Northwest Pear Research Review

Confluence Technology Center, Wenatchee

Wednesday, February 22

Time	Page	PI	Title
8:00		T. Schmidt	Welcome & housekeeping
8:05		Schmitten	Introduction
			Final Project Reports
8:15	1	Dhingra	Rootstock workshop
8:30	6	Einhorn	Cold hardiness of quince
8:45	16	Einhorn	Horner rootstock grower trials
9:00	24	Reed	Improved micropropagation and rooting of dwarfing pear rootstocks
9:15	31	Schmidt	Pear crop load management and rootstock field testing
9:30	39	Johnson	Evaluation of integrated fire blight control technologies
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11:00	76	Dhingra	Systems approach for ensuring superior pear fruit quality
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11:30	90	Evans	Pear rootstock breeding: ***Extension***

FINAL PROJECT REPORT

Project Title: International pear workshop

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Cooperators: WA, OR and CA Pear Industry, Richard Bell, USDA-ARS; US Pear Scientists, Joan Bonany, IRTA Spain; Stefano Musacchi, University of Bologna, Italy; Enrique Sanchez, INTA, Argentina; Marie-Helene Simard, INRA, France

Total Project Funding: $20,000

Budget History:

Item	**2011**		
Air Fare	$7700		
Food and incidentals	$5000		
Lodging	$4300		
Domestic travel	$3000		
Total	20,000		

OBJECTIVES

- Objective 1: Arrange for the pear researchers to interact directly with OR-WA industry folks in orchards and packing sheds across the production regions

As articulated by the pear research sub-committee, we would like to bring international researchers who have specialties in pear horticulture to the PNW. The focus in expertise would be in historic and current use of dwarfing/precocious rootstocks, genetics, training systems and unique horticultural manipulation of pear. Ideally, we would like to use their experience to translate similar benefits to the PNW industry. Adaptation of current foreign systems in our unique environments may have limitations but the PNW industry feels that they need to be exposed to the possibilities and use these researcher's experiences to view change objectively. Based on the current level of collaboration, California may also get involved adding on a visit from the researchers before or after the tour or simply have their representatives participate in the PNW tour.

- Objective 2: Arrange for focused researcher meetings around (but not limited to) a potential SCRI application.

As advised by the Pear Research sub-committee, the visit will end in a final day workshop/summary discussion at which all of the tour participants will summarize the activities and map future strategy focusing on White paper development and the proposed SCRI application. This activity would involve the presence and guidance of the Pear Industry Advisory Committee. This will be a logical progression of the momentum established during the discussion/meeting in Argentina where global pear researchers and industry came together for the common cause of increasing per capita consumption of pears.

SIGNIFICANT FINDINGS

Objective 1:

- Three of the four invited international researchers attended the workshop. Joan Bonany, IRTA Spain, Stefano Musacchi, University of Bologna Italy, and Enrique Sanchez INTA, Argentina were in attendance while Marie-Helene Simard, INRA, France was unable to travel due to health issues.
- Over 57 researchers and industry members participated in this workshop.
- The workshop attendees visited several orchards and packing operations in Hood River OR, Yakima WA and Cashmere WA.
- The workshop covered the following orchards and topics
  1. Reflective fabric test plots in low-density 'd'Anjou'
  Hosts: John Benton & Todd Einhorn; Location: Benton Orchards
  2. High-density 'Bartlett' V-trellis & productive low-density 'd'Anjou'
  Host: Ken Goe
  Location: Goe orchards
  3. 'Bosc', 'd'Anjou', 'Bartlett', and 'Comice', in-row-steep-V-system
  Host: Gorham Blaine; Location: Dog River Ranch
  4. Container nursery stock culture; new plantings of green 'd'Anjou' & 'Bosc' V-trellis; 'Red d'Anjou' decline; 'Forelle' & fire blight
  Host: Tim Annala; Location: Annala Orchards
  5. 'Bartlett' & 'Bosc' V-trellis; 'Horner' rootstocks; new pear plantings
  Hosts: Don Gibson & Mike Sandlin; Location: Mount Adams orchards
  6. Pear consumption – Gorge Delights value-added pear products
  Host: Gary Willis; Location: Willis Family Farms
  7. 'Bosc' & 'Red d 'Anjou' V-trellis; pear systems; spacing and training concepts

Host: John Wells; Location: Wells Orchards
8. Jerry Haak Orchard
9. Don Weippert, Firewood Orchard,
10. Dave Olsen Orchard
11. Peters Orchards
12. Matson Fruit, Selah, WA
13. Roundtable discussions; Hilton Garden Inn conference room, Yakima.
14. Classic pear production Cashmere area and assess problems.
Walk through the Pine Flats Area.
Schmitten/Cunningham 12 year old Red Anjou and Starkrimson OHXF 87
15. Blue Star Growers-Cashmere. Packing, Conditioning and MCP
16. Small group meeting with Crunch Pak – 8 participants (Lead: Ray Schmitten)
17. Higher density Orchards
    a. Koempel Blewett Pass Block
    b. Rudy Prey Block, Prey's Fruit Barn
    c. Schmitten Orchards, Turkey Shoot Orchard
18. Tim Smith Rootstock trial
19. Roundtable discussions around all the meetings. Location Schmitten residence.
20. Pear CGC meeting, Tree Fruit Research and Extension Center
21. Scientist and industry group meeting. Location WSU TFREC. Several Pear Bureau Members and CA Industry representatives attended.

- A brainstorming session was held in Yakima with industry representatives from the area

Objective 2:

- The final day of discussions were attended by several pear industry leaders and most pear researchers either in person or via phone
- A draft summary of pear industry's topmost issues was developed. A synopsis is appended to this document.

EXECUTIVE SUMMARY

The International Pear Workshop established a framework of priorities that has been taken up by researchers to draft a Pear Research Roadmap White Paper. A synopsis of this document is available at http://genomics.wsu.edu/pages/researchpear/index.html and a summary is appended to this document

During the workshop many participating members of the pear industry were interviewed. A short video was prepared to highlight the issues prevalent in the industry. The video can be accessed on you tube by visiting the following link: http://www.youtube.com/watch?v=mfJjjA_JtKc

Collaboration was maintained with the international pear researchers throughout the year; they are all participating in the developing SCRI application bringing both their expertise and the offer of valuable genetic resources. The impact of their visit also lead to them being invited back to attend and present at the Washington State Horticultural Show in Wenatchee 2011.

Several of these elements were included in the grant application that was prepared for submission to the USDA SCRI panel. The work on the roadmap and the SCRI application continues. The grant application will be submitted in FY 2013.

Appendix: Synopsis of the Pear Research Roadmap Document

Current situation, vital needs and research priorities for enhancing profitability and global competitiveness of the pear industry

This is a synopsis of a white paper that is being currently drafted. This document summarizes urgent pear industry needs and research priorities that will modernize the pear industry so that its profitability and global competitiveness can be enhanced.

Introduction: This document has been developed using real-time information gathered at WA, OR and CA pear orchards, processing and packaging sheds, and from pear marketing boards in the same three states since 2009. The WA, OR and CA are key players in pear production, with the West coast representing more than 95% of the US Pear industry however east coast researchers and industry will play an important role in the determined research priorities. A US pear industry-sponsored week-long International Pear Workshop in July 2011 served as a platform to document the information and prioritize near, medium and long-term goals of the industry. The workshop was attended by the US pear research community (east and west coast) and pear science experts from Spain, Italy and Argentina.

Current Situation: The US pear industry is economically stagnant. There is an urgent need to increase its profitability. Decreasing trends in pear consumption are matched by increasing concerns for a sufficient labor supply. This is especially relevant in the face of several other competitive products such as other fruits and fruit-containing health products on the market.
Pear orchards in the US are ageing and outdated resulting in decreased fruit quality with little vigor management. The old, three dimensional trees lack consistent fruit set and the resulting fruit size is highly variable. One of the critical reasons for this state is a lack of dwarfing and precocious rootstocks suited to the production environment in the US. There is also a lack of uniformity in trees for new plantings, which are few and far in between. There are several plant propagation and nursery-related issues linked to the non-availability of an adequate rootstock.

Due to the high variability in pear production systems, there is a lack of best management practices for pear production and crop load indices. Existing yields are driving down profitability, and furthermore, the current orchard architecture is not amenable to implementation of mechanization that could potentially provide cost-savings and reduce the issues of labor shortage. As production costs are rising, there is a need to increase productivity to recover those costs. Establishing further quantitative and qualitative economic information addressing the issue of time-value money for orchard production or time to return on investment is necessary. The industry and researchers conclude that the existing genetic diversity in Pyrus is not being exploited to address the above-mentioned critical production-related questions.

The ageing orchard infrastructure doesn't bode well for the safety of an already scarce labor force. There are major concerns related to ladder safety. Typically the ladders are 10 to 16 feet tall. The pear industry is unprepared for the near-term challenges of policy change in immigration regulations and customer expectations in labor safety. In addition, adverse environmental effects due to the use of pesticides, chemicals and water use come into question with the current infrastructure. The pear industry is extremely fragile and has a large carbon footprint.

At the consumer level, the pear industry has fallen short of providing a consistent organoleptic experience to the consumer. This is in part due to the high variability of fruit obtained from outdated production systems. Not much emphasis has been provided to fruit finish and promoting product uniformity. The lack of a ready-to-eat pear and variable ripening requirements confound the issue further. There are numerous post-harvest pathological and physiological disorders that plague the pear industry also raising issues about food safety. Further, the biology of pear fruit has not been carefully considered to devise appropriate handling and packaging throughout the value chain. There remains a clear disconnect between the customer and the retailer who sets high and

narrow standards for food quality. There are strict market constraints with current varieties and the situation is worsened by an inelastic demand curve. To make matters worse, there is strong resistance to new varieties at the retailer and packer level.

What the consumer wants in a pear fruit remains largely unknown. Consumer preference studies are inadequate for pears forcing the retailer to follow marketing strategies for other fruit such as apple. More information could be made available to the public on the health benefits of pear consumption; pears are known to be one of the most hypoallergenic fruits and recent data indicates their beneficial qualities for combating diabetes. Pears are rarely used in processed food markets which could be one avenue to boost domestic consumption and enhance profitability. In the US, there are only a handful of pear varieties available at the retail level consequently the consumer lacks the experience to sample the diversity in pear germplasm.

It was concluded that there is insufficient pear research being conducted in the public domain. There needs to be stronger integration of extension to translate the research outcomes into practice at the industry level. There is an urgent need for an excellent quality product aligned with consumer demand.

The pear industry ratified the research community's plan of action to address two major areas of pear research which can deliver enhanced profitability in the next five years while developing a research infrastructure for sustained progress in reinvigorating the pear industry over the next two decades. A research proposal is currently being developed to be submitted to a USDA SCRI panel with a team of over 40 US and International scientists.

FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Cold hardiness of quince

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Cooperators: Amit Dhingra, Kate Evans

Total Project Request: **Year 1:** $41,196 **Year 2:** $42,898 **Year 3:** $41,369

Other funding sources

Agency Name: National Plant Germplasm System
Amt. awarded: Requested amount of $12,192, Awarded amount, $9,750
Notes: USDA Plant Germplasm Evaluation Program approved funding March 19, 2010 to develop a precise system for determining the lowest survival temperatures of quince and pear accessions tested herein. We have successfully developed and implemented differential thermal analysis (DTA) techniques for measuring plant hardiness, and will be correlating those data with results from this project.

Budget 1 Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: Cynthia Cox**
Telephone: 541 737-3228 **Email address: cynthia.cox@oregonstate.edu**

Item	2009	2010	2011
Salaries	$18,000	$18,720	$19,469
Benefits	$10,942	$11,380	$11,835
Wages	$1,000	$1,040	$1,080
Benefits	$ 88	$ 92	$ 95
Equipment			
Supplies	$1,000	$1,500	$1,500
Travel	$500	$500	$500
Miscellaneous			
Total	$31,530	$33,232	$34,479

Footnotes: [1] **Salaries include ~ 50 % of a full-time Technician (salary and OPE) for project management, data collection, and equipment maintenance. Increases in years two and three reflect a 4 % rate increase.** [2] **Wages include approximately 90 hours of hourly labor @ $11/hr.** [3] **Travel is for one trip to the Plant Clonal Germplasm Repository, Corvallis, OR per year.**

Budget 2 Joseph Postman
Organization Name: USDA/ARS **Contract Administrator: Cynthia Cox**
Telephone: 541 737-3228 **Email address: cynthia.cox@oregonstate.edu**

Item	2009	2010	2011
Salaries			
Benefits			
Wages	$7,000	$7,000	$5,000
Benefits	$616	$616	$440
Equipment			
Supplies	$1,800	$1,800	$1,200
Travel	$250	$250	$250
Miscellaneous			
Total	$9,666	$9,666	$6,890

Footnotes: [1] **Salaries include 0.25 of a temporary part-time employee (8.8 % benefit rate) for sampling procedures Sept-April, and assistance in propagation of germplasm.** [2] **Travel is for one trip to the MCAREC, Hood River, OR per year.**

Objectives

1) Determine the depth of cold hardiness within the representative quince germplasm and identify changes in hardiness throughout dormancy and early and late season non-acclimated tissue in each of three years (Einhorn: lab analyses, Postman: sampling management).

2) Root quince cuttings in year one and transfer to containers for de-acclimation studies in years two and three (Postman: rooting and transplanting, Einhorn: de-acclimation studies).

3) Determine the tissue zone most sensitive to freeze injury (Einhorn).

4) Determine the value of electrolyte membrane leakage chambers for high-throughput cold hardiness screening (Einhorn).

Significant Findings Sep 2009-Feb 2012

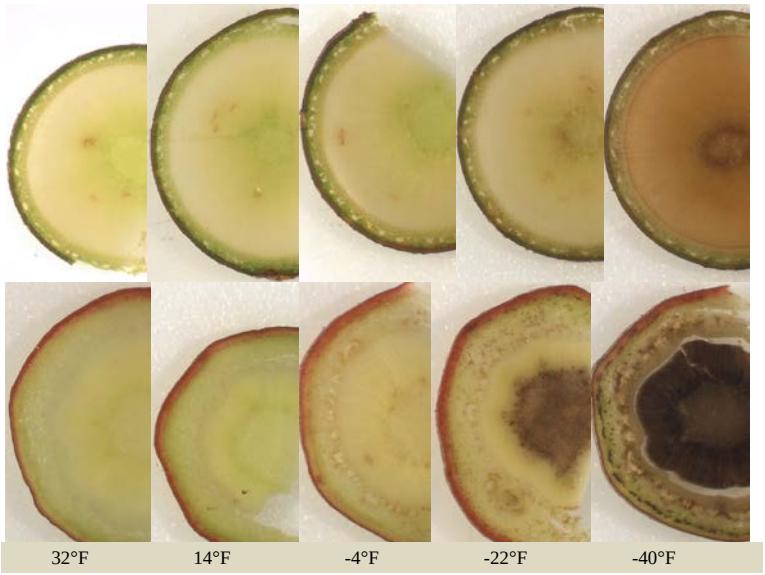
- Following cold acclimation 22 of the 56 quince accessions tested withstood -30 °C [-22° F] with less than 50% browning of tissues(i.e., data are means of three years).
- Two intergeneric hybrids (Quince x Pear, and Pear x Sorbus [i.e. Mt. Ash]) were hardy to -22 F.
- Three Amelanchier (Serviceberry) rootstock clones were hardy to -40 F, without observable tissue injury at peak hardiness. These clones have been budded to commercial pear cultivars and will be planted in trials spring of 2012.
- None of the pear accessions tested, including OHxF 87 and OHxF 97, and the scion clones 'd'Anjou', 'Bosc' and 'Bartlett' were hardy to temperatures below -22 F.
- In all years plants reached peak hardiness levels in December/January. Beginning in mid-late January, de-acclimation was evident as tissue injury was observed at higher temperatures.
- The cambial zone was observed to be more sensitive to cold injury throughout the measurement period, especially in early fall, and late spring, followed by xylem. Phloem developed the greatest hardiness in mid-winter. Differential thermal analysis (DTA) data confirmed these observations. We did not detect a low-temperature freeze event in phloem tissue within the measurement range (32 F to -50 F).
- The cold acclimation period from September through November varied among years. Frosts occurred earliest (October 6) in 2009. All years provided adequate acclimation conditions, though 2010 was fairly mild through mid-November. Irrespective, maximum cold hardiness levels were fairly consistent during December and January among test years (within 1 point on a six point tissue browning scale). No significant low temperature episodes were observed in any year, and it is likely that greater hardiness would be attainable in colder climates.
- Variation in rootability of different quince clones was high. Initial rootability tests were done using soft-wood cuttings. Many of the rootstock accessions (Pillnitz series, and Pigwa S series) were observed to root easily, while other fruiting clones did not.
- We have developed a DTA system to precisely identify low temperature freeze points of quince and pear tissue. Isolated tissue zones (phloem; xylem; pith) from stems showed that exotherms (i.e., freeze events) observed in the whole stem were related to freeze events in the xylem and pith, indicating these tissue zones as weak links to survivability.
- We have developed a novel grafting system to test survivability and regrowth of accessions following our controlled rate freeze tests. Evaluations will begin spring 2012.

Methods

Objectives 1 and 3: Mature, current season shoots from ten Pyrus (Pear) clones and 56 quince clones, were collected from trees located in the NCGR orchards (Corvallis, OR) and shipped next-day to

MCAREC. Tissue was sampled at ~three-four week intervals, beginning in late September, through bud-break (March-April), in each of three years (2009-2011). The protocol is briefly outlined below:

- Shoots were harvested from trees and shipped next-day to MCAREC. Upon receipt, samples were placed in 42° F storage, and sectioned into one-inch pieces. Samples were weighed, and their fresh weights recorded. Four replicate stem pieces per accession per treatment (i.e., temperature) were made. These replications also accounted for possible biological differences occurring within a shoot (i.e., rep 1 was always taken from the thicker, earlier growth at the basal portion of the one-year-old shoot, rep 2 with increasing distance toward the tip, rep 3 further, and rep 4 comprised the apical region, not including the terminal two inches of the shoot).
- Stem pieces were loaded into a programmable Tenney T2C Freeze Chamber, and subjected to freezing at a rate of 4° C per hour. Samples were removed following a one hour 'soak' at each of five treatment test temperatures (0, -10, -20, -30, and -40°C [32, 14, -4, -22, -40°F]), with the exception of the first sample period [Sep 2009], when samples were subjected to 0, -10, -25, and -40° C to account for a shortage of shoot material. Each of the four replicates was run on a separate date.



- Upon removal from the freeze chamber, stem samples were placed in sealed plastic bags with moistened paper towel, and allowed to incubate at room temperature for one week prior to microscopic evaluation.
- Transverse sections of stems were made midway into the one-inch sample, placed under a stereomicroscope, and individual tissue zones (phloem, cambium, and xylem) were rated according to the degree of oxidative browning observed using a six point scale, where 1, no damage [white]; 2, no damage [off-white]; 3, ~ 25% area lightly browned; 4, ~ 50% area browned; 5, >75% area browned; 6, 100% completely oxidized [black]. Visual assessment of freeze injury was performed by one technician, and all samples were prepared and rated in a double blind manner. The lowest exposure temperature which resulted in the absence of any observable levels of injury (i.e., a rating < 4) was termed the temperature prior to incipient damage. Photo shows examples of microtome sections under magnification of one cold-hardy quince accession (above) and OHxF 87 (below), following freezing and incubation. Browning within different tissue zones occurs with decreasing temperatures.
- Following analyses, sample pieces were dried in an oven at 70°C and weighed until a constant weight was attained (i.e., dry weight). Relative water content was derived from fresh and dry weights as, [(Fresh Weight - Dry Weight)/Fresh Weight] *100

Objective 2: In late May and early June, 2009, softwood cuttings were taken from 53 quince clones and one clone each of *Pyronia veitchii* (Pyrus x Cydonia) and *Sorbopyrus auricularis* (Sorbus x Pyrus), with the goal of generating 10 self-rooted trees of each. Sixteen cuttings were initially made for each genotype. Each cutting contained at least 3 nodes (~ 6 cm), and the base was dipped in a powdered rooting product containing 0.8% IBA before sticking in Oasis® Rootcubes and rooted

under mist with bottom heat to keep media temperature at about 24° C. For genotypes that failed to thrive or produce any roots after 4-6 weeks, a second set of cuttings was made in July, 2009.

We have developed a novel system to test survivability and regrowth following our controlled rate freeze tests. Four-node shoot pieces of several accessions were subjected to test temperatures of 32, 14, -4, -22, and -40 F (as described above). Upon removal from the freeze chamber, shoot pieces were cut in half; one half was incubated and evaluated for tissue browning (according to methods described above), the other half was stored at 38 F and will be grafted in late February 2012. Quince accessions will be grafted to Quince A rootstock (to avoid incompatibility). OHxF 87 was used as a control and will be grafted to OHxF 87 rootstocks. All grafts will be potted and placed in a greenhouse for evaluation of spring budbreak (date) and weekly measurement of shoot growth, throughout 2012. Shoot initiation and growth of buds from intact two-node shoot sections will provide a direct assessment of freeze injury to correlate with oxidative browning and DTA data.

Objective 4: Due to the previously determined precision of differential thermal analysis (DTA) we have substituted this technique for the electrolyte leakage studies that we originally proposed to develop. We leveraged funding awarded from the USDA to design a DTA system for detection of plant kill points (acknowledgements to Dr. Markus Keller's lab group at WSU-Prosser for sharing their experience and insights on DTA system design). The system was modified (Dr. David Gibeaut, OSU-MCAREC) from Mills et al. (2006). Briefly, shoots were harvested and shipped as described above. Samples were cut, wrapped first in a piece of damp paper towel then aluminum-foil, placed on thermo-electric modules (TEMs) and subjected to a 4 °C per hour freeze rate. The TEMs were wired into a data acquisition system (Keithley 2700-DAQ-40; Keithley Instruments, Cleveland, OH, USA) and data were recorded at 30 second intervals. The exotherm (heat release) associated with the phase change of water produces a temperature gradient across the TEM resulting in a voltage output (y-axis) that was plotted against temperature (x-axis). Temperature was measured via a thermocouple placed on a reference TEM without tissue. The height of the low temperature exotherm peak was then used to identify the temperature associated with the freeze event.

Results and Discussion

Objectives 1 and 3: Early fall 2010 temperatures recorded near the NCGR field site did not decline as quickly, nor did they result in frosts as observed in either 2009 or 2011 (Fig 1). In fact, no frost events were recorded prior to November 21, 2010, as compared to the light frosts observed on October 6 and 12, 2009, and October 30, 2011. A seasonal, gradual progression of declining minimum and mean temperatures followed by hard freeze events as observed in 2009 and 2011 are conducive to cold acclimation, a process by which plants acquire hardiness through exposure to increasingly lower temperatures, albeit, in the Willamette Valley (NCGR) this process occurs later than in most pear growing regions of the PNW. Mid-winter temperatures varied substantially among years (Fig 1). The only significant low temperature (<10F) episode was observed in December of 2009. Despite the differences among years and overall lack of cold, no consistent patterns emerged in the hardiness of the accessions tested among years. It is plausible that when established in colder environments, significant gains in hardiness would be observed.

For each sample date, we have determined the warmest temperature at which injury was observed (temperature of incipient damage), and report minimum hardiness level as that temperature which immediately preceded the temperature of incipient damage [i.e., lowest exposure temperature resulting in < 50% browning]; a point that is highly debatable since anecdotal evidence from cutting wood following winter freeze events suggests that pear re-growth the following spring is not impaired at such light browning levels. Subsequently, our estimates of maximum hardiness are extremely conservative.

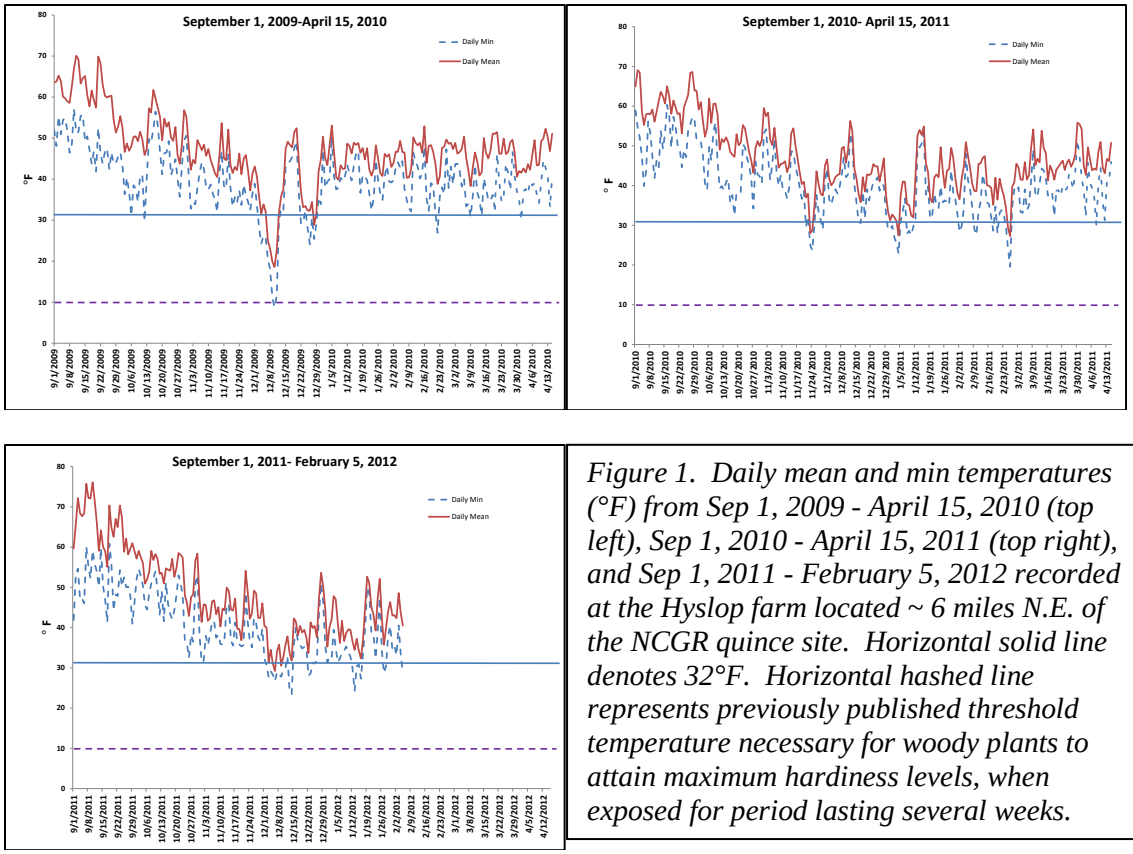


Figure 1. Daily mean and min temperatures (°F) from Sep 1, 2009 - April 15, 2010 (top left), Sep 1, 2010 - April 15, 2011 (top right), and Sep 1, 2011 - February 5, 2012 recorded at the Hyslop farm located ~ 6 miles N.E. of the NCGR quince site. Horizontal solid line denotes 32°F. Horizontal hashed line represents previously published threshold temperature necessary for woody plants to attain maximum hardiness levels, when exposed for period lasting several weeks.

We have identified a group of cold hardy taxa which have consistently performed well (<50% browning) following freeze tests. This group consists of 22 quince selections, 2 intergeneric hybrids, and 4 Amelanchier clones that were equal to, or hardier than the cold-hardy pear genotypes tested as controls (Tables 1-4). Data are maximum hardiness levels from December/January samples, and are means of three years of data. We intend to continue work with this subset of cold hardy selections, should funding be provided in 2012.

Following December sampling, maximum hardiness values decline, until hardiness is completely lost in April (not shown). As the season progressed, cambial tissue (meristematic tissue responsible for cellular division, lateral trunk growth and ultimately new xylem and phloem tissue) appeared to be consistently more sensitive to sub-freezing temperatures than either of the vascular tissues [i.e., phloem or xylem] (data not shown). At the maximum hardiness level [December] phloem tissue was hardier for most accessions, but differences between oxidative browning ratings for xylem and phloem rarely exceeded 1. Interestingly, DTA tests have shown that freeze events in the samples are occurring in the xylem and pith, and not in the phloem (Fig 2). This freeze resistance strategy has been documented in other Rosaceae species plants. It appears that phloem tissues avoid cellular freezing by a mechanism which facilitates the migration of water out of cells to extracellular ice. Accordingly, ice crystals in extracellular spaces will 'pull' water from cells, since the vapor pressure of water is higher over liquid than ice at the same temperature (free energy theory dictates that water will move down the energy gradient from a higher (liquid) to lower (ice) energy status. Further, the osmotic concentration of the cells is increased. This process, in turn, lowers the freeze point. In these cases, damage can be incurred by dehydration. Xylem, on the other hand, tolerates subfreezing temperatures by supercooling. However, supercooled liquids eventually freeze, as was observed near -38 F (Fig 2).

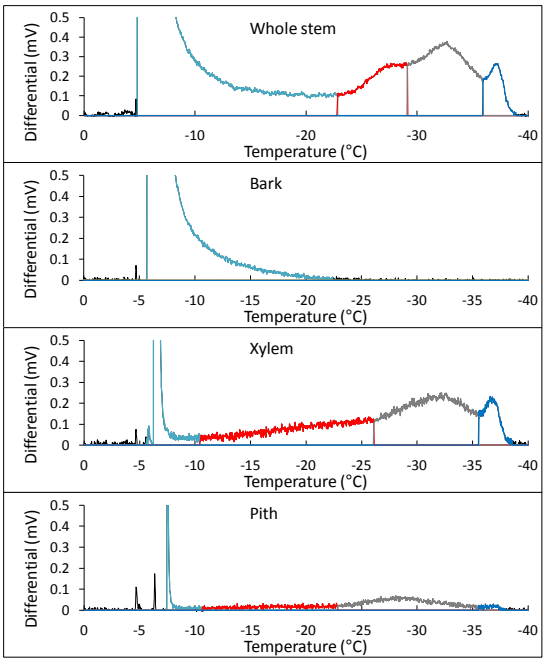


Figure 2. Differential thermal analysis (DTA) of quince cultivar "WF-17". Analyses were performed on first year stems cut from trees during their period of greatest cold hardiness. Bark samples included all tissues from the cambium outward. Xylem was stripped of all cambium then split lengthwise avoiding all pith. Pith was dissected by splitting stems lengthwise in four planes leaving a small amount of xylem.

Because the increment between measurement points is 10°, and in several cases the first temperature at which injury is detected results in quite significant browning (i.e., much higher levels of injury [score of 4-6]), the data reveal little about the qualitative nature of the temperature of incipient damage. Representative data collected from the quince accession 'Aiva from Gebeseud' is provided in Figure 3 to illustrate this point, and shows how hardiness is gained and lost (Fig 3). For example, in December oxidative browning was barely evident at -30 °C, and completely black (fully oxidized) at -40 °C (Fig 3). Once tissue damage is observed, we are unable to define whether the actual injurious event, or kill point, occurs following a 1° or a 9° lowering of the temperature from the previous test temperature.

