FINAL PROJECT REPORT

Project Title: Systems approach for ensuring superior pear fruit quality

PI	Amit Dhingra	**Co-PI**:	Todd Einhorn
Organization:	Washington State University	**Organization**:	Oregon State University
Telephone:	509 335 3625	**Telephone**:	541.386.2030 ext. 13
Email:	adhingra@wsu.edu	**Email**:	Todd.Einhorn@oregonstate.edu
Address:	Johnson 46	Address:	3005 Experiment State Drive
City:	Pullman	City:	Hood River
State/Zip:	WA 99164	State/Zip:	OR 97031

Co-PI:	Qin Zhang	**Co-PI**:	Kate Evans
Organization:	Washington State University	**Organization**:	Washington State University
Telephone:	509-786-9360	**Telephone**:	509-663-8181
Email:	qinzhang@wsu.edu	**Email**:	Kate_evans@wsu.edu
Address:	24106 N. Bunn Rd	Address:	1100 N. Western Ave
City:	Prosser	City:	Wenatchee
State/Zip:	WA 99350-8694	State/Zip:	WA 98801-1230

Cooperators: WSU - Matthew Whiting, Don Elfving, Tim Smith, Ananth Kalyanaraman, Carolyn Ross, Shyam Sablani; Marie-Helene Simard (Pear Breeder at INRA), Yves Lespinasse, Charles-Eric Durel, Elisabeth Chevreau, INRA at Angers, France; Richard Bell, USDA; Riccardo Velasco, IASMA, Italy; Gavin Ross, Plant and Food Systems, NZ, Toshiya Yamamoto, Japan, Stefano Tartarini, Italy, Josh Koempel, Nate Squire and Ray Schmitten.

Other funding sources – none

Total Project Funding: Year 1: 113,861 Year 2: 114,759 Year 3: 118,045

Budget 1 Amit Dhingra
Organization Name: WSU **Contract Administrator:** ML. Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	2010	2011	2012
Salaries[1]	55,002	46,765	43,871
Benefits	10,523	3,337	10,143
Wages	7,546	7,847	8,160
Benefits	724	753	783
Supplies	8,000	8,000	8,000
Travel	5,000	9,000	2,000
Consumer panel			5000
Miscellaneous – 454 sequencing		11,000	11,000
Total	86,795	86,702	88,957

Footnotes: [1] Salaries for agriculture research assistant (PhD-12 months) and agriculture research assistant (MS-9 months @ 65% of 0.50FTE) and visiting scholar for performing physiological and genomic profiling, all molecular work; sanitization platform and robotics respectively. The increase in salaries for years two and three reflects a 4 % rate increase.

Budget 2 Todd Einhorn

Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton
Telephone: 541 737-3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	2010	2011	2012
Salaries[1]	$21,662	$22,529	$23,430
Benefits[2]	$2,484	$2,608	$2,738
Wages[3]	$2,000	$2,000	$2,000
Benefits[4]	$170	$170	$170
Equipment			
Supplies			
Travel[5]	$750	$750	$750
Miscellaneous			
Total	$27,066	$28,057	$29,088

Footnotes:

[1]Salary is for a 0.49 FTE M.S. candidate calculated based on a 1.0 FTE salary rate of $44,208.
[2]MS OPE rate is $567/term * 4 terms/academic year
[3]Hourly wages for time-slip labor (~200 hours @ $9/hour) to assist with data collection and cultural practices
[4]Benefit rate for part-time employee is 8.5 %
[5]Travel includes transportation to off-station sites in OR, and one trip per year to WA sites at 0.59 cents/mile

OBJECTIVES

Summary Statement: This multi-investigator project represented multi-disciplinary activities aimed at ensuring superior pear fruit quality. Thanks to the vision of the PNW pear industry, over 68 scientists from US, Europe and South America representing diverse disciplines continue to work together with their respective industries on several aspects initiated as part of this project. A draft roadmap has been developed from our collective activities in collaboration with NW Hort Council. Further funding is being sought from USDA and NSF to build upon the foundation developed as part of this project.

Objective 1: Training systems: Evaluate, devise, and plant efficient orchard systems that are amenable to mechanized pruning and harvest using labor assist platforms. These will be located on both research station and grower cooperator sites.

