in graft-transmissible trait modification. This objective was deleted from project in 2005 due to significant funding cuts.

During the proposed three year period of the project the plants necessary for orchard trials will be genetically engineered and the ability of the transgenic rootstocks to cause trait modification in conventional scions will be evaluated using "micro-grafted" laboratory and greenhouse plants. The results of greenhouse and laboratory studies will be used to determine if orchard trials are

justified. If so, after the completion of the grant, trees will be propagated by standard commercial practices and grown under orchard conditions.

The goals for the next year (2006) are to:

- Complete genetic engineering and characterization of rootstocks and scions necessary for project.
- Produce micro-grafted plants for growth chamber and greenhouse tests.
- Evaluate trait modification through genetically engineered rootstocks in greenhouse and growth chamber tests.

Deviations from the original objectives and schedule: Due to a 75% reduction in funding in 2005 the resources available for the project were reduced. However, experiments were simplified to reduce cost but allow the primary goals of the project to be accomplished. Personnel changes were also made to reduce the cost of the project. The technician working on this project was terminated and her work to genetically engineer the apple plants required for the project was transferred to other technical staff, however only 20% of their time could be committed to the project. A part-time college student was hired to maintain and propagate the genetically engineered plants. **The continued support of the WTFRC is critical to the success of the project.**

- One of the markers (green fluorescent protein) for monitoring gene silencing in the scion was deleted from the project, the GUS marker and the native gene for sorbitol synthesis will be used.
- The number of rootstock and scion combinations to be evaluated was reduced.
- Objective 2 was deleted from the project.
- Full time technical support was reduced to 20% technical support and part-time student.

Significant accomplishments in 2005:

- 21 genetically engineered apple lines were produced for the project:
    - 5 M.26 rootstocks silenced for sorbitol production,
    - 12 M.26 rootstocks silenced for GUS (M.26-GUS),
    - 4 ‘Royal Gala’ lines engineered to produce GUS (‘Royal Gala+GUS’),
    - 1 ‘Royal Gala’ line silenced for sorbitol production.
- 131 genetically engineered apple plants were propagated for grafting.
- Grafting of ‘Royal Gala+GUS’ to M.26-GUS rootstock was initiated for greenhouse trials in 2006.

Methods:

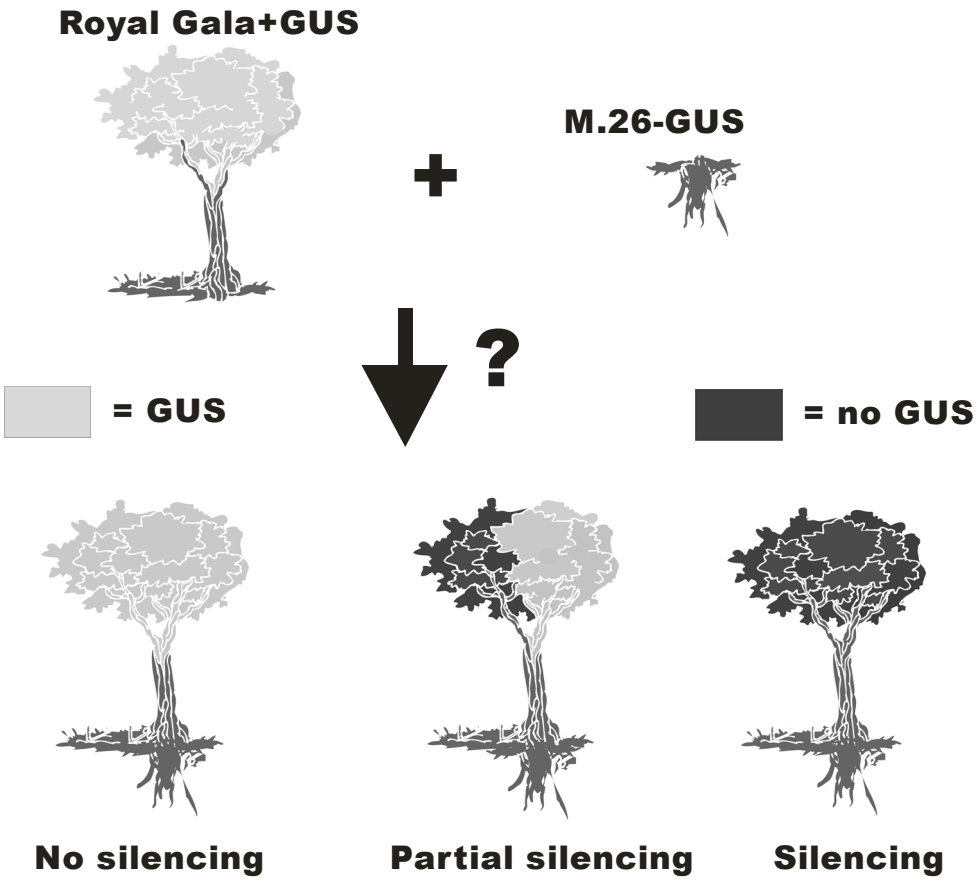
Experiments with GUS marker:

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:



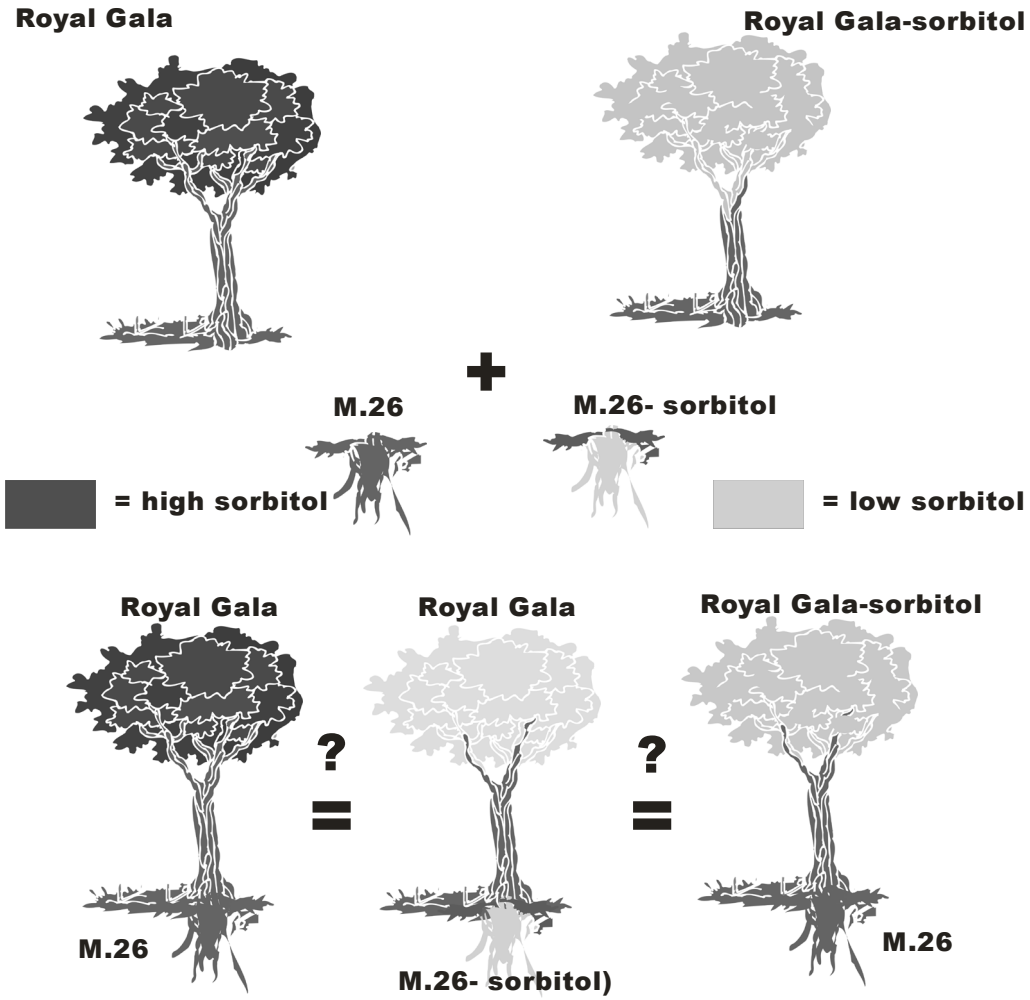
Experiments with sorbitol production:

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

Plans for 2006:

- complete work previously initiated in 2005 to genetically engineer 'Royal Gala' and M.26 to silence GUS and sorbitol production
- 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

Project Title: Trait modification through genetically engineered rootstocks
PI: Jay Norelli
Proposed project duration: 2004-2006
Current year: 2006 (year 3)
Project total (3 years): $75,388
Current year request: $22,719

Year:	**Year 1 (2004)**	**Year 2 (2005)**	**Year 3 (2006)**
Total:	**$42,669**	**$10,000**	**$22,719**

Current breakdown:

Item	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
Salaries	29,130[1]	0	5,826[2]
Benefits (30%)	8,739	0	1,748
Wages	0	6,163[3]	9,610[3]
Benefits (7.65%)	0	471	735
Equipment	0	0	0
Supplies	4,800	3,366	3,600[4]
Travel	0	0	1,200[5]
Miscellaneous	0	0	0
Total	**$42,669**	**$10,000**	**$22,719**

[1] Technician salary (GS6-1 level).
[2] 20% of technician salary (GS6-1).
[3] Wages for part-time student to propagate plants for greenhouse and growth chamber evaluations.
[4] Supplies to characterize and propagate plants is estimated at $300 per month.
[5] Four trips to transport genetically engineered plants from Kearneysville, WV to collaborator, Gennaro Fazio, in Geneva, NY and to jointly evaluate ability of genetically engineered rootstocks to cause trait modification in conventional scions.

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).

Other funding sources: There is no other current or pending external funding to support this project. However, the project is supported by in-house ARS CRIS project 1931-21220-014-00D "Management of Abiotic Stress in Fruit Crops".

CONTINUING PROJECT PROPOSAL **YEAR 1/3**
WTFRC Project #: AH-05-502 **WSU Project #:** 13C-3655-5260

Project title: Apple scion breeding
PI: Bruce H. Barritt, Horticulturist
Organization: WSU-TFREC, 1100 N. Western Avenue, Wenatchee
(509) 663-8181 ext. 233; etaplz@wsu.edu

Cooperators: Larry Pusey, USDA-ARS, Wenatchee

Contract administrators: Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

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 seedling precocity, fire blight and mildew resistance, productivity and fruit quality traits.

Significant findings:

From crosses made in 2004, 26,500 seeds were planted in the greenhouse in 2005 with healthy survivors (over 80%) transferred to the field nursery. A high proportion of the seedlings came from crosses with parents Cripps Pink, Aurora Golden Gala (BC8S-69-23), Honeycrisp, and an unnamed WSU selection (a Splendour X Coop 15 seedling). Over 4,000 seedlings were screened for fire blight resistance with the surviving seedlings (approximately 35%) transferred to the nursery. The proportion of seedlings with resistance to fire blight was greater for parents Enterprise and Aurora Golden Gala than with Honeycrisp and Arlet.

In 2005, 29,187 new hybrid seeds were produced from 28 crosses. Of these, 21,978 have been stratified for planting in the greenhouse in 2006. Cripps Pink was the most prominent parent (in 12 crosses) followed by Braeburn (9 crosses). Sundance and Enterprise, both resistant to fire blight, were each used in 7 crosses.

In evaluation orchards planted in 2002, 2003 and 2004 at TFREC, more than 17,000 seedling/M.9 trees were evaluated for fruit quality and tree health. Over 400 selections were made in the field (good eating quality and appearance) and their fruit was placed in cold storage. When removed from storage, over half of these selections did not retain their texture or firmness and were

discarded. The remainder will be evaluated again in a second year (2006) before being elevated to second test status.

Replicated second test trials of promising selections were established at three sites in 2004 and 2005, and trees have been propagated for a third planting in 2006. Approximately 45 promising selections were planted in a warm, long-season site, a cool, short-season site and an intermediate site. The trees were too young in 2005 to obtain meaningful data.

Based on fruit quality evaluations in the 2004 season, 12 promising selections were identified, and in 2005 each was budded onto M.9 in the nursery to produce trees for second test trials in 2007.

In an experiment to reduce the time and expense of propagating trees on M.9 in the nursery, older seedling/M.9 trees in the orchard were topworked in 2003 to new seedlings. None of the topworked trees fruited in 2005.

An assessment of important tree and fruit traits is underway to determine which will be suitable candidates for marker-assisted selection.

Methods:

1. Using classical fruit breeding methods, annually hybridize parents with desirable traits and produce hybrid seedling populations of over 20,000 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 that are maintained for four years, select approximately 1%, termed "selections," based on fruit quality objectives. Only seedlings that have fruit with acceptable appearance (size, shape, color) 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.

Results and discussion:

Apple breeding is a long-term activity made so by a long juvenile period and long propagation times. The current project has promising selections in trials at multiple sites in Washington. Conclusive data from these trials are two to three 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.

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.

Budget:

Project title: Apple scion breeding
PI: Bruce H. Barritt
Project duration: 2005-2007
Current year: 2006
Project total (3 years): $305,862
Current year request: $102,045

Year	2005	**2006**	2007
Total	$98,056	**$102,045**	$105,761

Item	2005	**2006**	2007
Salary[1]	$42,766	**$ 44,138**	$ 45,904
Benefits (34%)	14,540	**15,007**	15,607
Supplies[2]	34,500	**36,400**	37,500
Travel[3]	6,250	**6,500**	6,750
Total	$98,056	**$102,045**	$105,761

[1] Agricultural Research Technologist (Bonnie Konishi).
[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.

CONTINUING PROJECT REPORT **YEAR 1/3**
WTFRC Project Number: AH-05-508

Project Title: Employing Biological Elements of Orchard Ecosystems
PI: Mark Mazzola
Organization: USDA-ARS Tree Fruit Research Laboratory
Contract Administrator: Chuck Myers, USDA, ARS, PWA
800 Buchanan St. Albany, CA 94710
510-559-6019, EMAIL: cwmyers@PW.USDA.GOV

Brassicaceous seed meal amendments control certain elements of the biological complex that incites apple replant disease (Mazzola et al., 2001). In recent studies (Mazzola & Mullinix, 2005), on sites lacking significant lesion nematode populations, pre-plant *Brassica napus* seed meal amendment used in conjunction with a post-plant mefenoxam (RidomilGold EC) soil drench provided disease control and growth and yield responses which were equivalent to pre-plant soil fumigation. Preliminary studies with alternative brassicaceous seed meals have suggested that disease control can be effectively improved upon and may circumvent the need for the post-plant Ridomil drench. Such an outcome would allow for the implementation of such a strategy in organic production systems.

The overall objective of this program is to develop an integrated management strategy compatible to conventional and organic apple production systems that provides the shortest time frame to first commercial harvest on sites previously planted to apple. As similar biological entities appear to have a role in replant problems encountered in pear, peach and cherry (Mazzola, unpublished data; Browne et al., 2002), it is plausible that such a system would have utility across tree fruit production systems.

Specific objectives:
1.) Examine the capacity of Brassicaceae seed meals to suppress the biological complex inciting replant disease and enhance tree growth in replant orchard soils.

2.) Determine the mechanism(s) by which these soil amendments provide control of the various plant parasites and pathogens that incite replant disease development, with emphasis on *Rhizoctonia solani*.

3.) Assess the influence of rootstock genotype on composition of resident *Streptomyces* populations and the efficacy of RSM-induced disease suppression

Goals and Activities 2006:

- Evaluation of growth, yield and disease development will be conducted at the Gala/M26 block established at the CV orchard in May 2005, which seeks to determine the efficacy of different brassicaceous seed meal amendments relative to pre-plant soil fumigation, for the control of replant diseases.

- Studies will be conducted to determine the basis for the differential response of rootstocks to seed meals that were employed in 2005 experiments. Trials conducted in 2005 will be replicated in 2006 to assess whether the response can be induced consistently, and to determine whether disease control functions through direct pathogen suppression or induction of plant resistance.

- ❖ Isolates of Streptomyces recovered from the apple rhizosphere were screened for the abilty to produce Nitric Oxide (NO). This population varied widely in its ability to produce NO *in vitro*. As NO is known to stimulate host defense responses in other plant systems, experiments will be conducted to determine whether NO production is the means by which these bacteria induce plant resistance to certain fungal pathogens, including *R. solani.*

- ❖ Studies will determine whether enhanced plant growth observed at the CV orchard in response to mefenoxam treatment of *Brassica juncea* treated is consistent across orchard soils.

- ❖ Based on findings described below, seed meal mixtures will be evaluated as a means to obtain suppression of "problematic" components of the pathogen/parasite complex (e.g. *Pythium* & *Phytophthora*).

Significant Findings 2005:

Field trial: Evaluation of brassicaceous seed meal for soilborne disease control:

A field trial was established at the Columbia View Research and Demonstration Orchard, and was initiated with tree removal (Red Delicious/Seedling) from the site in October, 2004.

- ➢ Irrespective of seed meal type, pre-plant amendment in conjunction with a post-plant mefenoxam soil drench was as effective or more effective than pre-plant soil fumigation in promoting tree growth and suppressing disease development.

- ➢ Seed meal from the mustard *Sinapis alba* cv. IdaGold when used alone provided **initial** growth that was equivalent to pre-plant fumigation.

- ➢ Although no *Pythium* spp. were recovered from trees grown in *B. juncea* treated soils, mefenoxam soil drench unexpectedly provided a dramatic increase in tree growth.

- ➢ Molecular analysis of the fungal community recovered from roots of trees established in *B. juncea* treated soils indicated that these roots were heavily infested by *Phytophthora cambivora* and *Phytophthora megasperma.*

Greenhouse Trial: Impact of rootstock on seed meal efficacy for soilborne Disease Control

- ➢ For a majority of the rootstocks, *S. alba* cv IdaGold provided the greatest improvement in plant growth.

- ➢ For certain rootstocks, including G11 and G30, the bagging of *B. juncea* amended soils prior to planting, to simulate tarping in the field, had a deleterious impact on tree growth.

- ➢ Seed meal amendment was superior to soil pasteurization in the capacity to enhance tree growth.

- ➢ *Brassica juncea* seed meal was superior to other seed meals for the suppression of lesion nematode populations.

- ➢ A complete assessment of the impact of seed meal treatments on disease suppression is currently in progress.

Methods, Results and Discussion:

Update 2002 Field Trial: Initial field trials that employed *Brassica napus* seed meal, were established at the Columbia View (CV) Orchard and the Wenatchee Valley College-Auvil Orchard in 2002. Seed meal amendment in conjunction with a post-plant soil drench with the fungicide mefenoxam (RidomilGold) was as effective as pre-plant fumigation with Telone-C17 for the control of apple replant disease at the CV orchard. Through 2005, yields from the seed meal/mefenoxam treatment continued to yield at a level **equivalent** to that obtained from fumigated plots (Table 1). The site at the WVC-Auvil orchard was removed due to activities at Pangborn Field. However, the seed meal/mefenoxam treatment **did not** perform as well as pre-plant fumigation, and a biological assessment determined that this resulted from a re-infestation of treated soils and tree roots by the lesion nematode, *Pratylenchus penetrans*.

Significance to industry: This study indicates that the use of *B. napus* seed meal amendment in conjunction with post-plant mefenoxam drench may be an effective alternative to pre-plant soil fumigation **on sites lacking significant lesion nematode populations**. As seed meal amendment satisfied the N needs of trees at this site for the period of 2002-2004, based on leaf analysis, this may be an economically superior measure to pre-plant fumigation in conventional production systems where lesion nematode **does not** contribute to disease development.

Table 1. Cumulative (2003-05) fruit yields (kg tree^{-1}) from Gala/M26 apple established at the CV orchard, Orondo, WA in May 2002

Treatment	Yield
Control	13.0a
1,3-dichloropropene-chloropicrin	22.6b
RSM/mefenoxam[y]	23.6b

2005 Field Trial

A field trial was established at the Columbia View Research and Demonstration Orchard, and was initiated with tree removal (Red Delicious/Seedling) from the site in October, 2004. In addition to *B. napus* (rape seed), seed meal of *S. alba* cv IdaGold (yellow mustard) and *B. juncea* cv Pacific Gold (oriental mustard) were employed with or without a post-plant mefenoxam (Ridomil) soil drench. These seed meals were chosen based upon our data, and data from the literature, suggesting the relative activity of these materials toward the pathogens and parasites causing replant disease. In a preliminary greenhouse trial, *B. juncea* was superior to other seed meals for control of lesion nematode (Table 2).

Table 2. Impact of seed meal on soil and gala seedling root lesion nematode populations

Treatment[z]	*Pratylenchus*/g soil	*Pratylenchus*/g root
Control	217b	370c
DE	19a	79ab
Athena	5a	111b
IG	7a	105b
PG	1a	2a

[z]DE and Athena=*Brassica. napus*; IG=*Sinapis alba*; PG=*Brassica. juncea.*

B. juncea also seemed a suitable choice for organic systems as in greenhouse trials it did not stimulate *Pythium* populations resident to orchard soils (Cohen and Mazzola, 2006). In the field, *B. napus* and *S. alba* amendments increased *Pythium* populations by greater than an order of magnitude (Table 3), and caused significant infection of Gala/M26 roots. *Pythium ultimum* and *P. heterothallicum* were the primary species recovered from apple roots. All seed meal

amendments, but not pre-plant Telone-C17 fumigation, provided significant control of *Rhizoctonia* root infection (Table 3).

Table 3. Impact of soil treatments on soil populations and Gala/M26 root infection by fungal pathogens.

Treatment	*Pythium spp.* propagules per g soil	*Rhizoctonia* root infection (%)
Control	265b	14.0a
Telone C17	65a	11.7ab
Brassica juncea	75a	5.0b
Brassica napus	5320c	6.6b
Sinapis alba	4515c	2.8b

[z]Values followed by the same letter are not significantly different according to the Tukey test (P=0.05)

With the exceptions of *B. juncea* seed meal without post-plant mefenoxam and mefenoxam alone, all soil treatments resulted in **initial year** tree growth that was superior to the non-treated control (Table 4). All seed meal amendment treatments with mefenoxam resulted in tree growth that was as good, or ***significantly better***, than that attained in response to soil fumigation. *Sinapis alba* seed meal alone resulted in tree growth that was equivalent to Telone-C17. The lack of a significant increase in growth of trees established in *B. juncea* "alone" treated soils and the tremendous growth stimulation achieved in these same soils treated with mefenoxam were unexpected. No *Pythium* spp. were isolated from roots of trees grown in *B. juncea* amended soils. However, molecular analysis of the fungal community colonizing Gala/M26 roots revealed that all trees established in *B. juncea* alone treated soils were heavily infested with *Phytophthora cambivora* and *Phytophthora megasperma*. While *Pythium* dominated the oomycete root population in control and *B. napus* treated soils, *Phytophthora* dominated the population from tree roots in *B. juncea* treated soils based upon DNA sequence analysis of amplicons obtained by PCR using oomycete specific primers.

Table 4. Impact of pre-plant seed meal amendment and post-plant mefenoxam soil drench on first-year increase in trunk diameter (mm) of Gala/M26 apple established at the CV orchard, Orondo, WA in May 2005

Pre-plant soil treatment	No post-plant treatment	Post-plant mefenoxam
Control (no treatment)	1.32d[z]	2.04cd
Telone-C17	2.75b	-
Brassica juncea	1.96cd	4.75a
Brassica napus	2.34c	3.29b
Sinapis alba	2.71bc	4.49a

[z]Values followed by the same letter are not significantly different according to the Tukey test (P=0.05)

Significance to industry: While the initial finding from the *B. juncea* alone treatment was unexpected and disappointing, these data provide further information for developing effective and **consistent** control of replant disease in organic systems without the use of soil fumigation. This trial utilized the *Phytophthora*-susceptible root stock, M26. The fact that we determined **why** disease control failed in the absence of a mefenoxam post-plant soil drench will now enable us to effectively modify the system, for example through the use of a *Phytophthora* resistant rootstock in subsequent trials or modification of the irrigation protocol. For conventional production systems, these **initial** findings are very promising as growth promotion and disease control to date using *B. juncea* or *S. alba* seed meal in conjunction with mefenoxam was superior to pre-plant Telone-C17 fumigation. A significant motive for evaluating additional seed meals was the incomplete nematode control achieved using *B. napus* seed meal. Thus, trials **must** now be conducted on orchard replant sites possessing significant lesion nematode numbers and monitored through to the fruit production phase.

Rootstock Trials:

Trials were conducted to determine whether rootstocks respond differentially to brassicaceous seed meal amendment of orchard replant soils. Studies were conducted in the greenhouse using soils from the WVC-airport block, E. Wenatchee, and the GC orchard, Manson. At both sites, lesion nematode contributes significantly to disease development. The study utilized the following rootstocks: seedling, Bud9, G11, G16, G30, M7, M9 Pajam 2, M9 Nic 29, M26, MM106, and MM111. A replication of this study was planted at the CV orchard in July 2005 and will be harvested in 2006.

Seed meals of *Brassica napus* cv Dwarf Essex, *Brassica juncea* cv Pacific Gold, and *Sinapis alba* cv. IdaGold were used in these trials. Seed meal was added to soil at a rate of 0.3% (vol/vol), and for *B. juncea*, soils a "bagging"treatment was included to simulate tarping in the field or were left unbagged. Rootstocks were planted in April and harvested in December.

Analysis of disease control (suppression of plant parasitic nematodes and fungal pathogens) attained in the initial study is continuing to date. However, growth data (increase in tree diameter) suggest that seed meal amendments provided effective disease control. Rootstock growth performance in seed meal amended soils was consistently and significantly better than that attained in non-treated soil, and in many instances at least one seed meal outperformed the pasteurization soil treatment (Tables 5a and 5b); due to space consideration, only a representative portion of the data are reported). Among seed meals, growth was similar in GC soil, but across rootstocks the *S. alba* seed meal amendment resulted in significantly better (P<0.001) tree growth in WVC soil. Complete interpretation of these studies awaits collation of root and shoot biomass data. In certain instances (G11), rootstock growth performance consistently was numerically better in *B. napus* amended soils. The impact of "bagging" *B. juncea* amended soils was rootstock-dependent, and ranged from growth suppression to growth enhancement. In one instance, "bagging' enhanced growth of Seedling rootstock in *B. juncea* amended soil, while the growth of G30 was consistently and significantly suppressed by this treatment. Determination of whether this resulted from an impact on soil biology or was a phytotoxic response resulting from the activity of glucosinolate hydrolysis products will require further analysis.

Table 5a. Impact of seed meal amendments on diameter growth increment (mm) of apple rootstocks in GC orchard replant soils.

Treatment	M7	Seedling	M26	MM111	Nic29	G11	G16	G30	Bud9
Control	2.24	2.94	1.62	2.11	1.16	1.72	0.96	2.13	1.40
Pasteurization	2.46	4.53	2.71	3.73	2.45	2.47	2.64	2.40	2.12
B. napus	3.02	3.54	2.77	2.87	2.56	2.84	2.91	2.47	2.67
S. alba	2.94	3.19	3.49	3.12	2.49	2.63	2.79	3.60	2.02
B. juncea	2.81	3.40	3.15	2.88	2.51	2.68	2.65	3.46	2.27
B. juncea-bag	3.04	4.66	2.90	2.49	2.57	2.57	2.47	2.17	2.34
LSD (p=0.05)	0.52	0.87	0.74	0.63	0.80	0.61	0.79	0.74	0.66

Significance to industry:

Our studies have demonstrated that the efficacy of *B. napus* seed meal in the control of apple replant disease functions, in part, through elements of the microbial community resident to orchard soils (Cohen & Mazzola, 2005, 2006). As plant genotype significantly alters this community, the rootstock employed is apt to modulate the efficacy of this treatment. Therefore, matching the appropriate rootstock with the most suitable seed meal will allow for optimization of disease control. These greenhouse studies support this ascertion; whereas *S. alba* consistently was superior to other seed meal amendments in promoting growth of M26 rootstock in orchard soils, the *B. napus* and the bagged *B. juncea* treatment provided the greatest growth increment in

G11 and seedling rootstock, respectively. Again, direct implication of soil biology in this response awaits further analysis.

Table 5b. Impact of seed meal amendment on diameter growth increment (mm) of apple rootstocks in WVC orchard soils.

Treatment	M7	Seedling	M26	MM111	Nic29	G11	G16	G30	Bud9
Control	1.81	2.50	1.19	1.62	1.36	1.17	1.24	1.44	1.37
Pasteurization	2.37	3.27	2.66	2.83	2.35	2.12	2.53	2.64	1.39
B. napus	2.56	3.08	2.63	3.73	2.09	2.56	1.89	2.69	2.20
S. alba	2.81	3.61	3.58	4.22	2.49	2.48	2.62	3.20	2.83
B. juncea	2.45	3.62	2.84	3.52	2.05	2.16	2.45	2.49	2.52
B. juncea-bag	2.80	3.90	2.73	3.00	1.77	1.76	2.75	1.58	1.88
LSD (p=0.05)	0.52	0.68	0.77	0.76	0.48	0.54	0.57	0.71	0.81

Literature Citations:

Browne, G.T. et al. 2002. Pages 24.1-24.4, in Proceedings; International Research Conference on Methyl Bromide Alternatives. MBAO, Fresno, CA.

Cohen M. F., and Mazzola, M. 2006. Applied Soil Ecology. 31:(in press).

Cohen, M. F., and Mazzola, M. 2005. Soil Biology & Biochemistry 37:1215-1227.

Mazzola et al. 2001. Phytopathology 91: 673-679.

Mazzola, M. and Mullinix, K. 2005. Plant Dis. 89:1207-1213.

BUDGET:

PI: Mark Mazzola; **Project Duration**: 2005-2007; **Current Year**: 2006; **Project Total (3 years)**: $166,640; **Current Year Request**: $55, 522

Other Funding Sources: USDA-CSREES Grants Program 2006: $101,341 (direct & indirect costs)

Funds are requested for partial support (40%) of a postdoctoral research associate and a part-time laboratory research assistant. A graduate student funded entirely through a USDA grant will contribute to these studies, examining the efficacy and functional mechanism(s) of brassicaceae seed meals in controlling individual elements of the pathogen complex inciting replant disease. An additional graduate student, funded in part through the USDA grant is studying the ability of these seed meals to control weeds in orchard production systems, both in a pre-plant and post-plant setting.

Project duration: 2005-2007

Item	**2005**	**2006**	**2007**
[1]Salaries	22,800	23,940	25,137
Benefits (30%)	6,840	7,182	7,541
[2]Wages	12,000	12,000	12,000
Benefits (30%)	3,600	3,600	3,600
Equipment	0	0	0
[3]Supplies	8,000	8,000	8,000
[4]Travel	800	800	800
Miscellaneous	0	0	0
Total Proposed	$54,040	$55,522	$57,078

[1]Postdoctoral Research Associate, 0.4 FTE; 0.6 FTE of this position is funded through other external funds and will contribute to this program; [2]Research Assistant, 0.5 FTE; [3] Enzymes, media, plasticware, chemicals, gases, greenhouse supplies, rootstocks; [4]Travel to field sites to collect soil, manage field trials, collect growth and yield data.

CONTINUING PROJECT REPORT **YEAR 1/3**
WTFRC project#: AH- 05-509 **Organization Project #:** 3361-4542

Project Title: Synthetic and bio-nematicides for plant parasitic nematodes

PI: Dr. Ekaterini Riga, Nematologist
Organization: Washington State University, IAREC, 24106 N. Bunn Rd., Prosser, WA, 99350
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CO-PI(s): Dr. Mark Mazzola, Plant Pathologist, 104 North Western Ave., Wenatchee, WA (509) 664-2280 ext:245, mazzola@tfrl.ars.usda.gov;

Dr. Tom Forge, Nematologist, Agriculture & Agri-Food Canada
Aggasiz, BC, V0M 1A0, (604)796-2221, forgeta@agr.gc.ca

Cooperator(s): 1) R. Fuller, Apple Grower, Stormy Mountain Ranch, Chelan, WA; **2)** D. Anyan, G. S. Long Co., INC, Yakima, WA. **3)** B. Hiromoto, Technology Officer, ABR LLC, Puunene, Hawaii. **4)** C. Ishida, Field R&D Scientist, Valent Biosciences Co.; **5)** Mr. Dale Gies, Precision Seeds, Moses Lake, WA.

Contract Administrator: Stephanie Brock, WSU-IAREC, Prosser
sabrock@wsu.edu, Tel: (509)786-9224

ML. Bricker, mdesros@wsu.edu, 509-335-7667

Objectives: The objective of this proposal is to study the effect of the following novel nematicides, **(DiTera, SLS Enhanced Nematicide/Liquid Compost factor** and **NatureCur**) on plant parasitic nematodes, especially on lesion nematodes under both conventional and organic apple orchards. DiTera is registered for Certified Organic Apples, while the registration of the other bio-nematicides is pending. Presently, Organic Apple Growers do not have effective bio-nematicides to control plant parasitic nematodes while Conventional Growers have limited choice of synthetic nematicides. In addition, we will evaluate the potential of the above nematicides to enhancing beneficial free-living nematodes in the soil. The first field season has been completed and we are requesting funds for the 2nd field season. This is a 3 year project and in the end of the project we will provide apple growers with new tools to control plant parasitic nematodes. The schedule of activities for 2006 will be the same as 2005. So far, we have obtained data that shows trends i.e. there is a reduction in plant parasitic nematodes but the reduction is not significantly different than the controls. However, for 2006 we expect to obtain statistically significant data – in comparison to the untreated control trees. In addition to the above bio-nematicides, we are planning to incorporate a mustard meal (defatted meal pellets from *Brassica carinata*) in one of the two apple orchards to evaluate plant parasitic nematode response to this novel mustard meal.

Significant findings (data in Appendix I):
Rene Garcia Farm – NatureCur Treatment – 7 months old project:

- NatureCur did not have any negative effect to beneficial free-living nematodes (Fig.1). There is an increase of free-living nematodes 7 months post-application of NatureCur in comparison to the untreated controls but the increase is not significantly different.
- There is a significant increase of lesion nematodes in the soil around the roots 7 months post-application of NatureCur in comparison to the controls (Fig. 2). However, there is a significant decrease of the lesion nematode inside the roots of the treated trees (Fig. 3). Therefore, the roots are protected against the lesion nematodes.
- There is no significant difference in the trunk diameter between the controls and the treated trees after 7 months post-application of NatureCur. Similarly there is no

significant difference in fruit weight. However, trees treated with NatureCur produced heavier apples with an average weight of 0.36 lbs per apple while the untreated trees produced apples with an average weight of 0.34 lbs per apple (data not shown).

Ray Fuller Farm – DiTera and SLS+LCF Treatment - 17 months old project:

- There is a decrease in the lesion nematode - in the soil - in all DiTera treatments in all rootstocks, 17 months post-treatment (Fig, 4, 5, 6). However, the decrease in not significantly different in comparison to the untreated controls. No, lesion nematode root data has been collected as the seedlings are too young to collect root samples.
- There is a decrease in the lesion nematode - in the soil - in SLS+LCF in M-26 rootstock, 17 months post-treatment but not in Bud-9 and G-16 rootstock (Fig, 4, 5, 6).
- There is an increase of free-living nematodes 17 months post-application of Ditera and SLS+LCF on M-26 rootstock in comparison to the untreated controls. There is no reduction of free-living nematodes in Bud-9 and G-16 rootstocks (Fig. 7, 8, 9).
- There is no significant difference in the truck diameter of the treated trees in comparison to the controls (Fig 10). The trees are too young to produce fruit.

Methods: We have completed 17 months and 7 months, respectively, of a 3 year field project on the efficacy of novel nematicides on an organic apple orchard (R. Fuller, Stormy Mountain Ranch, Chelan) and a conventional apple orchards (R. Garcia, Naches). Field trials are used to determine rates and efficacy of the above nematicides on the lesion nematode, and on beneficial free-living. A randomized block design consisted of five trees per treatment and DiTera and SLS+LCF were replicated 3 times i.e. 30 trees per treatment and 15 trees per control. In addition, in Fuller's farm, three different rootstocks were used, so 135 trees were used in total. Apple trees in Fuller's farm were 1 year old, therefore, only truck diameter and nematode data was collected. In Garcia's farm, NatureCur was applied using a randomized block design. The treatment was replicated 4 times i.e. 5 trees per treatment and 5 trees per control. The apple trees in Garcia's farm were 6 years old, so fruit yield, trunk diameter and nematode data was collected. Nematode data was collected in the spring prior to applications, mid-season and at harvest. Both plant parasitic and free-living nematodes were extracted from the soil using standard Nematology elutriator extraction techniques. In addition, where possible, feeder roots were collected and nematodes were extracted from the roots as the lesion nematode spends part of its life cycle inside the roots. Nematodes were enumerated using a dissecting microscope and expressed as nematode #s per 250 cc soil and nematode #s per gram root. **In May 2006, *Brassica carinata* meal will be banded prior to root flash at 1 T/acre, in Rene Garcia's farm – new addition to the project.**

Application rates of nematicides

Treatment	**Rates**
LCF + SLS/CA[1]	2 quart/ acre at 1:400 dilution (LCF) and 1 quart / acre at 1% solution (SLS)
DiTera®[2]	15 pounds / acre
NatureCur[3]	5000 ppm
Brassica carinata meal[4]	1T/acre

[1]SLS/CA Enhanced Nematicide / LCF will be applied early spring (when soil temperature at 45^0 - 50^0F) and then monthly till October – watered in via micro-sprinklers.