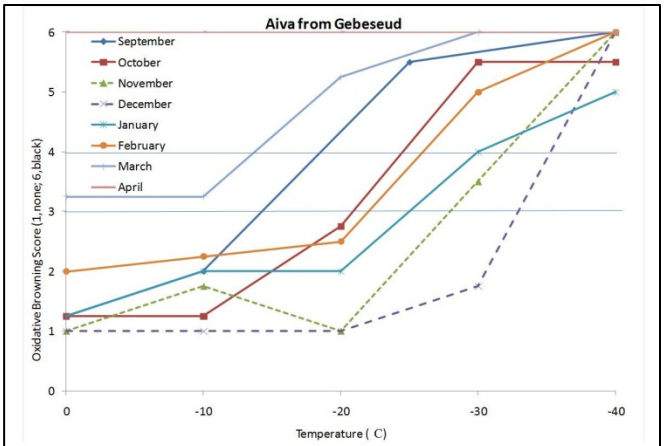
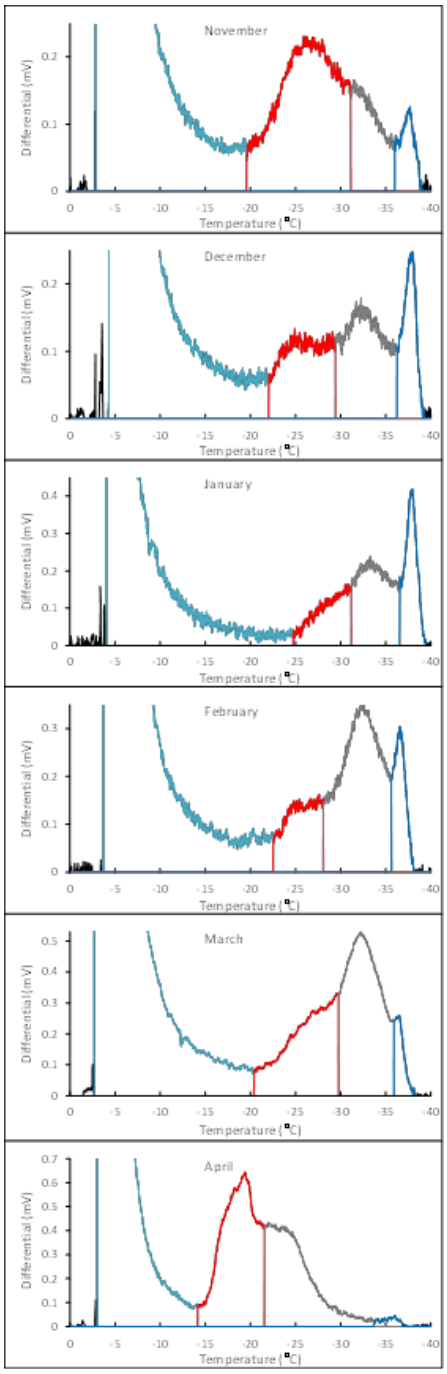


Figure 3. Oxidative browning rating of vascular tissue (see description of browning scale in methods) of the quince accession 'Aiva from Gebeseud' from Sep 2009-April 2010. Horizontal line on y-axis at 4, signifies the threshold for injury (i.e., ,50% tissue area lightly browned).

Overall, results are very encouraging. A large group of quince taxa exist with the apparent capacity to acclimate and attain sufficient levels of cold-hardiness for many regions of the PNW, and these data are supported from three years of sampling. Additionally, previous reports have suggested that full expression of hardiness is associated with exposure to temperatures below 10° F for several weeks (hashed line in Fig 1). Temperatures at the test orchards did not attain these values for any extended period of time, indicating that greater cold tolerance is entirely possible when planted in colder climates.

Objective 2: Roughly 300 rooted cuttings have been established from 31 quince genotypes (~half of the sample population) having an average of 8 rooted cuttings per clone. Plants were budded with one bud of 'd'Anjou' and one of 'Bartlett' for preliminary information regarding compatibility. However, symptoms expressed in shoots of 'Bartlett' suggested that the 'Bartlett' budwood was virus-infected Consequently, results are not reliable. Rooting of soft-wood cuttings varied substantially (data not shown). Further work will be required to develop effective hardwood, softwood and tissue-culture protocols to determine ease of propagation of these taxa.

Objective 4: DTA data show changes in hardiness behavior of an open pollinated quince seedling, Akhtubinskaya- Volgograd, Russia (Fig 4) from December through April. The first peak (occurring at



~ -5 °C) signifies the freezing of extracellular water in the tissue, and is termed the high temperature exotherm. It is a non-lethal, non-injurious event. The next series of peaks represent cellular freezing (low temperature exotherms), and these are highly associated with injury of tissue and, in the case of the low temperature exotherm at -38 °C, likely tissue death. Preliminary anatomical observations indicate that these separate freeze events appear to be unrelated to specific tissue zone injury, but further work is required to understand these events. Across the population of taxa evaluated, the low temperature exotherm consistently occurs near -40C, however intermediate peaks vary with cultivar. The changes as taxa acclimate in the fall, attain maximum hardiness in mid-winter, and de-acclimate in the spring agree with our oxidative browning results (data not shown in final report, but reported in earlier continuing reports).

We intend to align DTA data with results from regrowth assays this spring to gain a better understanding as to the nature of these freeze events.

Figure 4. Differential thermal analysis (DTA) of quince cultivar "Akhtubinskaya O.P. seedling - Volgograd, Russia". Analyses were performed on first year stems cut from trees approximately 30 days apart during the critical periods of dormancy, cold hardiness and break of dormancy.

Tables 1-4. The lowest temperature sustained resulting in oxidative browning levels less than 50% for all accessions tested, at their maximum hardiness level. Maximum hardiness levels were observed between December and January sampling periods in all years evaluated. Accessions highlighted in grey are those suggested to withstand most PNW production region climates.

Amelanchier (Serviceberry) accessions	Hardiness Temperature (°F)
A-2	-40
A-7	-40
A-10	-40

Intergeneric hybrid accessions	Hardiness Temperature (°F)
Pyronia veitchii (= IGC 9)	-22
Sorbopyrus 'Smokvarka'	-22

Pyrus (Pear) accessions	Hardiness Temperature (°F)
Harbin (P. ussuriensis) (cold hardy)	-22
Krylov (cold hardy)	-22
Anjou	-22
Bosc	-22
OHxF 97	-22
OHxF 87	-22
Bartlett (Hood River)	-22
Lesnaia Krasavitza (cold hardy)	-4
Bartlett (Corvallis)	-4
Pyrus pashia	14
Pyrus koehnei	14

Cydonia (Quince) accessions	Hardiness Temperature (°F)
Tashkent AR-232 seedling 2 (A)	-22
Tashkent AR-232 seedling 4 (B)	-22
C. oblonga - Arakseni, Armenia	-22
C. oblonga - Megri, Armenia	-22
Skorospelka O.P. seedling	-22
Aiva from Gebeseud	-22
C. oblonga - Seghani, Armenia	-22
Akhtubinskaya O.P. seedling (B)	-22
Akhtubinskaya O.P. seedling (A)	-22
Bereczki [Beretskiquitte]	-22
C. oblonga - Babaneuri, Georgia	-22
Kashenko No. 8	-22
Krukovskaya O.P. seedling	-22
Quince A	-22
Quince C7/1	-22
Quince S	-22
Quince W	-22
Teplovskaya O.P. seedling	-22
Trentholm	-22
Van Deman	-22
W-4	-22
WF-17	-22
Aiva from Kara-Kala No.9	-4
C. oblonga - Alema, Armenia	-4
C. oblonga - Dusheti, Georgia	-4
Cooke's Jumbo	-4
Ekmek	-4
Fontenay	-4
Karp's Sweet Quince - Majes Valley, Ara	-4
Kaunching	-4
Kichikara Dede 88-1 (virus?)	-4
Kichikara Dede 88-2	-4
Krimskaya	-4
Kuganskaya	-4
Le Borgeot	-4
Limon	-4
Maslenka Rannaya O.P. seedling	-4
Meech's Prolific	-4
Pigwa S-1 - Poland	-4
Pigwa S-2 - Poland	-4
Pigwa S-3 - Poland	-4
Pillnitz 1	-4
Pillnitz 2	-4
Pillnitz 3	-4
Pillnitz 5	-4
Pineapple	-4
Portugiesische Birnquitta	-4
Provence (BA 29-C)	-4
Quince E	-4
Quince Evalina	-4
Seker Gevrek	-4
TE-2-73	-4
Tekes	-4
Tencara Pink	-4
Yuz-Begi 89-1	-4
Zeakli 89-1	-4

Executive Summary

Over a three-year period, shoots of fifty-seven quince, two intergeneric hybrids, and ten pear accessions were tested monthly in a programmable freeze chamber to characterize freeze resistance and hardiness. Shoots were sampled from *in-situ*, own-rooted trees located at the USDA NCGR in Corvallis, Oregon between September and April of each year. In addition, we tested three Amelanchier rootstock clones developed in Germany, and maintained in containers at the OSU Mid-Columbia Agricultural Research and Extension Center in Hood River, Oregon.

Oxidative browning assays and differential thermal analysis (DTA) were used to quantify freeze events and injury sustained following controlled rate freezing experiments. All accessions acclimated and reached maximum hardiness levels in mid-December to early January, though the range of hardiness varied markedly among the sample population. Twenty-two of the quince accessions and both intergeneric hybrids tolerated -22°F without significant tissue browning (<50% browning) during the maximum hardiness period. The three Amelanchier clones tolerated -40°F with <50% tissue browning. Importantly, these results were consistently observed in all years. No pear accessions were capable of attaining greater hardiness levels, based on degree of browning or DTA. Commercial pear rootstocks, OHxF 87 and OHxF 97, tested hardy to -22°F.

Cambium tissue was observed to be slightly more sensitive than xylem or phloem throughout the test period, but DTA showed that freeze events in the stem were related to freezing in the xylem.

We will evaluate survivability and regrowth of shoots subjected to a range of sub-freezing temperatures using a novel grafting assay. Budbreak and growth rate analyses of grafts will be related to oxidative browning and DTA.

FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Horner rootstock grower evaluation trials

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Cooperators: Growers: Mike McCarthy and Eric Von Lubken (Hood River Trial), Chuck Peters (Wapato Trial), Bob Foyle and site manager Garrett Znan, (Bridgeport Trial), Mark Stennes (Methow Trial).

[1]**Budget:** **Year 1:** $15,370 **Year 2:** $16,958 **Year 3:** $18,552

Other funding sources: None

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Cynthia Cox
Telephone: 541 737-3228 **Email address:** Cynthia.cox@oregonstate.edu

Item	2009	2010	2011
Salaries[1]	2,905	3,021	3,142
Benefits	1,765	1,837	1,910
Wages			
Benefits			
Equipment			
Supplies			
Travel[2]	1,500	1,500	1,500
Miscellaneous			
Total	$6,170	$6,358	$6,552

Footnotes: [1] Salaries are calculated as 2 weeks of a Full Time Technician's salary and OPE, for oversight of planting, mapping, plant measurements, and data management. The increase in salaries for years two and three reflects a 4 % rate increase. [2] Travel includes 1 trip to WA sites/year at 0.58 cents per mile, one night lodging and two days per diem for PI and technician, and visits to OR orchard sites for data collection and support.

Budget 2: Tom Auvil
Organization Name: WA Tree Fruit Research Comm. **Contract Administrator:** Kathy Schmidt
Telephone: 509-665-8271 **Email address:** Kathy@treefruitresearch.com

Item	2009	2010	2011
Salaries[1]	4200	5280	6,000
Benefits[1]	1330	1672	1,900
Wages[1]	1475	2024	2,300
Benefits	425	624	700
Equipment			
Supplies			
Travel[1]	900	900	1000
Miscellaneous	800	100	100
Total	$9200	$10,600	$12,000

[1]Salary and benefits include WTFRC internal program's time for supervision, planning, logistics and data management for pear projects.

Objectives:

1. Determine the influence of Horner 4 and 10 on tree growth, yield, fruit size and quality for the cultivars, 'Bartlett', 'Golden Russet Bosc' and 'd'Anjou'. OHxF 87 will be used as the standard.

2. Compare rootstock/scion interactions among orchards at different geographic locations.

Significant Findings 2009-2011:

- Of the five trial sites planted, four are performing well. A fifth site was inadvertently subjected to herbicide damage in 2010. Trunk circumference is roughly 25-50% of that observed at other sites.
- The mortality rate for all sites was 6 %, but varied markedly among sites (e.g., range of 1% to 12%). Averaging across scion cultivars and sites, Horner 4 sustained the greatest rootstock mortality rate [10%], Horner 10 was intermediate [7%], and OHxF 87 had the fewest losses [3%] (Table 1). Causes of individual tree losses varied with site, and do not appear to be related to rootstock genotype.
- Third leaf 'Bartlett' bloom was slightly lower on Horner 10 than either Horner 4 or OHxF 87; however, Horner 10 fruit set was reduced by ~40%.
- First crop 'Bartlett' fruit size was small, irrespective of rootstock.
- Precocity of 'GR Bosc' and 'Anjou' was not observed through third leaf for any rootstock/cultivar combination.
- At the completion of 3rd leaf, 'Bosc' tree size was slightly smaller on Horner 10 than either Horner 4 or OHxF 87, which were roughly equivalent.
- At both 'Bartlett' sites, tree size was slightly, and non-significantly, smaller on Horner 10 than either OHxF 87 or Horner 4, which were roughly equivalent in size.
- For 'd'Anjou', OHxF 87 and Horner 10 produced trees similar in size, and ~ 40 % smaller than trees on Horner 4, not including data from the Bridgeport site.
- Root suckering was not observed.

Results and Discussion:

1. Sites.

Fumigated trial sites were planted spring 2009. All trees were headed and feathers removed at the time of planting. Planting methods included: 1) Shovel-planted (all WA sites), 2) Augured holes (Hood River), and 3) Tractor-drawn transplanter (Parkdale). Grower cooperators, researchers and technicians continued to collaborate on training system and plot management decisions. Information pertaining to individual sites is provided below:

Hood River

- Spacing: 17' x 6' (427 trees per acre)
- Scion: 'd'Anjou'
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Modified central leader/three wire support
- Replicates: Six, five-tree reps

Parkdale

- Spacing: 12' x 6' (605 trees/acre)
- Scion: 'd'Anjou'
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: In-line "V" fruiting wall/wire support
- Replicates: Six, five-tree reps

Bridgeport Anjou

- Spacing: 16’ x 6’ (OHxF87 and Horner 10), 16’ x 8’ (Horner 4)
- Scion: ‘d’Anjou’
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Perpendicular “V”/wire support
- Replicates: Five, five-tree reps

Bridgeport Bosc

- Spacing: 16’ x 5’ for OHxF 87 and Horner 10; 545 trees per acre), 16’ x 7’ (Horner 4; 389 trees per acre)
- Scion: ‘Bosc’
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Perpendicular “V”/wire support
- Replicates: Five, five-tree reps

Wapato

- Spacing: 10’ x 4’ (1089 trees per acre)
- Scion: ‘Bartlett’ and ‘Bosc’
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Tall spindle fruiting wall/wire support
- Replicates: Five, five-tree reps

Methow

- Spacing: 12’ x 4’ (907 trees per acre)
- Scion: ‘Bartlett’
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Tall spindle/wire support
- Replicates: Five, five-tree reps

2. Rootstock effects

Effects of rootstocks are presented relative to cultivar.

A. ‘d’Anjou’. Horner 4 produced a markedly larger tree than either Horner 10 or OHxF 87, at both Oregon sites (Table 1). Limited bloom and fruit set, and insignificant yields were observed at Parkdale (Table 2). Trees at both sites were vigorous, and precocity was not induced by any of the rootstocks evaluated. Bridgeport was initially characterized as a low vigor site due to poor soil fertility and the presence of gravel bars throughout the profile. There is value for including low-vigor sites in the trial, since preliminary data (Meilke and Sugar, 2004) indicated a substantial difference in vigor between the two ‘Horner’ rootstocks. Subsequently, a potentially vigorous rootstock, such as Horner 4, might result in superior performance under poor fertility conditions. However, herbicide-induced phyto-toxicity confounded results at the Bridgeport site. Interestingly, the inherent vigor of Horner 4 was observable at Bridgeport, despite herbicide damage (Table 1). Fruit set between the 4th and 7th leaf will be critical for management of Anjou in higher-density plantings.

Anjou mortality rates were high at the Hood River site (11%) following the 2010 season. Tree losses were highest for OHxF 87 and Horner 4; incidentally, tree size was significantly

smaller for both of these roots relative to those on Horner 4 at the end of year one. It is likely that these 'weaker' trees succumbed to a combination of environmental factors and disease pressure. However, no additional trees losses were recorded in 2011 (Table 5), and the general health of the planting is good. Notably, similar tree losses were not observed at the two other 'd'Anjou' sites, indicating that site specific issues were likely responsible for the higher mortality rates of Horner 10.

Table 1. 2011 trunk size [trunk cross-sectional area (cm^2)] and yield efficiency [kg of yield/cm^2 TCA] per rootstock-cultivar combination for all sites.

Rootstock	Cultivar	Site	Trunk Size TCA(cm^2)	Yield Efficiency Yield/TCA (kg/cm^2)
Horner 10	Bartlett	Wapato	9.5	0.7
		Methow	10.9	0.1
Horner 4		Wapato	11.2	0.8
		Methow	12.1	0.1
OHxF87		Wapato	10.9	0.9
		Methow	12.3	0.3
Horner 10	GR Bosc	Wapato	11.6	0.2
		Bridgeport	7.7	n.d.
Horner 4		Wapato	13.3	0.1
		Bridgeport	6.8	n.d.
OHxF87		Wapato	13.2	0.2
		Bridgeport	7	n.d.
Horner 10	Anjou	Bridgeport	5.2	n.d.
		Hood River	17.5	n.d.
		Parkdale	19.8	n.d.
Horner 4		Bridgeport	6.6	n.d.
		Hood River	27.2	n.d.
		Parkdale	27.1	n.d.
OHxF87		Bridgeport	5.1	n.d.
		Hood River	16.9	n.d.
		Parkdale	18.3	n.d.

n.d., no data due to insignificant yield.

Table 2. 2011 'Anjou' flowering (total clusters per tree), total fruit per tree, and fruit set (per 100 clusters) as affected by rootstock.

Rootstock	Site	Avg. Clusters (no./tree)	Avg. Fruit (no. at set/tree)	Fruit set (% [fruit/cluster])
Horner 10	Parkdale	9	0.4	4.4
	Bridgeport	n/a	n/a	n/a
	Hood River	n/a	n/a	n/a
Horner 4	Parkdale	17	1.7	10
	Bridgeport	n/a	n/a	n/a
	Hood River	n/a	n/a	n/a
OHxF87	Parkdale	7	0.4	5.7
	Bridgeport	n/a	n/a	n/a
	Hood River	n/a	n/a	n/a
		n.s.	n.s.	n.s.

[2]n/a (data not available)

B. 'GR-Bosc'. Interestingly, for 'GR Bosc', Horner 4 did not impart significantly greater vigor in the tree (Table 1), compared to the other rootstocks. Only slight differences in tree size were observed between Horner 10 (smaller), and the slightly larger OHxF 87 and Horner 4 (Table 1). Similarly to 'd'Anjou', the Bridgeport trees suffered from the combination of a low vigor site, and herbicide damage, consequently trees were much smaller at Bridgeport. Although 'GR Bosc' is more precocious than Anjou, significant flowering and fruit set were not observed (Table 3), regardless of rootstock. It is plausible that the 4' spacing between trees at Wapato might result in enhanced root competition between adjacent trees, but perhaps additional years will be required to observe such effects.

Table 3. 2011 'GR Bosc' flowering (total clusters per tree), total fruit per tree, fruit set (per 100 clusters), yield (lbs) and fruit size (g) as affected by rootstock.

Rootstock	Site	Avg. Clusters (no./tree)	Avg. Fruit (no. at set/tree)	Fruit per 100 clusters	Yield (lbs)	Fruit (no. at harvest)	Fruit Size[1] (g)
Horner 10	Wapato	28	10	39	4.3	10	197
	Bridgeport	n/a	n/a	n/a	n/a	n/a	n/a
Horner 4	Wapato	7	7	118	3	7	185
	Bridgeport	n/a	n/a	n/a	n/a	n/a	n/a
OHxF87	Wapato	18	10	103	5	10	218
	Bridgeport	n/a	n/a	n/a	n/a	n/a	n/a
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1]Fruit size taken on all fruit (including undersized culls).
[2]n/a (data not available)

C. 'Bartlett'. At both 'Bartlett' sites tree size was smallest, albeit non-significantly, for Horner 10 (Table 1). As observed for 'GR Bosc', Horner 4 tree size was equal to OHxF 87. The inherent precocity of 'Bartlett', relative to the other cultivars, was observed at both sites, though markedly more pronounced at Wapato (Table 4). Significant differences were observed in the number of fruit set at Wapato; Horner 10 had roughly 30% fewer fruit than either Horner 4 or OHxF 87, and nearly half the yield (Table 4). It appears that earlier canopy development, and subsequently greater canopy volume of Horner 4 and OHxF 87 resulted in higher productivity. Alternatively, relative fruit set data (fruit per 100 clusters)

imply that the more dwarfing Horner 10 did not enhance precocity. Fruit size was small (Table 4), as is often observed in first-year crops; however, fruit size was determined as the average of the total number of fruit, including non-marketable fruit, and therefore is not entirely representative of the crop. Future efforts will need to present size-class, distribution data, so rootstocks comparisons can be made using proportions of marketable and non-marketable fruit.

Table 4. 2011 'Bartlett' flowering (total clusters per tree), total fruit per tree, fruit set (per 100 clusters), yield (lbs) and fruit size (g) as affected by rootstock.

Rootstock	Site	Avg. Clusters (no./tree)	Avg. Fruit (no. at set/tree)	Fruit per 100 clusters	Yield (lbs)	Fruit (no. at harvest)	Fruit Size[1] (g)
Horner 10	Wapato	124	38 b[2]	36	13.9 B[3]	38.3 B	165
	Methow	40	6	19	2.3 b	6.5 b	161
Horner 4	Wapato	146	60 a	47	20.2 A	60.2 A	152
	Methow	67	8	11	3.1 b	8.1 b	176
OHxF87	Wapato	124	62 a	41	21.9 A	61 A	163
	Methow	63	17	29	7.4 a	16.9 a	176
		n.s.		n.s.			n.s.

[1]Fruit size is the average for all fruit (including undersized culls)

[2]Capital letters indicate significant differences at Wapato site within columns

[3]Lower-case letters indicate significant differences at Methow site within columns

Table 5. Mortality rates of rootstock clones. Data are cumulative through 2011.

Rootstock	Individual Tree Losses	Total trees planted	Mortality rate %
Horner 4	18	185	10
Horner 10	13	185	7
OH x F 87	5	185	3

Executive Summary
Five trial sites were established in 2009 to test effects of Horner 4 and Horner 10 rootstocks on 'GR-Bosc', 'd'Anjou', and 'Bartlett' performance. OHxF 87 was included as a control at each site. Trial sites were established in commercial orchards. Cultivar selection, planting design and training system varied from site to site.

In the third leaf (2011) only 'Bartlett' trees flowered and set a significant crop. Differences existed between the two 'Bartlett' sites. At Wapato, WA, Horner 4 and OHxF 87 had similar and higher fruit set, and yields than Horner 10. At Methow, WA, OHxF 87 had the highest yield, but first crop production was quite low for all rootstocks at this site. None of the rootstocks induced precocity in 'GR-Bosc' or 'd'Anjou', at any site.

Tree size was not significantly influenced by rootstock for either 'Bartlett' or 'GR-Bosc', though trees were slightly larger on Horner 4. In 'd'Anjou', Horner 4 produced a significantly larger tree than Horner 10 and OHxF 87, at two of the three 'd'Anjou' sites. Tree size of 'd'Anjou' on Horner 10 and OHxF 87 were similar. A third site was still recovering from inadvertent herbicide-induced phyto-toxicity.

Mortality rates were low at all sites (ranging from 3%-10%), and did not appear to be related to rootstock genotype. Suckering was not observed in any of the combinations.

FINAL PROJECT REPORT

Project Title: Improved micropropagation of dwarfing pear rootstocks

PI: Barbara M. Reed
Organization: USDA-ARS
Telephone: 541-738-4216
Email: Barbara.Reed@ars.usda.gov

Cooperators: Todd Einhorn, Oregon State University; Randall P. Niedz and Terrence J. Evens, USDA-ARS; Postdoc. Sugae Wada, Oregon State University

Other funding sources

Agency Name: California Pear Commission
Amt. awarded: $36,900
Notes: Improved Media for Micropropagation of Dwarfing Pear Rootstocks (to supplement FPC/PPC funding for this project; for post doctoral researcher)

Total Project Funding: $35,000

Budget History:

Item	2011
Salaries	20,000
Benefits	12,000
Wages	
Benefits	
Equipment	
Supplies	2500
Travel	500
Miscellaneous	
Total	$35,000

RECAP ORIGINAL OBJECTIVES (for original 3 year project)

This was designed as a 3 yr project and funded for 1 year.

1) Develop growth medium suitable for commercial micropropagation of dwarfing pear rootstock selections and cultivars. **Year 2011-12** Spring-Summer: Multiply stocks of available cultures for testing. Initiate cultures of additional dwarfing pear rootstock cultivars or selections from grafted or forced shoots. Summer-Winter: Initial test of available cultures for "mesos" elements ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$). Winter: Data analysis and preliminary report. Continue optimization studies in years 2 and 3.
2) Determine rooting potential of shoot cultures on new medium formulations (yr 2).
3) Finalize standard micropropagation and rooting protocols and transfer this information to commercial micropropagation facilities (yr 3).

SIGNIFICANT FINDINGS

- Initial growth on our improved medium allowed for enough propagation to start the experiments.
- Quality of the micropropagated shoots improved significantly for all eight genotypes with 1.5X or greater mesos ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$) compared to standard MS medium**.**
- Leaf spot and edge burn symptoms, hyperhydricity and leaf curl decreased with 1.5 or 2.0X mesos.
- In most cases shoot multiplication was only slightly influenced by mesos.
- Shoot length, leaf color and leaf size were best on mesos of 1.5X or greater.

RESULTS & DISCUSSION

We initiated shoot cultures of dwarfing pear rootstocks and multiplied them for a study of the effect of MS mesos concentrations ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$). Five of the eight genotypes were growing very poorly at the beginning of the experiment (OHxF69, OPR125, G28.120, Fox11 and Pyro 2-33) while the others were growing sub optimally. Increased mesos were required for moderate to good growth of all eight genotypes (Fig. 1). A range of MS medium mesos concentrations from 1.5X to 2.5 X gave the best "quality" ratings, the longest shoots, and the best leaf form and color for most genotypes (Fig. 2) but most still are very short and are not multiplying as rapidly as would be preferred. Additional genotypes were multiplied for future testing.

Quality: All eight genotypes had the best quality on mesos 1.5X to 2.5X although these were not always high ratings.

Shoot number: There were no significant differences in multiplication (this is often governed by nitrogen ratios).

Shoot length: In many cases all treatments were similar. For others (OHxF 97, Pyrodwarf and Pyro2-33) the higher mesos produced the longest shoots. Nitrogen ratios are known to affect shoot length.

Leaf color rating: Leaf color was darkest at 2.0 and 2.5X mesos. Red or yellow leaves were noted for 0.5 and 1.0 mesos plants.

Leaf size rating: Leaf size was moderate at 1.5X mesos.

Callus: Callus was not a serious problem on any of the cultivars.

The lowest mesos concentration (0.5X) gave an indication of true deficiency symptoms. In all cases the plants were stunted, with fat stems, pale in color and had reddened or spotted and curled leaves. The normal MS mesos (1.0X) plants were also small and many had leaf spotting or edge discoloration. At 1.5X shoots were slightly taller and leaves were a normal color and size. Plants on the 2.0X and 2.5X concentrations were darker green, with larger leaves and often longer stems. This experiment indicates that the rootstock cultivars and selections have a requirement for higher concentrations of 'mesos' (2.0 and 2.5X) than did the scion cultivars (1.5 and 2.0X). We will grow these selections for additional passages on the higher concentrations to determine if they are suitable for long-term propagation. This initial test shows that changes in mineral nutrition result in significant improvements in the shoots (as well as eliminating a number of the common physiological abnormalities) of the dwarfing pear rootstocks. However, most genotypes still require further improvements in shoot multiplication and shoot length (Fig. 3). Optimization of nitrogen and minor elements is needed to optimize growth and produce one or more improved growth media for use in commercial micropropagation, as well as protocols for the improvement of specific genotypes. Once commercial micropropagation is possible, these and newly introduced rootstocks would be more widely available for field testing and grower use.

A preliminary rooting test with two genotypes (OH x F 87 and Horner 51) produced 50-100% rooting for some treatments and all *in-vitro* rooted shoots survived under mist in the greenhouse. This preliminary test provided information that will be useful when designing the final rooting studies. The base medium used for multiplication and the base rooting medium both influenced the percent shoots that rooted. This will be further tested in additional studies.