Years 1-3	Years 1-3	Years 1-3	Year 3
Todd Einhorn	Todd Einhorn, Amit Dhingra	Kate Evans, Amit Dhingra	Qin Zhang, Todd Einhorn
1a.Develop cropload indices for the optimum productivity of target fruit.	1b.Plant progressive, high-density pear systems using both the physiological thresholds identified from objective 1a, and experience gained from recent high-density PNW pear plantings.	1c.Identify genotypic sources of dwarfing in rootstocks and collate information from Co-PIs project on potential rootstocks for pear.	1d.Assess the potential of mechanized pruning in high density, vertical trellis or inclined UFO pear orchards.

Objective 2: Vigor Control: Assess the effectiveness of vigor-retarding mechanical and chemical techniques.

Years 1-3	Years 1-3	Year 1-3
Todd Einhorn	Todd Einhorn, Amit Dhingra	Todd Einhorn
2a. Identify optimal limb orientation on vigor (shoot growth) precocity, fruit size and fruit quality in planar trellis systems.	2b. Perform a comparative analysis on the effect of vigor control chemistries on apple and pear.	2c. Assess different chemistries for vigor control and develop timing and rate recommendations for effective vigor control in pear.

Objective 3: Fruit Quality

Years 1 and 3	Years 1-3	Year 1-3
Amit Dhingra	Amit Dhingra	Amit Dhingra, Ray Schmitten, Josh Koempel, Nate Reed
3a. Study the impact of cuticle or fruit skin on fruit quality.	3b. Understand cork spot and russet using microscopy and genomic profiling under physiologically inductive conditions.	3c. Test the impact of chlorophyll stabilizing chemistries on scuffing and fruit quality.

Objective 4. Evaluate alternative fruit sanitization platforms

Years 1-3	Years 1-3	Year 1-3
Shyam Sablani and Karen Killinger	Qin Zhang	Shyam Sablani and Carolyn Ross

4a. Test alternate fruit sanitization methods to reduce pathogen load.	4b. Identify alternate methods of processing fruit on processing lines to prevent skin damage.	4c. perform a consumer preference study to assess consumer experience with alternately sanitized or processed pears.

SIGNIFICANT FINDINGS

Objective 1:

New plantings:

- One rootstock trial was established spring 2012 (Bartlett; Chuck Peters, Wapato, WA). A second (Anjou) will be planted in spring 2013 at MCAREC. Both sites will compare three training systems (single axe, bi-axe, and steep V). Each system will be evaluated on different rootstocks (OHxF 87, OHxF 69 and Pyro 2-33) and at three different in-row spacings (2, 4, and 6 ft.).

Rootstocks:

- More than 200 accessions of promising Pyrus rootstock material have been identified in Spain, France, Italy, UK and Argentina and are currently being imported in small groups. DNA based population structure analysis will be initiated shortly.
- In coordination with the Pyrus Crop Germplasm Committee, U.S. nurseries and national and international collaborators a selected list of desirable pear rootstocks and rootstock selections has been compiled.

Mechanized Pruning:

- Work with vertical trellis system in sweet cherry bodes well for its application in pears. Challenges for implementation of mechanized pruning in pears have been identified.

Objective 2:

Limb Training:

- ‘Bartlett’ and ‘Anjou’ scaffolds were initiated and trained to 0, 30 or 45 degree angles (from horizontal) in 2009 on an eight wire vertical trellis (18 scaffolds per tree). In 2011, Bartlett 3rd leaf scaffolds trained to 30 degrees from horizontal were significantly more precocious than those trained to 45 or 0 degrees from horizontal.
- Average fruit set per tree was highest on 30 degree limbs (216 fruit), intermediate on 45 degree limbs (141), and lowest on horizontal (0 degree) limbs (75).
- Total length of scaffolds decreased as the angle decreased. Heavy fruit loads of the 30 degree limbs significantly reduced the number and length of offshoots per scaffold relative to the other angles. Total canopy leaf area for the 30 degree trees was half that of the other two angles.
- In 2012, there were no significant differences among limb angles for Bartlett fruit set, yield (42 to 46 bins per acre projected) or fruit number (~130 fruit per tree). Fruit size at harvest was similar on 30 and 45 degree limbs (100s), and slightly smaller on 0 degree limbs (110s). In the dormant season (Feb, 2012) all scaffolds were pruned to 10 fruiting spurs, irrespective of their limb angle.
- Despite profuse bloom in 2011 and 2012 (130-240 clusters per tree), Anjou fruit set and yield was poor, and unaffected by limb angle treatments.