[2] DiTera® ES will be applied early Spring (when soil temperature at 45^0 - 50^0F) and then monthly till October - watered in via micro-sprinklers.

[3]NetureCur will be applied early Spring (when soil temperature at 45^0 - 50^0F) and then

monthly till October – watered in via micro-sprinklers.
[4]*Brassica carinata* meal will be applied, banded, early Spring (prior to root flash) – and watered in via micro-sprinklers.

Results and Discussion:
All data is shown in Appendix (below).
NatureCur significantly decreased lesion nematodes inside the roots of the treated apple trees in comparison to the controls – 7 months post-treatment. DiTera also reduced lesion nematodes in the soil in comparison to the controls for all rootstocks – 17 months post-treatment. SLS+LCF reduced lesion nematodes only in M-26 rootstock. So far, both NatureCur and DiTera have provided us with promising results in terms of lesion nematode control and apple root protection. In addition, NatureCur, DiTera and SLS+LCF did not have any negative effect towards beneficial free-living nematodes.
It will take at least 2 more years for the trees to significantly respond to the treatments and to obtain lesion nematode control.

Project title: Post-Plant Management of Lesion Nematodes in Apple Orchards in WA
PI: Ekaterini Riga
Project duration: 2005-2008
Current year: 2006
Project total (3 years): $45,000
Current year request: $15,000

Item	Year 1 (2005)	**Year 2 (2006)**	Year 3(2007)
Salaries			
Benefits (11%)			
Wages [1]	10,811	**10,811**	10,811
Benefits (%)	1,189	**1,189**	1,189
Equipment			
Supplies	1,500	**1,500**	1,500
Travel [2]	1,500	**1,500**	1,500
Miscellaneous			
Total	15,000	**15,000**	15,000

[1]Wages are for time slip help to collect soil samples and assist with nematicide application. Time slip benefits are at 11% arte. Supplies include pipette tips, gloves for precise nematicide applications. [2]Travel expenses include gas, mileage, meals and overnight accommodation to travel to the farms.

Additional support will be sought from CSANR, WSU, for 12,000. All nematicides and mustard meal are donated by the manufacturers. This information is provided to the commission for informational purposes only and does not constitute a cost-share obligation on the part of WUS. Moreover, there is no requirement for WSU to document this information as part of any cost-share or matching obligation.

Appendix

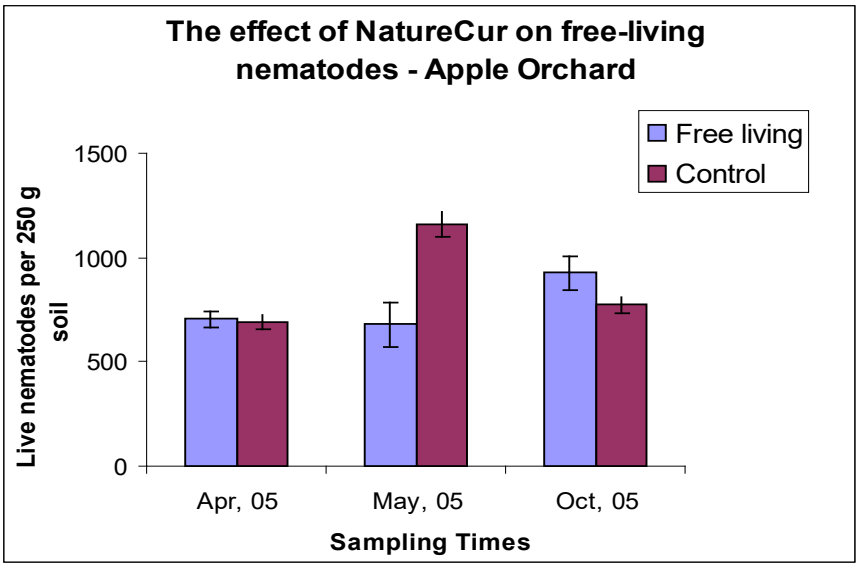


Figure 1.

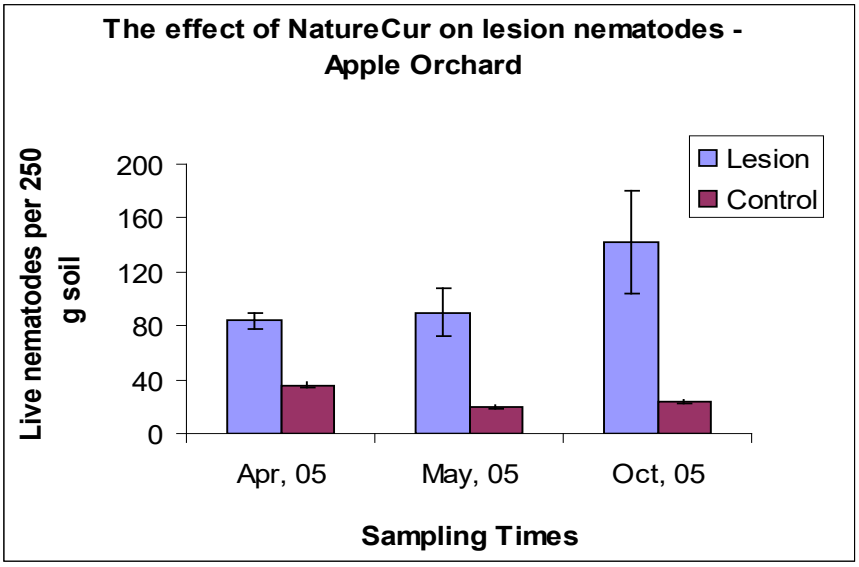


Figure 2.

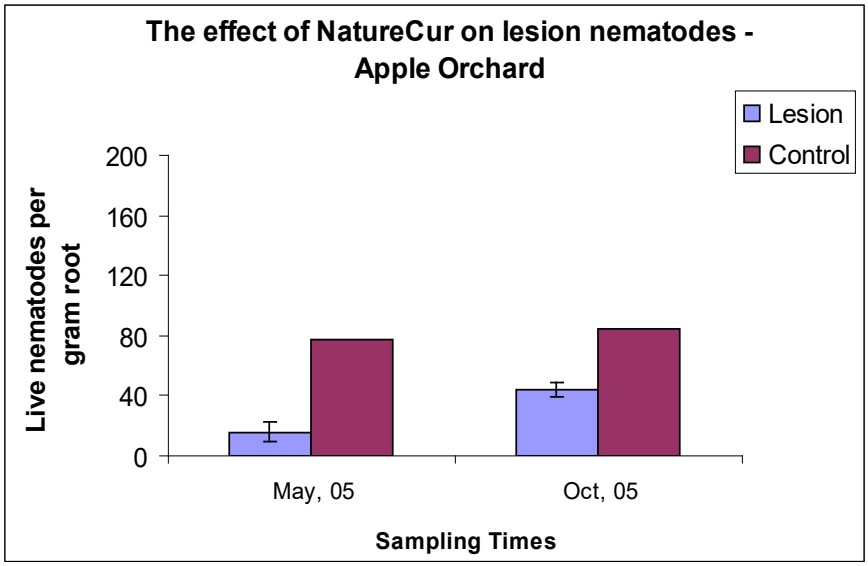


Figure 3.

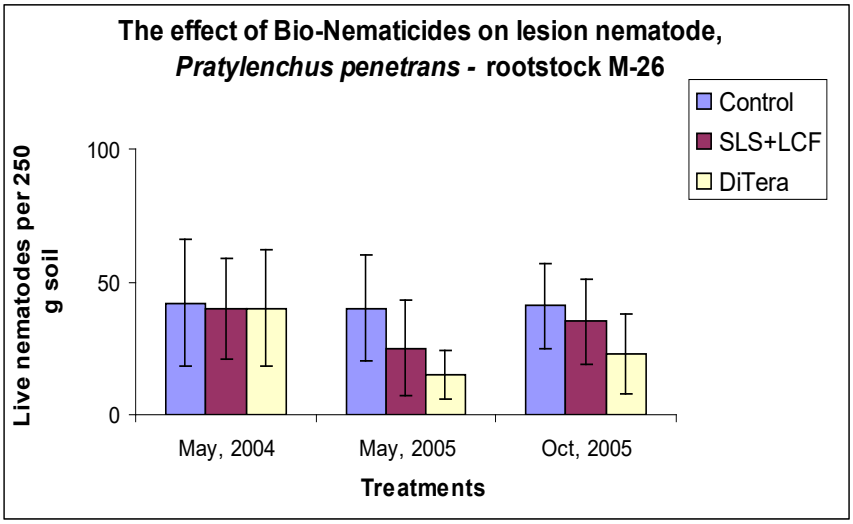
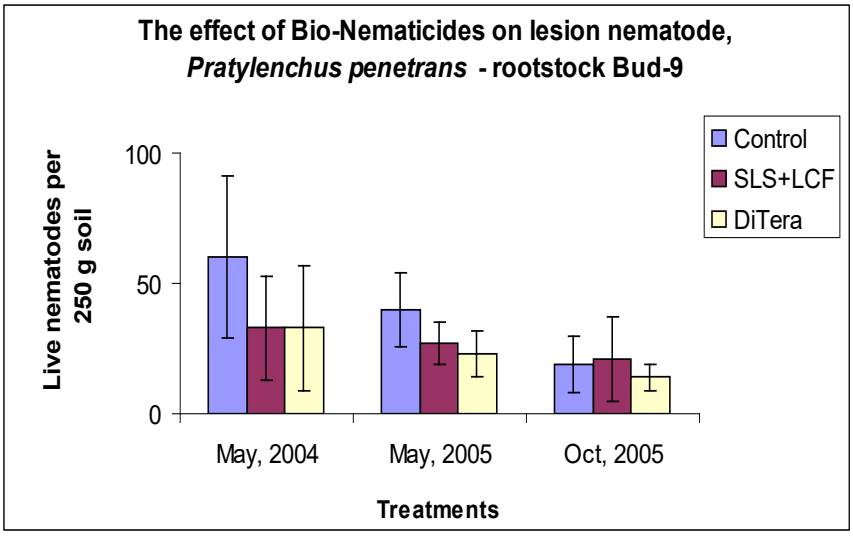


Figure 4.



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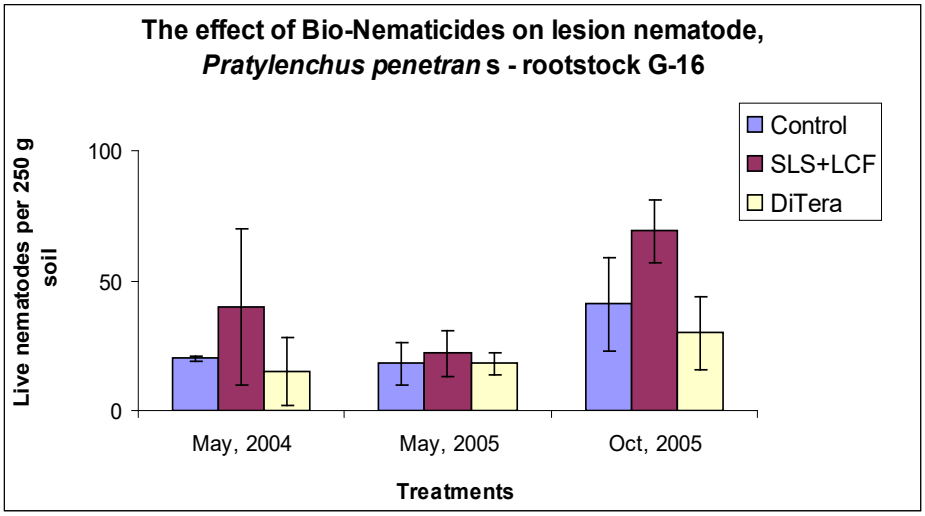

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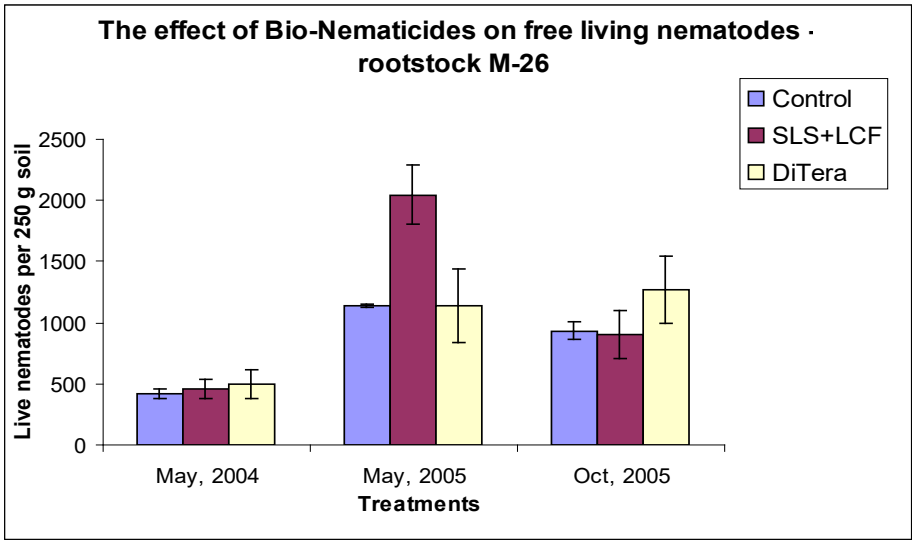

Figure 7.

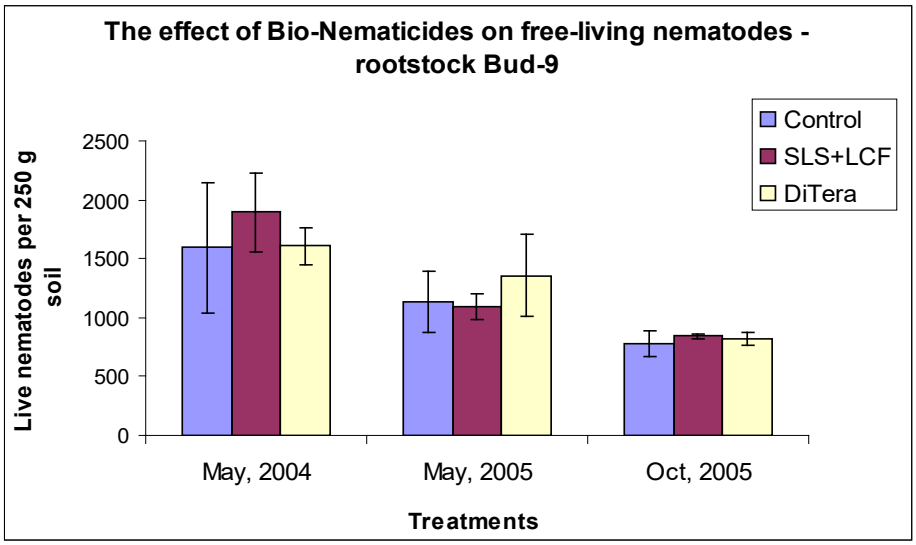

Figure 8.

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project # AH-04-419

Project title: Monitoring apple fruit growth for predicting chemical thinning response
PI: Duane W Greene
Organization: University of Massachusetts
CoPI and affiliation:
Alan N. Lakso, Cornell University, Geneva (anl2@cornell.edu)
Terence L. Robinson, Cornell University, Geneva (tlr1@cornell.edu)
Contact administrator: **Duane W. Greene, dgreene@pssci.umass.edu**
Phone (413) 545-5219

Objectives:

1. Confirm that fruit growth is an accurate and early predictor of final fruit set. Our goal is to be able to predict thinner response accurately within 7 to 8 days of thinner application.
2. Continue to incorporate a heating degree component in the predictions system and confirm its usefulness in predicting thinning. This past year we included heating degree units for the first time and found that it appeared to eliminate much of the year to year variability that we experienced in the past.
3. Refine and continue to develop a procedure for selecting fruiting spurs that will assure that spurs selected and measured will represent the response that occurs on a whole tree. This past year we did several studies that helped us define the number and location of spurs to provide an accurate prediction. Prediction of abscission of measured fruit was extremely accurate but extrapolation of these data to the whole tree was less accurate. A second years data to supplement the results this year will allow us to know how many spurs must be selected and measured.
4. Construct an Excel–based spreadsheet template that will simplify data input and automate many of the required calculations.
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 procedures necessary for accurate thinner response prediction.
6. Utilize the Phytech® fruit growth sensors to accurately and continuously monitor and record fruit growth information following thinner application. This activity will allow determination of the most precise information and earliest possible time that response to a thinner can be detected.

Significant findings:

- We continue to confirm that following fruit growth is an extremely accurate way to predict response to a chemical thinner application and weather.
- Weather following thinner application, especially temperature, influences the not only the extent of the response but also the time required to see a response. This past year we found that incorporating heating degree calculations will tell us when we can make an accurate prediction.
- The predictive system allows not only a prediction of a response but also provides a framework and guidelines for setting goals for achieving an ideal final fruit set. The system, as it will finally appear, will make thinning much more predictable, precise and science based than has been hitherto possible.
- The predictive system will be simple enough so that workers can be trained in a minimum amount of time to collect and process the appropriate data.

Methods Employed:

Fruit Growth Studies

Mature Ace spur Delicious/M.26 and Braeburn/M.26 trees were selected. Two limbs per trees were tagged and all blossom clusters counted. When fruit developed to the 7 to 9 mm stage, 20 spurs on 10 Braeburn trees and 15 spurs on 6 Delicious trees were tagged. Just prior to thinner application all fruit on the tagged spurs were marked and the diameters measured with a digital caliper. Marked fruit were then measured on the same location on the fruit at 2 to 3 day intervals for 16 days. Final set was taken at the end of June drop in July.

In a block of 3-year-old slender spindle trees on M.9 rootstock Braeburn, Pacific Rose Fuji and Buckeye Gala trees were selected. At the pink stage of flower development all blossom clusters were counted on each tree. The tree was divided into four equal sections from top to bottom and then 5 spurs were randomly tagged in each segment for a total of 20 spurs per tree. All fruit in each spur were marked and measured just before thinner application when fruit size averaged 8 mm. Previously all trees received a petal fall application of Sevin XLR at 1 pint/100 gal. Braeburn and Fuji received a dilute spray of 50 ppm MaxCel while Buckeye Gala received 100 ppm MaxCel. Final set was measured at the end of June Drop.

In another block, a thinning treatment of 7.5 ppm NAA + 1 pint/100 gal Sevin was applied to mature Delicious and Gala/M9 trees when king fruit diameters were 8-10 mm. Just prior to application 25 spurs per tree were marked and diameter of all the fruit were measured. Fruit were again measured 2, 4, 6, 8, 10, 13 and 19 days after application. Final set was assessed after June drop.

Spur Selection Studies

Six 6-year-old Honeycrisp/M.9 were selected. Three lower scaffold limbs, three upper scaffold limbs and the central leader were separately tagged and all blossom clusters counted at the pink stage of flower development. At the same time every other spur on the whole tree was tagged with a small tag. At the end of June drop in July fruit set was taken on all tagged limbs. In addition, set was determined on all spurs on the whole tree where fruit were identified as either being on a tagged or an untagged spur.

Desert Rose Fuji and Braeburn trees were selected in a block of 3-year-old slender spindle trees on M.9 rootstock. Trees were divided equally with tape into 4 sections: bottom, mid 25-50%, upper 50-75%, and the top of a tree. Bloom was counted and recorded on all tree segments and then every other flowering spur was tagged with a small tag. At the end of June drop all fruit in each tree segment were counted and fruit set on individual spurs was taken. Fruit were identified as being located on a tagged or untagged spur.

Heating Degree Day Studies

All studies involving predictive fruit set in the past 2 years and in 2005 were reviewed and weather data for the 12 days following application of thinners in each year was retrieved. From the temperature data the heating degree days were calculated for each thinning study in each year. The time after thinner application when an accurate prediction of final fruit set was also noted.

Results and Discussion

Fruit Growth and Prediction of Thinning

The goal in this project is to be able to predict thinner response within about 7 days of application. When NAA at 10 ppm + 0.5 lb/100 gal carbaryl was applied to Braeburn prediction of final set at 7 days after application was 16% when the final set ultimately was 14% (Figure 1). This is excellent agreement between predicted and final set. Ace spur Delicious received a petal

fall spray of 1 lb/100 gal carbaryl followed by a spray of 50 ppm MaxCel (TRV dilute equivalent) at the 8-9 mm stage of fruit development. Eight days after application prediction of final set based upon fruit growth rates was 16% whereas the final set at the end of June drop was 13% (Figure 2). Both of these predictions on mature trees show remarkable agreement between predicted and actual final set.

Three thinning experiments were done on slender spindle trees; Braeburn, Autumn Rose Fuji, and Buckeye Gala. All three varieties received a 1 pint/100 gal spray of Sevin XLR at petal fall. Braeburn and Autumn Rose Fuji received 50 ppm MaxCel at the 7-9 mm stage whereas Buckeye Gala received 100 ppm MaxCel. Prediction of final set at 8 days after thinner application was Braeburn 9% predicted, 4% actual (Figure 3); Autumn Rose Fuji 18% predicted, 16% actual (Figure 4); and for Buckeye Gala 4% predicted and 3% actual (Figure 5). Unexpected hot weather followed appplicaiton on Braeburn and Gala resulting in overthinning on these trees. These too represent very good predictions of final set within 8 days of application.

In NY, there was a very hot period (mid-90's/mid-60's day/night) immediately following the thinner application. This caused a very rapid response in the decline in fruit growth rate and a rapid drop in predicted final set to a stable value at 4 days (expected with such warm conditions) that was maintained at 6 days. In this case, however, an unusual response occurred with a later decline again. It appears that there were two effects occurring; first, the response to thinners by most fruit (dropping with diameters of 8-13 mm), then a later response to the heat (dropping with diameters of 18-22 mm). We feel that the prediction of the thinner effect was accurate by 4 days, but the final set was not predicted until 10 day after treatment when the heat-affected fruit stopped growing. In any case, this points out the value of monitoring fruit growth as it was not possible to distinguish this double effect by looking at final drop only.

Spur Selection Studies

There generally is excellent agreement between fruit growth on spurs and predicted set. There is less agreement between set on the whole tree and fruit growth. Three different studies were done in an attempt to determine how many spurs must be measured and to determine their location to have good prediction of set. Thinning on Gala was excessive so these data are not presented. Data in Table 1 shows the number of spurs sampled and the resulting prediction based upon the actual number of fruit on the tree. In this table 100% is perfect and represents what is actually on the tree. Percent figures above 100% are over prediction and values under 100 are under estimation. While there is some deviation around 100% it appears that if as low as 1 in 22 spurs on Honeycrisp is measured, that represents and excellent prediction. On these trees this would generally mean measuring 12 to 15 spurs per tree on 5 trees. On Desert Rose Fuji, a super spindle, a higher percent should be measured (1 out of 18) to give reliable results. However, these trees are smaller and this presents perhaps closer to 7 to 10 spurs per tree on 5 trees. Location of spurs in a tree influences final set. Percent set on the lower limbs of Honeycrisp was 32%, 42% on the upper scaffold limbs and 47% the central leader (Table not shown). Similarly, the percent fruit set on the bottom 25% of the slender spindle Fuji trees was 43%, 69% on the lower 25-50% of the tree, 84% on the upper 50-75%, and 110% on the central leader (Table not shown). The fact that fruit set is lower on the lower limbs in not surprising but it does illustrate the need to distribute spurs to sample on a tree that is representative of the bloom distribution of the tree. This was our first attempt to fine tune spur selection. The results were good, and they made good pomological sense . However, there was no clear break point that gives a definitive number that is required for a reliable result. A study such as this should be repeated to confirm these data and to establish definitively the number of spurs one needs to measure to make a reliable prediction.

Heating Degree Day Studies

The 2003 and 2005 seasons were characterized as having good and appropriate weather following thinner application for good thinner response. Consequently, prediction of thinner response was possible within 7 to 8 days of application. The 2004 season was cooler and thinners did not respond until 9 to 11 days after application. Heating degree days were calculated for the experiments done in the last three years to see if we could find a specific number of degree day units that should be accumulated before an accurate prediction can be made. We felt that it was necessary to be able to separate lack of thinner response from a delay in thinner response due to temperature. In all thinning trials done in 2003 and 2005 where accurate predictions were made, there was an accumulation of at least 130 or more heating degree days by 7 to 8 days after application table 2. In 2004 it required 11 to 12 days to accumulate 130 heating degree days, and this is approximately the same amount of time required to make an accurate thinning prediction. This must be repeated again and checked out again in other locations to confirm a definitive number of heating degree units a tree must be exposed to make an accurate prediction.

FIG. 1 BRAEBURN- NAA + CARBARYL

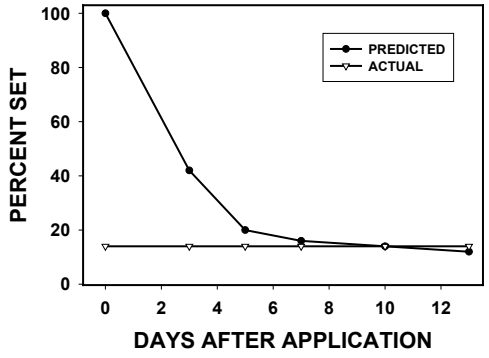

FIG. 2 DELICIOUS- MAXCEL + CARBARYL

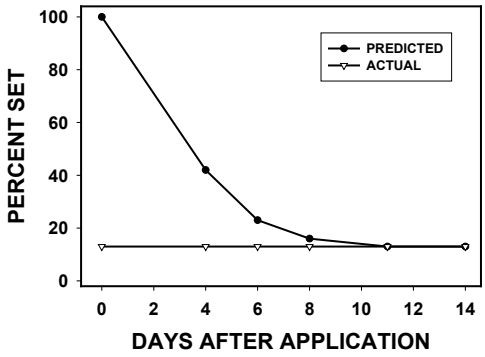

FIG. 3 BRAEBURN- CARBARYL, MAXCEL

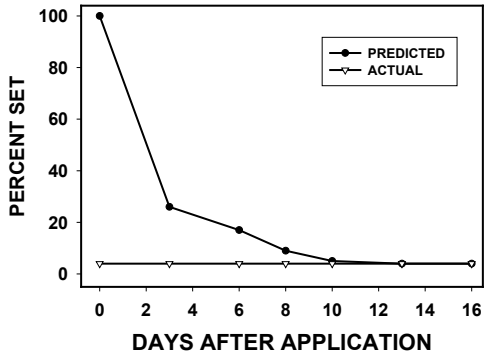

FIG. 4 FUJI- CARBARYL, MAXCEL

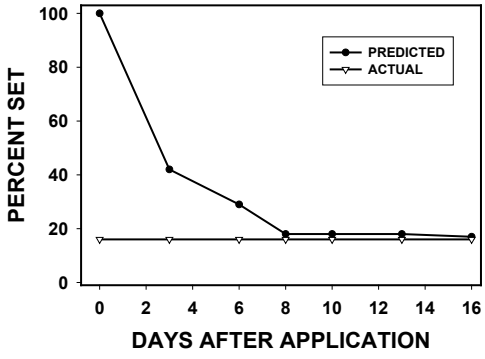

FIG. 5 GALA- CARBARYL, MAXCEL

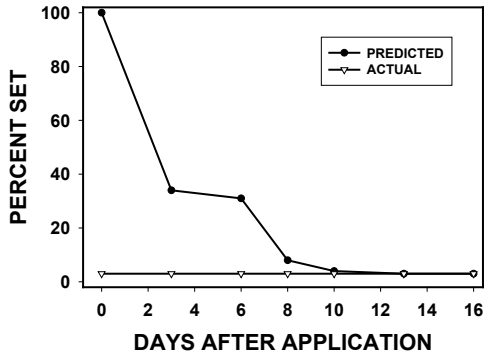

FIG. 6 GALA NY 2005- NAA + CARBARYL

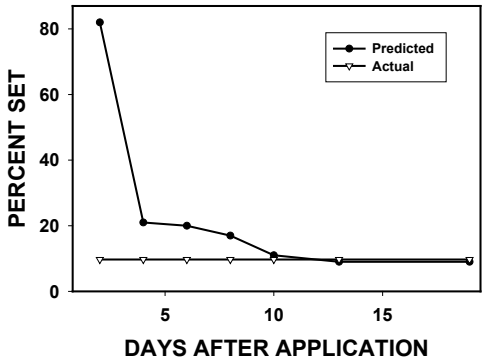

FIG. 7 GALA INFLUENCE OF THINNER & HEAT

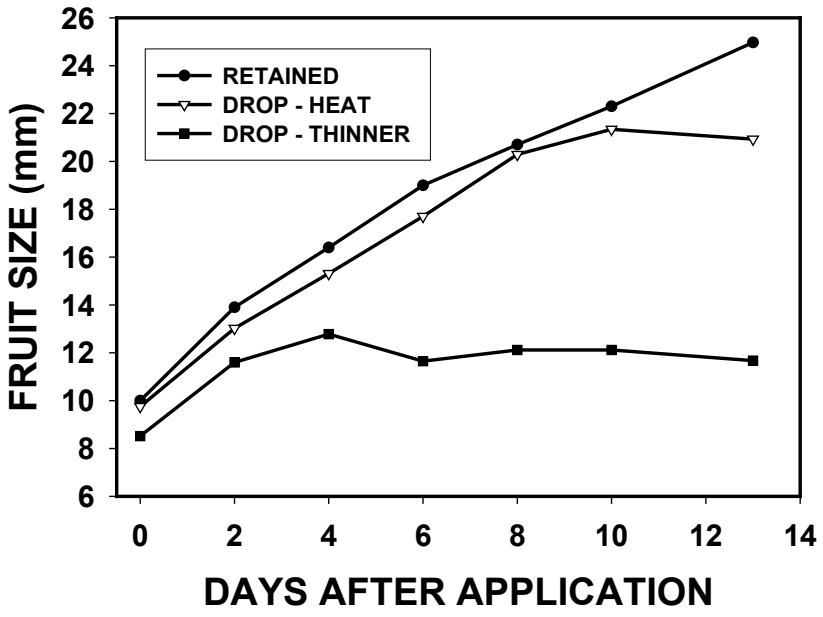

Figure 7. Examples of growth pattern following treatment with NAA/Carbaryl. Some fruits continued to grow and were retained (labeled Retained). Most fruits responded quickly and dropped when about 12-14 mm diameter (labeled Drop –Thinner). However, some fruits continued to grow after the thinner was applied, but later after about 5 days of heat, stopped growing and eventually dropped (labeled Drop – Heat).

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 sampled	Honeycrisp (%)	Desert Rose Fuji (%)
All spurs	100	100
1of 2	111	100
1 of 4	107	107
1 of 6	102	97
1 of 8	109	95
1 of 10	112	101
1 of 12	94	130
1 of 14	119	85
1 of 16	121	91
1 of 18	91	93
1 of 20	103	134
1 of 22	96	
1 of 24	68	138
1 of 30	131	

Table 2. Growing degree days (base 50) following application of thinners in 2003, 2004, and 2005. Thinning and prediction of thinner response could be accurately made in 2003 and 2005 at 7-8 days after application, whereas it required 10-11 days for a similarly accurate prediction in 2004.							
Days after application	2003		2004		2005		
	Delicious	Golden Delicious	Golden Delicious	Delicious	Braeburn	Delicious	Braeburn
	McIntosh			McIntosh	Fuji		
1	14.3	22.8	9.2	10.3	7.9	10	12.8
2	31.6	38.6	19.5	25.4	18.4	22.8	34
3	46.4	56	30.3	29	28.4	44	58.6
4	60.1	74.2	45.2	40.5	41.2	68.6	76.1
5	82.9	97.9	55.9	47.8	87	86.1	100.1
6	98.7	119.6	70.3	57	104.5	110.6	126.1
7	116.1	145.7	84.8	67.3	129	136.1	151.4
8	134.3	161.7	84.8	78.1	154.5	161.4	174.3
9	158	181.8	98.7	93	179.8	184.3	199.6
10	179.7	207.3	110.2	103.7	202.7	209.6	223.4
11	205.8	224.3	120.4	118.1	228	233.4	250.6
12	221.3	240.9	132.9	132.6	251.8	260.6	275.7

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

PI: Duane W. Greene

Project duration-3 years (2004-2006)

First year- 2004

Project total (3 years): $53,319

Current request: $23,319

Item	2004	2004	Total 2004	2005	2005	Total 2005	2006	2006	Total 2006
	Mass.	Cornell		Mass.	Cornell		Mass.	Cornell	
Wages[1]	6,600	6,500	13,100	6,700	0	6,700	7,250	7,750	15,000
Benefits[2]	2,310	2,668	4,978	2,345	0	2,345	2,538	3,181	5,719
Crop loss	600		600	600	0	600	600		600
Supplies[3]	490	832	1,322	355	0	355	500	1,100	1,600
Spreadsheet					0		400		400
Total	10,000	10,000	20,000	10,000	0	10,000			23,319

[1]Labor is for the maintenance of the experimental orchard, the flower counts, the applications of treatments, and manual monitoring of fruit development and fruit drop, and the setup, monitoring, logging and summarizing growth measurements and the electronic sensor information.
[2]Fringe benefit rate is 41.05% for Cornell and 35% for Massachusetts.
[3]Supplies and general expenses required for minimal supplies and expenses related to orchard maintenance, and general supplies for conducting experiments.

CONTINUING PROJECT REPORT **YEAR 1/2**

Project Title: Mechanisms of apple fruit growth under Washington conditions

PI: Peter M. Hirst

Organization: Purdue University

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OBJECTIVES AS STATED IN PROPOSAL

Our objective was to establish a baseline for the growth of Red Delicious and Gala fruit growing in Washington. High and moderate crop loads were established on trees of each cultivar. We aimed to describe fruit growth in terms of:

- Cell size
- Cell number
- The proportion of cells actively dividing

Using the above data we intended to calculate cell production rates for each cultivar/crop load combination.

SIGNIFICANT FINDINGS

- No effect of crop load on fruit diameter was found.
- Both varieties had a similar proportion of cells dividing up to 30 DAFB but cells in Red Delicious fruit continued dividing over a longer period than did those in Gala fruit.
- Crop load did not affect the proportion of cells dividing.

METHODS

Mature trees of Red Delicious and Gala will be selected in Washington by WTFRC staff. At full bloom, 10 trees of each cultivar will be hand thinned to a low-moderate crop load so that the genetic and environmental potential for fruit size is expressed. Sampling will be carried out according to the following schedule (Table 1)

On each sample date, 2 flowers/fruit per tree with pedicels attached will be sampled. This represents 20 fruit per cultivar and 40 fruit total (on each sampling date). Each group of 2 fruit from a tree should be tagged or placed in a small bag with cultivar and rep # on it.
Some samples will be used for flow cytometry analyses (to examine the proportion of cells actively dividing) and others will be used for the calculation of cell number and cell size using digital images captured of flower/fruit transverse sections. Samples will be shipped overnight

from Washington to Indiana for analysis the following day. Generally, we will follow the procedures developed during our previous work, which have proved to be both efficient and successful.

This study will enable us to provide a detailed description of fruit growth of Gala and Red Delicious fruit grown in Washington. Specifically we will describe:

- The rate of cell division
- The proportion of cells actively dividing
- The duration of cell division
- Cell size
- The cell production rate

Due to inherent year-to-year variation, we propose the study be conducted in each of 2 years to enable us to make conclusions with more certainty.

RESULTS AND DISCUSSION

Samples were collected from an orchard in Washington during the 2005 season according to the following schedule (Table 1).

Table 1. Schedule of flower/fruit sample collection during the 2005 season.

#	DAFB	Date	Gala	Red Del
1	4	5-May		
2	8	9-May		
3	10	11-May		
4	15	16-May		
5	18	19-May		
6	22	23-May		
7	25	26-May		
8	30	31-May		
9	36	6-Jun		
10	43	13-Jun		
11	50	20-Jun		
12	57	27-Jun		
13	72	12-Jul		
14	85	25-Jul		
15	99	8-Aug		
16	113	22-Aug		
17	128	6-Sep		
18	141	19-Sep		
19	155	3-Oct		

Sample collection began soon after bloom and continued until harvest. On each sampling date, fruit were shipped immediately from Washington to Indiana where fruit diameter was measured followed by preparation of samples for flow cytometry and dissection.

Fruit size (as estimated by fruit diameter) showed little difference due to crop load treatment. With Gala there was a slight tendency for one treatment to have larger fruit (Fig. 1) but the trend was reversed with Red Delicious (Fig. 2).

Figure 1. Fruit size of Gala apples growing in Washington in response to crop load treatments during the 2005 growing season.