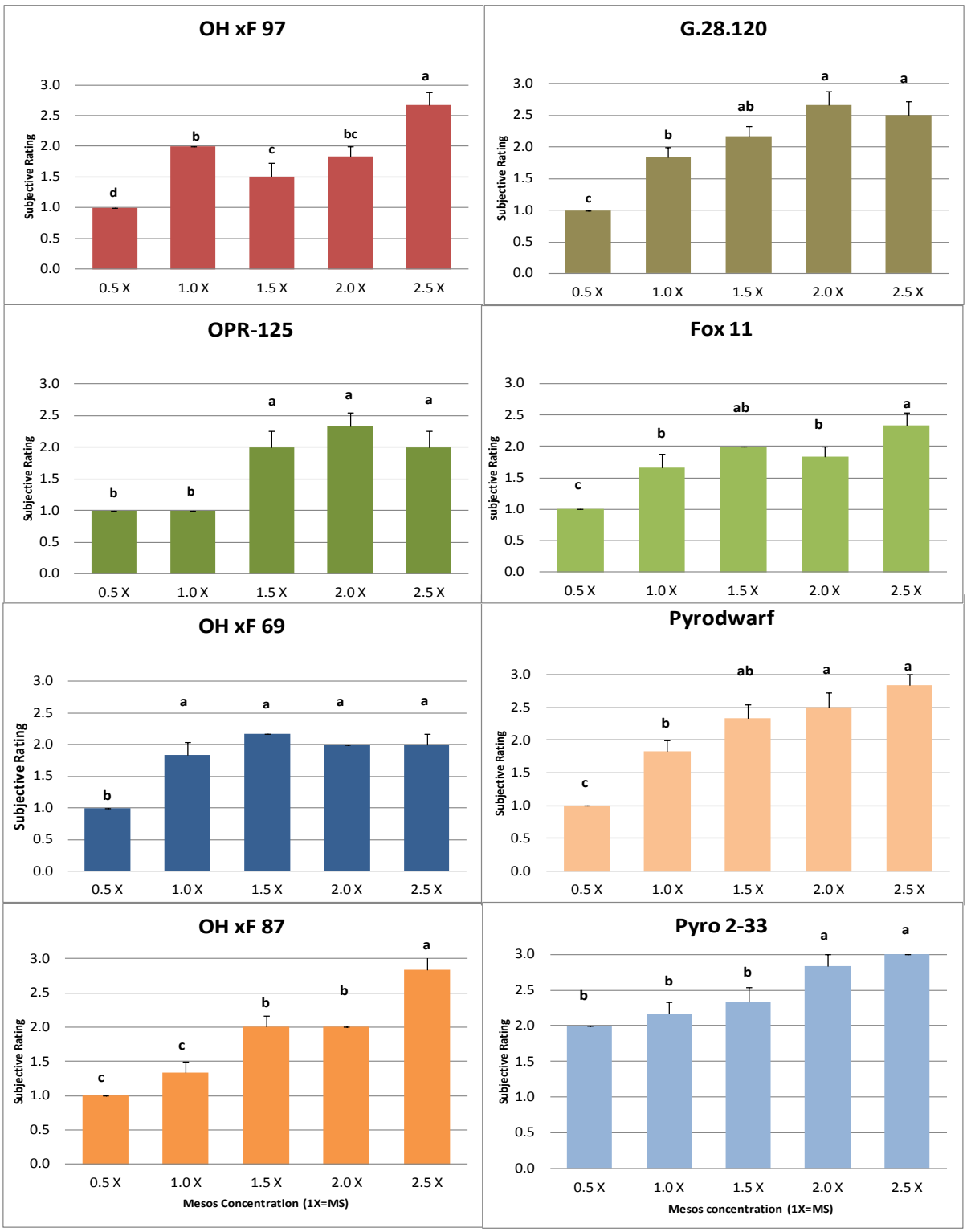

Figure 1. Mean quality ratings of shoots of eight pear rootstocks grown on MS medium with increasing concentrations of 'mesos' elements. Plants were rated for quality: 1 poor, 2 moderate, 3 good. Treatments with the same letter are not significantly different (α=0.05). n=6 shoots per treatment.

Fig. 2. Photographs of the pear shoots grown on five mesos concentrations. From left: 0.5X, 1.0X (MS), 1.5X, 2.0X, 2.5X mesos. Scale is in centimeters**.** The ideal plant would be 7-9 cm tall.

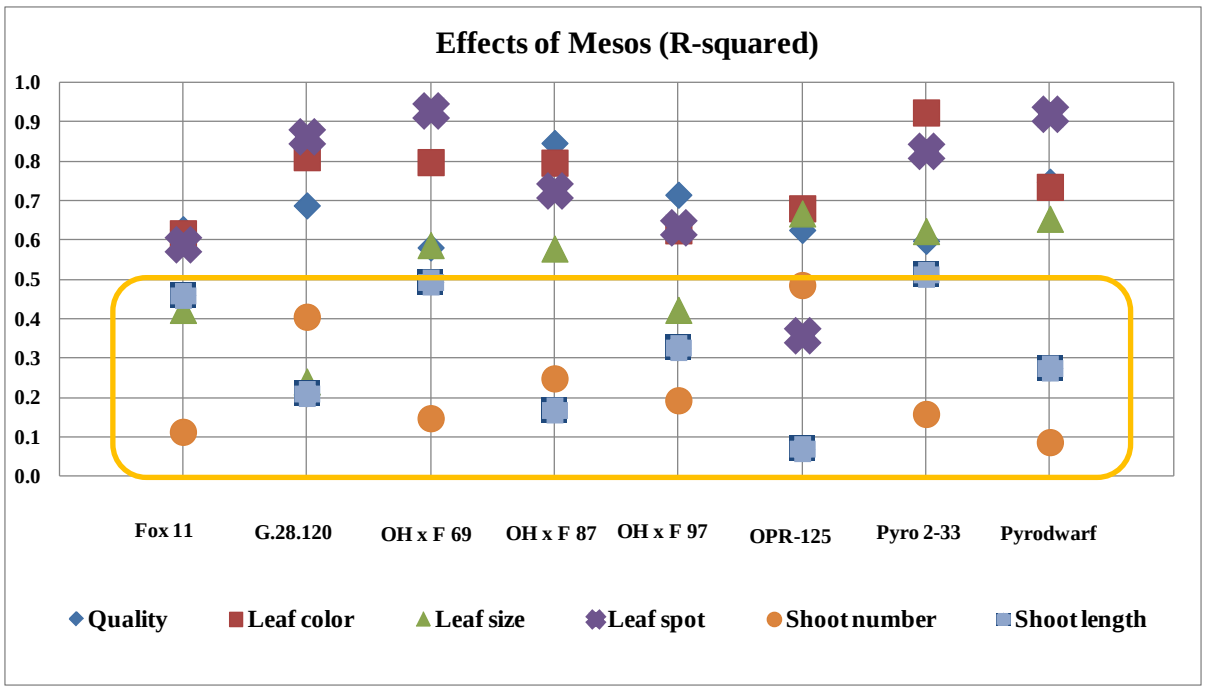

Fig. 3. This chart shows the statistical significance of the plant responses to increased mesos concentrations. Improvements resulting from increasing mesos are indicated by the top half of the chart (>0.5) while responses that did not improve are in the yellow box at the bottom. Shoot number (orange dots) and shoot length (blue square) still need improvement on most or all of the genotypes.

EXECUTIVE SUMMARY

The development and use of pear dwarfing rootstocks has been restricted by the lack of effective and rapid propagation systems. Dwarfing rootstocks are difficult to propagate both traditionally and *in vitro*. Many promising dwarfing rootstocks were abandoned because of difficulty with traditional propagation or poor growth *in vitro* (Proebsting, WTFRC reports 2003-7). Our laboratory conducted intensive studies of the mineral nutrition of *in-vitro* grown pear scion cultivars and species over the last four years. During this process we determined key nutrients in the growth medium that promote the growth of a range of cultivars and species that originally would not grow or grew poorly and slowly on standard medium (Reed et al., 2011b). Amazing improvements were seen in the 17 pears studied in these experiments. Initially most were in poor condition, but now all are showing excellent growth and multiplication with these mineral nutrient improvements. These earlier studies of scion pear cultivars indicated that the mineral nutrition factors with the most effect on plant appearance and growth were in the 'mesos' stock solutions ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$). In the current study we tested the 'mesos' concentration in medium for eight dwarfing pear rootstocks. Growth of all eight genotypes improved significantly (from poor to moderate or good) with increased 'mesos'. The best quality shoots, the longest shoots and the best leaf form and color were obtained with increased 'mesos' concentrations. Half of the tested plants were rated as good quality (rated>2 out of 3) on one of the higher 'mesos' concentrations. All the genotypes were greatly improved but are not yet of a quality high enough for routine micropropagation. Shoot number and shoot length are still subpar. Continued study of the effect of mineral nutrients, especially nitrogen and micronutrients, should result in medium formulations that will provide optimal micropropagation for all 15 genotypes in the test group. Development of growth media that can be transferred to commercial nurseries for production of the rootstocks will allow wider use of more rootstock types. Testing the most effective rooting treatments with shoots grown on improved medium formulations would also provide standard protocols for use by commercial micropropagation laboratories.

FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Pear crop load management and rootstock field testing

PI:	Tory Schmidt	**Co-PI (2):**	Tom Auvil
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Cooperators: Felipe Castillo, Ines Hanrahan, Jim McFerson, Dave Sugar, Todd Einhorn

Total Project Request: **Year 1:** 24,000 **Year 2:** 26,000 **Year 3:** 16,000

Other funding sources

All chemicals donated by companies
$2000 each from Valent and Fine Americas to support fruit set trials
$2400 from Fine Americas to support thinning trials

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

Item	2009	2010	2011
Salaries	10,500	12,000	6000
Benefits	3300	3800	1900
Wages	5500	5500	5500
Benefits	1500	1500	1500
Equipment			
Supplies			
Travel	3000	3000	1000
Miscellaneous	200	200	100
CLM Subtotal	14,800	15,400	16,000
Rootstock subtotal *	9,200	10,600	See Einhorn report
External funding		(3,000)*	(6,400)*
Grand Total	$24,000	$23,000*	$9,800

Footnotes: 2011 expenses related to Einhorn Horner evaluation project have been removed from this budget
*Note: original budget total for 2011 was $16,000; current figure has been revised to reflect contributions to project from Valent and Fine Americas

Objectives:

1. Continue development of effective crop load management programs for pear to reduce production costs, increase fruit size, and promote return bloom (Schmidt).

2. Provide consulting, logistical, labor, and data management support for Todd Einhorn's project for grower screening of Horner series rootstocks (Auvil).

Significant findings:

- ATS applied during bloom and BA applied at 10 mm fruitlet size effectively thin Bartlett pears; combined programs provide the best results
- Tank mixing of BA with other materials (oil, abamectin, phosphite, carbaryl) did not produce clear benefits
- Split applications of reduced rates of BA showed no benefit over single full rate applications in our studies
- Application of AVG (ReTain), GA_3 (ProGibb, Falgro), GA_4 (Novagib), GA_7, GA_{4+7} (ProVide), and BA + GA_{4+7} (Promalin, Perlan) did not improve fruit set of D'Anjou or Red D'Anjou in 7 trials over 3 seasons
- BA frequently improved harvest fruit size across chemical thinning and fruit set trials
- Budget and details for Horner rootstock evaluation in Einhorn's report

Methods:

Chemical thinning: From 2009-2011, we conducted chemical thinning trials in one D'Anjou and ten commercial Bartlett orchards; three Bartlett trials were applied by grower-cooperators using their own spray equipment, while the rest were applied by WTFRC staff with an AccuTech sprayer. Grower-applied trials were designed as randomized complete blocks with plots comprised of 2-3 whole rows to simplify spraying. WTFRC-applied trials generally featured smaller designs, generally consisting of 5-8 trees per plot, depending on tree size and spacing. Initial bloom counts were recorded on tagged sample branches in each plot. All trials were successfully treated at appropriate timings using 100 gal water/acre; treatments are detailed in Table 1. Fruit set counts were made on sample branches after June drop, but before green fruit hand thinning. Representative fruit from each plot were sampled within a few days of commercial harvest and evaluated in the WTFRC lab for size, firmness, sugar levels, acidity, and fruit finish.

Table 1. Pear chemical thinning programs evaluated. WTFRC 2009-2011.

Material	Concentration	Timing(s)
ATS	5%	20% & 80% bloom
NC99	10%	20% & 80% bloom
BA (MaxCel, Exilis Plus, Genesis 6-BA)	16 - 128 oz/A	8-10 mm, 14-16 mm
BA + carbaryl	128 oz + 64 oz/A	10 mm
BA + Superior oil	128 oz/A + 1%	10 mm
BA + Sysstem-CAL	32 oz + 64 oz/A	10 mm
BA + AgriMek + summer oil	32 oz + 20 oz/A + 1%	10 mm

Fruit set: Seven trials were conducted from 2009-2011 investigating the potential use of various plant growth regulators to increase fruiting in commercial D'Anjou and Red D'Anjou blocks with histories of poor fruit set. Materials were applied by WTFRC staff at 100 gal water/acre with our AccuTech sprayer; application timings and concentrations were determined based on reports of successful programs in Europe and input from the research staff of the respective chemical manufacturers (Table 6). Trials were designed as randomized complete blocks with 6-7 trees per plot. Initial bloom counts were recorded on tagged sample branches in each plot. Fruit set counts were made on sample branches after June drop. Representative fruit from each plot were sampled within a few days of commercial harvest and evaluated in the WTFRC lab for size, firmness, sugar levels, acidity, and fruit finish.

Results and discussion:

Chemical thinning: Starting in 2003, our research program began screening potential bloom thinners of Bartlett pears, including ammonium thiosulfate (ATS), an organic magnesium/calcium brine (NC99), urea, lime sulfur (LS), and combinations of horticultural oils and LS. As is typical of chemical thinning work in other crops, some products performed well in isolated cases, but their effects were unreliable. Over several years of trials, we found ATS to be more consistent in reducing fruit set than other products (Table 5). ATS was also appealing due to its relatively low cost and ease of handling, and became the standard bloom thinning treatment in the course of our investigations.

In contrast to the variability of our chemical bloom thinning results, we have been surprised by the relatively consistent performance of benzyladenine (BA) products like MaxCel (Valent), Exilis Plus (Fine), and Genesis 6-BA (GS Long), especially with respect to increasing fruit size. In fact, the long-term success rate of BA producing statistically significant gains in fruit weight in 53% our studies (Table 5) is unparalleled in our work with any growth regulator in pear, apple, cherry, or soft fruits. Not surprisingly, many of our best trial results in recent years have been from programs featuring the use of ATS during bloom and BA at 10 mm fruitlet size (Tables 2, 3). Chemical thinning programs can often be confounded by poor weather or imprecise application timings and we generally find it advantageous to make multiple applications using different materials to improve chances for success.

The primary focus of our 2011 chemical thinning trials was to explore modifications to use patterns of BA, whether by splitting the applications over time (Monitor, Wapato) or tank-mixing BA with other products which may increase efficacy by improving uptake by plant tissues (Rock Island). Unfortunately, abnormally cold spring weather in 2011 may have compromised the performance of BA products across all three trials. Harvest fruit size was not affected by any treatment in any trial,

and the only reductions in fruit set (Monitor) could be attributed to the use of ATS during bloom in those programs (Table 2).

Nonetheless, our 2011 results (Table 2) corroborate earlier studies which indicated that splitting an equivalent amount of BA over multiple applications does not offer clear advantages over a single high-rate application, although we are aware of anecdotal reports from Northwest pear growers and South American researchers suggesting the contrary. A logical case can be made that split applications may be advantageous when a single application would be made in poor weather (i.e. < 65F) and a second might be applied during warmer temperatures, but our trials may not have experienced the particular weather conditions to properly test that hypothesis.

Even though no treatment in our 2011 trial in Rock Island significantly reduced fruit set or improved fruit size (Table 3), we saw no additional response from adding either oil + abamectin (AgriMek) or phosphite (Sysstem-CAL) to the spray tank with BA. This pattern is consistent with results in 2010, when we observed no benefit from the use of carbaryl with BA. In both 2009 and 2010, we found that using 1% Superior oil with BA slightly increased thinning, but also hurt fruit size, perhaps due to increased photosynthetic stress on the tree (data not shown here). In summary, we have yet to document any benefit to Bartlett growers by deviating from the base program of applying 96-128 oz/A of BA at 8-10 mm fruitlet size during favorable weather conditions.

Table 2. Crop load effects of bloom (ATS) and postbloom (BA) chemical thinners on Bartlett pears. WTFRC 2011.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
Bartlett/Seedling	ATS; half rate BA 2x	38 b	69 a	23 ab	240 ns	83
- Monitor	ATS; full rate BA 1x	40 b	68 a	26 ab	241	83
	ATS; FAL 551	38 b	72 a	21 b	251	80
	Control	60 a	58 b	29 a	241	83
Bartlett/Seedling	16 oz BA	85 ns	43 ns	35 a	154 ns	130
- Wapato	32 oz BA	85	49	25 b	143	140
	32 oz BA 2x	92	48	24 b	147	136
	32 oz BA; 16 oz BA	84	48	27 ab	149	134
	64 oz BA	92	44	30 ab	149	134
	Control	83	47	30 ab	149	134

Table 3. Crop load effects of bloom (ATS) and postbloom (BA, oil, AgriMek, Sysstem-CAL) chemical thinning programs on Bartlett pears. WTFRC 2011.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
Bartlett/OHxF.97	ATS; BA	56 ns	60 ns	27 ns	215 ns	93
- Rock Island	ATS; BA + AgriMek + oil	76	48	32	214	93
	ATS; BA + Sysstem-CAL	64	55	29	217	92
	Control	73	56	24	226	88

Our lone attempt to chemically thin a pear variety other than Bartlett showed strong treatment effects, but ultimately undesirable results from a grower's perspective. Even with less aggressive chemical rates than in used in Bartlett (Table 1), both BA and the tandem of ATS and BA over-thinned our D'Anjou trial plots in 2010 (Table 4). These results reflect the conundrum of crop load management in D'Anjou (and to a lesser degree, Bosc): while improved fruit size is desirable and achievable, chemical thinning programs typically reduce total yield too much to be considered profitable for growers. As such, we have attempted to identify PGR programs that might allow the use of BA to increase fruit size while still preserving or improving yields of weak-setting pear varieties.

Table 4. Crop load effects of bloom (ATS) and postbloom (BA) chemical thinning programs on D'Anjou pears. WTFRC 2010.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
Anjou/OHxF.97	ATS	34 a	73 c	22 a	239 b	84
- Buena	ATS; BA	9 b	92 a	8 b	247 ab	81
	BA	16 b	86 b	11 b	257 a	78
	Control	45 a	70 c	19 a	235 b	85

Due to the inherent variability in chemical thinning research results, we advocate evaluation of trial results across seasons, cultivars, and geographic regions to more accurately assess the efficacy of crop load management programs. Table 5 summarizes all WTFRC pear chemical thinning trials conducted since 2003; entries indicate how often various thinning agents have successfully achieved each of our three basic chemical thinning goals:

1. reduced hand thinning of green fruit (reflected by decreased fruit set)
2. increased fruit harvest fruit size
3. improved return bloom in the season after treatment

In this broader view, it is clear that ATS and BA products are the most consistent materials for reducing fruit set, while BA products most often confer larger fruit size and occasional improvements in return bloom.

Table 5. Incidence and percentage of results significantly superior to untreated control. Pear chemical thinning trials WTFRC 2003-2011.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	9 / 31 (29%)	5 / 30 (17%)	3 / 27 (11%)
Urea	1 / 17 (6%)	3 / 17 (18%)	0 / 15 (0%)
Crocker's Fish Oil + lime sulfur	0 / 13 (0%)	1 / 13 (8%)	1 / 12 (8%)
Lime sulfur	1 / 13 (8%)	3 / 13 (23%)	0 / 13 (0%)
BA	4 / 19 (21%)	9 / 17 (53%)	3 / 16 (19%)
NAA	0 / 6	0 / 6	0 / 1

[1]Does not include data from 2011 trials.

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

Fruit set: As demonstrated by our 2010 chemical thinning trial (Table 4), D'Anjou pears can be highly sensitive to chemical thinners including BA. In fact, many pear growers would benefit from tools to help them increase fruit set, as many D'Anjou and Bosc blocks produce light yields despite apparently ample bloom and good pollination conditions. In 2009 we began screening a range of plant growth regulators for their capacity to increase fruit set in light-bearing pear blocks with the

ultimate goal of developing programs which would allow D'Anjou and Bosc to enjoy the fruit sizing benefits of BA applications without significant losses in yields. The programs we tested were based largely on successful European pear industry practices for mitigating reductions in fruit set following spring frosts.

Unfortunately, no treatment in seven trials over three years provided any significant increase in fruit set and some actually reduced it. Protocols for 2011 trials not only featured more aggressive rates of all materials tested in 2009 and 2010, but alternative formulations of gibberellic acid (GA) not previously assayed. The best result from any treatment in any of the seven trials was a 50% boost in fruit set from GA_7 applied to Dryden D'Anjous in 2011(Table 6), but even that increase was not statistically significant. GA_7 is an isomer of gibberellin which is expensive to formulate and not available in a commercial formulation, rendering further investigation an academic pursuit.

Scientists from Italy and Spain recently reported at a local meeting on European research demonstrating effective use of several plant growth regulators to promote pear set. Their growers utilize specific "cocktails" of materials that are often customized to individual pear blocks and sometimes feature chemistries not registered for use in the US. The researchers were unaware of programs that had been used on D'Anjou or Bosc and suggested these cultivars may behave differently than common European varieties.

In light of our poor results over three seasons with available growth regulators to promote pear fruit set, we have decided to forgo further work in this area until new materials or approaches offer greater prospects for success.

Table 6. Crop load effects of PGR programs to promote fruit set of pears. WTFRC 2011.

PGR material/acre	Application timing(s)	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
D'Anjou/unknown - Dryden						
12 ppm GA_7	20 & 80% bloom	61 a	58 b	27 ns	208 ns	96
12 ppm GA_4	20 & 80% bloom	43 ab	68 ab	22	205	97
10 ppm GA_3	20 & 80% bloom	51 ab	63 ab	25	215	93
15 ppm GA_3	20 & 80% bloom	51 ab	62 ab	26	203	98
8 oz Promalin	20 & 80% bloom	43 ab	71 ab	19	219	91
12 oz Promalin	20 & 80% bloom	40 ab	70 ab	22	207	97
8 ppm GA_{4+7}	20 & 80% bloom	36 b	72 a	21	205	97
12 ppm GA_{4+7}	20 & 80% bloom	53 ab	65 ab	21	223	90
333 g Retain	Late petal fall	45 ab	67 ab	23	200	100
Control	NA	42 ab	69 ab	21	197	101
Red D'Anjou/OHxF.97 - Cashmere						
12 ppm GA_7	20 & 80% bloom	8 ns	93 ns	7 ns	218 ns	92
12 ppm GA_4	20 & 80% bloom	8	92	7	232	86
8 oz Promalin	20 & 80% bloom	7	93	6	236	85
12 oz Promalin	20 & 80% bloom	6	95	5	235	85
8 ppm GA_{4+7}	20 & 80% bloom	7	93	6	222	90
12 ppm GA_{4+7}	20 & 80% bloom	9	91	8	236	85
333 g Retain	Late petal fall	5	95	5	227	88
Control	NA	7	93	6	225	89
D'Anjou/OHxF.97 - Monitor						
8 oz Promalin	20 & 80% bloom	60 ns	62 ns	22 ns	230 ns	87
12 oz Promalin	20 & 80% bloom	72	59	18	226	88
12 ppm GA_{4+7}	20 & 80% bloom	57	65	19	227	88
333 g AVG	Late petal fall	58	63	21	229	87
Control	NA	63	60	22	229	87

EXECUTIVE SUMMARY

Over three years, chemical thinning trials were conducted on ten Bartlett and one D'Anjou blocks in Washington. Results confirmed the efficacy of ATS applied during bloom for decreasing fruit set and increasing fruit size. BA products applied postbloom consistently increased fruit size and often contributed to additional thinning. Neither split applications of BA nor tank-mixing BA with several other materials demonstrated any clear advantages over a single application of BA by itself. The strongest results were obtained by programs featuring use of ATS at 20% and 80% bloom followed by one application of BA at 8-10 mm. Use of chemical thinners on D'Anjou significantly reduced harvest yields and is unlikely to help improve returns for Northwest growers.

Use of several plant growth regulators to improve fruit set in D'Anjou or Red D'Anjou proved unsuccessful. No treatments in seven trials over three years including several formulations of GA, BA + GA, or AVG were successful despite reports that similar programs are effective for European pear growers. This line of research does not offer sufficient promise to warrant further study at this point.

Horner rootstock evaluation has been divorced from this project and information on those studies may be found in Todd Einhorn's report.

FINAL PROJECT REPORT

Project Title: Evaluation of integrated fire blight control technologies

PI: Ken Johnson
Organization: Oregon State University
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City: Corvallis
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Cooperators:
Materials: Arysta Life Sciences, Syngenta

Other funding sources: None

Total Project Funding: $ 90,484

Budget History:

Item	2009	2010	2011
Salaries FRA 6mo	20,000	15,450	10,300
Benefits OPE 63%	12,600	9,734	6,489
Wages			
Benefits			
Equipment			
Supplies	4,000	3,800	2,111
Travel local	1,000	1,000	500
Plot fee	1,500	1,500	500
Miscellaneous			
Total	39,100	31,484*	19,900**

*Budget reduced from original proposal owing to shift of Obj. 4 to WTFRC Apple Crop Protection.

**Budget reduced from original proposal owing to near completion of objectives 1-3.

OBJECTIVES:

1. **Integrate a new material, Kasumin, for blossom blight control programs in conventional orchards.**

2. **Evaluate potential for the fire blight pathogen to become resistant to Kasumin.**

3. **Evaluate integrated biological/chemical control of fire blight with a spontaneous mutant of BlightBan C9-1 resistant to Kasumin.**

4. **Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets. (Apple crop protection funds)**

5. **Evaluate use of soil drenches of a systemic acquired resistance inducer as a fire blight management tool in diseased pear trees.**

SIGNIFICANT FINDINGS:

- The product Kasumin 2L (kasugamycin) provided outstanding control of fire blight of pear and apple; EPA registration is on track for 2012.

- Resistance management strategies for Kasumin -- i.e., mixtures with oxytetracycline and integration with biological control -- provided excellent fire blight control. These strategies should help to ensure longevity of the product.

- An effective non-antibiotic strategy for fire blight control of pear was developed. This strategy is being implemented for pears exported under the International Organic Program standard.

- Pot drenches and trunk paints of the SAR inducer, ASM (acibenzolar-*S* methyl), significantly slowed expansion of fire blight in inoculated shoots of potted 'Bosc' pear.

- In the field, ASM applied as a drench did not consistently slow the advance of running fire blight cankers, but an ASM paint used in combination with cutting of blight reduced the significantly the severity of 're-ignited' fire blight cankers.

RESULTS AND DISCUSSION

1) Integration of a new material, Kasumin, into blossom blight control programs for conventional orchards.

a) 2011 season. Treatments of Kasumin alone and in combination with other materials where tested in 2011, marking the fifth year of evaluation. Trees used in the 2011 study averaged 587 flower clusters per tree. Fire blight risk as determined by the COUGARBLIGHT model was low during the bloom period but disease intensity on inoculated trees was moderate with water treated trees averaging 78 blighted clusters per tree (14% of flower clusters) (Table 1). Each of the treatments significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree compared to the water-treated control. Kasumin 2L and 8L (kasugamycin), Firewall (streptomycin), and Fireline (oxytetracycline) provided excellent disease control. The integrated program of Bloomtime in early bloom followed by Fireline (200 ppm), or a mix of Kasumin (100 ppm) and Fireline (50 ppm) at full bloom also provided excellent control. The pathogen inoculum used in the study was 50% streptomycin-resistant and 50% streptomycin-sensitive *E. amylovora.* Firewall (streptomycin) suppressed disease by 85%, whereas Kasumin 2L provided 93% control.

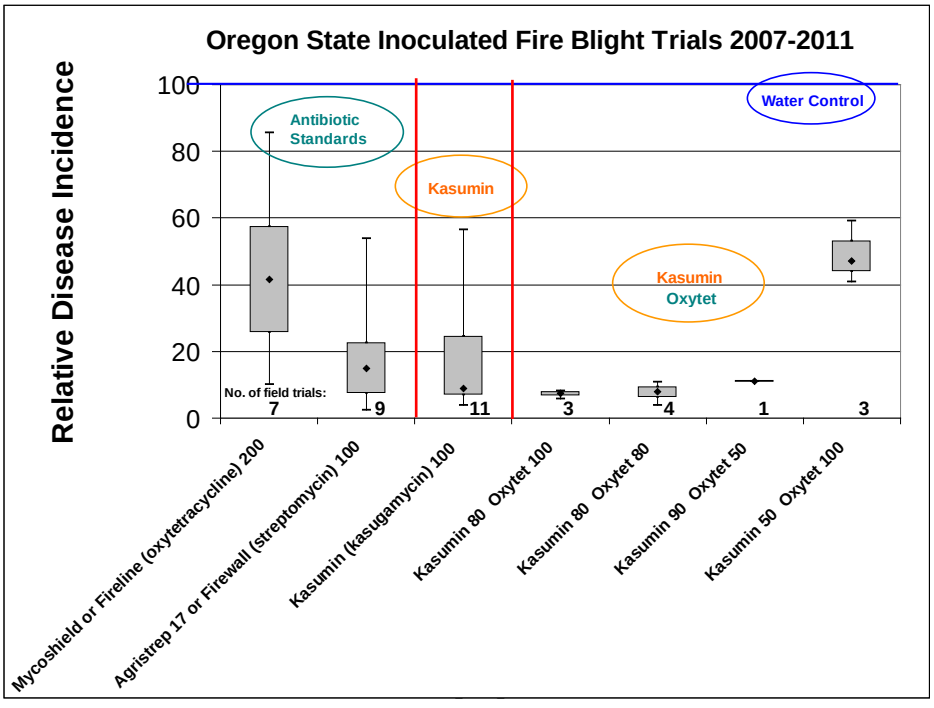
Table 1. Evaluation of Kasumin for suppression of fire blight of Gala apple, 2011

Treatment	Rate per 100 gallons water	Date treatment applied* 4 May 30% bloom	6 May 70% bloom	10 May Full bloom	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Water control	------	**X**[§]	**X**	**X**	**78**	**a**[#]	**14.1**	**a**[#]
Agri-mycin 100 ppm	8 oz.	---	---	X	13	b	2.1	b
Bloomtime then	5 oz.	X	X	---	10	bc	1.6	bc
Kasumin 2L 90 ppm	52 fl oz.	---	---	X				
plus Fireline 50 ppm	4 oz.	---	---	X				
Bloomtime then	5 oz.	X	X	---	8	bc	1.4	bc
Fireline 200 ppm	16 oz.	---	---	X				
Kasumin 8L 100 ppm	16 fl. oz.	---	---	X	8	bc	1.4	bc
Kasumin 2L 100 ppm	64 fl. oz.	---	---	X	5	c	1.0	c

* Trees inoculated on 9 May with 5 x 10^5 CFU/ml of a 50:50 mix of *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) and strain Ea153S (streptomycin-resistant). ** Transformed log (x + 1) prior to analysis of variance; non-transformed means are shown. *** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. [§] X indicates material was sprayed on that specific date; --- indicates material was not applied on that specific date. [#] Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at P = 0.05.

b) Summary Kasumin field trials from 2007 to 2011. Over the period, a total of nine orchard trials in pear and apple were conducted. Data were summarized in box and whiskers plots as 'Relative Disease Incidence', which for each trial is the number of fire strikes in the Kasumin (or comparative)

Fig. 1



treatment divided by the number of fire strikes in the water-treated control (expressed as a percentage) (Fig. 1). The median response for Kasumin was > 90% control, which was equivalent to streptomycin (targeted to streptomycin sensitive strains of *E. amylovora*) and better than the median 58% control obtained with oxytetracycline. [Note: trials in 2007, 2008, and 2009 had two treatments of each antibiotic, and had trials in 2010 and 2011 had one treatment.]

Mixtures of Kasumin and oxytetracycline were evaluated as a resistance management strategy. Use of effective chemicals in mixes has long been advocated as a means to delay the resistance development in plant pathogens, and in fact, the original formulation of streptomycin registered in the 1950s was amended with 10% oxytetracycline for this purpose (van der Zwet & Keil 1979). In the mid-1960s, the oxytetracycline formulated into this premix was dropped (because it didn't contribute control). Resistance in *E. amylovora* to streptomycin was first reported in California in the early 1970s, 13 years after first registration, but only a few years after removal of the oxytetracycline. In considering mixtures to evaluate in this study, we wanted effective amounts of each material with an eye toward the cost of the mixture to the grower and also toward previous observations that Kasumin applied multiple times at higher rates has been phytotoxic to pear (Adaskaveg et al., 2011). Thus, 80 ppm of both Kasumin and oxytetracycline was tested, and although many other mixtures could be evaluated, this particular mixture provided excellent fire blight control. Over the series of trials, however, phytotoxic effects of Kasumin were not observed in any trial at any of the tested rates.