PGR Vigor Control:

- The plant growth regulator abscisic acid (ABA) showed limited value for controlling shoot growth of pear due to its rapid metabolism (i.e., ~2 weeks after application).
- Apogee was extremely effective in controlling Anjou and Starkrimson shoot and tree vigor over nine separate trials between 2010 and 2012. Apogee markedly reduced shoot length (~50%) in all years compared to untreated controls.
- In 2012, we refined our spring application timing to occur when shoots were ~2 inches long.
- 250 ppm was the most effective Apogee rate for controlling shoot growth, and typically only required one application per year; however, in a few trials treated shoots resumed growth needing a second application (250 ppm) ~60 days after the first.

- This second flush of growth was not observed at any of the upper valley trial sites possessing shorter, cooler seasons, or in cooler seasons at lower elevations (Hood River).
- Apogee did not negatively affect yield or individual fruit size of 'd'Anjou' and 'Starkrimson' in any year. In fact, in 2012 whole tree Apogee applications significantly improved fruit set and yield (+70%) over controls.
- Apogee was shown to have a strong localized effect on shoot growth in a hedgerow planting. Protected, untreated shoots arising from the same scaffold as treated shoots showed ~2-fold more growth at the end of the season than treated shoots.
- Apogee had stronger control over growth from un-headed shoots compared to dormant headed shoots.
- Return bloom of Anjou spurs was reduced by ~20% on average from 2010 and 2011 trials. Starkrimson return bloom was not affected by Apogee applied in 2011. 2012 return bloom will be evaluated spring of 2013. Despite the reduction in Anjou bloom, fruit set and yield the year after application was not significantly different than controls, implying that reduced return bloom did not adversely affect fruit set.

Objective 3:

Fruit quality:

- Freeze fracture method was found to be an efficient method for determination of cuticle structure
- A standardized model to correlate cuticle thickness and fruit quality as it exists for apple could not be established for pear. This is primarily due to the separation of maturity and ripeness in pears.
- The cuticle thickness was highly variable within a fruit and also within fruit collected from different areas.
- There was some difference observed in amount of cuticular waxes however no correlations could be established between the site of collection and amount of wax.

Russet and Cork spot

- Physiological induction of cork spot and russet using published protocols was not successful.
- The pear homolog of apple bitter pit-related gene has been cloned and its expression will be tested in cork tissue in 2013 growing season to establish any correlations.

Pigment stabilization and fruit quality

- Pigment stabilizing chemistry has a positive effect on fruit storage quality as it maintains its firmness throughout the storage process.
- Expanded field tests were performed in Year 3. Fruit is currently under storage and will be analyzed from Feb –April 2013.

Objective 4:

Alternate fruit sanitization to reduce pathogen load:

- UV-C was effective in reducing generic *E. coli* and blue mold populations on intact and wounded pear surfaces.
- Efficacy of UV-C treatment was dependent on the type of microorganisms and fruit surface morphological profiles, for example generic *E. Coli* bacteria were more UV-C resistant than blue mold, and higher UV-C doses were required to reduce microorganism population on wounded surfaces compared to intact fruit surfaces.

Alternative fruit handling

- 1-MCP treated pear fruit appearance does not seem to be affected by processing line components.
- If 1-MCP can be utilized successfully in pears, any damage on the processing line can be countered.

Consumer preference study

- Sensory study is underway and will complete on February 11. Soon after the data will be analyzed and results will be reported.