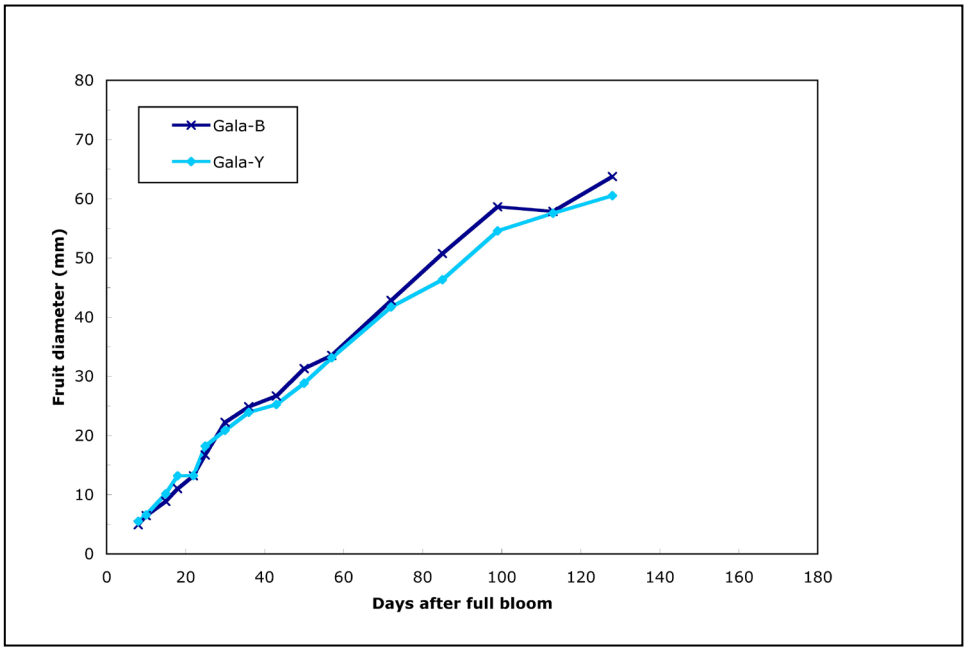
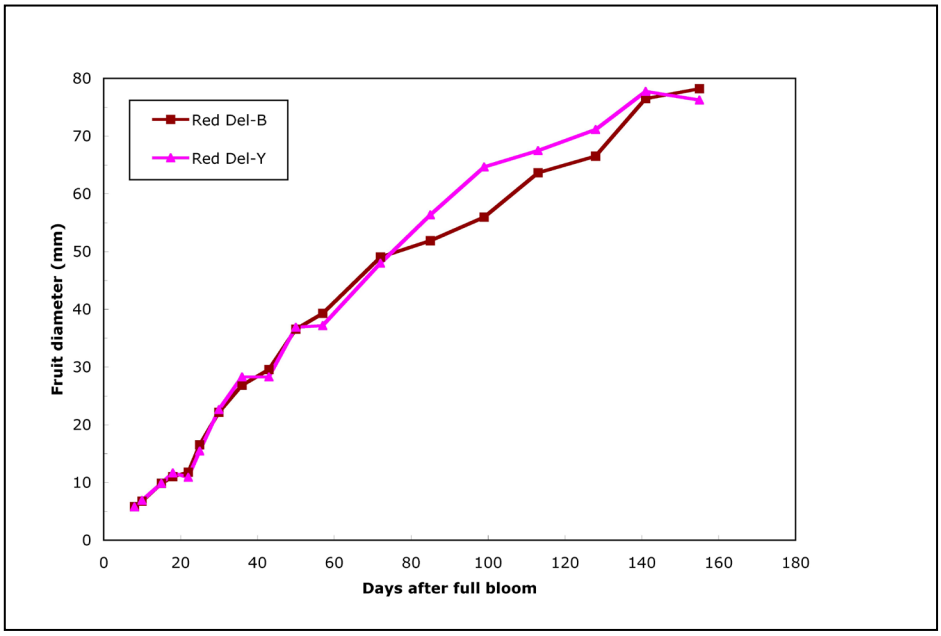
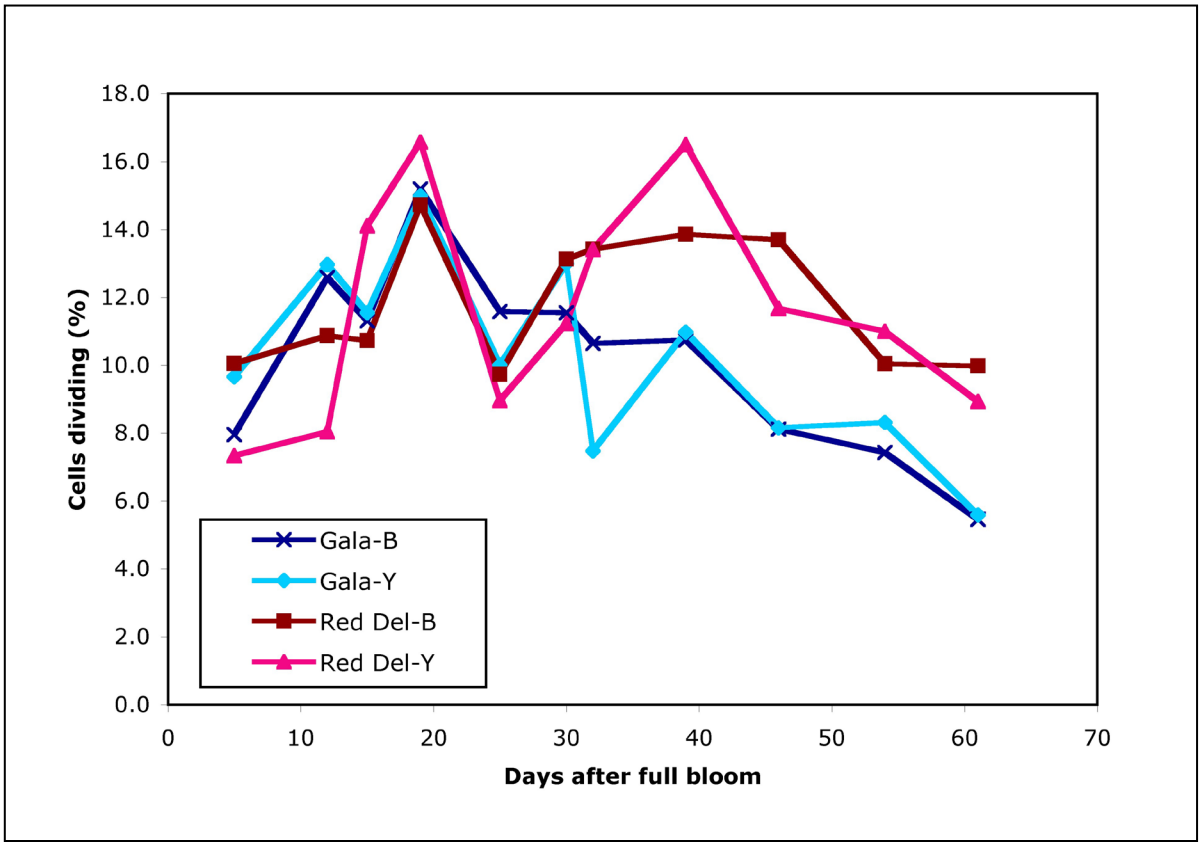


Figure 2. Fruit size of Red Delicious apples growing in Washington in response to crop load treatments during the 2005 growing season.



Flow cytometry analysis gives a precise estimate of the proportion of cells actively dividing. In terms of the proportion of cells dividing, there was little difference among the varieties during the early part of the season, but marked differences were noted from 30-60 days after bloom (Figure 3).

Figure 3. The proportion of cells actively dividing for Gala and Red Delicious fruit growing in Washington during the 2005 season. B and Y denote different crop loads.



Analysis of cell number and cell size from samples preserved during the season is still continuing, but a snapshot is presented below.

The number of cell layers in the radial axis of the fruit increased from the early part of the season due to rapid cell division (Figure 4). This rapid cell division also resulted in a rapid initial decrease in cell size (Figure 5).

Figure 4. Cell number of Gala and Red Delicious fruit growing in Washington during the 2005 season. B and Y denote different crop loads. Cell number was measured from the petal vascular bundle to the epidermis.

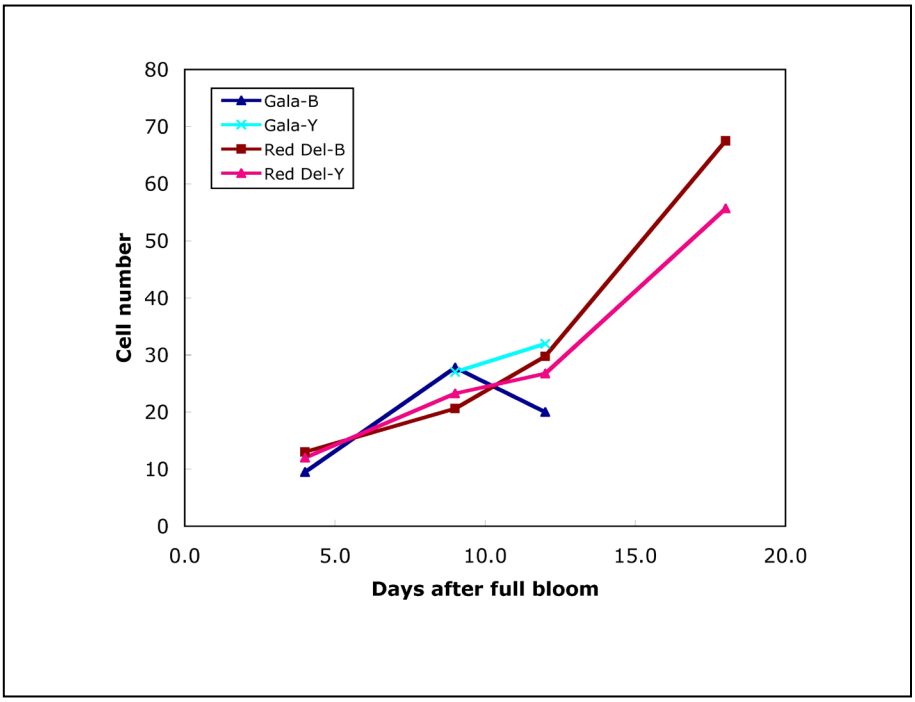
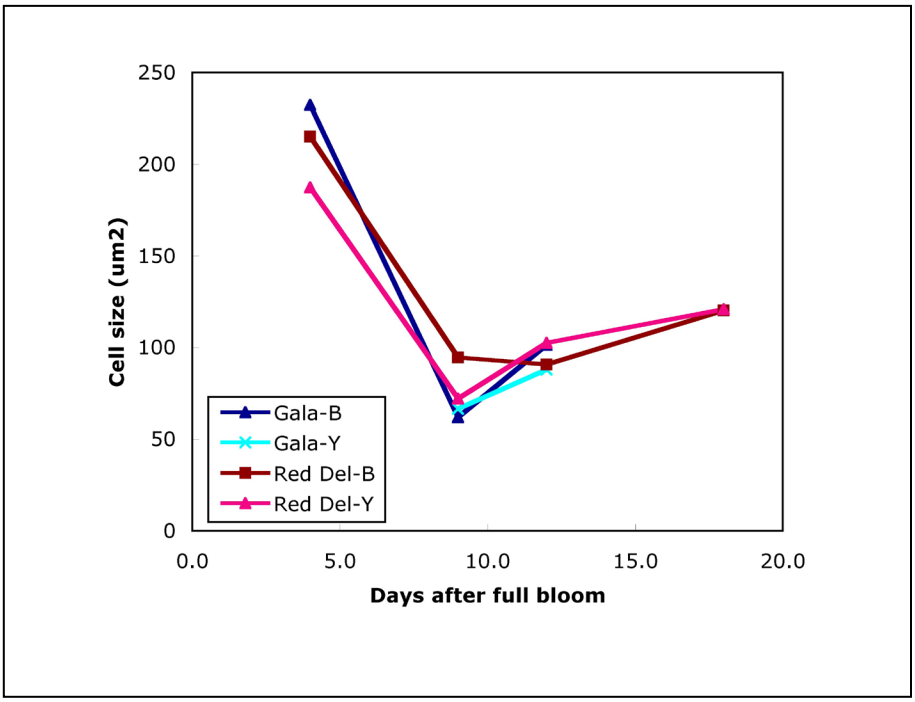


Figure 5. Cell size of Gala and Red Delicious fruit growing in Washington during the 2005 season. B and Y denote different crop loads.



BUDGET

Project Title: Mechanisms of apple fruit size response to environment and thinning

PI: Peter M. Hirst

Project duration: 2005-6

Current year: 2006

Project total: $38941

Current year request: $23941

Item	Year 1 (2005)	Year 2 (2006)	Total
Salaries[1]		3380	3380
Benefits		1159	1159
Wages	7494	9584	17078
Benefits	757	968	1725
Supplies[2]	6750	8850	15600
Travel			
Total	**15000**	**23941**	**38941**

[1] 5% of Hirst time spent on this project

[2] Flow cytometry costs

CONTINUING PROJECT REPORT **YEAR 1/3**

Project Title: Influence of temperature on pollen germination & tube growth

PI: Dr. Ross Byers, Professor Emeritus of Horticulture, VA Tech -AREC Winchester
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Phone: (540)-869-2560, Ext. 21; e-mail: ksyoder@vt.edu
Dr. Rongcai Yuan, Assistant Professor of Pomology, VA Tech,
Dr. Jim McFerson, Washington Tree Fruit Research Commission

Cooperating investigators:
Leon Combs, Research Specialist, VA Tech AHS-AREC; e-mail: lecombs@vt.edu
David Carbaugh, Research Specialist, VA Tech AHS-AREC, Winchester VA
Tory Schmidt, Washington Tree Fruit Research Commission, Wenatchee, WA

Organization: Virginia Polytechnic Institute and State University (VA Tech)
Contract Administrator: Sharron McElroy
Office of Sponsored Programs, 460 Turner Street, Suite 306,
Blacksburg, VA 24060
(540)-231-2068 e-mail: mcelroys@vt.edu

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.

2005 objectives: Our primary goal for 2005 was to compare the effective pollination period of "on-tree" flowers in a range of temperature and light conditions. Specific objectives were:

1) Study the effects of constant and alternating temperatures on tube growth of Snow Drift, Manchurian, and Golden Delicious pollen in Gala and Golden Delicious pistils, comparing optimal (75ºF) and sub-optimal temperatures (emphasis on 45ºF, 55ºF, 65ºF) under 12 hr light/12 hr dark rotations or continuous light (Exps. 1, 2, and 3), and determine the minimum time required for pollen tubes to grow to the stylar base of Golden Delicious and Gala pistils at optimum and sub-optimum temperatures.
2) Study pollinizer effect on fruit set in Gala and Fuji pistils pollinized in field with Golden Delicious, Manchurian, Snow Drift, and David (Exp. 4).
3) Test Gala stigma receptive period on tree pollinized with Golden Delicious 1, 3, 5, and 7 days after bloom and alternated at 55ºF light (12 hr) and 35ºF dark (12 hr) test (Exp. 5).
4) Study pollen germination and tube growth of selected crabapple pollens (Donald Wyman, David, Hyslop, S1173) applied to Golden Delicious pistils 'on tree' and evaluated after 24 hours at 75ºF under continuous light (Exp. 6).
5) Assimilate 2005 data into the early development of a functional model of pollen tube growth in selected apple cultivars for growers to use in conjunction with thinning programs to reduce chemical and labor costs.

Significant findings:

- Pollen tube growth to base of styles on Gala pistils occurred in less than 96 hours after pollination at alternating 55ºF 12-hr light/35ºF 12-hr dark (55/35º lt/dk) with all pollinizers tested.
- Pollen tube growth to base of styles on Gala pistils in tests at 65/40º lt/dk and 75/45º lt/dk 48 hours after pollination showed all with pollen tube growth to end of styles.
- Pollen tubes reached the end of some styles in less than 48 hours after pollination with Manchurian and Snow Drift pollen at 65/40º lt/dk and 75/45º lt/dk test trials. At 55/35º lt/dk pollen tubes reached the end of some Gala styles in less than 96 hours after pollination with Manchurian and Snow Drift pollen.
- There were no evident pollen tubes at stylar bases of Golden Delicious pistils pollinated with Golden Delicious pollen in any test.
- Some Golden Delicious pollen tubes growing on Golden Delicious stigmas and in styles developed bulbous ends which appeared to impede growth of tubes. This was not observed with Manchurian and Snow Drift pollen and may be related to pollen incompatibility.
- Stigma receptivity to pollen germination continued to 7 days after full bloom and pollen tubes grew to the end of styles after 96 hours at 55/35º lt/dk. Optimum receptivity occurred 3 days after full bloom with tube growth to stylar base in 100% of styles tested, 45% after 5 days, and 30% after 7 days.

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 which 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 (Experiments 1-3, 6 & 6): 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 were 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. 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. Ratings included abundance of pollen germination/tube growth (0-10) on the stigma surface, 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 (Experiment 4): Flowers on orchard trees were selected at late balloon stage for pollination. On the day of pollination, selected flowers were

tagged for identification and hand pollinated with pollen harvested 2005 by using a #2 brush to apply pollen to stigmas of flowers. All other flowers on trees were removed to prevent cross-pollination of test flowers. After pollination of test flowers and removal of non-treated flowers, selected limbs or whole trees used for experiment were covered with insect netting to prevent cross-pollination by insects or other vectors. 28 days after pollination treated limbs or trees were counted for fruit set and percentage was recorded.

RESULTS and DISCUSSION

EXP. 1-Gala pistils "on tree" at 45/32ºF, 55/35ºF, 65/40ºF, 75/45ºF alternating 12-hr lt/dk; Manchurian, Snowdrift, and Golden Delicious pollen 12, 24, 48, and 96 hr after pollination.

- At 45/32ºF lt/dk 96 hr after pollination of Gala pistils with Golden Delicious, Manchurian or Snow Drift pollen, none of the pollen tubes examined had grown to the end of the style.
- At 55/35ºF, 65/40ºF, and 75/45ºF lt/dk 96 hr after pollination of Gala pistils, pollen tubes of Golden Delicious, Manchurian or Snow Drift pollen had all reached the end of the style.
- At 65/40ºF, and 75/45ºF lt/dk 48 hr after pollination of Gala pistils, pollen tubes of Golden Delicious, Manchurian or Snow Drift pollen had all reached the end of the style.
- At 75/45ºF lt/dk the number of Golden Delicious pollen tubes reaching base of styles was more than twice the amount of Manchurian or Snow Drift pollinizers after 48 hr but no significant difference after 96 hr (Table 1).
- More pollen tubes growing into styles from the stigma does not necessarily assure more fertilization as indicated by comparing test 65/40ºF vs 75º/45ºF with Snow Drift (Table 1). A higher number of pollen tubes in the 65/40ºF test did not translate into higher numbers of pollen tubes reaching end of styles. This also occurred with Manchurian after 96 hr (Table 1) and to a lesser extent with both Snow Drift and Manchurian on Gala (Table 2).
- Pollen tubes reached the ovules within 48 hr at 75/45ºF, in estimated 80 hr at 65/40ºF, and approximately 85% of the style length at 96 hr at 55/35ºF.

EXP. 2- Golden Delicious pistils "on tree" at 45/32ºF, 55/35ºF, 65/40ºF, 75/45ºF alternating 12-hr lt/dk; Manchurian, Snowdrift, and Golden Delicious pollen 12, 24, 48, and 96 hr after pollination.

- Pollen germination/tube rating on stigmas pollinated with Golden Delicious pollen was as prevalent or better than Manchurian or Snow Drift pollinizers, but this did not increase the number of tubes growing into styles (Table 2).
- Golden Delicious pollen tubes growing into G. Del. styles were shorter than those from Manchurian or Snow Drift pollen.
- No G. Delicious pollen tubes reached the end of G. Del. styles in 96 hr at any test temperature.
- Some Golden Delicious pollen tubes growing on G. Del. stigmas and in styles developed bulbous ends which appeared to impede further growth of tubes. This was not observed with Manchurian and Snow Drift pollen and may be related to pollen incompatibility.
- Manchurian pollen does not appear to germinate as well as Snow Drift on Golden Delicious pistils, but a higher number of Snow Drift pollen tubes in G. Del. styles did not assure more tubes at end of styles than Manchurian.

EXP. 3- Golden Delicious pistils "on tree" in continuous light and temperature 45º, 55º, 65º and 75ºF; Manchurian, Snowdrift, and Golden Delicious pollen 12, 24, 48, and 96 hr after pollination (Table 3).

- Golden Delicious pistils pollinated with Golden Delicious pollen under continuous light/temperature was similar to alternating lt/dk: no tubes reached the ovules (Table 3). There were increases in number of pollen tubes growing into styles and average of tube

length at 55ºand 65ºF continuous light tests when compared to results from 55º/35ºF lt/dk and 65º/40ºF lt/dk inExp.2. Results from 75ºF continuous light test showed a marked decline in results from 55ºF, 65ºF continuous light tests. This is similar to the results from Exp. 2 also.

- After 24 and 48 hr, Manchurian and Snow Drift pollen tubes in Golden Delicious styles were 2-3X longer at 55ºF and 65ºF compared to results from 55º/35ºF lt/dk and 65º/40ºF lt/dk, in Exp. 2. This should be expected since trees and flowers were not subjected to darkness and temperature fluctuation during testing.

EXP. 4- Effect of pollinators on fruit set in Gala/M.9 and Fuji/M.27 pistils pollinized "on tree" in field with Golden Delicious, Manchurian, Snow Drift, and David pollen (data not shown).

- Fruit set of Fuji was 85% with Golden Delicious pollen, 77% with Manchurian pollen, and 87% with David pollen and 100% with Snow Drift pollen. All pollinizers showed very good pollination compatibility.
- Although some of the pollinizers tested in this experiment showed lower fruit set than others this may be misleading because even 13% or 53% fruit set would probably be sufficient for apple crop and could result in less bloom or fruit thinning by grower.

EXP. 5- Receptive period of Gala stigma to Golden Delicious pollen applied at 1, 3, 5, and 7 days after bloom; 55/35ºF lt/dk throughout test; sampled 96 hr after pollination (data not shown).

- Stigma receptivity to pollen germination continued to 7 days after full bloom with pollen tubes growing to end of some styles sampled at 3, 5, and 7 days after full bloom. Maximum pollination at this 55/35ºF lt/dk rotation was 3 days after full bloom. Stigma receptivity to pollen remained high at 5 days and even was receptive as long as 7 days after full bloom.

EXP. 6- Pollen germination and tube growth of Donald Wyman, David, Hyslop, S1173 crabapple pollens in Golden Delicious pistils. Evaluated after 24 hr at 75ºF, continuous light (data not shown).

- Donald Wyman pollen gave 100% of Golden Delicious styles with tubes past the base of the style after 24 hr at 75ºF, followed by David (43%), Hyslop (27%) and S1173 (13%).
- Donald Wyman appears better suited as a pollinizer for Golden Delicious at this temperature though David may set sufficient fruit for cropping. Hyslop and S1173 pollinizers do not appear to be highly compatible to this variety in this test procedure.

Model generation: Models projecting fertilization will be generated from data such as shown in Figure 1, using the length of time for pollen tubes to reach style bases at each test temperature.

CONCLUSIONS

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 continue to build on findings in 2004. These tests have yielded 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 can be used in developing a model for 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. Actual 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.

Original budget*:

Item:	2005	2006	2007
Salaries	20,000	20,000	20,000
Fringe benefits:	7,350	7,350	7,350
Supplies:	1,000	1,000	1,000
Contractual services and repairs	1,000	1,000	1,000
Total requested:	29,350	29,350	29,350

*** Note:** We will submit 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 we will request a $316 increase in supplies for cost of Fuji trees for research in '06.

Table 1. Effect of temperature and light on pollen tube growth of Golden Delicious, Manchurian, or Snow Drift pollen in Gala pistils on tree at 12, 24, 48, and 96 hours under indicated 12-hour temperature and light/dark (lt/dk) rotations after pollination (2005).

Time/temp. (ºF)/ Light exposure	Pollen tubes per style penetrating stigma base				Mean length, longest pollen tubes (mm)				No. of pollen tubes past end of style (per style)			
Snow Drift pollen[z]	12hr	24hr	48hr	96hr	12hr	24hr	48hr	96hr	12hr	24hr	48hr	96hr
45º (LT)/32º (DK)	0.4 d[y]	10.9 b	19.9 c	10.5 c	0.03 d	1.0 c	1.4 d	0.9 c	0.0 a	0.0 a	0.0 b	0.0 b
55º (LT)/35º (DK)	19.4 c	9.1 b	51.2 b	49.5 b	0.7 c	0.6 c	2.7 c	6.0 b	0.0 a	0.0 a	0.0 b	1.2 b
65º (LT)/40º (DK)	51.0 a	58.9 a	93.5 a	103.3 a	1.9 b	1.9 b	5.4 b	8.8 a	0.0 a	0.0 a	0.7 b	11.5 a
75º (LT)/45º (DK)	28.9 b	52.0 a	54.1 b	53.8 b	3.4 a	4.9 a	7.1 a	8.0 a	0.0 a	0.0 a	3.5 a	15.2 a
Manchurian pollen[z]												
45º (LT)/32º (DK)	23.3 ab	2.1 c	0.0 d	39.1 b	1.6 bc	0.4 d	0.0 d	2.3 c	0.0 a	0.0 a	0.0 b	0.0 c
55º (LT)/35º (DK)	12.3 b	21.4 b	36.3 c	48.8 b	0.9 c	1.1 c	2.5 c	5.6 b	0.0 a	0.0 a	0.0 b	1.9 c
65º (LT)/40º (DK)	31.9 a	51.7 a	76.9 a	76.2 a	2.3 b	2.8 b	4.5 b	8.2 a	0.0 a	0.0 a	0.2 b	8.2 b
75º (LT)/45º (DK)	27.8 ab	42.7 a	59.1 b	45.7 b	3.5 a	4.1 a	7.5 a	7.6 a	0.0 a	0.0 a	3.7 a	12.1 a
Golden Delicious pollen[z]												
45º (LT)/32º (DK)	16.8 bc	9.1 c	0.3 c	23.2 c	1.6 b	0.8 c	0.1 d	1.2 c	0.0 a	0.0 a	0.0 b	0.0 b
55º (LT)/35º (DK)	8.3 c	9.6 c	51.0 b	78.3 a	0.5 c	0.4 c	2.5 c	5.7 b	0.0 a	0.0 a	0.0 b	1.4 b
65º (LT)/40º (DK)	22.9 b	26.6 b	67.9 a	73.5 a	1.2 bc	2.2 b	4.8 b	8.3 a	0.0 a	0.0 a	0.1 b	14.6 a
75º (LT)/45º (DK)	39.0 a	47.2 a	70.6 a	55.4 b	2.8 a	3.9 a	7.7 a	8.7 a	0.0 a	0.0 a	8.9 a	17.1 a

[z]Pollen viability test (2 hr at room temperature-70ºF) = Golden Delicious-70%; Manchurian-50%; Snow Drift-40%, (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 ($P \leq 0.05$).

Table 2. Effect of temperature and light on pollen tube growth of Manchurian, Snow Drift or Golden Delicious pollen in Golden Delicious pistils on tree at 12, 24, 48, and 96 hours under indicated 12-hour temperature and light/dark (lt/dk) rotations after pollination (2005).

Time/temp. (ºF)/ Light exposure	Pollen tubes per style penetrating stigma base				Mean length, longest pollen tubes (mm)				No. of pollen tubes past end of style (per style)			
Snow Drift pollen[z]	12hr	24hr	48hr	96hr	12hr	24hr	48hr	96hr	12hr	24hr	48hr	96hr
45º (LT)/32º (DK)	0.0 b[y]	0.0 b	0.0 c	0.5 c	0.0 c	0.0 d	0.0 d	0.3 c	0.0 a	0.0 a	0.0 b	0.0 c
55º (LT)/35º (DK)	1.5 b	5.3 a	18.9 b	22.9 b	0.4 c	0.7 c	2.7 c	2.8 b	0.0 a	0.0 a	0.0 b	0.1 c
65º (LT)/40º (DK)	15.4 a	6.3 a	49.7 a	76.1 a	1.2 b	1.4 b	5.2 b	7.9 a	0.0 a	0.0 a	0.9 a	4.9 b
75º (LT)/45º (DK)	6.9 b	5.9 a	19.1 b	27.7 b	1.7 a	2.2 a	6.8 a	7.6 a	0.0 a	0.0 a	1.4 a	7.4 a
Manchurian pollen[z]												
45º (LT)/32º (DK)	0.0 b	0.0 b	1.9 b	1.3 c	0.0 b	0.0 b	0.3 c	0.5 d	0.0 a	0.0 a	0.0 b	0.0 c
55º (LT)/35º (DK)	4.7 a	6.0 a	11.7 a	20.8 b	0.6 b	0.4 b	1.4 bc	3.7 c	0.0 a	0.0 a	0.0 b	0.5 c
65º (LT)/40º (DK)	0.6 b	7.8 a	7.7 ab	36.7 a	0.4 b	1.5 a	2.1 b	6.6 b	0.0 a	0.0 a	0.3 b	4.0 b
75º (LT)/45º (DK)	2.5 ab	6.1 a	14.9 a	31.9 a	1.3 a	2.2 a	6.0 a	8.1 a	0.0 a	0.0 a	0.8 a	10.5 a
Golden Delicious pollen[z]												
45º (LT)/32º (DK)	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a
55º (LT)/35º (DK)	1.0 b	0.5 b	0.1 b	14.7 b	0.3 ab	0.1 b	0.1 c	1.1 a	0.0 a	0.0 a	0.0 a	0.0 a
65º (LT)/40º (DK)	0.6 b	0.2 b	8.0 a	13.3 b	0.1 b	0.1 b	0.6 b	1.6 a	0.0 a	0.0 a	0.0 a	0.0 a
75º (LT)/45º (DK)	3.5 a	3.8 a	6.3 a	27.2 a	0.5 a	0.7 a	1.0 a	1.5 a	0.0 a	0.0 a	0.0 a	0.0 a

[z]Pollen viability test (2 hr at room temperature-70ºF) = Golden Delicious-70%; Manchurian-50%; Snow Drift-40%, (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 ($P \leq 0.05$).

Table 3. Effect of temperature and light on pollen tube growth of Golden Delicious, Manchurian, or Snow Drift pollen in Golden Delicious pistils on tree at 12, 24, 48, and 96 hours under continuous temperature and light after pollination (2005).

Time/temp. (ºF)/ Light exposure	Pollen tubes per style penetrating stigma base				Mean length, longest pollen tubes (mm)				No. of pollen tubes past end of style (per style)			
Snow Drift pollen[z]	12hr	24hr	48hr	96hr	12hr	24hr	48hr	96hr	12hr	24hr	48hr	96hr
45º (Cont. light)	0.0 b[y]	0.0 c	0.2 c	1.0 c	0.0 b	0.0 d	0.1 c	1.0 c	0.0 a	0.0 b	0.0 c	0.0 c
55º (Cont. light)	0.0 b	18.1 b	26.6 b	70.1 a	0.0 b	2.0 c	5.2 b	7.5 a	0.0 a	0.0 b	1.6 c	14.7 a
65º (Cont. light)	35.9 a	41.7 a	84.9 a	83.1 a	2.4 a	4.3 b	7.1 a	7.4 ab	0.0 a	0.7 b	15.0 a	15.8 a
75º (Cont. light)	11.2 b	12.2 bc	26.7 b	16.1 b	2.2 a	5.8 a	6.8 a	6.5 b	0.0 a	4.3 a	7.5 b	4.1 b
Manchurian pollen[z]												
45º (Cont. light)	0.0 b	0.0 c	0.0 c	1.1 d	0.0 b	0.0 c	0.0 b	0.5 c	0.0 a	0.0 b	0.0 c	0.0 c
55º (Cont. light)	1.7 b	15.2 ab	18.3 b	40.6 b	0.2 b	2.0 b	6.8 a	7.6 a	0.0 a	0.0 b	2.3 b	9.0 b
65º (Cont. light)	2.1 b	27.8 a	51.7 a	63.1 a	0.5 b	2.7 b	5.6 a	7.1 ab	0.0 a	0.1 b	4.9 a	18.1 a
75º (Cont. light)	8.7 a	9.9 bc	12.1 b	15.7 c	1.7 a	5.4 a	6.0 a	6.2 b	0.0 a	3.1 a	3.7 ab	3.3 c
Golden Delicious pollen[z]												
45º (Cont. light)	0.0 b	0.0 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 c	0.0 c	0.0 a	0.0 a	0.0 a	0.0 b
55º (Cont. light)	0.0 b	1.3 b	11.3 b	27.4 b	0.0 b	0.2 b	1.5 ab	1.9 b	0.0 a	0.0 a	0.1 a	0.1 ab
65º (Cont. light)	2.0 b	14.4 a	36.5 a	61.0 a	0.4 a	0.7 a	2.0 a	3.2 a	0.0 a	0.0 a	0.0 a	0.3 a
75º (Cont. light)	6.1 a	1.9 b	10.2 b	19.1 b	0.4 a	0.2 b	0.7 bc	1.7 b	0.0 a	0.0 a	0.0 a	0.1 ab

[z]Pollen viability test (2 hr at room temperature-70ºF) = Golden Delicious-70%; Manchurian-50%; Snow Drift-40%, (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 ($P \leq 0.05$).

CONTINUING PROJECT PROPOSAL **YEAR 1/3**
WTFRC Project #: AH-05-507 **WSU Project #:** 13C-3655-5299

Project title: Growth and crop load management in apple trees with bioregulators
PI: Don C. Elfving, Horticulturist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Cooperators: Thomas D. Auvil, Research Horticulturist, WTFRC, Wenatchee, WA
Eric A. Curry, Horticulturist, USDA/ARS/TFRL, Wenatchee, WA
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Dwayne Visser, Agricultural Research Technologist II, WSU-TFREC, Wenatchee, WA

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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. The alternating flowering and yield effects observed this year in 'Fuji' trees treated with GA in 2003 were a direct result of altered flowering in 2004, **NOT** of altered fruit or crop load that year. Hence, flowering intensity alone in 'Fuji' apples can induce an alternating bloom and production cycle. It is likely that this fact explains the difficulty in controlling alternate bearing in 'Fuji' trees with thinning alone.
2. Even high concentrations of ethephon applied twice after the thinning period is over do not produce reliable improvement in flowering in cropping trees. Induction of good return bloom in cropping trees is very difficult.
3. New GA/alternate bearing trials established in 2005 will examine using GA, BA/GA or combinations of NAA/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.'
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.

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 double-tree plots in randomized complete block designs. One trial from 2004 was carried over in 2005 to evaluate return bloom responses to ethephon applied in 2004. 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 2005. New trials were established in 2005 to 1) examine fruiting and return-bloom responses to applications of gibberellic acid, 2) examine the relative merits of leaf removal and cyclanilide for branch induction in sleeping-eye trees and 3) evaluate TDZ for induction of bud activity in latent buds, which are extremely difficult to induce into growth activity.

Results and discussion:

A. Control of flowering with GA in alternating apple cultivars (Objectives 1,2)

1. In the third season following GA treatment in spring to control return bloom, young 'Fuji'/M.9 trees showed a continuing and opposite alternating bloom pattern induced by the 2003 GA applications (Fig. 1a).
2. GA_7, the strongest isomer for reducing return bloom, contributed to a greater decrease in flowering in 2004 that was reflected in a much stronger increase in bloom in 2005, still showing the concentration dependence that affected return bloom in 2004 (Fig. 1b).
3. Although reduced flowering was observed in 2004 as a result of the 2003 GA treatments, fruit numbers per tree and yield in 2004 were not affected by GA treatments in 2003 due to greater fruit set in lighter blooming trees in 2004 (Fig. 1b).
4. Significantly, the flowering and yield effects observed in 2005 were a direct result of altered **flowering** in 2004 (Fig. 1a), **NOT** of altered **fruit or crop load** (Fig. 1b). **Hence, flowering intensity alone in 'Fuji' apples can induce an alternating bloom and production cycle.** This observation may help explain why normal postbloom chemical thinning methods have reduced yield the year of treatment but have not proven effective for interrupting alternate cropping in 'Fuji' apple trees.

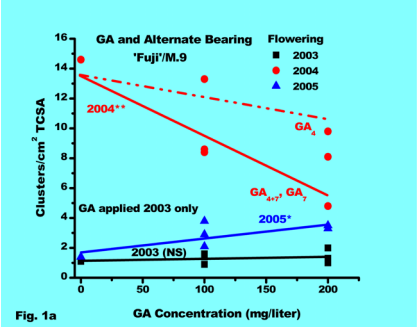

Fig. 1a

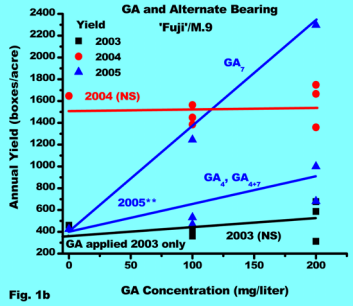

Fig. 1b

B. Induction of flowering with ethephon (Objective 3)

1. Post-thinning treatment of moderately-cropping, young 'Honeycrisp'/M.9 trees with ethephon once or twice at up to 600 ppm did not have a significant effect on return bloom in 2005.