In 2011, we observed that a 90:50 (ppm K:O) mixture also was effective when oversprayed in an integrated program with Bloomtime Biological (*P. agglomerans* E325) whereas in previous trials a 50:100 (ppm K:O) was not (Fig. 1). Although not observed in this research, work by others researchers (T. Smith, *unpublished*; Ngugi et al. 2011) has shown Kasumin is slightly less effective than streptomycin (against sensitive strains) when tested under extreme disease pressure (high inoculum). Moreover, as shown in Fig. 1, the effective mixtures of Kasumin and oxytetracycline showed less variability in control than Kasumin alone. Mixtures containing Kasumin at full label (100 ppm) with a partial rate of oxytetracycline (e.g., 50 ppm) could be advisable in high disease pressure situations as both a resistance management strategy and as a treatment to enhance control.

When registered, Kasumin will enhance and broaden the effective tool box for conventional fire blight management. Kasumin is not used in human medicine, and shows no cross resistance to streptomycin or oxytetracycline. Kasumin is likely not absorbed into pear or apple tissue as readily as streptomycin, which is considered locally systemic. Analogous to oxytetracycline, sprays of Kasumin should be timed at full to late bloom beginning when moderate (as opposed to high) levels of disease risk have been forecasted.

Objective 2) Evaluate potential for the fire blight pathogen to become resistant to Kasumin and 3) Evaluate integrated biological/chemical control of fire blight with a spontaneous mutant of BlightBan C9-1 resistant to Kasumin).

These objectives were addressed by a graduate student, Andrew Hubbard, M.S. His thesis is online at: http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/21845/completed.pdf?sequence=6**.**

By first treating trees with a biological agent (BlightBan C9-1 or Bloomtime Biological) followed by Kasumin, an 'integrated strategy' reduces the likelihood of selection for kasugamycin-resistance in the pathogen. The mechanisms that reduce selection pressure are suppressed pathogen populations via competition with the biological agent and limitation of Kasumin use (e.g., to one application as opposed to the two applications often typical in commercial production). Over the

2007 to 2011 period, the orchard trials showed that there was no statistical difference between integrated control with Kasumin compared to Kasumin alone (Table 1 (above) and Table 2 (below)).

Table 2. Integrated control with Kasumin and Kasumin-resistant BlightBan C9-1S

		Fire blight strikes per tree
2009 Bartlett Pear	Water	485 a
	P.v. C91S^{kr} then Kasumin	38 b
	P.v. C91S then Kasumin	42 b
	Kasumin twice	33 b
2009 Gala & Golden Delicious Apple	Water	132 a
	P.v. C91S^{kr} then Kasumin	18 b
	P.v. C91S then Kasumin	16 b
	Kasumin twice	8 b
2010 Gala Apple	Water	236 a
	P.v. C91S^{kr} then Kasumin	14 b
	P.v. C91S then Kasumin	16 b
	Kasumin once	20 b

Means within cultivar and year followed by same letter are not significantly different according to Fischer's protected least significance difference at *P* = 0.05. Experiments conducted in orchards located near Corvallis, Oregon. *Pantoea vagans* C9-1S^{Kr} is a kasugamycin-resistant selection of *Pantoea vagans* C9-1S (the organism in BlightBan C9-1 and related to *Pantoea agglomerans* strain 325 in Bloomtime Biological).

We also hypothesized that use of Kasumin could have a negative impact on populations of the biological agent on flowers and that this effect could be overcome by use of a kasugamycin-resistant strain of *Pantoea vagans* strain C9-1S, thereby improving the efficacy of the biological component of the integrated strategy. In the field, data collected on disease incidence and on population sizes of the biological agents on flowers failed to support this hypothesis. For example, in the 2009 'Bartlett' pear experiment, during full bloom, incidence of recovery for both the Kasumin-sensitive and -resistance strains of *P. agglomerans* (sensitive = strain C9-1S; resistant = strain C9-1^{Kr}) averaged > 75% of sampled flowers, and the population size of these antagonists on flowers from which it could be recovered ranged from 10^4 to 10^5 CFU per flower (Fig. 1). (Note: this is in contrast to pathogen populations, which are strongly suppressed by the Kasumi overspray [data not shown]). The results indicate that non-target effects of Kasumin on 'sensitive' *Pantoea agglomerans* are relatively small, and thus, use of Kasumin 2-3 days after a biological treatment would be expected to have minimal impacts on populations of bacterial biological control agents. In this regard, the effect of Kasumin on non-target bacteria on flowers was more like that observed with oxytetracycline than observed with streptomycin.

Fig. 2. **Strain C9-1S** **Strain C9-1^{Kr}**

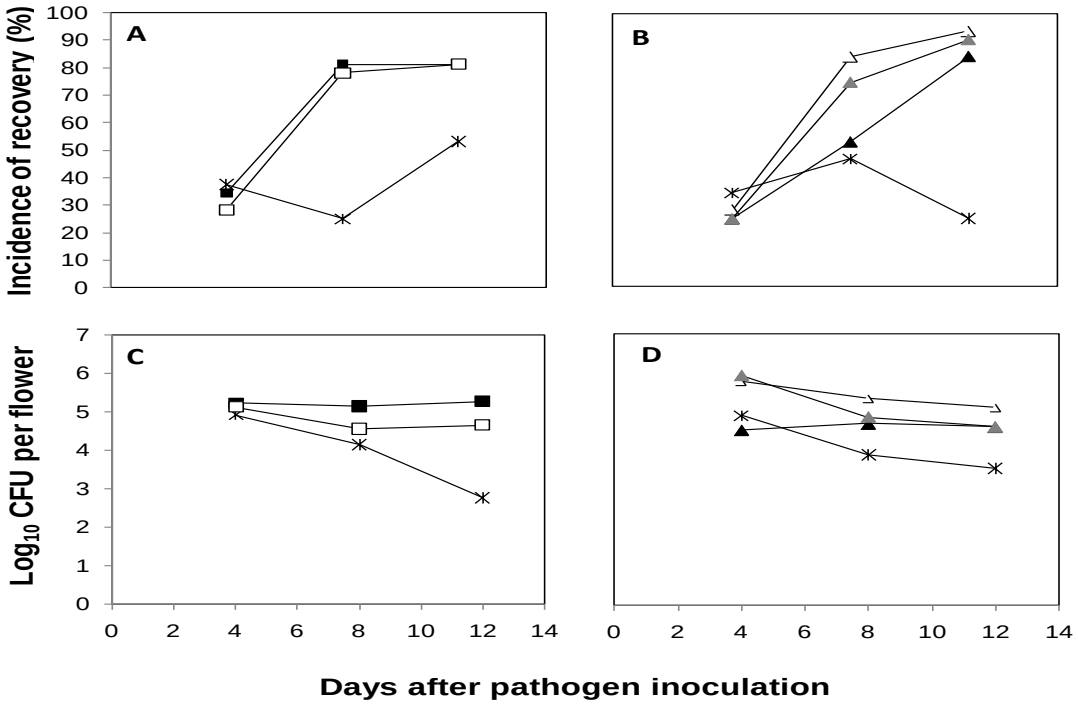


Fig. 2. Incidence of recovery (A, B) and population size (C,D) of *Pantoea vagans* strains C9-1S (A,C) and strain C9-1^{Kr} (B,D) on flowers of Bartlett pear treated with these bacterial antagonist and then oversprayed with antibiotics. The antagonist and antibiotic treatments were made in the context of integrated biological and chemical fire blight control and occurred in 2009 in an experimental orchard located near Corvallis, OR. Specific integrated antagonist strain and antibiotic treatments were: C9-1S with oxytetracycline (200 µg/ml, ■), C9-1S with Kasumin (100 µg/ml, □), C9-1^{Kr} with oxytetracycline (200 µg/ml ▲), C9-1^{Kr} with Kasumin 100 µg/ml (∆) and C9-1^{Kr} with oxytetracycline (80 µg/ml) and Kasumin (80 µg/ml, ▲). (∗) indicates each strain on water control.

In the years of this project, McGhee & Sundin (2011) characterized resistance in the fire blight pathogen to kasugamycin. Analogous to our results with biocontrol agent *Pantoea vagans*, selection of kasugamycin-resistant strains of the fire blight pathogen with the ability to grow at the maximum label rate of Kasumin (100 ppm) was observed to be a two-step mutation process (i.e., the initial selectable mutation (frequency 10^{-9} to 10^{-10} CFU) to resistant occurs at a sub-label rate (~50 ppm), then a second mutation is required for *E. amylovora* (or *P. vagans*) to achieve the ability to grow at 100 ppm (maximum label rate). In contrast, a spontaneous mutation in these bacteria to resistant to the maximum label rate of streptomycin is a one step process, and a spontaneous mutation to resistant to oxytetracycline has not been characterized (even from a laboratory selection process). Thus, because of the 2-step process, the risk of selecting resistance in *E. amylovora* to Kasumin is intermediate to the other registered antibiotics or fire blight control. Also analogous to our results, McGhee & Sundin (2011) found that kasugamycin had only minor effects on the non-target bacterial flora of apple flowers.

4) Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets.

Effect of frequency of treatment for integrated non-antibiotic control. In orchard trials, for both pear and apple, increasing the frequency of treatment of biological products improved fire blight control. For example, a stigma-colonizing *Pantoea agglomerans* product (Bloomtime Biological or BlightBan C9-1) applied at 30 and 70% bloom followed by two applications of the fermentation product of *Bacillus subtilis* QST 713 (Serenade Max) at full bloom and petal fall reduced the incidence of blighted flower clusters by an average of 71% (four orchard trials) compared to 47% when each component of this product combination was used only once.

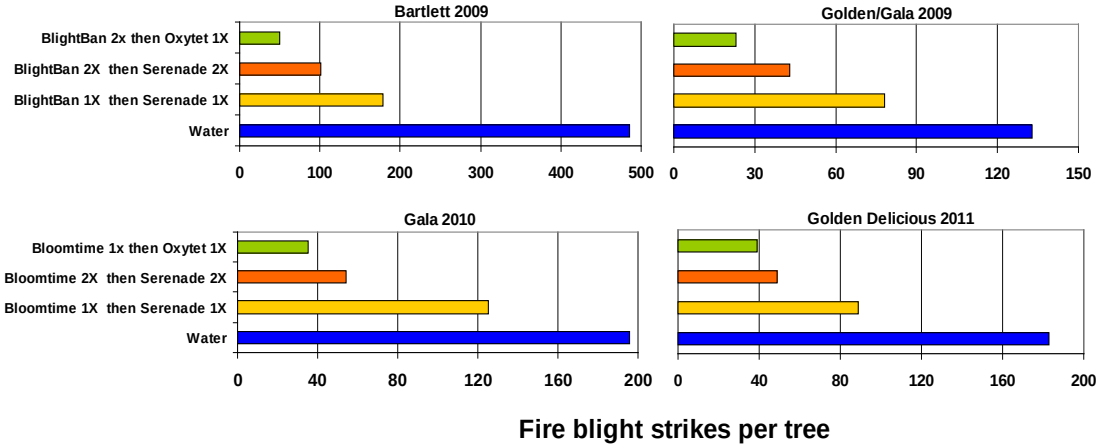


Fig. 3. Incidence of fire blight on pear and apple flower clusters as affected by integrated biological and antibiotic treatments in orchard trials conducted near Corvallis, Oregon from 2009 to 2011.

The integrated approach first demonstrated by Stockwell et al. (2008) and Lindow et al. (1996) utilized a gram negative bacterial antagonist (e.g. *P. agglomerans* and/or *Pseudomonas fluorescens*) to suppress the pre-requisite epiphytic phase of *E. amylovora* on floral stigmas followed by an oxytetracycline treatment later in bloom to prevent infection in the floral cup (nectary). The non-antibiotic program we modeled on this strategy involved substitution of the biological product, Serenade Max, for oxytetracycline. In this role, Serenade Max proved to be inhibitory to the fire blight pathogen, but obtaining levels of fire blight control closer to that achieved with oxytetracycline after a bacterial antagonist required doubling the frequency of application of the biological products (Fig. 2). This result indicates that non-antibiotic programs for fire blight will likely be more expensive than programs utilizing antibiotics because satisfactory disease control will require more treatments in the orchard. Spraying more often also means that orchardists will need to be more preventative in their approach to fire blight control (i.e., sprays required every few days) as opposed to reactive, where a single antibiotic spray could be applied based on an imminent infection event forecasted by a disease warning model (CougarBlight).

Obj. 5) Evaluate use of soil drenches of systemic acquired resistance inducers as a fire blight management tool in diseased pear trees.

a) Greenhouse studies on SAR induction. Greenhouse experiments were conducted in both 2009 and 2011 with Bosc pear. The experimental design was to inoculate the pathogen into the growing tip of the terminal shoot and then measure fire blight canker expansion through the summer. SAR treatments (drench. sprays, and paints of acibenzolar-*S* methyl (ASM)) were applied at inoculation, 1 month prior to inoculation, or one month after inoculation.

Drenches of ASM slowed expansion of running fire blight cankers in potted Bosc pear (Fig. 4 A, B). In non-treated trees, the canker expanded form the shoot tip to an average of halfway down the main trunk. In contrast, with the exception of the 1 mo delayed drench treatment in 2011, cankers on ASM-drenched trees expanded only a short distance into the woody trunk tissue; these trees remained alive and continued to produce new shoot growth. *Sprays* of ASM provided inconsistent responses with the treatments timed 'at inoculation' and 'delayed to one month after inoculation' providing a significant slowing of canker expansion in 2009, but with none of the spray treatments providing a significant reduction in 2011 (Fig. 4 C, D). *Paint* treatments of ASM (in 2011 only) slowed expansion of fire blight regardless of time of treatment; although between September 14 and October 20, running cankers on ASM painted trees tended to catch up with canker expansion on the untreated trees (Fig. 4 E).

2009 2011

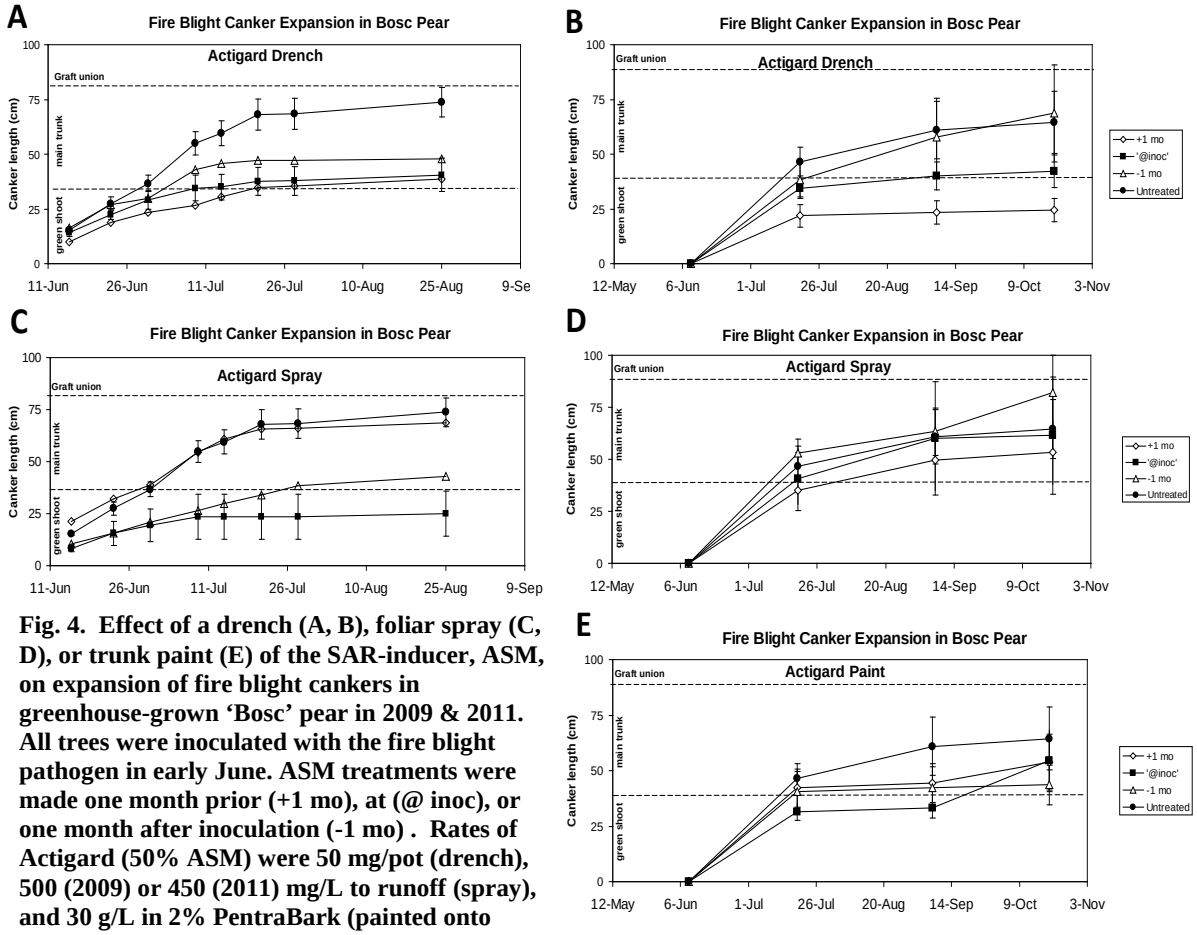


Fig. 4. Effect of a drench (A, B), foliar spray (C, D), or trunk paint (E) of the SAR-inducer, ASM, on expansion of fire blight cankers in greenhouse-grown 'Bosc' pear in 2009 & 2011. All trees were inoculated with the fire blight pathogen in early June. ASM treatments were made one month prior (+1 mo), at (@ inoc), or one month after inoculation (-1 mo) . Rates of Actigard (50% ASM) were 50 mg/pot (drench), 500 (2009) or 450 (2011) mg/L to runoff (spray), and 30 g/L in 2% PentraBark (painted onto woody trunk tissue). Each point is the mean of 6 trees except the 'non-treated' points, which represent 10 trees. Lines drawn through each point are +/- one standard error of the mean.

b) Field studies on SAR induction in pear. Similar to the greenhouse experiments**,** the general approach was to inoculate pear trees with the fire blight pathogen (10^9 colony forming units per ml); inoculum was either placed onto flowers (2011 experiments) or onto cut terminal ends of growing shoots (2010). After running cankers were established in the trees, experiments with SAR-

inducing treatments (drenches, sprays, paints, combinations, untreated control) were arranged randomly onto the diseased trees. Two types of experiments were performed: a) analogous to the greenhouse, measure the effect of ASM on expansion of running cankers, and b) measuring the severity of re-ignited cankers after removal (pruning) of disease symptoms. The pruning cuts to remove blight were made as *intentional short cuts* to ensure a high probability of canker re-ignition.

i. 2010 season: running cankers on 2-yr-old Bosc pear. Trees were inoculated on 9 June and ASM treatments were applied 23 June. A drench treatment combined with a foliar spray of ASM significantly slowed expansion of fire blight cankers. The final sizes of cankers in the drench/spray treatment were 33% smaller than in the untreated control (Table 3). Cankers on all trees stopped expanding in mid/late-July, which coincided with slowing of new shoot growth.

Table 3. Effect of ASM on length of fire blight cankers on 2-yr-old Bosc pear near Corvallis, OR in 2010.

Method of ASM application	Canker length (cm)									Reps
	Longest			Next longest			Total canker			
Drench	22.4	±	3.5[#]	7.3	±	1.3	27.9	±	4.1	28
Paint	23.0	±	3.3	7.4	±	0.6	29.7	±	3.6	28
Spray + drench	**13.6***	**±**	**3.2**	**5.7**	**±**	**0.9**	**19.2***	**±**	**4.2**	**14**
Spray + paint	19.4	±	4.3	10.2	±	2.4	27.5	±	5.7	14
Untreated	22.6	±	5.1	8.2	±	2.7	28.4	±	6.1	14

[#] Standard error of the mean

* Significantly different ($P < 0.05$) from the untreated control as determined by t-test.

ii. 2011 season: Running cankers on 11-yr-old Bartlett pear. Trees were inoculated on 21 April. ASM treatments were applied on 2 June when canker length was 3- 8"; ASM treatments were repeated on 10 June. Diseased branches were 'harvested' on 17 August; data were recorded as strikes per tree and weight of diseased branches removed by pruning. ASM treatments applied to trees with running cankers did not result in a significant reduction of disease severity (Fig. 5).

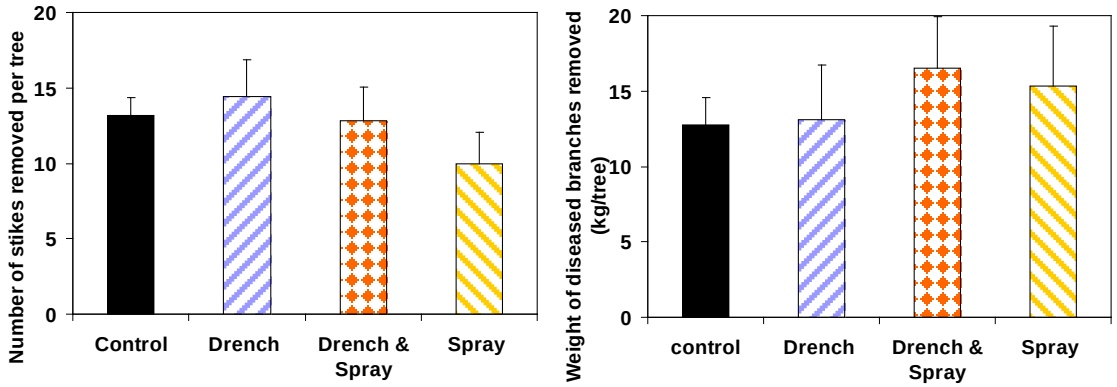


Fig. 5. Effect of drenches and sprays of the SAR-inducer, ASM, on severity of running fire blight cankers in 11-yr-old 'Bartlett' pear. Trees were inoculated with the fire blight pathogen in late April. Canker length was 3-8" on 2 June when they were treated with ASM in a drench (2 g Actigard in one liter poured into shallow collar dug around base of each tree) or sprayed (0.3 g Actigard per L sprayed to runoff (~3 L/tree)). Actigard treatments were repeated 10 June. Each bar is the mean of 5 trees arranged in a randomized complete block design.

iii. 2011 season: Pruned cankers on 11-yr-old Bartlett pear. This experiment was done in the same orchard block described under 'ii' above with a similar date of inoculation (4/22) and dates

Actigard spray treatments (6/2 & 6/10). Treatments were 'Cut only' and 'Spray, cut and paint'. Cuts made 6 and 14 June and 8 July at 5 cm (2") below canker margin – ALL CUTS WERE INTENTIONAL SHORT CUTS to ensure a high probability that cankers would re-ignite. Immediately after cutting, the ASM paint was applied to 25-30 cm (10-12") of symptomless branch below the cut. Compared to cut only, the severity of the re-ignited fire blight cankers was significantly reduced by the spray and paint combination of ASM treatments.

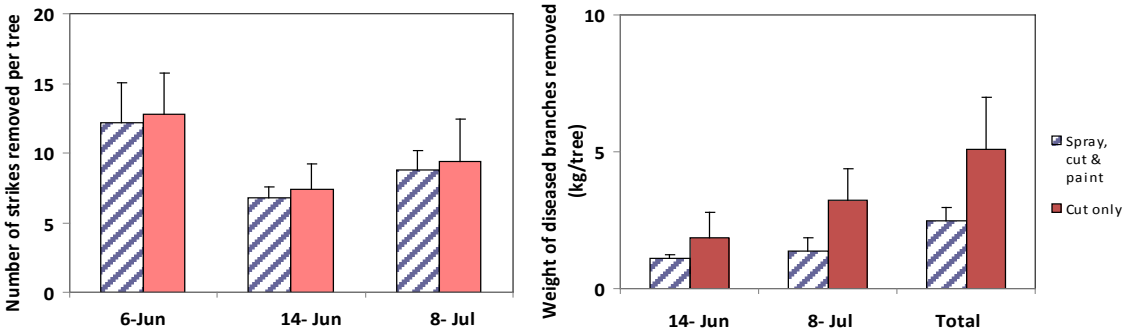


Fig. 4. Effect of branch paints and oversprays of the SAR-inducer, ASM, on re-ignition of fire blight cankers in 'Bartlett' pear. Trees were inoculated with the fire blight pathogen in late April. Canker length was 3-8" on 2 June when they were sprayed with ASM (0.3 g Actigard per L sprayed to runoff (~3 L/tree)); the spray was repeated 10 June. Fire blight cankers were cut 5 cm (2") below canker margin (intentional short cuts) on 6 and 14 June and 8 July. On 6 and 14 June, on sprayed trees, 25-30 cm of symptomless branch below each cut was painted with Actigard (30g/L) in 2% Pentrabark. Each bar is the mean of 5 trees.

iv. 2011 season: Pruned cankers on 2-yr-old Bosc pear. This experiment was planned to be a repeat of the 2010 Bosc pear experiment (described under 'i' above) but after initiating the cutting treatments in the Bartlett pear experiment (described under 'iii'), we decided to cut the fire blight out of the trees and then measure the severity of the re-ignited fire blight cankers. Flowers on trees were inoculated on 1 May. ASM treatments were applied on once June 6, and cankers were cut once on June 8 (an average of 20-25 cm (8-10") below margin of canker. Disease was allowed to re-ignite and severity of disease was assessed on 10 October as '% of the tree dead'.

After cutting once, severe running cankers re-ignited in most trees. The trees that received the ASM paint and the ASM spray/drench combination, however, showed reduced severity compared to the non-treated control, the drench and spray only treated trees.

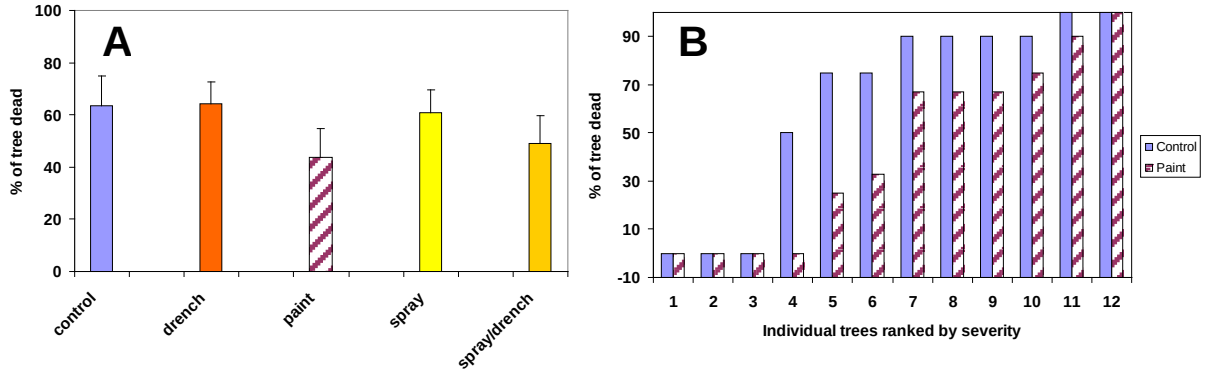


Fig. 4. Effect of a drench, spray, or paint of the SAR-inducer, ASM, on re-ignition of fire blight cankers in 'Bosc' pear. Trees were inoculated with the fire blight pathogen on 1 May. ASM treatments were applied on 6 June. ASM was applied as a drench (1 g Actigard in 500 ml poured into shallow collar dug around base of each tree), spray (0.45 g Actigard per L to runoff), or trunk paint (Actigard 30g/L in 2% Pentrabark). Cankers from the May inoculation were removed on 8 June; average canker length was 55 cm (22") at the time of removal. The percent of tree dead after canker re-ignition was assessed on October 8. A) Each bar is the mean and standard error of 12 trees; B) ranked comparison of the disease severity of individual ASM-painted trees to individual trees in the untreated control.

Discussion of SAR. Like apple, fire blight susceptible pear cultivars can respond to treatments of the SAR inducer, acibenzolar-*S* methyl (ASM), resulting in slowed canker expansion in diseased trees. The effect of ASM on suppression of fire blight was most dramatic when drenches were applied to potted greenhouse-grown trees. In contrast, drenches of ASM did not show a strong effect when applied in the orchard. Perhaps the confined root system in the pot allows for a more efficient uptake of ASM compared to drenches applied to field-grown trees, where the material was placed only at the base of the tree.

In the future, we intend to focus on branch and trunk paints in tree rescue-type treatments because this method of application provided the best responses in the field environment. Moreover, with greenhouse-grown apple (see 2012 apple crop protection report), trunk paints of ASM showed levels of PR-gene induction that were on par with the levels of induction achieved by pot drench. The measurement of PR-gene induction provides a marker on whether or not a SAR inducer is providing consistent induction of host defense genes. In contrast to pot drenches and trunk paints, foliar sprays showed a consistently low level of PR-gene induction.

For the body of data collected from pear and apple (see 2012 apple crop protection report), ASM treatments applied by paint and spray were most suppressive when the pathogen was present but the amount of active disease in the tree was small. For example, in the greenhouse, paint or spray treatments made at the time of inoculation (pathogen present, small amount of disease) were generally more effective that treatments made one month prior (no pathogen) or one month after inoculation (increased amount of disease). In the field, an ASM paint applied to symptomless branch below a cut canker provided a stronger response than trunk paints applied to trees where cankers were left to run.

For the reasons given above, ASM could prove practical as aid to cutting blight in pear, either reducing severity of re-ignited cankers (as demonstrated) or perhaps reducing the incidence of re-ignition. In this research, our rate of canker re-ignition was high because of intentional short cutting, which we deemed necessary to obtain consistent, measurable responses in a small plot experiment. A large commercial block with fire blight could provide a better test of the effect of ASM on canker re-ignition as the trees would be pruned properly and the larger scale of a commercial block would increase the number of cuts that receive the ASM treatment.

Literature cited

Adaskaveg, J. E., Förster, H., and M. L. Wade. 2011. Effectiveness of kasugamycin against *Erwinia amylovora* and its potential use for management of fire blight of pear. Plant Dis. 95:448-454.

Ngugi, H. K., Lehman, B. L., and Madden, L. V. 2011. Multiple treatment meta-analysis of products evaluated for control of fire blight in the eastern United States. Phytopathology 101:512-522.

Lindow, S. E., McGourty, and Elkins, R. 1996. Interactions of antibiotics with *Pseudomonas fluorescens* strain A506 in the control of fire blight and frost injury to pear. Phytopathology 86:841-848.

