Northwest Pear Research Review

Confluence Technology Center

Wednesday, 2/17/2016

Time	Page	PI	Title	Yrs
8:00		Gix	Welcome new members, housekeeping	
8:15	1	Willett	WTFRC update	
8:30		Moffitt	Pear Bureau	
8:45		McClain	California Pear Advisory Board	
9:00		Godwin	WSU Endowment Advisory Committee	
9:15		Heater	MCAREC report	
			Final Project Reports	
9:30	7	Horton	Tests of a sprayable pheromone formulation against winterform psylla	14-15
9:45	13	Johnson	Optimizing use of Actigard for post-infection fire blight control	14-15
10:00	23	Wang	Controlling postharvest disorders of pears during storage and export	13-15
10:15			Break	
10:30	34	Dhingra	Physiological, economic and consumer evaluation of sliced pears	15
10:45	39	Cooper	Bacterial endosymbionts of pear psylla	15
11:00	45	Einhorn	Improving fruit set, production efficiency, and profitability of pears	15
11:15	Tech	Tynan	Technology Committee: *See Reports in Appendix*	15
11:30			Committee Lunch Discussion - priority discussion/concerns	
12:30 - 2:30			Continuing Projects	
1	54	Cooper	Suppression of pear psylla using elicitors of host-defenses	14-16
1	60	Unruh	Pesticide resistance in pear psylla *No cost extension*	14
1	66	Beers	Miticide resistance in spider mite pests of pears *No cost extension*	13-14
1	73	Moffitt	Health role of pear for Metabolic Syndrome	14-16
2	78	Musacchi	Fall and summer pruning to control vigor and psylla in d'Anjou pear	14-16
2	85	Musacchi	Improving quality and maturity consistency of 'd'Anjou'	14-16
2	92	Wang	Delivering quality pear fruit to consumers	15-17
3	98	Dhingra	Establishing NW-acclimated Pyrus rootstock breeding material	14-16
3	103	Einhorn	Evaluation of potential new pear cultivars for the PNW	15-17
3	108	Neale	Development of marker-based breeding technologies: No-cost extension	14-15
3	114	Evans	Pear rootstock breeding*	15-17

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: MISC-15-100

Project Title: Tree fruit internships: work force development for the future

PI: Kim Kidwell
Organization: WSU
Telephone: (509)335-4562
Email: kidwell@wsu.edu
Address: P.O. Box 646243
Address 2:
City/State/Zip: Pullman, WA 99164-6243

Cooperators:

Total Project Request: **Year 1:** $15,000 **Year 2: $20,000** **Year 3:** $40,000

****Note: The portion of the pear request is 10% of the total project request*****
Other funding sources

Other support will be provided by CAHNRS: Year 2- $35,000, Year 3-$35,000

Agency Name: Washington State University's Center for Transformational Learning and Leadership
Amt. requested/awarded: $35,000/year committed as other support for Year 2 and 3.

(WSU is including the following information on other resources that are available in support of this activity. These resources are activities undertaken by the Principal Investigator (PI) and are not included as a commitment of cost share by WSU.)

Budget 1
Organization Name: CAHNRS CTLL **Contract Administrator:** Herb Lengel/Carrie Johnston
Telephone: 509-335-4562 335-4564 **Email address:** herbert_lengel@wsu.edu carriej@wsu.edu

Item	2015	2016	2017
Salaries			
Benefits			
Wages		$20,000[2]	$40,000[2]
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel	$15,000[1]		
Plot Fees			
Miscellaneous			
Total	$15,000	**$20,000**	$40,000

Footnotes: [1] Center for Transformational Learning and Leadership team members have traveled to the tree fruit region and held focus groups with industry representatives to identify industry mentors, provide orientation on program processes, and set the parameters of expectations for these internship experiences.

[2] Each CAHNRS-sponsored incoming freshman research opportunity will be funded at $2,500/year for Pullman-based experiences during the academic year and $4,000 per year for experiences at the R&E Centers during the summer. These funds can be used for wages, travel, and/or to assist with housing costs. We will attempt to recruit students who live within driving distance of the R&E Centers to defer summer housing costs if possible.

Tree Fruit Internships: Work Force Development for the Future

"A Partnership between the Washington Tree Fruit Research Commission and Washington State University's Center for Transformational Learning and Leadership."

OBJECTIVES

Purpose: Develop a comprehensive partnership among the Washington Tree Fruit Research Commission (WTFRC), the Washington tree fruit industry, and Washington State University's (WSU) Center for Transformational Learning and Leadership (CTLL) in the College of Agricultural, Human and Natural Resource Sciences (CAHNRS) to attract and develop the future workforce of the tree fruit industry. Our collective intention is to provide students with a strong scientific background and immersion-based internship opportunities designed to allow them to explore career opportunities in the tree fruit industry.

Background: The WTFRC approached the CTLL to develop a comprehensive strategy to engage prospective and current undergraduate students in experiential learning opportunities with the expressed intent of developing a future workforce. Based on those discussions, the following proposal will outline a 2 to 3 year plan to engage students in experiences, internships, and careers in the tree fruit industry.

Implementing a Two-Year Pilot Program for Undergraduate Research ($40,000)
Each CAHNRS-sponsored incoming student research opportunity will be funded at $2,500/year for Pullman-based experiences during the academic year and $4,000 per year for experiences at the R&E Centers during the summer. These funds can be used for wages, travel, and/or to assist with housing costs. We will attempt to recruit students who live within driving distance of the R&E Centers to defer summer housing costs if possible. We recommend the following approach for phasing in the pilot project.

Summer 2015 ($15,000 in funding from WTFRC)

Personnel from the CTLL (Kari Sampson, Assistant Director of Recruitment and Retention, Herb Lengel, Internship and Career Development Coordinator, and Kim Kidwell Acting Dean CAHNRS), CAHNRS Alumni and Friends (Chrissy Shelton, Dev. Officer, and Ben McLuen, Director of Development) and Jim McFerson, WSU-TFREC/Manager WTFRC, engaged with the WTFRC and industry representatives to develop the immersion-based industry internship program. Over a four day period we met with 15 Washington tree fruit industry businesses (over 50 individuals) in the greater Wenatchee and Yakima areas to develop a comprehensive partnership intended to attract and develop the future workforce of the tree fruit industry.

Companies visited:

- McDougall & Sons Inc.
- Van Doren Sales, Inc.
- Washington Apple Education Foundation
- Stemilt Growers
- Blue Star Growers Inc.
- Chelan Fruit Cooperative
- Gebbers Farms
- Zirkle Fruit Company
- Allan Bros., Inc.
- WA Fruit & Produce
- WA State Tree Fruit Association
- Borton & Sons Inc.
- Kershaw Companies
- Matson Fruit Company
- Legacy Fruit Company

SIGNIFICANT FINDINGS:

- **Desired attributes of future workforce**: technical aptitude, strong work ethic, effective communication and management skills, passion for position, teamwork, critical thinking, leadership, business savvy, Spanish speaking/bi-lingual, computer skills, vision for the future and adaptable to change.

- **Industry opportunities**: horticulturist, IT, computer science, business accounting/finance, marketing, fruit pathology/physiology, quality control systems, food safety, mechanical engineers, supply chain management, refrigeration/HVAC, and domestic/international sales.

- The Washington tree fruit industry has one point of contact, Herb Lengel, the Internship and Career Development Coordinator, to advertise internship and career opportunities.

- Market tree fruit internship and job opportunities directly to students at WSU.

- Assist with building customized recruitment plans for internship and career opportunities.

- Invite industry to participate in CAHNRS Internship & Career Networking Night.

    - Stemilt Growers and Zirkle Fruit Company attended on 10/05/2015.

        - Due to the event being held during harvest, not many companies could attend. We have also invited companies to attend the WSU Career Expo on 02/02/2016.

        - We are in the planning stages of doing a tree fruit only networking night in the spring.

        - Tree fruit industry site visits this spring with students to their facilities/orchards.

- Network and strengthen relationships with high schools, community colleges, technical schools, FFA, 4H, and the Washington Apple Education Foundation to continually recruit students to CAHNRS majors that are relevant to the tree fruit and allied industries.

    - Kari Sampson, CAHNRS Assistant Director of Recruitment and Retention heads our team of faculty, staff, and students that recruit and educate potential students on the benefits of attending WSU and the great opportunities that the tree fruit industry provides.

- Serve as a liaison for industry partners with the Carson College of Business and Voiland College of Engineering and Architecture to access students in these disciplines.

- Through this partnership, we will provide students with a strong technical background and immersion-based internship opportunities designed to allow them to explore career opportunities in the tree fruit industry. This new approach is designed to ensure that the Center for Transformational Learning and Leadership (CTLL) is creating student programs to meet industry needs.

Fall 2015-Summer 2016 ($20,000 in funding from WTFRC; $35,000 from CAHNRS)

Proposal: The CTLL, in close partnership with the WTFRC, will develop a three phase pilot plan with the intent of building applied technical skills, real work experience, and awareness for the career opportunities in the tree fruit industry[a]. If the pilot is successful, a third year of funding will be requested to expand the program.

Phase 1: Target six top-tier students who will be invited to participate in CAHNRS incoming undergraduate research program Ignite. CTLL personnel will target student recruitment areas to high schools and community colleges within concentrated tree fruit production regions of the state to identify potential students for this program ($15,000 provided by CAHNRS).

Phase 2: Target four top-tier sophomores and/or juniors who will be identified to work with co-investigators on four WTFRC funded projects. Students will spend at least one semester during the academic year working with the Pullman-based faculty member on the WTFRC-funded project, and will spend the summer of 2016 working with the R&E Center-based faculty member or WTFRC scientist. Cost estimates for the year long experience are $10,000 per student ($40,000 total), which will be shared between the WTFRC and CAHNRS.

Phase 3: Mature students with adequate experience will be recruited to participate in immersion-based industry internship experiences. Marketing and recruitment efforts will be managed for industry participants through the CTLL. Internships will be funded by industry partners.

[a] If adequate resources are available, this program can be accelerated by launching all three phases in year 1 with the caveat that students will not receive the benefits of scaffolding experiences across the phases.
[b] Mature, suitably focused and experienced students may skip phase 1 and/or 2 with the mentor's approval.

Results so far:

- **Phase 1**: The Ignite Program launched in the fall of 2015. The CTLL launched a student recruitment and retention effort to encourage top caliber incoming freshman and transfer students to engage in undergraduate research during their first year at WSU. Students with high test scores were offered a $2,500 stipend to support their participation in an undergraduate research or creative project. Funding, provided by Academic Programs and allocated to the faculty mentor, can be used for wages for the student to support work, supplies or travel associated with an undergraduate research project conducted with a faculty mentor.

- 16 high caliber students who selected Agricultural and Food Systems or Integrated Plant Sciences as a potential degree option were invited to participate via a formal letter.
- 9 students responded that they were interested in our program and we moved on to phase two of the recruitment process.
- 2 students accepted the mentorship and are participating in the program this school year.

- CAHNRS is in the process of identifying additional top-tier students to join this program.
- We will continue to try new ways to recruit and educate incoming students on the vast and highly technical careers that are available with the WA tree fruit industry.

- **Phase 2:** Target four top-tier sophomores and/or juniors.
  - Student matching phase, a database of AFS/IPS students in the greater Wenatchee and Yakima areas, has been compiled to market tree fruit internship and research project opportunities.
  - Faculty pairings for Pullman and R&E Centers have been established based on Pullman faculty who have or have had WTFRC funding. The Pullman faculty members will be key contributors in the recruitment and mentoring of our students to our WTFRC-funded projects at our R&E Centers.
  - Each semester, CAHNRS facilitates an internship application process to partner students with world-class faculty internship mentors to create a unique hands on learning experience in Pullman or at our Research and Extension and County Extension locations.
- **Phase 3[b]:** WA tree fruit industry partners working with the CTLL on internship and career opportunities for students.
  - Stemilt Growers utilizes our customized recruitment services. We advertise their internship opportunities directly to students at WSU and coordinate office space for them to conduct interviews on campus. While on campus we also set up classroom visits to get exposure for their company and to recruit additional applicants for their openings.
- Yakima Hort Expo: CTLL personnel and industry partners presented at the Expo to inform the Expo participants of the services that we provide at the CTLL for our industry partners and students.

Fall 2016-Summer 2017 ($40,000 in funding from WTFRC; $35,000 from CAHNRS)
If the pilot is successful, a third cycle of funding to support expansion of the program to create mentored internship experiences for a target of 8 WSU students as described above will be requested to expand the program. The CTLL Internship and Career Development Coordinator will be

responsible for assessing the success of this program and providing progress and output reports to the WTFRC.

Conclusion: We have met our initial goal of connecting with industry leaders in the greater Wenatchee and Yakima areas. Every employer consistently told us that they need help to build up the next generation of tree fruit employees. The CTLL has mapped out the services that we can provide the industry partners and how to execute an internship with WSU. The continued funding will allow the CTLL to further strengthen partnerships with these partners, faculty, staff, and its students. Second, we have identified faculty in Pullman, Prosser, Wenatchee, and Yakima who have WTFRC funding experience who our students can work with on WTFRC funded projects. Third, we will continue to execute an effective marketing plan to our current and potential students to recruit them to these great opportunities to participate in this program. Effective marketing coupled with extraordinary student experiences will secure the long term future of this strategy for creating an employee pipeline for the industry.

FINAL PROJECT REPORT

Project Title: Tests of a sprayable pheromone formulation against winterform psylla

PI: David Horton
Organization: USDA-ARS
Telephone: (509) 454-5639
Email: david.horton@ars.usda.gov
Address: 5230 Konnowac Pass Road
City: Wapato
State/Zip: WA 98951

Other funding sources

Agency Name: Western Region IPM Grants Program
Amount awarded: $23,844

Total Project Funding: $19,000

Budget History:

Item	2014	2015
Salaries	$11,250	$1,500
Benefits	$ 3,750	$ 500
Plot Fees[1]	$ 1,000	$1,000
Total	$16,000	$3,000

[1] Pruning, herbicide, horticultural oil

OBJECTIVE:
Develop and test a sprayable formulation of pheromone

1. Confirm pheromone retains activity in oil
2. Disruption trials
    a. Large cage study
    b. Large plot study

SIGNIFICANT FINDINGS:

- Trapping studies indicated that the pheromone applied as a sprayable in 1% horticultural oil retained its attractiveness to male winterform psylla.
- Large cage study showed that treatment of potted pear trees with sprayable formulation led to ***higher*** mating rates by females rather than a hoped-for lowered mating rate.
- Large replicated field plot trials in two years confirmed the large cage study that the sprayable pheromone formulation led to enhanced mating rates.
    - Hatch rates of field-collected eggs were similar in pheromone plots and control plots, as expected given absence of mating disruption.

RESULTS AND DISCUSSION:

1. Confirm pheromone retains activity in oil

Methods. Paired limbs (each 2-3 foot in length) were drenched with oil (1% in water) + pheromone or 1% oil, and then enclosed in a clear sticky mesh to trap male psylla (Fig. 1). Solutions were applied to drip using a salad spritzer. I prevented overspray from contaminating non-target parts of the tree by collecting overspray with a towel held behind target limbs. I estimate each oil + pheromone limb to have received about 50 female equivalents of spray (some of which was lost to overspray). Traps were collected after 3 days in the field. The trial was done on 6 dates during the winterform generation.

Results. Male winterforms preferentially accumulated on the oil + pheromone traps compared to the oil-alone traps (Fig. 2). These results indicate that the pheromone retained its modest volatility and attractiveness to males even when applied in 1% horticultural oil + water.

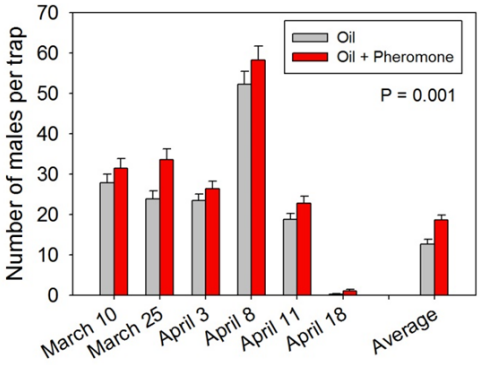

Figure 2. Bar chart showing capture of male winterforms on pheromone mesh traps and oil-alone mesh traps.

Figure 1. Mesh traps encircling oil + pheromone shoots or oil-alone shoots.

2a. Large cage study to examine effects of sprayable pheromone on mating

Methods. Cage studies were conducted out-of-doors with lab-reared (virgin) summerforms, using two large (6 x 6 x 6) ventilated cages (Fig. 3: photographs). Two fully leaved potted trees (approximately 4 foot in height) were set in the center of each cage and misted with either 1% oil in water, or with the pheromone solution (1% oil in water + pheromone; volume of solution approximately equal to 200 female equivalents). To both cages were added 100 females, followed 24 hours later with 100 males.

After 48 hours, 50 females from both cages were collected, and then dissected to determine number of matings.

Results. Results showed that probability of having been mated was actually higher in the pheromone-treatment than the control treatment (Fig. 3: bar graph). Thus, there is evidence that contact with the pheromone in 1% oil actually led to enhanced male success at locating and mating female psylla.

Figure 3. Cage study showing pear trees (2 per cage) and psyllid release vial. Bar chart shows percentage of females that were mated in oil alone trees (gray bars) and oil + pheromone trees (dark bars).

2b. Large plot trials to look for disruption of winterform mating

Methods. Twelve plots (each 16 trees in size) were set out at the Moxee research farm in February 2014 and 2015; six plots were designated oil + pheromone, and six were control (oil alone) plots (see Fig. 4 for design). Psylla were collected at approximately weekly intervals beginning in early February and dissected to determine onset of ovarian development. Once the first mature eggs were seen in dissected females and before mating had begun, plots were sprayed with the two solutions (early March both years). Each plot received approximately 4 gallons of solution applied through a 25 gallon weed sprayer (Scorpion Sprayer) attached to a 4 wheeler (Fig. 5). Tray samples were taken 1 week following application to determine adult densities and sex ratios. At 1 week following application, 40-50 females were collected from each plot and dissected to determine number of matings. Once eggs were seen in the field, spurs (enough to provide data on at least 100 eggs) were collected from each plot for monitoring egg fertility. Eggs were counted on each spur, cut ends of spurs were placed in water, and spurs were re-examined 1 week later to determine hatch rates (Fig. 6).

Results. Probability of being mated was higher both years in pheromone plots than control plots, a result consistent with results using the sprayable formulation in my large cage trials (Fig. 7). At this time, I cannot explain the increased mating rates in the pheromone plots. Sex ratios were the same in control and pheromone plots, thus there was no evidence that enhanced mating in the pheromone plots compared to control plots was due to a higher ratio of males to females in the pheromone plots. One possible explanation for the enhanced rates of mating in pheromone plots is that presence of pheromone stimulated males to increase their search efforts on treated trees, leading to increased rates of contact with females. However, controlled behavioral trials will be needed to examine this

hypothesis. Given the absence of mating disruption, I unsurprisingly found no hoped-for reductions of hatch rates of eggs in pheromone plots (Fig. 8).

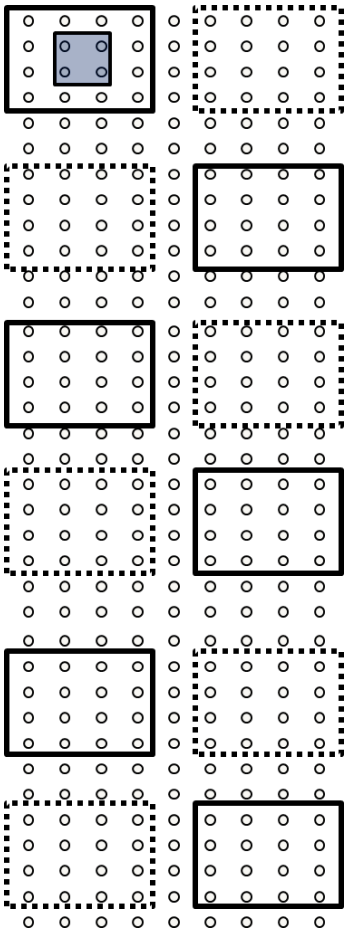

Figure 4. Design of large plot trial showing f oil-alone plots (solid line rectangles) and oil + pheromone plots (dashed line rectangles). Small square in one plot shows the 4 trees from which females were collected for dissection.

Figure 5. Applying oil + pheromone solution.

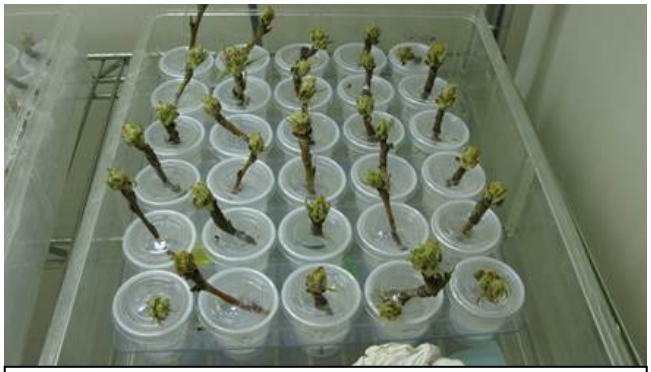

Figure 6. Field-collected spurs being monitored for hatch of psylla eggs.

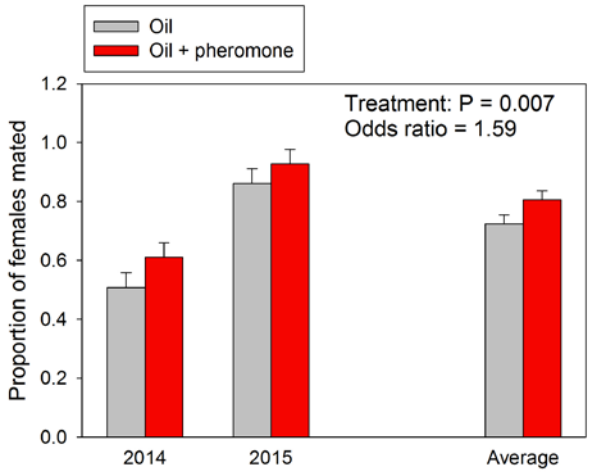

Figure 7 Bar chart shows percentage of females that had been mated in pheromone plots (dark bars) and oil-alone plots (gray bars). Mating was significantly enhanced in the pheromone plots both years.

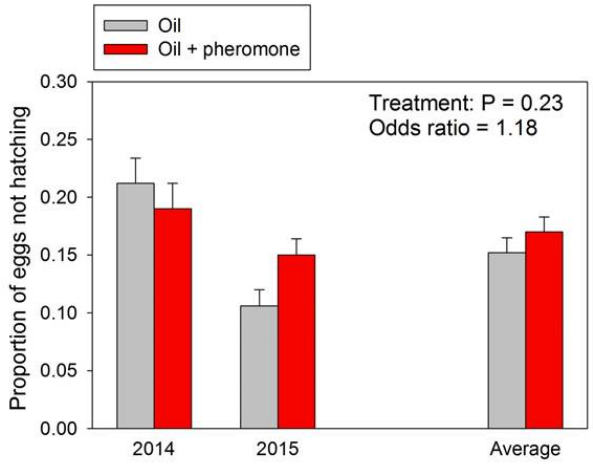

Figure 8. Percentage of eggs not hatching in oil plots (gray bars) and oil + pheromone plots (dark bars).

EXECUTIVE SUMMARY

Management of pear psylla requires some level of control of the post-wintering winterform generation. Pear psylla females overwinter in an unmated condition, and strategies that could be used to delay mating, leading to delays in egglaying combined with the production of early-season infertile eggs, would be useful. The sex pheromone of pear psylla is unfortunately highly non-volatile, making it logistically infeasible to saturate pear orchards by dispensing the compound through traditional dispensers. However, the compound is a hydrocarbon and is thus fully soluble in the horticultural oils used during the delayed dormant period in commercial orchards. This study examined whether using a sprayable, to saturate orchard with pheromone, would interfere with the male's ability to find unmated winterform females during the early post-winter generation.

A trapping study showed that the sprayable formulation is attractive to mate-seeking male winterforms, indicating that the pheromone retains its attractiveness when mixed with horticultural oil. However, cage studies and large field-plot trials failed to demonstrate that mating success declined on trees or in plots fully saturated with the sprayable formulation. Indeed, I observed the opposite effect in both types of trials: the sprayable formulation, when applied to whole-trees or to multi-tree plots, led to ***higher*** rates of mating than observed in control (oil alone) plots. The reasons for this response are not clear. One hypothesis is that the compound stimulated increased mate-searching activities of the male psyllids, possibly including an increase in acoustic signaling by males, which we now know (from a 2015 publication) is used by males of a very closely related pear psyllid to locate females for mating. This hypothesis merits attention, as disruption of mating through saturation of the environment with synthetically produced acoustic signals has been shown to reduce mating in acoustic-signally leafhoppers and in another pest psyllid (Asian citrus psyllid).

FINAL REPORT **YEAR**: 2 of 2

Project Title: Optimizing use of Actigard for post-infection fire blight control

PI: Ken Johnson
Organization: Dept. Botany and Plant Pathology, Oregon State University, Corvallis
Telephone/email: 541-737-5249 johnsonk@science.oregonstate.edu

Cooperators: Rachel Elkins UC-ANR, Lake County
Tim Smith WSU, Wenatchee
Steve Castognoli OSU, Hood River

Budget: **Year 1:** $21,400 **Year 2**: $22,042

Other funding sources

Agency Name: Syngenta Crop Protection ($5K)

WTFRC Collaborative expenses: None

Budget
Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Russ Karow
Telephone: (541) 737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	**2014-15**	**2015-16**	
Salaries Faculty Res. Assist.	12,000	12360	
Benefits OPE 58%	6,960	7168.8	
Wages undergrads	500	515	
Benefits OPE 12%	60	61.8	
Equipment			
Supplies	880	906.4	
Local Travel	500	515	
Miscellaneous			
Plot Fees	500	515	
Total	$21,400	$22,042	

OBJECTIVES

Obj. 1: In the field, evaluate the timing of Actigard paints to prevent running fire blight cankers and to suppress canker re-ignition.

Obj. 2: In the greenhouse, re-evaluate the concentration of Actigard in paints applied to slow fire blight canker expansion in pear.

Obj. 3: Evaluate alternative SAR inducers and surfactants.

SIGNIFICANT FINDINGS

- For a 5th season, a paint of concentrated acibenzolar-S-methyl (ASM, Actigard) used in combination with cutting reduced the severity of 're-ignited' fire blight cankers in Bosc and Concorde pear.
- In a greenhouse study, alternative SAR inducers did not suppress fire blight expansion to the same degree as ASM.
- A trial of silicone surfactants mixed with ASM found equivalent performance among surfactants.
- A summary of spray trials conducted in Wenatchee and Corvallis over the past five years demonstrated the addition of ASM to antibiotic sprays enhanced fire blight control over antibiotics alone.

Results

Objective 1: In the field, evaluate the timing of Actigard paints to prevent running fire blight cankers and to suppress canker re-ignition**.**

In 2015, this objective was addressed in two Bosc pear blocks (7-yr-old and a 5-yr-old) located at the Oregon State University Botany and Plant Pathology Field Laboratory near Corvallis, OR. , and in potted Concorde pear trees (3-yr-old in 3-gallon pots) located at the same facility. The experiments were arranged in a randomized complete block design with 20 to 23 replications. On 15 April, a cluster of flowers in three different areas of the trees were mist inoculated with a high dose of the pathogen. (Experimental details are in **Table 1** on the next page.) After running cankers were established in the trees, experimental units (trees) were randomized into blocks and treatments such that each block and each treatment had approximately the same number of strikes per tree. In the Bosc pear blocks, the first ASM treatment was timed to occur at 'most symptoms appeared' (mid-May), and in Concorde pear, the first ASM treatment was timed to occur at 'first symptoms'. The primary cut of fire blight strikes coincided with the first ASM treatment. Cankers were cut 15-20 cm (6-8") below canker margin. Treatments of ASM associated with the primary cut

Fig. 1. ASM treatments were 'painted' onto to central leaders of Bosc pear trees with a 1-liter Solo pump sprayer.

were applied to the central leader with a small Solo sprayer (**Fig. 1**); the length of leader treated was approximately 1 m (39 in.) and was located within the branching zone for Bosc trees and the lower trunk region for Concorde trees. In June, a second ASM treatment was applied to the central leader in the 7-yr-old Bosc block and to some of the Concorde pear trees. No secondary cuts were made during the summer. In late September/early October, treatment efficacy was evaluate by measuring length and weight of re-ignited fire blight cankers.

Table 1. Experimental details of 2015 ASM post-infection treatments applied to 7-yr-old and 5-yr-old Bosc pear in orchards near Corvallis, OR

Pear cultivar & year	Tree age (years)	Pathogen inoculation type and date	Treaments	Rate of ASM (a .i.)	Amount of ASM applied	Number of replicate trees	Cankers per tree (± s.e.) at 1° cut	Date(s) cankers removed 1°, 2° and 3° cuts	Cut distance below proximal edge of canker	Date(s) ASM painted	% Canker re-ignition after 1° cut	Cut canker yield 2° cuts kg (± s.e.)/tree	$P \leq 0.05$	Cut canker length 2° cuts m (± s.e.)/tree	$P \leq 0.05$
					On central leader:			Once		Twice		28-Sep		28-Sep	
Bosc	7	Flowers	Cut only	-	-	22	3.2 (0.4)	21-May	15-20 cm	-	24% on 28-Sept	2.0 (0.8)		0.28 (0.11)	
2015		1 x 10^6 CFU/ml on 15-Apr	Cut & Paint (sprayer)	15 g /L 1% Pentrabark	~750 mg in 50 ml	22	3.2 (0.4)	21-May	15-20 cm	21-May, 2-Jun#	7% on 28-Sep	0.2 (0.1)	yes	0.12 (0.06)	no
					On central leader:			Once		Once		8-Oct		8-Oct	
Bosc	5	Flowers	Cut only	-	-	23	3.2 (0.5)	21-May	15-20 cm	-	21% on 8-Oct	1.4 (0.5)		0.34 (0.10)	
2015		1 x 10^6 CFU/ml on 15-Apr	Cut & Paint (sprayer)	15 g /L 1% Pentrabark	~750 mg in 50 ml	23	3.2 (0.5)	21-May	15-20 cm	21-May	3% on 8-Oct	0.1 (0.1)	yes	0.04 (0.03)	yes
					On central leader:			Once		Once or Twice		28-Sep		28-Sep	
Potted Concorde	3	Flowers	Cut only	-	-	20	4.3 (0.6)	6-May	15-20 cm	-	13% on 28-Sept	0.13 (0.04)		0.38 (0.12)	
2015		1 x 10^9 CFU/ml on 15-Apr	Cut & Paint (sprayer)	15 g /L 1% Pentrabark	~60 mg in 4 ml	20	4.3 (0.6)	6-May	15-20 cm	7-May	7% on 28-Sep	0.02 (0.01)	yes	0.01 (0.01)	yes
			Cut & Paint (sprayer)	15 g /L 1% Pentrabark	~60 mg in 4 ml	20	4.3 (0.7)	6-May	15-20 cm	7 May, 22-Jun#	5% on 28-Sep	0.01 (0.1)	yes	0.01 (0.01)	yes

7-yr-old Bosc pear. Weather conditions were cool during pear bloom, which resulted in light infection as a result of the pathogen inoculation at full bloom. An average of 3.2 fire blight cankers developed on each tree (**Table 1**). ASM treatments were made on 21 May and 2 June; the primary cut occurred on 21 May. After cutting, running cankers re-ignited in 81% of non-ASM-treated trees which was 24% of cuts. The final evaluation (2º cut) of re-ignited cankers was made on 28 September. Compared to cut only, the ASM paint treatment significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers (yield of canker wood) (**Fig. 2**) but did not significantly reduce the lengths of secondary cankers (**Table 1**). Over the summer, 5 non-treated trees died as a result of re-ignited fire blight. Zero trees that received the ASM treatment died.

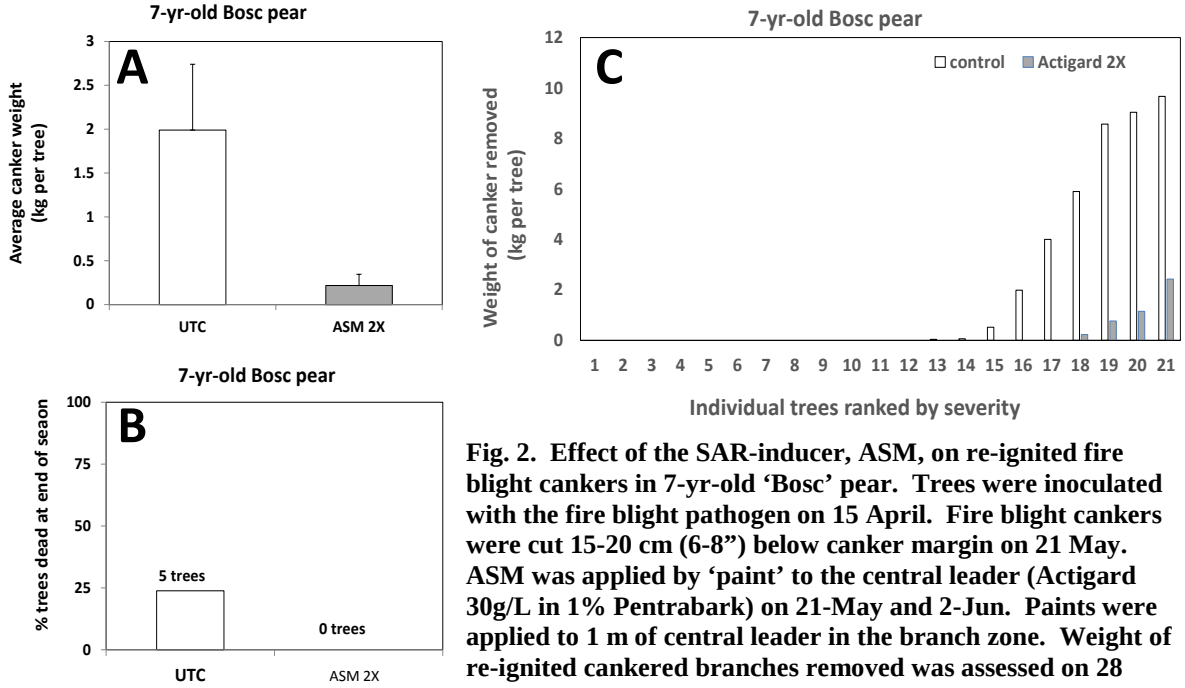

Fig. 2. Effect of the SAR-inducer, ASM, on re-ignited fire blight cankers in 7-yr-old 'Bosc' pear. Trees were inoculated with the fire blight pathogen on 15 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 21 May. ASM was applied by 'paint' to the central leader (Actigard 30g/L in 1% Pentrabark) on 21-May and 2-Jun. Paints were applied to 1 m of central leader in the branch zone. Weight of re-ignited cankered branches removed was assessed on 28 September. A and B: Each bar is the mean and standard error of 21 trees. C: Ranked comparison of the disease severity on individual 'cut and ASM-treated' trees compared to individual

5-yr-old Bosc pear. An average of 3.2 fire blight cankers developed on each tree as a result of the pathogen inoculation at full bloom (**Table 1**). The ASM treatment coincided with the primary cut on 21-May. Running cankers re-ignited in 60% of non-ASM-treated trees which was 21% of cuts. The final evaluation (2º cut) of re-ignited cankers was made on 8 October. Compared to cut only, the ASM paint treatment significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers (yield of canker wood) (**Fig. 3**) and also reduced the length of secondary cankers (**Table 1**)). Over the summer, 2 non-treated trees died as a result of re-ignited fire blight. Zero trees that received the ASM treatment died.