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 has already been 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; 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.

D. Programming the induction of lateral branching in sleeping-eye apple trees with bioregulators (Objective 6)

1. Cyclanilide (CYC) showed promise for aiding in the programmed development of lateral branches in a trial with sleeping-eye 'Honeycrisp'/M.9 apple trees planted in the orchard in 2004 and treated that year (Fig. 2).
2. In 2005, newly planted 'Honeycrisp'/M.9 apple trees showed no effect on height growth when young leaves were removed from the shoot apex at the time determined to be appropriate for branch-induction treatments.
3. Cyclanilide sprays applied to the shoot tips and nearby leaves did reduce height growth but only by 4-6 inches.
4. Leaf removal was effective for inducing the desired branching; cyclanilide alone at 100 or 150 ppm was more effective for branch induction and far more labor efficient. Combining leaf removal with cyclanilide produced no benefit for lateral branch development.
5. Two cytokinins, thidiazuron (TDZ, 500 ppm) and Promalin (PR, 5,000 ppm), were tested for efficacy for induction of lateral branching in one-year-old sleeping-eye trees of 'Honeycrisp'/M.9 that did branch properly in the first year in the orchard. Combinations of notching plus treatment with either product did not produce a useful branching response.

Fig. 2

E. Stimulation of bud activity on "blind wood" in apple (New Objective 1)

1. Thidiazuron (TDZ), a powerful cytokinin, was tested for efficacy in stimulating growth from latent buds on older limb sections (3- to 5-year-old wood) of 'Cameo' and 'Granny Smith' apple trees. TDZ at up to 1000 ppm did not produce significant changes in bud development on treated limb sections.

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 point 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.

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Publications 2005:

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Budget:

Project title: Growth and crop load management in apple trees with bioregulators
PI: Don C. Elfving
Proposed duration: Three years (2005-2007)
Current year: 2006
Project total (3 years): $54,615
Current year request: $17,956

Item	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)
Salaries (Technical)[1]	8,000	**8,400**	8,820
Benefits (34%)	2,720	**2,856**	2,999
Wages (Time-slip)[1]	2,000	**2,000**	2,200
Benefits (10%)	200	**200**	220
Equipment	0	**0**	0
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**	19,239

[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 throughout the south-central and north-central Washington fruit production areas.

CONTINUING PROJECT REPORT **YEAR 1/3**

PROJECT TITLE: Chemical thinning of apple
PI: Dr. Jim McFerson, WTFRC
Washington Tree Fruit Research Commission
1719 Springwater Ave, Wenatchee WA 98801
ph: 509-665-8271; email: mcferson@treefruitresearch.com

CO-INVESTIGATORS: Tom Auvil, Felipe Castillo, Tory Schmidt, WTFRC

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
- Expand collaborative efforts with other research programs

SIGNIFICANT FINDINGS:

Effective bloom thinning programs reduce hand-thinning and frequently improve fruit size, quality, and return bloom to levels of statistical significance (Table 3)

Oil + lime sulfur programs are the most efficacious options for bloom thinning (Table 3)

Many spray oils (dormant, summer, vegetable, fish) are effective in combination with lime sulfur; Crocker's Fish Oil programs are successful most often (Tables 2, 3)

Thinning programs of high rates of lime sulfur are gaining industry acceptance and are generally superior to ATS programs (Table 3)

Preliminary results with novel bloom thinning programs including Raynox, vinegar + oil, urea, and Pacific Natural fish emulsion + lime sulfur are inconclusive

All formulations of BA evaluated showed similar effects (Table 4)

In a majority of trials, BA + carbaryl thinning programs give results equal or superior to NAA + carbaryl or ethephon + carbaryl programs; BA shows a positive effect on fruit size (Table 5)

Timing trials with various postbloom thinners generally indicated better results with applications made at 5mm and 10mm fruitlet size, rather than 10mm and 15mm (Table 5)

In all three trials testing combinations of bloom and postbloom thinners, Crocker's Fish Oil + lime sulfur followed by carbaryl + BA demonstrated the best results (Table 6)

Isolated incidences of fruit russeting are more attributable to trial block environmental conditions than response to chemical thinners

Intensified collaborative efforts across disciplines, institutions, and regions will increase relevance and impact of all crop load management research (Table 7)

BACKGROUND:

The internal research program of the WTFRC conducted 19 apple chemical thinning trials in 2005 at eight commercial orchard sites around the state of Washington. In contrast to prior seasons, we deployed fewer trials at fewer sites 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 180 field trials since 1998 on eleven cultivars and ten rootstocks representing all important growing districts in the state. In 2005, 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.

Chemical thinning goals

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. These three parameters are highlighted in Table 3, which summarizes our trial results over the past several years.

BLOOM THINNING:

Our protocols generally call for two applications of each bloom thinning programs, at 20% and 80% full bloom. Programs investigated in 2005 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, postbloom chemical thinning programs were left to the discretion of individual growers-cooperators as long as all experimental plots received the same treatments.

Table 1. Chemical bloom thinning programs evaluated. WTFRC 2005.

3.4 gal Ammonium thiosulfate (ATS)/A
5 gal NC99/A
6-8% Lime sulfur (LS)
1-3% Crocker's Fish Oil (CFO) + 2-4% LS
1.5% JMS Stylet Oil (JMS) + 2-3% LS
0.5-1% Wilbur-Ellis Supreme Oil (WES) + 2-3% LS
2 pts Tergitol/A
8-10% Vegetable Oil Emulsion (VOE)
10-17% VOE + 17% Vinegar
40 lbs Urea/A
2% Pacific Natural Fish Emulsion + 2.5% LS
10-20% Raynox
3 pts Ethrel/A

Results from 2005 bloom thinning trials (Table 2) revealed that all treatments were effective at reducing fruit set at trials in Quincy and Chelan. Harvest fruit size was improved by most thinning

programs in Quincy, but no size differences were appreciable at the Chelan site. A third bloom thinning trial in Othello (data not shown) showed no thinning effects for any treatment, including oil + lime sulfur programs. Unfortunately, this trial included several novel treatments such as vegetable oil + vinegar, urea, Raynox, and a new fish emulsion (Pacific Natural) + lime sulfur. These new treatments need to be repeated in 2006 to fairly evaluate their efficacy.

Table 2. Crop load effects of bloom thinning programs. WTFRC 2005.

Trial	Thinning program	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit diam
			%	%	cm
Fuji / M.9	Tergitol	135 ab	25 ns	35 ab	7.63 ab
Quincy	Urea	137 ab	24	31 b	7.73 a
	Lime Sulfur	127 ab	22	29 b	7.61 ab
	ATS	107 b	21	30 b	7.41 ab
	Vinegar + VOE	111 b	22	30 b	7.66 a
	VOE	124 ab	22	28 b	7.53 ab
	WES + LS	119 ab	23	42 a	7.31 b
	CFO + LS	121 ab	23	33 ab	7.56 ab
	Control	151 a	26	28 b	7.42 ab

Results from individual trials may not accurately reflect the overall success rate of a given program. As with any type of research, we feel it is important to examine an entire body of work, rather than "selected results." Table 3 summarizes results from all apple bloom thinning trials conducted by the WTFRC since 1999, reflecting a very conservative standard by which to assess our most frequently studied programs.

Table 3. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials WTFRC 1999-2005.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit diam	Return bloom[1,2]
Ammonium thiosulfate	14 / 45 (31%)	9 / 48 (19%)	2 / 35 (6%)
NC99 (Mg^{++}/Ca^{++} Cl^{-} brine)	14 / 27 (52%)	7 / 29 (24%)	2 / 23 (8%)
Lime sulfur	25 / 50 (50%)	12 / 44 (27%)	9 / 41 (22%)
Crocker's Fish Oil + lime sulfur	51 / 76 (67%)	24 / 71 (34%)	13 / 53 (25%)
JMS Stylet Oil + lime sulfur	14 / 24 (58%)	8 / 23 (35%)	4 / 21 (19%)
Wilbur-Ellis Supreme Oil + lime sulfur	14 / 26 (54%)	4 / 25 (16%)	3 / 21 (14%)
Vegetable Oil Emulsion	13 / 22 (59%)	4 / 21 (19%)	2 / 18 (11%)

[1]Does not include data from 2005 trials.

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

This macro-level view of our work clearly shows that oil and lime sulfur combinations are consistently the most effective options for achieving our three chemical thinning goals.

POSTBLOOM THINNING:

Carbaryl continues to be the industry standard for postbloom chemical thinning in conventional systems, and research has yet to identify a viable alternative for it. Even so, we are encouraged by the

performance of benzyladenine (BA) products in combination with carbaryl in terms of their ability to provide modest thinning and increased fruit size. In roughly 3 out of every 4 trials, we have found BA + carbaryl programs to be as good as or superior to either NAA + carbaryl or ethephon + carbaryl programs.

BA products tend to be expensive, but the financial gains of growers reducing hand-thinning and shifting more fruit into target size ranges should more than offset any increased material costs. We are hopeful that market competition between alternative formulations will drive down the cost of all materials. In three 2005 trials, we were unable to distinguish between two commercial BA formulations (MaxCel and Exilis Plus) and two products in development (Genesis BA and Nufarm BA). Table 4 shows results typical of all three trials.

Table 4. Comparison of alternative BA formulations as postbloom thinners. WTFRC 2005.

Trial	Thinning program	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit diam
			%	%	cm
Gala / M.26	Sevin + Nufarm + Regulaid	31 bc	72 ab	25 bc	7.63 a
Baker Flats	Sevin + Exilis + Regulaid	32 b	70 b	28 b	7.58 ab
	Sevin + Genesis + Regulaid	28 bc	72 ab	27 bc	7.58 ab
	Sevin + MaxCel	33 b	68 b	31 b	7.58 ab
	Sevin + Regulaid	43 ab	60 bc	37 ab	7.56 ab
	Sevin + NAA + Regulaid	16 c	85 a	14 c	7.47 ab
	MaxCel	39 ab	63 bc	35 ab	7.57 ab
	Control	50 a	53 c	44 a	7.42 b

The inherent variability in crop load management make it difficult to draw any absolute conclusions, but one tried and true concept is that "earlier is better." We feel that aggressive, early thinning achieves four important objectives:

1. Thins off more fruit before it becomes more tenaciously set
2. Maximizes fruit size by reducing competition for limited nutrients and carbohydrates
3. Improves return bloom by eliminating sources of gibberellins (juvenile seeds)
4. Allows growers more time to assess action of thinners before making subsequent sprays

Results from selected postbloom thinner timing trials (Table 5) generally corroborate increased thinning with earlier applications, as well as frequent fruit size gains. These data further demonstrate that carbaryl + BA programs are effective thinners.

Table 5. Spray timing effects on postbloom thinning programs. WTFRC 2005.

Trial	Thinning program	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit diam
			%	%	cm
Pacific Rose / M.26	Sevin + Ethrel + Genesis 5mm, 10mm	34 b	68 a	31 ns	7.92 ns
Brewster	Sevin + Ethrel + Genesis 10mm, 15mm	49 ab	56 ab	39	7.84
	Sevin + Genesis 5mm, 10mm	44 ab	60 ab	36	7.72
	Sevin + Genesis 10mm, 15mm	43 ab	60 ab	36	7.75
	Sevin + NAA + Ethrel 5mm, 10mm	44 ab	58 ab	39	7.84
	Sevin + NAA + Ethrel 10mm, 15mm	51 ab	53 ab	43	7.89
	Genesis 5mm, 10mm	45 ab	59 ab	38	7.90
	Genesis 10mm, 15mm	54 a	53 b	41	7.76
	Control	51 ab	54 ab	41	7.87

COMBINED BLOOM AND POSTBLOOM PROGRAMS:

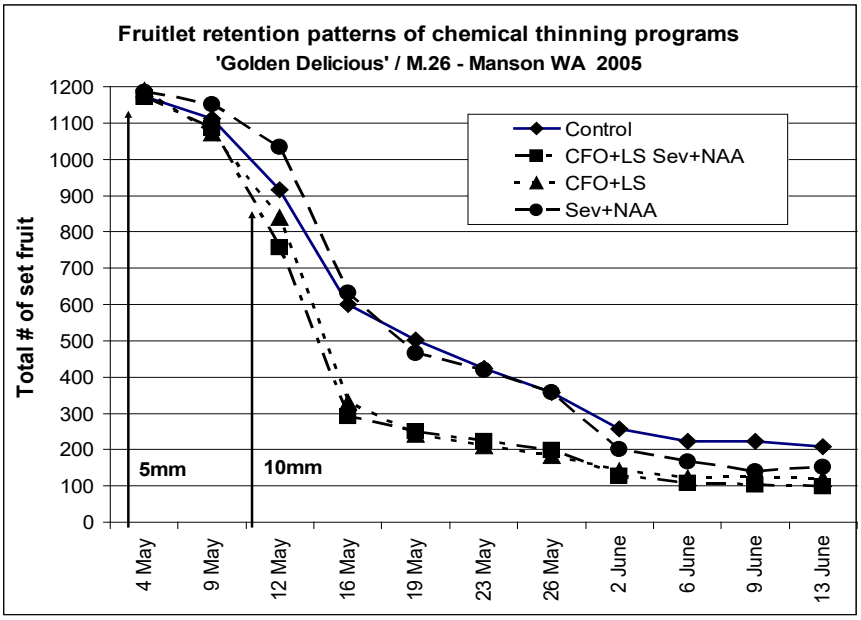
Even though many of the chemical thinners we have evaluated through the years have been consistently effective, they rarely reduce cropping to levels considered satisfactory by commercial growers. In 2005, we conducted three trials evaluating various combinations of popular bloom thinning programs (ATS; oil + LS) and postbloom thinning programs (carbaryl + NAA; carbaryl + BA). Table 6 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. Interestingly, the top treatment for all three trials was Crocker's Fish Oil + lime sulfur applied twice during bloom, followed by two applications of carbaryl + BA after petal fall.

Table 6. Crop load effects of bloom + postbloom thinning programs. WTFRC 2005.

Trial	Bloom thinner	Postbloom thinner	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit diam
				%	%	cm
Golden Del / M.26	ATS		89 a	37 c	44 abc	7.35 cd
Manson	ATS	Sevin + NAA	87 a	36 c	45 ab	7.65 ab
	ATS	Sevin + Exilis	58 bcd	50 abc	44 abc	7.33 d
	CFO + LS		78 abc	43 bc	40 abc	7.57 abcd
	CFO + LS	Sevin + NAA	51 cd	58 ab	34 bc	7.73 a
	CFO + LS	Sevin + Exilis	49 d	61 a	31 c	7.65 ab
		Sevin + NAA	85 ab	36 c	48 a	7.63 abc
		Sevin + Exilis	78 abc	41 bc	45 ab	7.28 d
		Exilis	81 ab	42 bc	41 abc	7.40 bcd
	Control		86 ab	39 c	42 abc	7.48 abcd

The following graph portrays the fruitlet abortion patterns of selected treatments from the trial described in Table 6. Treatments which did not receive bloom thinners (control and Sevin + NAA) were much later in aborting fruitlets than those which received CFO + LS treatments. Early thinning usually translates to larger fruit size and improved return bloom, which we will measure this spring.

Graph 1.



FRUIT FINISH

Historically, we have been unable to observe any clear relationship between fruit finish/russet and chemical thinning programs. Despite rigorous application of conservative grading standards (e.g. all fruit with any visible russet is graded as "russeted," regardless of degree) we have been unable to discern that any of our treatments have had a consistent effect, positive or negative, on fruit finish. At some 2005 trial sites, we did observe marginal increases in fruit russet across several trial blocks, but not associated with a particular thinning agent (data not shown). In other words, isolated cases of increased fruit russet in 2005 showed a clearer correlation with the orchard environmental conditions than with individual treatments.

COLLABORATIVE RESEARCH

Cooperative work between the WTFRC and other research programs has increased our understanding of many diverse aspects of apple crop load management (Table 7). Early success of these collaborations underscores the need to expand them in future studies.

Table 7. Ongoing WTFRC collaborative research on apple crop load management.

Scientist	Institution	Focus Area
Elfving	WSU	PGR effects on vegetative/reproductive site balance
Fallahi	Univ Idaho	Tergitol as a bloom thinner
Faubion / Lewis	WSU	Mobile platforms for human and potentially robotic thinners
Greene	Univ Mass	Predictive modeling of fruit set
Hirst	Purdue	Cell division / expansion models; genetic fruit size potential
McArtney	NC State	Mid-summer PGRs to promote return bloom
Rom	Univ Arkansas	Organic chemical thinning programs, pollen germination
Whiting	WSU	Physiological effects of bloom thinners (whole canopy Pn)
Yoder / Byers	Virginia Tech	Novel bloom thinners, pollen tube development

Project Title: Chemical thinning of apple
PI: Jim McFerson
3 year project total: $114,900

Item	2005	2006	2007
Salary			
Benefits			
Timeslip	30,000	30,000	30,000
Benefits (~16%)	4,800	4,800	4,800
Supplies	3,000[1]	3,000[1]	3,000[1]
Travel	500	500	500
Total	38,300	38,300	38,300

[1] Chemicals, fruit purchase
NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

CONTINUING PROJECT REPORT

PROJECT TITLE: Sprays to improve packouts and annual cropping in apple

PI: Dr. Jim McFerson
Washington Tree Fruit Research Commission
1719 Springwater Avenue, Wenatchee, WA 98801
ph: 509-665-8271; email: mcferson@treefruitresearch.com

CO-INVESTIGATORS: Tom Auvil, Felipe Castillo, Tory Schmidt, WTFRC
Don Elfving, WSU-TFREC, Wenatchee, WA
Steve McCartney, NCSU-MHCREC, Fletcher, NC
Larry Schrader, WSU-TFREC, Wenatchee, WA

OBJECTIVES:

1. Investigate chemical programs (GA, Raynox, Apogee) to improve fruit finish
2. Evaluate new sunburn protectants (Fruit Shield, Eclipse, others) for apple
3. Investigate spray programs to increase (ethephon, NAA) or diminish (GA) return bloom

SIGNIFICANT FINDINGS:

Application of Raynox had no positive effect on fruit finish in 2005 trials; ProVide (GA_{4+7}) and Novagib (GA_4) reduced russet in most trials

Results for treatments to improve fruit finish of Gala were inconclusive due to limited development of scarf skin in untreated controls

Surround WP and Raynox are industry standards for sprayable sunburn protection; prior research established these materials can reduce sunburn-related cullage by approximately 50%

All materials tested in 2005 trials were effective in suppressing sunburn; results in all trials were superior to those observed in 2004 and 2003

Calcium-based materials offer promise as low-cost alternatives to Surround WP and Raynox

All treatments tested but one were easily removed from fruit surface with standard packing line washes and brushing; Surround WP left some residue in calyx and stem bowls

In 6 Proptec-applied trials at three sites in 2004, no treatment of ethephon and/or NAA increased 2005 return bloom to levels of statistical significance vs. control, but trends were positive for most programs

Ethephon improved return bloom (Cameo) and GA diminished return bloom (Cameo, Fuji, Honeycrisp) in Schmidt master's research trials (data not shown); response was less prominent when trees were in severe alternation (either very high or very low crop loads)

FRUIT FINISH:

Fruit surface disorders cost the state apple industry millions of dollars a year. In particular, Fuji is prone to many maladies, some of which are obvious in the field and others which show up well into the storage season (see Schrader report). Fruit russet is typically induced early in the growing season and is likely aggravated by a combination of weather conditions, spray chemicals, and/or biotic pests on the fruit surface. However, few practical options are available to orchardists to suppress the

development of russet. Building on encouraging results from preliminary trials in 2004, we conducted several trials in 2005 evaluating various rates, combinations of, and spray regimes of ProVide (GA_{4+7}), Novagib (GA_4), Apogee (prohexadione-Ca), and Raynox to improve fruit finish.

Materials evaluated include:

ProVide (Valent) -- effective against fruit russet in trials and commercial application
Novagib (Fine) -- also effective in research trials, is not widely used by industry
Raynox (FruitGard) -- theorized to fill in cuticle cracks caused by rapid fruit growth
Apogee (BASF) -- effective when combined with ProVide against "scarf skin" of Gala in preliminary East Coast trials (McArtney, personal communication)

Results from 2005 trials confirm the efficacy of gibberellin products (ProVide and Novagib) at various rates and spray regimes (Table 1) for improving fruit finish. Treatments generally reduced russet on fruit shoulders and flanks. Gibberellins are known to be inhibitors of floral initiation in apple, especially materials containing GA_7, such as ProVide. We anticipate observing reduced return bloom in these treatments next spring.

Table 1. Fruit finish and weight effects of commercial gibberellin formulations. WTFRC 2005.

Trial	Treatment	Russeted fruit	RUSSET TYPE			Fruit weight
			Stem bowl	Shoulder	Flank netting*	
		%	%	%	%	g
Pacific Rose	Novagib 15ppm 4x	36 ab	8 ns	23 ab	6	218 ns
M.26	Novagib 20ppm 4x	36 ab	0	28 a	8	234
Brewster	Novagib 26ppm 3x	21 b	6	6 b	8	242
	Novagib 26ppm 4x	19 b	8	8 b	4	240
	ProVide 15ppm 4x	24 ab	3	18 ab	4	256
	ProVide 19ppm 4x	20 b	8	10 ab	3	261
	ProVide 25ppm 3x	25 ab	8	15 ab	3	222
	ProVide 25ppm 4x	35 ab	14	18 ab	4	223
	Control	50 a	9	28 a	14	240

*** values pooled from ratings of 5%, 10%, and 25% net-type russeting on flanks of fruit**

When applied by itself, Raynox did not improve fruit finish in any trials in 2005 (Table 2). Tank-mixing Raynox with a gibberellin increased fruit russet to a level of statistical significance at one site (Royal City), but positive data trends from other trials suggest that result may have been an aberration. Despite selecting a trial site notorious for scarf skin on Gala, control fruit in that block did not show enough of the disorder to properly evaluate treatments against it.

Table 2. Fruit russet and weight results. WTFRC fruit finish field trials 2005.

Trial	Treatment	Russeted fruit	RUSSET TYPE			Fruit weight
			Stem bowl	Shoulder	Flank netting	
		%	%	%	%	g
Fuji	Raynox	44 ns	6 ns	6 ns	32	225 ns
M.9	Novagib	38	9	8	21	227
Prosser	Raynox + Novagib	34	4	1	29	221
	Control	30	1	5	24	221

Golden Delicious	Raynox	63 ab	45 a	11 ns	6	252 ns
M.7	ProVide	65 a	58 a	6	1	254
Othello	Raynox + ProVide	55 ab	38 ab	14	3	256
	Control	44 b	21 b	18	3	261
Golden Delicious	Raynox	50 a	14 b	26 ns	10	240 ns
M.7	Raynox + Novagib	58 a	30 a	23	4	232
Royal City	Novagib	31 b	15 b	9	8	259
	Control	48 a	18 ab	19	12	252
Golden Delicious	ProVide	30 a	7 ns	22 ns	2	160 ns
Seedling	Raynox + ProVide	17 ab	8	8	0	163
Brewster	Control	15 b	3	8	3	183
Gala	ProVide	10 ns	7 ns	3 ns	0	155 ns
Seedling	Apogee; ProVide	7	5	2	0	157
Brewster	Control	13	7	7	0	155

SUNBURN SUPPRESSION:

After lackluster performances in 2003 and 2004, data from 2005 trials once again confirm that a number of products are effective against sunburn. Improved results may be attributed to more moderate sunburn pressure and/or more careful site selection and trial designs in 2005. Regardless, all materials tested reduced sunburn at several sites, often to levels of statistical significance. For ease of interpretation, data from multiple sunburn categories has been pooled in Table 3 to reflect how much fruit each treatment saved from cullage. Sunburn was especially prolific on Golden Delicious in Othello, where tree limbs throughout the trial block were unfortunately repositioned in August to accommodate tractor/sprayer travel, causing sudden exposure of unacclimated fruit. Overall, we are encouraged by good performances from newer calcium-based products (Eclipse and Fruit Shield), which have potential to increase market competition for Surround WP and Raynox and drive down prices for all materials. Unfortunately, we have yet to observe an increase in fruit calcium levels from treatment with Fruit Shield (Genesis Ag) or Eclipse (D & M Chemical).

Table 3. Sunburn-related cullage of exposed fruit. WTFRC 2005.

Trial	Treatment	Packable fruit	Sunburn cullage	Fruit weight
		%	%	g
Fuji	Raynox	89	11	192
M.26	Eclipse + SilWett	81	19	204
Azwell	Fruit Shield	84	16	197
	Surround WP	82	18	193
	Control	80	20	188
Fuji	Raynox	96	4	245
M.9	Control	90	10	238
Orondo				

Golden Delicious	Raynox	68	32	241
M.7	Eclipse + SilWett	68	32	232
Othello	Fruit Shield	71	19	238
	Surround WP	55	45	235
	Control	32	68	238
Fuji	Raynox	99	1	203
M.9	Eclipse + SilWett	98	2	205
Quincy	Fruit Shield	97	3	224
	Surround WP	95	5	222
	Control	92	8	212
Fuji	Raynox	95	5	247
M.9	Control	82	18	260
Royal City				

Note: all materials were applied approx. every 2 weeks from early July until harvest

For the sake of efficiency, sunburn counts have traditionally been conducted by WTFRC and other programs by assaying a sample of "exposed" fruit in selected trees. These counts have used sound sampling techniques and produced relevant results in that they accurately reflected how one treatment performed in proportion to another or to an untreated control. The primary drawback of this approach has been uncertainty regarding the correlation of sunburn percentages as reported with the true extent of damage to the entire population of fruit in a given tree or block.

Working with Schrader's group (WSU-TFREC), we assayed samples of only exposed fruit and then all fruit from those same whole trees in an effort to extrapolate what traditional counts might mean in terms of a packout of all fruit from a given block. Counts were conducted in two trials reflecting standard orchard systems with sunburn pressure judged to be moderate to significant. We found that percentages derived from sun-exposed fruit exaggerated the extent of damage by factors of 3-6x. Of course, this relationship is profoundly influenced by tree structure, crop load, and sampling criteria used by the data collector, but nonetheless points out that research data based on sampling rarely accurately reflect the absolute values to be expected for an entire population of fruit, trees, insects, or any other subject of study.

Table 4. Correlation of on-tree sunburn counts of exposed fruit with whole-tree counts of same trees. WTFRC 2005.

Trial	Treatment	Clean	Yellow 1	Yellow 2	Yellow 3	Tan	Black
		%	%	%	%	%	%
SUN-EXPOSED FRUIT							
Fuji	Raynox	75 ns	14 ns	9 ns	2 b	0 b	0
M.26	Eclipse + SilWett	63	18	14	5 ab	0 b	0
Azwell	Fruit Shield	68	16	13	3 ab	0 b	0
	Surround WP	64	18	13	5 ab	1 a	0
	Control	64	16	15	5 ab	0 b	0
Fuji	Raynox	86 ab	13 ab	1 c	0 b	0 ns	0 ns
M.9	Eclipse + SilWett	82 abc	16 ab	2 c	0 ab	0	0
Quincy	Fruit Shield	82 abc	15 ab	2 c	1 ab	0	0

	Surround WP	79 cd	16 ab	5 ab	0 ab	0	0
	Control	73 d	19 a	7 a	1 a	0	0
WHOLE TREE COUNTS							
Fuji	Raynox	96 ns	2 ns	1 ns	0 ns	0 ns	0 ns
M.26	Eclipse + SilWett	98	2	1	0	0	0
Azwell	Fruit Shield	95	3	1	1	0	0
	Surround WP	97	2	1	0	0	0
	Control	94	4	2	0	0	0
Fuji	Raynox	95 ns	3 ns	1 ns	0 ns	0 ns	0 ns
M.9	Eclipse + SilWett	95	3	2	0	0	0
Quincy	Fruit Shield	96	3	1	0	0	0
	Surround WP	94	4	1	1	0	0
	Control	93	5	2	1	0	0

Fruit treated with the above sunburn protectants was run across a research packing line with a standard wet dump tank and brush bed. No material residue was observed at the end of the line except Surround, which was still slightly visible in the stem bowl and calyx ends of fruit.

RETURN BLOOM:

Research trials from the Midwest and East Coast have suggested that serial application of low rates of NAA through the middle of the growing season can increase return bloom. Growers in Washington are interested in developing new programs which may augment or provide alternatives to ethephon-based programs which achieve those same goals. Six trials were conducted in 2004 evaluating various combinations and rates of NAA and ethephon applied at roughly two-week intervals starting after June drop. Table 5 highlights results from trials featuring combinations of ethephon and NAA, while Table 6 shows results from trials only utilizing NAA. Even though the data lack consistency and statistical clarity, we are encouraged by these results and look forward to evaluating our 2005 trials when we assess return bloom in 2006.

Table 5. 2004 fruit quality and 2005 return bloom. WTFRC return bloom trials 2004-2005.

Trial	Treatment	2005 return bloom	2005 return bloom / CSA	Fruit firmness	Fruit weight	Russeted fruit
		%	clusters / cm^2	lbs	g	%
Cameo	Ethrel 48 oz/A	39 ns	0.6 ns	14.1 ab	233 ns	26 ab
Bud.9	Ethrel 48 oz/A + NAA 5ppm 2x	40	0.9	13.8 ab	225	40 a
Tonasket	Ethrel 48 oz/A + NAA 5ppm 3x	36	0.7	13.7 ab	218	16 b
	Ethrel 72 oz/A	43	0.9	14.1 ab	218	25 ab
	K-Salt NAA 5ppm	19	0.6	13.2 b	224	26 ab
	NAA 5 ppm	39	1.0	13.7 ab	226	29 ab
	Control	66	0.9	14.1 a	228	34 ab
Pacific Rose	Ethrel 48 oz/A	311 a	19.2 ab	16.7 ns	269 ns	24 ns
M.26	Ethrel 48 oz/A +	271 ab	17.9 ab	16.3	268	38

	NAA 5ppm 2x					
Brewster	Ethrel 48 oz/A + NAA 5ppm 3x	342 a	20.0 ab	16.5	263	25
	Ethrel 72 oz/A	350 a	22.3 a	16.7	254	45
	K-Salt NAA 5ppm	159 b	9.7 c	16.6	276	24
	NAA 5 ppm	212 ab	10.6 c	17.1	268	38
	Control	274 ab	14.3 bc	17.6	272	31

Table 6. 2004 fruit quality and 2005 return bloom. WTFRC return bloom trials 2004-2005.

Trial	Treatment	2005 return bloom	2005 return bloom / CSA	Fruit firmness	Fruit weight	Russeted fruit
		%	clusters / cm^2	lbs	g	%
Cameo	NAA 10ppm 4x	70 ns	1.6 ns	14.4 ns	228 ns	19 ns
Bud.9	NAA 10ppm 8x	45	1.1	14.4	219	18
Tonasket	NAA 10ppm + Regulaid 8x	36	0.8	14.1	220	24
	NAA 5ppm 4x	60	1.3	14.2	226	25
	NAA 5ppm 8x	69	1.3	14.7	226	16
	K-Salt NAA 10ppm + Regulaid 8x	13	0.4	14.1	218	24
	Control	28	0.7	14.2	226	21
Pacific Rose	NAA 10ppm 4x	158 ns	12.5 ns	17.0 ns	259 ns	26 ab
M.26	NAA 10ppm 8x	212	14.6	16.9	251	26 ab
Brewster	NAA 10ppm + Regulaid 8x	224	12.8	17.5	242	20 b
	NAA 5ppm 4x	207	13.8	16.8	259	34 ab
	NAA 5ppm 8x	197	13.3	17.0	253	36 ab
	K-Salt NAA 10ppm + Regulaid 8x	237	15.9	16.6	261	24 ab
	Control	141	11.2	17.3	271	40 a

Project Title: Sprays to improve packouts and annual cropping in apple
PI: Jim McFerson
3 year project total: $110,400

Item	2005	2006	2007
Timeslip	30,000	30,000	30,000
Benefits (~16%)	4,800	4,800	4,800
Supplies	1,500[1]	1,500[1]	1,500[1]
Travel	500	500	500
Total	36,800	36,800	36,800

[1] Chemicals, fruit purchase

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

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project #AH-04-412 **WSU Project** #3355-6420

Project Title: Impact of cultural practices on apple canopy carbon physiology

PI: Matthew Whiting
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Co- Investigators: Horst Caspari, Department of Horticulture and Landscape Architecture, Colorado State University, Grand Junction, CO

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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:

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
- the 4-d spray interval appeared effective for maintaining a deficit carbon balance
- trees recovered quicker from SO treatment than from FOLS treatment

Deficit irrigation trial
- neither deficit irrigation regime (DI or PRD) affected significantly whole-canopy or leaf gas exchange or stomatal conductance
- water use efficiency did not vary among irrigation treatments
- 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 trial

- 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).

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

Chemical thinner trial

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 2005 we compared the effects of applying FOLS and soy oil emulsion (SO) at a 4-day interval. Our data from previous research suggested that the reductions in net photosynthesis from a 7-day interval were insufficient to elicit an adequate thinning response (see our report in 2003 Apple Hort/Path book).

Table 1. Summary of effects of thinning treatments fish oil + lime sulphur (FOLS) and soy oil (SO) on whole-canopy net CO_2 exchange rate (as a percent of untreated control) 5-yr-old 'Fuji'/'M.26' trees. NCERs are calculated over the period listed, air temperature is reported as the diurnal mean. 80%FB application was on 22-April, 80% FB+4 application was on 26-April, and 80%FB+8 application was on 30-April.

Treatment	22-Apr	23-Apr	24-Apr	25-Apr	26-Apr	27-Apr	28-Apr	29-Apr	30-Apr	1-May	2-May	3-May
Measurement period	80% FB Application	0600-1730	too windy	1000-2000	80%FB+4 application	1500-2000	0600-2000	0600-2000	1630-2000	0600-2000	0600-2000	0600-2000
Mean daily air temp (^{0}C)		16.3		16.6		15.1	12.7	13.5	12.5	16.2	14.7	17.1
Control		100		100		100	100	100	100	100	100	100
SO		68		87		72	58	86	82	69	82	87
FOLS		81		87		72	65	73	85	67	72	78

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.

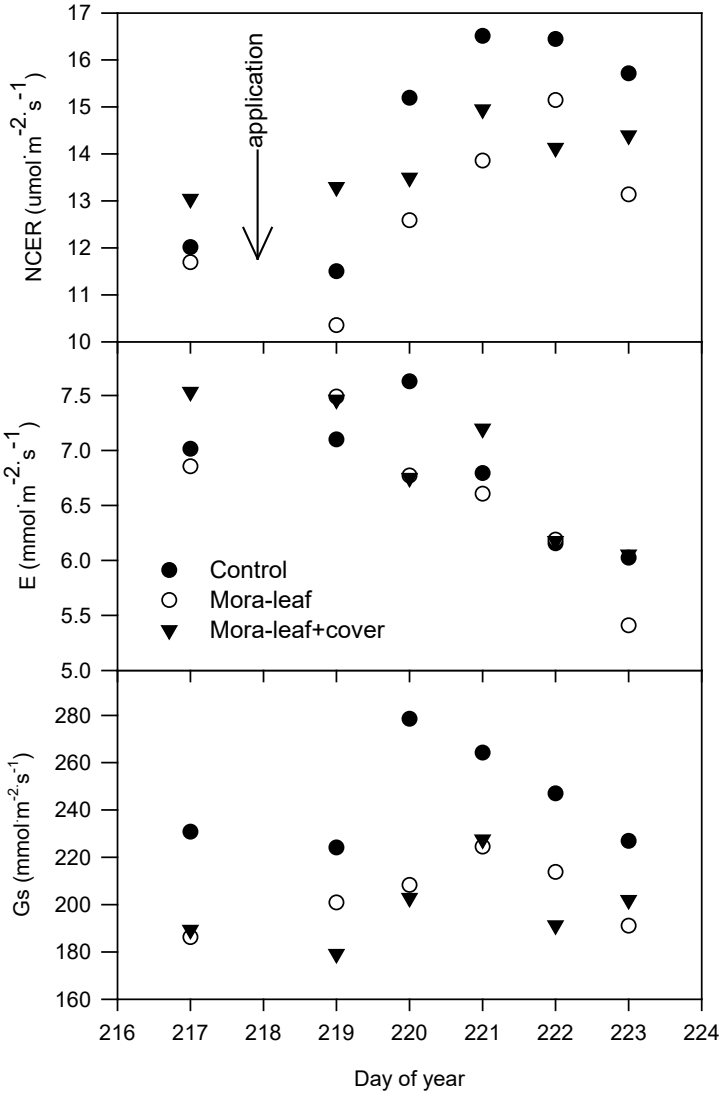

Figure 1. Effect of Mora-leaf® alone and in combination with insecticide cover spray on leaf net CO_2 exchange rate of 11-year-old 'Fuji' apple trees. Applications were made on 6 August (DOY 218). See Methods section for treatment details. n = 5.

Calcium + cover spray trial

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 (Fig. 1). 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 (Fig. 2). 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.