McGhee, G. C., and Sundin, G. W. 2011. Evaluation of kasugamycin for fire blight management, effect on nontarget bacteria, and assessment of kasugamycin resistance potential in *Erwinia amylovora*. Phytopathology 101:192-204.

Stockwell, V.O. Temple, T.N., Johnson, K.B., and Loper, J.E. 2008. Integrated control of fire blight with antagonists and oxytetracycline. Acta Hortic. 793:383-390.

van der Zwet, T., and Keil, H. L. 1979. Fire Blight: A Bacterial Disease of Rosaceous Plants. Agricultural Handbook Number 510. USDA Science and Education Administration, Washington, D.C.

EXECUTIVE SUMMARY

Project Title: Evaluation of Integrated Fire Blight Control Technologies

Investigator: Ken Johnson, Oregon State University

SIGNIFICANT FINDINGS:
Kasumin:

- The product Kasumin 2L (kasugamycin) provided outstanding control of fire blight of pear and apple; EPA registration is on track for 2012.

- Resistance management strategies for Kasumin -- i.e., mixtures with oxytetracycline and integration with biological control -- provided excellent fire blight control. These strategies should help to ensure longevity of the product.

Industry implications: When registered, Kasumin will enhance control and broaden the effective tool box for protection of pear flowers from fire blight in conventionally managed orchards. Kasumin is more effective than oxytetracycline, and we expect it to have a positive impact on fire blight management, particularly in high disease risk situations. The risk of resistance developing in the fire blight pathogen to Kasumin is intermediate to streptomycin (higher) and oxytetracycline (lower).

Organic fire blight control:

- An effective non-antibiotic strategy for fire blight control of pear was developed. This strategy is being implemented for pears exported under the International Organic Program standard.

Industry implications: The information we have been generating has been immediately implemented by growers in the International Organic Program (IOP). Furthermore, the issue of non-antibiotic control of fire blight has increased in importance because the USDA National Organic Program (NOP) has set a 2014 phase out (sunset) on use of streptomycin and oxytetracycline under the NOP standard.

Systemic acquired resistance:

- Pot drenches and trunk paints of the SAR inducer, ASM (acibenzolar-*S* methyl), significantly slowed expansion of fire blight in inoculated shoots of potted 'Bosc' pear.

- In the field, ASM applied as a drench has not consistently slowed the advance of running fire blight cankers, but an ASM paint used in combination with cutting of blight reduced the significantly the severity of 're-ignited' fire blight cankers.

Industry implications: Even with excellent products for prevention of fire blight, the disease still occurs and its clean-up can be difficult, especially in young orchards. Systemic acquired resistance (SAR) is an induced defense response in a tree, which when induced in pear and apple has the potential to slow/stop fire blight progression. Commercial products that induce SAR in pear have potential to be used as aids in cutting of fire blight to prevent re-ignition of advancing cankers and to enhance protective sprays when mixed with antibiotics.

FINAL PROJECT REPORT
WTFRC Project Number: PR-10-100

Project Title: Development of field applications for a pear psylla sex attractant

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Cooperators: Jocelyn Millar, University of California, Riverside

Total Project Request: **Year 1:** $20,000 **Year 2:** $20,000

Other funding sources

Agency Name: Binational Agricultural Research and Development (BARD)
Amount awarded: $280,000 (Oct 2011-Sept 2014); $88,000 for the Horton lab.

Total Project Funding: $40,000

Organization Name: USDA-ARS **Contract Administrator:** James Harris
Telephone: 509-454-6560 **Email address:** james.harris2@ars.usda.gov

Item	2010	2011
Salaries	$15,500	$15,500
Benefits	$ 4,500	$ 4,500
Wages		
Benefits		
Equipment		
Supplies		
Travel		
Miscellaneous		
Total	$20,000	$20,000

OBJECTIVES

Our objectives were to:

1. **Winterform:** Conduct simultaneous field and laboratory assays with 13-methylheptacosane to assess whether attractiveness of the chemical to male psylla changes seasonally.
2. **Winterform:** Conduct field assays to optimize the use of 13-methylheptacosane as a male psylla attractant (dose response, trap design, release rate).
3. **Summerform:** Conduct laboratory assays to determine response of male summerform psylla to 13-methylheptacosane and to blends of this compound with 2-methylheptacosane and 3-methylheptacosane.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- Proposal to BARD for the "Optimization and field-testing of synthetic sex attractants for two psyllid pests of pears"; $280,000 for 3 years was awarded.

Objective 1:

- Determined that there is a seasonality in attraction of winterform males to 13-methylheptacosane (13-MeC27) both in the field and in laboratory bioassays which seems to coincide with females reaching reproductive maturity and being mated in the field.
- A seasonality in attraction of winterform males to live females was also observed in the laboratory and was delayed in comparison to male attraction to 13-MeC27 in the laboratory.

Objective 2:

- Traps baited with 100 ug and 1000 ug of 13-MeC27 consistently caught more males than the 10 ug, 1 ug or 0.1 ug doses or the control for all 3 dates (two conducted in the winter and one in late summer), although this trend was not statistically significant, probably due to the low densities of psylla present at the time and/or location.
- The clear screen trap caught significantly more winterform males and females than the clear panel, the yellowish mesh, or the commercial delta trap. However, the number of psylla caught was again low.
- The release rate in the laboratory was not conducted because Christelle Guédot was laid off for the second half of 2011.

Objective 3:

- Identified 3 chemicals that were predominant in summerform female extract compared to male extract (13-MeC27; 2-methylheptacosane, 2-MeC27; and 3-methylheptacosane, 3-MeC27).
- Demonstrated attraction of males but not females to 13-MeC27 and to a blend of 13-MeC27+2-MeC27+3-MeC27.
- Demonstrated that summerform males are as attracted to 13-MeC27 as to blend of chemicals, and that 13-MeC27 and the blend are as attractive to males as an extract of females.
- Demonstrated in the field that summerform males are attracted to traps baited with 13-MeC27 and to traps baited with the blend of chemicals compared to control traps.
- Demonstrated in the field that summerform females are not attracted by either 13-MeC27 or the blend of chemicals in either laboratory or field assays.

METHODS

Seasonality of attractiveness of 13-methylheptacosane. 13-MeC27 was loaded into gray rubber septa and pinned to the center of sticky traps composed of sections of nylon mesh and covered with tanglefoot. Simultaneously, psylla were collected from the orchard and immediately assayed in the olfactometer. We assessed male response to live females vs. a blank (2011 only) and to 13-MeC27 vs. a blank (2010 and 2011). The seasonality of attractiveness was assessed from end of January to early April 2010 and 2011.

Optimization of 13-methylheptacosane. We field tested different doses (0.1 to 1000ug) of 13-MeC27 on sticky traps (**Figure 1 C)** to assess the most efficient dose for optimum male capture. We also tested different trap designs (**Figure 1 (A)** clear panel, **(B)** clear screen, **(C)** yellowish mesh trap, and **(D)** commercial delta trap) with 13-MeC27 as the attractant, to determine the most efficient trap for male capture.

Summerform response to sex attractants. Chemical analyses of whole-body washes were conducted with a GC-MS to confirm the identity and quantify the chemicals predominant in female washes. Compounds of interest were tested in the olfactometer to assess male and female response to these chemicals. Because 13-MeC27 was already shown to be a sex pheromone attractant for winterform males and because it is also the compound most abundant in females compared to males in the summerform, we tested psylla response to 13-MeC27 alone and in combination with the other 2 compounds identified. We tested the effect of combining all 3 compounds in a blend to assess whether the addition of the other 2 compounds would enhance male response to the 13-MeC27. Assays were conducted in the laboratory with a Y-tube olfactometer and in the field using sticky traps.

RESULTS AND DISCUSSION

Seasonality of attractiveness of 13-methylheptacosane. Attraction by winterform males to 13-MeC27 occurred from early to late February in 2010 and was consistent between laboratory (**Figure 2**) and field (**Figure 3**) assays. Females were not attracted to 13-MeC27 baited traps in the field (not shown). Beginning in March, males were no longer attracted to 13-MeC27 in laboratory or field assays (**Figure 2 and 3**), coinciding with females reaching reproductive maturity in the field (**Figure 4** dashed line) and being mated in the field (**Figure 4**). Furthermore, males assayed to live females in olfactometer tests on March 2nd, 2010 were not attracted to females when paired with a blank. In 2011, attraction by winterform males to synthetic 13-MeC27 in the laboratory occurred from mid-February to late March (**Figure 5**). Attraction by males to live females in the laboratory occurred from late February to late March (**Figure 6**). The delay in the onset of male attraction to live females compared to the onset of male attraction to 13-MeC27 suggests that males might become responsive before females become attractive. At the end of March, male were no longer attracted to 13-MeC27 and to live females in the laboratory, coinciding with males no longer being attracted to 13-MeC27 in the field (**Figure 7)**. Females were not attracted to 13-MeC27 baited traps in the field (not shown). Females reached reproductive maturity around mid-March with most females being mated in the field (**Figure 8**). In conclusion, the same trends were observed between 2010 and 2011 with a >2-week delay in 2011 probably due, at least in part, to the lower temperatures experienced in the winter of 2011 compared to 2010. This series of experiments will be conducted again in 2012 to confirm the trends.

Optimization of 13-methylheptacosane. The dose response experiment was conducted 3 times: in February, March and September 2011. However, due to low densities (< 2 males/trap) in February and September, only the data obtained in March 2011 is presented here (**Figure 9**). The traps baited with `100 ug and 1000 ug of 13-MeC27 consistently caught more males than the 10 ug, 1 ug, 0.1 ug

or the control for all 3 dates, although this trend was not statistically significant, probably due to the low densities of psylla present at the time of year and/or location (**Figure 9**). This experiment will be conducted again in March 2012 to confirm this trend. Regarding the trap design experiment, the clear screen trap (**Figure 1B**) caught significantly more winterform males and females than the clear panel, the yellowish mesh, or the commercial delta trap (**Figure 1C, D, and E** respectively**)**. However, the number of psylla caught was again low (**Figure 10**) and we plan on running this experiment again in March 2012. The release rate of 13-MeC27 from gray rubber septa over time in the laboratory was not conducted because Christelle Guédot was laid off for the second half of 2011. We intend to run this experiment in 2012.

Summerform response to sex attractants. Chemical analyses of whole-body washes of summerform psylla revealed that 13-MeC27, 2-MeC27, and 3-MeC27 were found to be considerably more abundant in females than males. Females did not respond to either 13-MeC27 or to the blend of chemicals, i.e. 13-MeC27+2-MeC27+3-MeC27 (**Figure 11**). On the other hand, males were attracted to both 13-MeC27 and to the blend, with no statistical difference between 13-MeC27 and the blend when presented in pair (**Figure 12: upper panel A**; filled bars and asterisks indicate significant preference). We then compared male attraction to 13-MeC27 and the blend vs. an extract of females. Males did not show a preference for 13-MeC27 when paired with the extract of females. Similarly, males did not show a preference for the blend when paired with the extract of females (**Figure 12: middle panel B)**. We also assessed the effect of chirality of 13-MeC27, i.e. (*R*)-13-MeC27 and (*S*)-13-MeC27 enantiomers, on male attraction. More males were attracted to the racemic blend containing both enantiomers than to the (*R*)-13-MeC27 or the (*S*)-13-MeC27 enantiomers (**Figure 12: lower panel C)**. Males did not show a preference for either enantiomer when presented in pair. Finally, in field assays, more males were caught on traps baited with 13-MeC27 alone and on traps baited with the blend than on unbaited traps, with no significant difference in trap catches between 13-MeC27- and blend-baited traps (**Figure 13**). Females were not attracted to 13-MeC27- or blend-baited traps compared to the control traps (**Figure 13**). These results suggest that 13-MeC27 is also a sex attractant pheromone for pear psylla males of the summerform.

PUBLICATIONS

Horton D.R., Guédot C., and P.J. Landolt. 2007. Diapause status of females affects attraction of male pear psylla, Cacopsylla pyricola, to volatiles from female-infested pear shoots. Entomologia Experimentalis et applicata 123: 185-192

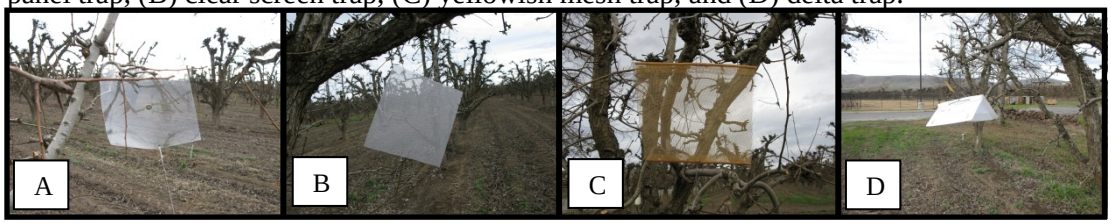
Horton, D.R, C. Guédot, and P.J. Landolt. 2008. Attraction of male summerform pear psylla to volatiles from female pear psylla: effects of female age, mating status, and presence of host plant. *Canadian Entomologist* 140: 184-191.

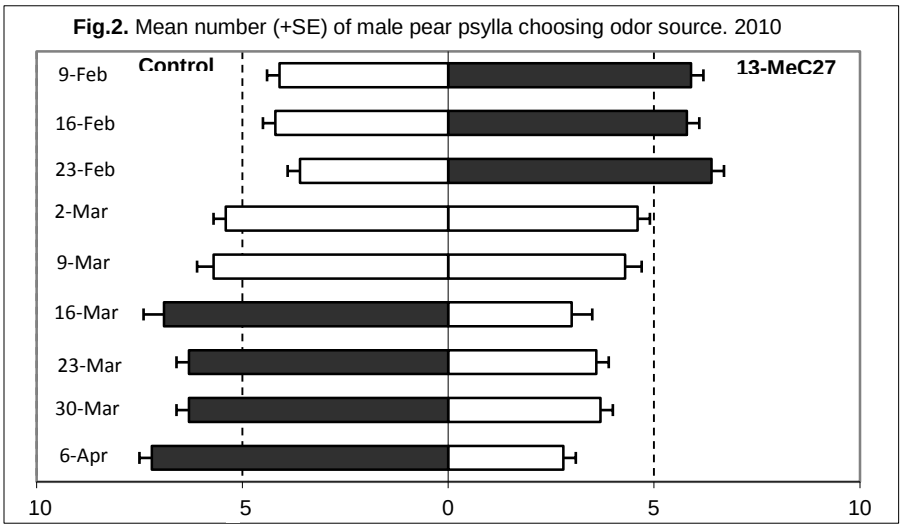
Guédot C., Horton D.R., and P.J. Landolt. 2009. Attraction of male winterform pear psylla to female-produced volatiles and to female cuticular extracts with evidence of male-male repellency. *Entomologia Experimentalis et applicata* 130: 191-197

Guédot C., Millar J.G., Horton D.R., and P.J. Landolt. 2009. Identification of a sex attractant pheromone for male winterform pear psylla, *Cacopsylla pyricola*. *The Journal of Chemical Ecology* 35: 1437-1447

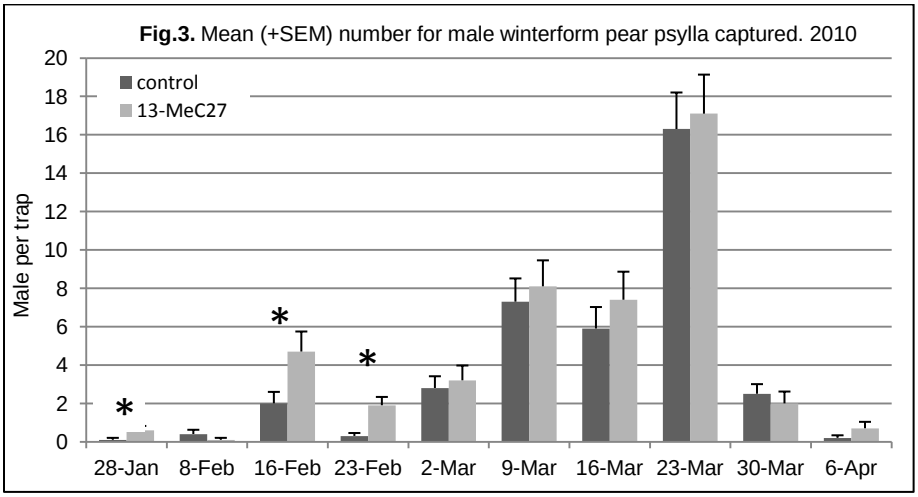
Guédot C., Horton D.R., and Landolt P.J. 2011. Response of summerform pear psylla (Hemiptera: Psyllidae) to male- and female-produced odors. *Canadian Entomologist* (In press)

Fig.1. Traps used with gray rubber septa loaded with 100 ug of 13-methylheptacosane. (A) clear panel trap, (B) clear screen trap, (C) yellowish mesh trap, and (D) delta trap.

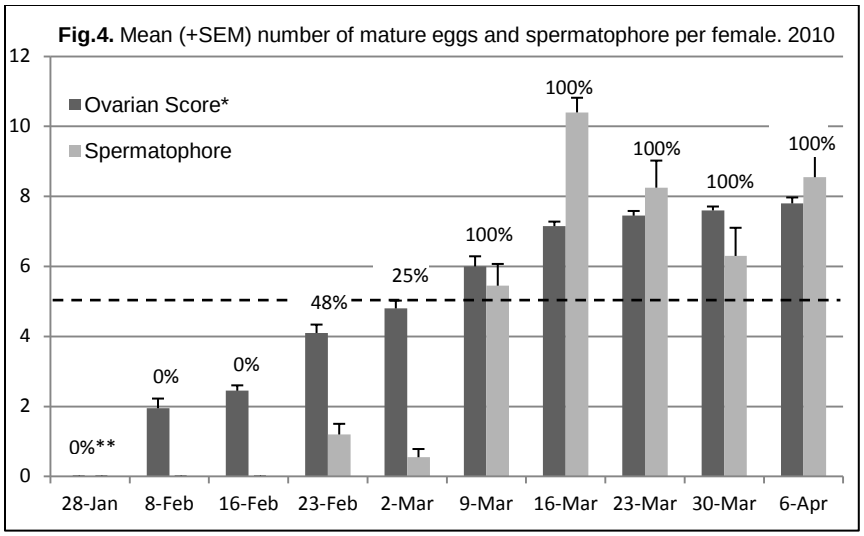




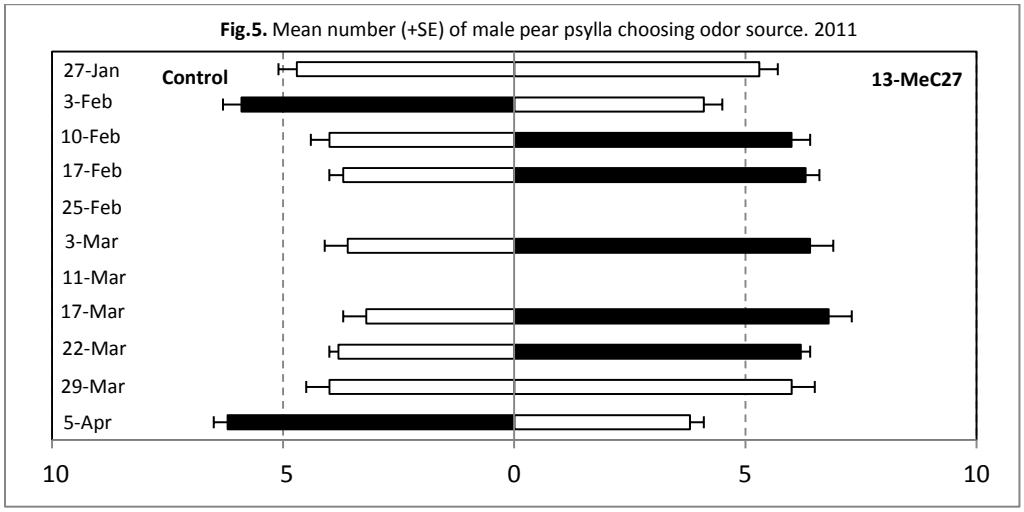
Black shading indicates significant preference for the odor source



* indicates significant preference

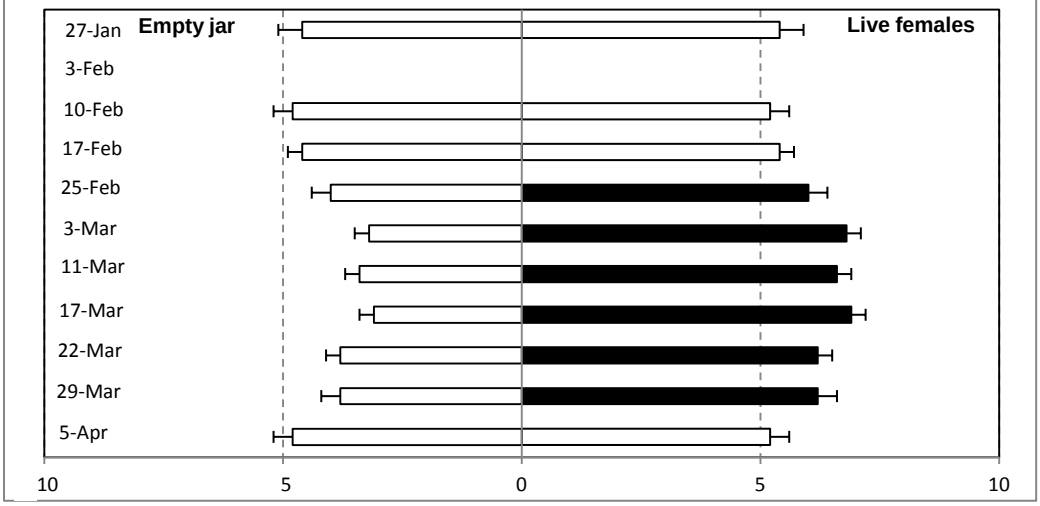


*Dashed line indicates the ovarian score (5) at which females have mature eggs (Krysan and Higbee 1990).
**Percent mated females



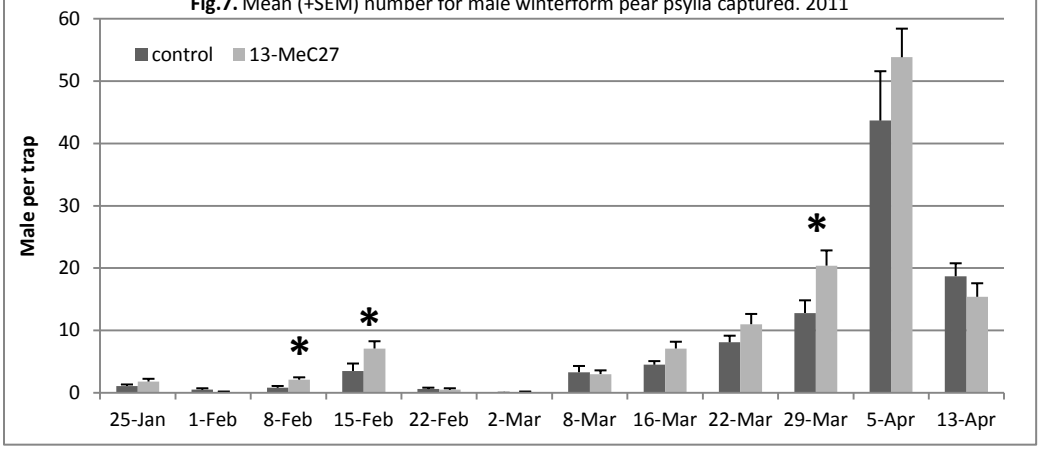
Black shading indicates significant preference for the odor source

Fig.6. Mean number (+SE) of male pear psylla choosing odor source. 2011



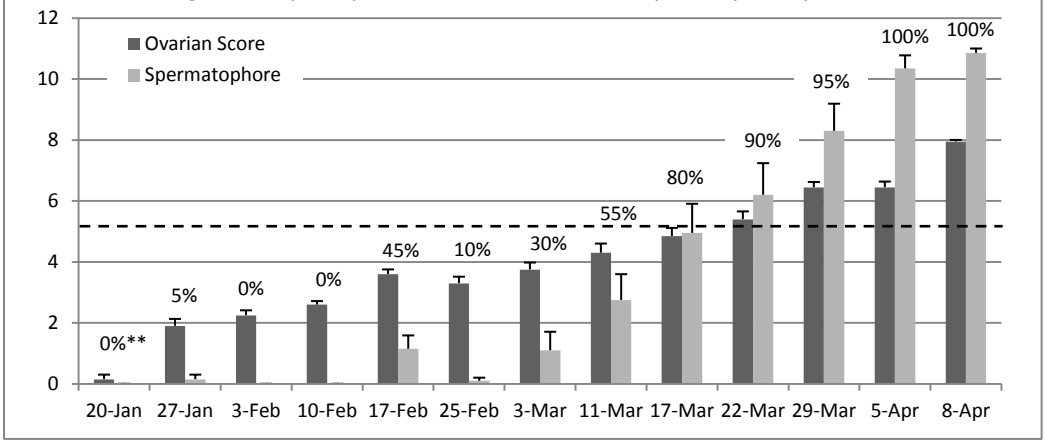
Black shading indicates significant preference for the odor source

Fig.7. Mean (+SEM) number for male winterform pear psylla captured. 2011

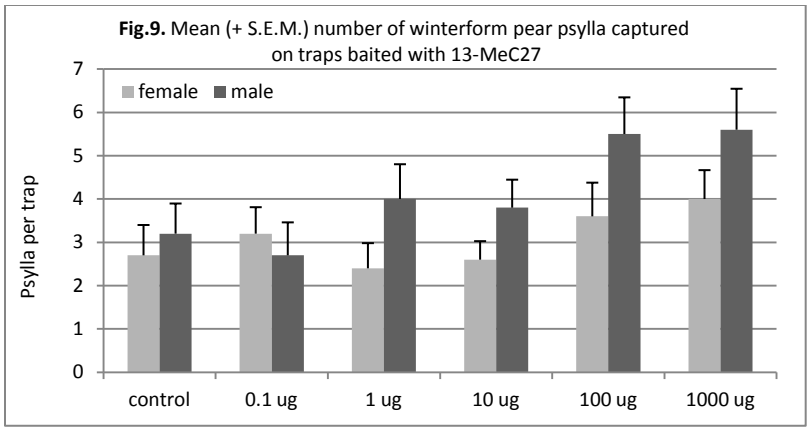


* indicates significant preference

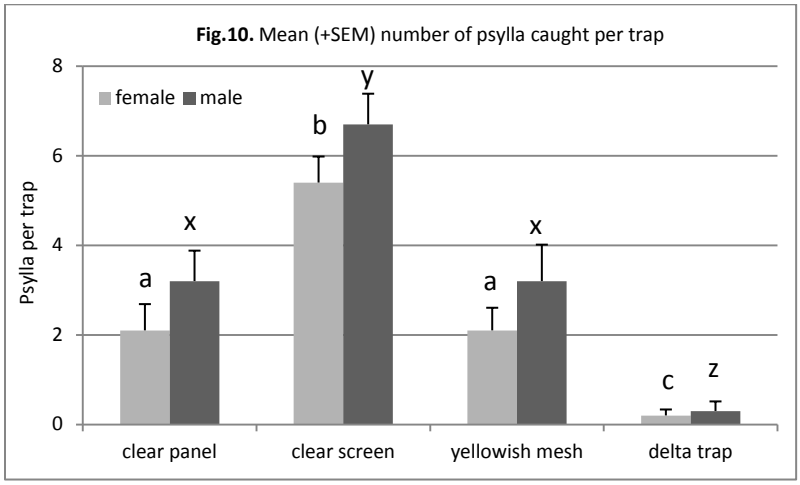
Fig.8. Mean (+SEM) ovarian score and number of spermatophores per female. 2011



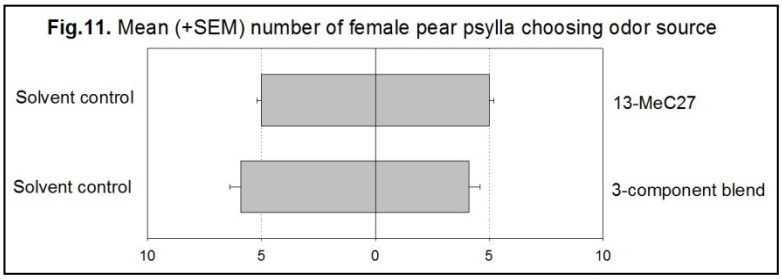
*Dashed line indicates the ovarian score (5) at which females have mature eggs (Krysan and Higbee 1990).
**Percent mated females.

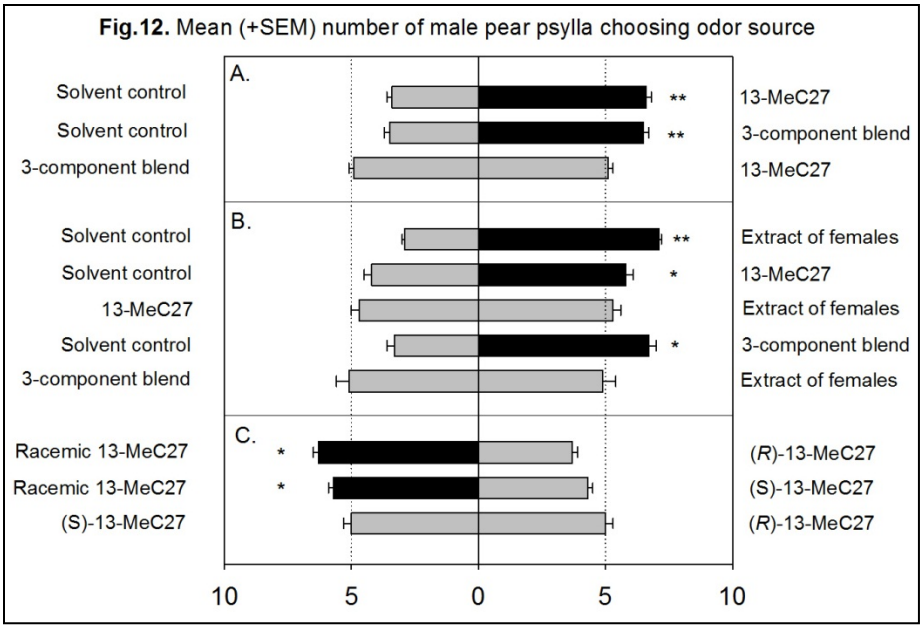


Traps baited with different amounts of 13-MeC27 dispensed from gray rubber septa (*n* = 10 traps per treatment).