3-yr-old potted Concorde pear. An average of 4.3 fire blight cankers developed on each tree as a result of the pathogen inoculation at full bloom (**Table 1**). The ASM treatments were made on 7 May and 22 June with the primary cut occurring on 6 May. Running cankers re-ignited in 55% of non-ASM-treated trees, which was 13% of cuts. The final evaluation (2º cut) of re-ignited cankers was made on 28 September. Compared to cut only, the ASM paint treatment significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers (yield of canker wood) (**Fig. 4**) and also reduced the length of secondary cankers (**Table 1**)). Over the summer, 9 non-treated trees died as a result of re-ignited fire blight. Two trees in each of the ASM treatments died.

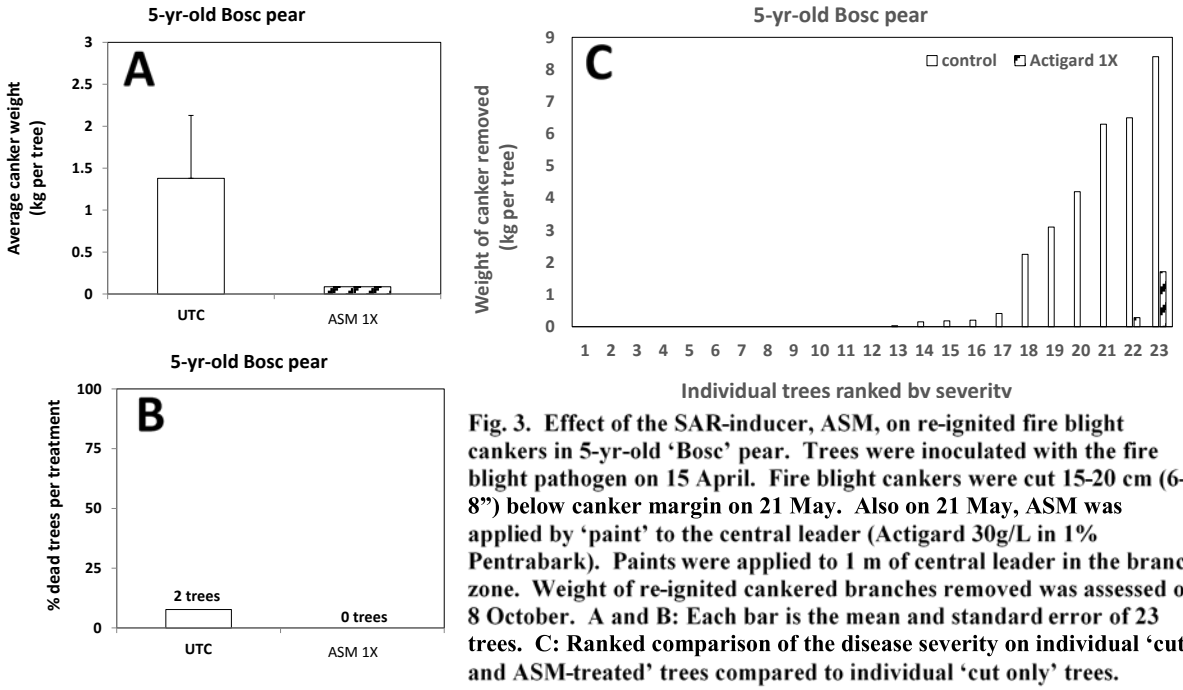

Fig. 3. Effect of the SAR-inducer, ASM, on re-ignited fire blight cankers in 5-yr-old 'Bosc' pear. Trees were inoculated with the fire blight pathogen on 15 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 21 May. Also on 21 May, ASM was applied by 'paint' to the central leader (Actigard 30g/L in 1% Pentrabark). Paints were applied to 1 m of central leader in the branch zone. Weight of re-ignited cankered branches removed was assessed on 8 October. A and B: Each bar is the mean and standard error of 23 trees. C: Ranked comparison of the disease severity on individual 'cut and ASM-treated' trees compared to individual 'cut only' trees.

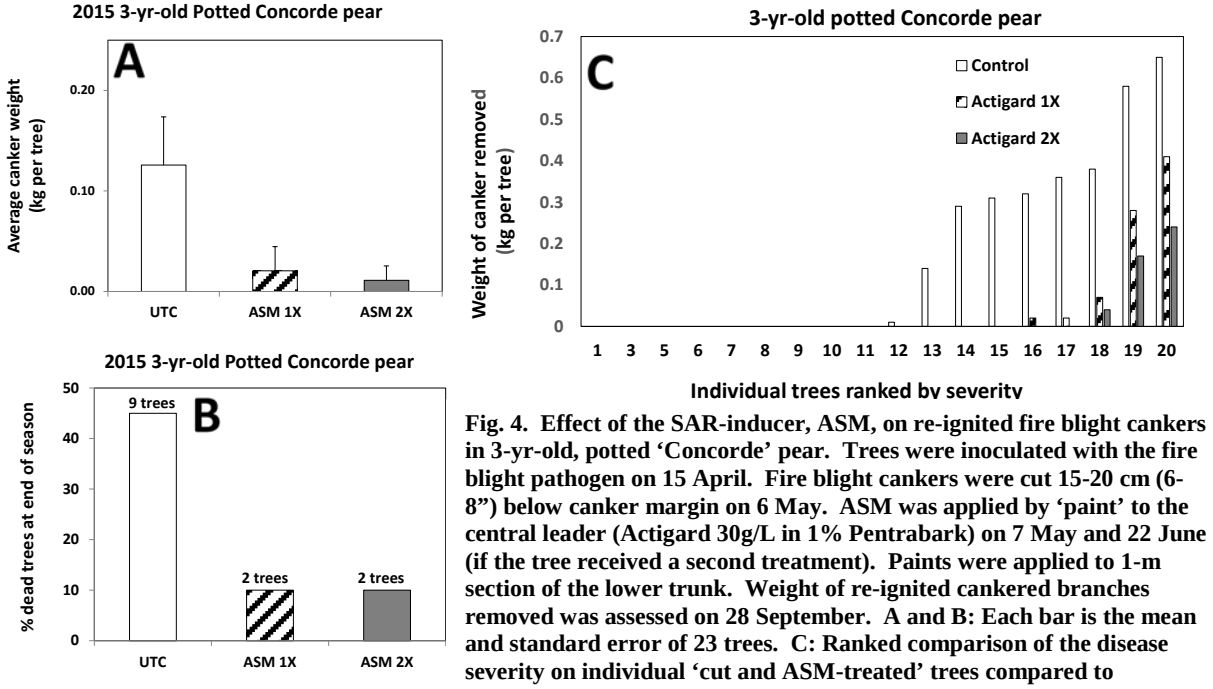

Fig. 4. Effect of the SAR-inducer, ASM, on re-ignited fire blight cankers in 3-yr-old, potted 'Concorde' pear. Trees were inoculated with the fire blight pathogen on 15 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 6 May. ASM was applied by 'paint' to the central leader (Actigard 30g/L in 1% Pentrabark) on 7 May and 22 June (if the tree received a second treatment). Paints were applied to 1-m section of the lower trunk. Weight of re-ignited cankered branches removed was assessed on 28 September. A and B: Each bar is the mean and standard error of 23 trees. C: Ranked comparison of the disease severity on individual 'cut and ASM-treated' trees compared to individual 'cut only' trees.

Discussion Actigard paints to prevent running fire blight cankers and to suppress canker re-ignition. For a 5th season, a paint(s) of concentrated acibenzolar-S-methyl (ASM) used in combination with cutting reduced the severity of 're-ignited' fire blight cankers in Bosc and Concorde pear. In contrast to previous seasons, the number of fire blight strikes on the trees as a result of pathogen inoculation was modest (3 to 5 strikes per tree), but this level of infection is more typical of a commercial orchards. In addition, we made the first ASM treatments within a day of the primary cut of cankers,

whereas last year we did not cut until a period of time (12 to 26 days) after the first ASM treatment (see 2014 report). Perhaps for these reasons, the observed effect of ASM on reducing secondary canker re-ignition and expansion was somewhat better than we have observed in previous experiments. The central leader 'paint' of ASM (applied by small sprayer) again provided results consistent with our earlier method of applying the ASM treatment to the 12-18 inches of healthy branch immediately below each cut canker (see previous reports). Treatment of the central leader requires much less time to implement than painting of specific diseased branches. But on larger trees, painting a branch (with a small sprayer) might be more practical.

The results we have had over the course of this research suggest that ASM therapy will be useful in commercial orchards after a fire blight infection event, especially during early years after orchard establishment (ages 2- to 10-yr-old) when clean-up from this disease has proven difficult to manage with therapeutic pruning only. The ASM treatment induces SAR in the living cylinder of non-symptomatic parenchyma and cambial tissues near the leading edge of the expanding canker. Personnel cutting fire blight cankers in commercial orchards also commonly use a disinfesting solution (e.g., bleach) to clean pruning tools between cuts, and therefore, could easily adopt the additional practice of painting a trunk or branch with ASM as cankers are removed. In fact, based on years of experience in inoculating the pathogen and cutting blight, we believe most secondary cankers that develop at the location of a primary cut are the result of inoculum that originated inside the tree and not from inoculum spread canker-to-canker by cutting tools. **Consequently, for young trees at risk of developing secondary (re-ignited) cankers, it is our opinion that treatment of the cut trees with ASM will provide greater benefits than disinfestation of tools between cuts.**

In the translation of results from small plot trials to commercial orchards, there are several caveats/issues that may only be resolved after commercial orchardists have gained experience with the ASM material and the painting technique. One issue is that trees in commercial orchards typically grow faster than our plot trees because of higher inputs of nitrogen fertilizers. Nitrogen is a known risk factor contributing to the susceptibility of the trees and development of secondary fire blight cankers. Consequently, we are concerned there may be an interaction between ASM-paint treatment efficacy and nutritional status of the tree (we are attempting to address this in 2016). A second caveat is that in order to obtain a reasonable amount of re-ignited cankers to work with, our primary cuts were 'short' (6 to 8" below the canker edge) compared to standard recommendations for cutting fire blight cankers (12 to 14 inches below the canker edge). Thus, our rate of re-ignition may be higher is typical with a good blight cutting crew, which could de-value an ASM treatment in marginal situations. [But note that the first two caveats potentially cancel each other out.] A last issue is the rate of ASM in a paint suspension compared to the amount that is legal to apply acre per day and per season (see label below **Fig. 5**). After a severe infection event, it would be easy to exceed these amounts if every tree in the orchard was diseased and painted with ASM. Therefore, this technique

Fig. 5. 2015 EPA section 3 registration for Actigard 50W outlining paint application after canker cut-out.

Crop	Pest	Rate per Application	Remarks
Apples **Pears**	Suppression of: Fire Blight *(Erwinia amylovra)*	Per Acre 0.5 - 3.2 oz/A	**Foliar Application:** Apply in a tank mix with a fire blight treatment (generally an antibiotic) that is standard in your area. This is generally 2-3 applications between 20% bloom and petal fall depending on the environmental conditions. Do not apply closer than a 7 day interval.
		1 oz/1 quart of 1% penetrant	**Paint application after canker cut-outs or grafts:** * Mix 1 oz Actigard in 1 quart of 1% Pentrabark or similar penetrant. Apply to the branch area immediately below canker after cutting to an area extending 1 – 1 1/2 feet. One quart will treat approximately 500 cuts. Do not apply within 60 days of harvest.
Pome Crop Group 11-10: Apple; azarole; crabapple; loquat; mayhaw; medlar; pear; pear, Asian; quince; quince, Chinese; quince, Japanese; tejocote; cultivars, varieties, and/or hybrids of these.			
Specific Use Restrictions: (1) Do not apply more than 3.2 oz (0.1 lb ai) Actigard 50WG per acre per application. (2) Do not apply more than 12.8 oz (0.4 lb ai) of Actigard 50WG per acre per season. (4) Do not apply within 60 days of harvest (60-day PHI).			

will be most useful in a well-managed commercial orchards (i.e., those with spring fire blight preventative program) where it is implemented by orchard workers pruning out fire blight at the level of the more sporadically distributed, individual diseased tree.

Obj. 2: In the greenhouse, re-evaluate the concentration of Actigard in paints applied to slow fire blight canker expansion in pear, and **Obj. 3:** Evaluate alternative SAR inducers and surfactants.

In 2014, greenhouse experiments under this objective failed because the 200 Bosc pear trees we purchased to address these objectives apparently had been frozen after digging in the nursery. In 2015, the nursery from whom purchased pear trees could fill only 60% of our order, which left us with too few trees to obtain sufficient replication in the experiments. Nonetheless, we conducted all treatments with reduced tree replicates, which resulted in higher than usual variability in the treatment means.

In conducting the greenhouse trials, we went back to experimental protocols first used in 2009. One-year-old trees pear cv. 'Bosc' were potted into 2 gallon containers containing growth medium and maintained in a greenhouse (70-85°F). Treatments were arranged onto experimental tree; 5 to 10 single-tree replicates per treatment. At inoculation (23 April), terminal shoots were ~48 cm (20 in.) in length; terminal shoots were inoculated by splitting the meristematic tip and mid-veins on the two youngest leaves longitudinally with a surgical scissors to distances of 1 to 2 cm. Wounded tissues were dipped into freeze-dried cells of *E. amylovora* strain Ea153N resuspended in distilled water (1 x 10^9 CFU/ml). After inoculation, a plastic bag was wrapped over the cut end and left in place for one week. Length of cankers on inoculated trees were measured every 6 weeks. Treatments included one to two paint treatments of ASM or another SAR inducer in combination with PentraBark or an alternative surfactant. These treatments were applied with a foam brush to a 60-cm length of trunk with the proximal edge of the treated area located just above the graft union.

Treatment effects are best viewed by distinguishing if mean canker length expanded into the woody trunk tissue (upper half of **Fig. 6**) or mean canker length was limited to the green shoot tissue produced earlier in the spring (lower half of **Fig. 6**). Using this criterion, treatments that included

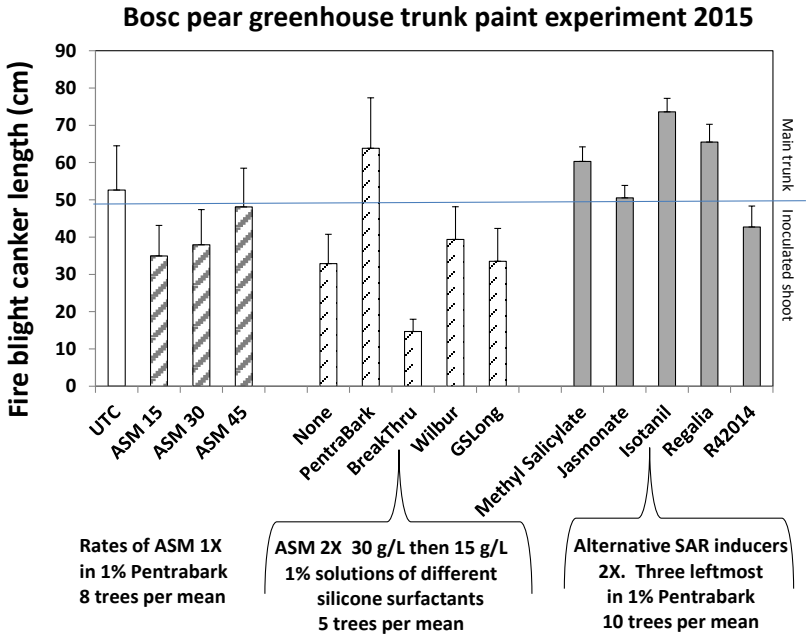

Fig. 6. Effect trunk paints of SAR-inducers and silicone surfactants on expansion of fire blight cankers in 1-yr-old Bosc pear. All trees were inoculated on 23 April 2015. Trunk paint treatments were made on 27 April and if a tree received a second treatment, 29 May. Each bar is the mean and standard error of the number of trees indicated in the legend.

ASM generally did not expand into woody trunk tissue. The exception was ASM with PentraBark, which has been our standard surfactant in SAR field trials (i.e., we know ASM mixed with PentraBark is an effective treatment). In contrast, ASM with the surfactant BreakThru yielded the smallest cankers. For the alternative SAR inducers, most of the mean cankers lengths extended into woody tissue which was also the case with the untreated control (UTC). For the 50 trees that received an alternative SAR inducer, mean canker length was 59 cm, and cankers, on average, extended into woody tissue. For the 49 trees that received an ASM treatment, mean canker length was 41 cm, and cankers, on average, did not extend into woody tissue.

Discussion of greenhouse SAR and surfactant experiments. Without benefit of multiple years of experiments, the greenhouse results indicate that ASM is the best (known) SAR inducer for post-infection therapy of pear and apple after a fire blight infection. Again, as mentioned above, there are too few replications for the individual treatments, and therefore, the result for any one specific treatment should be viewed cautiously. Nonetheless, results with ASM and alternative surfactants yielded a few surprises. For example, ASM with no surfactant performed similarly to the average of ASM with a surfactant, and ASM with PentraBark was a poor performer relative to ASM with BreakThru. Consequently, in field experiments in 2016, in at least one trial we will compare ASM with BreakThru and ASM with no surfactant to ASM with PentraBark.

Supplemental Results: ASM foliar spray trial research in 2010 to 2015.

Orchard studies on the integration of acibenzolar-S-methyl (ASM) with antibiotics for protection of pear and apple from fire blight have conducted in the west coast region (OSU, Corvallis and WSU, Wenatchee) for the last 5 years. In 11 pathogen-inoculated trials, a single treatment of

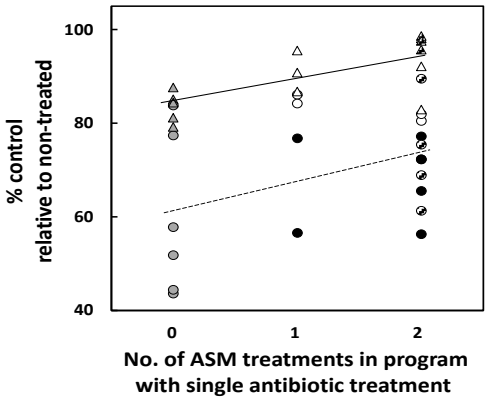

Fig. 7. Percent control of fire blight infection by one antibiotic treatment in combination with one or two applications of acibenzolar-S-methyl (2 oz. per 100 gallons). Points are from 11 orchard trials conducted in Wenatchee, WA (T. Smith) and Corvallis, OR (K. Johnson) from 2010 to 2014. In leftmost column, the shapes indicate the antibiotic used in each trial: triangle = streptomycin, and circle = oxytetracycline. In center and rightmost columns, color of the shapes indicate timing of ASM treatment(s): black = late bloom, white = early bloom, and striped = before and after the antibiotic treatment. Lines are regression of relative % control on number of ASM treatments.

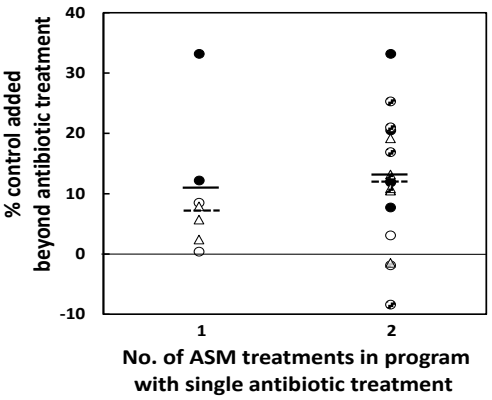

Fig. 8. Fig. 2. Percent added control of fire blight from one or two applications of acibenzolar-S-methyl (2 oz. per 100 gallons) beyond that achieved by one antibiotic treatment. Points are from 10 orchard trials conducted in Wenatchee, WA and Corvallis, OR from 2010 to 2014. In the columns, the shape used for each data point indicate the antibiotic used in the trial: triangle = streptomycin, and circle = oxytetracycline. Colors of the shapes indicate timing of ASM treatment(s): black = late bloom, white = early bloom, and striped = before and after the antibiotic treatment. Short horizontal bars in each column are the mean (solid) and median (dashed) responses.

streptomycin or oxytetracycline provided an average of 83 and 61% disease control, respectively. The addition of one or two treatments of acibenzolar-S-methyl (ASM) to the single antibiotic program contributed an additional 6 and 12% disease control, respectively, for both antibiotic materials. Among trials, ASM treatment timings were varied from early to late bloom but an effect of timing on disease control could not be determined. In commercial pear orchards, ASM treatments at full bloom and petal fall were superimposed onto the antibiotic program used in each orchards. For the 14 orchards that developed fire blight, the ASM-treated plots showed 38% fewer infections than an adjoining plots that received antibiotic program only. When integrated with antibiotics, ASM provides added disease suppression to fire blight control programs, but the modest degree of protection provided will likely limit its use to high disease risk situations, which includes orchards with a previous disease history, and those planted recently to highly susceptible cultivars.

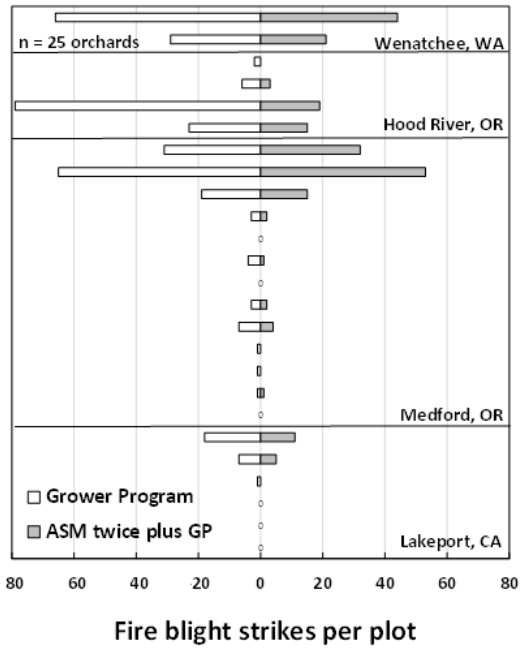

Fig. 9. Number of fire blight infections in 4-acre plots of commercial pear orchards located in northern California, southern and northern Oregon, and northern Washington as affected by grower's antibiotic program alone or grower's program plus two additional treatments of acibenzolar-S-methyl (ASM, 70 g a.i./ha). Trials were conducted in commercial orchards of cultivar 'Bartlett' located near the cities shown. ASM treatments were applied at full bloom and near petal fall with fire blight infections scored 3 to 5 week after full boom.

Discussion. ASM is a new addition to the toolbox for fire blight management. In spray trials, it continues to show value as program partner with antibiotics during bloom, which could prove to be cost effective in high risk/high value orchards. We speculate that the suppression achieved by ASM sprays in conjunction with antibiotics is due to a longer residual time (7-10 days) compared to antibiotics (~3 days). This property may extend its usefulness to suppression of rattail and shoot infection (see 2014 report), and of trauma blight (infection from storm-induced wounds), which is difficult to suppress with antibiotics only. The EPA section 3 registration of Actigard 50W for use on pome fruit was granted in late 2015; first commercial use in Washington State will occur in 2016.

Publications:

Johnson, K. B., and Temple, T. N. 2016. Comparison of methods of acibenzolar-S-methyl application for post-infection fire blight suppression in pear and apple. Plant Dis. 100: doi:10.1094/PDIS-09-15-1062-RE.

Johnson, K.B., Smith, T. J., Temple, T. N., Gutierrez, E., Elkins, R. E., and Castagnoli, S. 2016. Integration of acibenzolar-S-methyl with antibiotics for protection of pear and apple from fire blight caused by *Erwinia amylovora.* Crop Protect. (in review).

EXECUTIVE SUMMARY

Project Title: **Optimizing use of Actigard for post-infection fire blight control**
Investigator: Ken Johnson, Oregon State University

Significant findings:

- For a 5th season, a paint of concentrated acibenzolar-S-methyl (ASM, Actigard) used in combination with cutting reduced the severity of 're-ignited' fire blight cankers in Bosc and Concorde pear.
- In a greenhouse study, alternative SAR inducers did not suppress fire blight expansion to the same degree as ASM.
- A trial of silicone surfactants mixed with ASM found equivalent performance among surfactants.
- A summary of spray trials conducted in Wenatchee and Corvallis over the past five years demonstrated the addition of ASM to antibiotic sprays enhanced fire blight control over antibiotics alone.

Industry implications: Over the last six years, the goal of this project has been to identify a material and method(s) to induce systemic acquired resistance (SAR) in pear and apple trees as an aid to the restoration of tree health after fire blight infection. The need for an improved therapy arises because the current method of cutting fire blight cankers out of trees in late spring and early summer frequently fails to restore health, especially in the first 10 years after orchard establishment (i.e., multiple rounds of cutting are required and frequently, the trees die). We found that in conjunction with cutting blight, acibenzolar-S-methyl (ASM) applied as a branch or trunk paint induces SAR in a tree for a prolonged period (at least 2 months), places the material near where it is most needed in the tree, and is potentially adaptable to specific fire blight management situations in the orchard.

Based on our data, trunk paints were most effective at restoring tree health when applied at the time of cutting. The result of an ASM paint is that fewer cankers re-ignite and those cankers that do re-ignite are smaller than on non-ASM treated trees. Personnel cutting fire blight cankers in orchards commonly use a disinfesting solution (e.g., bleach) to clean pruning tools between cuts, and therefore, could easily adopt the additional practice of painting a trunk or branch with ASM as cankers are removed. In fact, based on years of experience in inoculating the pathogen and cutting blight, we believe most secondary fire blight cankers that develop at the location of a primary cut are the result of inoculum that originated inside the tree and not from inoculum spread canker-to-canker by cutting tools. Consequently, for young trees at risk of developing secondary (re-ignited) cankers, it is our opinion that treatment of the cut trees with ASM will provide greater benefits than disinfestation of tools between cuts.

In addition to post-infection treatments, we also have found that ASM provides value as program partner with antibiotics sprayed during bloom, which could prove to be cost effective in high risk/high value orchards. We speculate that suppression achieved by ASM sprays in conjunction with antibiotics is due to a longer residual time (7-10 days) compared to antibiotics (3 days). This property also may extend its usefulness to suppression of rattail and shoot infection (see 2014 report), and of trauma blight (infection from storm-induced wounds), which is difficult to suppress with antibiotics.

The EPA section 3 registration of Actigard 50W (ASM) for use on pome fruit was granted in September 2015; first registered uses in Washington and Oregon orchards will occur in 2016. All methods of application discussed in this report are on the section 3 label.

FINAL REPORT **YEAR:** 3 of 3

Project Title: Controlling postharvest disorders of pears during storage and export

PI: Yan Wang
Organization: OSU MCAREC
Telephone: 541-386-2030 (38214)
Email: yan.wang@oregonstate.edu
Address: 3005 Experiment Station
City/State/Zip: Hood River/OR/97031

Cooperators: Xingbin Xie, Todd Einhorn, Steve Castagnoli, David Sugar, Wade Root, Craig Mallon

Total Project Request: **Year 1:** $25,090 **Year 2:** $25,751 **Year 3:** $26,431

Other funding sources: None

Budget
Organization Name: Agricultural Research Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2013	2014	2015
Salaries	13,088[1]	13,481	13,885
Benefits	1,250[2]	1,300	1,352
Wages	6,715[3]	6,917	7,124
Benefits	537[4]	553	570
Equipment			
Supplies	3,000[5]	3,000	3,000
Travel	500[6]	500	500
Miscellaneous			
Total	25,090	25,751	26,431

Footnotes:
[1]Postdoctoral Research Associate (Dr. Xingbin Xie): 1/3 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 500hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 8% of the wage.
[5]Supplies: maintaining cold rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

OBJECTIVES

1. Control senescence disorders and extend storability of summer pears by ethylene inhibitors
2. Reduce pear scuffing by wax coatings
3. Evaluate the efficacy of a premix formulation of Difenoconazole + Fludioxonil on storage decays

SIGNIFICANT FINDINGS:

Objective 1. Control senescence disorders and extend storability of summer pears by ethylene inhibitors

1. Pre-harvest AVG efficacy on extending storability of 'Bartlett' is affected by application rate, timing, and fruit harvest maturity.

- AVG at 60-120ppm applied 1 week before harvest (WBH) extended storability of 'Bartlett' for H1 (H1=19lb) and H2 (12d after H1, H2=18lb) fruit. H3 (17d after H1, H3=17lb) fruit did not response to the AVG treatments.
- AVG applied 2 WBH1 had little effect on storability.
- AVG applied 1 WBH1 did not affect the initial harvest maturity (H1), but delayed fruit maturation on the tree about 5d for H2 and H3 fruit.

2. Postharvest 1-MCP efficacy on extending storability of 'Bartlett' is inconsistent at commercial application among lots and years. To ensure a consistent 1-MCP efficacy:

- Harvest fruit at ≥19lb, especially for fruit from higher production elevations (i.e., >1,000ft).
- Treat fruit within 10-12 days after harvest. Eliminate field heat quickly and store fruit at 30°F during the treatment delay.
- Vent out exogenous ethylene (if > 300ppb) in the treating room before 1-MCP treatment.

3. Pre-harvest AVG or postharvest 1-MCP treatments extend storage life of 'Starkrimson'.

- 'Starkrimson' produces a higher amount of ethylene and has a higher respiration rate and therefore a shorter storage life compared to other PNW pear cultivars.
- AVG at 60-120ppm applied 1 week before harvest extends 'Starkrimson' storage life without significant effect on ripening capacity following cold storage.
- 1-MCP at 300ppb extends 'Starkrimson' storage life to 4 months at 30°F. However, it took 2 weeks to ripen at 68°F following 4 months of cold storage.

Objective 2. Reduce pear scuffing by wax coatings

- 'Comice', 'Anjou', 'Bartlett', 'Bosc', and 'Starkrimson' are in the increasing order regarding susceptibility to anaerobic injury.
- Compared to the commercial standard rate of 5-6% solid, Carnauba wax coating at solid of 7-8% decreases friction forces and therefore reduce scuffing without causing anaerobic flavor of 'Comice' pear. Sugar-ester edible coating at 0.5-1.0% a.i. reduces scuffing and maintained green color without affecting ripening capacity and flavor of 'Bartlett' pear.
- Ethoxyquin at 1000ppm mixed in wax coating may slow down chlorophyll degradation and reduce scuffing expression of 'Comice'.

Objective 3. Evaluate the efficacy of a premix formulation of Difenoconazole + Fludioxonil

- The pre-mix formulation of Difenoconazole + Fludioxonil (Syngenta product) applied as drenching at 16 oz. per 100 gallons controls blue and gray mold decays at levels equivalent to Penbotech or Scholar alone.

- The different modes of action between Difenoconazole and Fludioxonil in the pre-mix may retard resistance development in the pathogens.

METHODS

Objective 1. Extend storability of summer pears by ethylene inhibitors

'Bartlett'. The effects of harvest maturity (19-16lb), delayed treatment (1-12d), production elevations (500-2,000ft), and exogenous ethylene (0-1ppm) on efficacy of 1-MCP were studied. A 40m^3 cold room at 32°F was used to treat fruit with 1-MCP at the commercial rate of 300ppb for 24h. Treated fruit were stored at 30°F. Fruit color, I_{AD}, IEC, FF, senescent scald, internal breakdown, and flavor were evaluated before and during storage for 4 months. The effects of pre-harvest AVG spray rate (30, 60, and 120ppm) and timing [1 and 2 WBH1 (weeks before H1)] on storability of 'Bartlett' fruit at three harvest maturities [H1: when control fruit firmness (CFF) ≈ 19lb; H2: 12 d after H1 when CFF ≈ 18lb; and H3: 17 d after H1 when CFF ≈ 17lb] were measured with respect to ethylene production, storage quality and ripening capacity during 5 months of storage at 30°F. ***'Starkrimson'.*** AVG at 30, 60, and 120 ppm was sprayed 1 week before commercial harvest. Fruit quality and ripening capacity were determined after 1, 2, 3, and 4 months of cold storage at 30°F. Postharvest treatment with 1-MCP at 300 ppb was as the same as described for 'Bartlett'.

Objective 2. Reducing pear scuffing by wax coatings and/or ethoxywuin

Effects of carnauba wax solids (0, 5%, 10%, 15%, and 20%) and sugar-ester edible coating a.i. (0.1%, 0.5%, and 1.0%) on fruit respiration and ripening physiology, anaerobic physiology, fruit quality, and scuffing of the major PNW cultivars ('Starkrimson', 'Bartlett', 'Bosc', 'Comice', and 'd'Anjou') were studied. The effect of ethoxyquin mixed in wax on scuffing was studied on 'Comice' pear

Objective 3. Evaluate the efficacy of a premix formulation of Difenoconazole + Fludioxonil on storage decay of pears

A pre-mix formulation of Fludioxonil + Difenoconazole, Difenoconazole alone, Scholar, and Penbotec were obtained from Syngenta. Artificially inoculated 'Bosc' pear fruits with spore solutions of *Botrytis cinerea* and *Penecillium expansum* were drenched with the fungicides at label recommended rates. Decay incidence, decay severity, and sporulation were evaluated after 3-5 months of storage at 30°F.

RESULTS

1. Ensure a consistent 1-MCP efficacy on extending storability of 'Bartlett' pears

1-MCP efficacy on inhibiting senescence of 'Bartlett' pears has been reported being inconsistent from year to year and from lot to lot in the PNW. The effects of harvest maturity, orchard elevations, delayed treatment after harvest, holding temperature during treatment delay, and exogenous ethylene concentration in treating room on 1-MCP efficacy were studied in 2012 and 2013.

1.1. Effect of 'Bartlett' harvest maturity on 1-MCP efficacy.

There were 3 harvest maturities: H1=19lb, H2=17.2lb, H3=16.5lb. 1-MCP treatment maintained fruit peel chlorophyll and FF for H1 and H2 fruit without senescent disorders (yellowing, senescent scald and internal breakdown) for 4-5 months of cold storage. However, 1-MCP treated F3 fruit lost chlorophyll and FF significantly after 2 months and developed senescent disorders after 4 months of cold storage at 30°F. Ripening capacity after storage was retarded especially for H1 and H2 (Fig. 1).

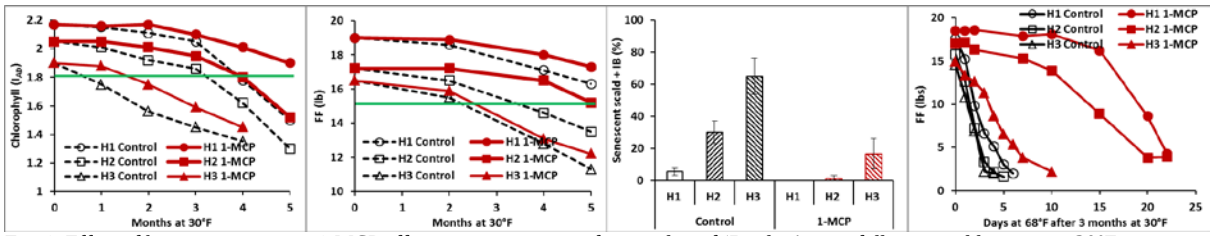

Fig. 1. Effect of harvest maturity on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' pears following cold storage at 30°F.