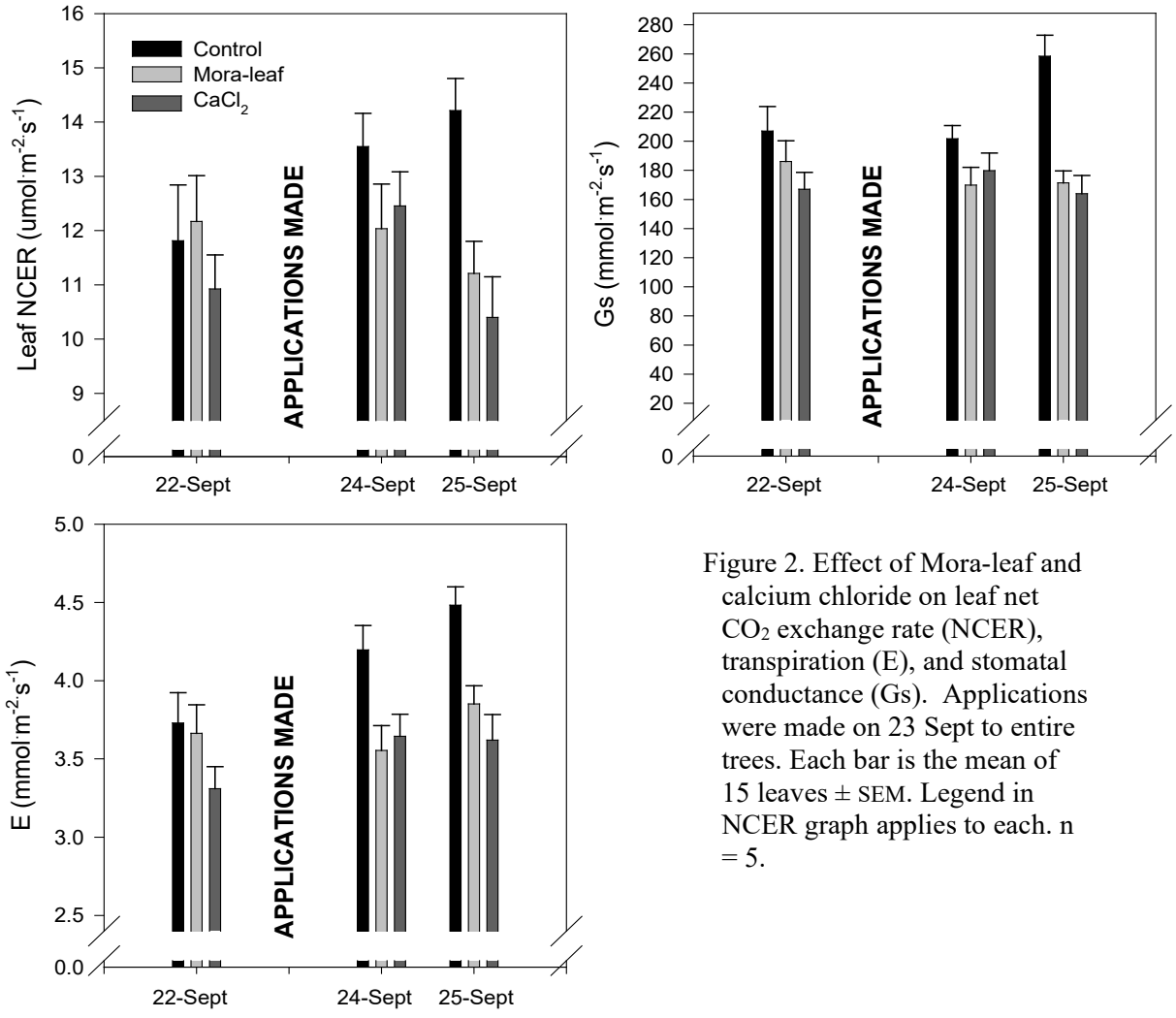

Figure 2. Effect of Mora-leaf and calcium chloride on leaf net CO_2 exchange rate (NCER), transpiration (E), and stomatal conductance (Gs). Applications were made on 23 Sept to entire trees. Each bar is the mean of 15 leaves ± SEM. Legend in NCER graph applies to each. n = 5.

Deficit irrigation trial

This project also has 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 this volume (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 water potential to improve our assessment of stress.

BUDGET:

Project duration: 2004-2006

	Year	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
	Total	$ 25,740	$ 25,740	$ 25,740

Current year breakdown:

	Item	Year 1 (2003)	Year 2 (2004)	Year 3 (2005)
01	Timeslip[1]	$ 14,000	$ 14,000	$ 14,000
07	Timeslip benefits (16%)	$ 2,240	$ 2,240	$ 2,240
03	Goods and Services[2]	$ 2,500	$ 2,500	$ 2,500
06	Equipment[3]	$ 4,000	$ 4,000	$ 4,000
04	Travel[4]	$ 3,000	$ 3,000	$ 3,000
	Total	$ 25,740	$ 25,740	$ 25,740

[1] Assistance with data collection - 4 persons part-time, for a total of approx. 1650 hours @ $8.50/hr).

[2] Mylar (approx. 250 ft @ $0.60/ft), Velcro (approx. 600 ft @ $0.48/ft), polyethylene, tubing, etc. for chamber construction, supplies (we construct new chambers every year).

[3] Portable generator and blowers.

[4] In-state travel to orchard research plots for data collection.

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project # AH-04-420

Project Title: Target-specific control of fungal pathogens by natural compounds
PI: Dr. Bruce C. Campbell
Organization & Address: Plant Mycotoxin Research Unit (PMR), Western Regional Research Center USDA-ARS, 800 Buchanan St., Albany, CA 94710 (510-559-5846 bcc@pw.usda.gov)
CO-PI(s) and affiliation(s): Dr. Jong Heon Kim, PMR, (510-559-5841, jhkim@pw.usda.gov)

Objectives:
Identify new natural compounds effective as antifungal or antimycotoxigenic. In apple orchards, controlling a phytopathogenic disease is problematic as chemical controls are currently not available for several fungal diseases. In the first year of the study, we identified a set of natural compounds with great promise for controlling fungal pathogens. In the second year we focused on determining an effective method for delivery of newly discovered natural compounds, leading to a target-specific strategy for an easy, safe and economic approach to pathogen control. We proved antioxidative stress response systems are essential for fungal tolerance to natural compounds identified and are potentially useful molecular targets for control of fungal pathogens such as *Penicillium*. These compounds are especially effective in significantly augmenting the biocidal activity of certain commercial fungicides (*e.g.*, strobilurins)

Significant Findings (Year 2):
- We used the yeast *Saccharomyces cerevisiae* as a model fungal system to discover vulnerable gene targets. Using gene deletion mutants lacking specific genes in the antioxidative stress response system, we identified four groups of molecular targets of certain natural phenolic compounds (*i.e.,* benzoic or cinnamic acid derivatives). The gene targets identified were: i) regulators of pH responsive transcription or glutathione transferase transporter, ii) vacuolar H(+)-ATPase (V-ATPase), iii) mitogen-activated protein kinase (MAPK) kinase and iv) antioxidative enzymes.
- By using sixteen yeast mutants lacking genes in six different MAPK signaling pathways, we confirmed mutants defective in cell wall construction were also sensitive to the test compounds.
- Selected phenolic agents were used in target-gene based bioassays to determine their impact on mitochondrial respiration. Targeting mitochondrial superoxide dismutase (Mn-SOD) with phenolic acid derivatives (*e.g.,* vanillylacetone) resulted in a 100 to 1000 fold greater sensitivity to strobilurin or carboxin fungicides. This synergism is significantly greater with strobilurin than with carboxin, suggesting that complex III of the mitochondrial respiratory chain is a better target than complex II for fungal control, using phenolics.
- Positive interaction between phenolics and concanamycin A, an inhibitor of V-ATPase, or berberine hemisulfate, an alkaloid targeting mitochondrial superoxide dismutase (Mn-SOD), was observed where combined application of test phenolics with either of these compounds greatly enhanced the inhibition of fungal growth.
- These results show certain natural compounds are effective synergists to commercial fungicides and can be used for improving control of food-contaminating pathogens. Use of such compounds for fungal control reduces environmental and health risks associated with commercial fungicides, and lowers cost for control and the probability for development of resistance to these fungicides.

Methods and Results:
***In vitro* susceptibility bioassays.** Cinnamic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, caffeic acid, ferulic acid, benzaldehyde, veratraldehyde, vanillin, benzoic acid, vanillic acid, 4-hydroxybenzoic acid, 3,4,5-trimethoxybenzoic acid, 3-chlorobenzoic acid, salicylic acid, vanillylacetone, thymol, carboxin (inhibits

complex II of the mitochondrial respiratory chain), strobilurins (inhibits complex III of the mitochondrial respiratory chain) and berberine hemisulfate were examined in fungi. For yeast assays, ≈10^6 cells were cultured in YPD (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) and serially diluted from 10-fold to 10^5-fold in SG (0.67% Yeast nitrogen base w/o amino acids, 2% glucose with appropriate supplements: 0.02 mg/ml uracil, 0.03 mg/ml amino acids). Cells from each serial dilution were spotted adjacently on SG agar medium incorporated with each phenolic to be tested. Cells were grown at 30 °C for 7 days. Numerical scoring is: 6= colonies were visible in all dilutions, 0= no colonies were visible in any dilution, 1= only the undiluted colony was visible, 2= the undiluted and 10-fold diluted colonies were visible, *etc.* For fungal assays ~200 spores were diluted in Phosphate-Buffered Saline (PBS) and spotted in the center of Potato Dextrose Agar (PDA) plates containing phenolic reagents and/or inhibitors of mitochondrial respiration and observed after 7 days at 28 °C. Colony growth was measured based on percent radial growth compared to control colonies.

Enhanced growth inhibition of fungi by co-application of test compounds (Positive interaction). Phenolic compounds were added to the medium with strobilurin, carboxin, concanamycin A or berberine hemisulfate. The cell growth was monitored for 5 to 7 days at 30 °C or 28 °C for yeast or fungal pathogens, respectively. The types of medium, culture condition and measurement of the cell growth were as described in the *in vitro* susceptibility section.

To test the positive interaction with concanamycin A, o-*coumaric acid was chosen and combined in the medium for target fungi (*Penicillium, *aspergilli, and yeast). Positive interaction between berberine hemisulfate (0.5 mM) and phenolics (vanillylacetone 10 mM, veratraldehyde 5 mM, vanillic acid 3 mM, vanillin 1 mM, cinnamic acid 0.1 mM,* m-*coumaric acid 5mM) were tested in the yeast as described in the* in vitro *susceptibility bioassay section. For other fungi,* i.e*., aspergilli and* Penicillium*, berberine hemisulfate (0.5 or 1 mM) and vanillylacetone (5 or 10 mM) were given together in the PDA medium, and the cell growth was monitored for 5 to 7 days.*

Results and discussion:

Identification of molecular targets of phenolic agents in *S. cerevisiae.* As an approach to identify vulnerable gene targets in pathogenic fungi, we first tested the levels of sensitivity in our model fungal yeast system. We examined forty-six *S. cerevisiae* deletion mutants defective in the antioxidative stress response system against seventeen phenolic agents (Kim *et al.* 2005, In press). We chose minimum effective concentration (MEC) for each compound where the growth of the wild-type yeast was almost not affected but reduced colony size. We reasoned molecular target(s) most crucial for cellular tolerance/detoxification against the antifungal phenolics could be identified under this condition. Four classes of mutants showing hypersensitivity to many of the benzoic or cinnamic acid derivatives were identified, as follows: i) regulators of pH responsive transcription (*rim101*Δ) or glutathione transferase transporter (*ure2*Δ), ii) V-ATPase system (*tfp1/vma1*Δ, *vph2*Δ), iii) mitogen-activated protein kinase kinase (*hog4*Δ; MAPKK), and iv) antioxidative enzymes for glutathione biosynthesis (*gsh1*Δ, *gsh2*Δ)/superoxide dismutation (*sod1*Δ, *sod2*Δ). Different levels of sensitivity with the structure-activity relationship were also observed between compounds, while benzaldehyde or 4-hydroxybenzoic acid at the given concentrations did not affect the growth of most of the strains tested. Genes identified are suggested to be important targets for control of target phytopathogenic fungi. The levels of antifungal activity of phenolics were not correlated to their acidic nature, but were due to certain structural characteristic(s).

Identification of an effective antifungal target on the mitochondrial respiratory chain. Our previous bioassays using the S. cerevisiae tsa1Δ mutant showed phenolics such as vanillylacetone affect the normal function of mitochondrial respiration of yeast (Kim et al. 2005, In press). To target mitochondrial respiration and the antioxidative response system more efficiently for fungal control, we applied vanillylacetone to synergize the effects of the fungicides carboxin or strobilurin. Application of vanillylacetone enhanced the level of growth inhibition by these fungicides (**Table 1**). There was greater synergism in activity of

strobilurin than that of carboxin by vanillylacetone. This result suggests complex III of the respiratory chain is an efficient target for fungal control. Thus, using vanillylacetone as a synergist may significantly reduce potential for development of resistance to these types of fungicides that inhibit mitochondrial respiration; a frequent problem with conventional fungicides.

Table 1. Effects of different concentrations of fungicides (μM) carboxin and strobilurin combined with vanillylacetone (mM) on growth of fungi *Penicillium expansum* (*Pe*), *Aspergillus niger* (*An*) and *A. flavus* (*Af*)[a].

Vanillylacetone (mM) / Fungal species / Fungicide (μM)	0 *Pe An Af*	5 *Pe An Af*	10 *Pe An Af*	15 *Pe An Af*	20 *Pe An Af*
None 0	100,100,100	80,100,100	64, 60, 91	40, 19, 58	24, 0, 29
Carboxin 50	75, 100, 98	60, 89, 91	29, 32, 64	~0, 17, 29	0, 0, 0
100	71, 100, 96	52, 83, 85	19, 24, 47	0, 0, 17	0, 0, 0
150	52, 100, 96	~0, 70, 70	0, 20, 32	0, 0, 0	0, 0, 0
Strobilurin 50	71, 100,100	24, 28[b], 62	0, 0, 18	0, 0, 0	0, 0, 0
100	69, 100, 98	0, 0, 55	0, 0, 0	0, 0, 0	0, 0, 0
150	63, 100, 96	ND[c]	ND[c]	ND[c]	ND[c]

[a] Growth of fungi is represented as a percentage of radial growth of the fungal mat of treated compared to control (no inhibitors of mitochondrial respiratory chain). Values are means of three replicates, standard deviations of all measurements were <2% except where noted. ~0 means barely germinated.; [b]SD: 17%; [c]Not detected due to precipitation.

Identification of berberine derivative as a natural synergist for targeting mitochondrial superoxide dismutase. The alkaloid berberine, or its derivatives, was previously shown to inhibit the growth of fungi or selectively inhibit MAPKK Wis1 of the stress-activated protein kinase pathway (SAPK) in *Schizosaccharomyces pombe* (Jang *et al*. 2002). Since we identified the *pbs2*Δ (MAPKK) mutant of *S. cerevisiae* to be sensitive to most of the test compounds, we also tested the effect of berberine hemisulfate on the antioxidative stress response system, especially on the activity of the MAPK signaling pathway of *S. cerevisiae*. We observed that, instead of MAPK mutants, *sod2*Δ strain (deleted Mn-SOD) showed ~10 times higher sensitivity to this compound compared to the wild-type cells (1 to 3.5 mM; data not shown). This result strongly suggests berberine hemisulfate targets the activity of mitochondrial superoxide dismutase. We, then tested the effect of berberine hemisulfate as a synergist for fungal control by co-applying it with five phenolic agents, *i.e*., vanillylacetone (10mM), vanillin (1mM), veratraldehyde (5mM), cinnamic acid (0.1mM) and *m*-coumaric acid (5mM) to which the *sod2*Δ mutant showed sensitivities in our previous tests. We found co-application of berberine hemisulfate with vanillylacetone or veratraldehyde greatly enhanced (>10, 000 times) the growth inhibition of both wild-type and *sod2*Δ strains where *sod2*Δ mutant was ~10 times more sensitive than the wild-type strain (data not shown). Our study also indicated that, like antimycin A or strobilurin, which are inhibitors of complex III in the mitochondrial respiratory chain, vanillylacetone, veratraldehyde and berberine hemisulfate affect normal mitochondrial function under oxidative stress conditions (data not shown; See Kim *et al*. 2005 for *tsa1*Δ bioassay). Our results strongly suggest these compounds negatively affect the function of the common molecular target, *i.e*., Mn-SOD. We further tested

the positive interaction between berberine hemisulfate and vanillylacetone in *P. expansum* and different aspergilli (*i.e., A. ochraceous*, *A. niger*, *A. nidulans, A. fumigatus*, *A. flavus*, *A. parasiticus*), and observed the positive interaction of the compounds in most of the fungal strains tested; where the levels of sensitivities varied depending on fugnal species (**Table 2**; *A. fumigatus, A. nidulans* > other aspergilli, *P. expansum*).

Table 2. Enhanced growth inhibition of aspergilli and *P. expansum* with the co-application of vanillyl acetone and berberine hemisulfate[a].

Vanillylacetone (mM)	0			5			10		
Berberine hemisulfate (□M)	0	0.5	1.0	0	0.5	1.0	0	0.5	1.0
A. fumigatus AF293 (WT)	*100*	*63±5*	*15±26*	*100*	***0***	***0***	46	**0**	**0**
A. flavus NRRL 3557 (WT)	100	100	93	91	**64**	**57**	64	**25**	**21**
A. parasiticus NRRL 5862 (WT)	100	100	96	86	**63**	**57**	65	**16**	**0**
A. ochraceous NRRL 5175 (WT)	100	83	78	67	**40**	**38**	31	**24**	**21**
A. oryzae FGSC A815 (WT)	100	90	86	82	**51**	**45**	53	**0**	**0**
A. niger NRRL326 (WT)	100	81	79	114	**77**	**72**	46	**0**	**0**
A. nidulans FGSC A4 (WT)	100	71	58	78	**0**	**0**	39	**0**	**0**
P. expansum NRRL 974 (WT)	100	83	83	83	**65**	**61**	61	**52**	**43**

[a] Fungal growth is represented as a percentage of radial growth compared to control colonies grown on PDA plates receiving only DMSO. Values where positive interaction of the test compounds occurred are in red characters. Values are means of three replicates. Standard deviations of all measurements are <5% except where noted. WT: wild-type.

Targetng fungal V-ATPases using concanamycin A and phenolic agents. One of the promising tools to disrupt cellular pH homeostasis is targeting the V-ATPase system. The V-ATPases are multi-enzyme complexes located on the vacuolar membrane (Graham *et al.* 2003). Acidification of vacuoles is necessary for the generation of a proton gradient, leading to intracellular pH homeostasis. Vacuolar compartmentalization of toxic substances or xenobiotics is also important for cellular detoxification, where dysfunction in V-ATPase system results in severe defects in mitochondrial respiration. Results in the yeast bioassay showed co-application of concanamycin A (V-ATPase inhibitor; 2 μM) and *o*-coumaric acid (5 mM) highly enhanced the level of growth inhibition of all yeast strains tested (wild-type and mutants defective in pH homeostasis; **Table 3**).

Table 3. Enhanced inhibition of the growth of *S. cerevisiae* wild-type and *rim101*Δ/V-ATPase mutants with the co-application of *o*-coumaric acid and concanamycin A [a].

Concanamycin A (μM)	0			2			4 to 10		
o-Coumaric acid (mM)	0	5	10	0	5	10	0	5	10
Wild-type	6	5	1	6	4	1	6	4	0
*tfp1*Δ/*vma1*Δ□□□□	6	3	1	6	2	0	6	2	0

*vph2*Δ	6	3	1	6	2	0	6	2	0
*rim101*Δ	6	4	1	6	2	0	6	2	0

[a] Responses for yeast growth are represented as a logarithmic number. Values are means of two replicate.

Targeting stress responsive MAPK pathways of fungi using phenolic agents. As mentioned above, the *pbs2*Δ, lacking the MAPKK gene in the oxidative/osmotic stress response MAPK pathway, was sensitive to many of the phenolic agents tested. In *S. cerevisiae*, there are six different MAPK signaling pathways identified, *i.e.*, pathways for sprorulation, mating response, morphological switch, cell wall integrity, cell wall construction and oxidative/osmotic stress response. To see if the sensitive response to phenolic agents is unique to the anti-oxidative stress system, we examined eighteen mutants lacking key genes in the six MAPK signaling pathways by applying the test compounds described above. We observed that, in addition to the *pbs2*Δ mutant (MAPKK mutant; oxidative stress/osmoregulation pathway), *slt2*Δ (MAPK mutant; cell wall construction pathway) and *bck1*Δ (MAPKKK mutant; cell wall construction pathway) mutants showed sensitivities to *o*-coumaric acid, veratraldehyde, vanillylacetone or thymol. The *SLT2* and *BCK1* genes were also shown to respond to environmental stress (Garcia-Rodriguez *et al.* 2005; Harrison *et al*. 2004), strongly indicating responses to the phenolic agents exerted mainly by the stress-activated MAPK (SAPK) pathways.

Since thymol possesses antifungal activity by altering both the cell wall and membrane of *S. cerevisiae* (Bennis *et al*. 2004), we further tested *pbs2*Δ, *slt2*Δ and *bck1*Δ mutants in the presence of Congo Red, an agent interfering with cell wall assembly. We observed that the *slt2*Δ and *bck1*Δ mutants showed hypersensitivity to Congo Red, whereas the *pbs2*Δ mutant (like the wild-type strain) did not (**Figure 1**). Defects in cell wall construction will, therefore, result in a hypersensitive response to certain phenolic agents. Co-application of Congo red with thymol greatly enhanced the sensitivity of *S. cerevisiae slt2*Δ and *bck1*Δ strains to these compounds (See **Figure 1**).

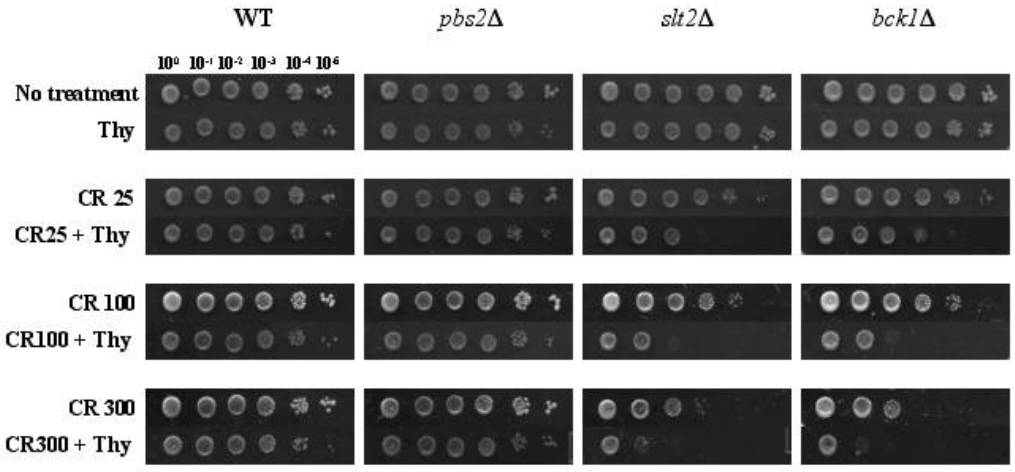

Figure 1. Enhanced inhibition of the growth of *S. cerevisiae slt2*□ and *bck1*□ mutants with the co-application of Congo red and thymol. Cells were grown at 30 °C for 7 days. CR: Congo red (□g/ml), Thy: Thymol (0.5mM).

Summary. During this second-year cycle we identified a potentially effective approach to fungal control using newly discovered natural compounds that have a target-specific basis of activity. Antioxidative stress response systems of fungi can be an efficient molecular target of phenolics for pathogen control. We proved positive interaction between phenolics and conventional fungicides or berberine hemisulfate significantly augment the fungicidal effects of commercial fungicides by reducing the costs of application, development of resistance, or contamination of the environment. We conclude natural compounds such as phenolic agents that do not have any significant medical or environmental shortcomings could be useful in control programs involving conventional antifungal or antimycotoxigenic agents.

Budget:

Project Title: Target-specific control of fungal pathogens of tree fruit by natural compounds
PI : Bruce C. Campbell, PMR, WRRC
CO-PI: Jong Heon Kim, ibid, (510-559-5841, jhkim@pw.usda.gov)
Project duration: 2004-2006

Current year: 2005
Project total (3 years): $66,966
Current year request: $21,856

Item	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
Salaries	$14,192[1]	$14,618[1]	$15,056[1]
Equipment	$5,000[2]	$3,000[2]	$2,800[2]
Supplies	$2,700[3]	$4,000[3]	$4,000[3]
Travel	$800[4]	$800[4]	
Total[5]	$22,692	$22,418	$21,856

[1]Salary for one half-time GS5 Biological Lab. Tech. with 3% projected salary increase for FY05 and 06. [2]2004-Upgrade DNA sequencer; 2005- Plant growth chamber; 2006- PCR thermo-cycler [3]2004- Yeast & fungal strains, kits (nucleic acids work), oligos, plasmids; 2005 & 2006- kits (nucleic acids & protein works), oligos, plasmids, fungal growth media, chemicals. [4]2004 & 2005- Washington State, field trip to orchards to isolate pathogens. [5]Technical support, equipment and supplies are being requested in this proposal for added ability to research on functional genomics and control of phytopathogenic fungi of orchard crops.

References:

Bennis, S., Chami, F., Chami, N., Bouchikhi, T. and Remmal, A. 2004. Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. *Lett. Appl. Microbiol.* 38, 454-458.

Garcia-Rodriguez LJ, Valle R, Duran A and Roncero C. 2005. Cell integrity signaling activation in response to hyperosmotic shock in yeast. *FEBS Lett*. 579:6186-6190.

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Jang, M.J., Jwa, M., Kim, J.H. and Song, K. 2002. Selective inhibition of MAPKK Wis1 in the stress-activated MAPK cascade of *Schizosaccharomyces pombe* by novel berberine derivatives. *J. Biol. Chem.* 277, 12388-12395.

Kim, J.H., Mahoney, Chan, K.L., Molyneux, R.J. and Campbell, B.C. 2005. Controlling food-contaminating fungi by targeting their antioxidative stress-response system with natural phenolic compounds. *Appl. Microbiol. Biotechnol.* DOI 10.1007/s00253-005-0123-6 *In press.*

CONTINUING PROJECT PROPOSAL **YEAR 1/3**
WTFRC Project #: AH-05-506 **WSU Project #:** 13C-3655-6325

Project title:	Improving fruit finish and fruit quality in apples
PI:	Larry Schrader
Organization:	WSU Tree Fruit Research and Extension Center, Wenatchee; (509) 663-8181 x265; schrader@wsu.edu
Cooperators:	Jim Mattheis, USDA-ARS Tree Fruit Research Lab, Wenatchee Jianshe Sun, David Felicetti, Jianguang Zhang, and Jun Tian, WSU Tree Fruit Research and Extension Center, Wenatchee Jim McFerson and Tom Auvil, Tree Fruit Research Commission, Wenatchee Dana Faubion, Extension Horticulturist, WSU Extension, Yakima John Fellman, Professor, WSU-Pullman Gordon Brown, Scientific Horticulture P/L, Tasmania, Australia
Contract administrators:	Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

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 regular and controlled atmosphere 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 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, and other stress-induced disorders. Fruit will be evaluated before storage and thereafter at intervals of cold storage in regular atmosphere.

Significant findings:

- Observations of fruit development during the entire growing season revealed that Fuji flecking and Golden Delicious russet were induced before 7 weeks after full bloom (WAFB) (Fig. 1). Similarities between the two disorders suggest that flecking is a type of russet.
- To protect fruit from chemical injury, fruit were bagged at intervals after blooming. The incidence of russet and flecking increased significantly as date of bagging was delayed, indicating that Fujis and Goldens are most susceptible to induction of russet early in the season (Fig. 2).
- Induction of flecking and russet was enhanced by chemical thinners (Sevin + NAA, ATS, and lime sulfur). Incidence of the disorders was often highest at ends of rows (more spray applied).
- Damage of cluster leaves near fruit at early stage was strongly related to the occurrence of flecking and russet. At harvest, 96% of fruit with flecking were located near some damaged cluster leaves. Thus, induction of flecking can be strongly related to chemical damage.
- Overhead irrigation and evaporative cooling increased expression of flecking and russet.
- Flecking/russet were not reduced in studies this year with GA, RAYNOX, and GA + RAYNOX.
- Pigment changes were studied in Gala, Cameo, Red Delicious, and Golden Delicious. Changes resulting from sunburn damage appear to be cultivar specific.

- Chlorophyll a of sunburned Cameo, Red Delicious, and Golden Delicious apples was significantly lower than in non-sunburned apples, and Chlorophyll b of sunburned Red Delicious and Golden Delicious was significantly lower than in non-sunburned apples.
- The sunburned area and the halo of the sunburned area of Red Delicious showed a significantly higher amount of carotenoids when compared to non-sunburned apples (Fig. 4).
- Gala apples showed a significantly lower concentration of carotenoids in the halo area than in non-sunburned apples.
- Significantly less anthocyanin was found in sunburned Gala and Red Delicious apples and in the halo area of Cameo and Gala as compared to their non-sunburned counterparts.
- Preliminary results on fruit quality of Gala apples from cold storage suggest that firmness and soluble solids increased in fruit with more serious sunburn, whereas titratable acidity decreased (Fig. 5). Studies on Fujis that are showing stain follow the same trends.

Methods:

Objective I on Fuji flecking and Golden Delicious russeting.

- In order to determine the susceptible stages of Fuji flecking and Golden Delicious russet, the occurrence of flecking or russet was tracked with digital photos that were taken (20 apples of each variety) at 3-day intervals during early stages of fruit development (until 2 WAFB) and thereafter at 2-week intervals. Every sampled fruit was labeled. In a second experiment, about 100 fruit were bagged from 2 WAFB at various intervals (about 10-day intervals at early stage and 1-month intervals after July). Bagged fruit were sampled at 7-day intervals for microscopic observation. Final evaluation of flecking and russet was done after harvest. Percent and index of flecking and russet were used for statistical analyses. A statistical method using index of flecking was also established with photos and grade values.
- Study on effect of chemicals (thinners) on Fuji flecking and russet. Four thinners were selected for field trials (lime sulfur, Ethrel, Sevin + NAA, and ATS). In three commercial orchards, flecking and russet were evaluated. Risk of flecking and russet was compared between trees located at end of rows and within rows.
- Flecking with and without EC was evaluated and relative humidity was also measured during the growing season.
- Cooperative studies initiated during 2004 with McFerson and Auvil from the WTFRC were continued to study the effect of early applications of gibberellic acid (GA) alone, RAYNOX alone, and a tank mix of GA + RAYNOX.

Objective II on pigment changes of fruit with stress-induced disorders.

- Methods for extraction and assay of the pigments were described in detail in the new proposal submitted last year. Analyses to separate the various components within a class of pigments with the HPLC were also described, as were non-destructive pigment analyses with a reflectometer.

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 before and after winter storage under regular atmosphere conditions. Soluble solids, titratable acidity, and firmness are being determined using commonly adopted methods. Gala apples that were sunburned to varying degrees are being examined. Fuji stain and lenticel breakdown that appear during cold storage will be recorded, and fruit quality will be determined on these fruit at the end of the storage period.

Results and discussion:

Objective I. Fuji flecking and Golden Delicious russet.

1. Digital images revealed that induction of Fuji flecking and Golden Delicious russet occurs very early. It was visible at 5 WAFB in Fuji and at 7 WAFB in Goldens (Fig. 1). The result was confirmed by a bagging experiment in the field. In fruit bagged later than 7 WAFB, the incidence of flecking was still at a very high rate (about 90%) (Fig.2). Thus, the disorders were induced before 7 WAFB.

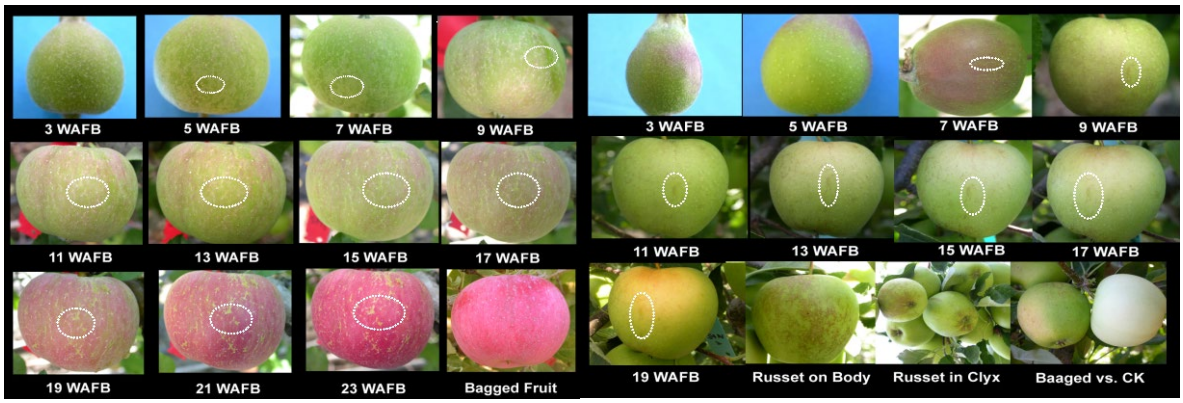

Fig. 1. Appearance of flecking and russet on Fuji (left) and Golden Delicious (right) from exposure to chemicals (thinners, minerals or pesticides).

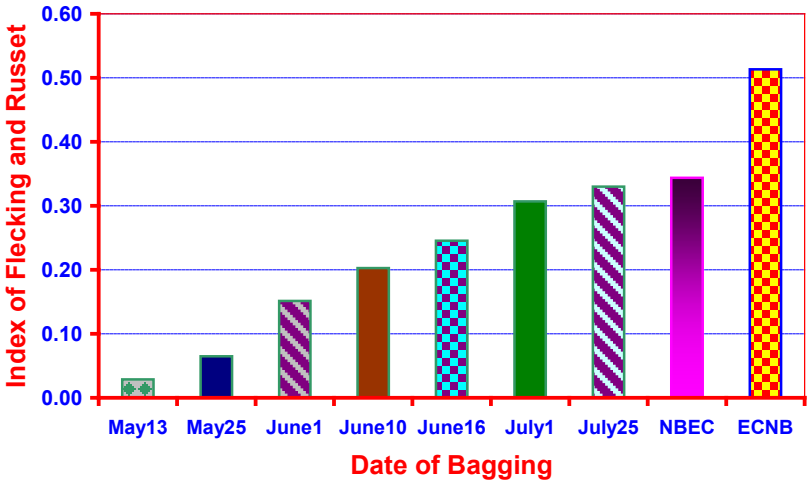

Fig. 2. Effect of bagging date and EC on Fuji flecking and russet (NBEC: no bagging or EC; ECNB: EC without bagging. Bloom date was April 22, 2005. Bags were applied at dates shown. All bags were removed at 3 weeks before harvest. Apples were assessed at harvest).

2. Microscopic studies showed that russet occurring in the calyx resulted mainly from certain thinners. It appeared at an early stage (before 7 WAFB) and may have been induced before fruit

turned downward. Heavy russet in the stem bowl appeared because more water and chemicals were retained for longer duration. In early stages, russet on the fruit body was similar to that on the stem bowl. As fruit surface area expanded, russet became scattered and looked like a net on the fruit body. This is referred to as Fuji flecking.

3. Effects of thinners. Investigation in a commercial Fuji orchard indicated that improper chemical spraying not only damaged young leaves but also significantly enhanced the risk of Fuji flecking and russet. More flecking appeared in some orchards at the ends of rows as compared to trees within the orchard, suggesting that higher levels of chemicals applied at the ends of rows were detrimental. At harvest time, 96% of fruit with flecking were located near some damaged cluster leaves. Thus, occurrence of flecking is strongly related to damage from chemicals. Bagging fruit at very early stage decreased the risk of flecking and russet because bags protected fruit.
4. The incidence of Fuji flecking was increased in those orchards that used overhead irrigation and in those where excessive evaporative cooling was used.
5. Attempts to reduce Fuji flecking and russet with early applications of GA, RAYNOX, and a combination of the two were not successful during 2005. This was not consistent with the promising results obtained during 2004.

Objective IIA. Spectrophotometric analyses of pigments.

1. Pigment changes resulting from sunburn damage appear to be cultivar specific.
2. Chlorophyll a concentration of sunburned apples was significantly lower than in non-sunburned apples for Cameo, Red Delicious, and Golden Delicious. The non-sunburned apples and the halo around sunburned areas showed no significant difference.
3. Chlorophyll b concentration of sunburned Red Delicious and Golden Delicious apples was significantly lower than non-sunburned Red Delicious and Golden Delicious.
4. Carotenoids were significantly higher in the sunburned area and the halo of the sunburned area of Red Delicious, whereas carotenoids in Gala were significantly lower in the halo area than in non-sunburned apples (Fig. 4).
5. Significantly less anthocyanin was found in sunburned Gala and Red Delicious as compared to their non-sunburned counterparts. The halo area of Cameo and Gala had significantly less anthocyanin than their non-sunburned counterparts.
6. Gala showed no significant change in any of the pigments after one month of RA storage.