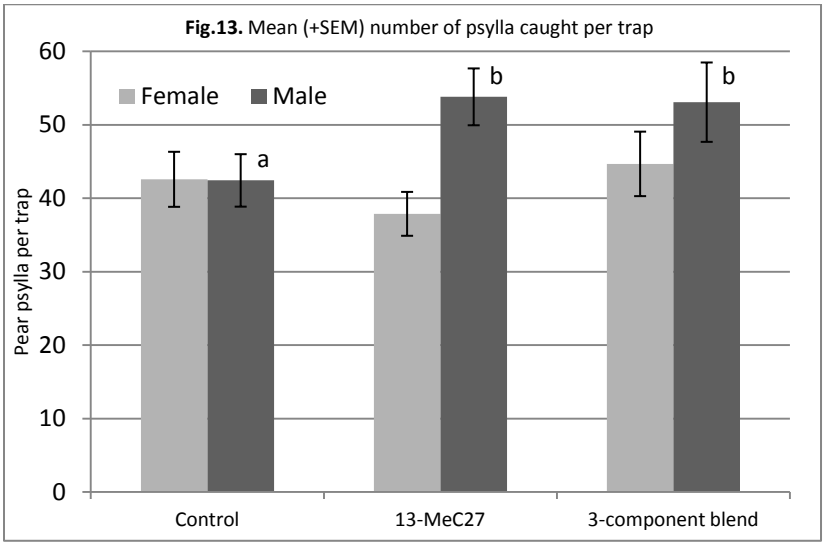


Different types of traps baited with 13-MeC27 (100 ug) dispensed from gray rubber septa (*n* = 10 traps per treatment). For male trap catches and female trap catches, treatments with different letters above them are significantly different (Tukey test, adjusted $P \leq 0.05$).





Black shading indicates significant preference for the odor source



Traps baited with 13-MeC27 (100 ug), or 3-component blend made of 13- MeC27 (100ug), 2- MeC27 (100ug), and 3-MeC27 (30ug), dispensed from gray rubber septa (n = 10 traps per treatment). For male trap catches, treatments with different letters above them are significantly different (Tukey test, adjusted $P \leq 0.05$).

EXECUTIVE SUMMARY
WTFRC Project Number: PR-10-100

Project Title: Development of field applications for a pear psylla sex attractant
PIs: Christelle Guédot, David Horton, and Peter Landolt
Organization: USDA-ARS
Email/Telephone: David.Horton@ars.usda.gov (509) 454-5639
Address: 5230 Konnowac Pass Road, Wapato, WA 98951

Outside Funding: $280,000 (BARD)

Total Project Funding: $40,000
Budget History:

Item	Year 1: 2010	Year2: 2011
Salaries	$15,500	$15,500
Benefits	$ 4,500	$ 4,500
Total	$20,000	$20,000

SUMMARY

- There is a seasonality in attraction of winterform males to the sex attractant pheromone 13-MeC27, both in the field and in laboratory bioassays which seems to coincide with females reaching reproductive maturity and being mated in the field.
- Seasonality in attraction of winterform males to live females was also observed in the laboratory and was delayed in comparison to male attraction to 13-MeC27 in the laboratory.
- Traps baited with 100 ug and 1000 ug of 13-MeC27 consistently caught more males than the 10 ug, 1 ug or 0.1 ug doses or the control.
- The clear screen trap caught significantly more winterform males and females than the clear panel, the yellowish mesh, or the commercial delta trap.
- Identified 3 chemicals that were predominant in summerform female extract compared to male extract (13-MeC27; 2-methylheptacosane, 2-MeC27; and 3-methylheptacosane, 3-MeC27).
- Summerform males are as attracted to 13-MeC27 as to blend of chemicals, both in the laboratory and in the field.
- 13-MeC27is a female-produced sex attractant pheromone that is attractive to winterform and summerform pear psylla males.

Plans for 2012

- Confirm the trend observed with the seasonality in male attraction to live females and to 13-MeC27 in the laboratory and the field
- Confirm the trend observed with the dose response experiment and the trap design experiment
- Determine the release rate of 13-MeC27 from gray rubber septa in the laboratory

FINAL PROJECT REPORT

Project Title: Ripening capacity and decay control in Winter Pears

PI: David Sugar
Organization: Oregon State University
Telephone: 541-772-5165 x 222
Email: david.sugar@oregonstate.edu

Cooperators: E.J. Mitcham, A. Dhingra

Other funding sources: None

Total Project Funding:

Item	2010	2011
Salaries	19,500	19,500
Benefits	12,090	12,090
Wages		
Benefits		
Equipment		
Supplies	2,000	2,000
Travel		
Miscellaneous		
Total	33,590	33,590

OBJECTIVES

This project had two overall objectives:

1. Characterize appropriate conditioning regimes for ‘Anjou’ and ‘Comice’ pears based on fruit maturity at harvest, ethylene conditioning, and intermediate temperature conditioning. Appropriate conditioning regimes will result in fruit with (1) early capacity to ripen to good quality, (2) adequate shipping firmness, and (3) a useful post-conditioning storage life before shipping.

2. Advance the development of orchard-based programs for postharvest decay control, integrating new materials, timings, and modes of application with effective techniques identified previously.

SIGNIFICANT FINDINGS

Objective 1 (Ripening Capacity):

1. The most efficient temperature for inducing ripening capacity in ‘Anjou’ and ‘Comice’ pears was 50 °F.
2. The duration of temperature conditioning needed by ‘Anjou’ and ‘Comice’ pears to develop ripening capacity, at all temperatures tested, decreased linearly with advancing harvest maturity. Conditioning time can be calculated based on the harvest date relative to the orchard block reaching the top of the firmness range for maturity.
3. ‘Anjou’ pears did not have the capacity to ripen after 24 or 48 hours in ethylene, unless given further temperature conditioning. Temperature conditioning after ethylene exposure can be completed faster at 50 °F than at 31 °F. Little or no further conditioning was needed after 72 hours in ethylene.
4. Identifying useful ethylene-temperature combinations to induce ripening capacity involves balancing eating quality (increases with longer conditioning) and shipping firmness (decreases with longer conditioning).
5. The storage potential at 31 °F of ‘Anjou’ and ‘Comice’ pears after conditioning decreases with increasing time in ethylene, warmer post-ethylene conditioning temperatures, and later harvest.
6. Smaller pears softened faster in response to ethylene treatment than did larger pears, but this effect was most pronounced with extreme size differences and marginal ethylene exposure.

Objective 2 (Postharvest Decay):

1. Decay control efficacy was compromised when application of Bio-Save 10 as a postharvest line-spray was delayed until 3 or more weeks after harvest, and of Scholar fungicide when delayed until 6 weeks or more after harvest.
2. Calcium chloride summer sprays resulted in strong reduction in decays caused by *Cladosporium* and *Alternaria* fungi, but not in decay caused by *Botrytis* (gray mold).
3. Pristine fungicide applied one week pre-harvest reduced all types of natural infection in these experiments, while Luna Sensation was effective in reducing *Botrytis* infection but not *Cladosporium* / *Alternaria* infections.
4. Potential organic decay control programs with yeast orchard sprays followed by a Bio-Save 10 line spray was did not provide significant decay reduction.
5. A single-bin drench with Scholar applied in the orchard reduced decay at a level similar to applying Scholar as a packinghouse line-spray between 3 and 6 weeks after harvest.

METHODS

This project uses the series of research-size CA-style rooms at the Southern Oregon Research and Extension Center for controlled temperature and ethylene treatments. All experiments are replicated four times, with replication based in the orchard; that is, replicate lots of fruit will come from distinct areas in the orchard to account for variability among orchard locations. Fruit firmness for maturity, shipping firmness, and storage quality measurements are determined using a Fruit Texture Analyzer. Ethylene is introduced from a compressed ethylene cylinder and concentrations verified using a gas chromatograph.

Studies of the interaction of fruit maturity, ethylene exposure, and temperature conditioning, including follow-up factors of shipping firmness and storage life require detailed scheduling of the movement of fruit and the measuring of firmness and evaluation of quality. A technician supported by this project has daily responsibilities for fruit tracking and firmness measurements. The Principal Investigator is responsible for application of ethylene treatments, temperature management, weekend fruit movement and measurements, and quality evaluations.

RESULTS & DISCUSSION

Objective 1 (Ripening Capacity):

1. Surprisingly, the most efficient temperature for inducing ripening capacity ("satisfying the chill requirement") among the temperatures studied was 50 °F. A range of potential conditioning temperatures for 'Anjou' pear were studied in 2009 and 2010; combined results shown in Fig. 1.

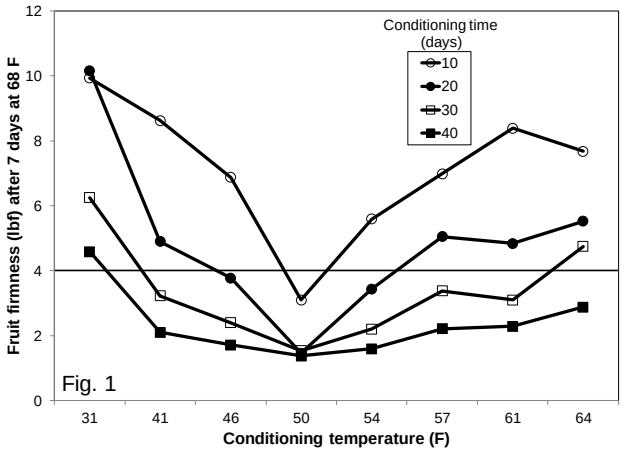
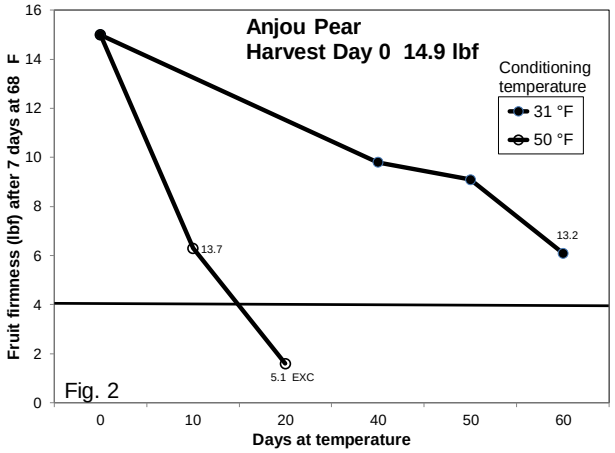


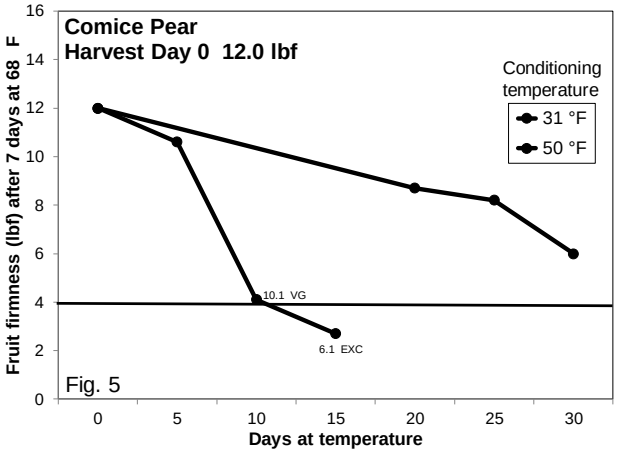
Fig. 1

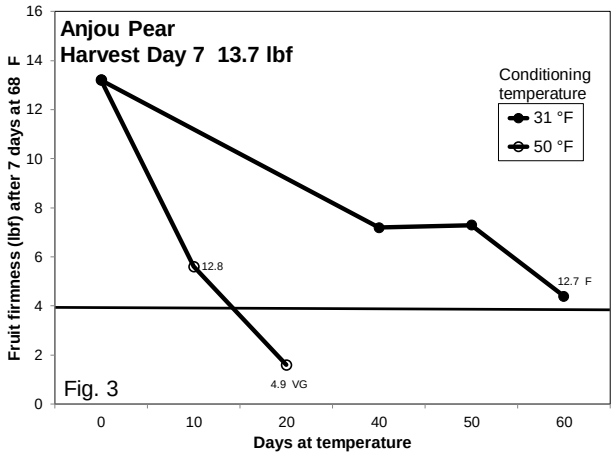
Similar results were found for 'Comice' pear. This confirms the potential of using exposure to 50 °F as a tool for conditioning winter pears much faster than at 31 °F. Preliminary results from colleagues at UC Davis show that ripened 'Comice' pears that had been conditioned at 50 °F had more intense sweet pear aroma than those that had been conditioned at 31 °F or in ethylene for 72 hours. Because peak conditioning efficiency occurs at 50 °F, detailed work on integrating harvest maturity, temperature conditioning, and ethylene conditioning in 2011 focused on 50 °F.

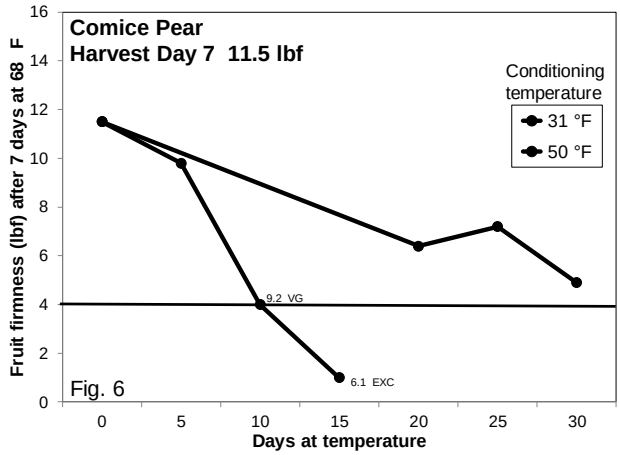
2. Experiments concluded in 2010 found a linear decrease in conditioning time with advancing harvest maturity, regardless of conditioning temperature. The conditioning time at any temperature can be calculated from the equation for the line describing the relationship. Experiments in 2011 for 'Anjou' and 'Comice' included three harvest dates: 0, 7, and 14 days after the average fruit firmness in the orchard reached the top of the maturity range. The efficiency of both temperature and ethylene conditioning will always reflect interaction with the fruit harvest maturity. The duration of conditioning at 31 °F and 50 °F from the three harvest dates is shown in Figs. 2-4 for 'Anjou' and Figs. 5-7 for 'Comice'. In these and other charts in this report, the data points reflect fruit firmness after 7 days of ripening time at 68 °F. Values falling below the horizontal line at 4 lbf are considered "ripe" in being at the onset of a buttery-juicy texture. Numbers next to data points indicate the fruit firmness at the end of conditioning, before ripening. Letters next to data points below 4 lbf indicate overall eating quality of the ripe fruit.

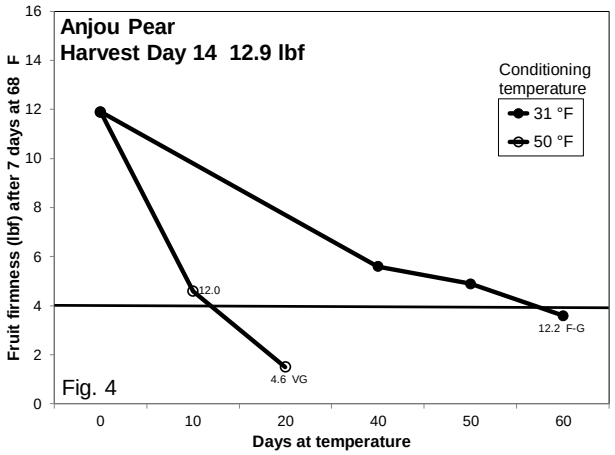
3. ‘Anjou’ pears did not have the capacity to ripen after 24 or 48 hours in ethylene, unless given

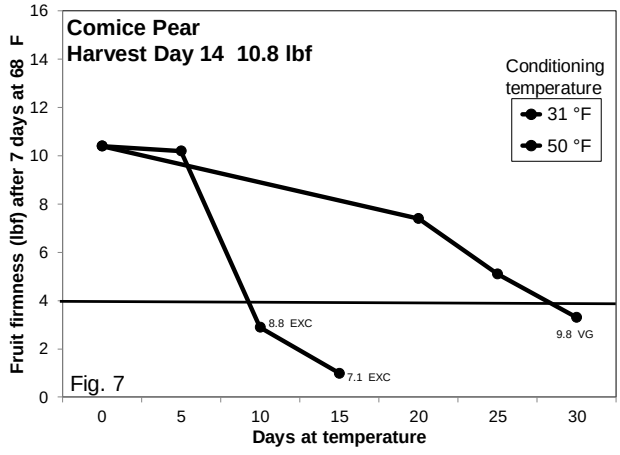


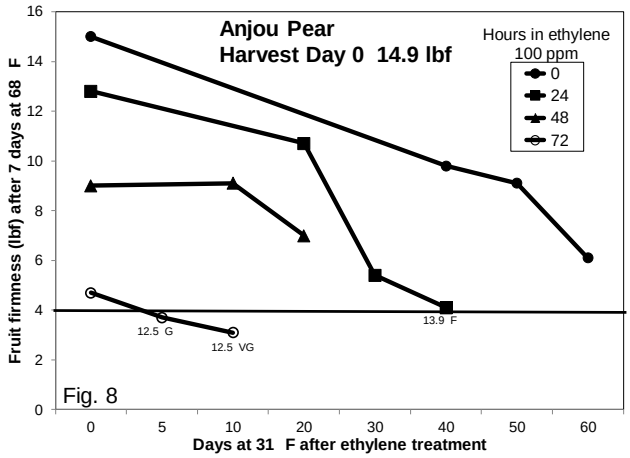


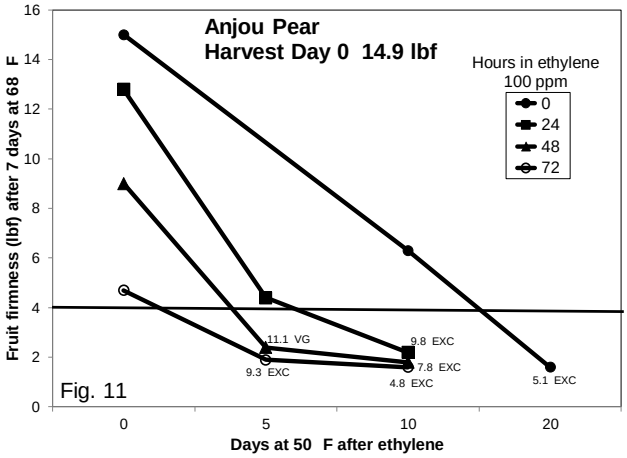


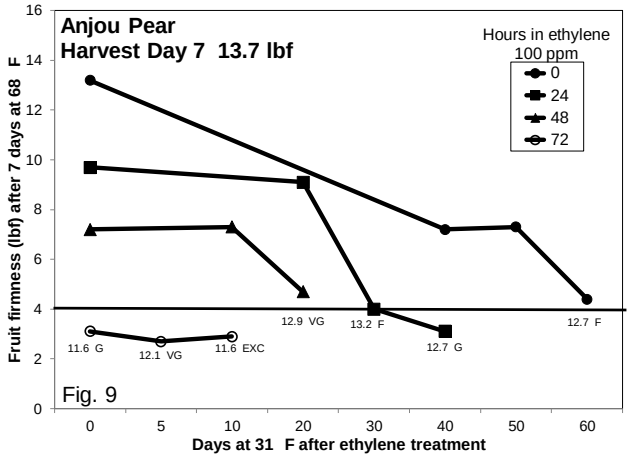


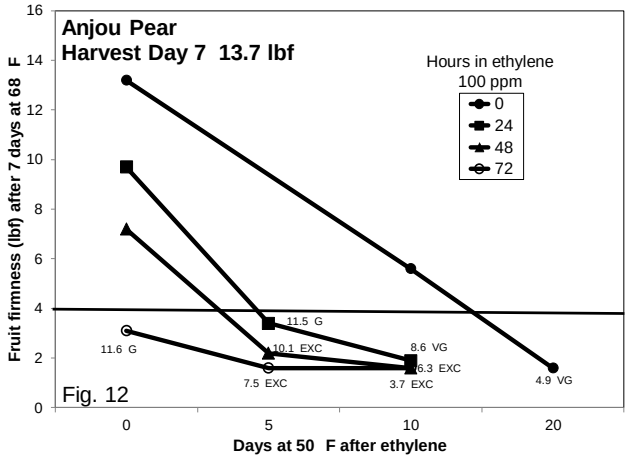


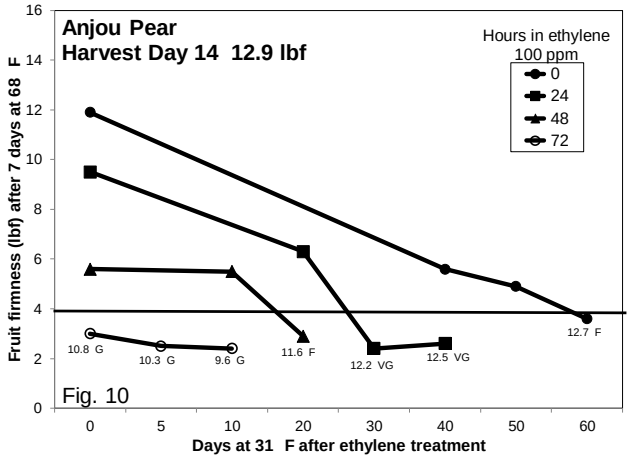


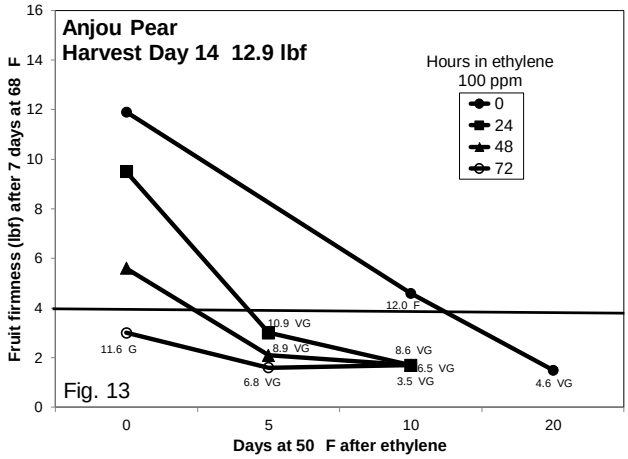






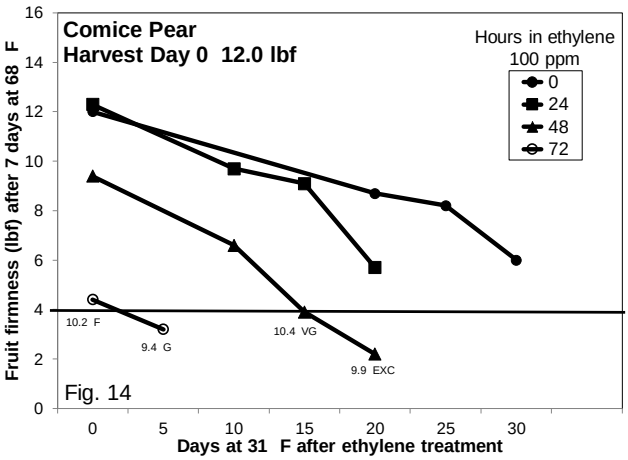


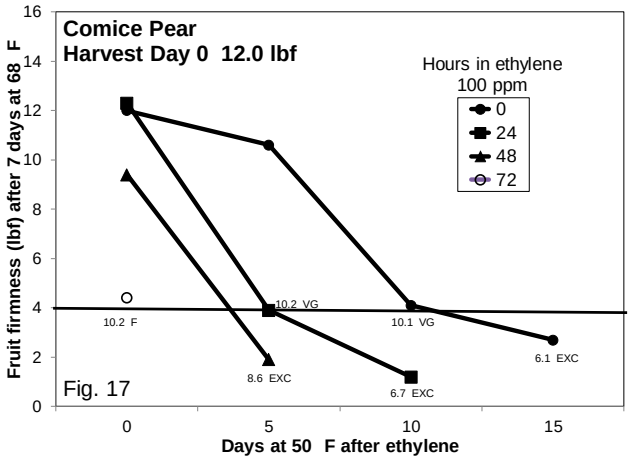


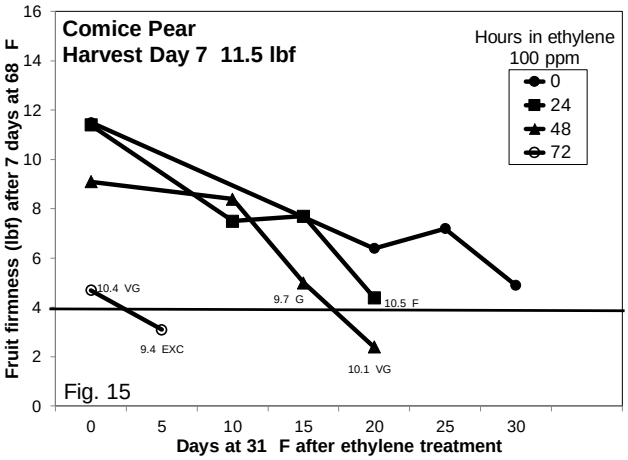


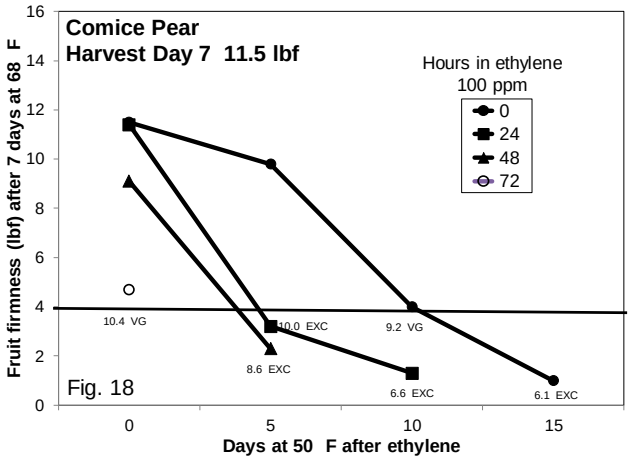
further temperature conditioning. After 24 hours in ethylene, 'Anjou' pears needed an additional 25-40 days at 31 °F to develop ripening capacity, depending on maturity at harvest (Figs. 8-10). After 48 hours in ethylene, 'Anjou' needed 15-30 days at 31 °F (Figs. 8-10). After 72 hours in ethylene, 'Anjou' pears softened to nearly 4 lbf without further temperature conditioning (Figs. 8-10). When the post-ethylene conditioning temperature was 50 °F, induction of ripening capacity proceeded significantly faster. Typically, 5 days at 50 °F following 24 or 48 hours in ethylene was sufficient to complete induction of ripening capacity (Figs. 11-13). For all three harvest dates of 'Anjou', the fruit firmness at the end of 10 days conditioning at 50 °F was equivalent to the fruit firmness at the end of 60 days conditioning at 31 °F. Similar response patterns, although on a shorter time scale, were found

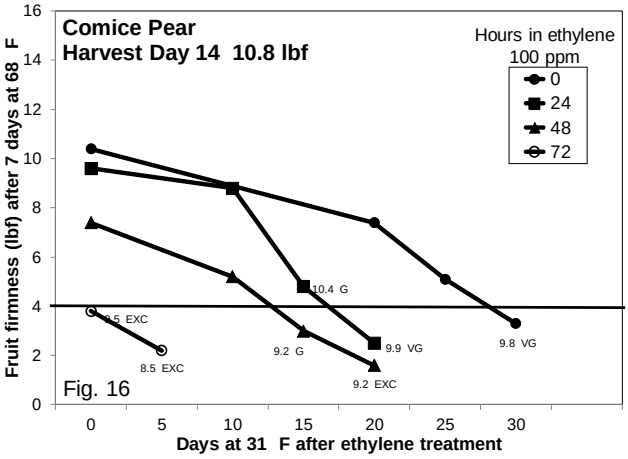
for ‘Comice’ pears when ethylene conditioning was followed by temperature conditioning at 31 °F (Figs. 14-16) or at 50 °F (Figs. 17-19). An element of this project that was lacking was to re-cool the fruit after conditioning and before ripening, which would have better simulated industry practices. Thus treatments which came close to softening to 4 lbf firmness within 7 days at 68°F might have actually done so if given further conditioning time through the re-cooling and shipping process.

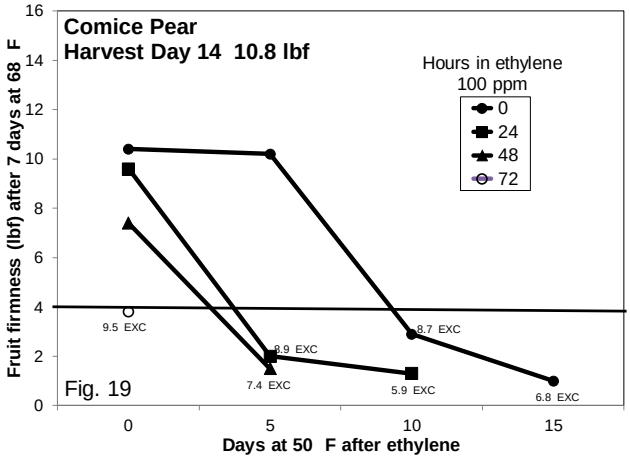








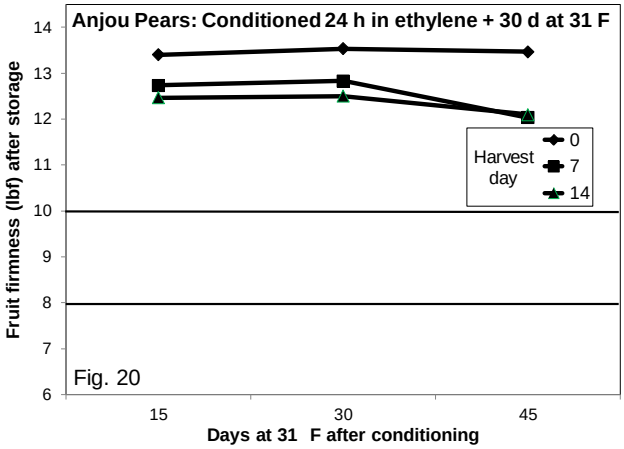


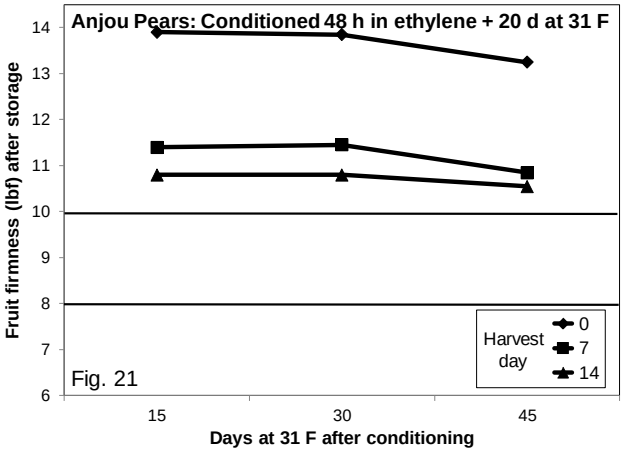


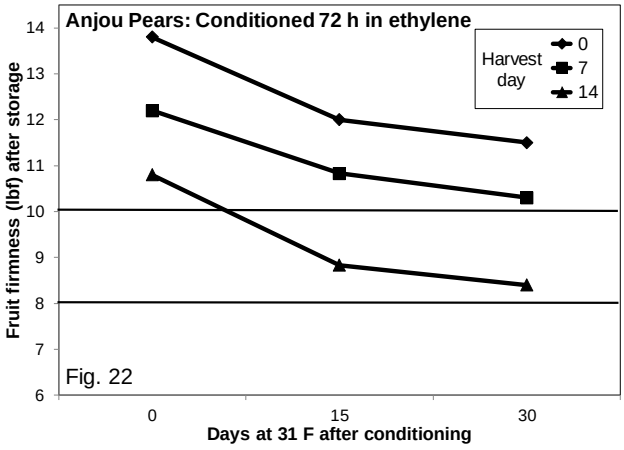
4. With 5 days at 50 °F following 24 or 48 hours in ethylene, fruit firmness at the end of conditioning (shipping firmness) varied from around 9 to 11.5 lbf, depending on harvest maturity and length of

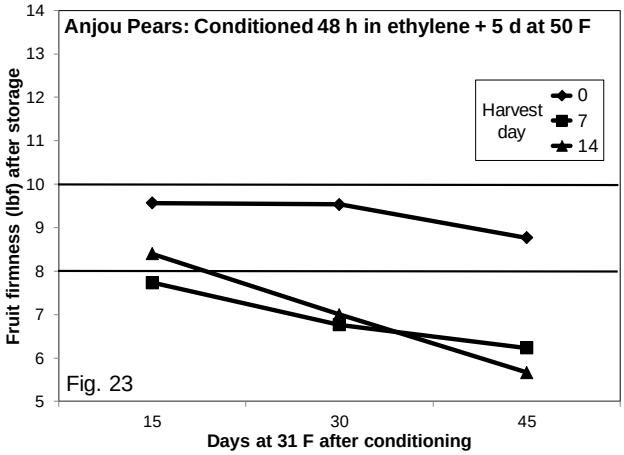
ethylene exposure (Figs. 8-19). From informal discussions with pear shippers, it appears that a lower threshold for shipping firmness may be between 8 and 10 lbf. Following some conditioning treatments in this project, fruit firmness was too soft for the fruit to be expected to ship without injury. Post-ethylene temperature conditioning, especially at 50 °F, needs to be managed to avoid excess fruit softening while gaining the ripening and eating quality benefits. In general, eating quality of ripe fruit improved with longer ethylene exposure and longer post-ethylene temperature conditioning time.