RESULTS AND DISCUSSION

Objective 1 and 2 (combined for simplicity of presentation and overlap of horticultural issues)

<u>PGR vigor control</u>. ABA proved to be ineffective at controlling vegetative vigor of pear trees (data not shown). Apogee®, on the other hand, was very effective at controlling vigor of Anjou and Starkrimson. Previous research demonstrated that Bartlett fruit size was directly limited by Apogee® in the year of application, while Bosc return bloom and yields were markedly reduced the year following application; Anjou fruit growth and return bloom, however, were not similarly affected (Elfving, Sugar and Mielke). Between 2010 and 2012 we conducted 9 Apogee experiments; six Anjou trials and three Starkrimson trials. In each trial we observed an approximate 50 percent reduction in the annual growth of shoots relative to untreated trees. The strongest response occurred when applications were made in early spring when shoots were ~2 inches (5cm) in length at a rate of 250 ppm (Figs 1 and 2). In 2012 we also combined Apogee with Ethrel based on previous research with sweet cherry showing a synergistic effect of these compounds on vegetative growth (Elfving and Lang). The combination did lead to slightly greater growth control than Apogee alone (Fig 1). Interestingly, Ethrel alone did not reduce vegetative growth (Fig 1). In most cases only one application was required to control Anjou shoot growth for the entire season, but in several cases a second application at the same rate was needed ~ 60-80 days after the first (Fig 1). This application coincided with a marked increase in the rate of shoot growth (Fig 1), presumably due metabolism of Apogee® in the plant. Favorable environmental conditions, however, likely play an important role in stimulating this regrowth, since we did not observe it in most years or trials. Strakrimson trees did not require multiple applications of Apogee® (Fig 2). In all years, total tree yields of Anjou and Starkrimson were either slightly improved on trees sprayed with Apogee® or similar to untreated trees (Tables 1 and 2). Strarkrimson fruit size was unaffected by Apogee®; Anjou fruit were smaller, though we considered this to be an indirect effect of the significantly higher croploads on Apogee® treated trees (Table 1). In years when yields were unaffected by Apogee, fruit sizes were equivalent to those of control trees (Table 2). In the seasons following applications, Anjou return bloom was on average 15 percent reduced (Fig 4), but this did not translate to similar reductions in yield. Return bloom of Starkrimson trees was not reduced by Apogee® (Fig 4). In 2012, Ethrel was applied ~60 days from bloom (corresponding to the flower induction period for pear) to determine if Ethrel at this timing could lead to improved return bloom in 2013.

In a separate trial, Apogee® was applied in early spring to individual Anjou shoots of a planar, hedgerow system that were either dormant headed or left unpruned. Strong control of growth was achieved for Apogee® treated shoots while growth of adjacent untreated shoots, often originating on the same scaffold as their treated counterparts, was unaffected, indicating limited transport within trees (Fig 3). The localized effect of Apogee® is notable since it offers the ability to precisely manage portions of the canopy that are imbalanced, such as is often observed with increasing canopy height, or in the tops of trees that have been headed during the dormant season. Good control of Anjou growth from dormant heading cuts to tops of mature Anjou trees has been previously shown (Elfving). Apogee® was more efficacious when applied to unheaded shoots, but significantly reduced shoot length of headed shoots relative to untreated headed shoots as well. Although Apogee® is not presently labeled for pear we have contacted the manufacturer to discuss the next steps to achieving a label for Anjou and possibly Starkrimson.

<u>Limb training</u>. In ‘Bartlett’, training scaffolds to 30° from the horizontal markedly improved precocity (2011 flowering, fruit set, and yield of 3rd leaf limbs) compared to scaffolds trained to 45° or 0° from horizontal (Table 3). Scaffolds trained to 30° also had the least vegetative growth relative to other branch angles (data not shown). The high cropload associated with the 30° angle resulted in smaller fruit size in 2011. The high early fruit set, and higher yields were effective at controlling vigor, but perhaps the balance was shifted too much in favor of fruit. In 2012, scaffolds were pruned in the dormant season to 10 fruiting spurs removing thin wood with weak fruiting buds on the ends of scaffolds. Pruning to 10 spurs also maintained scaffolds in their allotted canopy space (trees are planted at 4 ft. in-row, so each scaffold has ~2 ft. to develop). Some overlap from scaffolds of

adjacent trees was permitted. Yield was not affected by angle of scaffold in 2012; all trees had relatively good yields averaging 53 lbs per tree (projected production of ~45 bins per acre). Horizontal branch angles produced smaller fruit in 2012, presumably because the wood was markedly weaker. The situation was not the same for Anjou trees. As is typically observed with Anjou, profuse bloom in third and fourth leaf limbs did not translate to significant fruit set or yield, irrespective of limb angle treatment (data not shown).