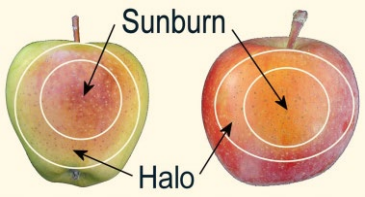

Fig. 3. Illustration depicting the sunburned area and the halo around the sunburned area. The area inside the inner circle is considered the sunburned area. The area between the two circles is considered the halo around sunburned area.

Objective IIB. HPLC analyses.

HPLC analyses to separate the various pigments within a pigment class (e.g. carotenoids) of sunburn and stain samples are incomplete. Samples of sunburned and non-sunburned Red Delicious, Fuji, Gala, and Granny Smith were collected and are in liquid N awaiting analyses.

Objective IIC. Non-destructive pigment analyses.

Preliminary work suggests a strong correlation between pigment concentrations and specific

reflectance ratios. Strong correlations were observed for chlorophyll a, b, carotenoids, and flavonoids at certain peel reflectance ratios (e.g. peel reflectance ratio of 703 nm/680 nm for chlorophyll). This technique shows promise for use as a non-destructive assay of pigments.

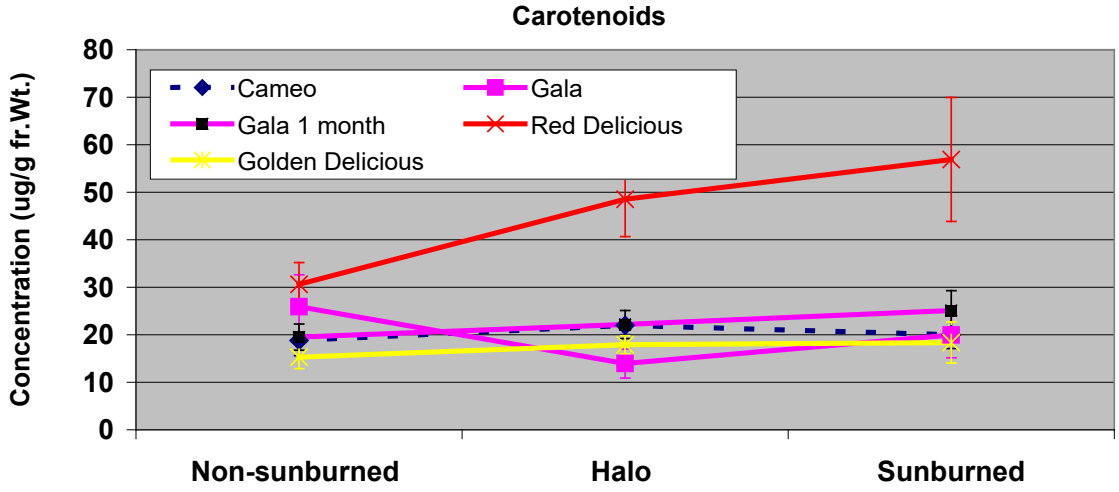

Fig. 4. Total carotenoid concentration of the sun-exposed peel of non-sunburned apples, sunburned apples and the halo around sunburned areas.

Objective III. Fruit quality analyses of fruit with heat and light-induced disorders.

Firmness of Gala apples increased as the severity of sunburn damage increased (e.g. Sb-0 with no sunburn had the lowest firmness and Sb-4 with serious sunburn browning had the highest firmness at harvest and at every later evaluation of firmness (Fig. 5). Soluble solids followed the same trends, but titratable acidity was highest in Sb-0 and lowest in Sb-4 (data not shown). For Fuji stain (fruit harvested October 13), apples on which the stain disorder has appeared have higher firmness and lower titratable acidity than apples with no stain appearing to date. These studies are still in progress.

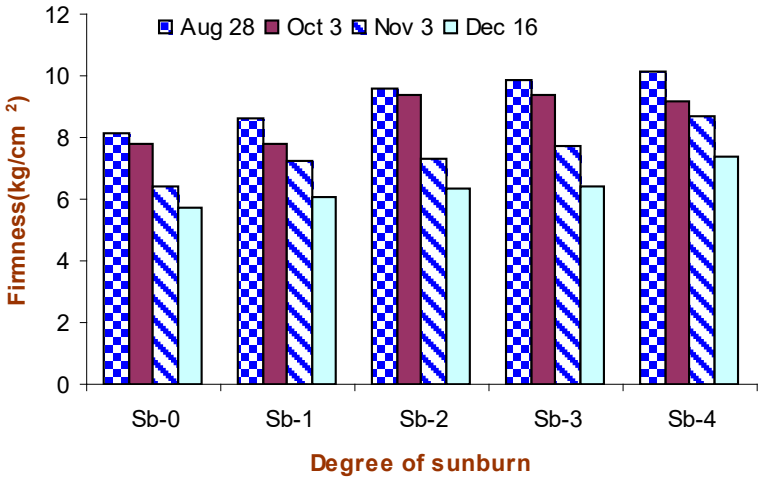

Fig. 5. Changes in firmness of Gala apples that had different degrees of sunburn when placed in cold storage. Apples were harvested August 22. Firmness was determined August 28, October 3, November 3, and December 16.

Project title: Improving fruit finish and fruit quality in apples
PI: Larry Schrader
Project duration: 3 years (2005-2007)
Current year: 2006
Project total (3 years): $344,106
Current budget request: $115,337

Year	Year 1 (2005)	**Year 2 (2006)**	Year 3 (2007)
Total	111,822	**115,337**	116,947

Current year breakdown

Item	Year 1 (2005)	**Year 2 (2006)**	Year 3 (2007)
Salaries[1]	74,336	**76,378**	79,433
Benefits[2]	22,586	**23,859**	24,814
Wages			
Benefits (10%)			
Equipment	0	**0**	0
Supplies[3]	8,500	**8,500**	6,000
Travel[4]	3,400	**3,500**	3,500
Miscellaneous[5]	3,000	**3,100**	3,200
Total	112,000	**115,337**	116,947

[1] Salaries: for Ag Project Assistant ($25,758); for Research Associate ($31,000); for Associates in Research ($4,320—10% appt. +15,300—50% appt.). David Felicetti is the Ag Project Assistant and is a Ph.D. candidate who has completed course work and is working full time on research in Wenatchee. Mr. Felicetti characterized the third type of sunburn and is now working on color development and other fruit finish issues in apples. Dr. Xu (Agricultural Univ. of Hebei) has been offered the Research Associate position (and is awaiting a visa). He will work primarily on the Fuji flecking disorder, evaporative cooling issues, and other fruit finish issues.

A new half-time Associate in Research (to replace Jun Tian) will be employed. A new Senior Associate in Research (to replace Leo Jedlow) will be employed (10% appt. on this project).

[2] Benefits: Ag Project Assistant ($1,932); Research Associate @ 44% ($13,640); Associate in Research @ 44% ($6,732); Senior Associate in Research @ 36% ($1,555).

[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”; cell phone charges; and $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.

[5] 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 advantages of two crops per year and fosters collaboration among scientists with common interests. Only $3,100 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.

Salary for PI and 65% 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.

Continuing PROJECT PROPOSAL **YEAR 1/2**

WTFRC PROJECT #: AH-05-510 **WSU PROJECT #:** 13C-3661-5366

Project title:	Sphaeropsis Rot in Apple
PI:	Chang-Lin Xiao, Assistant Plant Pathologist
Organization:	WSU Tree Fruit Research and Extension Center
Address, phone, e-mail:	1100 N. Western Avenue, Wenatchee; 509-663-8181 ext. 229; clxiao@wsu.edu
Cooperators:	Yong-Ki Kim, Postdoctoral Researcher, WSU TFREC, Wenatchee Dana Faubion, WSU Extension, Yakima Packinghouses in major apple-producing areas in Washington
Contract administrators:	Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

Objectives:

1. Determine the sources and availability of inoculum of *Sphaeropsis pyriputrescens* in the orchard during apple-growing season.
2. Determine seasonal susceptibility of apple trees to infection by *S. pyriputrescens* in the orchard.
3. Determine susceptibility of apple fruit at different growth stages to infection by *S. pyriputrescens* in the orchard in relation to Sphaeropsis rot during storage.
4. Test sensitivity of the fungus to various fungicides including new postharvest fungicides.
5. Determine the prevalence and incidence of Sphaeropsis rot as well as other postharvest diseases on major apple varieties under various handling systems.
6. Evaluate effectiveness of preharvest fungicides and postharvest drench with fungicides in controlling Sphaeropsis rot.

Significant findings:

- In a Fuji orchard infected by *S. pyriputrescens*, 60-90% of the sampled apple trees infected by the fungus and 27-53% of the sampled dead fruit spurs or twigs were infected. Sources of inoculum of the fungus responsible for fruit infection included dead fruit spurs, dead pedicle of flowers or fruit, and twigs with dieback symptoms or cankers. In a Red Delicious orchard, 20-40% of the sampled trees were infected by the fungus. Dead tissues on fruit spurs appeared to be a common source of inoculum for fruit infection. Viable fruiting bodies of the fungus were available throughout the fruit-growing season.
- Cankers and dieback twigs of crabapple trees were not the only sources of inoculum. Crabapple trees, if present, likely facilitated spread of the fungus from crabapple trees to apple trees in the orchard. Fruiting bodies of the fungus on apple trees were the immediate inoculum responsible for infection of fruit in the orchard leading to Sphaeropsis rot during storage.
- Significant cankers developed on twigs inoculated in November and early spring. No significant cankers developed on twigs inoculated in June. It appeared that trees were more susceptible to infection during the dormant period and that Fuji trees seemed to be more susceptible to infection than Red and Golden Delicious. These observations need to be confirmed in the ongoing study.
- Fruit of Fuji, Red Delicious and Golden Delicious were susceptible to infection from May to harvest (September or October), but disease symptoms developed only during storage. On Red Delicious, stem and calyx infections both were common; on Golden Delicious, stem infection

was more common than calyx infection; on Fuji, calyx infection was more common than stem infection. These observations need to be confirmed in the ongoing study.

- Scholar, Mertect and Pristine were highly effective in inhibiting mycelial growth of the fungus, and Penbotec was effective only at higher rates. The information has been used for developing pre- and postharvest fungicide programs for control of Sphaeropsis rot.
- Sphaeropsis rot varied from lot to lot but accounted for approximately 20% of the decay on Red Delicious. Instances of severe Sphaeropsis rot were again observed on Fuji and Red Delicious fruit. Our three-year survey for postharvest diseases on apples indicated that gray mold, blue mold and Sphaeropsis rot should be considered major targets for decay control. Bull's eye rot should also be considered a target disease on Golden Delicious.
- Ziram applied at two weeks before harvest was effective in reducing losses by Sphaeropsis rot in one year but not the following year. A postharvest drench with Mertect reduced losses by Sphaeropsis rot. We are currently evaluating new pre- and postharvest fungicides (Pristine, Topsin M, Scholar and Penbotec) for control of Sphaeropsis rot.

Methods:

Sources and availability of inoculum of *Sphaeropsis pyriputrescens* were monitored in two commercial apple orchards during the apple-growing season in 2005.

Experiments were conducted four times a year to determine susceptibility of trees of Fuji, Red Delicious and Golden Delicious to *S. pyriputrescens*. Three isolates of the fungus and a non-inoculated control were included in the tree inoculation experiments.

To determine susceptibility of apple fruit to infection by *S. pyriputrescens*, fruit of Fuji, Golden Delicious and Red Delicious were inoculated with the pathogen four to five times during the growing season. Fruit were harvested and stored at 32ºF for decay development. Decay incidence and infection sites on the fruit were recorded.

Effectiveness was tested of new fungicides (Scholar, Penbotec, Pristine) and other fungicides in inhibiting mycelial growth and spore germination. Ten representative isolates were included.

Decayed apple fruit were sampled from commercial packinghouses to determine the prevalence and incidence of Sphaeropsis rot and other diseases under the current postharvest handling practices.

An experiment was conducted in a commercial Red Delicious orchard with a history of severe Sphaeropsis rot to evaluate effectiveness of preharvest fungicides and postharvest drench with fungicides in controlling Sphaeropsis rot.

Results and discussion:

Sources and availability of inoculum in the orchard

In 2005, we monitored inoculum availability of the Sphaeropsis fungus in two commercial orchards (Table 1). In the Fuji block, sources of inoculum likely responsible for infection of fruit are as follows: (1) Twig dieback and dead fruit spurs. In 2005, 60-90% of sampled apple trees were infected by the Sphaeropsis fungus; 27-53% of the sampled dead fruit spurs or twigs were infected by the fungus. (2) Fuji fruit mummies on the trees. Fifty-five percent of the mummies sampled in mid-May were infected by the fungus. (3) Cankers, twig dieback and infected fruit of crabapple. Over 90% of sampled crabapple trees were infected by the Sphaeropsis fungus. At Fuji harvest, 48% of the crabapple fruit sampled from the trees was infected by the fungus, and 3% of the infected fruit exhibited fruiting bodies of the Sphaeropsis fungus.

In the Red Delicious orchard, crabapple trees were not commonly planted. The focus was on dead fruit spurs or twigs and dead bark. In 2005, 20-40% of the sampled trees were infected by the fungus; 1-16% of the sampled fruit spurs had pycnidia of the fungus.

The results indicate that dead tissues on fruit spurs, dieback twigs or cankers, and crabapple trees were important sources of inoculum responsible for infection of apple fruit in the orchard leading to Sphaeropsis rot during storage. The results also indicate that viable inoculum of the fungus was

available throughout the fruit-growing season.

Table 1. Sources and availability of inoculum of the Sphaeropsis fungus in two apple orchards in 2005.

Date	Orchard	Variety	Type of samples[1]	%Trees with pycnidia	%Samples with pycnidia
13-May	1	Crabapple	Twigs	100	86.7
		Fuji	Spurs/Twigs	90	53.3
	2	Red Delicious	Bark	20	2
			Spurs	40	8
31-Jun	1	Crabapple	Twigs	100	73.3
		Fuji	Spurs/Twigs	60	26.7
	2	Red Delicious	Bark	0	0
			Spurs	10	2
4-Aug	1	Crabapple	Twigs	100	96.7
		Fuji	Spurs/Twigs	60	26.7
	2	Red Delicious	Bark	0	0
			Spurs	40	16
16-Sep	1	Crabapple	Twigs	100	100
		Fuji	Spurs/Twigs	60	40
	2	Red Delicious	Bark	30	3
			Spurs	20	6

[1] In the Fuji orchard, at each sampling time, 3 dieback twigs from each of 10 crabapple trees and 3 dead fruit spurs or twigs from each of 10 Fuji trees were sampled. In the Red Delicious orchard, 5 pieces of dead fruit-spur tissues and 10 pieces of dead bark tissues from each of 10 trees were sampled.

Seasonal susceptibility of apple trees to cankers caused by the Sphaeropsis fungus

We inoculated trees of Fuji, Golden Delicious and Red Delicious at four different times each year. Canker sizes at 6 months after inoculation are presented in Table 2. Data from other inoculation dates will be forthcoming in the next report. Significant cankers developed on twigs inoculated in November and early spring. No significant cankers developed on twigs inoculated in June. It appeared that trees during the dormant period were more susceptible to infection and that Fuji trees seemed to be more susceptible to infection than the other two varieties. These observations need to be confirmed in the ongoing study.

Table 2. Susceptibility of apple trees to infection by the Sphaeropsis fungus.

Inoculation Date	Variety	Isolate	Canker Size (mm)	
			Average	Range
6-Apr-04	Fuji	Check	7.9	5.0 −11.0
		Sphaeropsis	8.4	6.0 − 12.0
	Golden	Check	5.6	5.0 − 7.0
		Sphaeropsis	7.2	5.0 − 11.0
	Red	Check	6.9	5.0 − 10.0
		Sphaeropsis	8.7	6.0 − 23.0
11-Jun-04	Fuji	Check	8.3	7.0 − 11.0
		Sphaeropsis	8.5	6.0 − 11.0
	Golden	Check	7.1	6.0 − 9.0
		Sphaeropsis	8.1	6.0 − 10.0
	Red	Check	7.4	6.0 − 11.0
		Sphaeropsis	8.9	6.0 − 12.0
19-Nov-04	Fuji	check	6.6	6.0 − 8.0
		Sphaeropsis	30.8	8.0 − 110.0
	Golden	check	7.1	6.0 − 9.0
		Sphaeropsis	9.7	7.0 − 18.0
	Red	check	6.9	6.0 − 10.0
		Sphaeropsis	18.2	7.0 − 61.0
22-Mar-05	Fuji	check	8.8	6.0 − 12.0
		Sphaeropsis	12.6	6.0 − 25.0
	Golden	check	6.9	6.0 − 10.0
		Sphaeropsis	10.8	6.0 − 18.0
	Red	check	8.6	6.0 − 13.0
		Sphaeropsis	12.1	7.0 − 24.0

Susceptibility of apple fruit to infection by the Sphaeropsis fungus in the orchard in relation to Sphaeropsis rot during storage

In 2004 and 2005, we inoculated with the fungus fruit of Fuji, Golden Delicious and Red Delicious at different growth stages. The 2004-05 data are presented in this report. The fruit from the 2005 inoculation study are currently in storage for decay development, and the data will be presented in the next report. We observed that Sphaeropsis rot developed on early-inoculated fruit as well as late-inoculated fruit, indicating that when conditions were met the fungus was able to colonize the fruit even in the early season and to remain latent throughout the fruit-growing season. We also observed that on Red Delicious, stem and calyx infections both were common; on Golden Delicious, stem infection was more common than calyx infection; on Fuji, calyx infection was more common than stem infection. These observations need to be confirmed in the ongoing study.

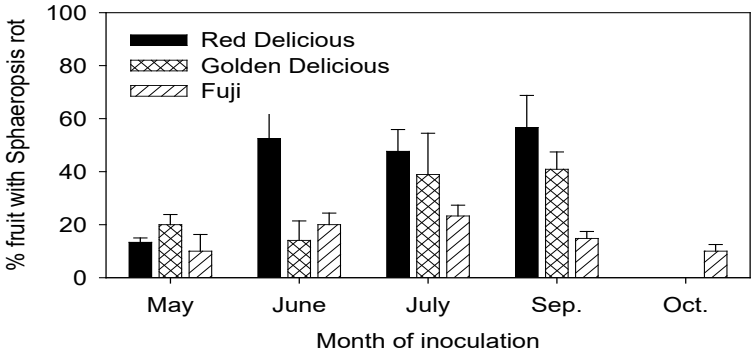

Fig. 1. Development of Sphaeropsis rot on apple fruit after 9 months of storage at 32ºF. Fruit were inoculated with *Sphaeropsis pyriputrescens* in the orchard during the fruit-growing season.

Sensitivity of the Sphaeropsis fungus to newly registered pre- and postharvest fungicides

In 2004 we tested sensitivity of the fungus to various preharvest fungicides. In 2005, we tested sensitivity of mycelial growth and conidial germination to new fungicides fludioxonil (Scholar), pyrimethanil (Penbotec) and Pyraclostrobin+boscalid (Pristine). Scholar, Mertect and Pristine were highly effective in inhibiting mycelial growth of the fungus, and Penbotec was effective only at higher rates. Pristine was also effective in inhibiting spore germination. The information has been used for developing pre- and postharvest fungicide programs for control of Sphaeropsis rot.

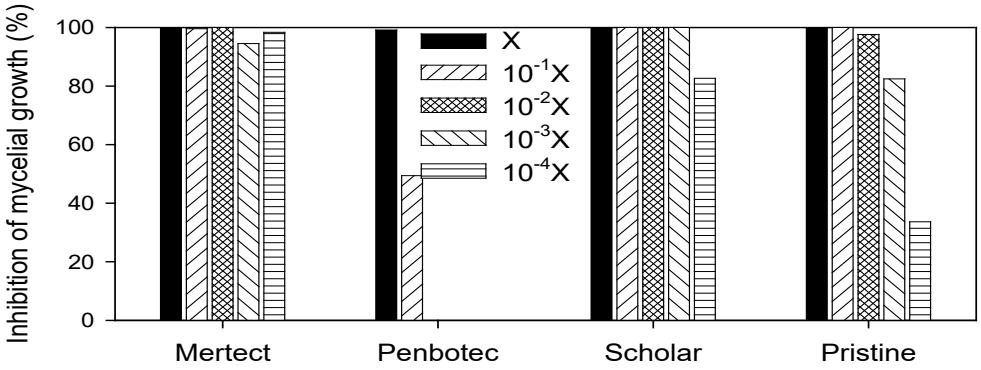

Fig. 2. Sensitivity of mycelial growth of the Sphaeropsis fungus to Pristine and three postharvest fungicides. "X" represents ¼ label rates for postharvest fungicides and the label rate of Pristine.

Prevalence and incidence of Sphaeropsis rot and other diseases in 2005

In 2005, 81 grower lots (37 lots of Red Delicious, 19 lots of Golden Delicious, 25 lots of Fuji) were sampled. Gray mold, blue mold, Sphaeropsis rot and Bull's eye rot were the four major postharvest diseases on apples in 2005 (Fig. 3). *Phacidiopycnis washingtonensis* was responsible for 5% of the total decay on Red Delicious, but it has the potential to cause significant economic losses. In one case, this fungus caused 23% losses in a Red Delicious lot. The percentage of each disease in the total decay varied from lot to lot and also was dependent on varieties and postharvest handling practices. The incidence of gray mold was higher on nondrenched fruit than on drenched fruit, whereas blue mold was higher on drenched fruit (Fig. 4). Sphaeropsis rot varied from lot to lot but accounted for approximately 20% of the decay on Red Delicious. Instances of severe Sphaeropsis rot was again observed on Fuji and Red Delicious fruit. Our three-year survey for postharvest diseases on apples indicated that gray mold, blue mold and Sphaeropsis rot should be considered major targets for decay control. Bull's eye rot should also be considered a target disease on Golden Delicious.

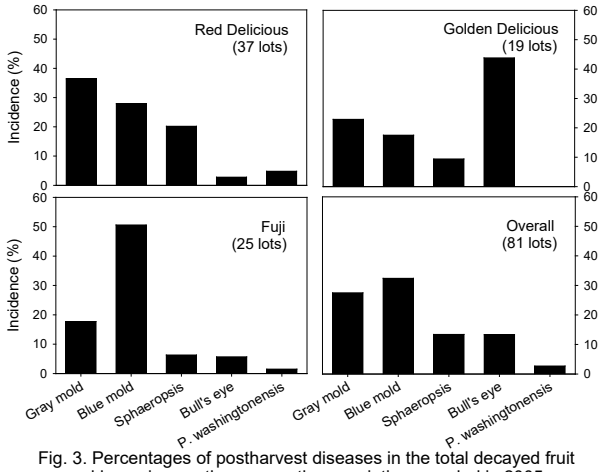

Fig. 3. Percentages of postharvest diseases in the total decayed fruit caused by various pathogens on three varieties sampled in 2005.

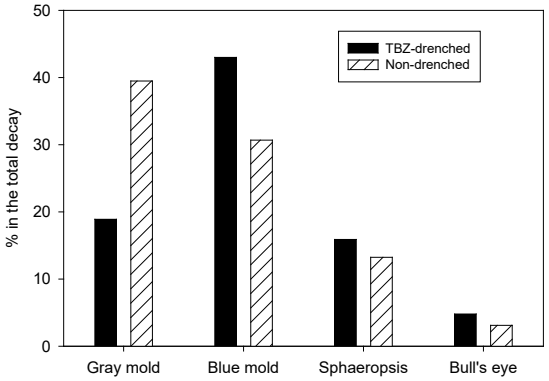

Fig. 4. Occurrence of postharvest diseases (% in the total decay) caused by various pathogens on TBZ-drenched and non-drenched fruit sampled in 2005 (based on 37 grower lots of Red Delicious and 25 lots of Fuji).

Chemical control of Sphaeropsis rot

In 2004-05, we evaluated pre- and postharvest fungicides for control of Sphaeropsis rot. The result is summarized in Table 3. Significant control of Sphaeropsis rot was seen on Mertect-drenched fruit; only 0.7% of the fruit was lost to Sphaeropsis rot. None of the preharvest fungicides provided statistically significant control in comparison with the nontreated control. This was inconsistent with what we observed from the last year's trial in which ziram applied at two weeks before harvest significantly reduced Sphaeropsis rot compared with the nontreated control. This needs to be further evaluated. We have tested sensitivity of the Sphaeropsis fungus to newly registered pre- and postharvest fungicides (Pristine, Scholar and Penbotec). In 2005, we conducted experiments to evaluate these new fungicides for control of Sphaeropsis rot. The results will be forthcoming when experiments are completed.

Table 3. Control of Sphaeropsis rot in apple with fungicides (7 months after harvest).

Treatment	Yield loss by Sphaeropsis rot (%)
Nontreated control	4.3 a[1]
Flint at 2 weeks before harvest	3.6 a
Ziram at 2 weeks before harvest	3.2 a
Thiram at 1 week before harvest	2.2 a
Postharvest drench with Mertect	0.7 b

[1] Values with the same letter in the same column are not significantly different based on the LSD test ($P = 0.05$)

Budget:

Project title: Sphaeropsis rot in apple
PI: Chang-Lin Xiao
Project duration: 2 years
Current year: 2006
Project total (2 years): $103,881
Current year request: $57,029

Year	2005	**2006**
Total	$46,852	**$57,029**

Current year breakdown:

Item	2005	2006
Salaries[1]	27,037	**35,020**
Benefits (40% for year 1 and 42% for year 2)	10,815	**14,709**
Wages[2]		**3,000**
Benefits (10%)		**300**
Supplies[3]	6,000	**3,000**
Travel[4]	3,000	**1,000**
Total	$46,852	**$57,029**

[1] Salary for Dr. Y. K. Kim, Postdoctoral Research Associate, who is on the position already. Funds are being requested for 10 months of Y. K. Kim's salary for year 1 and 12 months for year 2.

[2] Part-time technical helpers.

[3] This project deals with a lot of isolations of fungal pathogens from decayed apple fruit sampled from packinghouses as well as testing sensitivity of the Sphaeropsis fungus to various fungicides on Petri plates. Supplies include isolation media, chemicals, Petri dish plates, cryogenic vials and other supplies for ultra-low temperature storage of fungal isolates, and fungicides. In addition, $4,000 to $5,000 is needed to cover the cost of fruit from a commercial orchard for a trial testing pre- and postharvest fungicides for control of Sphaeropsis rot. I will seek support from the Washington State Commission on Pesticide Registration or agrichemical companies. Cell phone charges are allowed on this project.

[4] We will be using a leased vehicle.

Other Support:

This project was funded ($19,800) in 2005 by the WSU Safe Food Initiative funding program. In 2005, part of Dr. Kim's salary was covered through the WSU SFI grant. WSU SFI funding program is no longer available. In addition, Robin Boal (Scientific Assistant, a partially state-supported tech position) will also be working on this project (0.2 FTE) and this is not shown in the project.

Continuing PROJECT PROPOSAL **YEAR 2/3**

WTFRC PROJECT #: PH-04-446 **WSU PROJECT #:** 13C-3661-7368

Project title:	Holistic Approach to Decay Management
PI:	Chang-Lin Xiao, Assistant Plant Pathologist
Organization:	WSU Tree Fruit Research and Extension Center
Address, phone, e-mail:	1100 N. Western Avenue, Wenatchee; 509-663-8181 ext. 229; clxiao@wsu.edu
Cooperators:	Dana Faubion, WSU Extension, Yakima Gene Kupferman, WSU Wenatchee
Contract administrators:	Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

Objectives:

1. Evaluate effectiveness and timing of preharvest fungicides in controlling postharvest decay and their effects on fruit finish.
2. Evaluate various pre- and postharvest integrated programs for control of postharvest diseases.
3. Establish baseline sensitivity of major postharvest pathogens to new postharvest fungicides and assess the potential risk of fungicide resistance development in the orchard/storage system.
4. Evaluate new technologies for decay control.

Significant findings (as of mid-December 2005):

a. On Red Delicious apples, Pristine applied at 7 and 14 days before harvest reduced gray mold by 68-78% and blue mold by 70% in comparison with the nontreated control. Ziram applied at 2 weeks before harvest significantly reduced gray mold but not blue mold, indicating that a higher residue level may required for protecting wounds from infection by blue mold.

b. On Fuji apples, Pristine applied at 1 and 7 days before harvest was equally effective and reduced gray mold by 93-99% and blue mold by 87-95% as compared with the nontreated control. Topsin M reduced gray mold by 61% and blue mold by 64%. Thiram reduced gray mold but not blue mold.

c. Pristine was labeled in April 2005 for use on pome fruits. Based on the results from trials on Fuji and Red Delicious, Pristine could be a promising fungicide used as a preharvest treatment to control postharvest gray mold and blue mold on apples.

d. In a trial on Fuji, fruit that had been drenched with fungicides and DPA tended to have higher levels of lenticel marking compared with nondrenched fruit. The underlying mechanisms for this phenomenon are not yet known. These observations and the magnitude of this impact on fruit finish need to be further evaluated.

e. In 2004-05, three postharvest fungicides in various combinations as either a drench or an online treatment were tested for control of blue mold. We observed that Penbotec and Scholar had some good residual protection at packing even when they were applied as pre-storage drench treatments.

f. In 2004 and 2005, 70-90% of the *Penicillium expansum* isolates recovered from decayed apple fruit that had been drenched with Mertect prior to storage were resistant to TBZ, whereas <3% of the isolates from nondrenched fruit were resistant. Research is being conducted to determine sources of TBZ-resistant isolates in the production process from orchard to storage.

g. Based on three assays (mycelial growth, spore germination and germ-tube elongation), we have established baseline sensitivity patterns of *P. expansum* to the two new postharvest fungicides, Scholar and Penbotec. The information will be used for monitoring sensitivity shifts in the pathogen populations.

h. We have generated fludioxonil-resistant and pyrimethanil-resistant mutants of *P. expansum* in the laboratory. We are currently using these mutants to examine potential cross resistance of new postharvest fungicides with other fungicides. This would help us develop strategies for management of fungicide resistance in postharvest pathogens.

i. Research is being conducted to evaluate thermofogging pyrimethanil for control of postharvest diseases on apples.

Methods:

Preharvest fungicides were evaluated for control of postharvest gray mold (*Botrytis cinerea*) and blue mold (*P. expansum*) on Red Delicious and Fuji apples. Fungicides were applied within two weeks before harvest. After harvest, fruit were immediately wounded and inoculated with either *B. cinerea* or *Penicillium expansum*. Fruit were tray packed and stored in cardboard boxes in air at 32ºF. The percentages of fruit that developed gray mold and blue mold were recorded, and lesion diameters were measured after 4 and 8 weeks of storage.

Three preharvest fungicides and three postharvest fungicides were evaluated for control of postharvest diseases on Fuji apples from a commercial orchard. Elevate, Thiram and Topsin M were applied within 3 days before harvest. Fruit were harvested and part of the fruit from each preharvest treatment was drenched with Mertect and DPA. In a separate test, Fuji fruit harvested from the orchard was drenched with DPA in combination with one of the three postharvest fungicides (Mertect, Scholar and Penbotec). Fruit were stored in CA for seven months. Fruit were run through a commercial packingline. Decayed fruit were removed from the packingline and decay incidence was recorded. Two boxes of fruit from each treatment were used for fruit quality and finish (lenticel marking) assessment at packing and 7 days post-packing at room temperature.

Three postharvest fungicides alone or in various combinations as either drench or online treatments were evaluated for control of decay before packing as well as after packing.

Isolates of *P. expansum* were collected from various apple-related sources, including fruit in the orchard, bins at harvest, soil or organic debris on the bottom of bins, drench solutions, etc. Isolates are being identified to species. Isolates of *P. expansum* from various sources are being tested for resistance to thiabendazole. Fludioxonil-resistant and pyrimethanil-resistant mutants of *P. expansum* have been generated in the laboratory. These mutants are being used to examine potential cross-resistance of new postharvest fungicides with other fungicides.

A trial to evaluate the efficacy of thermofogging pyrimethanil for control of postharvest diseases was conducted on Red Delicious. Commercially harvested fruit and fruit inoculated with pathogens were included in the study.

Results and discussion:

Preharvest fungicides for control of postharvest gray mold and blue mold.

In the trial conducted on Red Delicious apples, when applied at 7 and 14 days before harvest, Pristine reduced gray mold by 68-78% and blue mold by 70% in comparison with the nontreated control (Fig. 1). Ziram applied at 2 weeks before harvest significantly reduced gray mold but not blue mold, indicating that a higher residual level may required for protecting wounds from infection by blue mold. Topsin M applied at 3 and 7 days before harvest reduced gray mold by approximately 44% and blue mold by approximately 65%.

In the trial conducted on Fuji apples, when applied at 1 and 7 days before harvest, Pristine was equally effective and reduced gray mold by 93-99% and blue mold by 87-95% as compared with the nontreated control (Fig. 2). Topsin M reduced gray mold by 61% and blue mold by 64%. Thiram reduced gray mold but not blue mold.

In April 2005, Pristine was labeled for use on pome fruits. Based on the results from trials on Fuji and Red Delicious, Pristine appeared to be a promising fungicide when used as a preharvest treatment to control postharvest gray mold and blue mold on apples.

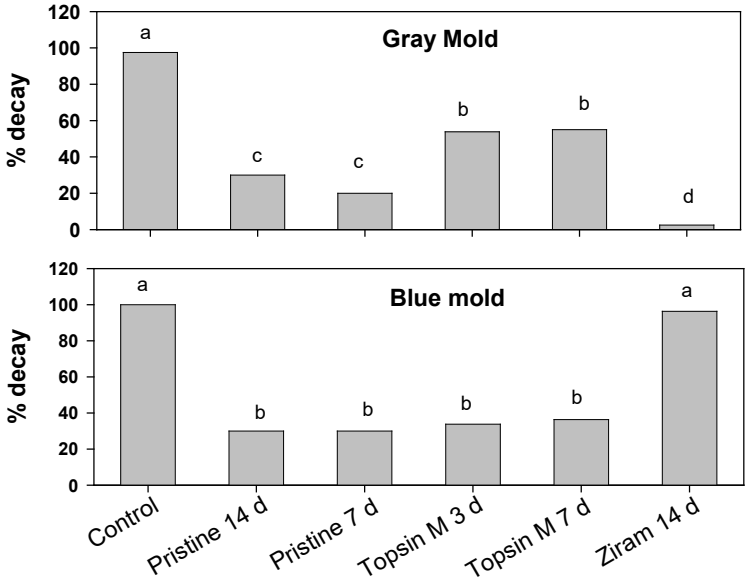

Fig. 1. Effectiveness of preharvest fungicides applied near harvest (Pristine 14.5 oz/A at 7 and 14 days, Topsin M 1 lb/A at 3 and 7days and Ziram 8 lb/A at 14 days before harvest) in controlling postharvest gray mold and blue mold on the 2005 crop of Red Delicious apples.

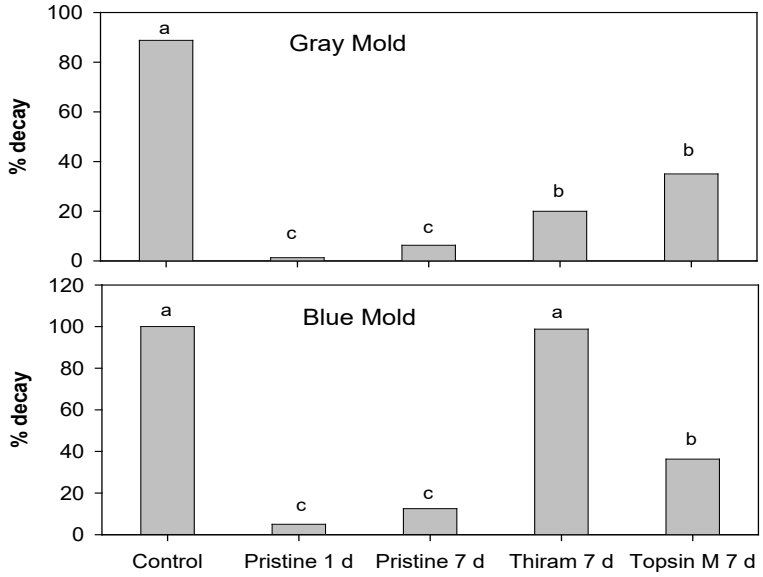

Fig. 2. Effectiveness of preharvest fungicides applied near harvest (Pristine 14.5 oz/A at 1 and 7 days, Topsin M 1 lb/A and Thiram 6.8 lb/A at 7 days before harvest) in controlling postharvest gray mold and blue mold on the 2005 crop of Fuji apples.

In 2004-05, we conducted three trials in commercial orchards. The first trial was conducted on Red Delicious, and results were presented in the progress report submitted to the Commission in July 2005. The other two trials were conducted on Fuji. Results are summarized in Tables 1 and 2.