After 3 months of cold storage, fruit were transferred to a cold room at 41°F for 3 weeks to simulate export transit conditions. 1-MCP treatment maintained higher peel chlorophyll content and FF for only H1 fruit after the simulated export transit. Both H2 and H3 fruit treated with 1-MCP lost chlorophyll and FF significantly and developed senescent scald and IB after the simulated transit (Fig. 2).

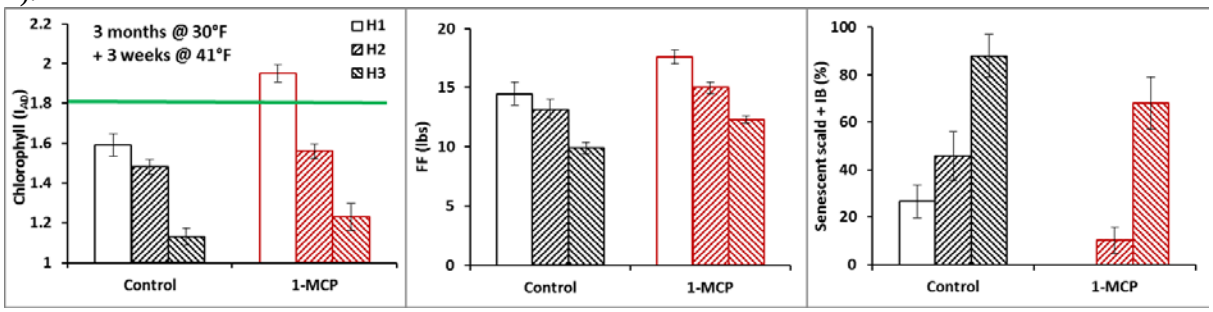

Fig. 2. Effect of harvest maturity on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' pears following 3 months of cold storage at 30 °F plus 3 weeks at 41 °F.

1.2. Effect of delayed treatment after harvest on 1-MCP efficacy.

It may take 10-12d or longer time to fill a storage room. 'Bartlett' fruit were harvested at 19-17lbs. Fruit were stored at cold rooms at 30, 37, and 41°F for 12d until 1-MCP treatment. IEC did not increase at 30°F, but increased at 37 and 41 °F during the 12d delay. A delayed treatment of fruit that have been stored at 30 °F for 12d did not reduce 1-MCP efficacy on maintaining fruit peel chlorophyll and FF of 'Bartlett' pears after 4 months of cold storage, compared to treating fruit immediately after harvest. However, storing fruit at 37 and 41 °F for 12d after harvest reduced 1-MCP efficacy on maintaining quality of 'Bartlett' after 4 months of cold storage (Fig. 3).

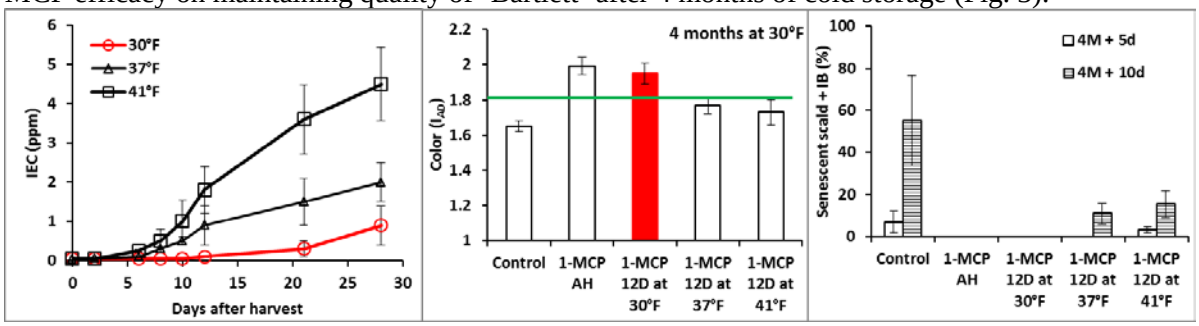

Fig. 3. Effect of treatment delays on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' after 4 months of cold storage at 30 °F.

1.3. Effect of production elevation on 1-MCP efficacy.

'Bartlett' fruit were harvested at 3 maturities based on FF from two elevations: 500ft and 2000ft. at the same FF, fruit from the higher elevation (2000ft) had higher IEC, especially for H2 (17lbf) and H3 (16.5lbs). H2 and H3 from elevation of 2000ft reduced 1-MCP efficacy on maintaining color and FF after 4 months of cold storage, compared to H1.

1.4. Effect of exogenous ethylene in treating room on 1-MCP efficacy.

Exogenous ethylene at 300ppb in treating room reduced 1-MCP efficacy on maintaining chlorophyll and FF and senescent scald-free after 4 months of cold storage (Fig. 4).

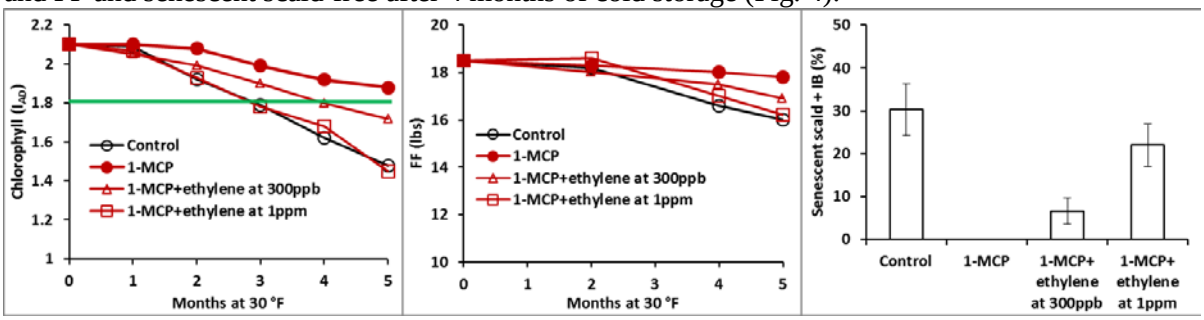

Fig. 4. Effect of exogenous ethylene in treating room on 1-MCP efficacy on maintaining fruit quality of 'Bartlett' following 4 months of cold storage at 30°F.

2. Pre-harvest ReTain® spray efficacy on extending storability of 'Bartlett' pears is affected by application rate, timing, and fruit harvest maturity

2.1. Ethylene production

In H1, the control fruit started to produce significant amount of ethylene after 2 months of cold storage (Fig. 5A). AVG at 60 and 120ppm applied 1 WBH1 reduced ethylene production rate significantly during 2-5 months of storage. Compared to 60ppm, AVG at 120ppm 1 WBH1 further reduced ethylene production rate numerically but not at statistically significant level (p = 0.05) during the experimental period. In contrast, AVG at 30ppm applied 1 WBH1 and AVG at 120ppm applied 2 WBH1 did not inhibit ethylene production compared to the control. For H2 fruit following 4 months of storage, similar to H1 fruit, AVG at 60 and 120ppm applied 1 WBH1 reduced ethylene production, but AVG at 30 mg L^{-1} applied 1 WBH1 and 120 mg L^{-1} applied 2 WBH1 did not affect ethylene production (Fig. 5B). Ethylene production in H3 fruit following 4 months of storage was not affected by the AVG treatments (Fig. 5B).

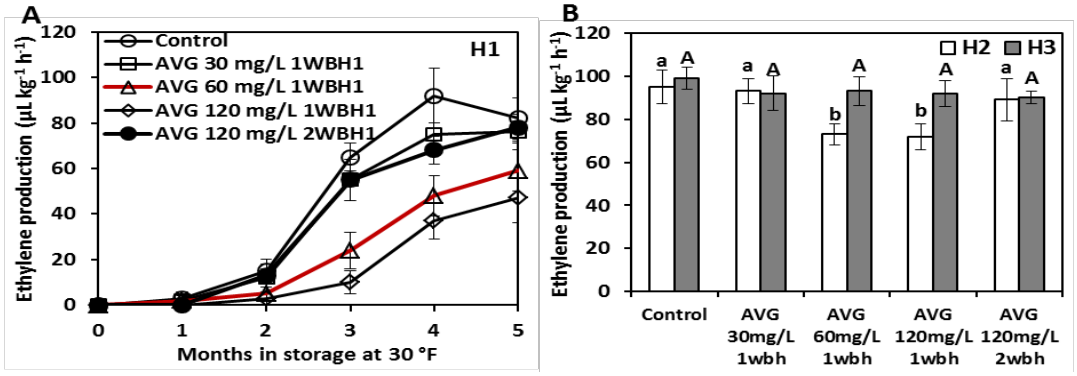

Fig. 5. Effects of pre-harvest AVG sprays on ethylene production of 'Bartlett' pears with three harvest maturities (H1, H2, and H3) on day 1 at 68°F following cold storage at 30°F for 1-5 months in H1 and 4 months in H2 and H3.

2.2. Fruit storage quality

In H1 fruit, FF, I_{AD}, and TA decreased gradually in all the treatments. Their losses were not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1, but slowed down significantly by AVG at 60 and 120ppm applied 1 WBH1 during 5 months of storage (Fig. 6). In H2 fruit following 4 months of storage, AVG at 60 and 120ppm applied 1 WBH1 maintained higher FF, I_{AD}, and TA, but AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1 did not affect the losses of FF, I_{AD}, and TA. H3 fruit did not response to the AVG treatments in terms of the losses of FF, I_{AD}, and TA

(data not shown). SSC increased in a small magnitude in each of the three maturities during storage, but it was not affected by the AVG treatments.

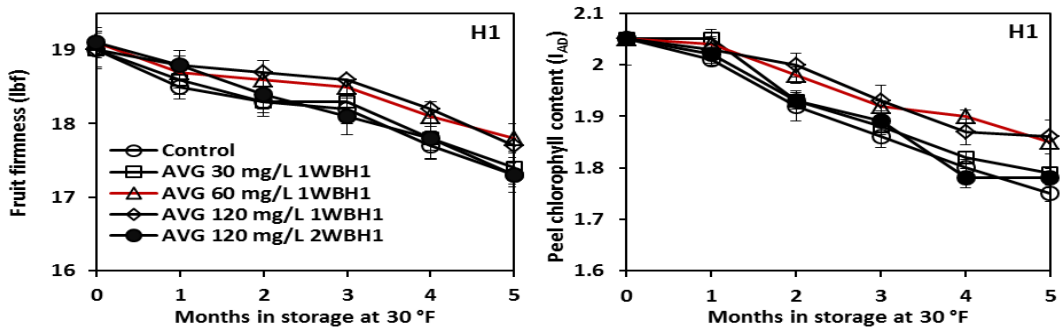

Fig. 6. Effects of pre-harvest AVG sprays on fruit flesh firmness and peel chlorophyll content (I_{AD}) of 'Bartlett' pears on day 1 at 68°F following cold storage at 30°F for 1-5 months in H1.

2.3. Senescence disorders

In the control, H1 and H2 fruit developed senescence disorders of 30.3% and 35.0% after 5 and 4 months of storage, respectively (Fig. 7). AVG at 60ppm applied 1 WBH1 reduced senescence disorders to 5.6% and 16.1% in H1 and H2 fruit after storing for 5 and 4 months, respectively. Senescence disorders in H1 and H2 fruit were not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1. Compared to AVG at 60ppm, AVG at 120ppm did not improve its efficacy on reducing senescence disorders. H3 control fruit developed 49.5% senescence disorders following 4 months of storage and the AVG treatments did not affect the senescence disorders significantly.

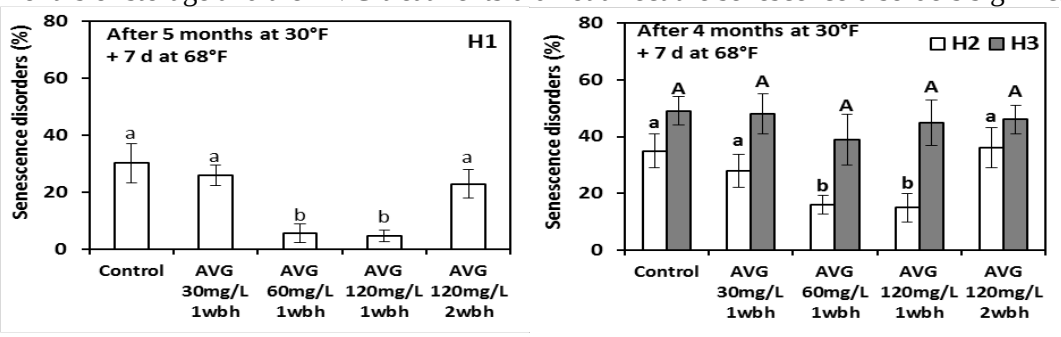

Fig. 7. Effects of pre-harvest AVG sprays on senescence disorders of 'Bartlett' pears with three harvest maturities (H1, H2, and H3) on day 7 at 68°F following cold storage at 30°F.

2.4. Ripening capacity

In H1, the control fruit developed ripening capacity following 1-4 months, but developed mealy texture with increased EJ > 650 mL kg^{-1} within 7d at 68°F following 5 months of cold storage (Fig. 8A&C). Following 1 month of cold storage, fruit treated with AVG at 60ppm applied 1 WBH1 could ripen to FF = 5.5lb with EJ = 649 mL kg^{-1} in 7 d at 68°F, however, fruit treated with AVG at 120ppm applied 1 WBH1 did not develop ripening capacity. Fruit treated with AVG at 60 and 120ppm applied 1 WBH1 developed ripening capacity with FF < 5lb and EJ < 650 mL kg^{-1} after 7 d at 68°F following 2-5 months of cold storage. Compared to the control, the ripening capacity was not affected by AVG at 30ppm applied 1 WBH1 and 120ppm applied 2 WBH1. Following 4 months of storage, while H2 fruit in all the treatments developed ripening capacity, the fruit treated with AVG at 60 and 120ppm applied 1 WBH1 had less EJ after 7d at 68°F compared to the control and fruit treated with AVG at 30 mg L^{-1} applied 1 WBH1 and 120 mg L^{-1} applied 2 WBH1 (Fig. 8D). In contrast, H3 fruit, regardless of the control and AVG treatments, developed mealy texture with EJ > 650 mL kg^{-1} after 7d at 68°F following 4 months of cold storage (Fig. 8D).

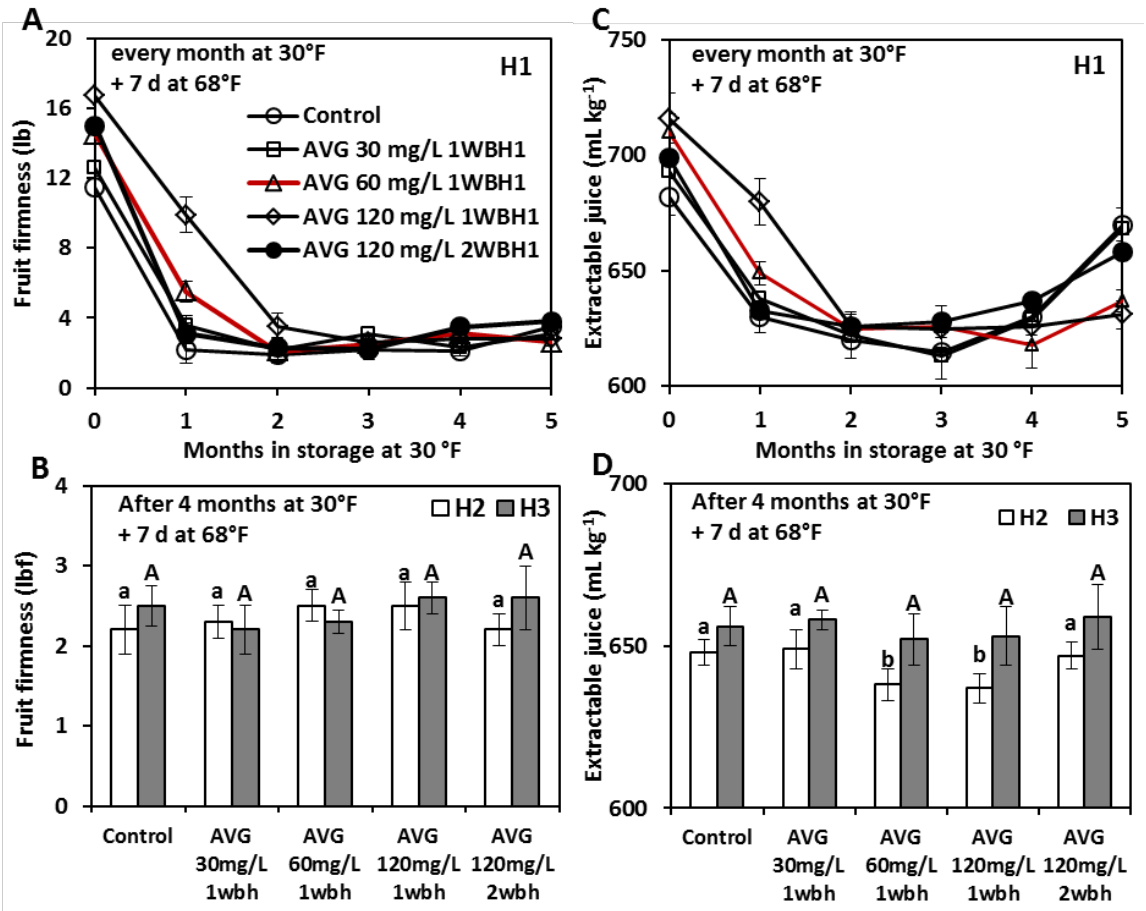

Fig. 8. Effects of pre-harvest AVG sprays on fruit ripening capacity expressed as flesh firmness and extractable juice on day 7 at 68°F following cold storage at 30°F for 1-5 months in H1 and 4 months in H2 and H3.

3. Extend storability of 'Starkrimson' by preharvest ReTain® or postharvest 1-MCP

3.1. Ethylene production and respiration rate

Control fruit started accumulating IEC at about 0.6ppm after 4 weeks. Thereafter, IEC increased gradually and reached the highest amount of 4.8ppm at 16 weeks of storage at 30°F (Fig. 9). Fruit treated with AVG at 30, 60 and 120ppm started accumulating IEC at 1.7, 0.9, and 0.6ppm, respectively, after 8 weeks and IEC peaked at 4.7, 3.3, and 3.2ppm, respectively, after 16 weeks of storage. There was no difference between AVG at 60 and 120ppm on IEC accumulation. Compared to the AVG treatment, 1-MCP was more effective in inhibiting the IEC during storage. 1-MCP treated fruit started accumulating IEC at 0.1ppm after 8 weeks and had IEC lower than 1.0ppm for the 16 weeks of storage. Ethylene production rate (EPR) in control fruit increased significantly after 4 weeks, increased thereafter and reached a maximum value after 16 weeks. Fruit EPR was not affected by AVG at 30ppm but decreased significantly by AVG at 60 and 120ppm during 4-16 weeks. 1-MCP prevented EPR during 16 weeks of storage period (Fig. 9). The respiration rate (RR) of control fruit increased during 16 weeks of storage and was generally higher than that of AVG at 60 and 120ppm. AVG at 30ppm did not affect RR compared to control. 1-MCP treated fruit maintained the lowest RR which decreased in the first 4 weeks and then increased during 4–16 weeks of storage (Fig. 9).

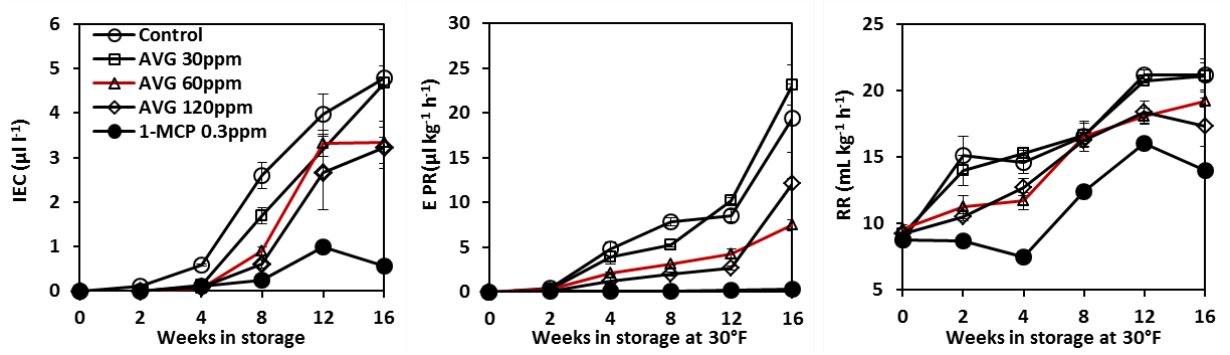

Fig. 9. Effects of AVG and 1-MCP on internal ethylene concentration (IEC), ethylene production rate (EPR) and respiration rate (RR) of 'Starkrimson' pears during 16 weeks of storage at 30°F.

3.2. Fruit storage quality

AVG sprayed one week before harvest at 60, but not 30 and 120ppm slowed down the FF reduction of fruit on the trees compared to control. At nearly the commercial harvest date, FF of fruit treated with AVG at 0, 30, 60, and 120ppm were 58.7, 58.0, 62.6, and 59.9 N, respectively (Fig. 10). Control fruit decreased FF from 58.7 to 53.1 N and maintained SSC at about 11.5% for 12 weeks of storage at -1.1 °C (Fig. 10). AVG and 1-MCP applications did not affect FF and SSC. TA decreased gradually and lost 40% in control fruit after 16 weeks of storage (Fig. 10). AVG and 1-MCP treatments inhibited TA reduction (Fig. 10). For example, AVG at 30, 60, and 120 µL L^{-1} and 1-MCP reduced TA loss from 40% to 28, 20, 28, and 23%, respectively, after 16 weeks of storage.

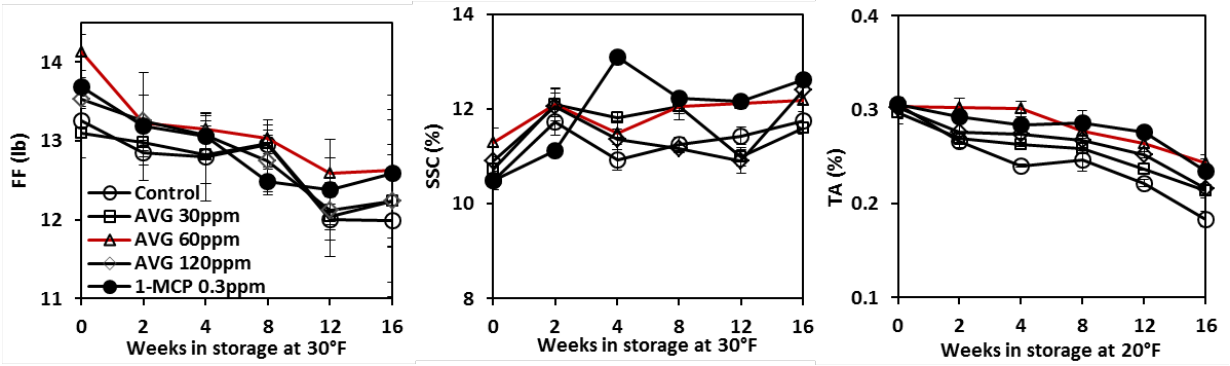

Fig. 10. Effects of AVG and 1-MCP on FF, SSC, and TA of 'Starkrimson' pears during 16 weeks of storage at 30°F.

3.3. Senescence disorders

After 16 weeks of cold storage, control fruit developed internal breakdown (IB) and decay at 12.3 and 7.1%, respectively. AVG at 30, 60, and 120 µL L^{-1} and 1-MCP at 0.3 µL L^{-1} reduced IB to 10.5, 2.5, 3.3, and 0%, and decay to 6.6, 1.2, 3.3, and 1.1%, respectively (Fig. 11).

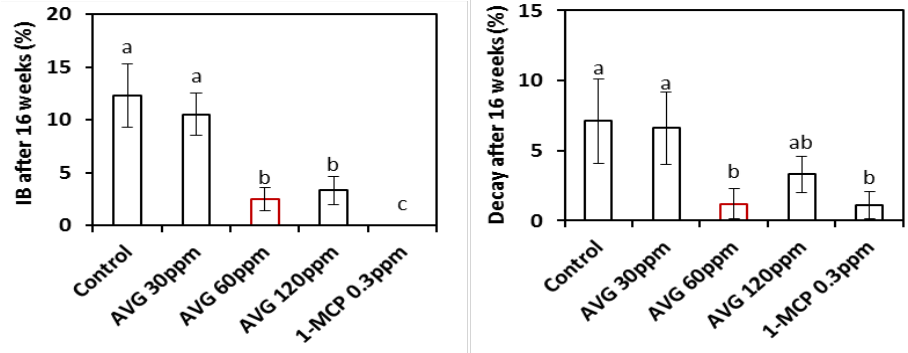

Fig. 11. Effects of AVG and 1-MCP on internal breakdown (IB) and decay of 'Starkrimson' pears after 16 weeks at 30°F.

3.4. Ripening capacity

The control fruit could not ripen immediately after harvest, but developed ripening capacity within 5d at 68°F after 2 weeks of storage at 30°F. Ripening capacity was not affected by AVG at 30 and 60ppm. Fruit treated with AVG at 120ppm developed ripening capacity after 4 weeks of cold storage. Both control and fruit treated with AVG at 30ppm maintained low EJ (i.e., < 600 mL kg^{-1} FW) and high eating quality (i.e., > 7) between 2–8 weeks of storage and increased EJ and lost eating quality thereafter. Fruit treated with AVG at 60 and 120ppm maintained low EJ and high eating quality between 2–16 and 4–16 weeks of cold storage, respectively. 1-MCP treated fruit could not develop ripening capacity within 5d at 68°F for 16 weeks of storage, but could ripen in 15d at 68°F with high eating quality following 4–16 weeks of cold storage (Fig. 12).

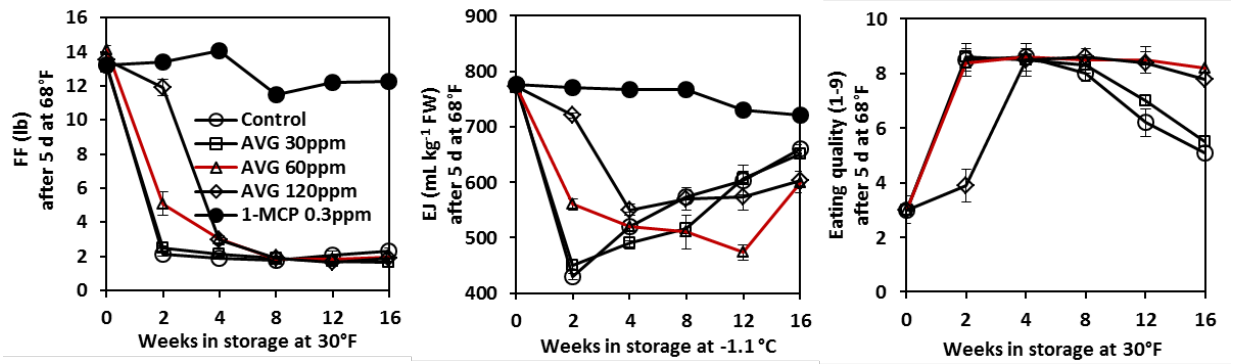

Fig. 12. Effects of AVG and 1-MCP on FF, extractable juice (EJ), and eating quality of 'Starkrimson' pears after 5 d at 68 °F following 16 weeks of storage at 30°F.

4. Reducing pear scuffing.

Pears are more sensitive to high CO_2injury. Therefore, waxes (e.g., carnauba) for pears are lower in solids (5-6%) than those for apples (18-22%). However, low solid waxes are less effective in minimizing scuffing. Fruit were waxed after harvest using a commercial carnauba wax coating at solids of 0, 5, 10, 15, and 20%. After satisfying chill requirement plus 7d at 68°F, 'Starkrimson' and 'Bosc' developed anaerobic metabolism at wax solid of 20%; 'Starkrimson', 'Bosc', 'Bartlett', and 'd'Anjou' developed IB or abnormal ripening at wax solids higher than 10%. Injury was not found in 'Comice' at any of the wax solid treatments (Fig. 13).

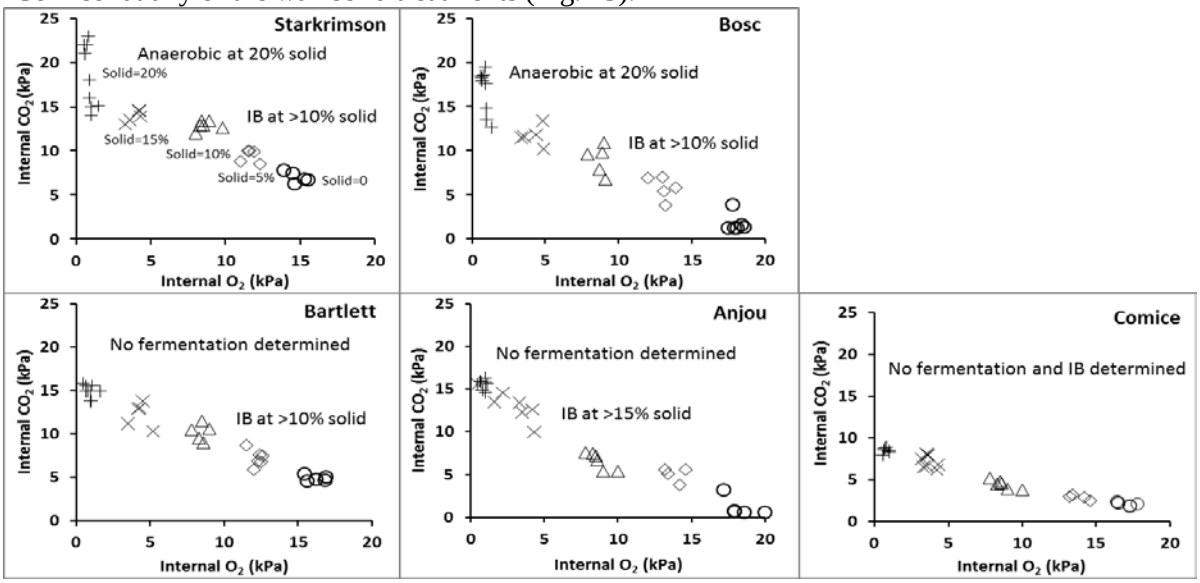

Fig. 13. Internal O_2 and CO_2 concentrations affected by wax solids of a commercial carnauba wax coating after fruit ripening of 5 European pear cultivars.

Compared to the commercial wax solids of 5-6%, wax solids at 7-8% plus ethoxyquin at 1000ppm reduced scuffing without negative effect on fruit quality of 'Comice' pear. Carnauba wax coating at higher solids may reduce abrasion force on fruit peel during online processing and ethoxyquin may reduce the enzymatic reaction and therefore expression of the discoloration. Semperfresh at 0.5-1% a.i. reduced scuffing and maintained green color without affecting ripening capacity of 'Bartlett' after long-term storage (Fig. 14).

Fig. 14. Effect of Semperfresh at 0-1.0% a.i. on 'Bartlett' pear appearance after 6 months storage at 30°F.

5. The efficacy of a premix formulation of Difenoconazole + Fludioxonil on storage decay

The pre-mix of Fludioxonil + Difenoconazole, applied as drenching within 18h after inoculation, was very efficient and comparable with Penbotec and Scholar on controlling both blue and gray molds of pears during cold storage (Fig. 15). The rate at 16 oz was more efficient than 11.4 oz on gray mold. Both rates were equal and efficient on blue mold.

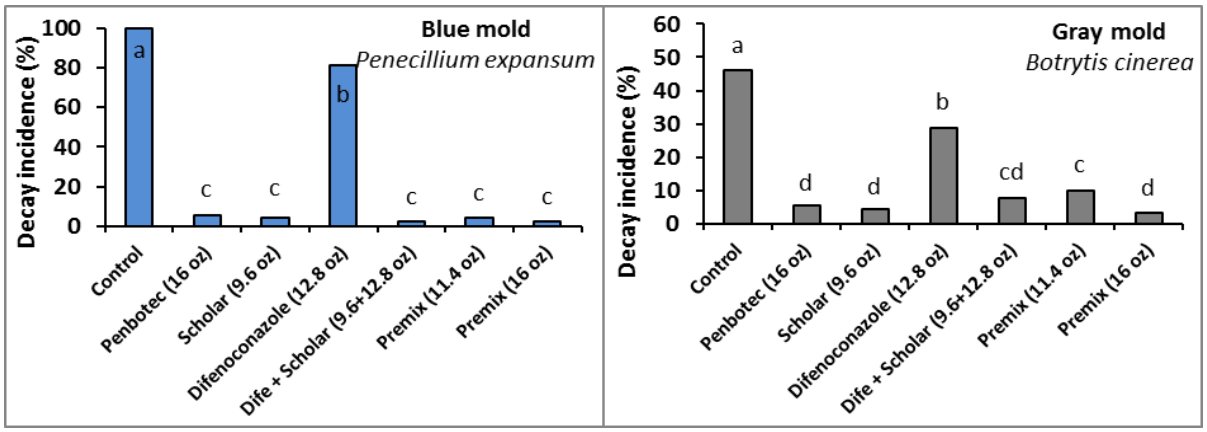

Fig. 15. Blue mold and gray mold decays in inoculated 'Bosc' pears treated by experimental and standard fungicides and evaluated after 3 and 5 months of cold storage at 30°F. Rates are in fluid ounces per 100 gallons of water.

EXCUTIVE SUMMARY

Project title: Deliver 1-MCP treated ‘d’Anjou’ pears with predictable ripening capacity

‘Bartlett’ and ‘Starkrimson’ are summer pears with relatively short storage life. Significant losses may occur after long-term cold storage or long-distance shipping due to senescence disorders. For ‘Bartlett’, a recent trend toward greater fresh market utilization has increased the need for extending ‘Bartlett’ storage life to prolong the packing and marketing season. For ‘Starkrimson’, increased export demand has resulted in new challenges for maintaining quality during long-distance transport. The senescence disorders and relatively short storage life of summer pears are the result of increased ethylene production induced by cold storage. Ethylene inhibitors AVG and 1-MCP have the potential to extend storability and reduce senescence disorders of ‘Bartlett’ and ‘Starkrimson’.

1-MCP efficacy on extending storability of ‘Bartlett’ is inconsistent at commercial application in PNW among production lots and years. This research indicated that to ensure a consistent 1-MCP efficacy, (1) harvest fruit at ≥19lb, especially for fruit from higher production elevations (i.e., >1,000ft); (2) treat fruit with 1-MCP within 10-12 days after harvest and eliminate field heat quickly after harvest and store fruit at 30°F during the treatment delay; (3) vent out exogenous ethylene (if > 300ppb) in the fumigation room before the 1-MCP treatment. Pre-harvest ReTain® spray efficacy on improving storability of ‘Bartlett’ pears is affected by application rate, timing, and fruit harvest maturity. To maximize AVG efficacy, (1) apply AVG at 60-120ppm at 1 week before harvest 1 (WBH1); (1) harvest fruit at H1 (H1=19lb) and H2 (12d after H1, H2=18lb), H3 (17d after H1, H3=17) fruit did not response to the AVG treatments; (3) AVG applied 2 WBH1 had little effect on any of the storage responses measured; (4) AVG applied 1 WBH1 doesn’t delay H1 but extend harvest window for 5d.