New Plantings. One new Bartlett planting was successfully established in Wapato Washington (Chuck Peters) spring 2012; an identical planting of Anjou will be planted in Hood River (OSU-MCAREC) spring 2013. The trials were designed to evaluate Bartlett and Anjou performance on OH × F 87, OH × F 69 and Pyro 2-33 trained to three different systems: Tall spindle/single-ax; bi-ax (parallel to the row); and, a steep, perpendicular V (each side ~10-15° from the vertical). For the V, each tree is bent to the opposite side of the tree row. For each rootstock/training system combination, three within row spacings will be evaluated: 2ft.; 4ft.; and, 6ft. Between row spacing is 12ft. Rootstocks were raised from tissue culture (North American Plants, LLC.) and delivered to Willow Drive Nursery spring of 2011. Rootstocks were budded to Anjou late summer 2011. Double budding to establish bi-axe trees was performed in the nursery. The bi-ax system has the advantages of splitting vigor over two axes, and provides a larger proportion of future bearing surface at planting compared to single leader trees, or trees that are headed at planting to create V systems. Finished trees will be delivered to MCAREC spring of 2013 and planted in fumigated ground.

Figures and Tables:

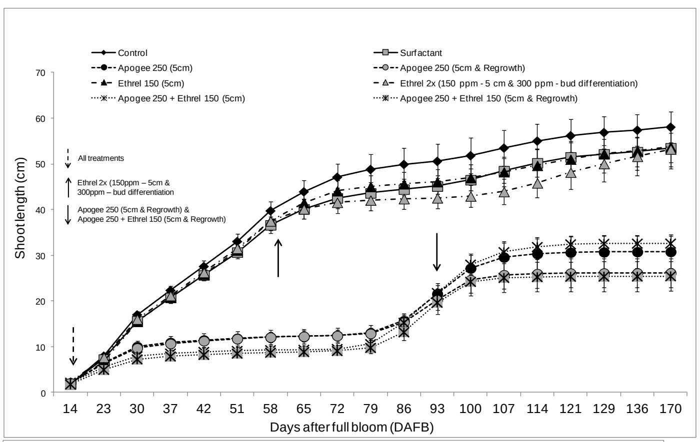

Figure 1. Shoot growth [length (cm)] of 'd'Anjou' pear trees sprayed with plant growth regulators, either alone or combined, when shoots were ~5cm long. Treatments were applied to whole trees (6 replicates) of similar trunk circumference (n=12 shoots per tree). MCAREC, 2012.

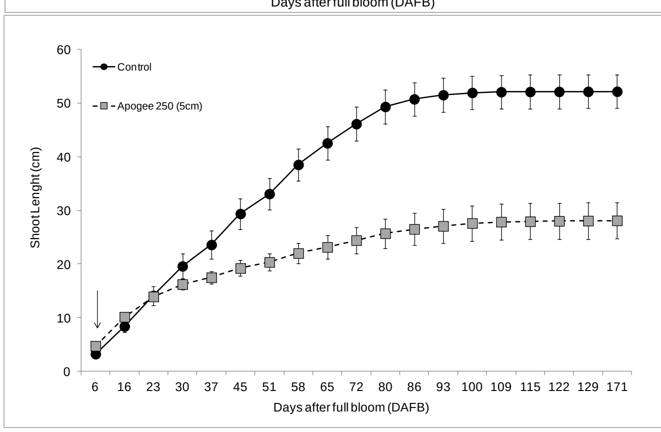

Figure 2. Shoot growth [length (cm)] of 'Starkrimson' pear trees sprayed with Apogee (250 ppm) when shoots were ~5cm long. Treatments were applied to whole trees (5 replicates) randomized within blocks (n=14 shoots per tree). MCAREC, 2012.