On the 2004 crop, the amount of decay resulting from natural infections in the nontreated control was low (Tables 1 and 2). Only the fruit from the treatment with preharvest Elevate and postharvest drench with Mertect and DPA had a higher level of decay compared with other treatments. This was likely because Elevate was not effective to control blue mold, and a postharvest drench with Mertect increased the likelihood that fruit could be inoculated by TBZ-resistant *Penicillium* during the drenching process.

Interestingly, the fruit that had been drenched with fungicides and DPA tended to have higher levels of lenticel marking (a fruit-finish parameter rated on a 0-3 scale) compared with nondrenched fruit (Table 1).

In a separate trial, three postharvest fungicide-drench treatments significantly reduced the amount of decay (Table 2). About 3% decay developed on nondrenched, packed fruit, whereas no decay developed on Scholar- or Penbotec-drenched fruit at 7 days post-packing at room temperature, indicating that Scholar and Penbotec might have some residual protection at packing even when they were applied as pre-storage drench treatments. These observations need to be confirmed in the ongoing study. Again, in this trial the fruit that had been drenched with fungicides and DPA tended to have higher levels of lenticel marking compared with non-drenched fruit (Table 2). The underlying mechanisms for this phenomenon are not yet known. The magnitude of this impact on fruit finish needs to be further evaluated.

Table 1. Control of postharvest decay and effects of pre- and postharvest fungicide treatments on fruit finish on Fuji apples in 2004-05.

Treatment	% Decay at packing	% Decay at 7 days post-packing at room temperature	Fruit finish (0-3 scale) at packing	Fruit finish at 7 days post-packing at room temperature
Control	0.8 b	4.92 a	0.24 c	0.31 b
Preharvest Elevate	0.7 b	4.54 a	0.34 c	0.39 b
Preharvest Elevate+ postharvest Mertect+DPA	1.7 a	7.58 a	0.70 ab	0.71 ab
Preharvest Thiram	0.7 b	2.65 a	0.31 c	0.43 b
Preharvest Thiram+ postharvest Mertect+DPA	1.1 b	3.41 a	0.79 a	0.93 a
Preharvest Topsin M	1.1 b	7.95 a	0.39 bc	0.51 b

Table 2. Control of postharvest decay and effects of postharvest fungicide treatments on fruit finish on Fuji apples in 2004-05.

Treatment	% Decay at packing	% Decay at 7 days post-packing at room temperature	Fruit finish (0-3 scale) at packing	Fruit finish at 7 days post-packing at room temperature
Control	1.1 a	3.03 a	0.75 b	0.66 c
Mertect+DPA	0.9 bc	1.89 a	1.22 a	1.30 a
Penbotec+DPA	1.0 b	0.00	1.08 a	1.20 a
Scholar+DPA	0.9 bc	0.00	1.03 a	0.99 b

Integrated postharvest fungicide programs.

In 2004-05, three postharvest fungicides in various combinations as either a drench or an online treatment were tested for control of blue mold. We observed that Penbotec and Scholar had some good residual protection at packing even when they were applied as pre-storage drench treatments. The results were presented in the previous report submitted to Commission in July 2005.

On the 2005 crop, we had set up an experiment to repeat this study. The fruit are currently in CA and various tests will be conducted in spring 2006. Results from this study will be presented in the next report.

Management of fungicide resistance in postharvest pathogens.

In 2004 and 2005, we collected *Penicillium expansum* isolates from decayed fruit sampled from orchard floors and from commercial packinghouses. Resistance of these isolates to TBZ was tested (Table 3). We selected 120 isolates of *P. expansum* for a baseline sensitivity study. Based on three assays (mycelial growth, spore germination and germ-tube elongation), we have established baseline sensitivity patterns of *P. expansum* to the two new postharvest fungicides, Scholar and Penbotec. These results were presented in the July 2005 report.

In 2005, we collected isolates of *Penicillium* spp. from various apple-related sources, including fruit in the orchard, bins at harvest, soil or debris on the bottom of bins, drench solutions, etc. We have obtained hundreds of isolates of *Penicillium* spp. and are currently identifying them to species. Isolates of *P. expansum* will be tested for resistance to TBZ. The goal of this study is to understand the sources of TBZ resistance of *P. expansum* in the apple-production process from orchard to storage. This would help us implement strategies to minimize the likelihood of development of resistance to new postharvest fungicides.

We have generated fludioxonil-resistant and pyrimethanil-resistant mutants of *P. expansum* in the laboratory. We are currently using these mutants to examine potential cross-resistance of new postharvest fungicides with other fungicides. This would help us develop strategies for management of fungicide resistance in postharvest pathogens. Results from this ongoing study will be presented in the next report.

Table 3. Sources of TBZ-resistant strains of *Penicillium expansum* collected in 2004-05.

Origin	Pre-storage treatment	Collection year	Total isolates	TBZ-resistant isolates (%)
Orchards		2004	303	2.64
Packinghouses	TBZ-drenched	2004	75	69.3
		2005	45	91.1
	Non-drenched	2004	22	0
		2005	48	2.1

New technologies for decay control.

In 2004-05, we evaluated biofumigation with the Muscodor fungus for control of postharvest blue mold and gray mold. Results were presented in the 2005 July report. The company is trying to improve the formulation of the biofumigant inoculum.

On the 2005 crop, we have set up a thermofogging trial to evaluate the efficacy of fogging pyrimethanil for control of postharvest diseases. The trial was conducted on Red Delicious. Commercially harvested fruit as well as fruit inoculated with pathogens were included in the study. Results will be forthcoming when the experiment is completed.

Budget:

Project title: Holistic Approach to Decay Management
PI: Chang-Lin Xiao
Project duration: Three years
Current year: 2006
Project total (3 years): $212,514
Current year request: $75,816

Year	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
Total	**$61,510**	**$75,188**	**$75,816**

Current year breakdown:

Item	2004	2005	**2006**
Salaries[1]	$31,000	$41,152	**$42,313**
Benefits[2]	12,710	16,486	**16,953**
Wages[3]	5,000	5,000	**5,000**
Benefits (11%)	800	550	**550**
Equipment			
Supplies[4]	10,000	10,000	**9,000**
Travel[5]	2,000	2,000	**2,000**
Miscellaneous			
Total	$61,510	$75,188	**$75,816**

[1] Salaries for a Postdoctoral Research Associate at 1.0 FTE (Hongxia Li) and Robin Boal at 0.2 FTE. The increase in the request of salary for 2005 and 2006 is because I have to cover part of Robin Boal's salary. Robin Boal (0.2 FTE Scientific Assistant, partially state-supported tech position) is working on this project.

[2] 41% for Hongxia Li and 37% for Robin Boal

[3] Temporary or hourly workers.

[4] Including:

(1) $4,000 (in both years 1 and 2) and $2,000 in year 3 for isolation media, chemicals, petri dish plates for isolation of fungi and fungicide sensitivity tests, cryogenic vials and other supplies for ultra-low temperature storage of fungal isolates;

(2) $6,000 for cost of fruit bought from commercial orchard for trials. Additional fruit needed for other experiments will be covered by either gift support from agrichemical companies or a grant from the Washington State Commission on Pesticide Registration.

(3) Cell phone charges are allowed on this project.

[5] Travel to orchards and packing houses across the state is required for sampling. We will be using a leased vehicle.

Project title: Molecular biology-based assay for improved fruit thinning
PI: Steven van Nocker
Project duration: 2002-2004

Project update at poster session only. No written report required.

CONTINUING REPORT
WTFRC Project #AH-04-416

Project Title: Role of Sorbitol in Sugar and Acid Accumulation in Apple Fruit
PI: Abhaya M. Dandekar
Department of Plant Science, University of California, Davis, CA 95616

Collaborators: Yasuo Suzuki, Gianni Teo and Sandie Uratsu
Department of Plant Science, University of California, Davis, CA 95616

Objectives:

1 Defining and validating the role of sorbitol related enzymes in the accumulation of Sugar (Fructose) and Acid (Malic Acid) in apple fruit.
2 Determining the role of sorbitol in the development of water core in apple fruit.

Significant Findings:

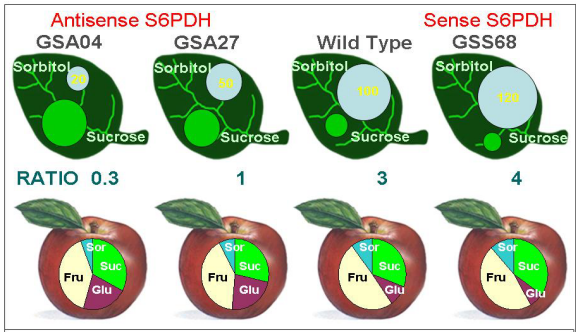

Fig. 1: Metabolic phenotypes of major transgenic genotypes with alterations in sorbitol metabolism due to antisense/sense expression of cDNA encoding S6PDH.

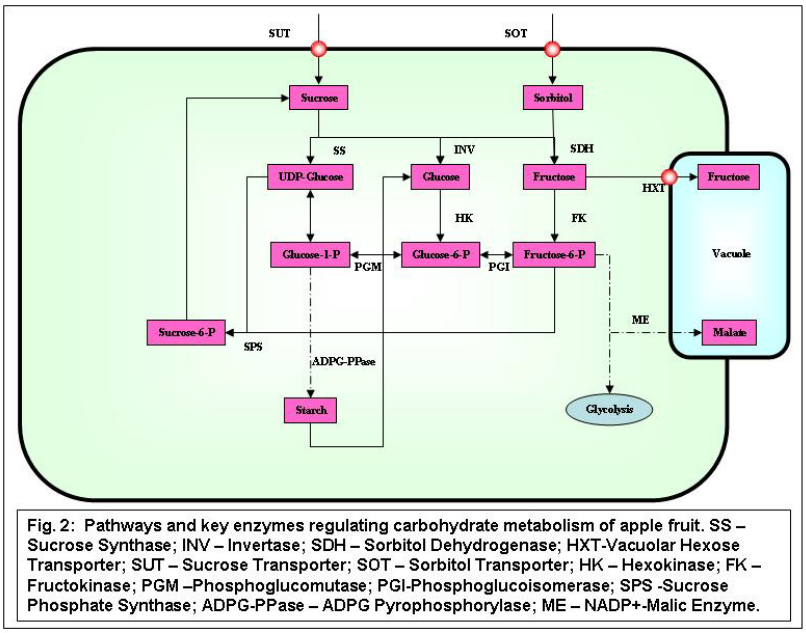

Fig. 2: Pathways and key enzymes regulating carbohydrate metabolism of apple fruit. SS – Sucrose Synthase; INV – Invertase; SDH – Sorbitol Dehydrogenase; HXT-Vacuolar Hexose Transporter; SUT – Sucrose Transporter; SOT – Sorbitol Transporter; HK – Hexokinase; FK – Fructokinase; PGM –Phosphoglucomutase;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

1. Sorbitol/Sucrose ratio regulates the amount of malic acid accumulation and thus the level of acidity in fruit
2. Sorbitol partitioned into fruit regulates the level of nonstructural carbohydrates but does not significantly affect firmness.
3. Higher sorbitol levels are responsible for water core development.
4. Sorbitol level in the fruit regulates sorbitol related enzymes and thus the metabolism in fruit tissues during development and in the final product.

Methods:

Field planting of transgenic apple plants: Transgenic plant materials utilized for this project were collected from two fields:
Field A: Source planting of all transgenic apple plants expressing sense/antisense S6PDH. This planting contains all of the source materials of different transgenic clones planted in a randomized design on their own roots at a 15x15 ft spacing. The plot contains 354 apple trees of which 53 are control trees of the golden delicious apple cultivars, Greensleeves (GS; 26) and Ginger Gold (GG; 27). The other trees are transgenic apple trees of which 106 trees express an anti-S6PDH, 111 trees express a sense-S6PDH and

the remainding 74 trees express other constructs not relevant to this present study.

Field B: This is a transgenic production orchard planted in 2001 contains 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 with each replicate being 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 being 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. In the case of 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 by reading the 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 by reading the 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 assayed by determining the amount of glucose produced 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 assayed by determining the amount of 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 30^{o}C, and the reaction was stopped with the addition of 2.5 N NaOH. Production of sucrose 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 by reading the changes in absorbance at 340 nm at 25^{o}C. 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 by reading the changes in absorbance at 340 nm at 25^{o}C.

Results and Discussion:

This proposal focuses on understanding the biochemical mechanisms that are involved in the metabolism and partitioning of sorbitol in apple fruit. We have outlined the key role that sorbitol plays in: 1) sugar composition, 2) starch accumulation 3) malic acid metabolism and 4) the development of the watercore disorder. For these reasons, understanding the metabolism and partitioning of sorbitol in fruit tissues should remain an important priority for an industry dependent on the production of high quality apple fruit. Unfortunately, defining the role of sorbitol in normal apple fruit is complicated by presence of sucrose that is simultaneously partitioned along with sorbitol. Both sorbitol and sucrose are synthesized from the very same products of photosynthesis creating two pathways of carbon flow into the fruit where they are metabolized to common sugars and other products that determine fruit quality. For example fructose in fruit can therefore come either from sorbitol or sucrose. We have solved this problem by creating transgenic apple trees that are specifically altered in their ability to make and translocate sorbitol. These plants behave like specific mutants and provide us with a unique opportunity to evaluate the role of sorbitol in fruit quality. Shown in Fig. 1 are the phenotypes of the different transgenic lines that we currently using for this study. The sorbitol to sucrose ratio (So/Su) is an indication of the differences among these lines. Normal apple trees have a ratio around 3 and as can be seen in Fig 1 we have lines that have a ratio more than a fold lower. The ratio of sorbitol to sucrose decreases from 3.4 to 0.3 and 0.2 in leaves of antisense clones GSA27 and GSA04. In sense clone GSS68, it increases to 3.8. The ratio in fruit pedical phloem exudates is also closely related to the ratio found in the leaves (previous reports). As a result, some factors, which determine fruit quality, including fruit growth, fruit size and firmness do not change, but others, including sugar composition, acid content and starch content, change (Fig. 1). This means sorbitol plays an important role in fruit quality relating with flavor (previous report). The object of this current year's research is to determine key enzymes in fruit which regulate fruit quality, especially flavor, through analyses of activities and the expression of their encoding genes. In our previously funded project (WTFRC-AH-01-69) we analyzed one of these lines (GSA04) in detail and were able to demonstrate that sorbitol plays a key role in the sugar composition, starch distribution and malic acid accumulation in fruit tissues. In this study we are extending our analysis of sorbitol metabolism to identify the proteins/enzymes and genes that regulate sugar acid composition and starch accumulation (Fig 2). In this regard we have begun to analyze the enzymes important for the development of the sugar-acid content (Fig 2).

The most important enzyme relating in apple fruit is SDH which is responsible for the catabolism of sorbitol the principal translocated sugar in fruit. SDH activity is the highest at the late stages of 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 its peak appeared in premature fruit at 91 DAF. At harvest, activity was detected at very low levels (Fig. 3). In GSS68, SDH activity was not also detected at 30 DAF. The pattern of SDH activity was as the same as that of control, but the activity tended to be higher than that of control through the fruit development. In antisense clones, GSA04 and GSA27, activities were lower than that of control. Especially, activities were significantly lower at 91 DAF, when the activity was highest in control fruit, and were not detected any more at harvest. Earlier work has shown the interruption of assimilate by girdling led to a decrease in SDH activity in apple fruit (Beruter and Feusi 1997). Low SDH activity of apple fruit with girdling was recovered by in vitro sorbitol treatment (Archbold, 1999). These reports suggest that sorbitol supply regulates SDH activity in fruit. Our results further support this hypothesis by presenting direct genetic evidence with in vivo experiment using transgenic apple fruit. In cultured celery cells, gene expression of mannitol dehyrogenase (MDH) was repressed by hexose and it was suggested that HK and sugar phosphorylation are involved in signaling its repression (Prata et al., 1997). Further studies

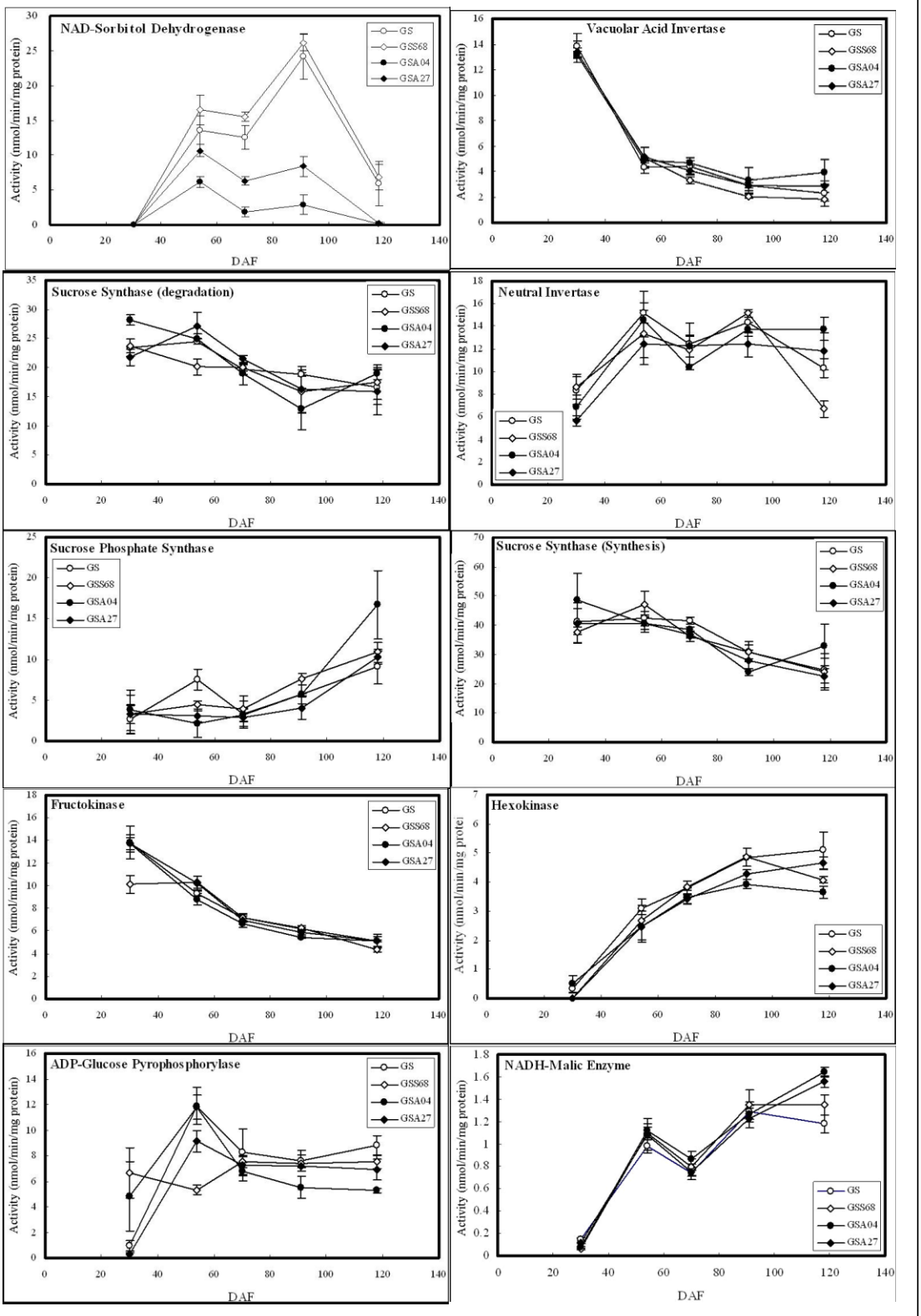

Fig. 3: Analysis of key enzymes regulating carbohydrate metabolism in normal and transgenic apple fruit at various stages of development.

on SDH gene expression by sugars are necessary since its activity was also shown to be increased not only by sorbitol but also by hexose in sliced tissues of Japanese pear fruit (Iida et al., 2004). SS and invertases have been shown to play an important role in a sink organ, since they catabolize sucrose which is the other translocating sugar in apple. In general, these enzymes contribute to sequential stages of sink initiation, expansion, and storage/maturation (Koch, 2004). Girdling treatment during the period of active starch synthesis as mentioned above also led to a decrease in neutral invertase activity (Beruter and Feusi 1997), which might suggests that neutral invertase is important at this particular stage. In this present study, differences of these enzymes were not detected when control fruit were compared to transgenic fruit, though sucrose contents in phloem exudates were different. Fructokinase, hexokinase, sucrose synthase (synthesis), sucrose phosphate synthase, and ADP-glucose pyrophosphorylase are crucial enzymes in sugar metabolism. We did not detect prominent differences of these enzymes in control and transgenic fruit (Fig. 3).

It has been suggested that ME is related with a decrease in malic acid in apple fruit (Yoshioka et al. 1989). ME activities tended to increase during the development (Fig. 3). They were not different among control and transgenic plants until 91 DAF, but at harvest activities of antisense clones, GSA04 and GSA27, were higher than those of control and/or sense clone, GSS 68. Acid contents of antisense clones are lower than those of control and/or sense clone (previous report). This suggests that low acid content is due to high activity of ME.

Gene expression of carbohydrate enzymes, as well as sugar transporters, will be further investigated this coming year to further investigate the enzyme levels that we obtained this year. Taken together this would provide a comprehensive view of sugar-acid metabolism and its regulation in apple fruit.

Literature review:

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Yamaki, Y. 1980. Sorbitol oxidase converting sorbitol to glucose in apple leaf. Plant Cell Physiol. 69:117-121.

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Significance to the Industry and Potential Economic Benefits:

The long term goal of this research is to develop the genetic tools necessary to optimize, predict or screen genetic determinants that regulate apple fruit quality and shelf-life. The following are specific deliverables:
-Define the genetic determinants that regulate the accumulation of fructose and malic acid in developing apple fruit.
-Define the genetic determinants that regulate watercore formation in apple fruit.
-Development of diagnostic tools to optimize and predict sugar-acid development in the field during apple growth and production.
-Develop unique diagnostic tools to predict apple fruit disorders in the field.
-Develop diagnostic tools to efficiently evaluate natural variation in the germplasm available for breeding programs directed at improving apple fruit quality parameters and reducing fruit related disorders.

Budget:

Title:	Role of Sorbitol in Sugar and Acid Accumulation in Apple Fruit
PI:	Abhaya M. Dandekar
Project Duration:	2004 to 2006
Current year:	2006
Project Total (3 years):	$120,000
Current year request:	**$42,000**

Year	**Year 1 (2004)**	**Year 2 (2005)**	**Year 3 (2006)**
Total	**$38,500**	**$39,500**	**$42,000**

Budget breakdown:

	2004	2005	**2006**
Salaries PGRIII (58%)	$21,364	$22,342	**$23,554**
Benefits	$ 3,843	$ 4,035	**$ 4,237**
Equipment			
Supplies	$12,493	$12,233	**$13,409**
Travel	$ 800	$ 800	**$ 800**
Miscellaneous			
Totals	$38,500	$39,500	**$42,000**

CONTINUING REPORT **YEAR 2/3**
WTFRC Project #PH-04-0442 Organizational Project #58-3635-5-438

Project Title: Hyperspectral Reflectance and Fluorescence for Assessing Apple Maturity

PI: Renfu Lu, Agricultural Engineer
USDA ARS, Michigan State University, East Lansing, MI
Co-PI: *Randolph M. Beaudry, Professor, Horticulture*
Michigan State University, East Lansing, MI

Contract Administrator: Susan Shrout, Authorized Departmental Officer
USDA/ARS/MWA Agreement Office
1815 North University St., Peoria IL 61604
Phone: 309-681-6631; Email: shrouts@mwa.ars.usda.gov

Objectives:

The overall objective of the project is to develop a sensing technique of integrating hyperspectral reflectance and fluorescence for measuring multiple apple quality parameters including skin and flesh color, fruit firmness, soluble solids, starch, and acid. In the past one and one half years, we assembled and tested two sensing systems for collecting hyperspectral reflectance and fluorescence images and spectra from apples. One was the hyperspectral reflectance and laser-induced fluorescence (LIF) imaging system (or *the R/LIF system*) and the other was the reflectance and UV-induced fluorescence spectroscopic system (or *the R/UV-F system*). Since July 2005 (last report), we made further improvements in the hardware of the two systems so that they could acquire both reflectance and fluorescence more efficiently and rapidly.

Our goal for the next year is to improve the efficacy of integrating reflectance and fluorescence for better measurements of apple quality attributes. Specific objectives are as follows:

- Continue to test and evaluate the improved reflectance and fluorescence systems for measuring the multiple quality parameters of apples before and after storage;
- Develop algorithms that can more effectively utilize both reflectance and fluorescence data for improved predictions of fruit quality parameters;
- Assemble and test a prototype that is compact, portable, and fast in acquiring reflectance and fluorescence for measuring the maturity indexes of apples.

Significant Findings:

- The improved R/LIF and R/UV-F systems can acquire both reflectance and fluorescence instantaneously without time delay for each sensing mode.
- The R/UV-F system gave excellent measurements of flesh and skin color, good measurements of fruit firmness, soluble solids, and starch index and poor measurements of titratable acid.
- Compared to the single sensing mode of reflectance or fluorescence, the integrated sensing mode of reflectance and fluorescence from the R/UV-F system achieved consistently better measurements for all maturity indexes except for flesh color; the measurement errors were reduced between 4% and 100%. Further improvements are expected through improving the algorithm of analyzing the integrated data of reflectance and fluorescence.
- A mathematical method of incorporating the apple size/shape effect for more accurate quantification of reflectance and fluorescence has been developed, which improved the performance of the reflectance and laser-induced fluorescence imaging system (Results are not presented in this report).
- A calibration method was developed to compensate for the effect of light source instability on fluorescence measurements for both systems.

Methods:

In the past six months, we made several improvements in the R/LIF system and the R/UV-F system. For the R/LIF system, we added a mechanical shutter that is operated via computer to accurately control laser illumination for instantaneous acquisition of hyperspectral fluorescence images. The improved R/LIF system was able to acquire hyperpspectral reflectance and fluorescence images from each fruit in 0.025 s and 0.1 s, respectively. For the R/UV-F system, we changed the reflectance measurement mode from reflectance to interactance. The interactance mode allows light to better penetrate into the fruit flesh, which would lead to better measurements of soluble solids content and possibly other quality indexes. We also added a mechanical shutter to the system for better control of the UV light source and accurate measurement of fluorescence spectra. In addition, a calibration method was developed for using a standard reference material to compensate for the effect of UV or blue light source fluctuations on fluorescence measurements.

During the 2005 harvest season, 750 'Golden Delicious' apples were harvested over a period of four weeks from an orchard of Michigan State University Horticultural Teaching and Research Center in East Lansing, Michigan. On each week, 150 apples were picked on a specific day, and they were tested in the following day. Reflectance and fluorescence measurements were first taken from each fruit. After reflectance/fluorescence images and spectra were collected from individual apples, the standard reference methods were then used to measure skin and flesh color, fruit firmness, soluble solids content (SSC), starch pattern index, and titratable acid. Flesh and skin colors were expressed in hue and chroma; hue defines the color attribute (i.e., red, blue, yellow, etc.) whereas chroma reflects the intensity or saturation of a particular hue. Fruit firmness was measured using the Magness-Taylor (MT) puncture tester. SSC was measured by a digital refractometer from the juice released during the MT measurement. Starch pattern index (SPI) was measured using an iodine stain method. Finally, fruit acid content was measured using a titration method. Cubic pieces were cut from individual apples and they were then frozen in a freezer. Juice was extracted from the thawed apple cubes and the titration test was performed with 0.50% malic acid as a reference.

After all data were collected, we performed image and spectral analyses. As done in the previous year, we developed prediction models for individual quality parameters from either reflectance or fluorescence data. Prediction models that integrated the reflectance and fluorescence data were then developed. Single sensing models and the integrated sensing models were compared to determine how they measured fruit quality parameters. A new method was developed for analyzing the data of reflectance and fluorescence and their combinations. We first applied a mathematical method (called principal component analysis) to separately extract essential information (or principal components or PCs) for each set of data. Artificial neural networks were used for developing calibration models. Once the neural networks were properly trained, they were then used to predict maturity indexes of a separate group of samples. So far we have only completed analyzing the data collected from the R/UV-F system. We are still working on the data collected from the R/LIF system. A method of correcting for the effect of fruit size/shape on the hyperspectral reflectance and fluorescence images collected by the R/LIF system has been developed.

For the coming year, our research tasks will include the following:

1. Developing calibration models for the R/LIF system We have collected hyperspectral reflectance and fluorescence image data from 'Golden Delicious' apples during the 2005 harvest season (the maturity study). We will develop calibration models for each sensing mode as well as for the integrated mode of reflectance and fluorescence. Our previous year study showed that apple size/shape affected the fluorescence and reflectance images. We have developed a mathematical method that will take into consideration the effect of fruit size/shape in the image analysis. A mathematical function will be used to describe the reflectance and scattering profiles at each wavelength, from which we obtain function

parameter spectra. We will then apply the principal component analysis method to compress the function parameter spectra. Principal components from the reflectance and fluorescence images are then input into the neural networks for predicting individual maturity indexes. After completing the training of the neural networks, we will test and evaluate their performance with a separate group of apple samples.

2. Collecting data for apples after storage Apples from commercial packinghouses will be used for further evaluation of the two systems. Studies from our laboratory and others have showed that near-infrared spectroscopy generally works better with apples after storage than with freshly harvested apples. We expect similar results for our systems. To be consistent with our maturity study, we will continue to use 'Golden Delicious' apples for our study. The same measurement procedures, as described in the maturity study, will be followed for collecting spectral and image data and maturity index data. Once the data collection is completed, the same calibration method will be used for analyzing the storage apple data.

3. Assembling and testing a prototype We will start to assemble a prototype that is compact, portable, and convenient for measuring both reflectance and fluorescence from apple fruit. We will consider a different fluorescence light source from the one used in our current R/UV-F system. The new light source will be LED (light emitting diode) based and compact. The algorithm that has shown to be effective in integrating both reflectance and fluorescence will be used in the prototype. The prototype will be tested during the 2006 harvest season with one or two apple cultivars. Further tests of the prototype will be carried out with apples from storage.

Results and Discussion:

The statistics of the maturity indexes for the 750 Golden Delicious apples measured by the standard destructive methods are summarized in Table 1. The firmness range for the apples was relatively small with the mean Magness-Taylor force of 75 N (or 16.9 lbf) and the standard deviation of 10.8 N (or 2.4 lbf).

Table 1. Statistics of standard destructive quality measurements of 750 Golden Delicious apples

Quality Parameters*	Max	Min	Mean	S.D.**
Firmness (N)*	105.5	39.4	74.7	10.8
Soluble Solids (%)	17.1	9.4	13.7	1.4
Starch Pattern Index	8.0	1.0	4.8	2.2
Titratable acid (%)	0.67	0.14	0.42	0.09
Skin Chroma	54.2	34.9	44.9	2.5
Skin Hue	-1.13	-1.54	-1.24	0.07
Flesh Chroma	40.8	21.2	28.2	2.5
Flesh Hue	-1.20	-1.48	-1.34	0.05

* Firmness is expressed in the unit of Newtons or N (1 lbf = 4.45 N). The color parameters, chroma and hue, have no unit.

** S.D. = standard deviation.

We are still in the process of analyzing the data collected with the R/LIF system. Hence this report only presents the results from the R/UV-F system.

Table 2 summarizes neural network predictions of the maturity indexes of Golden Delicious apples for each sensing mode (i.e., reflectance, fluorescence, and integrated). In a single sensing mode, the reflectance mode performed better than the fluorescence mode in predicting all maturity indexes except for skin color, in which the two sensing modes had about the same results. The correlation coefficient (standard error) for firmness and soluble solids content (SSC) predictions from the reflectance mode were 0.77 (7.23 N or 1.62 lbf) and 0.74 (0.94), respectively. While these correlations are not great, they are fairly typical for freshly harvested apples with near-infrared spectroscopy. Our study and other studies have shown that near-infrared reflectance spectroscopy had consistently low predictions of apple fruit firmness and SSC for freshly harvested apples than for apples after storage. This could be due to the fact that 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 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 poorer predictions of skin and flesh chroma. Reflectance and fluorescence had similar results for predicting the starch pattern index with values for the correlation coefficient of 0.82 and 0.81, respectively. Poor results were obtained for titratable acid for both sensing modes.

Table 2. Neural network prediction results (correlation coefficient/standard error) of the maturity indexes of Golden Delicious apples for each sensing mode (reflectance, fluorescence, and integrated) from the reflectance and UV-induced fluorescence spectroscopic system.

Quality Parameter	Reflectance*	Fluorescence*	Integrated*
Firmness (N)	0.75/7.23	0.62/8.51	0.77/6.93
Soluble Solids Content (%)	0.74/0.94	0.67/1.04	0.77/0.90
Starch Pattern Index	0.82/1.26	0.81/1.27	0.89/0.97
Titratable Acid (%)	0.60/0.08	0.53/0.08	0.62/0.07
Skin Hue	0.97/0.02	0.97/0.02	0.99/0.01
Skin Chroma	0.74/1.78	0.74/1.75	0.77/1.69
Flesh Hue	0.96/0.01	0.87/0.02	0.96/0.01
Flesh Chroma	0.79/1.63	0.47/2.30	0.77/1.71

* The first number in each pair denotes the correlation coefficient and the second number is the standard error. Firmness is expressed in Newtons or N (1 lbs = 4.45 N). The color parameters are dimensionless.

Figure 1 shows neural network predictions of fruit firmness, soluble solids content, starch pattern index, titratable acid, and skin and flesh hue for Golden Delicious apples from the integrated data of reflectance and fluorescence. The integrated sensing mode had consistently better predictions of all maturity indexes than either reflectance or fluorescence, with the exception for flesh color. Compared to the reflectance mode, the prediction errors (standard error) for the integrated sensing mode were reduced by 4% to 100%. Hence, the integration of reflectance and fluorescence can lead to better and more consistent predictions of apple fruit maturity indexes than either reflectance or fluorescence. The results from this year's study are comparable to those from the previous year. In our previous year's study, we used steady-state fluorescence to predict apple quality indexes. It takes about 3-5 minutes for fluorescence to stabilize. With the improvements made to the R/UV-F and R/LIF systems this year, we

were able to acquire fluorescence instantaneously (1 s for the R/UV-F system and 0.1 s for the R/LIF system). Since the results reported here are from the apples that were tested within one day after harvest, we expect that the R/UV-F system should perform better for apples after cold storage.

Budget:

Since the second year funding of $32,788 will cover the one full year period ending on July 31, 2006, we request for funding of $16,852 to cover the six month period from August 1, 2006 to January 31, 2007.

Current year: 2006
Project total (2.5 years): $80,740
Current year request: $16,852
Budget breakdown:

Year / Items	Year One	Year Two	Year Three (Six Months)
Research Associate*			
Salary (0.4)	$ 18,400	$ 18,952	$ 9,760
Benefits (36%)	6,624	6,823	3,514
Student Employee (0.2)**	4,576	4,713	2,428
Travel		800	400
Supplies	1,500	1,500	750
Total	$ 31,100	$ 32,788	$ 16,852

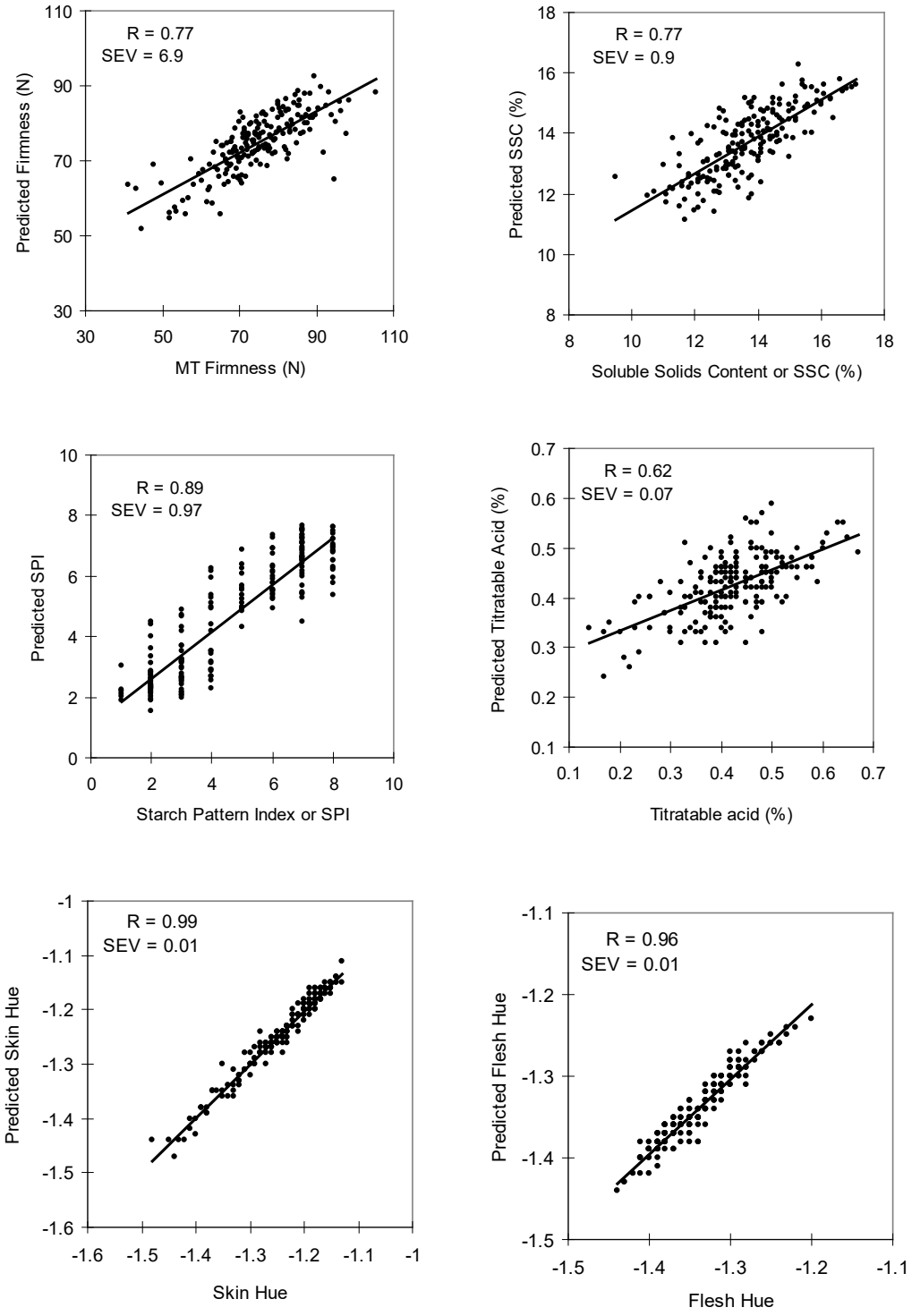

Figure 1. Neural network predictions of the selected maturity indexes of Golden Delicious apples from the integrated data of reflectance and fluorescence acquired by the reflectance and UV-induced fluorescence spectroscopic system. (Labels in the graphs: R = correlation coefficient; SEV = standard error for independent samples.)