‘Starkrimson’ produces a significant amount of ethylene and has a higher respiration rate and therefore a shorter storage life compared to other PNW pear cultivars. The present study indicated that pre-harvest ReTain® or postharvest 1-MCP treatments extend storage life of ‘Starkrimson’. To ensure efficacy, (1) apply AVG at 60-120ppm at 1 week before harvest and harvest fruit at 15-14lb; (2) 1-MCP at 300ppb extend ‘Starkrimson’ storage life to 4 months at 30°F, however, it takes 2 weeks to ripen at 68°F following 4 months of cold storage.

Sugar-ester edible coating (i.e., Semperfresh) at 0.5-1.0% a.i. or carnauba wax coating at solid of 7-8% decrease friction forces and therefore reduce scuffing without negative effects on ripening and flavor of ‘Bartlett’ and ‘Comice’ pears, respectively. Ethoxyquin at 1000ppm mixed in wax coating slows down chlorophyll degradation and reduce scuffing expression of ‘Comice’ pear.

The pre-mixed formulation of Difenoconazole + Fludioxonil (Syngenta product) applied as drenching at 16 oz. per 100 gallons control blue and gray mold decays at levels equivalent to Penbotech or Scholar alone. The different modes of action between Difenoconazole and Fludioxonil in the pre-mix may retard resistance development in the pathogens.

FINAL PROJECT REPORT

Project Title: Physiological, economic and consumer evaluation of sliced pears

PI: Amit Dhingra
Organization: Washington State University
Telephone: 509 335 3625
Email: adhingra@wsu.edu

Co-PI: Carolyn Ross
Organization: Washington State University
Telephone: 509 335 2438
Email: cfross@wsu.edu

Co-PI: Karina Gallardo
Organization: Washington State University
Telephone: 253 445 4584
Email: Karina_Gallardo@wsu.edu

Cooperators: Crunch Pak: Tony Freytag and Ozgur Koc
WSU: Seanna Hewitt, Christopher Hendrickson, Scott Mattinson and Frank Younce
Pear Bureau – Kevin Moffitt

Other funding sources

Agency Name: Washington State Department of Agriculture
Amt. awarded: $204,466
Notes: **"Sliced Pears – A novel avenue for pear consumption"**. Support for a scientist, graduate student and organized taste panels to perform large scale evaluation of sliced pears in the market.

Agency Name: NIH Protein Biotech Training Program
Amt. awarded: $52,234
Notes: Support for Seanna Hewitt, Ph.D. student includes stipend, travel, medical, tuition and fees

Agency Name: Crunch Pak
Amt. awarded: $30,000
Notes: Support for pear slicing, packaging, purchase of fruit, labor and fruit quality analysis

Agency Name: USA Pears
Amt. awarded: $6,895
Notes: Support for economic analysis and consumer surveys

Total Project Funding: $69,921

Budget History

Item	2015
Wages[a]	19,200
Benefits	8,221
Equipment[b]	29,500
Supplies[c]	8,500
Travel[d]	1,500
Miscellaneous[e]	3,000
Total	**69,921**

Footnotes: a: Wages for technician to assist in fruit processing, quality assessment and gas analysis; **b**: Purchase of two separate pieces of equipment for analysis of respiration and ethylene in whole and sliced fruit; **c**: Purchase of ripening compounds, lab reagents and consumables; **d**: Travel costs for picking up of whole fruit and sliced fruit; **e**: Cover the cost of fruit

OBJECTIVES

1. Physiological evaluation of sliced pears derived from 1-MCP fruit treated with ripening compounds.
2. Economic analysis – willingness to pay for sliced pear product will be conducted.
3. Trained panel surveys will be conducted to accurately quantify organoleptic preferences.

This project requires post-harvest fruit which starts becoming available late January/early February after the controlled atmosphere rooms begin to open up. We have received some fruit from Blue Star Growers and conducted a taste panel during the annual Washington State Tree Fruit Association meeting in Yakima in December 2015.

SIGNIFICANT FINDINGS

1. Physiological evaluation of sliced pears derived from 1-MCP fruit treated with ripening compounds.

    - Ethylene levels were observed to increase during storage in modified atmosphere bags.
    - Sliced fruit lasted for over 20 to 30 days in the bags exceeding the shelf life requirements.

2. Economic analysis – willingness to pay for sliced pear product will be conducted.
    - To be conducted on March 2 and 3, 2016 by Karina Gallardo at the OSU Food Science Innovation Center

3. Trained panel surveys will be conducted to accurately quantify organoleptic preferences.
    - Consumer panel at the annual WSTFA meeting ranked the Ripening Compound-treated 1-MCP fruit (sliced) as the most acceptable in all categories tested.
    - Additional surveys are scheduled to be conducted in March 2016 at the WSU Food Science Sensory lab.

RESULTS & DISCUSSION

Objective 1: Physiological evaluation of sliced pears derived from 1-MCP fruit treated with ripening compounds.

Smartfresh-treated Fruit was obtained from Blue Star Growers and sliced in the lab. Sliced fruit was treated with various concentrations of ripening compound which was directly mixed in with the non-browning mix (Crunch Pak) and packaged in modified atmosphere bags provided by Crunch Pak. Fruit was stored at 40 deg F and monitored visually for browning for 20 days. A total of 20 bags per treatment were prepared and measurement of ethylene and respiration was performed on 4 bags each after every 5 days. Figure 1 demonstrates the release of ethylene from sliced fruit directly measured from modified atmosphere bags. Respiration was also measured in the modified atmosphere bags. It was noteworthy to observe that the respiration levels remained similar however there was a sharp increase on day 20 (Figure 2). This implies that the modified atmosphere bags were able to maintain the carbon dioxide levels and avoid anaerobic respiration. Overall, these results confirm previous observations that sliced pears can be maintained in modified atmosphere bags with an extended shelf life.

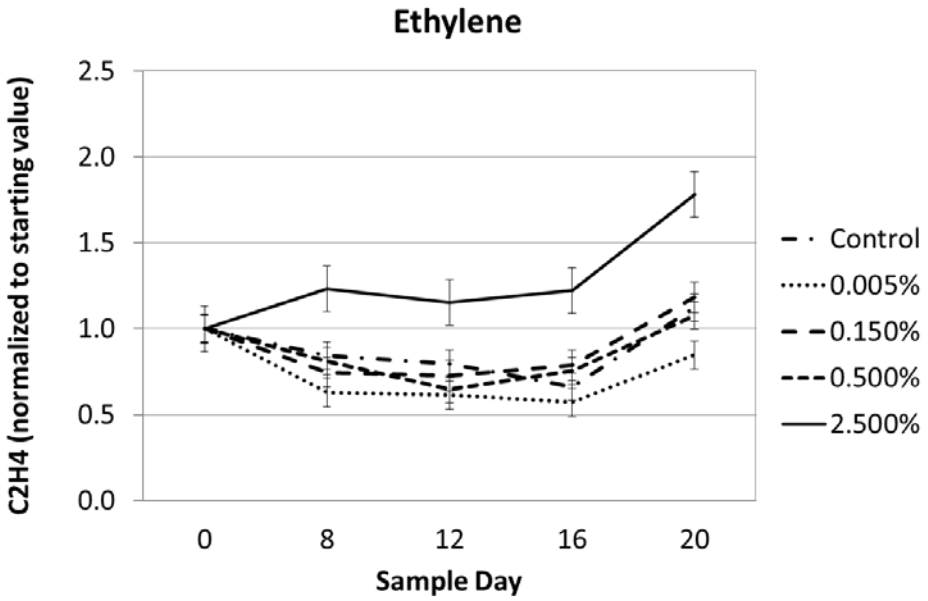

Figure 1: Ethylene released by sliced fruit in modified atmosphere bags. All the fruit used was Smartfresh treated and was further treated with ripening compound post-slicing.

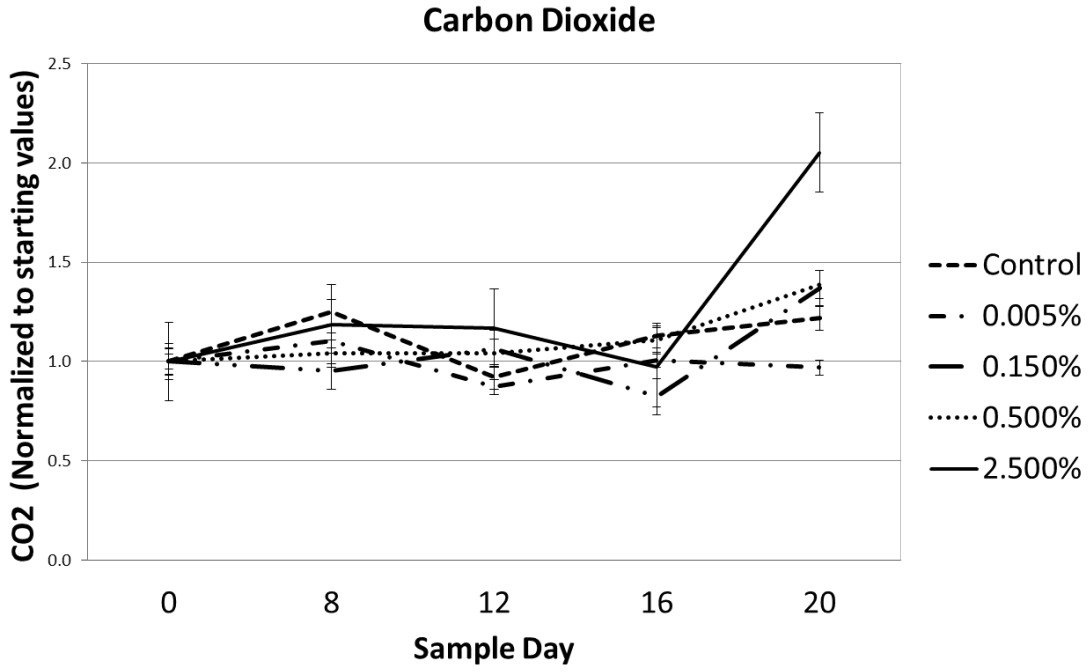

Figure 2: Carbon dioxide (respiration) levels in modified atmosphere bags.

Objective 2: Economic analysis – willingness to pay for sliced pear product will be conducted.

- To be conducted on March 2 and 3, 2016 by Karina Gallardo at the OSU Food Science Innovation Center

Objective 3: Trained panel surveys will be conducted to accurately quantify organoleptic preferences.

While the trained panel surveys are scheduled to be conducted in March 2016, we conducted a consumer taste panel at the annual WSTFA meeting in December 2015 in Yakima. The results are summarized in Table 1 and indicate that Smartfresh treated pears when sliced and treated with the ripening compound are favored over control Smartfresh treated pears. The 3% RC treated fruit was most acceptable in all categories except for appearance, it indicates that there is a need to standardize the amount of non-browning mix and the type of modified atmosphere bag.

Table 1: Summary results of a consumer taste panel conducted at the annual WSTFA meeting in December 2015.

	Ranking 2015 - Anjou			
	Overall acceptance	Appearance	Taste/ Flavor	Texture
Most acceptable	3% RC	1%	3% RC	3% RC
	2%	Control	2% RC	2% RC
	Control	2%	1%	1%
Least acceptable	1% RC	3% RC	Control	Control

OUTREACH

- Good Fruit article - **http://www.goodfruit.com/sliced-pears-show-potential/** Sliced pears show potential Published September 25, 2015

- Woot Fruit – Established connection with Kim Gaarde at Woot Fruit, CA to establish collaboration for sliced pears.

- Naumes fruit – Amit Dhingra visited Naumes fruit to discuss the feasibility of producing sliced pears with Comice and Bosc varieties.

EXECUTIVE SUMMARY

Enhancement of per capita consumption of pears has remained a long-desired goal of the pear industry. This has been recorded repeatedly in every annual pear industry research priority document since George Ing published his seminal summary in 1994 (Ing 1994). Sliced pears offer a novel avenue for enhancing pear consumption. In this project we have demonstrated that sliced pears produced by using Smartfresh treated pears which are then sliced and treated with the ripening compound last on the shelf for over 20 days. The commercial requirement is about two weeks. While we have engaged Crunch Pak in the Wenatchee valley, collaborative arrangements are underway with Woot Fruit in CA to evaluate the ripening compounds with them. Woot Fruit has already introduced sliced pears in the market.

In the immediate future, sliced pears are expected to add an additional 10% resulting in a potential value of $40M to the current U.S. Pear Market. The customer's willingness to pay a premium for a novel product will be evaluated in 2016 and 2017 and this information is expected to be useful for adoption of the product by the retail market.

This project will contribute in increasing per capita consumption and will specifically enable the utilization of fruit ranging in size from 120 to 135, which is currently underutilized or undervalued. Further, the application of 1-MCP prior to slicing, and then having the ability to reverse the effect of 1-MCP, can enable longer storage of the fruit, resulting in expansion of the marketing timeline. This is expected to contribute to further economic benefits to the pear industry.

Final Project Report

Project Title: Bacterial endosymbionts of pear psylla

PI: W. Rodney Cooper
Organization: USDA-ARS-YARL
Telephone: 509/454-4463
Email: rodney.cooper@ars.usda.gov
Address: 5230 Konnowac Pass Road
City/State/Zip: Wapato, WA 98951

Cooperators: S. Garczynski, USDA-ARS, R. Hilton, Oregon State University, D. Horton, USDA-ARS, P. Shearer, Oregon State University

Total Project Request: Year 1: $12,000

Other funding sources: None

Budget 1
Organization Name: USDA-ARS-YARL **Contract Administrator: Chuck Myers**
Telephone: 510/559-5769 **Email address: Chuck.Myers@ars.usda.gov**

Item	**2015**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$12,000		
Travel			
Miscellaneous			
Plot Fees			
Total	$12,000		

Footnotes: Supplies include fluorescence in situ hybridization reagents, PCR and qPCR reagents, TA cloning supplies, gene sequencing costs, and shipping costs.

OBJECTIVES

Summary statement: The overall goal of this one-year study was to document the bacterial endosymbionts of pear psylla.
Objective 1: Compare the prevalence of *Arsenophonus* among pear psylla populations collected pre-budbreak, mid-summer, and autumn from orchards located near Wenatchee, WA, Yakima, WA, Hood River, OR, and Medford, OR.
Objective 2: Document the localization of *Arsenophonus* in specific organs/tissues of pear psylla.
Objective 3: Survey pear psylla collected from various pear growing regions of the Pacific Northwest for undocumented endosymbionts.

SIGNIFICANT FINDINGS

Objective 1: *Arsenophonus* was widespread among pear psylla populations. The prevalence of *Arsenophonus* did not differ among locations.
Objective 2: *Arsenophonus* was predominantly located in the bacteriomes of pear psylla in close proximity to the obligate endosymbiont, *Carsonella ruddii*. *Arsenophonus* was present in the oocytes of each female sampled indicating a high rate of mother to offspring transmission. *Arsenophonus* appeared also capable of colonizing the salivary glands, which may permit plant-mediated horizontal transmission of this endosymbiont.
Objective 3: A survey for additional bacteria associated with pear psylla found that about 20% of pear psylla were carriers of *Phytoplasma pyri*, the pathogen associated with pear decline disease and peach yellow leaf roll disease. The phytoplasma appeared to be more prevalent in Yakima Valley compared with other locations. The endosymbiont *Profftella*, which provides Asian citrus psyllid with protection against parasitoids, may be present in some pear psylla adults.

RESULTS AND DISCUSSION

Objective 1. *Arsenophonus* is widespread among insects and is associated with a wide-range of extended phenotypes expressed in their hosts (Gherna et al. 1991, Novakova et al. 2009, Rana et al. 2012) including providing the red gum lerp psyllid with protection against parasitoids (Hansen et al. 2007). In a preliminary survey of endosymbionts of pear psylla, we identified a strain of *Arsenophonus* that was present in psylla populations sampled from Washington, Oregon, and West Virginia. Sequences of 16S from *Arsenophonus* revealed that this is a strain that is specific to pear psylla.

Based on our preliminary survey, we screened pear psylla populations from various locations for the presence of *Arsenophonus*. This bacterium was prevalent in pear psylla populations at each location (Figure 1). *Arsenophonus* tended to be more prevalent in winterform populations (2014 and spring 2015) than in summerform populations. We are still

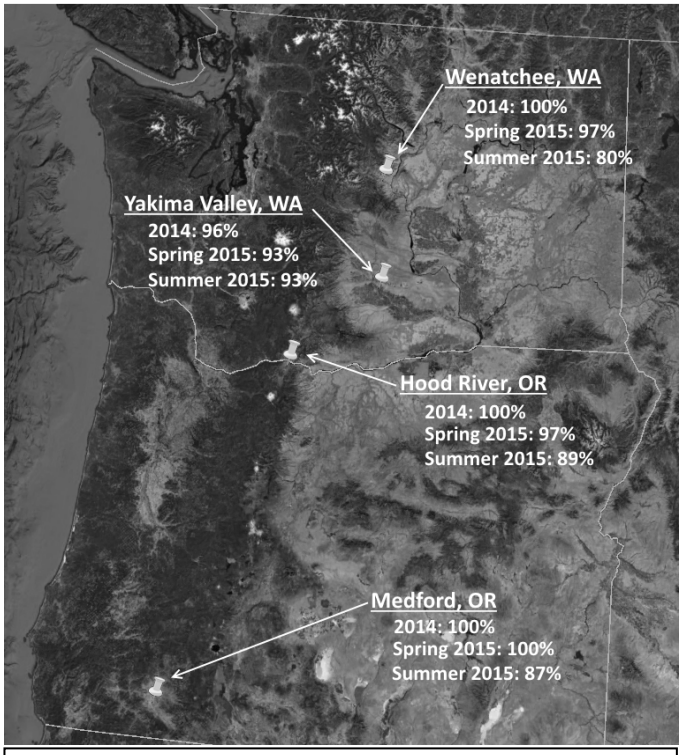

Figure 1. Percent of pear psylla adults harboring *Arsenophonus*

obtaining data for *Arsenophonus* in autumn populations, which will include a mixture of winterform and summerform. We are the first to report the presence of *Arsenophonus* in the pear psylla species that occurs in the United States. The high proportion of psylla carrying *Arsenophonus* suggests that either 1) *Arsenophonus* provides psylla with a selective advantage over psylla lacking this bacterium, or 2) this bacterium readily spreads throughout pear psylla populations without having negative effects on psylla fecundity or survival.

Objective 2. Bacteriomes are specialized organs in insects that house bacterial endosymbionts including the obligate endosymbiont, *Carsonella ruddii*, and many facultative endosymbionts. Using flourescence *in situ* hybridization, we observed *Arsenophonus* in the bacteriomes and oocytes of pear psylla (Figure 2). The occurrence of *Arsenophonus* in oocytes confirms that this endosymbiont is readily transmitted from mother to offspring.

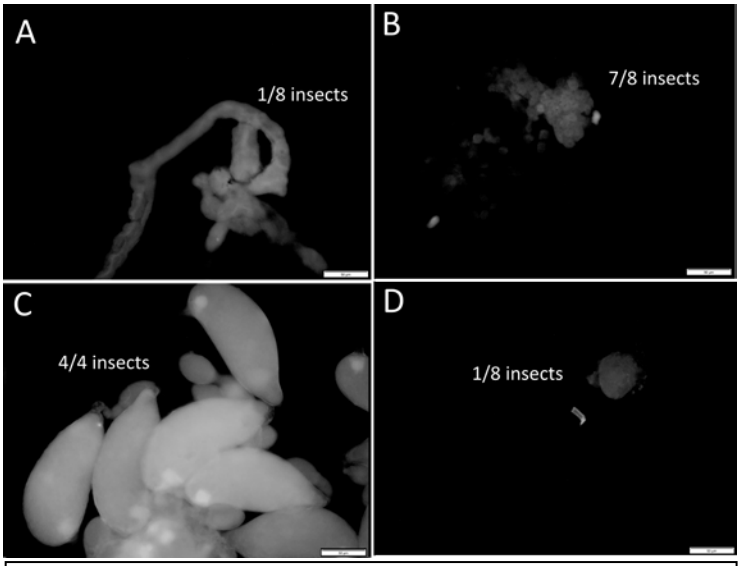

Figure 2. Fluorescent in situ hybridization to identify location of Arsenophonus in pear psylla adults: Alimentary canal (A), bacteriome (B), oocytes (C), and salivary glands (D). Visible tissues appear green through the fluorescent microscope indicating the presence of Arsenophonus. Values represent the number of the total samples positive for the bacterium.

Certain strains of *Arsenophonus* are plant pathogens, and are transmitted to new host plants by colonizing the insects' salivary glands (Novakova et al. 2009). Colonization of insect salivary glands may permit plant-mediated horizontal transmission of endosymbionts even if the bacteria cannot infect the plants (Caspi-Fluger et al. 2012, Gonella et al. 2015, Torres et al. 2015). For plant-mediated transmission to occur, the endosymbiont must be 1) colonize the insect salivary glands, 2) persist in the phloem long enough to be acquired by other insects, and 3) pass through the midgut to colonize the insect's hemolymph (blood). We observed *Arsenophonus* in the salivary glands and the gut of one psylla (Figure 2), but further research is required to confirm this observation and to determine whether colonization of the salivary glands of pear psylla by *Arsenophonus* leads to plant-mediated horizontal transmission.

Objective 3. An in-depth survey of endosymbionts in pear psylla populations did not reveal any new credible associations. In fact, the only bacteria identified using universal PCR primers were *Carsonella, Arsenophonus, Phytoplasma pyri*.

Since sequencing results indicated that *Phytoplasma pyri* was abundant in pear psylla, we used *Phytoplasma*-specific PCR primers to screen populations from different pear growing regions for the presence of this bacterium. This plant pathogen was most abundant in Yakima Valley, and was absent from Hood River. In general, the bacterium was more abundant in winterform populations (Spring 2015) than in summerform populations. We are still obtaining data for *Phytoplasma* in autumn populations, which will include a mixture of winterform and summerform. *Phytoplasma pyri* is the pathogen associated with pear decline disease, and is controlled by grafting pear to *Phtyoplasma* resistant rootstocks. While the use of resistant rootstock prevents the pathogen from overwintering in trees and prevents annual increases in bacterial titers, it is not known whether yearly reinfection with *Phytoplasma* affects tree health and yield. *Phytoplasma pyri* is also associated with peach yellow leafroll disease, which is transmitted to peach by winterform psylla that disperse from pear orchards during the winter (Purcell and Suslow 1984, Blomquist and Kirkpatrick 2002).

Many insect-vectored plant pathogens can alter insect behavior, especially their host preference and propensity to disperse. Further research is required to determine whether *Phytoplasma* alters the behavior of pear psylla.

The use of universal PCR primers will usually only detect the most abundant bacteria. We therefore are screening psylla populations using PCR primers specific for two endosymbionts known to occur in other psyllids, *Wolbachia* and *Profftella*. *Wolbachia* is a common endosymbiont of insects that causes reproductive manipulations. For example, female potato psyllids without *Wolbachia* are not capable of producing offspring with males with *Wolbachia* (Cooper et al. 2015). *Wolbachia* could provide mechanisms of novel psylla control strategies if our ongoing experiments confirm the absence of *Wolbachia* in pear psylla, or reveal a strain different from that of potato psyllid.

PCR amplicons associated with *Profftella* were observed from four pear psylla adults. *Profftella* is abundant in Asian citrus psyllid, and is thought to provide that psyllid with protection against parasitoids (Nakabachi et al. 2013). *Profftella* may also have important interactions with psyllid-vectored plant pathogens (Ramsey et al. 2015). Unfortunately, sequencing data was inconclusive so the product identity could not be confirmed. Further investigation is underway to confirm that PCR bands are associated with the presence of *Profftella*.

Conclusions

This study was first to investigate bacterial endosymbionts of pear psylla in the United States. This initial study is strongly related to our ongoing research on endosymbionts of potato psyllid. Results will be used to justify requests for research funds from other grant funding organizations. Further research on endosymbionts could lead to improved management decisions if results indicate that *Arsenophonus* provides pear psylla with protection from parasitoids or insecticides, as has been found for at least one other psyllid (Hansen et al. 2007). In addition to helping growers make informed pest management decisions, knowledge of psyllid endosymbionts could lead to the development of new control strategies that target endosymbionts to control psylla (Rio et al. 2004, Douglas 2007, Crotti et al. 2012). Researchers are searching for ways to target endosymbionts to control Asian citrus psyllid, aphids, and other insects (Rio et al. 2004, Douglas 2007, Crotti et al. 2012, Bouffard 2014). Our research on endosymbionts of pear psylla could allow these developing technologies to also be applied to pear psylla.

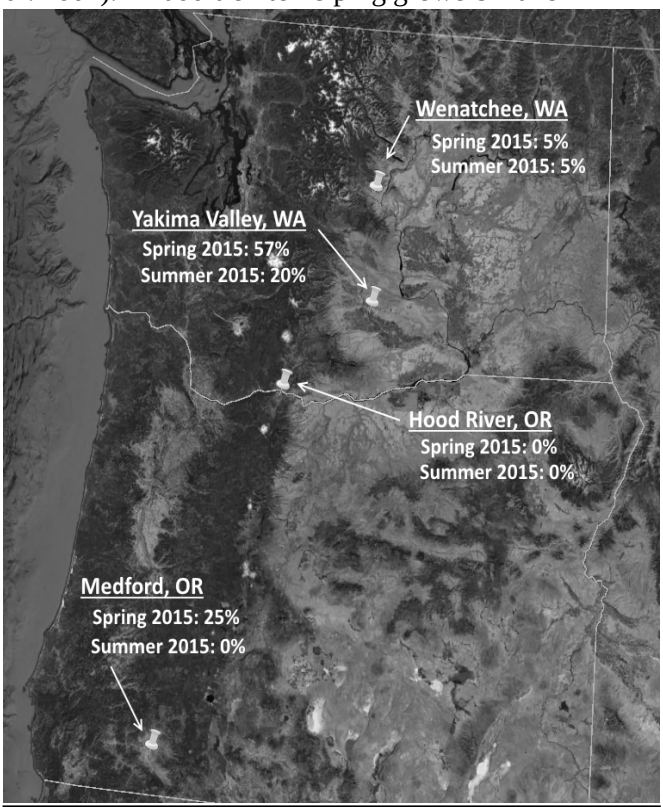

Figure 3. Percent of pear psylla adult harboring *Phytoplasma pyri*

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EXECUTIVE SUMMARY

The objective of this study was to investigate the bacterial endosymbionts of pear psylla, with an emphasis on the endosymbiont, *Arsenophonus*. The information learned from these experiments will help justify requests for research funding from other sources.

Summary of Findings

Results of this study indicate that *Arsenophonus* is prevalent in pear psylla populations throughout the Pacific Northwest. This endosymbiont is predominantly transmitted from mother to offspring, but the observation of *Arsenophonus* in the salivary glands and alimentary canal suggests that plant-mediated transmission is also possible. A survey of bacteria associated with pear psylla did not reveal new associations, but found that *Phytoplasma pyri* is present in some pear psylla populations. *Phytoplasma* appeared to be more prevalent in Yakima Valley than in other regions, and more prevalent in winterform psylla than in summerform psylla.

Future Directions

Bacterial endosymbionts, including plant pathogens, can often alter the behavior and susceptibility to parasitoids and insecticides of their insect hosts. Further research is required to determine whether *Arsenophonus* provides pear psylla with protection against parasitoids as has been demonstrated for another psyllid, or whether *Phtyoplasma* alters flight behavior of pear psylla. Ongoing research efforts seek to control Asian citrus psyllid and potato psyllid by manipulating their bacterial endosymbionts. Further research is needed to determine whether these developing technologies will apply to pear psylla and its endosymbionts.

FINAL PROJECT REPORT **YEAR**: 1 of 1

Project Title: Improving fruit set, production efficiency, and profitability of pears

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

Cooperators: Yan Wang, Stefano Musacchi, Don Kiyokawa

[1]Budget: **Year 1:** $41,885
Other funding sources: None

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Russell Karow
Telephone: 541 737-4866 **Email address:** Russell.Karow@oregonstate.edu

Item	2015	2016	2017
Salaries[1]	10,997		
Benefits	7,368		
Wages[2]	18,200		
Benefits	1,820		
Equipment	0		
Supplies[3]	2,500		
Travel[4]	1,000		
Miscellaneous	0		
Total	41,885		

Footnotes: [1]Salaries are calculated as 3 months of Full Time Technician's salary and associated OPE using actual rates; duties include management of all experimental designs and field plots, operation of root pruner, PGR applications, plant measurements, and data management. [2]Wages are for 2 part-time employees to work a combined total of 1,400 hours ($13/hr) to aid in plot maintenance, plant measurements, and harvest; actual benefits rate is 10%. [3]Supplies intended to cover ethylene gas, carrier gases for GC, plant growth regulators, and electrical costs associated with operating growth chambers. [4]Travel is to cover weekly trips to the root pruning site in objective 2.

Objectives:

1. Develop ReTain, NAA and Ethephon protocols for increasing return bloom and fruit set in pear. Determine the effects of each of these on flowering, fruit set, and yield.

2. Complete a 3-year evaluation of root pruning in a high-density 'd'Anjou' planting. Characterize the effects of root pruning and potassium fertilizer on production and growth.

3. Evaluate the efficacy of metamitron as a thinner for 'Bartlett' pear.

Significant Findings

Objective 1:

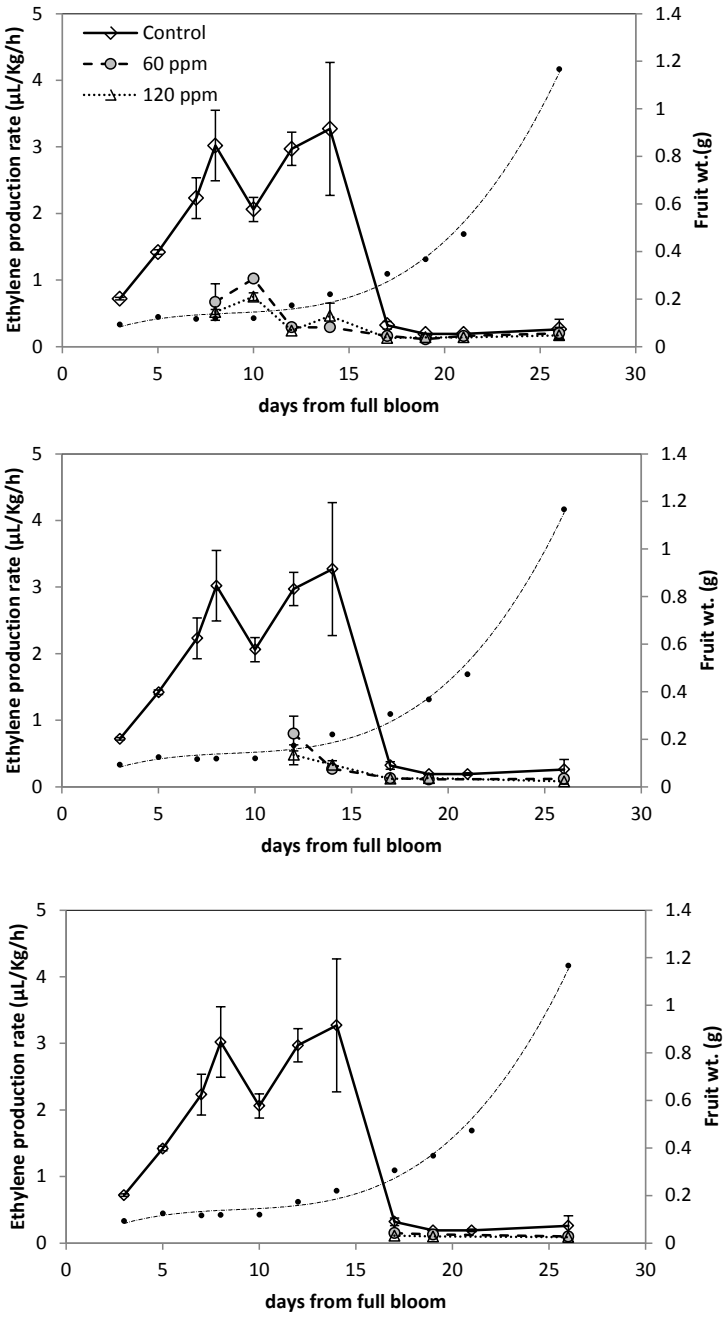
- ReTain applications improved fruit set and yield of mature Anjou trees by ~20% when applied just prior to, at, or after petal fall (i.e., 8, 12 or 16 days after full bloom).
- Application rates of ½ and 1 full pouch per acre were equally effective at increasing fruit set and yield.
- Natural ethylene production of untreated flowers and fruitlets increased ~4-fold from negligible production rates at bloom to maximum production rates ~ 14 days after full bloom, then declined sharply to values near 0 by 20 days after bloom.
- Applications of ReTain at 8, 12 or 16 days after full bloom markedly reduced ethylene production rates of flowers and fruitlets (i.e., ~30% of untreated levels).
- Ethephon 300 ppm applied 45 days after full bloom (performed in 2014) resulted in a 30% increase in return bloom and yield in 2015. Applications of 150 ppm did not result in a significant yield increase compared to untreated controls and 450 ppm had no benefits over the 300 ppm rate. These data support our earlier findings from 2013/2014.
- Ethephon 300 ppm applied 45 days after full bloom completely reversed the ~20% reduction of Anjou return bloom and yield from 2014 pro-hexadione calcium treatments (i.e., Apogee or Kudos).
- Four, weekly applications of NAA 5 ppm beginning 45 days after full bloom (performed in 2014) did not increase return bloom, fruit set or yield in 2015.

Objective 2:

- Root pruning both sides of 5th leaf Anjou tree-rows in 2014 at 1.5 ft. depth and distance from trees increased 2015 fruit set by 46% and resulted in a 40% increase in tree yield compared to controls. These data were similar to results reported in 2013/2014.
- Root pruning 4th leaf Anjou trees in a separate fertilizer trial in 2014 increased 2015 yield by ~35%. However, 2014 differential potassium applications were nullified by a grower decision to apply an aggressive fertilizer plan to the block in 2015.
- Fruit size of root pruned treatments was not significantly reduced in either experiment compared to controls as previously observed.

Objective 3:

- Metamitron effectively thinned Bartlett pears in a rate-dependent manner when applied at ~12 mm timing. The most efficacious rates required little to no follow-up hand-thinning. An earlier application (~6 mm) was not effective and did little to improve thinning when combined with the 12 mm timing.
- Metamitron reduced photosynthesis by 50% to 90% (relative to rate) for a two-week duration. This strong reduction of photosynthesis was associated with fruit abscission.
- The high levels of fruit drop from metamitron resulted in significantly larger fruit size compared to controls. Fruit quality at harvest and after storage was unaffected by metamitron.