5. The firmness of 'Anjou' and 'Comice' pears after a range of durations of ethylene treatments and post-ethylene conditioning temperatures also indicates the potential storage life after the fruit have experienced various conditioning strategies. While the storage potential at 31 °F of 'Anjou' and 'Comice' pears after conditioning decreases with increasing time in ethylene, warmer post-ethylene conditioning temperatures, and later harvest, fruit from several conditioning regimes and harvest dates could be stored at 31 °F for 15-45 days while retaining suitable shipping firmness (Figs. 20-23). In general, 'Anjou' and 'Comice' pears conditioned in ethylene only maintained high shipping firmness during post-conditioning storage, although 'Anjou' pears conditioned for 72 hours in ethylene from the latest harvest were close to 8 lbf after 30 days of post-conditioning storage at 31 °F (Fig. 22). Harvest date was a critical factor in the post-conditioning storage potential of fruit conditioned for 48 hours in ethylene followed by 5 days at 50 °F (Fig. 23).

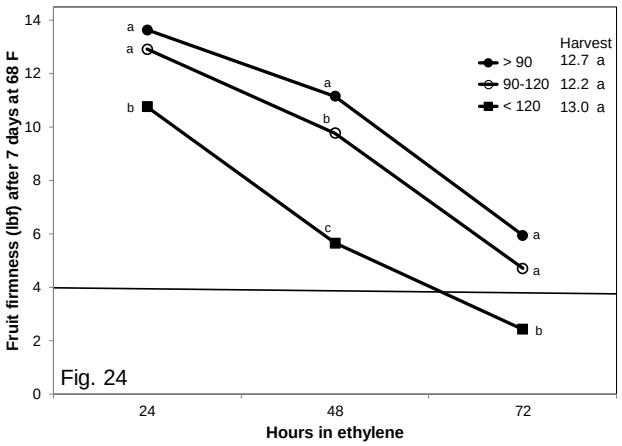


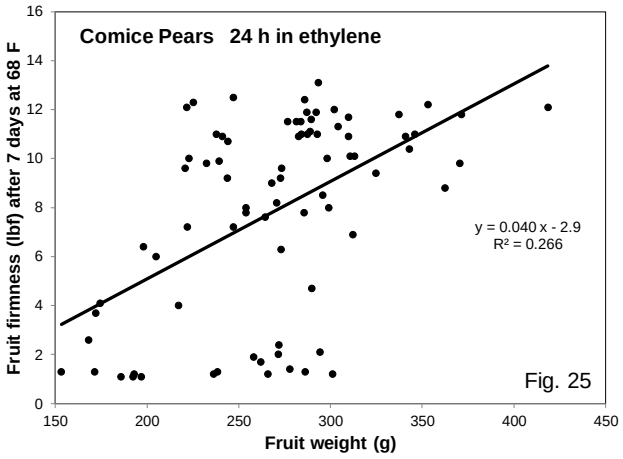


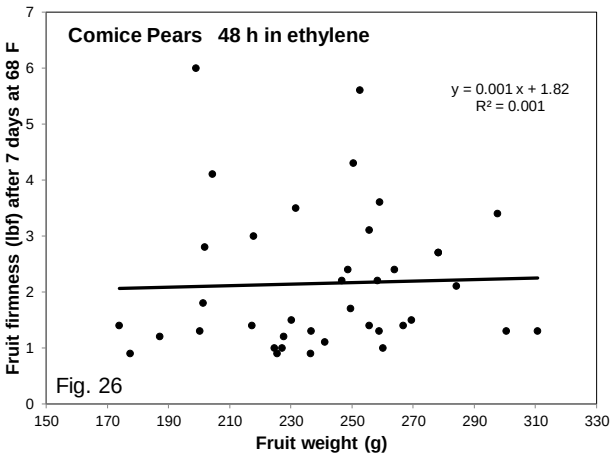




6. ‘Comice’ pears of three size categories (larger than 90, 90-120, and smaller than 120) were harvested and fruit firmness was measured after the fruit were exposed to ethylene for 24, 48, and 72 hours. When very small fruit were selected and compared to medium and large-sized fruit, they indeed responded to ethylene more quickly than the larger fruit (Fig. 24). Using a natural range of fruit sizes exposed to ethylene for 24 hours, there was a slight trend for smaller fruit to ripen more fully than larger fruit (Fig. 25). However, with longer ethylene exposure (48 hours), there was no relation between fruit size and ability to soften (Fig. 26). Thus fruit size may have a role in the variability of some lots of varying fruit size exposed to ethylene, but this is not expected to have a significant role in determining degree of variability conditioning ethylene that was not there was a high differences in fruit in fruit response to accounted for by size.







Objective 2 (Postharvest Decay):

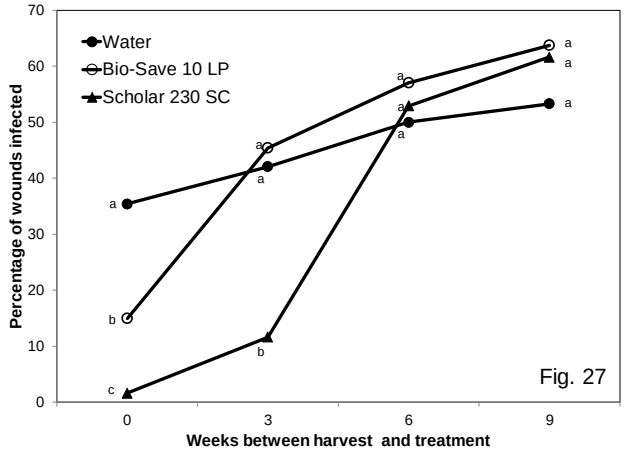


Fig. 27

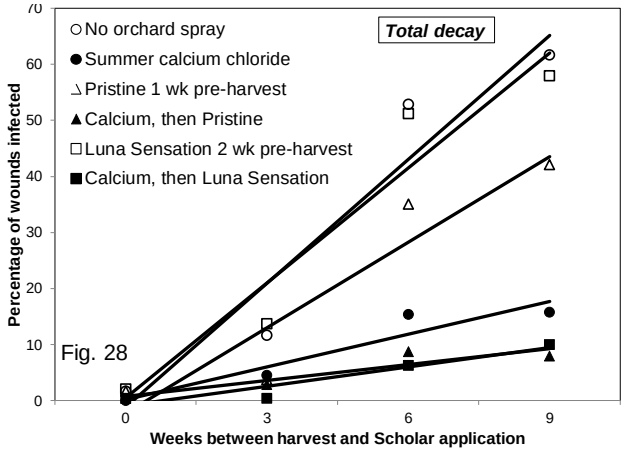


Fig. 28

1. This research addressed the common industry situation in which a large portion of the winter pear crop intended for mid-to-long-term storage may not receive postharvest fungicide treatment promptly after harvest, and thus postharvest fungicide treatment may be inadequate. Relying solely on postharvest treatments, the ability to control infections by decay fungi at wounds made at harvest was largely lost when postharvest treatment with Scholar fungicide was delayed until 6 weeks or more after harvest, and when postharvest treatment with Bio-Save 10 biocontrol agent was delayed until 3 weeks or more after harvest (Fig. 27). Treatment materials that may be effective when applied promptly after harvest may be of little value for decay control if applied a few weeks later, even if the fruit are kept cold between harvest and treatment. Thus integration of orchard treatments with postharvest treatments as key elements of a comprehensive decay control strategy may be critical to reducing economic losses due to postharvest decay.

2. Calcium chloride summer treatments can serve as a backbone for subsequent pre- and postharvest fungicide treatments. In our experiments, calcium chloride sprays were most effective in reducing "side rot" types of wound infections, caused by fungi such as *Cladosporium* and *Alternaria* (Fig. 28). Calcium chloride sprays were not effective in controlling gray mold (*Botrytis cinerea*) (Fig. 29).

3. Pristine fungicide applied one week pre-harvest was effective in reducing total decay incidence, while Luna Sensation applied two weeks pre-harvest did not appear to be effective (Fig. 28). However, when only the *Botrytis* infections were considered, Luna Sensation treatments reduced decay (Fig. 29). Experiments on pre-harvest fungicide and other orchard-based decay control options performed during in 2011 growing season will be evaluated in February, 2012.

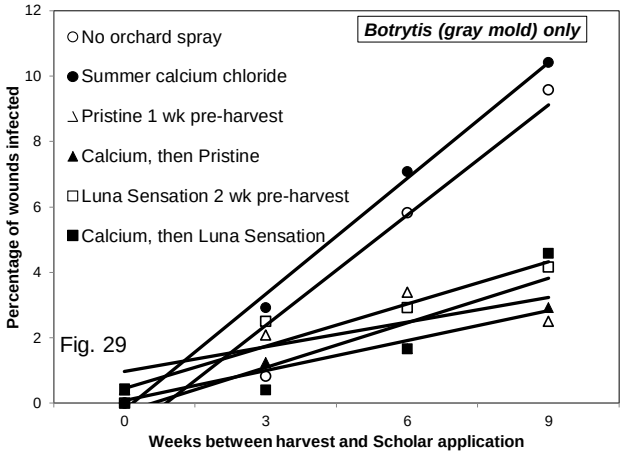


Fig. 29

4. Potential organic decay control strategies were evaluated, involving two yeast-based products applied before harvest and the bacterial-based biocontrol product Bio-Save 10 applied as a postharvest line. In general, the biocontrol programs based on either yeast followed by Bio-Save performed similarly to the check in decay control (Fig. 30), while the most effective fungicide program (Pristine followed by Scholar) was highly effective when applied promptly after harvest.

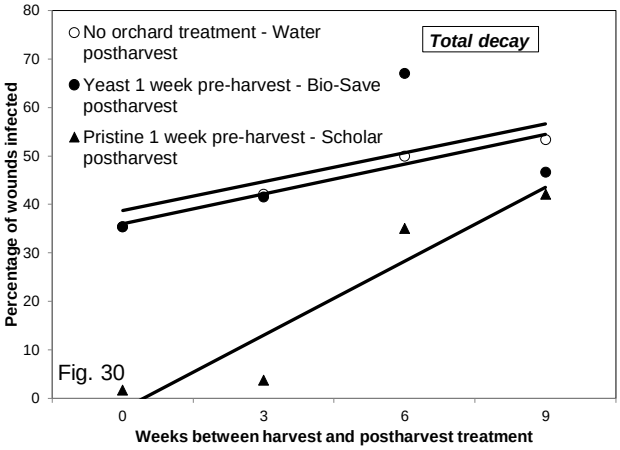


Fig. 30

5. As an alternative or additional to pre-harvest fungicide treatments, a single-bin drench system for applying fungicide or biocontrol agents to harvested bins of fruit is being evaluated. Scholar fungicide applied through the single-bin drench system further reduced decay in fruit that had been treated in orchard with calcium and/or Pristine (Fig. 31). Overall, applying Scholar through this system appears to provide decay control at a level similar to applying Scholar as a packinghouse line-spray between 3 and 6 weeks after harvest (Fig. 32). Biocontrol agents applied through the single-bin drench system in 2011 will be evaluated in February, 2012.

EXECUTIVE SUMMARY

The most efficient temperature for satisfying the chill requirement (inducing ripening capacity) in winter pears appears to be 50 °F. A range of potential conditioning temperatures for ‘Anjou’ and ‘Comice’ were compared, confirming the potential of using 50 °F as a tool for conditioning winter pears much faster than at 31 °F. In addition to speed of conditioning, preliminary results from UC Davis show that ripened ‘Comice’ pears that had been conditioned at 50 °F had more intense sweet pear aroma than those that had been conditioned at 31 °F or in ethylene for 72 hours.

‘Anjou’ pears did not have the capacity to ripen after 24 or 48 hours in ethylene, unless given further temperature conditioning. After 24 hours in ethylene, ‘Anjou’ pears needed an additional 25-40 days at 31 °F to develop ripening capacity, depending on maturity at harvest. After 48 hours in ethylene, ‘Anjou’ needed 15-30 days at 31 °F. After 72 hours in ethylene, ‘Anjou’ pears softened to nearly 4 lbf within 7 days at 68 °F without further temperature conditioning. When the post-ethylene conditioning temperature was 50 °F, induction of ripening capacity proceeded significantly faster. Typically, 5 days at 50 °F following 24 or 48 hours in ethylene was sufficient to complete induction of ripening capacity. For three weekly harvest dates of ‘Anjou’, the fruit firmness at the end of 10 days conditioning at 50 °F was equivalent to the fruit firmness at the end of 60 days conditioning at 31 °F. The same response patterns, on a shorter time scale, were found for ‘Comice’.

After 24 or 48 hours in ethylene followed by 5 days at 50 °F, fruit firmness at the end of conditioning (shipping firmness) varied from around 9 to 11.5 lbf, depending on harvest maturity and length of ethylene exposure. Following longer post-ethylene conditioning at 50 °F, fruit were too soft to ship without risk of injury. Post-ethylene temperature conditioning, especially at 50 °F, needs to be managed to avoid excess fruit softening while gaining the ripening and eating quality benefits. In general, the eating quality of ripe fruit improved with longer ethylene exposure and longer post-ethylene temperature conditioning time.

How long can conditioned pears be stored before shipping? While the storage potential at 31 °F of ‘Anjou’ and ‘Comice’ pears after conditioning decreases with increasing time in ethylene, warmer post-ethylene conditioning temperatures, and later harvest, fruit from several conditioning regimes and harvest dates could be stored at 31 °F for 15-45 days while retaining suitable shipping firmness. ‘Anjou’ and ‘Comice’ pears conditioned in ethylene generally maintained high shipping firmness during post-conditioning storage. ‘Anjou’ pears conditioned for 72 hours in ethylene from the latest harvest were close to 8 lbf after 30 days of post-conditioning storage at 31 °F. The post-conditioning storage potential of fruit conditioned for 48 hours in ethylene followed by 5 days at 50 °F was highly dependent on harvest date; earlier harvest provided the best storage potential.

The ability to control postharvest infections by decay fungi at wounds made at harvest was largely lost when postharvest treatment with Scholar fungicide was delayed until 6 weeks or more after harvest, and when postharvest treatment with Bio-Save 10 biocontrol agent was delayed until 3 weeks or more after harvest. Summer calcium chloride and pre-harvest Pristine were effective treatments for postharvest decay reduction. As an alternative or addition to pre-harvest fungicide treatments, Scholar fungicide applied to harvested bins in the orchard through a single-bin drench system further reduced decay in fruit that had been treated in the orchard with calcium and/or Pristine. Overall, applying Scholar through this system appears to provide decay control at a level similar to applying Scholar as a packinghouse line-spray between 3 and 6 weeks after harvest.

FINAL PROJECT REPORT

Project Title: Synthetic honey bee brood pheromone to enhance pear pollination

PI: Ramesh Sagili
Organization: Oregon State University
Telephone: 541-737-5460
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Address: 4017 ALS Building, Oregon State University
City: Corvallis
State/Zip: Oregon 97331

Cooperators: Pear growers and beekeepers in Oregon
Contech Inc., B.C, Canada

Other funding sources: None

Total Project Funding: $4000

Budget History:

Item	2010	2011	Year 3:
Salaries	3300		
Benefits			
Wages			
Benefits			
Equipment			
Supplies	225		
Travel	475		
Plot Fees			
Miscellaneous			
Total	4000	Did not apply for 2nd year funding	

OBJECTIVES

Primary goal of the project was to enhance pollination efficiency of honey bee colonies rented by pear growers for pollination, by using synthetic honey bee brood pheromone that has the potential to increase foraging stimulus of honey bees.

Specific objective: Examine and compare synthetic brood pheromone-induced foraging activity of treated colonies with controls in pear orchards.

SIGNIFICANT FINDINGS

Honey bee colonies treated with synthetic brood pheromone (SuperBoost®) had significantly greater number of foragers when compared to control colonies. The ratio of pollen to non-pollen foragers entering colonies was significantly greater in pheromone-treated colonies after brood pheromone treatment.

RESULTS & DISCUSSION

The ratio of pollen to non-pollen foragers entering colonies was significantly greater in pheromone-treated colonies after brood pheromone treatment. Foragers in pheromone-treated colonies returned with pollen load weights that were significantly heavier than controls. Pollen returned by foragers from pheromone-treated colonies was 47 % more likely to originate from the target crop (pear).

The mean sum of foragers entering colonies in a 5-min period was also significantly different between treatments (ANOVA: $F_{1,10} = 8.1$; $P < 0.01$). A significantly greater proportion of pollen foragers were observed returning in pheromone treated compared with control colonies (chi-square = 7.9, df = 1, $P < 0.01$). The mean ratio and standard error of pollen to non-pollen foragers was 0.3±0.1 in pheromone-treated colonies and 0.06 ± 0.002 in controls. That is, in pheromone-treated colonies there were about 3 times the numbers of non-pollen to pollen foragers, whereas in control colonies there were about 16 times the numbers of non-pollen foragers. Pollen load weight was significantly greater in the pheromone-treated colonies (ANOVA: $F_{1, 10} = 24$; $P < 0.01$). Pollen loads returned by bees from pheromone treated colonies were 47 % more likely to originate from the target crop (pear) (chi-square = 62, df = 1, $P < 0.0001$).

Results from this study suggest that synthetic brood pheromone increases total number of foragers and pollen foraging activity in honey bee colonies treated with brood pheromone in pear orchards. This increase in foraging may be a result of enhanced stimulation of foraging behavior by the

synthetic brood pheromone. This increase in foraging and especially pollen foraging is potentially beneficial for pear pollination keeping in view the fact that many times it is challenging to have adequate honey bee foraging activity during pear bloom.

EXECUTIVE SUMMARY

Adequate pollination is the key for high fruit quality and yield. One of the challenges facing pear growers is ensuring adequate pollination. Honey bees are principal pollinators of pear. Honey bees may be easily lured to flowering plants that are more attractive and rich in resources (Delaplane and Mayer 2000). Hence there is a need to explore tools or strategies that enhance pollination efficiency of honey bee colonies and increase overall pollination in pear. Brood pheromone (BP) released by honey bee larvae is an excellent apicultural tool that has the potential to increase pollination by manipulating foraging stimulus of honey bee colonies. In this study we examined if synthetic BP can be used to enhance pollination in pear.

Results from this study indicate that honey bee colonies treated with synthetic brood pheromone (SuperBoost®) in pear orchards had significantly greater number of foragers when compared to control colonies. The ratio of pollen to non-pollen foragers entering colonies was significantly greater in pheromone treated colonies after brood pheromone treatment. Future research should focus on documenting increase in crop yield and fruit quality resulting from increased pollination, as a result of synthetic honey bee brood pheromone use.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-10-103

Project Title: Systems approach for ensuring superior pear fruit quality

PI	Amit Dhingra	**Co-PI**:	Todd Einhorn
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Cooperators: WSU - Matthew Whiting, Don Elfving, Tim Smith, Ananth Kalyanaraman, Carolyn Ross, Shyam Sablani, Karen Killinger; 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, Nate Reed, AgroFresh.

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

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1 Amit Dhingra
Organization Name: WSU **Contract Administrator:** Carrie Johnston
Telephone: 509-335-4564 **Email address:** carriej@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 project represents multi-disciplinary activities aimed at ensuring superior pear fruit quality. Over 68 scientists from US, Europe and South America representing diverse disciplines continue to work together on developing a research roadmap for pears and developing grant applications to be submitted to various funding agencies within USDA and NSF.

Objective 1: (Year 1-3) 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.
Significant findings/Progress			
• These measurements will be recorded in year 3.	• An experiment to compare single axe, bi-axe and steep V trees at different in-row spacings, and on three rootstocks (OHxF 87, OHxF 69 and Pyro 2-33) will be established in WA and OR, spring 2013. Trees were either budded traditionally, or for the bi-axe system [two-buds, opposite sides on the rootstock] late summer 2011. • Trees planted in a replicated block of Bartlett, Starkrimson grafted on to OHF 87 planted in 2010 in Koempel block to the UFO training style suffered from frost damage. Some trees were replanted. We plan to observe the growth and development of this block in the coming season.	• More than 300 accessions of promising Pyrus rootstock material have been identified in Spain, France, Italy, UK and Argentina. • The PIs (Evans and Dhingra) have been coordinating with the Pyrus Crop Germplasm Committee, U.S. nurseries and national and international collaborators to compile a selected list of pear rootstocks and rootstock selections. First selections will be imported into the Clean Plant Network, Prosser during year 3.	• This assessment will be performed in year 3.

Methods: **1b**. All rootstocks were raised from tissue culture, in sufficient quantity, and delivered to the nursery in spring of 2011. Plant growth was adequate in the nursery throughout 2011, and rootstocks were budded late summer 2011. Double budding (nursery establishment of bi-axe trees) was successful, and we expect to have finished trees delivered following growth this season in the nursery (2012). Plots will be ripped, fumigated and prepared for planting in 2013.

1c. Most of the information has been gathered through personal contact and during the International Pear Workshop organized during summer 2011.

Results and Discussion: The UFO style planting has not established itself very well. It could be due to the quality of trees highlighting the need to have a robust rooting system prior to planting. In addition, tying down the trees immediately after planting may not be the best strategy for pears. This observation needs to be verified further.

The collaborators have committed to providing the rootstock genetic materials as part of the Pear Revival SCRI application. While the application will be submitted in 2013, work on identifying and importing first selections will continue. The selections will be imported via NCPN, Prosser.

Objective 2: (Year 1 and 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 (physiological and gene-level) 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.

Significant findings/Progress

- '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 (scaffolds in 3rd leaf), 30 degree scaffolds had significantly greater bloom than the other treatments (280 clusters per tree, vs. 175 and 130, for 45 and 0 degree angles, respectively).
- Total tree fruit set 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 relative to the other angles. Total canopy leaf area for the 30 degree trees was half that of the other two angles.
- Despite profuse bloom (130-240 clusters per tree), Anjou fruit set and yield was insignificant for all limb treatments (<20 fruit per tree).
- Testing of abscisic acid (ABA) for management of pear vigor was not pursued in 2011 due to its ineffectiveness at controlling shoot growth in 2010.
- Apogee was quite effective as a vigor control agent for all pear varieties tested. Apogee markedly reduced shoot length between 30% and 60 % of the untreated control in 'Bosc', 'd'Anjou' and 'Starkrimson'. Similar results were observed in both 2010 and 2011.
- In 2011 only 1 application of Apogee (250 ppm) at ~10 cm shoot length was required to control vigor for the entire season (i.e., shoots were not observed to have a second growth flush).
- As similarly observed in 2010, Apogee did not have any negative effects on yield, or individual fruit size of 'd'Anjou' or 'Bosc'. Results for 'Starkrimson' were inconsistent.
- Anjou 2011 return bloom of spurs was reduced by ~30% from two apogee applications of 250 ppm in 2010. Bloom developing on the tips of one-year-old wood was significantly reduced for both 'Anjou' and 'GR-Bosc'. For two sites in upper valley flower buds were killed from low temperature events. In 2012 we will evaluate return bloom of 4 research sites treated in 2011.
- Growing shoot tips have been collected for genomic analysis.

METHODS: 1. PGR vigor control. Entire primary scaffold limbs of 'Starkrimson', 'Golden Russet Bosc' and 'd'Anjou' [two sites for 'd'Anjou'; one lower and one upper Hood River valley] were

treated with Apogee beginning ~ 20 days after full bloom [DAFB] when shoot elongation was < 4 inches. Treatments were: 1) Control [water] 2) water + surfactant, 3) one application of 250 ppm Apogee when shoots were ~10 cm (i.e., 4 inches) long, 4) spring application of 250 ppm Apogee at roughly 4 inches of shoot elongation + 250 ppm Apogee with recurrence of shoot growth, and 5) spring application of 250 ppm Apogee at ~ 4 inches shoot elongation + 250 ppm P-Ca on a 30 day calendar schedule. For each treatment, five or six replicate scaffolds [depending on site] were treated. Four newly emerged shoots per scaffold were measured weekly. Following bud set in late summer, all shoots on the scaffold were assessed for growth. Yield and fruit size was collected at harvest. Scaffold circumference was taken in fall and compare to spring circumference.

2. Limb training. All primary shoots were removed from four-year-old, eight tier central leader trellised trees in spring of 2009 using Dutch-cuts. Three limb angle treatments (0, 30 and 45° from horizontal) were established once new shoots emerged. For each treatment, five single tree replicates, each with 16 shoots (2 per tier), were trained to their respective angles. The experiment was applied to both 'd'Anjou' and 'Bartlett' trees. Total length of each primary shoot, and all new shoots (watersprouts and lateral branches) initiating from the primaries were measured in 2009 and 2010. Number of flowers and fruit borne in 2010 ('Bartlett' only) were counted. Bloom and fruit set will be assessed in 2011.

RESULTS AND DISCUSSION

1. PGR vigor control.

Apogee resulted in good vigor control of Anjou, GR-Bosc, and Starkrimson (Fig 1). Shoot growth was controlled in 2011, by one application of 250 ppm for all varieties. Lower valley Anjou trees required multiple applications in 2010, since growth resumption was strong after the bio-regulator was metabolized. These results were the rationale for testing a 30 day, interval application in 2011. It is plausible that the cool growing season of 2011 contributed to the stronger growth control observed. Importantly, Anjou fruit size was not

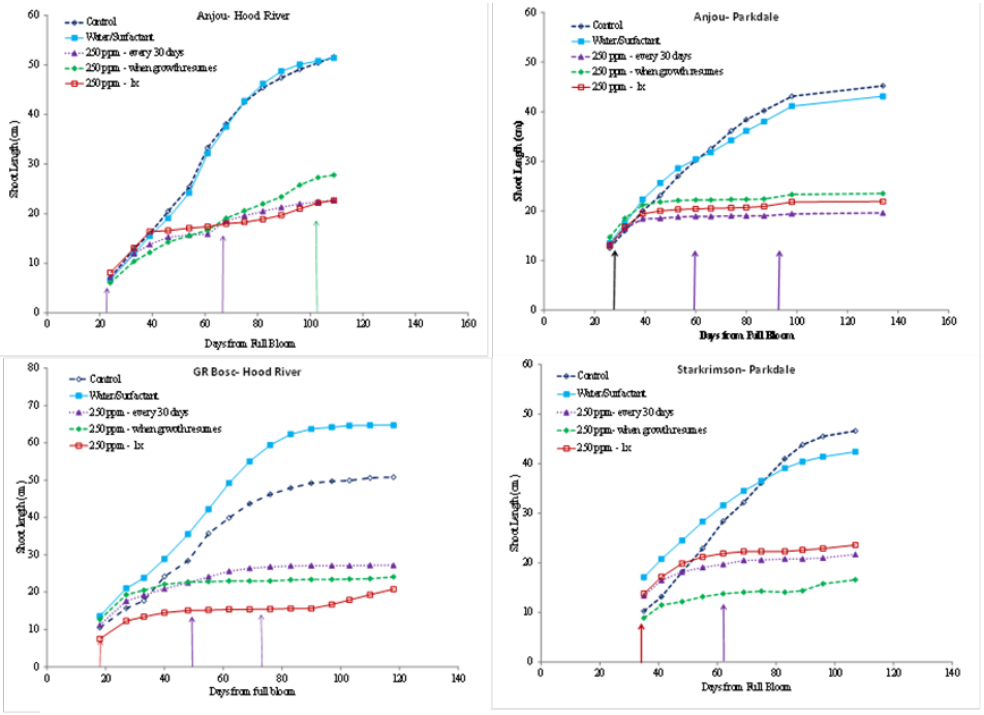


Figure 1. Effect of Apogee on shoot growth [length (cm)] of 'd'Anjou' lower Hood River Valley [top left]; 'd'Anjou' upper Hood River Valley [top right]; 'GR Bosc' lower Hood River Valley [lower left]; 'Starkrimson' upper Hood River Valley [lower right]. Arrows on the x-axis indicate application timings in spring (first arrow in each plot), and multiple applications for the calendar treatments. A second application for the growth resumption treatment was made at ~100 days from full bloom only for the Hood River Anjou plot. Data points are the means of 6 replications (n=4).

affected by any of the treatments (Table 1). However, 2011 return bloom was reduced by 2010 Apogee treatments (Table 2). Although, pear fruiting on the tips of last season's growth is characteristic of 'Bartlett', we observed a fair amount of bloom on these shoots for Anjou (Table 2). Further, this bloom was significantly limited by Apogee treatments the season prior, albeit spur bloom disproportionately comprises total tree bloom. Return bloom of 'Bosc' was similarly affected by Apogee (Table 2), as previously shown. We will examine return bloom dynamics from the 4 sites treated in 2011, spring of 2012.