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project # PH-04-0443 **Organization Project** # 5350-43000-004-09T

Project Title: Regulation of apple fruit ripening

Pi: James Mattheis, Plant Physiologist
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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.

Significant Findings:

1. Nitric oxide (˙NO) production increases during 'Delicious' apple maturation.

2. Whole apples exposed to ˙NO or NO_2^- have reduced ethylene production and respiration rate.

3. 'Braeburn' apples exposed to ˙NO at harvest had reduced incidence of internal browning after storage in 1.5% O_2 / 3% CO_2.

4. Delicious apples exposed to ˙NO at harvest did not develop superficial scald through 6 months storage.

5. Delicious apples repeatedly exposed to ˙NO during 6 months CA storage had higher firmness than fruit not exposed to ˙NO or exposed to ˙NO only at harvest.

Methods:

1. ˙NO production during fruit development. Apple fruit will be harvested sequentially starting at least two weeks prior to anticipated commercial harvest. Fruit will be placed into sealed vessels (typically glass jars) from which gas samples will be removed and analyzed. ˙NO gas evolved from intact fruit will be analyzed using a chemiluminescent analysis system (Sievers 280i). For maturity studies, fruit will also be analyzed for ethylene production, starch clearing, firmness and other quality attributes to establish relationships between known indices of maturity and ˙NO. Similar studies will be conducted with fruit previously treated with 1-MCP, ethylene, stored in air or CA, or during ripening after removal from storage. Resources for fruit storage, handling and analysis are in place and no new equipment will be required for this work.

2. Response of apple fruit to ˙NO applied after harvest or during storage. ˙NO gas has a relatively short half-life in air due to its reactivity with O_2. Previous studies have exposed fruit to ˙NO for short periods under anaerobic conditions, a protocol that is undesirable from a commercial standpoint. Experiments will be conducted where fruit is exposed to ˙NO over a range of ˙NO concentrations, durations, temperatures, and O_2 concentrations. Treated fruit will then be held in cold storage in air or CA with cultivar specific O_2 and CO_2 concentrations. ˙NO applications will be made at harvest and/or during storage to evaluate optimum treatment timing and duration of effects. Treatment effects will be established by analysis of fruit ethylene production, respiration, incidence of physiological disorders, and objective fruit quality. Equipment for these treatments and analyses are in place and only consumable items (fruit, gas) will need to be purchased.

3. A number of compounds have been demonstrated to influence ˙NO production in plant tissue. Some of these compounds (i.e. s-nitrosoglutathione, sodium nitroprusside) release ˙NO when in contact with living cells. Preliminary work using discs cut from 'Golden Delicious' apples showed ethylene production decreased when discs were floated on solutions containing these materials. We have also observed that ˙NO evolution by 'Granny Smith' apples increases upon exposure to high CO_2. Further studies with ˙NO donors, production inhibitors and activators will be conducted to characterize what if any impacts on fruit quality are induced by inhibiting or stimulating fruit ˙NO production. Studies will also be conducted to evaluate whether existing components of the apple postharvest system (temperature, O_2, CO_2, ethylene, 1-MCP, AVG) impact fruit ˙NO production. Equipment for these treatments and analyses are in place and only consumable items (fruit, ˙NO donors, inhibitors) will need to be purchased.

Results and Discussion:

Experiments initiated during the 2005 season focused on Objective 2 were designed to confirm 2004 results related to reduced development of superficial scald and internal browning following whole fruit exposure to ˙NO. Studies initiated in 2005 used 'Braeburn' (2 lots), Delicious (3 lots), and 'Granny Smith' (3 lots) fruit, harvested at commercial maturity. At harvest, fruit from each lot was exposed to 10 ppm ˙NO in air or 0.5% O_2 for 2 hours at room temperature. Fruit was then stored in air or CA at 33 ^{o}F. Some fruit stored in CA is being exposed to˙NO at regular intervals throughout an 8 month storage period. Fruit will be evaluated at 3 month intervals during the storage period for incidence and severity of disorders as well as fruit quality. No results are available at the time this report is being prepared.

Studies related to Objective 3 were conducted 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, or sodium ferrocyanide [$NaFe(CN_6)$], a SNP control. 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 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. 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. These results indicate ethylene production in apple disks can be manipulated by the presence of ˙NO and will lead to additional studies with whole fruit.

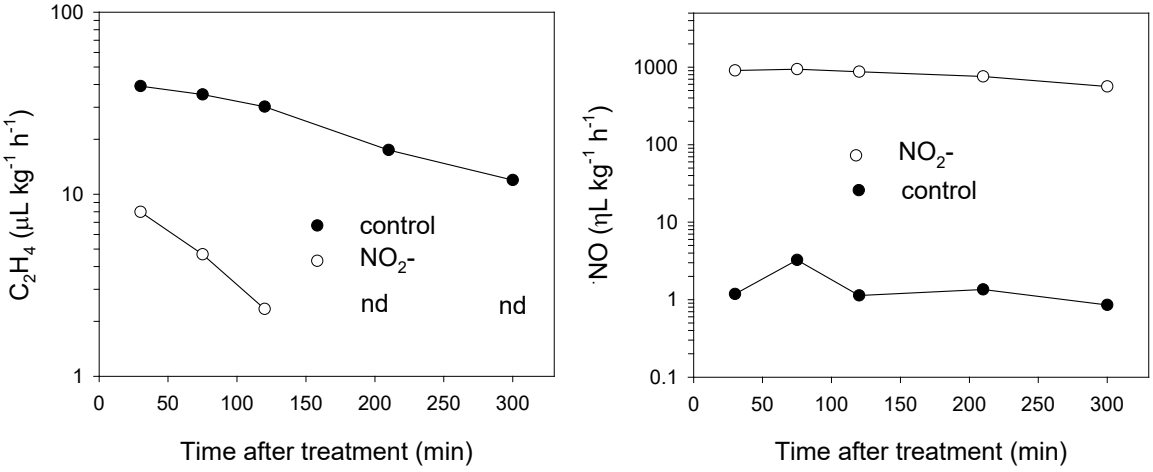

Figure 1. Ethylene and ˙NO production by 'Golden Delicious' apple fruit disks during exposure to nitrite. nd: not detected.

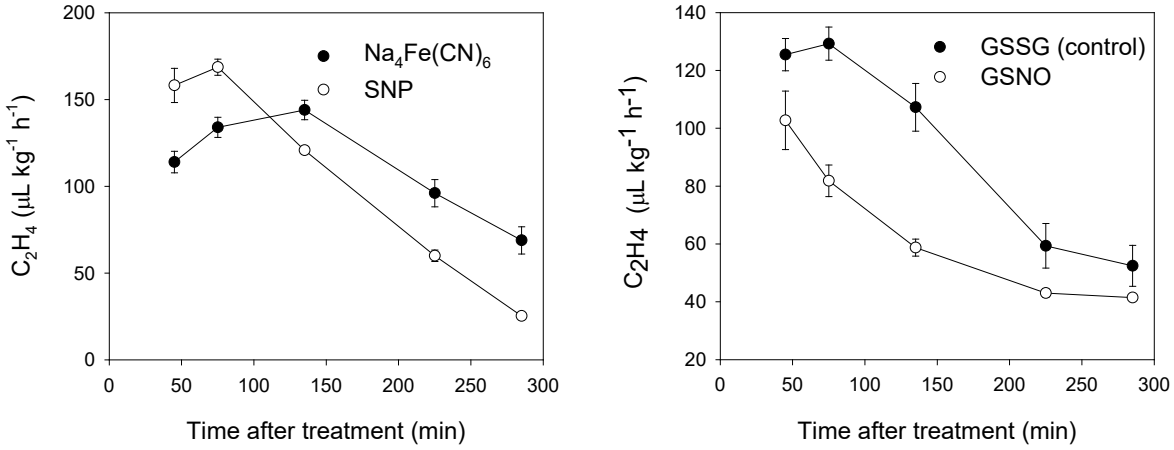

Figure 2. Ethylene (C_2H_4) production by 'Golden Delicious' apple fruit disks. Disks were exposed to solutions containing ˙NO releasers sodium nitroprusside (SNP), S-nitrosoglutathione (GSNO), or controls containing sodium ferrocyanide [$Na_4Fe(CN)_6$]or oxidized glutathione (GSSG).

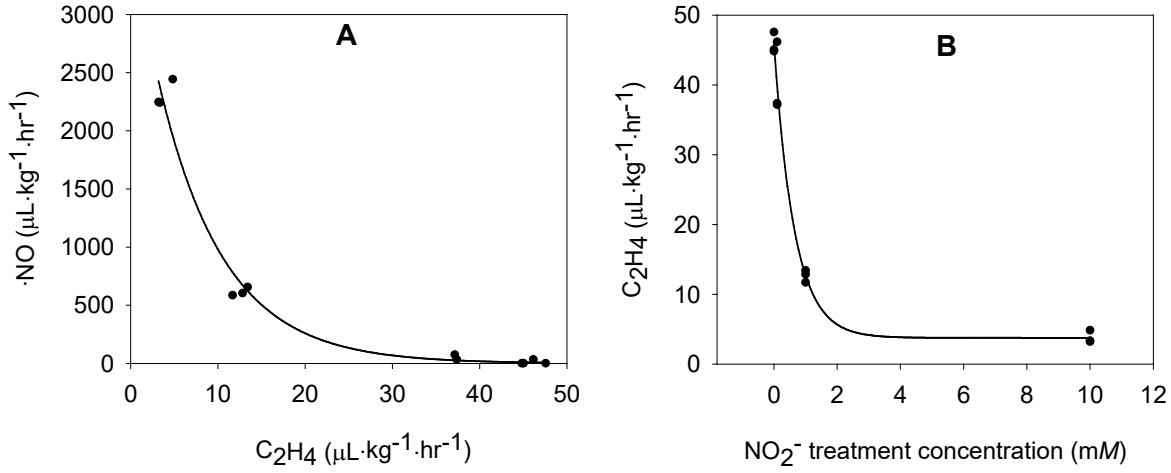

Figure 3. Relationships between ˙NO and ethylene production in response to treatment of 'Golden Delicious' apple disks with increasing concentrations of nitrite.

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.

BUDGET:

Project Title: Regulation of apple fruit ripening and development of physiological disorders by volatile compounds.
Principal Investigator: James Mattheis, Plant Physiologist
Project duration: 2004-2006
Current year: 2006
Project total (3 years): $101,500*
Current year request: $ 36,250

Year	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
Total	$41,500	$23,750	$36,250

Current year breakdown

Item	Year 1 (2004-05)	Year 2 (2005-06)	**Year 3 (2006)**
GS-11 Postdoctoral Research Associate	$40,000	$0	**$0**
GS-9 Biological Research Technician	$0	$19,750**	**$34,750*****
time-slip labor	$0	$2,500	**$0**
Supplies and materials	$ 1,500	$ 1,500	**$1,500**
Total	$41,500	$23,750	**$36,250**

*Total originally proposed was $138,750.
**Balance paid from a trust fund due to expire in 2005.
***Salary/benefits for 6 months only due to change in funding cycle.

CONTINUING PROJECT REPORT **YEAR 1/3**
WTFRC Project#: PH-05-504

Project Title: Defining the ethylene regulation of apple fruit quality

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Cooperators:
Ana Maria Ibanez and Sandie Uratsu, Department of Plant Science, Pomology, University of California, Davis, CA 95616

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

Objective 1. Identify specific transcripts regulated in transgenic apple fruit silenced for ethylene synthesis or perception and correlated with flavor and texture development - The primary aim of this objective is to compile and annotate the most regulated transcripts expressed during apple fruit development. The transcriptome of developing apple fruit will be temporally sampled with particular emphasis on transcripts expressed in two tissues, flesh and peel. Microarrays will be used to analyze the expression pattern of genes identified in the apple database that are regulated by ethylene. Expression patterns will clustered to identify and profile the different metabolic pathways.

Objective 2. Functional validation of pathways via the analysis of key metabolites an enzymes regulated by ethylene - Gene functional correlation will be derived for genes and pathways identified in Objective 1 by validating with metabolite and biochemical analysis. Tissues obtained from transgenic plants silenced for ethylene biosynthesis will be used for the biochemical and metabolite analysis. The transgenic plants will be treated with ethylene to validate the ethylene responsiveness and recovery of metabolites and enzymes regulated by ethylene.

Significant findings/accomplishments

1. Transgenic apple lines that are suppressed for either ACS (ACC Synthase) or ACO (ACC Oxidase) expression produced very low ethylene.
2. External color and firmness are regulated by ethylene.
3. Firmness was measured by both destructive and non destructive methods.
4. ACS and ACO suppressed lines show that individual sugars in fruit tissues are differentially regulated in ethylene suppressed lines, with sucrose and fructose showing

an ethylene dependent behavior. Down-regulation of ethylene biosynthesis do not reduces loss of acids.

5. Web accessible database has been developed and accessible to all WTFRC and other apple researchers through the Core Genomics facility (CGF) website (http://cgf.ucdavis.edu/) available by clicking on the apple icon to examine all publically accessible (GenBank, NCBI) genetic information.
6. Microarrays that contain the publically accessibly 'unigene set' have been designed and will be used to analyze the expression pattern of genes regulated by ethylene. Messanger RNA purification from apple flesh and peel is in progress.
7. Metabolomic analysis has been initiated to identified the full metabolite profiles in apple flesh and peel tissues from treated or untreated control and transgenic fruit apples.

Methods:

Plant material: The proposed experiments utilized 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 μL 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: Fruits from selected Greensleeves apples lines including transgenic 68G (ACO-antisense), 103Y (ACS-sense) and non-transformed 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 line 68G and 103Y lines were treated with ethylene. Half of the fruit was kept at 20ºC in an ethylene-free atmosphere and the other half was stored at 20 ºC under the flow of air containing 80 μL L^{-1} ethylene during storage. Because of the differences in enzymatic activities that have been 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 was 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 content (by a refractometer), and titratable acidity (by an automatic titration system), flesh firmness (by a Guss fruit texture analyzer and an Aweta Acoustic Firmness Sensor). All of these were used as characteristics in defining apple fruit maturity phenotype and provide a "quality" reference for samples tested for comparison with ethylene-silenced fruit.

Microarray analysis of the transcriptome: We have initiated the design phase of the development of an oligo microarray platform made by Combimatrix using their bioinformatics pipeline and design criteria utilizing the apple unigene set information from GeneBank. They will synthesize the desired oligonucleotides on the chip surface and then send us the chips. We will label RNA and hybridize 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 enzymes 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 acids using a high resolution GC/MS equipment. Enzyme that regulate sugar-acid balance will be assayed using methods described by Dey and Harborne (1990).

Additional Metabolite analysis: Metabolite analysis was carried out at the Metabolomic facility of the Davis campus (http://fiehnlab.ucdavis.edu/) under the direction of Prof. 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 their high resolution GC/MS equipment they resolved 400 compounds for each of our samples. We will then used their existing software to resolve these into pathways so that we can verify the pathway data obtained from the microarray analysis.

Results and discussion

1.- Transgenic apple lines that are suppressed for either ACS (ACC Synthase) or ACO (ACC Oxidase) expression produced very low ethylene.

The application of 1-MCP to GS (control) apple fruit produce a total suppression of ethylene production. The application of exogenous ethylene to 68G and 103 transgenic lines did not produce an increase in ethylene biosynthesis.

Ethylene production rate (µL ethylene Kg^{-1} h^{-1})		
Line	**1day storage at 20 ºC**	**14 days storage at 20 ºC**
GS control	2.0	115.3
GS-1-MCP treated		0.8
68G	1.9	2.0
68G-ethylene treated		1.9
103Y	2.4	0.8
103Y-ethylene treated		1.5

Values are means of 3 replicates of 5 fruits each. G= ACO antisense, Y=ACS sense.

2. External color and firmness are regulated by ethylene.

Maturity and quality parameters including external color and firmness were affected when ethylene biosynthesis was suppressed or inhibited. On the other hand parameters like soluble solids and titratable acidity were not affected by the reduction in ethylene biosynthesis. However, these results are preliminary and the final conclusions can be made after two years of field data.

Quality attributes after storage for 1 and 14 days at 20 ºC						
Line	**Soluble solids (ºBrix)**		**Acidity (Malic acid)**		**Color (hue angle)**	
	1 day	**14 days**	**1 day**	**14 days**	**1 day**	**14 days**
GS control	10.8	12.6	0.61	0.41	100	93
GS-1-MCP treated		13.0		0.54		100
68G	11.8	15.4	0.65	0.46	108	110
68G-ethylene treated		14.9		0.40		96
103Y	11.9	15.2	0.63	0.45	110	117
103Y-ethylene treated		15.8		0.37		88

3.- Firmness was measured by both destructive and non destructive methods.
Firmness, one of the main maturity and quality parameters is regulated by ethylene biosynthesis, most notably affected in the 1-MCP treated fruit, the effect of ethylene suppression on firmness is less obvious in transgenic lines. However, these results are preliminary and the final conclusions can be made after two years of field data. 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 measurement method) and using same fruits firmness was measure later by the Guss Fruit Texture Analyzer.

Firmness after storage for 1 and 14 days at 20ºC			
Line	**Firmness (Newtons), Fruit TA**		**Firmness Index, AFS**
	1 day	**14 days**	**14 days**
GS control	17.1	8.8	21.3
GS-1-MCP treated		16.7	23.9
68G	22.1	10.5	23.5
68G-ethylene treated		8.1	23.1
103Y	20.6	10.7	24.3
103Y-ethylene treated		6.8	22.6

Fruit TA = Guss fruit texture analyzer, AFS = Aweta Acoustic Firmness Sensor

4.- ACS and ACO suppressed lines show that individual sugars in fruit tissues are differentially regulated in ethylene suppressed lines, with sucrose and fructose showing an ethylene dependent behavior. Down-regulation of ethylene biosynthesis do not reduces loss of acids.
Sugars like fructose, glucose, sucrose and sorbitol are the major metabolites that determine apple fruit sweetness. In the lines suppressed for ethylene biosynthesis fructose and sucrose do not accumulate at the levels of that observed in the GS controls. However, when the suppressed lines were exposed to exogenous ethylene they reached control GS levels, except for glucose and fructose for line 103Y ethylene treated. The application of 1-MCP did not reduce sugar accumulation. However, final conclusions can be made with two years of field data. Individual sugars and malic acid were determined in flesh and peel tissues.

Sugars and Malic acid (µg/µL) levels in apple flesh tissue stored for 1 & 14 days at 20ºC										
Line	**Sucrose**		**Glucose**		**Fructose**		**Sorbitol**		**Malic Acid**	
	1d	**14d**	**1d**	**14d**	**1d**	**14d**	**1d**	**14d**	**1d**	**14d**
GS control	201	531	415	1182	375	593	24	27	50	41
GS-1MCP		512		1065		640		36		69
68G	211	121	400	139	380	120	73	13	58	9
68G-ethylene		401		697		538		61		46
103Y	137	157	484	45	267	118	89	14	66	11
103Y-ethylene		484		54		187		38		17

Sugars and Malic acid (µg/µL)s in peel tissue from apple fruit stored for 14 days at 20ºC					
Line	**Sucrose**	**Glucose**	**Fructose**	**Sorbitol**	**Malic Acid**
GS	14.8	3.9	6.7	0.5	0.5
GS-1MCP	12.8	1.0	8.3	0.6	0.6
68G	4.3	0.9	2.1	0.2	0.1
68G-ethylene	6.2	1.3	3.0	0.2	0.1
103Y	2.6	0.5	1.2	0.2	0.1
103Y-ethylene	6.8	1.3	2.6	0.5	0.1

5. Web accessible database has been developed and accessible to all WTFRC and other apple researchers through the Core Genomics facility (CGF) website (http://cgf.ucdavis.edu/) available by clicking on the apple icon to examine all publically accessible (GenBank, NCBI) genetic information.

We have assembled a cDNA anlaysis pipline at the Collage of Agriculture and Environmental Science Core Genomics Facility (CA&ES CGF) at Davis. This pipeline consists of a series of programs that help examine all of information stored in the GenBank public database. What we have been able to accomplish with this pipline is to access the raw sequence information present for apple ESTs which represent a terminal DNA sequence representing either the 5' of 3' end of an apple mRNA. These sequences are down loaded and stored in an Oricle database at Davis then they are sorted to remove extraneous sequences, sequences that represent E.coli, chloroplast or mitochondria DNA sequence information are removed and only high quality sequence information is retained for further analysis. Then these sequences are compared among them selves and with other sequences present in GenBank all of this is used to sort and to cluster these sequences based upon similarity and possible gene function. We have examined 160620 of the currently 198,663 entries in the public database for Apple (GenBank, NCBI). We have made our analysis web accessible to all apple researchers at WTFRC through the Core Genomics facility (CGF) website (http://cgf.ucdavis.edu/) available by clicking on the apple icon. We have currently analyzed 160,620 ESTs that correspond to a unigene set of 45,414 (28.3% discovery rate) genes with a majority (25,232; 15.7%) being singletons (represented once in our database) and 20,182 (12.6%) of these being 'contigs' (represented more than once in our online database).

6.- Microarrays that contain the publically accessibly 'unigene set' have been designed and will be used to analyze the expression pattern of genes regulated by ethylene. Messenger RNA purification from apple flesh and peel is in progress.

DNA microarrays provide a useful platform to evaluate the coordinate expression of groups of genes that may play a role in fruit development and in the ethylene mediated regulation of genes expressed in apple fruit. Recently microarray analysis was used to identify SAAT (Strawberry Alcohol Acetyltransferase) a gene involved in strawberry flavor development (Ahroni et al., 2000). We will use microarray analysis to profile all of the ESTs that we have identified in specific aim 1. And since quality traits like flavor, aroma and texture of fresh fruits like apple are genetic components of a complex phenotype that are regulated directly or indirectly by ethylene in the field during the growing season and during post harvest storage, microarrays data will also used to understand the role that ethylene plays in the different metabolic pathways. The clustering of expression patterns through apple development will be used to discover and link other unknown genes and pathways that may encode or regulate flavor and texture and to cluster known with unknown ESTs that show coordinate regulation. Validation of the pathway analysis will be accomplished by analyzing metabolites and enzymes. We have initiated the design phase of the development of an oligonucleotide based microarray platform made by Combimatrix using their bioinformatics pipeline and design criteria. We have begun this process by using the widely accepted apple unigene set information from GeneBank. Currently, they are 15,274 genes listed and we will be using all of them on our microarrays.

7.- Metabolomic analysis has been initiated to identified the full metabolite profiles in apple flesh and peel tissues from treated or untreated control and transgenic fruit apples.

Average metabolites detected in flesh from 1 day and 14 days after storage at 20 were 155 and 136 respectively. Average metabolites in peel from 1 day and 14 days were 132 and 125 respectively. In both cases the average metabolites observes included sucrose, glucose, fructose, sorbitol and malic acid. Complex statistical analysis will be used to evaluate the differences in values between each genotype and between treatments. Metabolite profiles will be used later to resolve these into pathways and verify pathway data obtained from the microarrays analysis.

Significance to the industry and potential economic benefits

Understanding the metabolic network (pathways) involved in apple fruit could facilitate extending post harvest life based on flavor, aroma and texture to match that of appearance, which, in turn, would promote consumption of fresh apple fruits. reduce losses during post harvest storage to stimulate the 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 and breeding and selection of existing and new cultivars.

Literature review:

Aharoni, A., Keizer, L.C.P., Bouwmeester, H.J., Sun, Z., Alvarez-Huerta, M., Verhoeven, H.A., Blaas, J., L. van Houwelingen, M.L., De Vos, R.C.H., van der Voet, H., Jansem, R.C., Guis, M., Mol, J., Davis, R.W., Schena, M., van Tunen, A.J. and O'Connell, A.P. 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarray. The Plant Cell 12:647-661.

Dey, P.M. and Harborne, J.B. 1990. Methods in Plant Biochemistry. Enzymes of Primary Metabolism. Lea, P.J. ed. Volume 3

Defilippi, B.G., Dandekar, A.M., Kader, A.A. 2004. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica Borkh*) fruits. J. Agric. Food Chem. 52:5694-5701.

Project title: Defining the ethylene regulation of apple fruit quality
PI: Abhaya M. Dandekar
Project duration: 2005-2007 (3 years)
Current year: 2005
Project total (3 years): $128,295
Past year request: $39,935
Current year request: $43,907

	Year 1 (2005)	**Year 2 (2006)**	Year 3 (2007)
Salaries PGRVI (50%)	$19,326	**$20,292**	$21,307
Benefits	$10,109	**$10,651**	$11,146
Equipment			
Supplies	$ 9,500	**$12,000**	$11,000
Travel	$ 1,000	**$ 1,000**	$ 1,000
Miscellaneous			
Totals	$39,935	**$43,907**	$44,453

Project Title: Identifying disease prevention benefits of apple consumption
PI: Preston Andrews, Department of Horticulture and Landscape Architecture
WSU Pullman

No report submitted

CONTINUING PROJECT REPORT **YEAR 1/3**

WTFRC Project # 2005-10 **Organization Project #** 3031

Project Title: Ethylene Measurement in Post-Harvest Storage
Principal Investigator: Reza Shekarriz, Ph.D., reza.shekarriz@microet.com; 503-234-2747, x110
Organization: MicroEnergy Technologies, Inc., 3525 S.E. 17th Ave., Portland, OR 97202
Co-PI: Dr. Lloyd Allen, MicroEnergy Technologies, Inc.; lloyd.allen@microet.com
Contract Administrator: Ms. Dawn Nelson, dawn.nelson@microet.com; Phone: 503.234.2747; x101

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 (Option): 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

As previously scheduled, the bulk of this work will begin in January of this year and we have currently been using our matching funding from USDA to develop the prototype, without accruing any charges against the current project. We anticipate to be working with Dana Faubion for some of our testing and evaluations of the prototype in the coming quarter, to characterize single apple ethylene respiration rate. Section IV of this report provides some of our current progress toward the development of the prototype for our testing.

III. Methods: Electrochemical sensing

The current approach for post-harvest ethylene sensing is based on electrochemical oxidation of ethylene. 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.

IV. Task Progress

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

For the design and development of the gold electrode, a test matrix has been made with which the key parameters in the plating process will be evaluated. Some of these parameters include plating chamber volume for the reducing solution and the oxidizing solution, the option of stirring and pre and post treatment methods. In order to evaluate these parameters, new plating apparatus had to be designed and fabricated. The rendering and photo below show the design and the fabricated chamber along (left and middle) with chamber inserts (right) which allow for changing the plating chamber volume.

Figure 1. Plating apparatus for development of the nanoporous gold catalyst.

The new plating chamber also has optional caps with holes for stoppers, as well as latches which make assembly and disassembly quick and easy.

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.

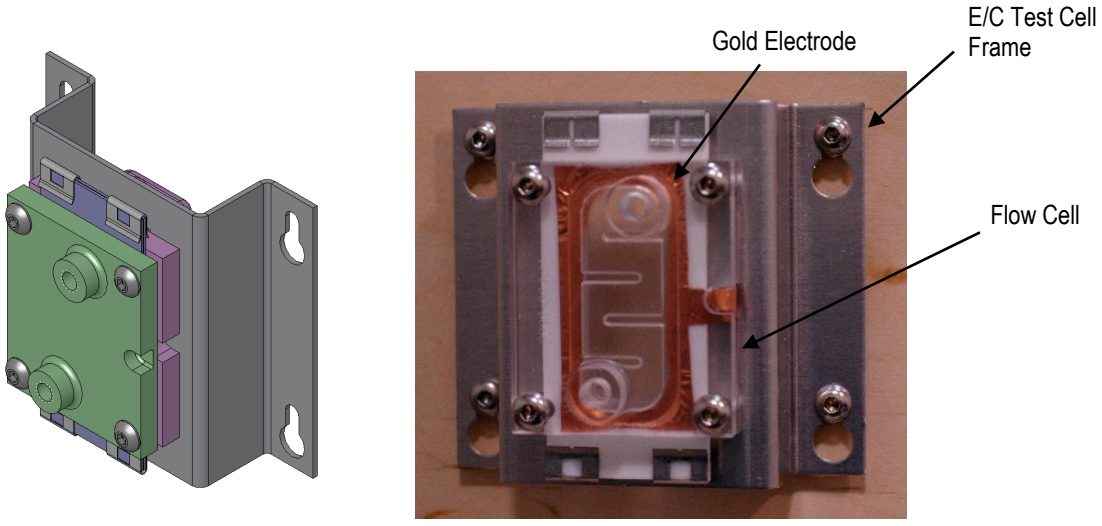

Figure 2. Electrochemical prototype for single apple respiration rate tests.

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 make it easily tested in different gas inlet configurations or environments. The catalyst exposed surface area is approximately 2.5 times larger than any previously tested surface area which should make 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. The frame is shown below.

Figure 3. Catalyst membrane frame.

This frame will make it possible to handle the membranes without worrying about damaging them and will make it much easier to use a membrane multiple times.

During the next reporting period we plan to use the new test cell to evaluate plated electrodes. We will also be working on the sensing electronics that will eventually be used in place of the potentiostat for current sensing and cell potential control.

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

Although actual prototype testing has not yet started, the test apparatus has been designed and constructed. The test apparatus was constructed in such a way that our target ethylene concentrations can easily be achieved with less error than was previously achievable. The new test apparatus also gives us the flexibility to run a range of flow rates from 0 to 2000 sccm through the test cell with ease through the use of quick disconnect fittings. Another advantage that the new test apparatus gives us is the ability to purge the system with a test gas prior without exposing the test cell, then quickly switching from purge mode to test mode via solenoid valves. This will help eliminate question about whether cell response is due to the system time constant or the cell itself as the only system time constant remaining will be due to a very short length of tubing. A picture of the new test apparatus is shown below.

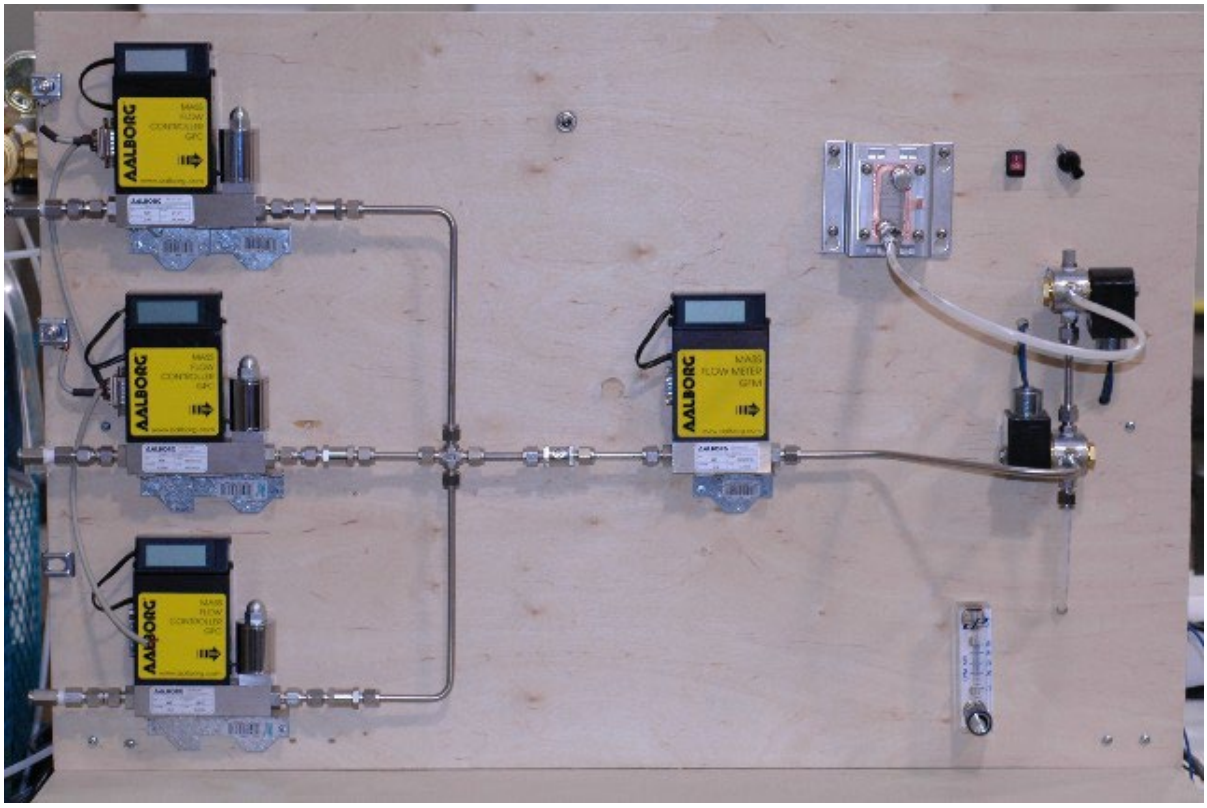

Figure 4. Electrochemical prototype testing apparatus at MicroET.

During the next reporting period, we plan to optimize the gold electrode, qualify our prototype in our laboratory, and carry out single apple respiration rate testing in laboratory and field.

V. Schedule of accomplishments

As discussed, there are three important tasks during year 1, namely, the electrode development, prototype design and fabrication, and testing. As the progress bars show, we have some progress in task 1, significant progress in task 2, and no progress in task 3. Task 3 cannot begin until we have completely assembled our prototype and tested it internally, which is expected to take place within the first quarter of 2006. Based on our progress, we are currently ahead of our original schedule.

ID	Task Name	2006 Qtr 4	2006 Qtr 1	2006 Qtr 2	2006 Qtr 3	2006 Qtr 4	2007 Qtr 1	2007 Qtr 2	2007 Qtr 3
1	**Ethylene Measurement in Post-Harvest Storage**								
2	**Year 1: 0.1 ppm Ethylene Sensor Development and Demonstration**								
3	Task 1: Gold Electrode Optimization and Characterization								
4	**Task 2: Prototype Design and Fabrication**								
5	**Task 3: Prototype Performance Testing and Evaulation**								
6	Year 2: Low Cost Prototype Development and Demonstration								

VI. Budget

ITEM	Year 1	Year 2
1. Salaries	25000	16000
2. Fringe Benefits	10000	6400
3. Fabrication	5000	3200
4. Equipment	3000	1500
5. Supplies	1000	2000
6. Travel	500	1500
7. General and Administrative	8900	6120
8. Fee	3738	2570
Total	**$57,138**	**$39,290**

Explanation:

1. Salaries are for the PI, Co-PI, and 2 other engineers.
   Detailed salaries can be provided upon request.
2. Fringe benefits are 40% and do not include OH.
3. Fabrication costs are calculated at a standard shop rate of $60/hr.
4. Low Flow Mass Flow Meters, Low Current Measurement System
5. Gold for Catalyst, Nafion, Fittings, Device Electronics
6. Initial travel during year 1 is to Richland, WA (working with PNNL)
   Travel during year 2 is for travel to field testing sites and travel to Richland, WA.
7. G&A rates are calculated at 20%.
8. MicroET fee is at 7%.

The budget for this project is provided in the table above. We have currently not spent any of our available budget and we expect to start accruing charges against the current project in January 2006.