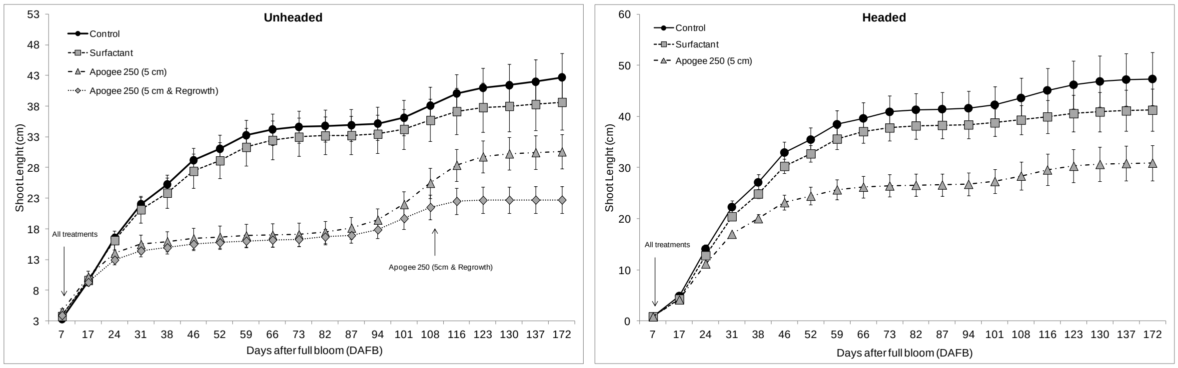

Figure 3. Shoot growth [length (cm)] of 'd'Anjou' single shoots (unheaded & headed at dormancy) following application of Apogee (250 ppm) when shoots were ~5cm long. Shoots were randomly selected in five-tree plots (5 replicates; n=10 shoots). Plots were selected in a high-density (906 trees/acre) planar system at MCAREC, 2012.

Table 1. The effect of plant growth regulators on 'd'Anjou' fruit number, yield and average fruit size. Treatments were applied to whole trees when shoots were ~5cm long and for certain treatments, again when a second growth flush was observed (data are means of 6 replicates). MCAREC, 2012.

Treatment	Yield		Avg. Fruit size
	(No. Fruit)	(lb per tree)	(g)
Control	266.8 c	160.7	273.0 a
Control + surfactant	296.8 bc	178.6	273.8 a
Apogee 250 ppm at 5 cm	447.8 a	229.5	234.5 cd
Apogee 250 ppm + Ethrel 150 ppm at 5 cm	396.8 ab	214.9	247.7 bcd
Ethrel 150 ppm at 5 cm	357.5 abc	200.1	257.3 ab
Apogee 250 ppm + Ethrel 150 (at 5 cm + regrowth)	349.2 abc	186.9	242.9 bcd
Apogee 250 ppm (at 5cm + regrowth)	345.3 abc	188.6	250.2 bc
Ethrel 150 ppm at 5 cm + 300 ppm at bud differentiation	323.0 bc	159.3	228.3 d

Within columns means with different letters are significantly different at P<0.05

Table 2. The effect of Apogee (250 ppm) on 'Starkrimson' fruit number, yield and average fruit size. Treatments were applied to whole trees when shoots were ~5cm (data are means of 5 replicates). MCAREC, 2012.

Treatment	Yield		Avg. fruit size
	(no.fruit per tree)	(lbs per tree)	(g)
Control	125	54.2	199.4
Apogee 250 ppm at 5cm	118.2	53.3	206.1