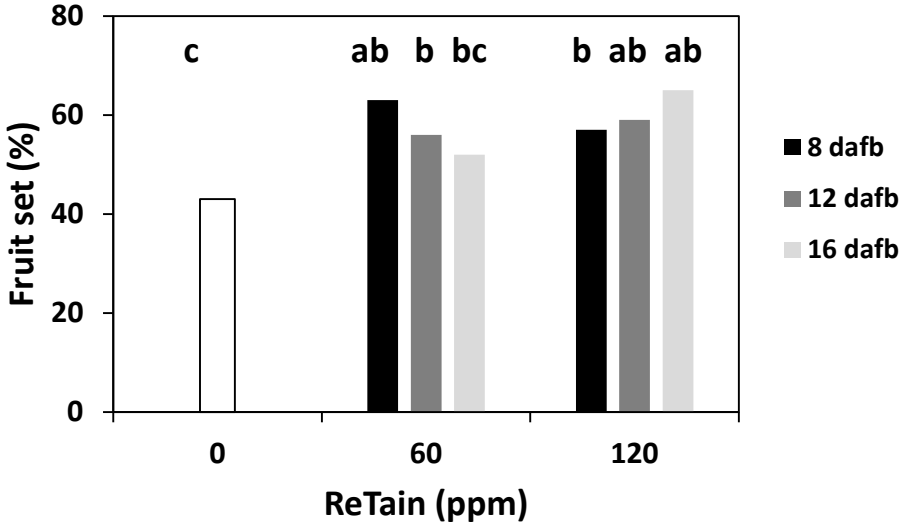
Results and Discussion

Objective 1 (PGRs):

ReTain: The active ingredient in ReTain (AVG) disrupts ethylene synthesis. Ethylene is a natural plant hormone that plays a strong role in senescence processes. The objective of using ReTain to improve fruit set is to reduce the production of ethylene in fruitlets that might otherwise induce abscission if left unchecked.

In past reports, we have documented a similar pattern of ethylene production from untreated pear flowers in different years (2013 and 2014) and of varying cultivars ('d'Anjou' and 'Comice') as steadily increasing from bloom to a maximum rate near 14 days after bloom, then rapidly declining to undetectable levels over the next few days. The application of ReTain significantly reduced ethylene production within 1 day in all cases; however, we observed a marked difference in the duration of the response induced by ReTain between years. In 2013 the response was strong and persisted for ~20 days, but in 2014, ethylene was only reduced for a few days after treatment. Despite the potential for long-lasting activity, we have never observed an increase in fruit set when ReTain was applied near full bloom. Collectively, these data informed us to target applications between petal fall and the peak of ethylene production (around 14 days after bloom). In 2015, we focused on a narrow range of timings using either ½ pouch (~60 ppm) or full-pouch (~120 ppm) rates per acre: 8 days after bloom; 12 days after bloom; and, 16 days after bloom. Whole trees were sprayed to runoff with a pressurized handgun. In all experiments a surfactant (Sylgard 309) was added to ReTain at 0.1% (v:v). ReTain markedly reduced, but did not completely inhibit, ethylene production of flowers and fruitlets at all timings (please see figure above). Consistent differences in the ethylene production rate were not observed for the half or full pouch ReTain rates; therefore, it appears that ½ pouch per acre saturates the response. The growth rate of untreated fruit is provided in all panels of the above figure for reference (dark circles with hashed line). ReTain did not reduce the growth rate of fruit (data not shown). Given the data, we

would have expected no effect of ReTain on fruit set when applied at 16 days after bloom (lower panel) since ethylene production of untreated fruit was nearly undetectable at this time.



Fruit set was improved for all rates and timings of ReTain (see figure directly above). The full-pouch rate did not significantly increase the fruit set achieved with half rates at any timing. These data agree mostly with our previous findings, with the exception of a few trials where the full rate provided a slightly improved response than the half rate.

Treatment (timing)	(ppm ReTain)	Yield (lbs/tree)	Fruit wt. (g)	Fruit firmness (lbs f)	Seeds (no./fruit)
Control	0	347 b	208.3 a	13.7	4.6
8 dafb	60	421 a	212.8 a	13.8	5
	120	399.3 ab	208.4 a	13.7	5
12 dafb	60	390 ab	205.9 a	14.1	4.6
	120	393.2 ab	196.5 ab	14	5.1
16 dafb	60	434 a	207.2 a	13.7	4.9
	120	420.3 a	187 b	13.6	5.3

Higher fruit set resulted in greater yields for most ReTain treatments relative to the untreated control (please refer to Table above). The fact that the 16 dafb application led to higher fruit set than controls is not clear. However, treatment timings were classified as days after full bloom and thus represent the average condition of the tree. While fairly uniform flower and fruitlet samples were collected for ethylene detection, the distribution of flower phenology would have comprised some portion of delayed blooms (bell-shaped curve), rendering that faction of flowers at the perfect stage for ReTain action resulting in greater set relative to the controls. Generally, ReTain increased fruit production by ~20%. Fruit weight, flesh firmness and seed count per fruit were largely unaffected by ReTain at harvest. The lack of difference in seed count among treatments indicates that ReTain did not set parthenocarpic (seedless) fruit, supporting our earlier observations.

Since beginning work with ReTain in 2012, we have documented an increase in fruit set and production in ~65% of trials.

In a separate series of experiments, we attempted to test the effect of temperature on AVG absorption and uptake using programmable temperature chambers. This work was designed to address the influence of application temperatures on the efficacy of ReTain. Shoots with sufficient flowers were sampled from the field and placed in test chambers held at either 35° F, 45° F, 55° F, 65° F, or 75° F then treated with ReTain. This approach was meant to mimic the range of temperatures likely when spray applications are made in the field, and to determine whether or not the temperature of plant tissue influences the uptake of ReTain. After drying, shoots were removed from chambers and flowers were weighed and placed in incubation tubes, sealed and held for 12 HRs at three temperatures designed to elicit a range of ethylene production rates (45° F, low ethylene rate; 65° F, moderate ethylene rate; 85° F, high ethylene rate). This portion of the experiment served to model post-application field conditions to describe their effect on uptake and activity of ReTain and characterize ethylene response to temperature. In all cases, treatments were compared to an untreated control (placed in separate chambers). Unfortunately, incubation temperatures (especially the two higher temps) led to increased humidity in the tubes which made detection on a gas chromatograph (GC) extraordinarily difficult. Because of the shift in retention time, the data were not reliable and are not reported. We are considering options to add a dehydration column upstream of the injection port so that we can attempt this work in 2016.

Ethephon and NAA: We have been evaluating ethephon for a few years to improve flower initiation and hence 'return' bloom the year subsequent to applications. For 'd'Anjou', flower initiation appears to occur around 50 dafb, hence our timing of 45 dafb. In 2012, 300 ppm ethephon applied at 50 dafb significantly increased 2013 yield by ~28%. The rate of 300 ppm was selected from reports using different cultivars and in different regions. Therefore, using a different set of trees in 2014, we repeated the experiment but tested several ethephon rates (150, 300 and 450 ppm). Return bloom, fruit set and return yield in 2015 were highest for 300 ppm ethephon (i.e., ~31% increase in production compared to controls; please refer to table below). Increasing the ethephon rate to 450 ppm did not improve the response. Conversely, the low rate of 150 ppm was not efficacious; hence, 300 ppm ethephon is the appropriate rate to increase return bloom of Anjou in the mid-Columbia region. Further, we applied ethephon to trees treated with two applications of prohexadione-calcium (active ingredient in Apogee and Kudos) to reduce vigor. In the past, we have documented reduced return bloom associated with prohexadione-calcium. Ethephon completely reversed the adverse effect of Kudos on return bloom (see table below) resulting in strong vigor control without sacrificing return bloom.

Treatments	Return bloom		Return yield and fruit wt.		
	Spurs	1-yr shoots	Yield per tree	Fruit per tree	Avg fruit wt
	%	%	lbs	no.	g
Control	43 b	42 b	251 b	534 b	212
Kudos 250 ppm	**17 c**	**13 c**	**184 c**	**365 c**	227
Kudos + 450 Eth	43 b	34 b	246 b	504 b	220
Kudos + 300 Eth	**57 ab**	**28 bc**	**274 ab**	**610 a**	203
Ethephon 150 ppm	49 b	50 ab	262 b	546 b	217
Ethephon 450 ppm	**48 b**	**49 b**	**305 a**	**659 a**	209
Ethephon 300 ppm	**64 a**	**60 a**	**330 a**	**634 a**	235

The use of NAA, applied weekly at low concentrations (5 ppm) beginning 45 dafb did not improve return bloom or yield of 'd'Anjou' trees (data not shown). This protocol has been successfully applied to apple

Objective 2 (Root Pruning):

Root pruning was performed in commercial orchards prior to bloom when ~10% of the flowers were open. The implement (fabricated by Mr. Herbie Annala, Hood River producer) was tractor mounted and pulled in low gear ~1.5 ft. from tree trunks down either one or two sides of the tree row. Root pruning treatments were compared to untreated control trees in randomized complete block designs, replicated four times throughout the orchard. Whole rows were treated in experiment 1; in experiment 2, replicates comprised 8 contiguous trees. The depth of the steel shank was 1.5 ft. and the angle was 5 degrees off from the vertical (angle facing into the tree row). All other cultural practices were performed according to commercial standards.

Experiment 1: 2014/2015 5th and 6th leaf 'd'Anjou'/OHxF 87 (4 ft. x 12 ft.) Trial- In 2014, we documented a two-fold increase in the return yield of root pruned 'd'Anjou' pear trees (root pruned in 2013 [4th leaf]) - this represented a per acre improvement of ~ 20 bins. Root pruning reduced shoot length by ~ 20% the year of application, and trunks were 30% smaller after the second year. Hence, the yield efficiency of root pruned trees in 2014 was markedly higher than untreated trees, fulfilling the primary objective of root pruning. These results were in contrast to an earlier experiment whereby root pruning negatively affected fruit set, yield, and fruit size of 6th leaf 'd'Anjou' trees in the year of application. In that trial, root pruned trees had higher return bloom, fruit set and yield in the subsequent year but not enough to compensate for the yield reductions of year 1. We surmised that root pruning was too severe for the age of the trees and, ideally, should be performed earlier in the life of the orchard.

In 2015, we either root pruned trees in consecutive years (i.e., 2014 and 2015) or applied root pruning to previously untreated, 6th leaf trees. In all cases root pruning was performed to both sides [i.e., 2xRP] of the tree row at a depth and distance from trees of 1.5 ft. In contrast to previous experiments, we observed a ~20% increase in yield the year of application of root pruning when trees had not received previous root pruning treatment (see table below). The effect of consecutive years of root pruning resulted in a 40% yield improvement relative to control trees. While a slight reduction in fruit weight was observed for trees receiving root pruning for the first time in 2015 (as previously shown) the reduction was not significant.

Treatment 2014/2015	Tree yield (lbs/tree)	Fruit per tree (no.)	Avg. fruit wt. (g)
Control/Control	49.1 b	98.2 b	223.9
Control/2x RP	59.4 ab	137.4 a	199.3
2x RP/2x RP	68.8 a	143.6 a	216.2

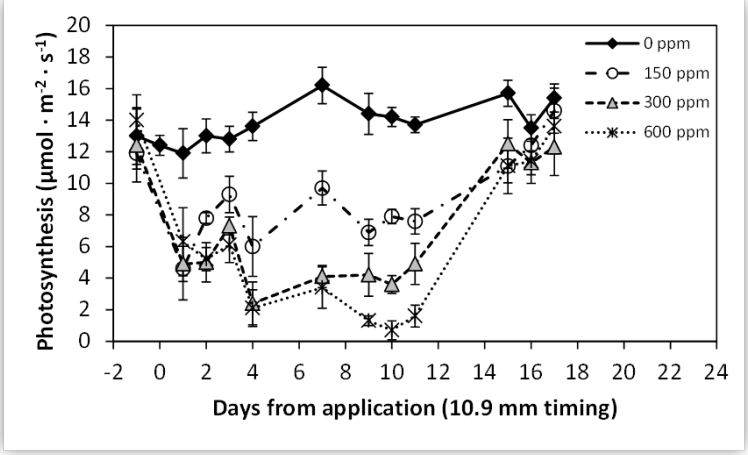
We did not quantify the response on shoot growth or vigor, since we have thoroughly documented these responses in previous reports, though shoots were visibly shorter in trees root pruned for the first time in 2015 (i.e., estimated to be ~1/4 to 1/3rd reduced).

Experiment 2: 2015 5th leaf 'd'Anjou'/OHxF 87 (4 ft. x 12 ft.) Potassium Trial- We have also been evaluating the effects of potassium fertilizer in combination with root pruning. Potassium is mobile in soils but could potentially become limiting when severely reducing the rhizosphere (i.e., root pruning). Moreover, potassium has been positively associated with fruit size and fruit size is often compromised by root pruning. In 2014, three levels of potassium (low, moderate and high) were applied with and without root pruning. Root pruning was applied as described above in Experiment 1. In 2015, we compared trees that were root pruned in 2014 but not root pruned in 2015 and trees that were root pruned consecutively (i.e., 2014 and 2015 2x RP) to untreated controls. We

intended to continue disparate potassium fertilizer treatments but, unfortunately, the grower entered a contract with a fertilizer company to treat the block uniformly using a different approach. Consequently, we were not able to evaluate the effects of potassium and its interaction with root pruning on yield and fruit relations. We did, however, harvest the block and compare the production of control trees to those root pruned. Root pruning resulted in a 35% increase in yield relative to untreated controls, irrespective of whether root pruning was re-applied in 2015 or not (see table below).

Treatment 2014/2015	Yield (lbs/tree)	Fruit no. (fruit/tree)	Fruit wt. (g)	Fruit firmness (lbf)
Control/Control	33.9 b	69.7 b	220.6	12.4
2x RP/Control	47.2 a	89.2 a	239.3	12.8
2x RP/2x RP	45.5 a	85.2 a	241.8	12.4

With the exception of one trial, root pruning has consistently led to greater 'd'Anjou' yields when applied to 4th, 5th and 6th leaf trees. Our previous experiments indicated that root pruning one side only was largely ineffective. Fruit size has typically been reduced the year of application, though we did not observe this in 2015.

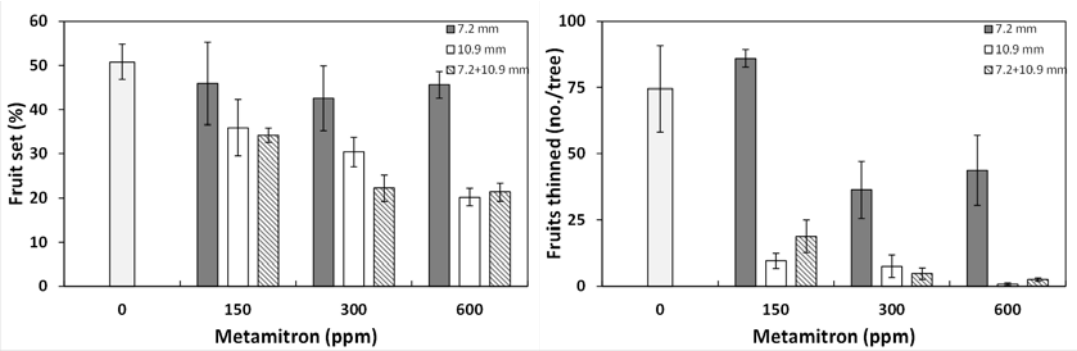


Objective 3 (Thinning):

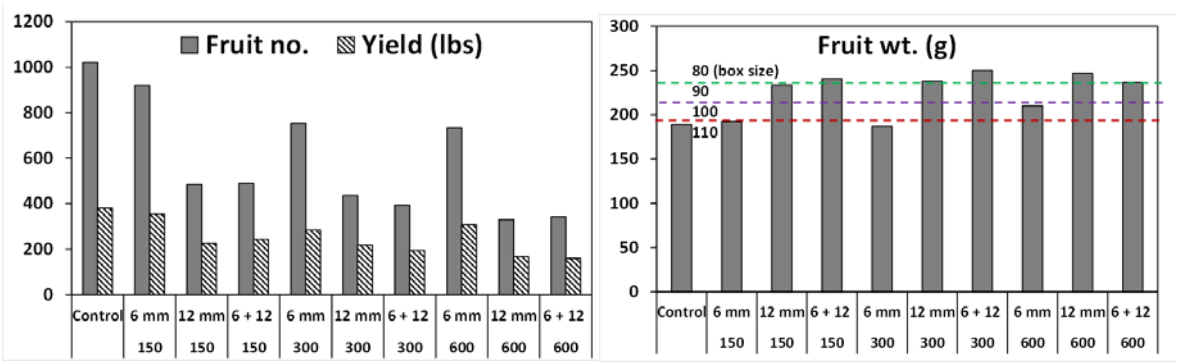
Rates of metamitron (150, 300, 600 ppm) were chosen based on a previously published trial using 'Conference' pear in The Netherlands that produced a range of fruitlet abscission from relatively little to excessive. For each of the three rates evaluated, we tested two application timings (6 mm and 12 mm), alone and combined. Our selection of a mature block of Bartlett trees (i.e., ~40-year-old trees) was predicated on our previous thinning work whereby older trees with presumably high reserve carbohydrates were able to withstand short periods of reduced photosynthesis (induced by alternative thinning compounds) without a concurrent increase in fruitlet thinning, compared to younger trees. From this work we hypothesized that a potentially large reserve carbohydrate pool might supply sufficient carbon to meet fruit growth demands despite a reduction in incoming carbon from photosynthesis. Metamitron reduced photosynthesis by ~50% to 90% depending on rate (please refer to figure at left). Interestingly, all rates reduced photosynthesis for approximately two weeks. The lack of measurements between 11 and 15 days from application, however, may have obscured actual differences among rates in the duration of the effect.

Fruit abscission was strongly associated with metatmitron rate (see upper figure on next page). Rates of 150 and 300 ppm reduced the crop load of untreated control trees by ~25% to 40%. The 6 mm timing (actually 7.2 mm) had relatively no thinning efficacy; therefore, the combination of early and late timings differed little from the 12 mm timing (actually 11.2 mm), which thinned exceptionally well. After evaluating fruit set and thinning efficacy, we lightly hand-thinned all trees to reduce clusters of 4 or more fruit. The level of hand thinning required was proportional to the thinning efficacy of the different rates and timings (see upper figure on next page). Treatment yields reflected

the relative number of fruits removed by chemical and hand thinning (see lower figure next page). Fruit size was improved for all 12 mm application rates and was clearly a function of crop load (see lower figure next page). We note, however, that a commercial level of hand thinning was not applied to untreated control trees. Therefore, a crop level between the control and 150 ppm (11.2 mm timing) may have been optimal to achieve good balance between fruit size and yield. Future work is proposed to refine application rates. No adverse effects were observed for any fruit quality attributes (fruit firmness, soluble solids, titratable acidity, and fruit finish (i.e., russet) evaluated at harvest and after 3 months of cold storage (data not shown).



Caption to above figure. The effect of metamitron rate (0, 150, 300 and 600 ppm) and timing (7.2 mm, 10.9 mm and 7.2 mm + 10.9 mm) on fruit set of 'Bartlett' pear flowers (expressed as % fruits per 500 clusters) left, and fruits removed by a light, follow-up hand thinning ~40 days after full bloom (right). Bars are the mean of 5 single-tree replicates +/- standard error.



Caption to above figure. The effect of metamitron timing (upper x-axis; 7.2 mm, 10.9 mm and 7.2 mm + 10.9 mm) and rate (lower x-axis; 150, 300 and 600 ppm) on the total number of fruits and yield per tree (left), and fruit size at harvest (right), compared to a control. Box sizes (no. of fruit per 44 lb box) are provided for comparison using hashed horizontal lines in right panel. Bars are the mean of 5 single-tree replicates (n= 100 [individually weighed fruit per tree]). Harvest began when flesh pressures reached 18 lbf.

Executive Summary:

PGRs

•ReTain applications improved fruit set and yield of mature 'd'Anjou' trees by ~20% when applied just prior to, at, or after petal fall (i.e., 8, 12 or 16 days after full bloom).

•Application rates of a half pouch per acre were sufficient to optimize the effect on fruit set and yield.

•Natural ethylene production of untreated flowers and fruitlets increased ~4-fold from negligible production rates at bloom to maximum levels ~ 14 days after full bloom, then declined sharply to values near 0 by 20 days after bloom.

•Applications of ReTain at 8, 12 or 16 days after full bloom markedly reduced ethylene production rates of flowers and fruitlets (i.e., ~30% of untreated levels).

•Ethephon at a rate of 300 ppm applied 45 days after full bloom (performed in 2014) increased 2015 return bloom and yield by ~30% compared to untreated controls. 150 ppm ethephon did not affect flowering or production in 2015 and 450 ppm ethephon had no appreciable benefits compared to the 300 ppm rate.

•Ethephon at a rate of 300 ppm applied 45 days after full bloom in 2014 completely reversed the ~20% reduction in return bloom and yield caused by 2014 pro-hexadione calcium treatments (i.e., Apogee or Kudos).

•Four, weekly applications of NAA (5 ppm) beginning 45 days after full bloom (performed in 2014) did not increase return bloom, fruit set or yield of 'd'Anjou' in 2015.

Root Pruning

•Root pruning both sides of 5th leaf Anjou tree-rows in 2014 at 1.5 ft. depth and distance from trees increased 2015 fruit set by 46% and resulted in a 40% increase in tree yield compared to controls. These data were similar to results reported in 2013/2014.

•Root pruning 4th leaf Anjou trees in a separate fertilizer trial in 2014 increased 2015 yield by ~35%. However, 2014 differential potassium applications were nullified by a grower decision to apply an aggressive fertilizer plan to the block in 2015.

•Fruit size of root pruned treatments was not significantly reduced in either experiment compared to controls as previously observed.

Thinning

•Metamitron effectively thinned Bartlett pears in a rate-dependent manner when applied at ~12 mm timing. The most efficacious rates required little to no follow-up hand-thinning. An earlier application (~6 mm) was not effective and did little to improve thinning when combined with the 12 mm timing.

•Metamitron reduced photosynthesis by 50% to 90% (relative to rate) for a two-week duration. This strong reduction of photosynthesis was associated with fruit abscission.

•The high levels of fruit drop from metamitron resulted in significantly larger fruit size compared to controls. Fruit quality at harvest and after storage was unaffected by metamitron.

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

Project Title: Suppression of pear psylla using elicitors of host-defenses

PI: W. Rodney Cooper
Organization: USDA-ARS-YARL
Telephone: 509/454-4463
Email: Rodney.Cooper@ars.usda.gov
Address: 5230 Konnowac Pass Road
City/State/Zip: Wapato, WA 98951

Cooperators: David R. Horton, USDA-ARS, 5230 Konnowac Pass Road, Wapato, WA

Total Project Request: **Year 1:** $25,000 **Year 2:** $25,000 **Year 3: $5,700**

Other funding sources: None

Budget 1
Organization Name: USDA-ARS-YARL **Contract Administrator: Chuck Myers**
Telephone: 510/559-5769 **Email address: Chuck.Myers@ars.usda.gov**

Item	2014	2015	2016
Salaries	$16,000	$16,000	$5,000
Benefits	$1000	$1000	$200
Wages			
Benefits			
Equipment			
Supplies	$5000	$5000	
Travel			
Plot Fees	$3000	$3000	$500
Miscellaneous			
Total	$25,000	$25,000	**$5,700**

Footnotes:
[1] Partial funding for a temporary employee to help with field studies

OBJECTIVES

1) Test the effects of commercially available elicitors of host defenses on pear psylla population growth in pear orchards.
2) Test the effects of defense elicitors on recruitment of natural enemies.
3) Test the combined effects of defense elicitors and potassium or magnesium fertilization on pear psylla performance.
4) Test the effects of defense elicitors on obligate bacterial symbionts of pear psylla.

SIGNFICANT FINDINGS

1) Both Actigard and ODC reduced pear psylla nymph populations by about 20% during peak populations of both study years.
2) Magnesium sulfate treatment reduced pear psylla numbers under greenhouse conditions, but did not enhance Actigard-activated defenses against psylla.
3) Adults collected from pear trees treated with Actigard had significantly reduced titers of the obligate symbiont, *Carsonella ruddii*, than did adults collected from untreated trees.

METHODS

Objectives 1. Test the effects of commercially available elicitors of host defenses on pear psylla population growth in pear orchards.

Experiments will be conducted in a Bartlett pear orchard located at the USDA-ARS experimental farm near Moxee, WA. The orchard was planted in 2001 with 16 × 16-ft spacing. The orchard will be divided into six main plots each with four subplots (one for each treatment). Each subplot will be randomly assigned a foliar treatment: Employ, Actigard, ODC, or untreated control. Foliar treatments will be applied according to the product labels every four weeks beginning mid-March and ending mid-August.

Populations of pear psylla will be monitored weekly beginning one week before the first application of defense elicitors and ending at fruit harvest. Populations of adult pear psylla will be estimated using beat tray samples from each of the four cardinal directions around each tree. Populations of pear psylla nymphs will be estimated by counting the numbers of nymphs on the ten most terminal leaves from ten shoots per tree. Fruit will be harvested from each tree in the fall to assess fruit downgrading due to honeydew as described by Pfeiffer and Burts (1983. Environmental Entomology 12: 895-901). The experiment will be repeated each year for three years. Data will be used to estimate the effects of each defense elicitor on populations of pear psylla and fruit damage caused by honeydew production.

Objective 2. Test the effects of defense elicitors on recruitment of natural enemies.

Experiments will be conducted using the same trees used for objective 1. Populations of common orchard predators (Coccinellids, Anthocorids, *Deraeocoris*, and lacewings), and the common parasitoid of pear psylla (*Trechnites* spp.) will be estimated based on samples obtained using a standard beat-tray. Nymphal stages of predators and parasitized pear psylla nymphs (mummies) will be sampled by observing the ten most terminal leaves of ten shoots per tree.

Objective 3. Test the combined effects of potassium and magnesium fertilization on induced defenses against pear psylla.

This objective is finished.

Objective 4. Test the effects of defense elicitors on the obligate bacterial symbiont of pear psylla.

Bartlett seedlings infested with early instar nymphs will be treated with Actigard or water. Once the nymphs have developed to adults, mated females will be transferred to untreated seedlings to obtain eggs. *Carsonella ruddi* will be quantified in the resulting nymphs from each female using qPCR and FISH. Data will be used to assess whether Actigard reduces transovarial transmission of

Carsonella from mother to eggs.

RESULTS AND DISCUSSION

Objectives 1. Test the effects of commercially available elicitors of host defenses on pear psylla population growth in pear orchards.

Year 1: Peak populations of pear psylla nymphs were observed on weeks 5-6 (1-7-May) and on weeks 11 and 12 (18-25 June) of our study (Figure 1A). Analyses indicated a significant treatment by week interaction, indicating that the effects of treatment were not consistent among weeks (Figure 1A). Untreated trees supported significantly more nymphs than did trees treated with Actigard, Employ, or ODC from weeks 11-15 (11-June to 9-July), and on the final sampling day (6-Aug). Averaged over all weeks, there were numerically more nymphs present on untreated trees (mean ± SE nymphs per shoot, 33.1 ± 1.97) than on trees treated with Actigard (23.9 ± 1.97), Employ (25.6 ± 1.97) or ODC (26.8 ± 1.97). Although the observed effects of defense elicitors on pear psylla nymphs were consistent with our previous study, the effects of elicitors were not as great as previously observed in the laboratory, where Actigard, Employ, and ODC reduced populations by nearly 50% compared with untreated trees.

We did not observe a significant treatment effect on pear psylla adults, or a significant treatment by week interaction (Figure 1B). Results were consistent with our laboratory studies, which also suggested that adults are not affected by plant responses to applications of defense elicitors. However, the numerically (but non-significant) greater number of adults observed on control trees compared with trees treated with elicitors on week 15 (9-July) and weeks 17-19 (23-July-6-Aug) suggest that more nymphs reached adulthood on untreated trees (Figure 1B). As expected, adult populations varied by week (Figure 1B). Peak adult populations occurred on week 8 (21-May) and weeks 14-15 (2-9-July), which was about 3-4 weeks later than peak nymph populations (Figure 1).

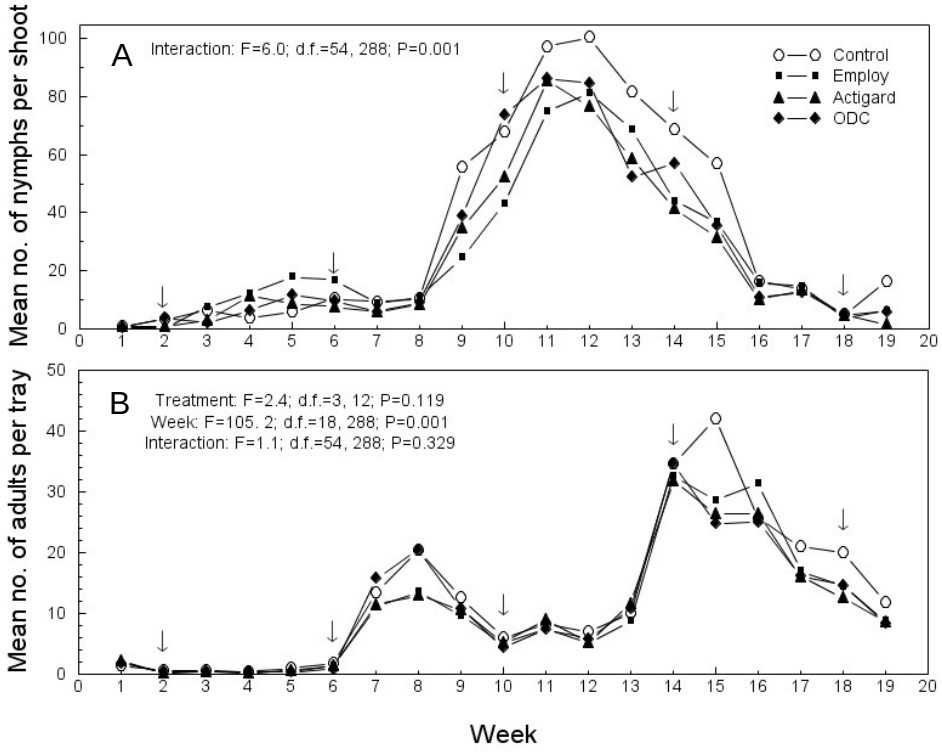


Figure 1. Effects of defense elicitors on pear psylla nymphs (A) and adults (B). Arrows denote treatment application dates (9-April, 7-May, 4-June, 2-July, and 30-July).

A rainstorm several days before harvest washed honeydew from fruit, so we were not able to compare honeydew accumulation on fruit among treatments. Severity of fruit russetting was ranked based on the percentage of each fruit (10 per tree) marked by russetting where 1=0-25%, 2=26-50%, 3=51-75%, and 4=76-100%. We did not observe significant differences in damage among treatments (Figure 2; χ^2=1.56; df=3; *P*=667). Although not significant, a greater percentage of fruit from trees treated with any of the three elicitors were ranked with the lowest damage rating than fruit from untreated trees (Figure 2).

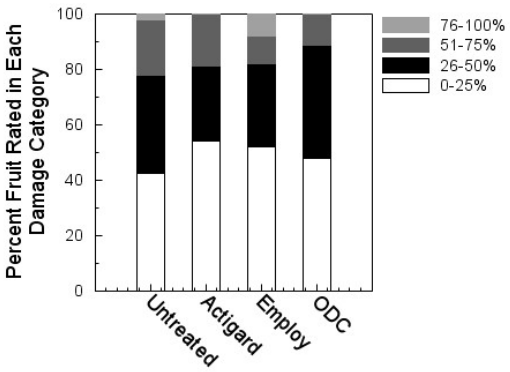


Figure 2. Fruit damage caused by fruit russetting.

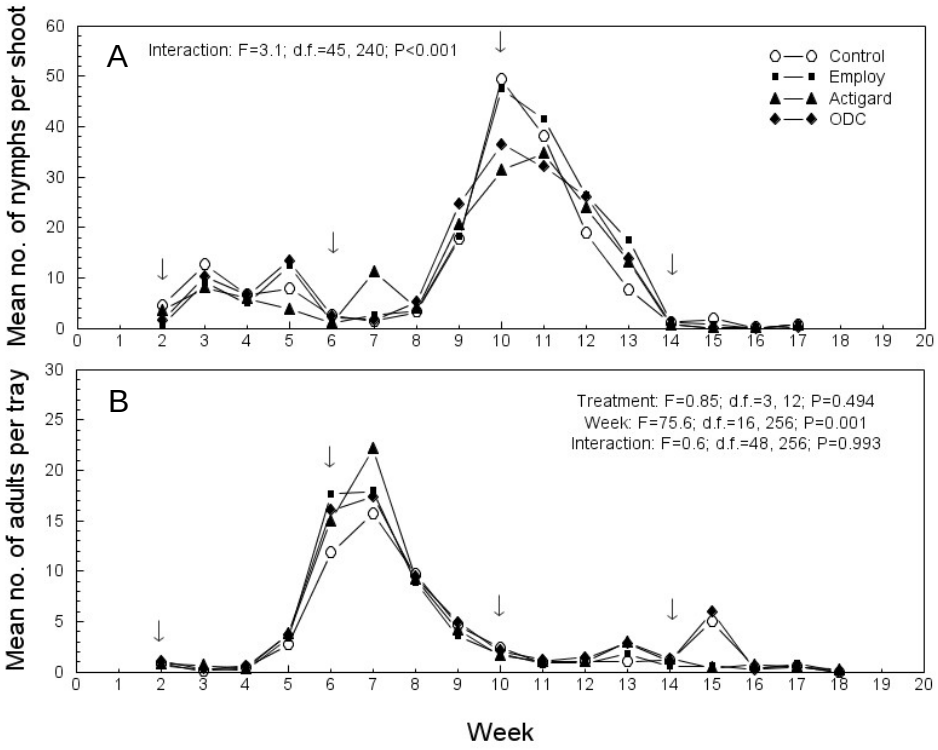


Figure 3. Effects of defense elicitors on pear psylla nymphs (A) and adults (B) in 2015. Arrows denote treatment application dates (15-April, 15-May, 10-June, 8-July). Weeks were arranged to correspond with dates in Figure 1.

Year 2. Pear psylla populations were substantially lower in 2015 than in 2014, and peak populations occurred about two weeks earlier (Figure 3). Analysis indicated a significant treatment by week interaction (Figure 3). Fewer pear psylla nymphs were observed on trees treated with elicitors on weeks 2 (14-April) and 3 (21-April), suggesting that treatments from 2014 had an effect on early season 2015 populations. Trees treated with Actigard and Employ both had fewer nymphs on week 10 (9-June). There were no significant differences in treatments on any other date. As observed in 2014, foliar treatments did not affect numbers of adults on trees (Figure 3).

Trees were treated with the same treatments in both years of the study. It is therefore possible that the trees became less responsive to the chemical elicitors. In other words, the less consistent effects of elicitors in 2015 compared to 2014 may be due weaker induction of defenses in

response to foliar treatments. In 2016, we will compare defense responses activated by Actigard between naive trees (trees that have not previously been treated with elicitors) and trees that were treated with Actigard in 2014 and 2015.

Objective 2. Test the effects of defense elicitors on recruitment of natural enemies.