2. Limb training. In 'Bartlett', 30 degree from the horizontal resulted in the most flowering, fruit set, and yield, and least vegetative growth relative to other branch angles (Fig 2; Tables 3 and 4). However, 30 degree treatments had the smallest fruit size. Although thinning was performed at standard 'Bartlett' timing, too many fruit were left for the 30 degree treatment (projected yields of 41 bins/acre on 3rd leaf scaffolds; Table 4). 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. Horizontal branch angles were not effective in inducing precocity in 'Bartlett'. As is typically observed with Anjou profuse bloom did not result in significant fruit set irrespective of limb angle treatment (data not shown).

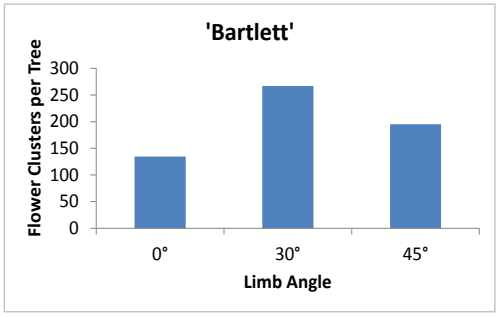
Table 1. Effect of Apogee on Hood River 'd'Anjou', 'Starkrimson' and 'GR-Bosc' yield and final fruit size. Freeze events confounded upper Valley 'd'Anjou' fruit set resulting in insignificant yield (data not shown). Treatments were made on entire primary scaffolds selected for uniformity in size and potential cropload. Data are means of 6 replications (n=1 for yield).

Treatment	Anjou		Starkrimson		GR-Bosc	
	Yield (lb)	Fruit Size (g)	Yield (lb)	Fruit Size (g)	Yield (lb)	Fruit Size (g)
Control	19.4	202.9	32.8	237.3a	9.2	214.1
Water+Surfactant	19.8	209.2	40.7	216.6a	5	228
250 ppm P-Ca every 30 days	23.4	199.7	34.5	183.2b	9.1	211.3
250 ppm P-Ca with growth resumption	24	209.8	34.7	204.5ab	6.6	212.9
250 ppm P-Ca 1x	21.4	197.4	45.4	214.8a	7.4	233.7
Significance	n.s.	n.s.	n.s.	*	n.s.	n.s.

Table 2. Effect of 2010 Apogee applications on 2012 Hood River 'd'Anjou', and 'GR-Bosc' return bloom. Freeze events limited bloom of the two upper Valley sites ('d'Anjou' and 'Starkrimson'). Consequently, data was not collected.

2010 Treatments	d'Anjou' (Hood River)		GR-Bosc'	
	Spurs	Shoots	Spurs	Shoots
	% of Spurs or Shoot Tips with Bloom in 2011			
Control	74	46	35	28
250 ppm Apogee	57	18	30	9
500 ppm Apogee (250 + 250)	49	9	22	6

Figure 2. Effect of branch angle (from the horizontal) on flowering and fruit set of Bartlett pear scaffolds (means are based on 5 replications (n=1).



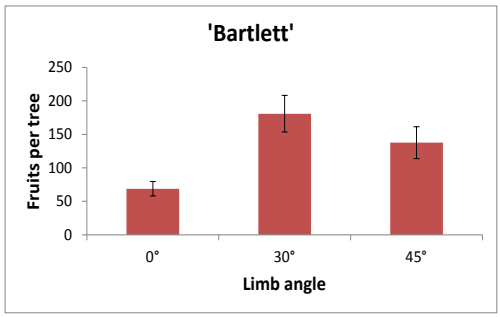


Table 3. Effect of branch angle

(from the horizontal) on growth (length) of primary scaffolds, initiation and length of secondary offshoots on primary scaffolds, and whole canopy leaf area.

Limb Angle	Primary Scaffolds Per Tree		Secondary Shoots Per Tree			Whole Canopy
(° from Horiz.)	Avg Length (m)	Ttl Length (m)	Number	Avg. length (cm)	Ttl annual growth (m)	LA (m^2)
45	1.6	24.9	93	54.6	57.8	21.6
30	1.2	19.7	64	58.1	39.9	8.1
0	1.2	17.1	89	67.4	62.7	21.1

Table 4. Effect of primary scaffold branch angle (from the horizontal) on initial fruit set, yield, and final fruit size.

Treatment	Fruit Set	Yield		Avg. Fruit Size	
Limb Angle (° from Horiz.)	Fruit per tree (# before Thinning)	Per Tree (lb)	Per Acre (1,100 lb bins)	Wt. (g)	Box Size (# per 44 lbs)
45	141	38	31	196	100
30	216	50	41	179	110
0	75	27	22	202	90

Tree spacing is 4 ft. x 12 ft. (906 trees per acre). System is an eight wire (13 ft tall) vertical trellis.

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.
Significant findings/Progress		
• After successful establishment of protocols this work will be carried out in Year 3.	• Physiological induction of cork spot and russet using published protocols was not successful. • Bitter pit related gene has been cloned from apple and work is ongoing to assess the role of its homolog in pear and test if it is involved in corking.	• Pigment stabilizing chemistry has a positive effect on fruit storage quality as it maintains its firmness throughout the storage process. • Expanded field tests will be done in collaboration with AgroFresh in Year 3.

METHODS: Induction of cork spot formation in pears was tested using protocols that are used to induce bitter pit in apples. Fruit were vacuum infiltrated with magnesium chloride as described (Burmeister and Dilley, 1993). However, this treatment was ineffective in inducing corking.

DM Burmeister and DR Dilley (1993). J. Agri. Food Chem. 41: 1203-1207.

RESULTS AND DISCUSSION: 3b. Corking and bitter pit are often considered to be similar issues since calcium treatment is used to alleviate these physiological disorders. The failure to induce corking raises question on this long-held belief. Profiling of genes in the corked vs. non-corked tissues can provide some answers as to the mechanism by which this disorder manifests itself.

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/Progress		
• UV-C is effective in reducing blue molds and generic *E. coli* populations on fresh fruit surfaces • Efficacy of treatment is dependent upon the type of microorganisms and fruit surface physiological and morphological profiles.	This study will be performed in Year 3.	This study will be performed in Year 3

METHODS: The effectiveness of UV system was tested on blue mold and general *Escherichia coli* ATCC 23716, a nonpathogenic surrogate strain inoculated on wounded and intact pear surfaces. Pear surfaces were exposed to UV-C light ranging from 0 to 7.93 kJ/m^2 UV doses. Reaction kinetics equations were employed to describe UV-C inactivation of generic *E. coli*.

RESULTS & DISCUSSION: UV-C dose of 1.17kJ/m2 corresponding to 1 min treatment was able to reduce 2.38 log of blue mold on intact pear surface (Figure 3). Maximum reductions of 3.7 log CFU/g was achieved for *E. coli* on plain pear surfaces ($P < 0.05$), with lesser reduction on wounded pear (3.1 log CFU/g) after UV-C exposure at 7.93 kJ/m^2 UV dose. The Weibull scale factor (α) values of UV-C inactivation kinetics of *E. coli* on intact pear and wounded pear were 0.001 and 0.002 minutes, respectively. The time required for a reduction in the number of *E. coli* was smaller in comparison to treatment time for wounded pear suggesting that UV-C light was more effective in inactivating *E. coli* on plain pear surface (Figure 4). The wounds on pear surfaces helped shield and protect the microorganisms against UV-C radiation. In addition, Fourier transform infrared (FT-IR) spectroscopy was employed to investigate the mechanism of *E. coli* injury and inactivation on fruit surfaces under UV-C treatment. Second derivative transformations of FT-IR spectra demonstrated bacterial membrane damage and DNA/RNA variation, resulting in *E. coli* inactivation on fruit surfaces by UV-C treatment. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to clearly segregate bacterial untreated samples from UV-C treated samples, suggesting significant ($P < 0.05$) biochemical compositional variations of bacterial cells after UV-C treatment.

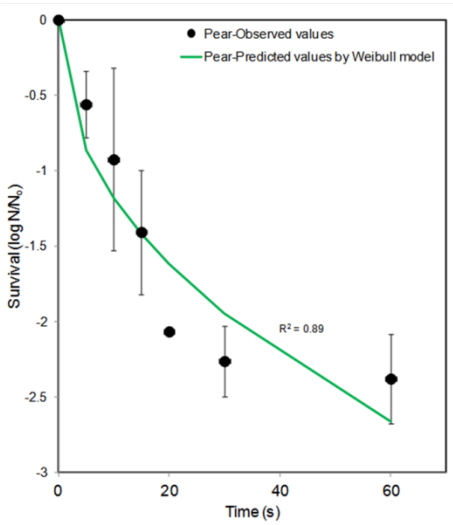


Figure 3: UV-C inactivation kinetics of blue mold on intact pear surface

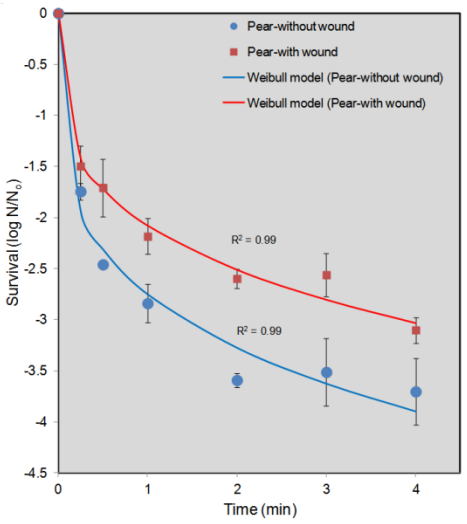


Figure 4: UV-C inactivation kinetics of generic *E. coli* on pear **surface**

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-10-104A

Project Title: Physiological genomics of pear ripening

PI: Amit Dhingra
Organization: Washington State University
Telephone: 509 335 3625
Email: adhingra@wsu.edu
Address: 149 Johnson Hall
City/State/Zip: Pullman WA 99164-6414

Co-PI (2): Todd Einhorn
Organization: Oregon State University
Telephone: 541-386-2030 (x.13)
Email: todd.einhorn@oregonstate.edu
Address: 3005 Experiment Station Drive
City/State/Zip: Hood River OR 97031

Cooperators: Blue Star Growers, David Sugar (Oregon State University), Tim Smith, WSU, Chris Hendrickson, Graduate Student, WSU and Kate Evans, WSU

Total Project Request: **Year 1:** $ 48,062 **Year 2: $** 64,785 **Year 3: $ 56,575**
Other funding sources: None

Budget 1
Organization Name: Washington State University **Contract Administrator:** Carrie Johnston
Telephone: 509 335 4564 **Email address:** carriej@wsu.edu

Item	2010	2011	2012
Salaries[1]	29,255	30,426	**31,643**
Benefits			
Wages	6,500	6,760	**7,030**
Benefits	310	322	**335**
Equipment			
Supplies	6000	7000	**7000**
Travel	2000	1,000	**2,000**
Plot Fee	0	0	**0**
Miscellaneous – 454 sequencing		11,000	
Total	$40,065	$56,508	**$48,008**

Footnotes: [1] Salaries for agriculture research assistant for performing physiological and genomic profiling and all molecular work. The increase in salaries for years two and three reflects a 4 % rate increase.

Budget 2
Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton
Telephone: 541 737 3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	2010	2011	2012
Salaries[1]	4,140	4,306	**4,478**
Benefits[2]	2,857	2,971	**3,089**
Wages			
Benefits			
Equipment			
Supplies	1,000	1,000	**1,000**
Travel			
Plot Fee	0	0	**0**
Miscellaneous			
Total	$7,997	$8,277	**$8,567**

Footnotes: [1] Salary is based upon as 0.15 FTE Technician for harvest, cold storage and ethylene room maintenance, fruit quality attribute measurements, and data management. The increase in salaries for years two and three reflects a 4 % rate increase. [2] OPE rate is 69 %. Supplies largely include overnight shipping costs.

Objectives: We proposed to understand ripening in winter pear and characterize the newly identified cold-induced pear ripening gene through the following objectives.

1. (Year1) Test the correlated activity of ethylene production genes along with the cold induced ripening gene identified in our program in response to cold treatment:

We will study how all the known ethylene genes work especially in relation to the cold-induced ripening master switch gene. Another intriguing question to be addressed: *what duration of cold-treatment triggers ethylene burst and corresponding expression of the genes involved in ripening?*

2. (Year 2 and 3) Establish a relationship between ripening in winter pear and activity of the master switch gene:

Tissues collected in Objective 1 will also be subjected to a gene-level comparative analysis to identify other genes involved in this phenomenon during ripening inductive conditions with ethylene and cold treatment.

3. (Year 2 and 3) Genetic diversity of the cold-induced ripening gene in pears:

We will test the diverse summer and winter pear varieties to identify gene-level differences in the ripening gene. This information could serve as a target for site-specific mutation or sport generation for improvement of existing varieties or a molecular marker in future breeding efforts.

Significant Findings (Objectives 1 and 2)

- Established unique patterns of ethylene biosynthesis gene ACS, and ethylene receptor gene expression in Anjou pears, consistent with expectations based on other winter pear varieties.
- Isolated, cloned and sequenced an allele of an ACS gene whose expression is known to drastically increase at the onset of System 2 ethylene induction which marks the onset of ripening in pears.
- Demonstrated significant increase in gene expression of the proposed pear-ripening master-switch gene in Anjou pears during conditioning.
- Narrowed candidate ripening regulation list of 165 genes from 9 signaling pathways in fruits, to 36 genes from 5 pathways.
- Established preliminary evidence of a role for calcium in affecting time and temperature conditioning requirements for System 2 induction in pear fruit.
- Established reduction in expression of genes directly implicated in repressing above-mentioned ACS, ethylene receptor and numerous genes' expression. These genes are exclusive to fruit that have gained ripening ability during conditioning treatment. These, and future findings establishing a relationship between the proposed pear ripening master-switch and these critical System 2 ethylene-related genes offer strong evidence to the underpinnings of ripening ability and System 2 ethylene induction, as sought by the objectives of this proposal.

Methods

A flow-through respiration system and climate-controlled rooms (held at 31, 41, and 50°F) were used to treat 24 cases (each) of Bartlett and Anjou pears harvested at maturity, and obtained from Blue Star Growers (Figures 1 and 2). Comice were harvested and treated in a similar manner at OSU-Medford, and OSU-MCAREC respectively. Peel and core tissue sampling was performed among 10 fruit at 8 intervals during the course of conditioning, then 7 days of ripening. Flesh firmness was also taken of 10 fruit at each interval using an 8 mm probe. Samples were taken of fruit treated with 100 ppm ethylene for 48 hours at 68°F. Ethylene treatment was calibrated using microcontroller valves controlling flow from cylinders of compressed air and concentrated ethylene, and checked by gas chromatograph against a certified 100 ppm ethylene standard. Actively expressed RNA was then extracted from sampled tissue. This was then used for quantitative real-time PCR (qPCR) analysis of expression of over 165 targeted genes involved in 9 signaling pathways central to, or closely correlated to-ethylene responsiveness, and ripening in general. As our data is collected, we have (and will continue to) compared gene expression between varieties, tissues, ethylene treatments, and ripening competency stages. This work is currently underway.

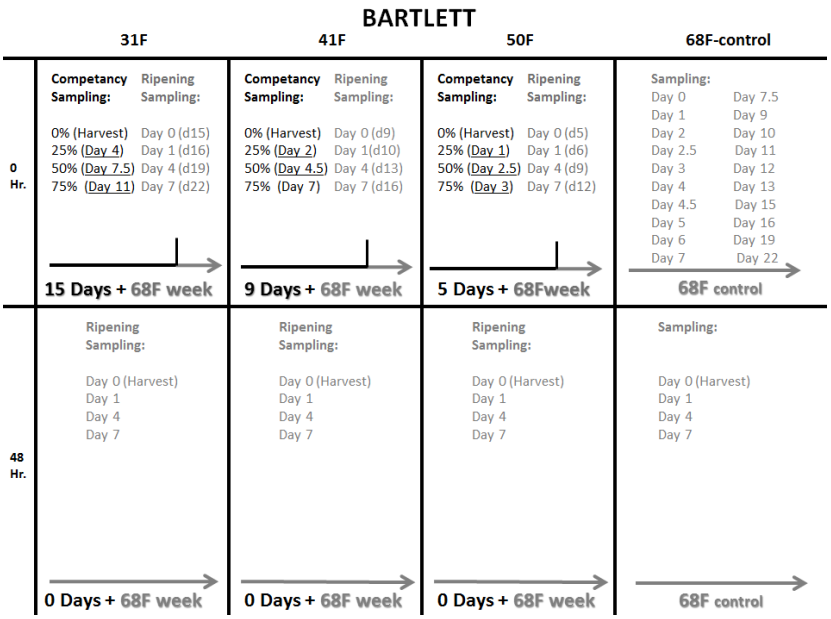


Figure 1- Bartlett treatment and sampling scheme of 2000 total fruit harvested at maturity, and obtained Blue Star Growers (Cashmere, WA). Eight subsampling events per treatment combination took place. At each event, 10 fruit were tested for flesh firmness, and 10 fruit peel and core tissues were obtained. This material was then immediately frozen for gene expression analysis. Similar conditioning regimes were followed for Comice and Anjou.

Results and Discussion

We have established a pear conditioning pipeline using the postharvest infrastructure at WSU Pullman campus (Figure 2), applying protocols described by David Sugar and Eugene Kupferman (2009). Results from work over the prior year have confirmed the conditioning responses based on prior work (Sugar and Einhorn, 2011; Bai and Chen et al., 2005), and have begun to reveal novel insight into understanding of pear conditioning, and ripening and System 2 ethylene production. This infrastructure and the reproducibility of results in this set-up is critical for subsequent

Figure 2- Flow-through respiration system using existing climate-controlled rooms, and infrastructure in Pullman.

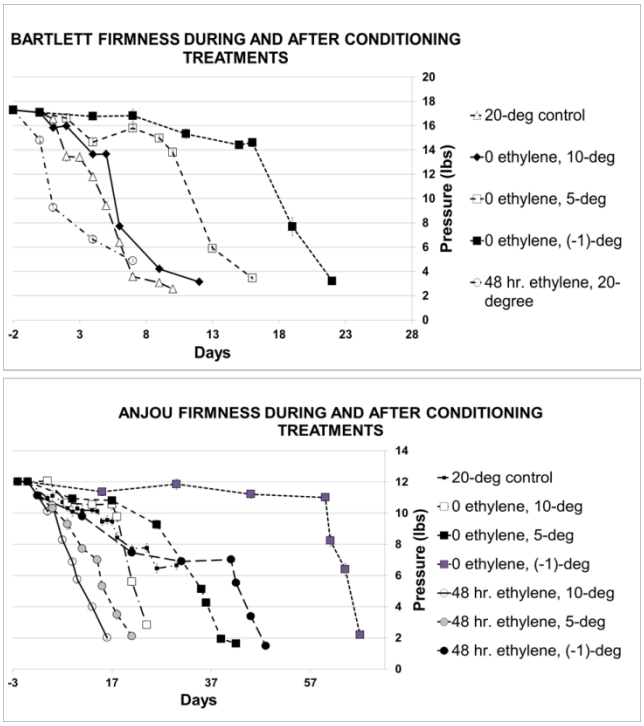


Figure 3: Conditioning and ripening related drop in fruit firmness in Bartlett and Anjou fruit.

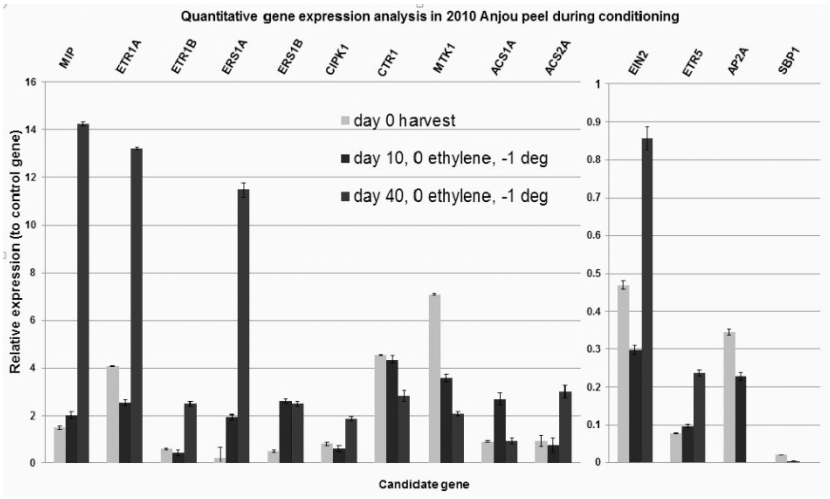


Figure 4- Quantitative real-time PCR gene expression analysis of selected ethylene perception, signaling and production genes in peel tissue of winter pears conditioned at 31°F (-1°C). Error bars represent standard error. Increased expression of the proposed master-switch ripening regulatory, System 2 ethylene-specific and ethylene responsive genes is seen during fruit conditioning progress.

genomics work. Fruit firmness dropped after conditioning and ripening experiments as expected (Figure 3).

Flesh softening of ungassed 41°F-stored fruit occurred more rapidly than some ethylene-treated fruit, indicating presence of ethylene independent, chilling-dependent genetic elements in regulation of ripening onset in pear. Further, we have demonstrated drastic changes in gene expression activity of the proposed pear ripening master-switch gene (MIP) in winter pears during conditioning and ripening progression, supporting the important role it is hypothesized to have in ripening induction (Figure 4). Increase in MIP expression intensity appear to precede or coincide with expression of System 1-to-System 2 transition and System 2-specific gene expression events known from research in pear, tomato and other climacteric systems. These findings correlate well with additional knowledge of this gene family. Similar MIP-related genes were found to move to plant cellular locations in which auxin is imported (Swarup et al., 2004), and whose expression increases after chilling-exposure (NCBI, 2011; Jia et al., 2004). *This strengthens evidence that MIP may be associated with the rise in endogenous auxin known to be required for, and immediately preceding-System 2 ethylene production associated with fruit ripening onset* (Karlova et al., 2011; Osorio et al, 2011; El-Sharkawy et al., 2004).

Additional work in the laboratory has allowed generation of pear genome sequence foundational knowledge which can greatly accelerate our understanding of how this and other candidate ripening regulatory genes tested in the upcoming years' research function in pear fruit under various conditioning treatments. *This resource has allowed identification*

of at least two unique forms of MIP present in pears, with nucleotide variation that may have critical roles in function. Further genotypic and functional characterization of MIP in summer and winter pears will be a primary goal of work for this year.

This provides a long-term economic benefit to growers, breeders and packhouse managers by providing an mechanistic understanding of uncontrolled ripening and related physical damage issues, which remain one of the most persistent and significant problems to the pear industry (Ing et al., 2002). With this, marker development and numerous post-harvest management studies can be developed to significantly reduce incidence of crop loss due to over-ripened and damaged fruit.

System 2 ethylene induction in pear, and its critical role in allowing full, proper ripening to occur is an autocatalytic process in which perception and communication (signaling) of the ethylene signal in fruit causes production of the hormone. With this in mind, it is helpful to think of System 2 ethylene production as a circuit, in which disruption of function of any genetic element of perception, signaling and production causes malfunction, and would impair ripening (Lelievre et al., 1997). Studies using various approaches in other chilling-dependent and independent climacteric fruits have concluded that the System 1-to-System 2 transition involves complex coordination of multiple hormone and stress-signaling genetic pathways (Chaabouni et al., 2009).

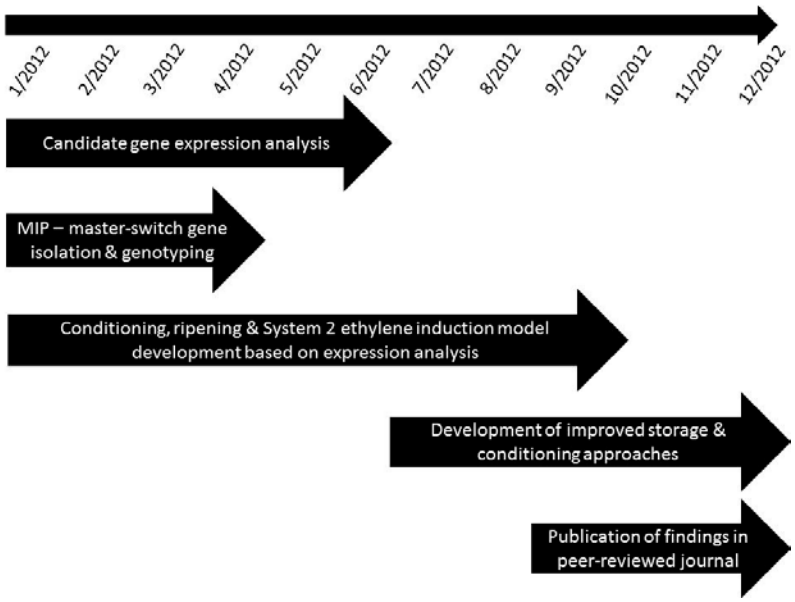
Based on this, and extensive knowledge of interacting genetic elements with the core elements of System 2 ethylene production, we have applied a genetic approach in which we have sought to correlate differences in conditioning requirement phenotypes to differences in gene expression. We have begun screening over 165 ripening regulatory candidate genes from 9 different hormone-related and cold-stress signaling pathways in plants in identification of gene expression differences in pears undergoing conditioning and ripening treatment. Our work this year has allowed us to continue the upcoming years research efforts into a narrowed list of 36 genes (some of which are featured in Figure 4) with high potential for directly controlling the onset of System 2 ethylene production, and ripening in response to various conditioning treatments in winter pear. Subsequent qPCR analysis of summer and winter pear tissue treated and collected this year will allow us to definitively examine this reduced list of candidate genes' expression in greater detail. In the next 12 months, this work will produce an enhanced base of genetic knowledge from which long term pear breeding, storage and management and disorder research can build upon.

In the face of stagnant growth and consumer demand, the foundational knowledge beginning to be revealed through efforts of this project offer a means of providing long-term solutions to the industry. With the full complement of available technologies, delivery of high-quality, uniformly ripened fruit to market becomes a possibility. However, these goals are dependent on a complete genetic understanding of conditioning treatments induce ripening and System 2 ethylene production in the full spectrum of chilling-dependent pear varieties. Results of the prior two years, and upcoming year will offer a far greater model of the relationship between chilling-dependence, ethylene applications, and unlocking of ripening capacity in pears. In concluding this study, we aim identify the causal genetic events underlying chilling-dependent ripening of summer and winter pears of the PNW. This will allow identification of gene-assisted breeding, chemical and other management strategies to reduce fruit-quality loss throughout the supply chain, allowing more control over fruit ripening. This will address of the most persistent challenges facing the pear industry (Ing et al., 2002).

Timeline and activities for 2012

In 2012, we will complete gene expression analysis of a smaller, narrowed list of candidate ripening regulatory network of genes in the tissue collected from summer and winter pear treated, conditioned, and ripened this year in our laboratory. Identified differences in ripening-regulatory gene expression between summer and winter pears will be characterized in greater detail, generating an increased

understanding of the genetic mechanism of chilling and ethylene treatments, and System 2 ethylene induction in pear conditioning. This will include development of ripening-related gene-specific markers unique to varietal patterns of conditioning requirements.



References

1. Sugar, D. October, 2009. Good Fruit Grower.
2. Sugar and Einhorn. 2011. Postharvest Biol. and Technol. 60(2): 121-124.
3. Bai and Chen. 2005. Acta Hort (ISHS) 671:325-331.
4. Swarup et al., 2004. Plant Cell. 16: 3069-3083.
5. Jai et al., 2004. Plant Cell Rep. 23:159–166.
6. Karlova et al., 2011. Plant Cell. 23: 923-941.
7. Osorio et al., 2011. Plant Physiol. 157(1): 405-425.
8. El-Sharkawy et al., 2004. Plant, Cell and Environ. 27(10): 1197-1210.
9. Ing, G. 2002. Pear production and utilization in North America.
10. Lelievre et al., 1997. Physiologia Plantarum. 101(4): 727-739.
11. Chaabouni et al., 2009. J. Exp. Bot. 60(4): 1349-1362.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (with extension)
WTFRC Project Number: PR09-905

Project Title: Pear rootstock breeding

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Cooperators: Timothy Smith, WSU Wenatchee; Amit Dhingra, Cameron Peace, Doreen S. Main, WSU Pullman; Todd Einhorn, OSU MCAREC; Gennaro Fazio, USDA-ARS

Total Project Request: Year 1: $4,500 **Year 2:** $12,300 **Year 3:** $3,500

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnston and Kevin Larson
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Item	2009	2010	2011	2012 (extension)
Travel	1,000	2,500	500	**0**
Propagation	3,500	8,800	2,000	**0**
Plot Fees	0	1,000	1,000	**0**
Total	4,500	12,300	3,500	**0**

Objectives:
1. Establish a pear rootstock advisory committee.
2. Review literature and search national and international collections for pear rootstock accessions.
3. Initiate propagation and planting of a new pear rootstock collection in Washington State.
4. Develop strategy for pre-selection of seedling populations.

Significant Findings:

As some of the parental trees are still in the nursery and not due to be planted until Spring 2013, it was agreed with the pear bureau to extend this project for one year at no further cost.

1. Rootstock germplasm was selected from the interspecific pear collection in Puyallup and budded for the parental collection at Sunrise orchard.
2. Pear rootstock breeding and selection techniques are included as an area of study in two new SCRI proposals.

Methods:
1. A pear rootstock advisory committee made up of industry and research experts will provide input on the objectives, activities and future planning for a pear rootstock research project.
2. Use internet searches, literature and informed contacts to review wide-ranging pear germplasm to identify possible accessions for a new rootstock parental collection.
3. Access germplasm for propagation from collections and other breeding programs, arrange for importation and propagation at commercial nursery.
4. Meet with rootstock experts to discuss possible methods of pre-selection of pear rootstock progenies and develop strategies for handling progenies in a cost-effective, efficient manner.
5. Establish a pear rootstock parental germplasm collection with at least two standard trees of each selection to facilitate future crossing programs.

Results & Discussions:
Further germplasm was assessed during a visit to WSU Puyallup in August. A large collection of interspecific *pyrus* hybrids, originally produced by Westwood, was established at the research and extension center in Puyallup for assessment and possible selection of urban ornamental trees by Dr. Rita Hummel. Trees were selected principally on vigor and represent combinations of 11 different *pyrus* species.

Budwood was supplied to Willow Drive Nursery where the trees were propagated onto OHF 87 rootstock. Trees will be ready to plant at Sunrise orchard in 2012/13.

Ground has been fumigated at Sunrise orchard ready to plant the first batch of parental trees propagated in 2010 this spring.

Discussions continue regarding possible protocols for rootstock selection in seedlings. Attendance at the Washington Pear workshop and the Eucarpia Fruit Breeding and Genetics Symposium provided opportunities to discuss traits of importance as well as selection techniques. Pear rootstock breeding selection techniques have also been included as a research area in the SCRI rootstock proposal Root2Fruit currently being prepared by Dr. Greg Lang. A second SCRI proposal (Dr. Amit Dhingra) will include a wider-scale sourcing of possible rootstock (and scion) germplasm. Both proposals include establishing a pear rootstock breeding program at WSU.