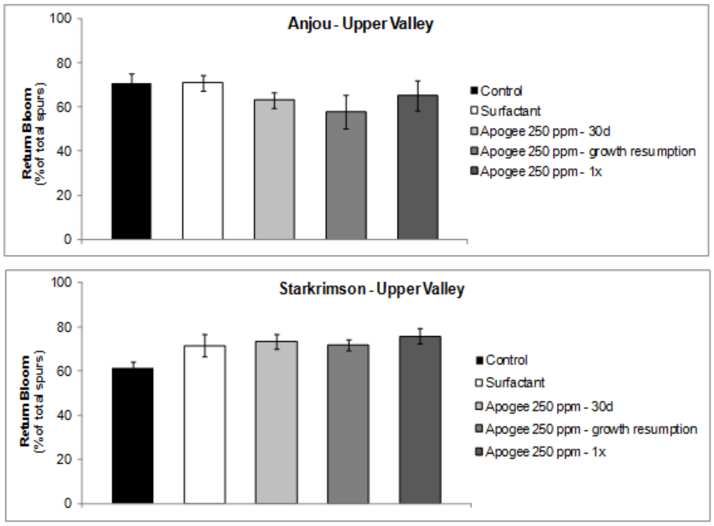

Figure 4. ‘d’Anjou’ and ‘Starkrimson’ return bloom in 2012 following 2011 Apogee applications. In 2011, Apogee was applied at a rate of 250 ppm when shoots were <10 cm long either once over the entire season (1x), twice (when shoots were <10 cm long and again when shoot growth resumed) or every 30 days, beginning when shoots were <10 cm long.

Table 3. Effect of primary scaffold branch angle (from the horizontal) on fruit set, yield, and average fruit size at harvest of Bartlett.

Limb Angle (° from Horiz.)	**Fruit Set** Fruit per tree (# before Thinning)	**Yield** Per Tree (lb)		Per Acre (1,100 lb bins)		**Avg. Fruit Size** weight (g)		Box Size (# per 44 lbs)	
	2011	2011	2012	2011	2012	2011	2012	2011	2012
45[#]	141	38	51	31	42	196	198	100	100
30	216	50	55	41	46	179	194	110	100
0	75	27	56	22	47	202	183	100	110

[#]2011 and 2012 are 7th and 8th leaf for trees, but all previous scaffolds were removed in 2009. New, angled scaffolds were initiated in 2009. Tree spacing is 4 ft. x 12 ft. (906 trees per acre). System is an 8-wire vertical trellis, with a max height of 13 ft.

Objective 3:
Chlorophyll stabilizing chemistry:
The chlorophyll stabilizing chemistry shows an affect in d’Anjou pears which is a repeat of what was observed last year (Figure 5). Lower brix levels after CA storage can be exploited for delivering better pears. Since this chemistry does not interfere with the ethylene pathway it may provide an alternative to MCP in maintaining firmness in pears.

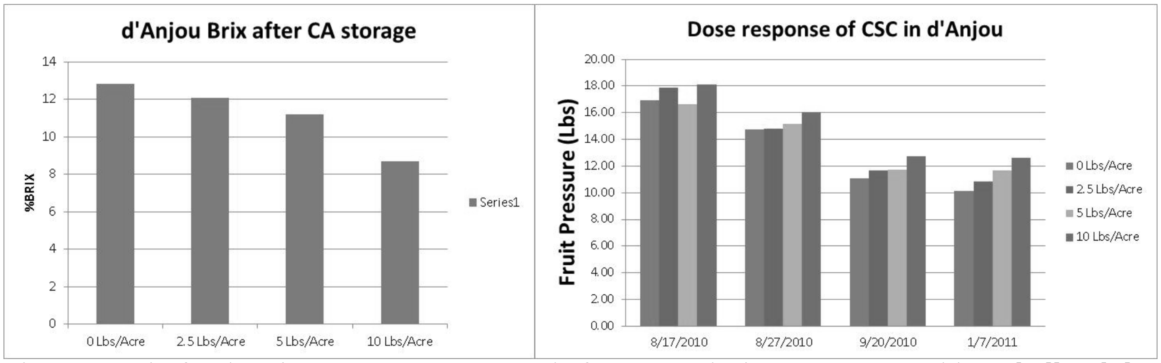

Figure 5: Brix for d'Anjou pears was measured after 3 months in CA storage. Chlorophyll stabilizing chemistry shows a clear dose response in maintaining fruit pressure similar to at harvest levels (right panel). Pears were stored in McDougal and Sons CA storage rooms.