Year 1. Populations of natural enemies were generally low throughout the year, and most varied by week. We did not observe significant differences in natural enemy populations among treatments, but a significant treatment by week interaction for lacewing adults indicated that the effects of treatment were not consistent among weeks. On week 8 of our study (21-May), which coincided with the first psylla adult population peak (Figure 1B), there were more lacewing adults captured on trees treated with Actigard or Employ than on untreated trees or trees treated with ODC. A similar trend was observed on week 11 (11-June) when more lacewing adults collected from trees treated with Employ than on other trees. Previous studies have shown that activation of acquired defenses, including salicylic acid-dependent defenses, can lead to increased recruitment of natural enemies (Thaler et al., 2001. Ecological Entomology 26: 213-324). However, our results do not provide substantial evidence that natural enemy populations increase on pear trees treated with defense elicitors. It is possible that pear trees with induced defenses do not release volatiles that are attractive to natural enemies, or that volatile signals from single pear trees are not strong enough to attract adequate numbers of natural enemies. It is also possible that natural enemy populations were too small at the Moxee farm in 2014 to reliably compare their populations among treatments.

Year 2. Although natural enemy populations changed over time, there were no significant differences in natural enemy populations among trees treated with elicitors or water.

Objective 3. Test the combined effects of potassium and magnesium fertilization on induced defenses against pear psylla.

Year 1. Experiments were setup in the screenhouse located at the experimental farm in Moxee, and each potted tree was inoculated with psyllids. However, all of the trees were infected with fire blight, and many of the trees died before the study was completed 31 days after foliar treatments. Averaged over all treatments, trees treated with foliar applications of magnesium sulfate had 220 pear psylla compared with 570 psylla on untreated trees, but these values were not significantly different.

Year 2. Greenhouse assays confirmed our previous results that Actigard treatments reduce pear psylla performance (Figure 4). Results also revealed that foliar application of magnesium sulfate also reduced pear psylla performance (Figure 4), which is consistent with anecdotal reports on aphids. Adding magnesium sulfate did not improve plant protection provided by Actigard (Figure 4). We found no evidence that potassium fertilization influences pear psylla performance. We do not plan to continue these experiment in year 3.

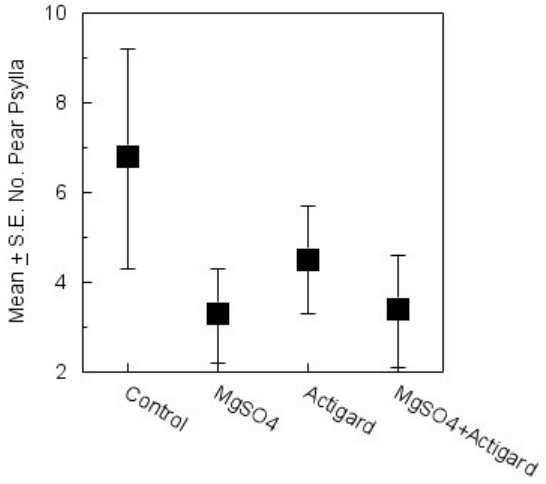


Figure 4. Effects of Magnesium sulfate and Actigard applications on pear psylla performance

Objective 4. Test the effects of defense elicitors on the obligate bacterial symbiont of pear psylla.

Year 1. We first developed methods to compare populations of the obligate symbiont of pear psylla, *Carsonella*, among different insects. One method uses fluorescence *in situ* hybridization (FISH) to visually detect *Carsonella* in bacteriocytes, specialized insect cells which harbor the bacteria. This method was largely based on our FISH assay to detect *Liberibacter* in specific tissues of potato psyllid (Cooper et al., 2014. Annals of the Entomological Society of America. 107: 204-210). Using FISH, we labeled *Carsonella* with a fluorescent probe and measured the intensity of fluorescence to estimate relative bacteria densities in individual bacteriocytes (Figure 5A inset). Our second method relies on quantitative real time PCR (qPCR) to estimate bacteria densities in whole insects.

Using these methods, we showed that *Carsonella* was more abundant in females than in males (Figure 5). These results confirmed that our methods are suitable for comparing *Carsonella* among pear psylla, and showed that insect sex should be controlled in our future studies. In addition, preliminary results suggested that the acquired elicited by Actigard lead to reduced populations of the obligate endosymbiont of pear psylla.

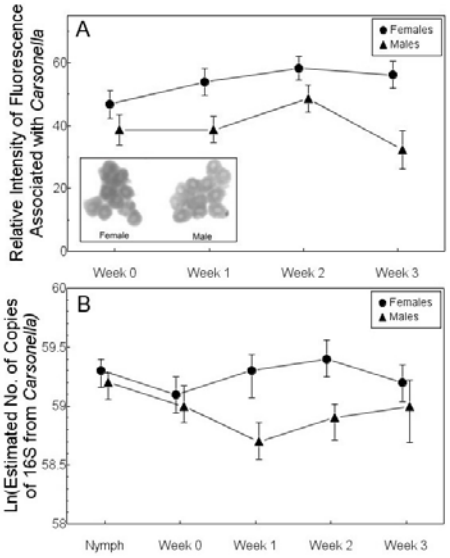


Figure 5. Comparison of *Carsonella* densities among females and males of using FISH (A) and qPCR (B). Inset shows samples of bacteriocytes containing *Carsonella* labeled with a fluorescent probe; the darker cells corresponds with a greater density of *Carsonella*.

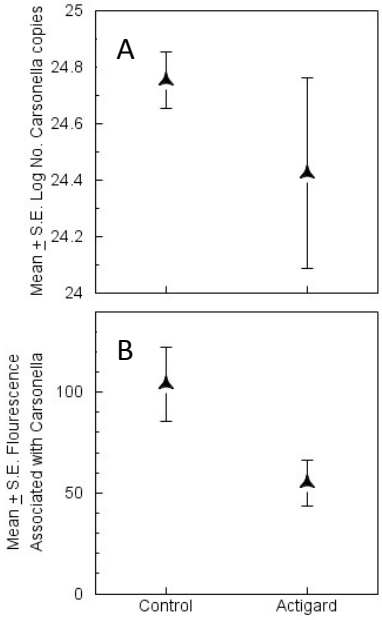


Figure 6. Effects of Actigard on *Carsonella* densities in whole pear psylla adults (A) and within individual bacteriocytes (B).

Year 2. In year 2, experiments were conducted to confirm preliminary results obtained in year 1 on the effects of defense elicitors on the obligate endosymbionts, *Carsonella*. Methods developed in year 1 (Figure 6) were used to measure *Carsonella* densities in whole adult insects and in individual bacteriocytes of pear psylla adults collected from trees treated with water (control) or Actigard. Both measures of *Carsonella* indicated that Actigard treatment led to significant decreases in *Carsonella* densities in pear psylla adults (Figure 6). Because nymphs acquire *Carsonella* from their mothers, this decrease in *Carsonella* in adult females may explain why nymphs are especially susceptible to defenses activated by Actigard treatment. In the final year of this study, we plan to test whether reductions in *Carsonella* in mothers leads to reduced titers of *Carsonella* in offspring.

PUBLICATIONS:

1) Cooper, W.R., and D.R. Horton. 2015. Effects of elictors of host plant defenses on pear psylla, *Cacopsylla pyricola*. Entomol. Exp. Appl. 157: 300-306.

2) Cooper, W.R., S.F. Garczynski, and D.R. Horton. 2015. Relative abundance of *Carsonella ruddii* (Gamma Proteobacterium) in females and meals of *Cacopsylla pyricola* (Hemiptera: Psyllidae) and *Bactericera cockerelli* (Hemiptera: Triozidae). J. Insect Sci. 15: 65.

CONTINUING PROJECT REPORT **YEAR**: 2016 (No-Cost Extension)
WTFRC Project Number: PR-14-103 (A, B&C)

Project Title: Pesticide resistance in pear psylla and identification of resistance related genes

PI:	Tom Unruh	**Co- PI**:	Peter Shearer
Organization:	USDA-ARS	**Organization**:	OSU-MCAREC
Telephone:	509-454-6563	**Telephone**:	(541) 386-2030-x215
Email:	thomas.unruh@ars.usda.gov	**Email**:	Peter.Shearer@oregonstate.edu
Address:	USDA-ARS	**Address**:	OSU-MCAREC, Horticulture
Address 2:	5230 Konnowac Pass Rd	**Address 2:**	3005 Experiment Station Drive
City/State/Zip:	Yakima, WA 98951	**City/State/Zip**:	Hood River, Oregon, 97031-9512

Co-PI:	Richard Hilton	**Co-PI**:	Joanna Chiu
Organization:	OSU-SOREC	**Organization:**	U. California, Davis
Telephone:	(541) 772-5165-x227	**Telephone:**	(530) 752-1839
Email:	Richard.Hilton@oregonstate.edu	**Email**:	jcchiu@ucdavis.edu
Address:	OSU-SOREC, Horticulture	**Address**:	U.C. Davis, Entomology
Address 2:	569 Hanley Road	**Address 2**:	Storer Hall 6348,
City/State/Zip:	Central Point, OR 97502	**City/State/Zip**:	Davis, CA 95616 USA

Total Project Request: $48,700 **Year 1**: $48,700

Budget 1 (Unruh)
Organization Name: USDA-ARS **Contract Administrator:** Charles W. Myers
Telephone: 510-559-5769 **Email address:** Chuck.myers@ars.usda.gov

Item	2014	NA	NA
Salaries			
Benefits			
Wages	$13800		
Benefits	$ 1200		
Equipment			
Supplies[1]	$ 1000		
Travel			
Miscellaneous			
Plot Fees[2]	$ 1000		
Total	$17,000		

Footnotes:[1] Insecticides, collection materials, computer program for DNA analysis. [2]Moxee farm pears-fertilizer

Budget 2 (Shearer and Hilton)
Organization Name: OSU MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2014	NA	NA
Salaries[1]	$5,215		
Benefits[1]	$3,454		
Wages[2]	$5,787		
Benefits[2]	$1,739		
Equipment	$0		
Supplies[3]	$346		
Travel[4]	$459		
Plot Fees	--		
Miscellaneous	--		
Total	$17,000		

Footnotes: Footnotes: [1]Salary and Benefits: Faculty Research Assistant 0.75 mo. Bioscience Research Technician 0.75 mo. [2]Wages and Benefits: Summer Technician(s), 10 weeks [3]Supplies: Lab supplies for assay and rearing [4]Travel to field. 0.556/mi.

Budget 3 (Chiu)
Organization Name: University of California Davis **Contract Administrator:** Guyla Yoak
Telephone: (530) 752-3794 **Email address:** gfyoak@ucdavis.edu

Item	2014	NA	NA
Salaries[1]	$5,896		
Benefits[1]	$2,252		
Wages	--		
Benefits	--		
Equipment	--		
Supplies[2]	$3,552		
Travel	--		
Miscellaneous[3]	$3,000		
Plot Fees	--		
Total	$14,700		

Footnotes: [1]Salary and Benefits: Technician (2 months of full time); [2]Supplies: Lab supplies for generating transcriptome sequencing libraries and library quality control including NEB Next Ultra RNA library Prep Kit for Illumina, NEB Next Multiplex Oligos for Illumina, NEB Next Poly(A) mRNA Magnetic Isolation Module, Biorad Experion Nucleic Acid Analysis Kit, and consumables such as pipet tips and microcentrifuge tubes
[3]Miscellaneous: Transcriptome sequencing costs at the UC Davis Genome Sequencing Center

OBJECTIVES:

1. **Conduct resistance survey of winter form pear psylla in 16 orchards, 8 in WA and 8 in OR**

We will measure resistance status using the slide dip bio-assay well suited to for pear psylla adults We will attempt to assay Warrior and Pounce (pyrethroids), Delegate (spinosyn), Admire (provado, a neonicotinoid), Nexter (METI interferes with mitochondrial energy cascade), sulfur (inorganic), Manzate (bis-dithiocarbamate), Agri-Mek (avermectin), and one or two more compounds to be determined. ($34,000)

2. **Produce and analyze transcriptomes from 6 populations of pear psylla to identify genetic variations that confer insecticide resistance**

We (Dr. Chiu) will identify inter-population variation at genes related to insecticide resistance using transcriptomes of expressed genes from pear psylla population samples collected from two isolated populations that have a history of minimal chemical control and evidence of low resistance from bioassays. We will compare these to four distinct populations chosen because they have shown significant resistance in bioassays and are from orchards with a history of significant pesticide use. The transcriptomes will be shared with colleagues to support other applications of this valuable genetic information. ($14,700)

Deviations from the Objectives

1. Collaborators in Northern Oregon were unable to collect adequate numbers of pear psylla to conduct studies in autumn of 2014 due to uncommonly low psylla abundance, conflicting travel restrictions and a significant and disruptive freeze in autumn. No Pear -WTFRC funds were spent in OR and a reduced number of collections and assays were conducted in Medford. Plans are to complete studies in autumn of 2015 and extending existing funding .
2. Dr. Chui has just received preserved pear psylla for transcriptome work, which has delayed transcriptome work by a month or two.
3. Sulfur and manzate are not suitable for slide dip assays and we will not proceed with those important materials unless we can put together a better assay.

SIGNIFICANT FINDINGS

- Six of the eight pesticides were successfully assayed against psylla at multiple sites, including: Admire (9 sites), Agrimec (9 sites), Delegate (12 sites), Nexter (8 sites), Pounce (12 sites) and Warrior (7 sites).
- Sites included: West Valley, Lombard Loop, Tieton, Sunnyside, Sawyer, Cashmere, Mesa, Omak, Orondo, Yakima in WA and six sites were in or near Jackson Co. OR.
- **Good News:** Nexter and Delegate show little evidence of resistance or tolerance
- **Mediocre News:** Agrimec and Admire are showing moderate efficacy but some sites appear to have some level or resistance to these two materials.
- **Bad news:** Psyllas at all sites show extreme resistance to Pounce and Warrior
- Pear psylla is ridiculously difficult to collect in numbers in autumn in organically managed sites (if you know of organic orchards that have psylla post-harvest please contact TRU.)
- John Dunley and Bruce Greenfield have provided psylla bioassay data from the Wenatchee region for 2000 and 2006 which will give a historical context to the data we are analyzing.

MATERIALS and METHODS

Objective 1

We will continue to evaluate resistance status of adult winter form psylla from several orchards in WA collected in autumn prior to leaf fall. The standard slide dip bio-assay will be used. Up to eight products will be tested for each of the 16 populations: Warrior and/or Ambush/Pounce (pyrethroids), Delegate (spinosyn), Assail and/or Provado (neonicotinoids), Nexter (METI), Agri-Mek (avermectin), and one or two more compounds may be tested. *Development of an assay to evaluate sulfur and manzate are being considered but may not work for this proposal.*

Objective 2

We will identify inter-population variation at genes related to insecticide resistance utilizing deep sequencing of transcriptomes. Specifically we will compare DNA sequence differences seen in the transcriptomes in psylla collected from 2-3 sites that are determined to be susceptible to 3-4 sites determined to be resistant. Transcriptome libraries will be sequenced using 100bp paired-end Illumina HiSeq at the UC Davis Genome Center Sequencing facility.

Our bioinformatic analyses will yield transcriptomes for the different psylla populations will uncover genetic variations that confer insecticide resistance or susceptibility among test populations. We will perform comparative sequence analysis against all available genomes in the public database to identify pear psylla genes that have similarity to genes from other insects known to be associated with insecticide target site or metabolic resistance Psyllidae and the Hemiptera.

We will examine both metabolic resistance genes omong psylla populations to determine if there is differential expression (upregulation) and if they show genetic substitutions that may increas detoxification efficiency. Similarly we will look for target site substitutions that would render specific pesticides ineffective because they are tolerated as opposed to being degraded. Results from gene expression should correlate with results of bioassays and will provide new basic understanding of resistance in this insect group. In addition, the psylla transcriptome produced will be shared with the scientific community to facilitate basic and applied research using the myriad of other genes of pear psylla captured by a transcriptome, providing molecular for other trait variations.

RESULTS & DISCUSSION

While we are still analyzing the bioassay data and will a brief summary supporting our good, mediocre and bad news stated above. Below we provide a listing of the 50% and 90% probabilities for mortality for each pesticide group as measured for each orchard and date of the collection. For example the Marec site, for Admire in 2015 (**see Table**) you will see: 0.50 2.8-**5.3**-12.9. The number, **5.3**, represents the amount of the chemical as a multiple of the high dose of the pesticide that is estimated to cause 50% mortality. The surrounding numbers represent the range of variation on this number based on the standard error from the data. The numbers: 0.90 473-**2468**-34193 represent the same for 90% predicted mortality. Please note that a multiple of high rate of 2468 indicates that this product is not capable of producing high rates of mortality of psylla, at least for those psylla collected from the study orchard.

The table below becomes clear if you have digested the previous paragraph. You will observe in the table that the efficacy of products (high efficacy to low) are approximately Delegate>Nexter> Admire+Agrimec>Warrior+Pounce.

The Probit Procedure

Admire

Mcarec Date=100815
Probability dose 95% FL
0.50 2.8-**5.3**-12.9
0.90 473-**2468**-34193

PineGrov Dt=110415
0.50 11288
0.90 1.40065E13

ten Dt=102914
0.50 1.29393
0.90 58.80743

Moxee Date=102815*
0.50 9.5
0.90 517.9

E. Zilla Date=102814
0.50 3858
0.90 54852202

Cashmere Date=102214
0.50 0.23- **2.32** - 809
0.90 12-**74** -1.2E15

Mesa Date=110614
0.50 67.3
0.90 1282

Omak Date=110514
0.50 37
0.90 1111

Orondo Date=102114
0.50 32.4
0.90 196.2

SITE=Sawyer Date=102314
0.50 4-**12**-1222
0.90 29-**246**-38728029

Tieton Dt=120414
0.50 88
0.90 16692

Agrimec

East Zilla Dt=101714
0.50 13.14
0.90 18.9

West Valley Dt=103014
0.50 925
0.90 214088

Cashmere Dt=102214
0.50 244
0.90 60523

Mcarec Dt=101515
0.50 21-**29**-43
0.90 107-**190**-525

Mesa Dt=111414
0.50 18-38-7041582
0.90 71-465-2.1E17

Moxee Dt=102815
0.50 3-**4**-5
0.90 9.2-**11.5**-15.5

Orondo Dt=101814
0.50 8.6-13-29
0.90 40-103- 1213

Sawyer Dt=102314
0.50 5-9-20
0.90 116-414-4413

TFREC Dt=102915
0.50 22184
0.90 31367862

Tieton Dt=120414
0.50 10 **15** 36
0.90 36- **86-** 1333

Delegate

East Zilla Dt=101414
0.50 **0.05** 0.07 0.11
0.90 **0.6** 0.9 1.5

West Valley Dt=103014
0.50 0.8 **1.2** 1.7
0.90 9 **15** 33

Cashmere Dt=102214
0.50 0.001 **0.008** 0.02
0.90 1.1 **2.49** 8.9

Mesa Dt=110614
0.50 1.1 **1.6** 2.2
0.90 8 **14** 27

Omak Dt=110514
0.50 0.54 **0.72** 0.86
0.90 1.27 **1.46** 1.79

Orondo Dt=101814
0.50 0.66 **1.31** 2.16
0.90 3.05 **5.24** 15.4

Sawyer Dt=102314
0.50 2.05 **2.53** 3.14
0.90 9 **12.5** 19.4

Nexter

Mcarec Dt=100615
0.50 0.26 **0.70** 2.19
0.90 14.2 **78.4** 3925

East Zilla Dt=101714
0.50 1.44
0.90 3.8

West Valleyl Dt=110314
0.50 0.64 **0.88** 1.31
0.90 2.5 **4.4** 15

Cashmere Dt=102914
0.50 0.01 1.12 2.7
0.90 1.8 4.0 8049
Mesa Dt=110614
0.50 0.7 **1.2** 3.9
0.90 5.3 **20.2** 2427

Omak Dt=110514
0.50 3.3
0.90 4.01

Orondo Dt=101814
0.50 0.05 **1.21** 2.1
0.90 1.6 **2.7** 201

Sawyer Dt=102914
0.50 0 0.04 0.16
0.90 0.11 **0.46** 86584289

Pounce

Cashmere Dt=102914
0.50 16 **46** 364
0.90 810 **8444** 1393745

Mesa Dt=110614
0.50 44
0.90 90

Moxee Dt=11415
0.50 49
0.90 76

Moxee Dt=102815
0.50 80957
0.90 637337949

Omak Dt=110514
0.50 640817
0.90 3.80823E12

Orondo Dt=102014
0.50 13 **18.5** 24
0.90 59 89 187

Sawyer Dt=102914
0.50 59
0.90 77

TFREC Dt=102915
0.50 68 **138** 1071
0.90 454 **2245** 460896

Tieton Dt=120414
0.50 5462018930
0.90 1.0 E18

Meyer Dt=102014
0.50 14.24 16.5 19.1
0.90 34.5 28.4 46.27

Royal Dt=102214
0.50 13.25
0.90 22.16

beeson Dt=10914
0.50 14.8 17.6 20.8
0.90 35.2 43.8 59.3

corlis Dt=102014
0.50 15.2 17.4 19.9
0.90 29.8 36.1 48.7

feath Dt=101614
0.50 1.7 10.7 37.3
0.90 15.6 29.8 7476122

frink Dt=10614
0.50 4.5 6.5 9.9
0.90 24.0 46.5 185

ten Dt=101414
0.50 11 12 14

0.90 22 28 40

Warrior 2

East Zilla Dt=102814
0.50 2490712779
0.90 4.3 E16

West Valley Dt=103014
0.50 630
0.90 197570

Cashmere Dt=102214
0.50 25
0.90 193

Mcarec Dt=101515
0.50 271
0.90 5481

Mesa Dt=111414
0.50 185 **12705** 5.2E74 0.90
22129 **109584416** 1.9E157

Omak Dt=111414
0.50 19
0.90 34

Orondo Dt=102014
0.50 14 **24** 42
0.90 243 **662** 4604

PineGrov Dt=110415
0.50 11636
0.90 3155997

Sawyer Dt=102314
0.50 334
0.90 925406

Okanagon Dt=11215
0.50 $8.3E^{-79}$
0.90 $2.9E^{-93}$

Agrimex

ten Dt=102914
0.50 3.97 **6.03** 9.32
0.90 22.6 **49.06** 469

Delegate (Medford)

Royal Dt=102214
0.50 1.65 **2.98** 1.2E-15
0.90 2.8 **5.13** 3.2E28

beeson Dt=101014
0.50 0.86
0.90 1.29

feath Dt=101514
0.50 1.22
0.90 6.84

meyer Dt=101514
0.50 0.41715
0.90 6.61522

ten Dt=101514
0.50 0.11 **1.3** 2.5
0.90 2.5 **4.6** 549

CONTINUING PROJECT REPORT **YEAR: 2 of 2 (2nd no cost extension)**
WTFRC Project Number: PR13-106

Project Title: Miticide resistance in spider mite pests of pears

PI:	Elizabeth H. Beers	**Co-PI (1):**	David Crowder
Organization:	WSU-TFREC	**Organization:**	WSU Pullman
Telephone:	509-663-8181 ext 234	**Telephone**:	(509) 335-7965
Email:	ebeers@wsu.edu	**Email**:	dcrowder@wsu.edu
Address:	1100 N. Western Ave	**Address:**	PO Box 616382
City/State/Zip:	Wenatchee/WA/98801	**City/State/Zip**:	Pullman/WA/99164

Cooperators: None

Total Project Funding: Year 1: 23,696 Year 2: 24,614

Other funding sources: None

Budget:

Item	2013	2014
Salaries	12,000	12,480
Benefits	4,666	4.853
Wages	5,720	5,949
Benefits	555	577
Equipment	0	0
Supplies	500	500
Travel	255	255
Plot Fees	0	0
Miscellaneous	0	0
Total	$23,696	$24,614

Note: This is a second no cost extension due to a mite colony being lost to contamination by predators and the objectives have not been completed.

Objectives

1. Survey resistance status of spider mite populations on pear to key miticides.

2. Examine population genetics of resistance in spider mites.

3. Develop recommendations for effective control of spider mites and a resistance management plan.

Significant Findings

- Twospotted spider mite populations were highly resistant to Agri-Mek, moderately resistant to Acramite, and slightly resistant to FujiMite
- Agri-Mek and Acramite are predicted to provide little control in the field (with the exception of Acramite for the Yakima population, where efficacy was much higher).
- FujiMite shows only incipient resistance, but field performance may still be retained.
- The ovicides (Onager, Zeal and Envidor) were less affected by resistance than the adulticides, with resistance to Onager and Zeal currently only found in one region. Where resistance occurred, it occurred at an extremely high level. No evidence of resistance to Envidor was found in any population.
- There is substantial evidence for cross-resistance between the Group 10 miticides, Onager, Apollo (10A), and Zeal (10B).

Methods

A total of 66 probit bioassays were performed on nine twospotted spider mite populations, eight collected from eastern Washington pear orchards, and one susceptible reference colony obtained from Cornell's Geneva Laboratory in New York. The latter has been reared in the laboratory for >15 years without exposure to pesticides. The bioassays were performed using commercial formulations of six acaricides (Table 1), including three adulticides and three ovicides. The acaricides chosen represent six different modes of action (MOAs); however, Onager and Zeal (10A and 10B, respectively) are considered closely related MOAs.

Table 1. Acaricides tested against populations of twospotted spider mites from pear

Trade name	Common name	Group	MOA	bioassay type
Agri-Mek	Abamectin	avermectins	6	adulticide
Acramite	bifenazate	N/A	unknown	adulticide
FujiMite	fenpyroximate	METI	21A	adulticide
Envidor	spirodiclofen	tetronic/tetramic acid derivatives	23	ovicide
Onager	hexythiazox	mite growth inhibitors	10A	ovicide
Zeal	etoxazole	mite growth inhibitors	10B	ovicide

The eight commercial orchard populations were collected over two growing seasons (four per season), representing pear orchards in the Chelan, Douglas, Okanogan and Yakima Counties. Initiating a colony from the field was made by transferring individual mites with a fine-tipped paintbrush, taking care to avoid transferring other arthropods. The populations were reared on bean plants, *Phaseolus vulgaris* L,. at a constant temperature of ca. 75 °F, and 16:8 light:dark photoperiod. Colonies were kept isolated in different rooms, and supplied with fresh bean plants every ≈2 weeks.

Each bioassay consisted of four to six concentrations of the acaricide and a distilled water check. All bioassays were conducted on bean leaf disks (3 cm/1.18 inch diam) with the lower surface facing up in a 3.25 oz plastic cup with cotton and water. Acaricide concentrations were mixed by serial dilution of a 1 liter stock solution, and sprayed in a Potter Spray Tower with 2 ml (0.06766 fl oz) of mixture at 6.5 psi.

Adulticide bioassays used 20 adult female mites/disk and were evaluated after 24, 48, and 72 h (the 72 h data are shown throughout this report). For ovicidal bioassays, 10 adult females were transferred to the disks and allowed to lay eggs for 24 h. Eggs were counted, and their positions marked with a felt-tip pen, and the females removed. The initial number of eggs was standardized to 20/disk by removing excess eggs. Eggs were treated and then held at 25°C (77°F) in a growth room for 10 days, when they were evaluated for treatment mortality (unhatched eggs). These methods are essentially the same as have been used historically in collecting information on mites from Washington tree fruits, allowing for comparisons across time.

The dose-response curves were calculated with POLO-Plus (LeOra software), which provided LC_{50}s (the concentration needed to kill 50% of mites) and associated 95% confidence intervals.

An additional calculation was made using the probit regression parameters (slope, intercept, natural response). Using the maximum label field rate, the predicted percentage mortality of the various populations was estimated. It should be noted that these are relative indicators of activity because of the differences between laboratory studies and field conditions. However, they provide an index of predicted activity in the context of actual use rates, which is difficult to ascertain from the degree of change in the LC_{50}.

Rate ranges for the bioassays were chosen based initially on historical data, and adjusted if mortality was too high or too low to produce an LC_{50} using probit analysis. Because of the variable (and much higher than anticipated) levels of resistance, many of the bioassays failed probit analysis, and were re-run. Only those bioassays with six concentrations, an acceptable level of check mortality (<20%) and valid estimates of the LD10, 50, 90 and 99 with 95% confidence intervals were retained (Table 2, Figs. 1a, b). Resistance ratios were (LC_{50}/baseline) calculated from the LC_{50} of the New York susceptible colony as the baseline; historical data are shown for reference. Resistance ratios are useful metrics in assessing the degree of resistance and likelihood for it to spread in the field. Values < 3 indicate no resistance, values 3-10 represent low levels of resistance that may spread in the field, values between 10-100 represent statistically significant resistance that may or may not cause field failure, and values > 100 indicate high levels of resistance that are likely to lead to field failure of the acaricide.

Table 2. LC_{50}s and resistance ratios (LC_{50} of tested field-derived colony divided by LC_{50} of susceptible laboratory colony) of six acaricides tested against eight populations of twospotted spider mites collected from commercial pear orchards in eastern Washington, 2013-2014.

Acaricide	TSM population	New York TSM baseline	Calc LC50	95% CI lower	95% CI upper	RR (New York)
Agri-Mek	C1-2013	0.004	271.20	142.38	409.74	**67,801**
Agri-Mek	C2-2013	0.004	503.04	413.28	604.14	**125,760**
Agri-Mek	C3-2013	0.004	389.33	277.54	508.77	**97,332**
Agri-Mek	Y1-2013	0.004	37.56	24.13	51.14	**9,391**
Agri-Mek	C1-2014	0.004				
Agri-Mek	C2-2014	0.004	116.05	64.10	170.53	**29,012**
Agri-Mek	D1-2014	0.004	165.67	117.072	230.602	**41,417**
Agri-Mek	O1-2014	0.004	11.31	6.235	18.371	**2,827**
Acramite	C1-2013	2.29	1213.51	982.09	1476.08	**531**
Acramite	C2-2013	2.29	2165.29	1730.02	2626.31	**947**
Acramite	C3-2013	2.29	687.14	599.95	789.71	**300**
Acramite	Y1-2013	2.29	10.59	0.00	53.65	**5**
Acramite	C1-2014	2.29	739.75	520.39	994.44	**323**
Acramite	C2-2014	2.29	2845.92	2299.65	3533.12	**1,244**
Acramite	D1-2014	2.29	125.23	86.34	188.59	**55**
Acramite	O1-2014	2.29	3.47	2.64	4.35	**2**
FujiMite	C1-2013	1.29	8.94	7.95	10.02	**6.93**
FujiMite	C2-2013	1.29	11.68	8.87	14.39	**9.05**
FujiMite	C3-2013	1.29	20.82	13.77	26.29	**16.14**
FujiMite	Y1-2013	1.29	1.35	0.13	3.37	**1.04**
FujiMite	C1-2014	1.29				
FujiMite	C2-2014	1.29	15.19	13.09	17.43	**11.77**
FujiMite	D1-2014	1.29	4.43	3.49	5.46	**3.43**
FujiMite	O1-2014	1.29	3.84	2.57	5.24	**2.98**
Zeal	C1-2013	0.062	5.02	2.81	7.25	**81**
Zeal	C2-2013	0.062	5.77	5.00	6.47	**93**
Zeal	C3-2013	0.062	[y]			--
Zeal	Y1-2013	0.062	1.57	1.29	1.83	**25**
Zeal	C1-2014	0.062	[y]			--
Zeal	C2-2014	0.062	[y]			--
Zeal	D1-2014	0.062	0.313	0.443	0.639	**5**
Zeal	O1-2014	0.062	1.418	1.012	1.879	**23**
Onager[x]	C1-2013	0.014	0.51	0.37	0.74	**36**
Onager	C2-2013	0.014	0.39	0.34	0.45	**28**
Onager	C3-2013	0.014	1785.18	1573.97	1995.69	**127,513**
Onager	Y1-2013	0.014	0.42	0.29	0.51	**30**
Onager	C1-2014	0.014				**0**
Onager	C2-2014	0.014	1182.94	1019.41	1367.30	**84,496**
Onager	D1-2014	0.014	0.15	0.11	0.18	**10**
Onager	O1-2014	0.014	0.24	0.18	0.28	**17**

Acaricide	TSM population	New York TSM baseline	Calc LC50	95% CI lower	95% CI upper	RR (New York)
Envidor	C1-2013	5.96	9.76	5.57	13.32	**1.64**
Envidor	C2-2013	5.96	11.41	9.20	14.15	**1.91**
Envidor	C3-2013	5.96	8.22	6.24	10.09	**1.38**
Envidor	Y1-2013	5.96	9.70	6.08	12.94	**1.63**
Envidor	C1-2014	5.96	4.083	1.668	6.131	**0.68**
Envidor	C2-2014	5.96	6.43	5.66	7.21	**1.08**
Envidor	D1-2014	5.96	9.277	8.038	10.539	**1.56**
Envidor	O1-2014	5.96	11.08	6.24	14.70	**1.86**

[x]Baseline LC_{50} is from contemporary bioassays on the susceptible New York colony
[y]Unable to obtain significant mortality at 200,000 ppm AI (near limits of solubility).

Results and Discussion

Agri-Mek. Resistance ratios (RR) for this material were extremely high for all populations tested (Table 2), ranging from ca. 2,827 to 125,760- fold increase in the LC_{50}. Of the mite populations examined, the lowest RRs were from Okanogan and Yakima counties; all those from Chelan and Douglas Counties were uniformly high. This high level of resistance is the probable cause for field failure as a miticide for spider mites. However, it may still be useful for rust mites and pear psylla. The elevated resistance levels reflect its continued and frequent use since the late 1980s in Washington's pear industry.

Acramite. The RRs for Acramite were considerably lower than those for Agri-Mek (4.63-947). This material has been used for a much shorter period of time. However, with the exception of the Y1-2013 colony from Yakima, RRs were still very high, indicating a major shift in the LC_{50}s.

FujiMite. The RRs were lower for FujiMite than the other two adulticides (1.04-16.14); the Yakima colony showed no increase in resistance, and the other three colonies a moderate increase.

Zeal. The RRs for Zeal all indicated that a moderate level of resistance has occurred, including the Yakima population. One of the Wenatchee River Valley populations (C3-2013) apparently had extreme levels of resistance, such that no significant mortality was measured at 200,000 ppm AI, making the RR > 3.2 million. The population with this high level of resistance is the same one with high (but measurable) levels of resistance to Onager, the other IRAC group 10 material. The predicted percentage mortality with Onager at the maximum label rate (Fig. 1b) is 100%, with the exception of the highly resistant population (0% predicted mortality). Although it has not yet been tested, the 2014 population that was highly resistant to Onager will likely show a high level of resistance to Zeal.

Onager. The RRs were quite variable for this material. Two of the populations (both from the Wenatchee River Valley, and essentially contiguous, although under different management) were very high (8,450 and 12,751). All other populations had very low RRs, well within the range of variation for bioassays.

Envidor. None of the populations tested showed any measureable resistance to Envidor; all RRs were <2. Envidor is one of the more recent materials to be used on pear. It is classed as IRAC MOA group 23, the same MOA as Ultor, which is routinely used on pears for psylla, and also has mite activity. All populations tested had a predicted mortality of 100% based on probit regression (Fig. 1b).

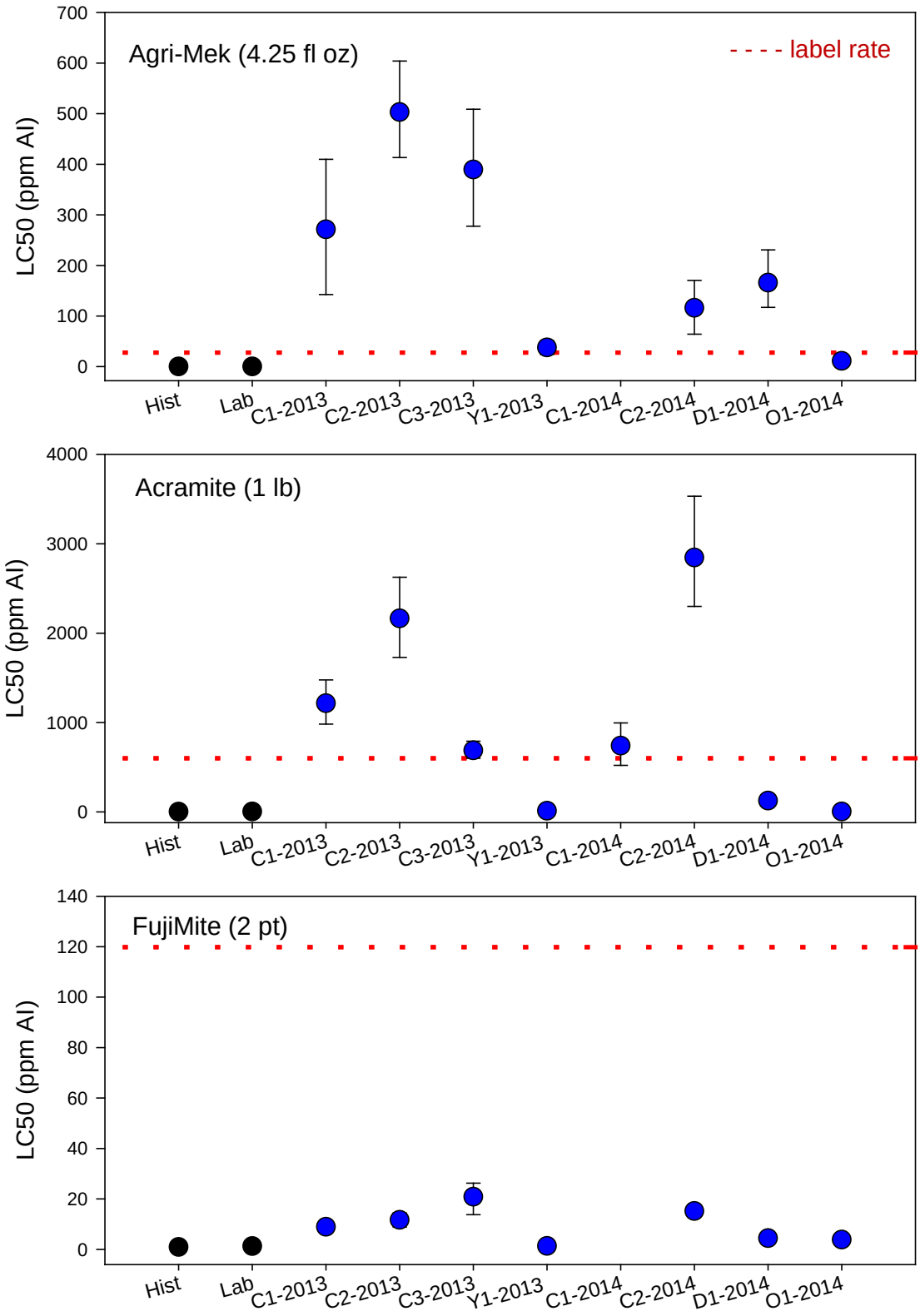


Fig. 1a. LC_{50}s of adulticidal acaricides for populations of twospotted spider mite from pear.

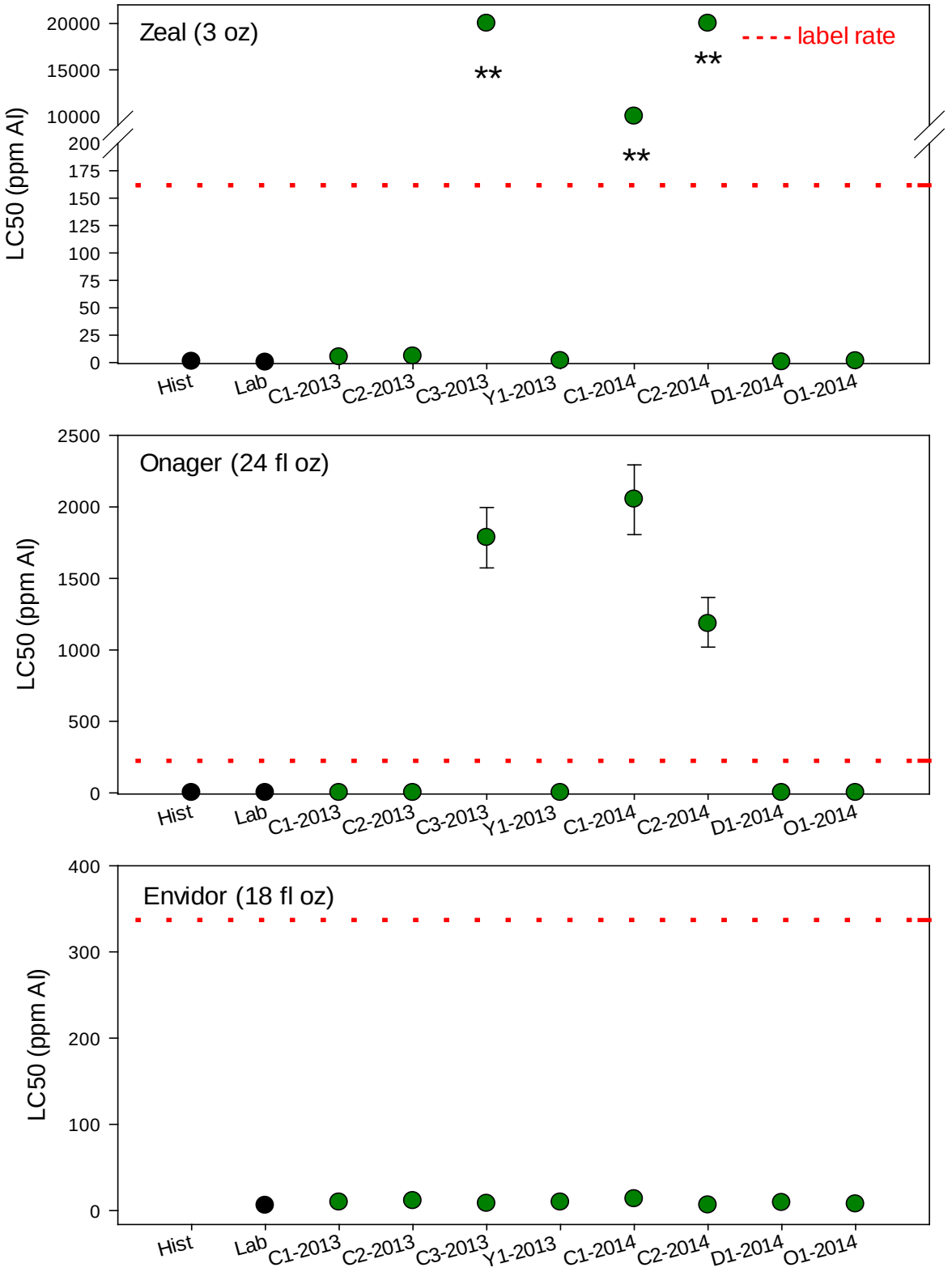


Fig. 1b. LC_{50}s of ovicidal acaricides for populations of twospotted spider mite from pear.

CONTINUING PROJECT REPORT **YEAR**: Year 2 of 3
WTFRC Project Number: PR-14-100

Project Title: Health role of pear for Metabolic Syndrome

PI: Bahram H. Arjmandi, PhD, RD
Organization: Department of Nutrition, Food and Exercise Sciences, Florida State University
Telephone: (850) 645-1517
Email: barjmandi@fsu.edu
Address: 412 Sandels Building, 120 Convocation Way, Tallahassee, Florida 32306-1493

Co-PI: Sarah A. Johnson, PhD, RD, CSO
Organization: Department of Food Science and Human Nutrition, Colorado State University
Telephone: (970) 491-3807
Email: sarah.johnson@colostate.edu
Address: 206 Gifford Building, 502 W. Lake St., Fort Collins, Colorado 80523-1571

Cooperators: Pear Bureau Northwest

Total Project Request: **Year 1:** $32,185 **Year 2:** $29,871 **Year 3: $18,000**

Other funding sources

Agency Name: Pear Bureau Northwest
Amt. requested: Year 1: $32,185 **Year 2**: $29,871 **Year 3:** $18,000
Notes: Pear Bureau Northwest will match the amount funded by the Pear Marketing Order 927 to bring the total funded amount to $64,370 for Year 1 and $59,742 for Year 2.

Budget 1
Organization Name: Florida State University
Contract Administrator: Gina Wells, Grants Compliance Analyst
Telephone: (850) 644-3658 **Email address:** glwells@fsu.edu

Item	2014	2015	2016
Salaries	$16,457.50	$16,951	$0
Benefits	$2,688	$2,853	$0
Wages	$0	$0	$0
Benefits	$0	$0	$0
Equipment	$0	$0	$0
Supplies	$12,539.50	$9,067	$18,000*
Travel	$0	$0	$0
Miscellaneous	$500	$1,000	$0
Plot Fees	$0	$0	$0
Total	$32,185	$29,871	**$18,000**

***We are requesting an additional $18,000 to cover the remaining costs of the proposed measurements as the total cost to carry out this study was underestimated at the time of submission.**

A. OBJECTIVES

The ***central hypothesis*** of the proposed study is that the daily consumption of 2 pears (medium sized Green Bartlett and/or Green Anjou pears weighing ~166 g each) for twelve weeks will improve blood pressure, lipid profiles, glycemic control and insulin resistance, inflammatory and oxidative status in men and women with MetS. Because pears are high in pectin, a soluble and fermentable dietary fiber, we propose two ***ancillary hypotheses*** as follows: **1)** regular intake of pears will promote gastrointestinal health (GI); and **2)** will improve measures of body composition. The hypotheses of the study will be tested in a randomized, crossover design study using 2 pears or 50 g isocaloric control drink powder with 50 men and women between the ages of 45 and 65 years with three of the five features of MetS using the following four ***specific aims***:

Specific Aim 1: To investigate the extent to which daily pear consumption reduces blood pressure and improves lipid profiles by measuring total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A1 and apolipoprotein B100 levels will be measured. Atherogenic risk ratios (TC/HDL-C, LDL-C/HDL-C, HDL-C/LDL-C) will also be assessed.

***Specific Aim 2*:** To determine the degree to which daily pear consumption will improve biochemical markers of **a)** inflammation [C-reactive protein (CRP), leptin, and adiponectin]; **b)** antioxidant defense [total antioxidant capacity (TAC)]; **c)** oxidative stress [oxidized low-density lipoprotein (LDL) and 8-hydroxy-2'-deoxyguanosine (8-OHdG)]; and **d)** insulin sensitivity [(fasting glucose, insulin, the homeostatic model assessment-insulin resistance (HOMA-IR), and hemoglobin A1c (Hgb A1c)].

Specific Aim 3: To investigate the ability of pear consumption to improve GI health using a validated Seven-Day Bowel Movement Questionnaire and serum levels of short-chain fatty acids.

***Specific Aim 4*:** To examine whether pear consumption has positive effects on body weight and composition including lean body mass (LBM), fat mass (FM) and percent body fat (%BF) using dual-energy x-ray absorptiometry (DXA).

The goals for 2016 are as follows:

1. To finish analyzing blood and urine samples for the analysis of biomarkers. Our goal is to have all laboratory analyses finished by March 31, 2016.
    a. We initially projected that laboratory analyses would start in October 2015 and be completed by January 2016; however, this was delayed until mid-December 2015 in order to wait for the 40th subject to finish the study. Additionally, due to financial constraints, measurement of certain blood biomarkers was delayed until funding could be secured.
2. To statistically analyze all data collected for the abovementioned specific aims.
    a. All statistical analyses will be completed after laboratory analyses are completed. We anticipate this will be completed by April 30, 2016.
3. To prepare abstract(s) for presentation at the 2016 Food & Nutrition Conference & Expo from October 15-18th – annual meeting of the Academy of Nutrition and Dietetics.
    a. We submitted one abstract to the American Society for Nutrition for presentation at their annual meeting at Experimental Biology from April 2-6th.
4. To prepare manuscript(s) for publication in peer-reviewed journals. Our goal is to have the first manuscript ready for submission to a high-quality journal (e.g. American Journal of Clinical Nutrition, Journal of the Academy of Nutrition and Dietetics) by June 1, 2016.

B. SIGNIFICANT FINDINGS

- Subject recruitment is finished and overall subject retention has been excellent (**Figure 1**).
- Summary of subject enrollment
    - 50 total enrolled participants
        - 7 participants dropped from the study due to health and personal reasons

- 43 participants have completed the first 12 weeks of the study
- 39 participants have completed the second 12 weeks of the study
  - The remaining participants will complete the study on January 26, February 23, and April 16, 2016.

- Based on discussions with the Pear Bureau Northwest and the Washington Tree Fruit Research Commission, it was decided that we would proceed with blood and urine biomarker analysis for the 40 participants who completed the study starting in December 2015. This was decided based on the fact that the final sample size was originally projected to be 40 and the time it would take for the final 3 participants to complete the entire study.
- We are currently in the process of analyzing data and laboratory biomarkers. Preliminary analyses of 36 participants indicates that after 12 weeks of fresh pear consumption, systolic blood pressure (– 5 mmHg, $P < 0.05$) and pulse pressure (– 5 mmHg, $P < 0.01$) were significantly lower than baseline levels whereas there were no changes in the control group.

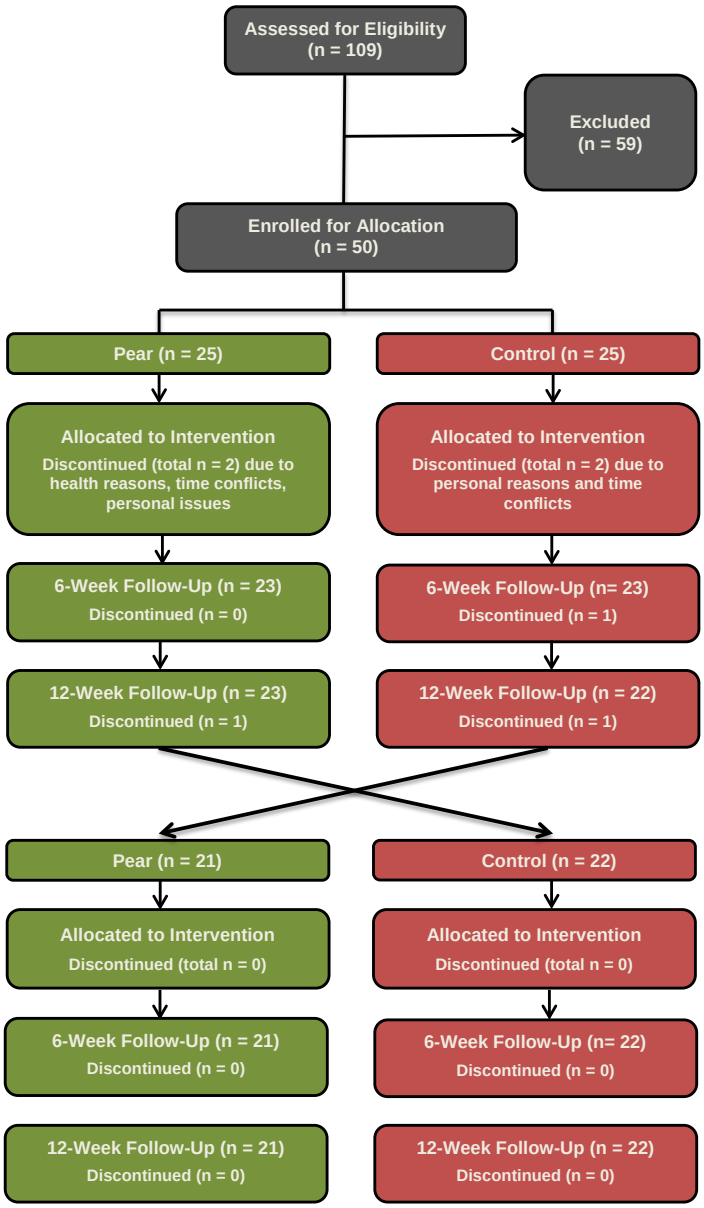


Figure 1. Flowchart of Enrollment

C. OUTLINE OF METHODS

A total of 50 men and women between the ages of 45 and 65 years who have three of the five features of MetS as defined by the ATP III will be included in the study (see Subjects Inclusion Criteria below). After a two-week run-in phase, eligible men and women will be randomly assigned to receive one of two treatments daily for twelve weeks: 1) Two medium-sized pears or 2) 50 g isocaloric maltodextrin-based pear-flavored control drink powder. After an initial ***telephone screening***, all participants will be requested to report to the study site for their first visit. On the **first visit (screening)**, the Study Coordinator, Sarah A. Johnson, PhD, RD, CSO, will provide the potential subjects with verbal and written explanation of the project and will answer any questions regarding the study. Then the individual will be asked to sign an informed consent form, followed by measuring waist circumference, resting brachial blood pressure, fasting serum triglycerides, HDL cholesterol, and glucose levels using the Cholestech LDX® System (Waltham, MA) to confirm MetS. Baseline assessments will be performed for medical history, medications use, dietary intake, and physical activity. If volunteers meet the study criteria they will be scheduled for their second visit two weeks later (actual baseline data collection) and randomly assigned to their treatment group. They will be given a three-day food record to take home and bring back on the second visit. Additionally, subjects will be asked to collect 25-50 ml their first void on the morning of the **second (baseline) visit (2-weeks)** and bring this with them to the clinical research facility. During this visit between the hours of 7-10 A.M., blood pressure will be measured followed by blood draw (20 ml venous blood). Subjects' anthropometrics including height, weight, and waist and hip circumferences will be measured. Participants will be asked to complete Physical Activity and Bowel Movement Questionnaires. Next participants will undergo a DXA scan for body composition measurements. They will be provided with their assigned treatment and will receive standard instructions on how to fill out daily diaries for their treatment, and for food records. Urine collection, blood pressure, blood draw, and anthropometric, body composition, diet, physical activity, and bowel movement assessments will be repeated at **6- (third visit) and 12-week (final visit)** intervals. Participants will be provided with light breakfast items before leaving the clinical research facility. After completing the assigned 12-week intervention, subjects will undergo a 4-week washout period before crossing over to the other intervention and all respective procedures will be followed at baseline, 6- and 12-week visits.

Study Procedures	**Screening**	**Baseline**	**6-Weeks**	**12-Weeks**
Informed Consent	X			
Medical History	X			
Three-Day Food Record	X	X	X	
Physical Activity Questionnaire		X	X	X
7-Day Bowel Movement Questionnaire		X	X	X
Anthropometrics	X	X	X	X
DXA		X		X
Blood Draws	X	X	X	X
Urine Collection		X	X	X
Blood Pressure	X	X	X	X
Assess Compliance	Ongoing throughout the study.			

Table 1. Study Flowchart

Data Analyses and Management:

An initial sample size of 50 participants, with attrition rate of 20% will produce a sample size of approximately 20 participants per group in a crossover design with greater than 80% power of more than 0.85 at an $\alpha = 0.05$ to detect a significant difference ($P < 0.05$). Statistical analysis will be performed using SAS Version 9.3 (SAS Institute, Cary, NC). Descriptive statistics will be calculated for all variables and will include means, standard deviations, medians, minima and maxima. Distributions of outcome variables will be examined graphically for asymmetry and for outliers. If a lack of symmetry is noted, the variable will be transformed before analysis. Baseline characteristics for the study groups will be compared and if differences occur in variables that could influence the results, subsequent analyses will adjust for the effects of these variables. Baseline values of blood pressure, serum, plasma, and urine biomarkers, anthropometric variables, body composition, and questionnaires for the two experimental groups will be compared using two-sample t-tests. The effects of dietary treatments on primary outcomes of interest (blood pressure and serum markers of lipids, insulin sensitivity, inflammation, oxidative and antioxidative status), and secondary outcome variables (body composition and gastrointestinal function) will be evaluated by 2 (group) x 3 (time) repeated measures ANOVA applied to changes in these measurements during the treatment periods. The effectiveness of the washout phase will be tested by comparing baseline values to the values at the end of the washout phase, and also by evaluating the change in the response during the washout phase. Appropriate multiple comparisons will be employed to investigate main or interaction effects. Some covariates such as age, initial BMI, and baseline characteristics identified in the preliminary analysis will be included. Other factors that might affect the results, such as physical activity and dietary intakes will also be examined.

D. RESULTS AND DISCUSSION

As mentioned in the Significant Findings section, subject recruitment is finished and overall subject retention was excellent with only 7 participants dropping from the study (14% attrition). Reasons for dropping from the study included personal reasons such as lack of time or moving, not wanting to take the placebo powder, and not wanting to give blood. Tolerance to daily pear consumption was generally reported as good; however, there were reports of taste fatigue towards the end of the 12-week pear interventions. With the exception of 3 subjects who are in the process of finishing the study, we are finished with data collection and are in the process of analyzing blood and urine biomarkers.

We submitted an abstract for poster presentation at the 2016 annual meeting of the American Society for Nutrition at Experimental Biology. The title of this abstract is "Fresh pear (*Pyrus communis*) consumption may improve blood pressure in middle-aged men and women with metabolic syndrome." This abstract presents preliminary analyses of 36 participants which showed that after 12 weeks of fresh pear consumption, systolic blood pressure (– 5 mmHg, $P < 0.05$) and pulse pressure (– 5 mmHg, $P < 0.01$) were significantly lower than baseline levels whereas there were no changes in the control group. No changes were noted in diastolic blood pressure or heart rate for either group at any time point. With aging, systolic blood pressure tends to increase while diastolic blood pressure tends to decrease. The difference between these two numbers is called pulse pressure. A high systolic blood pressure and pulse pressure suggest the presence of aortic stiffness and dysfunction of the arteries. The results of our preliminary analyses indicate that regular fresh pear consumption may improve blood pressure and vascular function in older men and women with MetS. However, these findings will be reexamined using data from the 40 participants who have completed the study to confirm this. Additionally, the effects of pears on vascular function needs to be tested in future studies using other measures of vascular function such as arterial stiffness (pulse wave velocity) and endothelial function (flow-mediated vasodilation).

CONTINUING PROJECT REPORT **YEAR: 2 of 3**
Project Number: PR14-104

Project Title: Fall and summer pruning to control vigor and psylla in d'Anjou pear

PI: Stefano Musacchi
Organization: WSU/ TFREC
Telephone: 509-663-8181 x236
Email: stefano.musacchi@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (1): Elizabeth H. Beers
Organization: WSU/ TFREC
Telephone: 509-663-8181 x234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (2): Jim Mattheis
Organization: USDA, ARS
Telephone: 509-664-2280 x249
Email: james.mattheis@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Sara Serra (WSU/TFREC)

Total Project Request: Year 1: $72,707 **Year 2**: $71,589 **Year 3**: **$71,170**

Other funding sources:

Agency Name: USDA/ARS
Amt. awarded: Harvest and postharvest quality analyses conducted by Jim Mattheis to be supported with base USDA, ARS funds.

WTFRC Collaborative Expenses: None

Budget
Organization Name: WSU **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509-335-4564/509-663-8181 **Email:** carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2014	2015	2016
Salaries[1]	36,480	37,939	39,456
Wages[2]	11,440	11,898	12,374
Benefit[3]	14,130	14,695	15,283
Travel[4]	757	757	757
Goods and Services[5]	9,900	6,300	3,300
Total	72,707	71,589	**71,170**

Footnotes:
[1] Salary for a new hire Research Intern (Musacchi), a Research Intern (Beers).
[2] One non-Student temporary for 13 wks: 40/wk at $11/hr (Musacchi) and one non-Student temporary for 13 wks: 40/wk at $11/hr (Beers).
[3] Benefits at 9.7% (Musacchi and Beers).
[4] 676 miles/year for domestic travel to go to the orchard (Musacchi) and 676 miles/year for domestic travel to go to the orchard (Beers).
[5] Fruit mineral analyses, data loggers, light bar, laboratory supplies for fruit quality analyses (Musacchi).

OBJECTIVES

1. *Control vigor through pruning practices in a mature Anjou orchard while maintaining yield and quality, and reduce psylla densities throughout the tree.*

SIGNIFICANT FINDINGS

Vigor control

- More material was removed from trees during fall pruning in comparison to winter and summer pruning.
- Wood and leaves removed from summer/fall pruning was double that which was cut from winter pruning.
- Pruning weight did not differ among rootstocks (OHF97, OHF69, and OHF87) in summer and winter; however, more material was cut in fall from OHF69 rootstocks, followed by OHF97 and OHF87.
- The number and weight of fruitlets removed in June with summer pruning was not statistically different between the three rootstocks.
- Trunk area was greater in the winter pruned trees than in the fall+summer ones.
- OHF97 was confirmed to be the most vigorous rootstock among the three in trial; OHF97 had the greatest trunk area, followed by OHF69 and then OHF87.
- At the end of the season, trees fall+summer pruned and grafted on OHF87 were the least vigorous accordingly to the trunk area.

Physiological measurements

- Fall+summer pruned trees tended to have more flower buds/m^3 than winter pruned trees, but the difference was not statistically significant.
- Winter pruned trees reported a higher SPAD value (chlorophyll reading) than the fall+summer ones, but there was no difference among the rootstocks.

Quality and Yield

- Fruit from 2014 harvest pulled out from air storage after 4 months showed some differences. Fruit picked from the winter pruned treatment lost more weight, had a faster ripening (accordingly to the DA values), higher firmness, soluble solid content (SSC) and titratable acidity than the fall+summer fruit.
- Winter pruning resulted in higher yield and larger fruits in 2015, but sunburn incidence in winter pruning was triple the incidence recorded for fruit harvested from fall+summer pruned trees.
- The three rootstocks did not show any significant difference in terms of productivity, average fruit weight.
- Frost damage occurrence was 6-7% in both treatments without any significant difference neither among rootstocks.

Psylla and Mite Densities

- With the exception of the pre-spray delayed dormant count, adult psylla densities were low throughout the season.
- No differences in psylla nymph densities were found among pruning treatments or rootstocks.
- Mite densities were low throughout the season, never exceeding 0.4/leaf.
- Fruit damage from insects (psylla, mealybugs, rust mite) was very low, although significantly higher pear rust mite russetting occurred in the winter-pruned treatment.

METHODS

The trial was carried out in an Anjou orchard trained at central leader and planted in 1998 on three different rootstocks: Old Home x Farmingdale (OHF) 97, 69, and 87. OHF 97 is considered a vigorous rootstock in comparison with the other two (semi-vigorous). The three combinations of Anjou on different rootstocks are fully randomized inside the orchard.

Vigor and physiological measurements

Half of the experimental rows were winter pruned (13 Mar 2015) following the traditional farm style. The other half of the rows were summer pruned (5 Jun 2015) to remove vigorous watersprouts, with the intent of reducing nutrient competition between shoots and fruit, reducing psylla presence and increasing canopy light penetration. The summer pruned rows were trimmed again in the fall after harvest (20 Oct 2015) with the aim to remove big and vertical branches, competing limbs and to promote flower buds for the following years' production. These pruning treatments were repeated exactly as done the previous year. For each pruning time, cut wood (and leaves for fall+summer pruning) from each tree was collected and weighed. Trunk circumference at 20 cm above ground was measured per single tree to calculate TCSA (trunk cross sectional area) in February and in October. In March 2015, counting of flower buds per m^3 on both sides of the trees was performed on 10 trees per rootstock and per pruning technique to assess if the fall+summer pruning technique had an effect on the flower bud formation. A 1 m^3 PVC structure was used and hanged on the tree at the same height from the ground to assess the buds counting. In late July 2015, fully expanded, healthy and mature leaves (not belonging to water sprouts) were selected and clamped with a portable SPAD 502-meter for a non-destructive leaf chlorophyll estimation. Literature studies reported a strong correlation between SPAD values and leaf N content.

Quality and Yield

Fruit belonging to 2014 harvest were pulled out after two (T1) and four months (T2) of storage at -1°C, fruit quality and maturity were assessed keeping fruit divided accordingly to I_{AD} classes (A, B, C, and D, $1.60< I_{AD}<1.79$, $1.80<I_{AD}<1.89$, $1.90< I_{AD}<1.99$ and $2.00< I_{AD}<2.19$ respectively. Skin color parameters (L, a, b), red blush overcolor percentage, weight, firmness, soluble solids content (SSC), exogenous ethylene concentration, cork incidence, acidity, and pH were assessed at each pull out after 7 days of ripening at room temperature. Only T2 quality results will be presented in the results section. Samples of pear flesh tissue were analyzed for calcium, nitrogen and other nutrients content by enzymatic digestion (Best Test Analytics, Moses Lake, WA).

Pre-harvest assessment of 2015 fruit maturity was carried out one week before harvest (harvest on Aug 24th) on one tree per each pruning treatment (OHF87 as reference) to determine differences between them. Harvest 2015 was done by tree, in 10 trees per each rootstock, for a total of 60 trees. A sample of 60 fruit per each tree (70-75 mm Ø) were collected just from OHF87 in both pruning treatments for following storage and quality analysis (1200 fruit total). Fruit sorting into I_{AD} classes was carried out in the same way as the previous year, planning a quality analysis at harvest (not reported) and two pullouts for 2016 at 5 month and 7 months after storage in normal air at +0.5°C.

Psylla and Mite Sampling

Psylla adults. Adult psylla were sampled with a beating tray (10 taps/subplot, or 20 per treatment x rootstock x replicate combination) every 2-3 weeks from mid-March through the end of September. The number of adult psylla falling on the tray was recorded, and the average of the 20 taps was used for analyses.

Psylla eggs and nymphs. Pear psylla eggs and nymphs were counted from late-April through the late August. After leaves had fully expanded, leaf samples were used to assess psylla and mite densities. Four leaves per each tree in the subplot (40 leaves total) were collected and kept cool during transportation and storage. Leaves were brushed with a leaf-brushing machine (Leedom Mfg, Mi-Wuk Village, CA) and collected on a revolving glass plate coated with undiluted dishwashing liquid. Psylla nymphs were recorded as either young (1st, 2nd or 3rd instar) or as old (4th or 5th instar). Psylla eggs and nymphs on spur and leaf samples were counted using a stereoscopic microscope.

Mites. The most common orchard mite species were also counted on the same leaf samples used for pear psylla starting on 28 April. All stages and species of phytophagous and predatory mites were recorded, including the eggs and motile stages of European red mite (ERM), *Panonychus ulmi* (Koch); twospotted spider mite (TSM), Tetranychus urticae Koch; McDaniel spider mite (MCD), *Tetranychus mcdanieli* McGregor [the eggs of TSM and MCD could not be distinguished, and were recorded as a group]; western predatory mite, *Typhlodromus* (=*Galendromus*) *occidentalis* (Nesbitt); and

Fruit damage. Fruit damage was assessed on 24 August on 46 fruit per subplot. Each fruit was rated for russet and the source of the russet (pear psylla, grape mealybug or pear rust mite) was noted. The russet rating was based on a severity scale of 0 = no russet, 1 = 1 to 10% of the fruit surface with russet, 2 = 11 to 20% russet, and 3 = 21 to 30% russet. In addition, the absence or presence of grape mealybug in the calyx of each fruit was noted.

RESULTS AND DISCUSSION

Vigor and physiological measurements

Differences in amount of wood cut from pruning were found among fall, summer, and winter pruning times. More material per tree was removed during fall pruning in comparison to winter and summer pruning (Fig. 1). The material removed from winter pruning was half the amount removed from fall+winter pruning (11.07 and 22.06 kg/tree, respectively; Fig. 1).The comparison among rootstocks, regardless of the pruning treatment, showed that OHF97, OHF69, and OHF87 did not differ in pruning weight removed by pruning treatment both in summer and winter, while more material was cut in fall from OHF69 followed by OHF 97 and 87 (Table 1).

During summer pruning, an average of 6-7 fruitlets were removed per tree, weighing around 20 g per fruit. The number and weight of those fruitlets was not statistically different when comparing the three rootstocks.

Trunk diameter of winter pruned trees measured at the end of the season (October 2015) resulted in a significantly higher trunk cross sectional area (TCSA) than in the fall+summer ones (Fig. 1), confirming the same trend observed at the beginning of the season (data not shown).

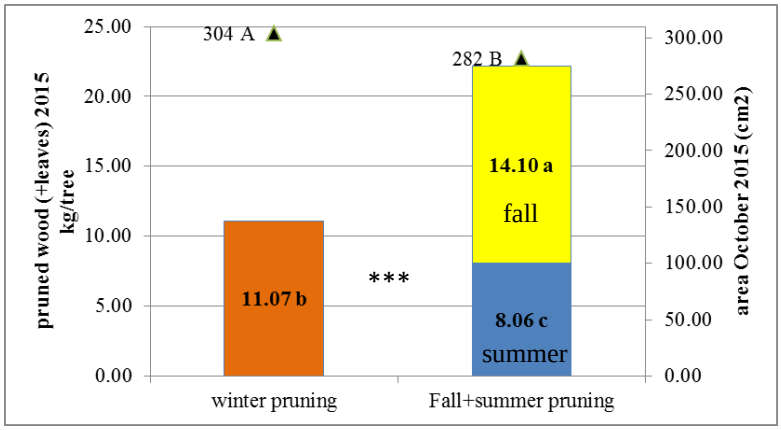


Figure 2: Winter and Fall+summer pruning treatments at the end of the 2015 growing season: comparison between cut material and TCSA (area) in October 2015. Significance:* p<0.05, ** p<0.01, ***p<0.001, ns= not significant; *post-hoc* Bonferroni test for mean separation.

Table 1. Average material removed per tree at each pruning date and number of fruit removed with summer pruning. Significance:* p<0.05, ** p<0.01, ***p<0.001, ns= not significant; *post-hoc* Bonferroni test for mean separation.

Rootstock	winter pruned wood (kg/tree)	num fruit cut w/summer pruning/tree	average weight cut fruit (g)	summer pruned wood+leaves (kg/tree)	fall pruned wood+leaves (kg/tree)	
	March 13th	June 5th			October 20th	
OHF69	**11.08**	**6.5**	**21.97**	**8.32**	**16.88**	**a**
OHF87	**10.30**	**6.1**	**21.15**	**7.07**	**11.03**	**b**
OHF97	**11.85**	**7.1**	**22.49**	**8.87**	**14.41**	**ab**
Significance	**ns**	**ns**	**ns**	**ns**	**	

OHF97 confirmed to be the most vigorous rootstock between the three in trial with a statistically higher TCSA, followed by OHF69 and then OHF87 (data not shown).
At the end of the season, trees fall+summer pruned and grafted on OHF87 were the least vigorous accordingly to their TCSA (data not shown).
In March 2015, flower buds per m^3 were counted because we observed a more abundant presence of flower buds in the Fall+summer treatment. Values revealed the Fall+summer pruned trees had an average of 32 flower buds/m^3 while winter pruned trees averaged just 25 flower buds/m^3; even so, the difference was not statistically significant (Fig. 2). Similarly, no meaningful differences in flower bud density were found when comparing the effect of rootstock.
The measures of SPAD units in the leaves showed a higher value in winter pruned trees than fall+summer ones, this can be explained with more N available and higher vigour. No differences were found among rootstocks, regardless of the pruning technique (Fig. 2).