Objective 4:

Alternate fruit sanitization with UV-C:

Maximum reductions of 3.70±0.13 log CFU/g were achieved for generic *E. coli* on intact pear surfaces, with lesser reduction on wounded pear (3.10±0.329 log CFU/g) after 4 minutes UV-C exposure at 7.56kJ/m^2. The time required for a 90% reduction in *E. coli* cell numbers for intact pear surfaces (0.019±0.009 min) was smaller than for wounded pear (0.062±0.013 min), suggesting that the wounds on pear surfaces helped to shield and protect microorganisms from UV-C radiation. Results indicated that blue mold inactivation on pear surface required lower UV-C doses than generic *E. coli* to reduce similar level of population (Figure 6). Fourier transform infrared (FT-IR) spectroscopy indicate that bacterial membrane damage (phospholipids, protein secondary structures and polysaccharides) and changes to DNA/RNA in *E. coli* resulted from UV-C treatment. UV-C can reduce microorganism populations on fresh pear but the efficacy of UV treatment is dependent upon the type of organism and morphological properties of the fruit and surface integrity.

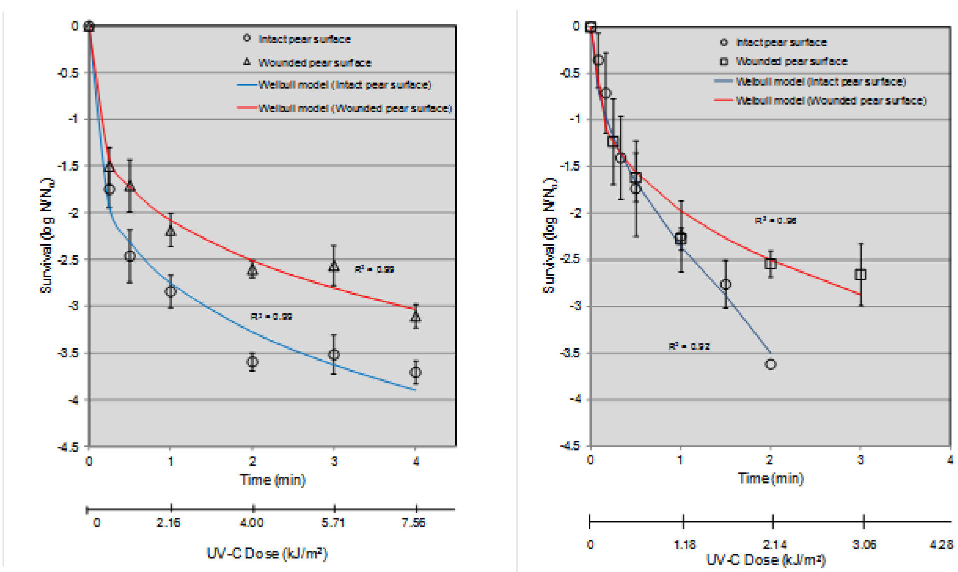

Figure 6. UV-C inactivation of generic *E. coli* (left figure) and blue mold (right figure) on intact and wounded pear surfaces

EXECUTIVE SUMMARY

This aim of this project was to conduct coordinated research in using a systems approach to ultimately improve fruit quality. In pears such an approach was needed to connect the sparse researchers and establish a core community. A network of researchers has been established that has contributed to the drafting of a pear research roadmap.

Significant progress has been made towards better understanding of horticultural management of the crop to impact fruit quality. A global network of pear breeders is already exchanging information, DNA and plant material that can be immediately implemented in the PNW in particular for rootstock improvement. A chemical has been identified to improve fruit quality along with promising results for alternate sanitization of fruit.

Summary of finding

Leaf scaffold angle regulated precocity in the new training systems. ABA was found not to be very effective in regulating plant vigor. However, Apogee was found to be highly effective not having any negative impact on yield or fruit size.

Chlorophyll stabilizing pigment continues to be promising in improving fruit quality and use of UV-C in sanitizing fruit has shown promising results.

Future directions

Some aspects of this research will be continued by individual investigators. In particular the impact of vigor controlling chemicals will be pursued further. Also, the efforts are ongoing to bring pigment stabilizing chemistry to market in collaboration with industries already working in this space. Additional funds are being obtained to continue research with UV-C. (Ultraviolet Light based Hybrid Technologies to Control Foodborne Pathogens on Fresh Produce, USDA AFRI Food Safety Program, $424,907, (Sablani, Rasco, Killinger and Syamaladevi, Pending**).**