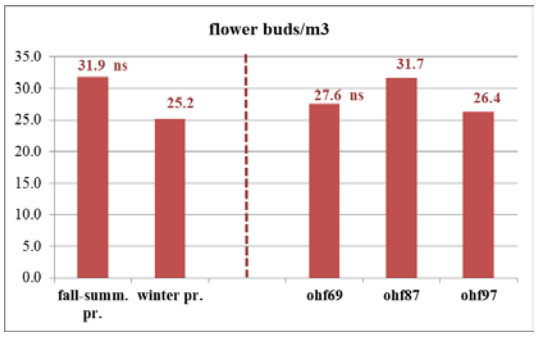


Figure 2: flower buds/m3: comparison between the two pruning technique and between the three rootstocks. Significance:* p<0.05, ** p<0.01, ***p<0.001, ns= not significant. SNK test for mean separation.

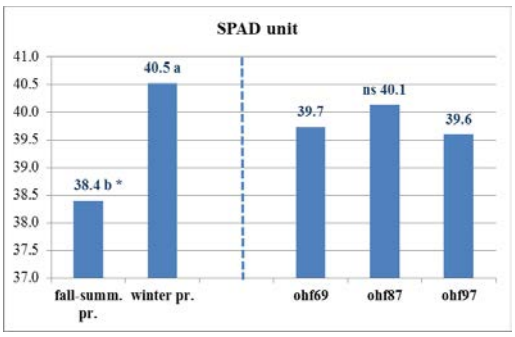


Figure 3: SPAD unit: comparison between the two pruning technique and between the three rootstocks. Significance:* p<0.05, ** p<0.01, ***p<0.001, ns= not significant. SNK test for mean separation.

Quality and Yield

Fruit from 2014 harvest pulled out from normal air storage after 4 month s at -1˚C showed some differences between the pruning treatments for OHF87 rootstock. Fruit picked from the winter pruned treatment lost more weight (5.9 g vs. 4.4 g of fall+summer treatment) and had a faster ripening (accordingly to the I_{AD} values) than the "fall+summer" fruit (Table 2). The color parameter revealed that fall+summer fruit had a higher red blush surface than the winter ones; this may be the effect of more light penetrating the canopy in response to the pruning treatment. The background color was greener for fruit harvested from fall+summer pruned trees than fruit harvested from winter pruned trees. After 4 months in storage, fruit harvested from winter pruned trees showed higher firmness,

higher soluble solid content (SSC) and higher titratable acidity than fruit harvested from fall+winter pruned trees (Table 2). Cork incidence was not different between the two pruning treatments. Comparing the four I_{AD} ripening classes within each pruning treatment we noticed that they remained distinguished from harvest to after 7 days of ripening after storage. In both pruning treatments, the I_{AD} class representing the most ripe fruit (class A) showed statistically higher SSC (Brix) values in comparison to classes representing less ripe fruit. Class D (the least ripe), on the other hand, reported the lowest drop in $I_{AD,}$ the greenest background color and the lowest percentage of overcolor in comparison with the other classes (data not shown).
Samples of pear flesh tissue from T1 and T2 were analyzed for calcium, nitrogen and other nutrients and there were no significant differences between winter pruned fruit and fall+summer fruit except for the nitrogen content that was higher in the winter fruits (data not shown).
Yield 2015 was higher on winter pruned trees with bigger fruit than fall+summer pruning. The difference between the two treatments was around 11 kg/tree (Table 3) equal to approximately 34 fruit/tree. The average fruit weight of winter pruning treatment was 25 g higher than the fall+summer one; they were commercially sized as 80 fruit/box and close to 90 fruit/box, respectively. A late spring frost episode occurred and caused significant damage to 2015 production.
In the comparison between the two pruning techniques and among rootstocks, no significant difference in frost damage incidence appeared; the damage was estimated to hit 6.7% of picked fruit. Sunburn incidence was more severe in the winter pruned trees, probably because of the greater top exposure of the production in comparison to fall+summer pruned trees, where more production was located in the middle-low part of the canopy (Table 3). The three rootstocks did not show any significant difference in terms of productivity, average fruit weight, or frost and sunburn incidence.
Figure 4 shows sampled fruit from OHF87 rootstock and both pruning treatments sorted in I_{AD} classes; the majority of the fruit were represented by class A and B ($1.60< I_{AD}<1.79$ and $1.80<I_{AD}<1.89$, respectively).

Table 2. Anjou/OHF87 quality analyses after 4 month of storage (T2), harvest 2014: comparison between pruning techniques (fruit harvest 2014). Proc GLM in SAS; type III sums of squares significance: *, p <0.05, **, p < 0.01; ***, p <0.001; post-hoc SNK test for mean separation.

T2 2014	I_{AD} harvest	I_{AD} (4 M storage)		I_{AD} drop in 4 M		I_{AD} after 7 days ripening		I_{AD} drop in 7 days		Red blush overcolor (% area)		Hue_T2		Chroma_T2		Firmness (lb)		Soluble solids (Brix)		Titratable acidity (% malic acid)		pH	
Fall+summer pr.	1.92	1.74	a	0.181	b	1.65	a	0.088	b	8.92	a	111.13	a	44.17	a	10.94	b	12.45	b	0.19	b	4.44	a
Winter pr.	1.92	1.70	b	0.219	a	1.60	b	0.095	a	5.21	b	110.61	b	43.70	b	12.55	a	13.07	a	0.23	a	3.93	b
Significance	ns	***		***		***		**		**		***		**		***		***		***		***	

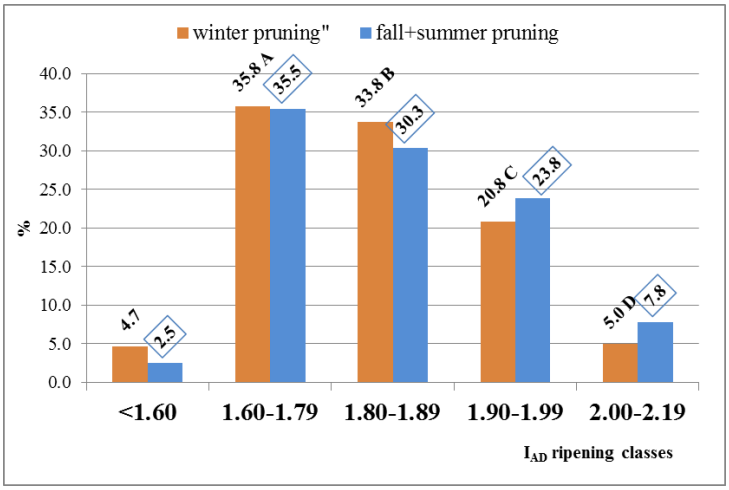


Figure 4: I_{AD} distribution at harvest in the different classes for fruit picked on Aug 24-25th, 2015 from Anjou/OHF87 in both the pruning treatment. Letters indicate the short name for the different classes.

Table 3. Anjou yield in 2015, Cashmere, WA. Productive parameter and disorder incidence (%) are compared for the pruning techniques and for the rootstocks. Proc GLM in SAS; type III sums of squares significance: *, p <0.05, **, p < 0.01; ***, p <0.001; *post-hoc* SNK test for mean separation.

	yield (kg/tree)	Yield (lb/tree)		num fruit/tree		averaage fruit weight (g)		frost incidence (%)	sunburn incidence (%)	
Fall+Summer	31.08	68.53	b	158	b	198.64	b	6.07	0.66	b
Winter	42.45	93.59	a	192	a	224.40	a	7.39	1.83	a
Significance	***	***		**		**		ns	***	
OHF87	37.43	82.51		182		207.66		7.21	1.10	
OHF97	33.13	73.03		159		210.31		6.44	1.26	
OHF69	39.75	87.63		184		216.58		6.55	1.37	
Significance	ns	ns		ns		ns		ns	ns	
*Sign. trt*root*	ns	ns		ns		ns		ns	ns	

Psylla and Mite Densities

Overwintering psylla adult densities were moderate (13-19/tap) on the first two sampling dates (18 February and 11 March) before the first insecticide applications were made. They remained low (<1/tap) throughout the season, rising slightly (5-6/tap) in September. Few treatment or rootstock differences occurred on any of the count dates, nor was the treatment x rootstock interaction significant.

Tetranychid mites remained low throughout the season, never exceeding 0.4 mites/leaf.

Fruit damage from psylla, mealybug and rust mite were low, with an average damage rating in all cases of <1 (1-10% of the fruit surface russetted). However, the damage from pear rust mite in the winter-pruned treatment was significantly higher than that in the summer/fall pruned treatments.

CONTINUING PROJECT REPORT **YEAR: 2 of 3**
Project Number: PR14-108A

Project Title: Improving quality and maturity consistency of 'd'Anjou'

PI: Stefano Musacchi
Organization: Washington State University/ TFREC
Telephone: 509-663-8181 x236
Email: stefano.musacchi@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (1): David Rudell
Organization: USDA, ARS
Telephone: 509-664-2280 x245
Email: david.rudell@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Co-PI (2): Jim Mattheis
Organization: USDA, ARS
Telephone: 509-664-2280 x249
Email: james.mattheis@ars.usda.gov
Address: 1104 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Sara Serra (WSU/TFREC)

Total Project Request: **Year 1**: $65,992 **Year 2**: $67,272 **Year 3**: **$68,602**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509-335-4564/509-663-8181 x221 **Email:** carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2014	2015	2016
Salaries[1]	24,000	24,960	25,958
Benefit[1]	7,992	8,312	8,644
Travel[2]	500	500	500
Goods and Services[3]	3,000	3,000	3,000
Total	35,492	36,772	**38,102**

Footnotes:
[1]Salaries and benefits for 50% Ag. Research Assistant (Musacchi).
[2]Travel to different orchards and farm where the different trials will be conducted (Musacchi).
[3] Consumable lab ware and mineral analyses.

Budget 2
Organization Name: USDA, ARS **Contract Administrator:** Chuck Myers
Telephone: 510-559-5769 **Email address:** Chuck.Myers@ARS.USDA.GOV

Item	2014	2015[2]	2016[2]
Wages[1]	15,000	15,000	15,000
Goods and Services[2]	15,500	15,500	15,500
Total	30,500	30,500	**30,500**

Footnotes:
[1] $12,500 for 25% annual instrument service contracts. $3,000 for consumables
[2]Add proposed same amount for year 1 if work is to be performed in years 2 or 3.

OBJECTIVES:

1) *Determine maturity and quality variation as impacted by tree and orchard management regimes.*
2) *Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.*

SIGNIFICANT FINDINGS

1) *Determine maturity and quality variation as impacted by tree and orchard management regimes.*

- Variability of fruit maturity was confirmed within large open vase canopies in the second year.
- External fruit were larger and had higher dry matter % and SSC than internal ones, both after 3 months and 8 months of CA storage.
- The drop in I_{AD} during 8 months CA storage was greater in external fruit, confirming they were more ripe and ripening faster during storage.
- Internal fruit were greener than external ones.

2) *Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.*

- Peel chemical analysis indicates that tree position will have a major impact on relative storability and eating quality. Pear ester (ethyl 2,4-decadienoate), an important varietal aroma component of Anjou, was different between canopy positions by 8 months storage
- Fruit aroma and compounds associated with quality were different depending upon tree position.
- Natural chemicals levels correlated with DA class at harvest.

METHODS

1) Determine maturity and quality variation as impacted by tree and orchard management regimes.

Fruit storage and quality (harvest 2014)
Upon removal from CA storage (3, 6 and 8 months) we subdivided all fruit into two groups for evaluation at 0 and after 7 days at room temperature to assess quality and ripening of all treatments. This included, by canopy position and I_{AD} class subdivision, percentage of blush over-color surface, background color (Minolta colorimeter), exogenous ethylene, I_{AD} (DA meter), and fruit weight, followed by fruit firmness, fruit diameter, cork spot incidence, dry matter percentage, percentage of viable seeds, soluble solid content (SSC), titratable acidity (TA) and pH.

Pre-harvest assessment
The same orchard (open vase, 20 ft x 20 ft, 109 trees/acre) from the 2014 trial was used for the 2015 trial. On August 17th, we picked a representative tree and approximated the total number of fruit, yield per tree, average fruit weight, frost damage and sunburn incidence. The variability of fruit maturity across the whole canopy was assessed using I_{AD} (ripening stage determined by the DA meter) of each individual fruit.

PAR measurement per single fruit (2015)

At the beginning of August, 19 trees were selected in the same orchard to categorize all of their fruit by two canopy positions by percentage of actual light intercepted (internal: <30% light and external: 70-100% light).

More than 500 fruit per canopy position were labeled on the stem with a number and position on the tag as well as the peel. Intercepted light was estimated using the PAR quantum Q53292 sensor (Licor) by placing the sensor perpendicular to the ground at the equatorial level of the south face of each pear (Fig. 1). Measurements (expressed in µmol s^{-1} m^{-2}) were carried out on multiple sunny days at solar noon ± 1 h. Every 20 measurements a reference PAR was recorded at full sun from above the canopy (>3.5m) as 100% light interception. The PAR reference value was used to calculate the percentage of light intercepted by each labeled fruit as: Light interception $_{[fruit]}$ (%) = ($PAR_{[fruit]}$ / $PAR_{[reference]}$ *100). Fruit not belonging to the two light level categories were harvested for storage and/or quality analysis.

Figure 3: Measurement of PAR of an internal fruit (<30% light interception) using the Q53292 quantum sensor (Licor). .

2015 harvest and fruit sorting

Fruit from each of the two light levels were harvested on the August 31. Fruit from internal and external canopy regions were picked separately. Fruit from each light condition were separated into two bins and immediately moved to 40°F for fruit maturity distribution analysis and categorization into two groups containing 460 external and 486 internal pears.

Within each group, fruit were again classified using I_{AD} into 5 classes (I_{AD}<1.60, 1.60<I_{AD}<1.79, 1.80<I_{AD}<1.89, 1.90<I_{AD}<1.99, 2.00<I_{AD}<2.19). The first or last class was only included in the external or internal canopy, respectively. Fruit belonging to each class were, then, equally divided into 3 groups for 0, 6, and 8 months CA storage. Fruit were stored in a research CA room (31°F, 2% O_2 and 0.8% CO_2). Fruit quality analysis at T0 2015 was performed in the same manner as 2014 except fruit were analyzed without post-harvest ripening step.

2) Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.

For the 2014 fruit chemistry analysis, peel and cortex samples were collected from fruit at 33 F (to minimize ripening during sampling) from "unripe" fruit following each pull out (3, 6, and 8 months CA storage). For sampling, three fruit per rep (5 reps/class) were used from each DA class and washed briefly with deionized water. Fruit were selected according to the following criteria: similar I_{AD} (after storage), I_{AD} drop following harvest (e.g. $I_{AD\ [T1]}$ - $I_{AD\ [T0]}$), weight, and relative absence of surface defects. After fruit were sampled for metabolic analysis, SSC was analyzed on the remaining fruit. The composite fruit from each rep (3 fruit) were juiced and assessed for pH and TA the following day. Peel and cortex samples were excised, chopped, and flash frozen using liquid nitrogen (80 samples per postharvest pull out). Samples were stored at -112 F until processing and analysis. Sample processing and untargeted metabolite analysis were performed using GC and LC-MS (modified from Leisso et al., 2015). Data extracted and analyzed using mzMine coupled and in-house metabolite libraries as well as the Unscrambler (CAMO, Trondheim, Norway) multivariate statistical processing package. Our untargeted peel chemical analysis evaluated additional compounds to include volatile that comprise fruit aroma and polar compounds that comprise both flavor, appearance, and structure. A total of 1538 individual chemicals were evaluated from the peel. Statistical analyses were employed to indicate which orchard and storage factors had the most impact

on how peel chemistry changed in reference to fruit quality and ripening and which peel chemicals primarily reflected these changes or were associated with I_{AD} class.

RESULTS AND DISCUSSION

1) Determine maturity and quality variation as impacted by tree and orchard management regimes.

2014 fruit storage and quality

After 3 months CA storage (T1) external fruit were larger (higher average fruit weight and diameter) and had higher dry matter % and SSC than internal fruit (Fig. 2). Internal fruit had greener peel (hue and "a" coordinate). A higher percentage of blushed fruit was observed on external fruit at 12-13% (data not shown). I_{AD} was still different between the two positions (> 0.20 for internal fruit) in both fruit analyzed immediately upon removal from storage and those held at 7 days at room temperature (Fig. 2). The I_{AD} drop after 3 months was greater for external fruit, confirming their advanced maturity status (data not shown). Ethylene production was detected but not different between external and internal fruit immediately upon removal from 3 month CA or after 7 days at room temperature (data not shown). There was no difference of firmness between fruit from the two positions immediately upon removal from CA (Fig. 2A); however, after 7 days of ripening, internal were firmer than the external fruit (Fig. 2B). Titratable acidity and percentage of viable seeds were not different between canopy positions (data not shown). When all ripening classes and canopy positions were compared as a group, significant differences of fruit weight, I_{AD} drop, dry matter %, firmness and soluble solid contents, were found at harvest and following 7 days ripening. Class $1.90<I_{AD}<1.99$ and $2.00<I_{AD}<2.19$ internal (the least ripe of the all classes) showed the lowest SSC and DM% values significantly different from class $I_{AD}<1.60$ external reporting the highest values. The firmest fruit were from the external $1.90<I_{AD}<1.99$ and internal $2.00<I_{AD}<2.19$ classes before and after post-storage ripening (data not shown).

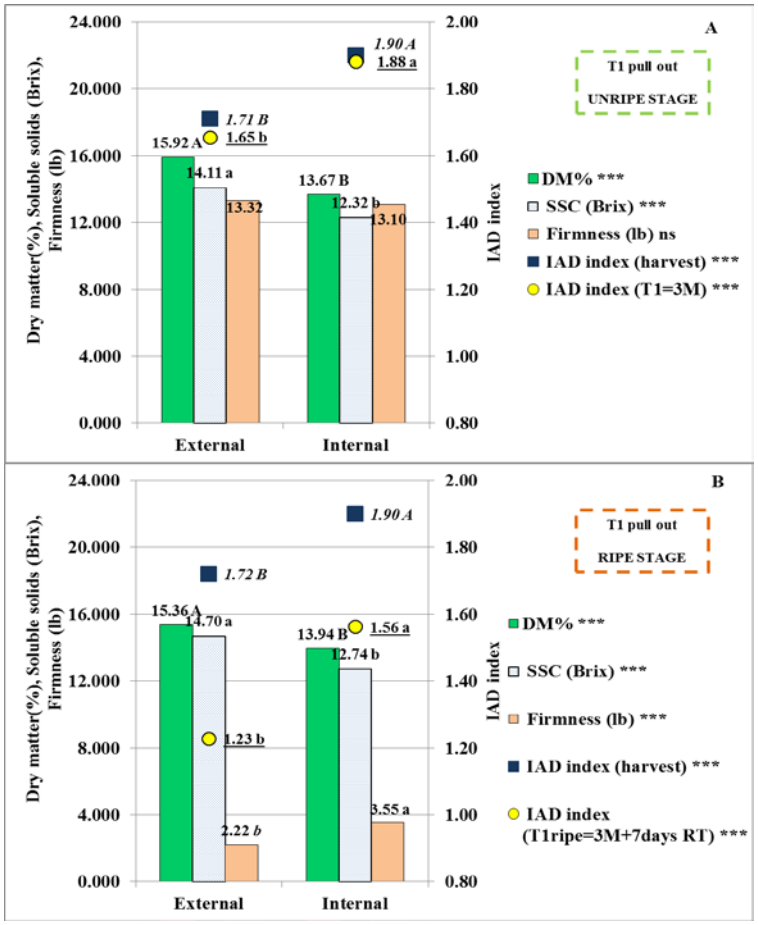


Figure 2: Anjou firmness, soluble solids, dry matter %, I_{AD} index at 0 days (A) and 7 days at room temperature (B): following 3 month CA storage* (p<0.05, ** p<0.01, ***p<0.001, ns= not significant; post-hoc SNK test for mean separation).

After 8 months of CA (T3), external fruit were larger (higher average fruit weight and diameter) and had a higher dry matter % and SSC than internal ones (Fig. 3). Internal peel was greener than external peel (hue and "a" coordinate). External fruit had a higher percentage of blush (14-17%).

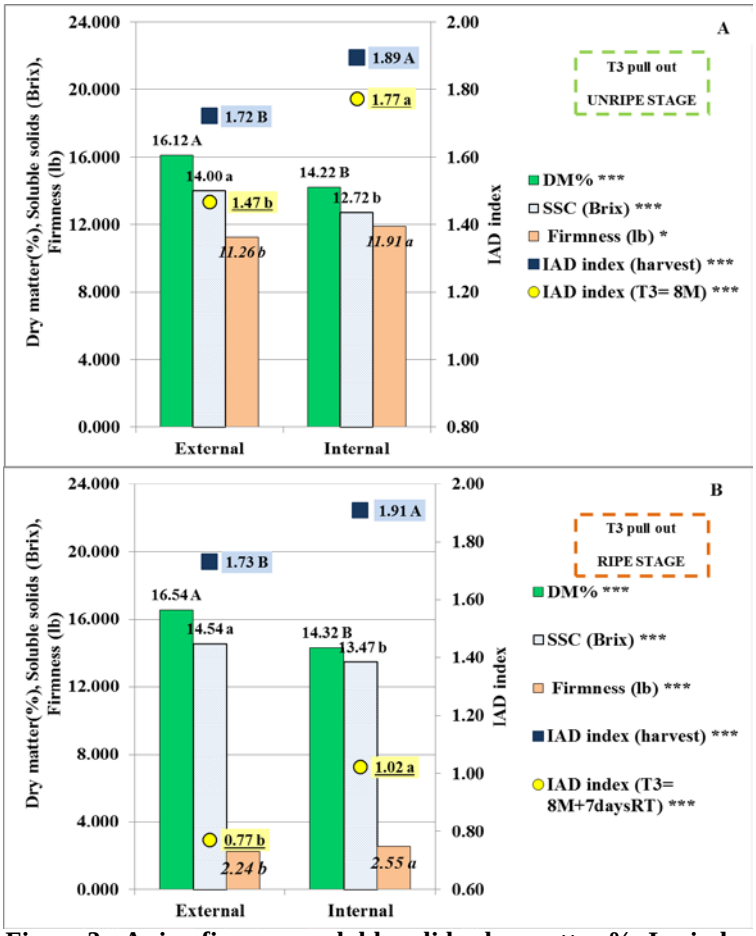


Figure 3: Anjou firmness, soluble solids, dry matter %, I_{AD} index at 0 days (A) and 7 days at room temperature (B) following 8 months CA storage (* p<0.05, ** p<0.01, ***p<0.001, ns= not significant; post-hoc SNK test for mean separation)

I_{AD} after 8 months CA storage indicated that fruit from the two positions was still different (≥ 0.31 higher values for internal fruit) both with and without post-storage ripening (Fig. 3). I_{AD} drop during 8 months CA was greater for external fruit, confirming their more advanced maturity status. Ethylene production was detected but not different at most sampling points; however, more ethylene was detected from the external fruit after 7 days of ripening compared to internal fruit (data not shown). Firmness was always higher for internal than external fruit upon removal from storage (Fig. 3 A) and following post-storage ripening (Fig. 3B). Titratable acidity was different between the two positions immediately upon removal from storage but not after post-storage ripening (data not shown). When comparing I_{AD} classes, internal fruit from classes $2.00<I_{AD}<2.19$ and $1.90<I_{AD}<1.99$ decreased the least in I_{AD} both before and after post-storage ripening, indicating different ripening behavior compared to external fruit from class $I_{AD}<1.60$, $1.60<I_{AD}<1.79$ (data not shown). DM% was different between external fruit from the 2 extreme I_{AD} classes ($I_{AD}<1.60$ and internal $2.00<I_{AD}<2.19$), this difference was ranging from 3.3% upon removal from storage to 2.9% after 7 days of ripening which was higher than external fruit from class $I_{AD}<1.60$ (data not shown).

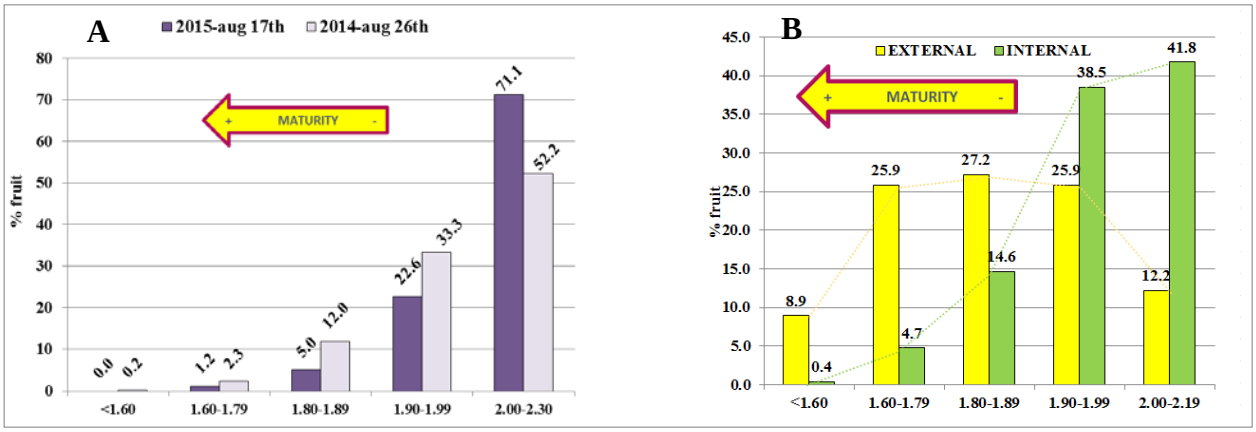


Figure 4: Pre-harvest assessment of fruit maturity distribution across the canopy of an open vase tree in 2014 and 2015 (before the August 17 harvest) (A) and the distribution of fruit picked categorized by canopy position and I_{AD} class as well as external and internal in 2015 (B).

Pre-harvest assessment and fruit maturity distribution

Fruit picked as part of our representative tree totaled 962 (included 911 good fruit and 51 of <60 mm size and/or with defects), with a total yield of 180 kg/tree and average fruit weight of 198 g. Sunburned incidence was 2.3% and frost damage, 0.11%.

Total 2015 fruit number was half of that in 2014, but the average fruit weight was higher (> 20g). By measuring I_{AD} before harvest, we confirmed the variability of maturity within a tree: 71% of fruit were classified in the least mature I_{AD} classes (over 2.00 I_{AD}) and only a small percentage (1.2%) of fruit were classified in the more ripe classes (below 1.80 I_{AD}, Fig. 4A). Fruit maturity distribution within I_{AD} classes at harvest was different. Forty-two % of internal fruit fell in the least ripe class, while only 12% of external fruit belonged to that class (Fig. 4B). Almost 8.9% of the external fruit were classified in the most ripe categories (<1.60 I_{AD}), while only 0.4% of the internal ones resided in the same classes (Fig. 4B).

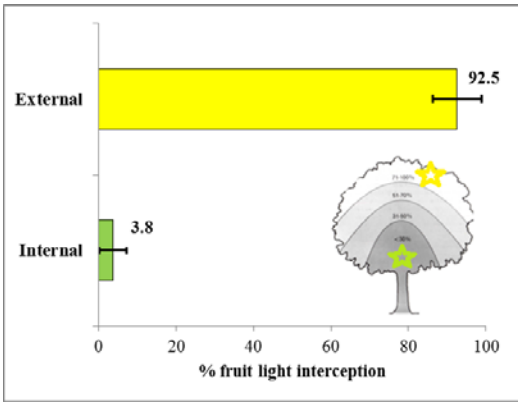


Figure 5: Percent difference of light interception of fruit from the two canopy position as determined by PAR measurement using the Q53292 quantum sensor (Licor). Values are average (N=500) ± stdDev.

PAR measurement per single fruit (2015)

PAR measurements of fruit marked for sampling allowed us to accurately choose fruit from the two canopy positions. The percentage of light intercepted by external fruit averaged 92.5% while only 3.8% by internal fruit (Fig. 5).

2) Correlate pear quality, maturity, and chemistry with DA meter evaluation and storability.

In our second year, peel chemistry changed alongside fruit appearance and other quality traits. Differences of peel chemistry were most dramatic with tree position which changed as fruit ripened during storage (Fig. 6). Results indicate the greatest impact on fruit ripening and chemistry results from tree position more than any other factor in the experiment and, accordingly, it is the greatest source of quality and ripeness variability. Differences were detected at harvest as well as throughout storage indicating the final product on the store shelf may also be different.

Differences of quality traits, including natural aroma and flavor, are clear within the chemical profile. These include sugars (sweetness), malic acid (tartness), phenolics (bitterness), and aroma volatiles (unripe flavors or ripe flavors). Pear ester (ethyl 2,4-decadienoate) is a particularly important aroma component of Anjou that was different between canopy positions, even at 8 months storage (Fig. 7). I_{AD} classification was reflected in the overall peel chemistry at harvest but this relationship declined with storage duration (data not shown). Peel chemical analysis results to date indicate that tree position will have a major impact on relative storability and eating quality.

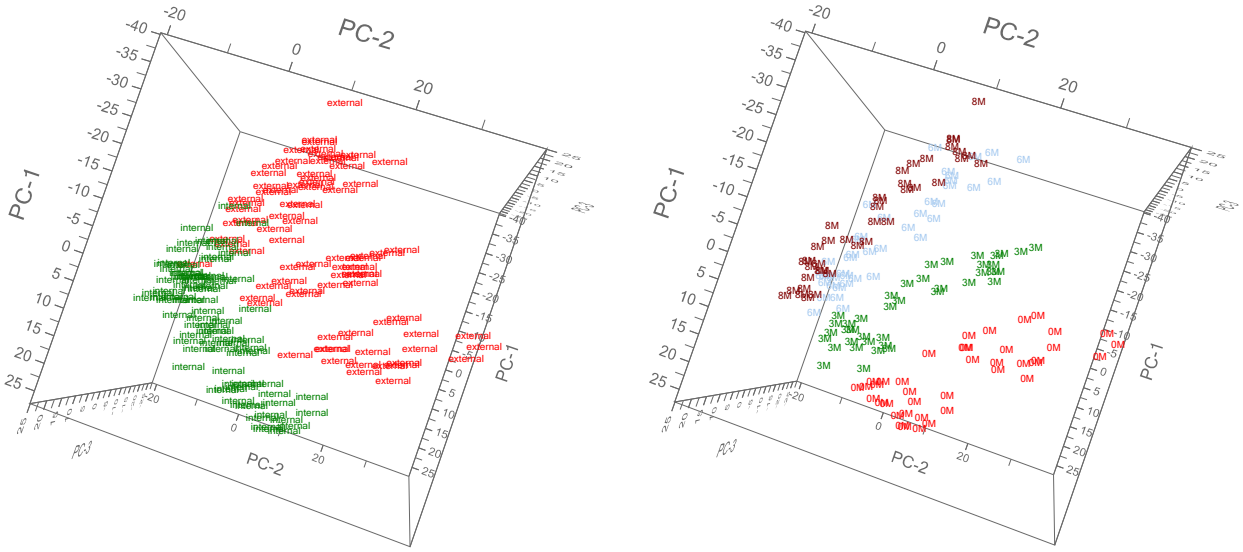


Figure 6: Principal components analysis (PCA) scores plot illustrating differences in overall natural chemical levels from Anjou pears harvested from the external or internal canopy and stored for up to 8 months in CA storage. Each point represents a summary of over 1400 natural peel chemicals for a single peel sample. On the left: green circle=internal, red square=external. On the right: red square= T0 harvest, green circle= T1 (after 3 months CA storage), blue cross=T2 (after 6 months of CA storage) and crimson diamond=T3 (after 8 months in CA storage).

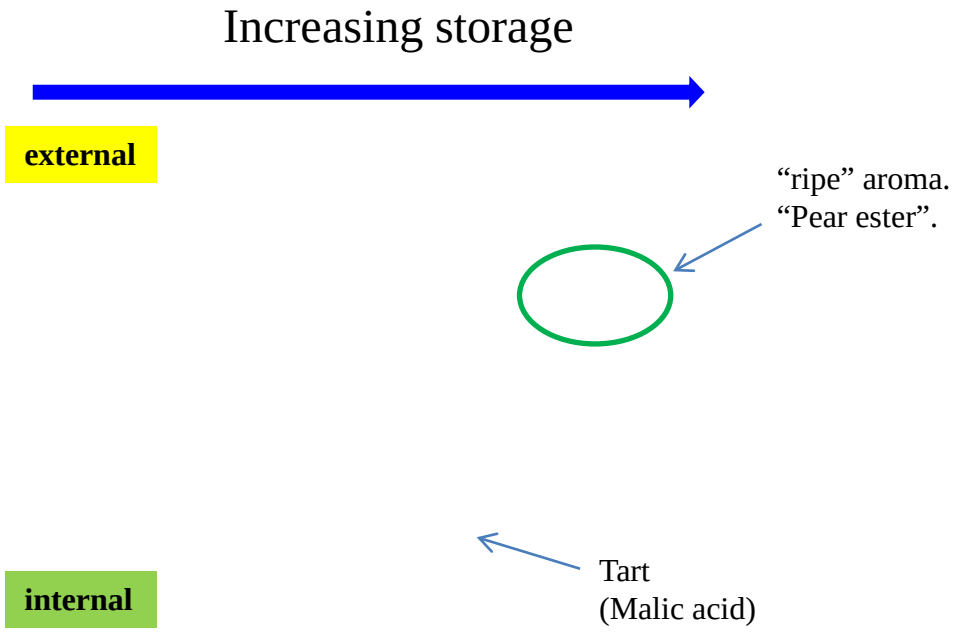


Figure 7: Partial least squares discriminate analysis (PLS-DA) loading plot illustrating natural chemicals associated with internal (below line) or external (above line) fruit over 8 months CA storage (0 months, left to 8 months, right). Triangles indicate known peel chemicals that are related to quality. The larger the triangles or circles, the more they are associated with either internal or external fruit.

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

Project Title: Delivering quality pear fruit to consumers

PI: Yan Wang
Organization: MCAREC
Telephone: 541-386-2030 x38214
Email: yan.wang@oregonstate.edu
Address: 3005 Experiment Station Dr
City/State/Zip: Hood River, OR97031

Cooperators: Dr. Yu Dong, Steve Castagnoli, Todd Einhorn, David Sugar, Paul Chen

Total Project Budget: **Year 1**: 25,725 **Year 2**: **26,390** **Year 3**: 27,073

Other funding sources
none

Budget 1
Organization Name: Agricultural Research Foundation **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2015	2016	2017
Salaries	13,088[1]	13,481	13,885
Benefits	1,250[2]	1,300	1,352
Wages	6,715[3]	6,917	7,124
Benefits	672[4]	692	712
Equipment			
Supplies	3,500[5]	3,500	3,500
Travel	500[6]	500	500
Miscellaneous			
Total	25,725	**26,390**	27,073

Footnotes:
[1]Postdoctoral Research Associate: 1/3 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 500hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 10% of the wage, with a 3% annual increase.
[5]Supplies: maintaining cold rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

Objectives:

1. Elucidating the cell metabolic mechanism underlying pear ripening and eating quality by:
    a. determining how pear ripening capacity and eating quality are related to cell wall chemistry.
    b. determining how cell wall pectin chemistry is regulated by ethylene and pre- and post-harvest factors.
    c. objectively characterizing the buttery-juicy texture using commercially feasible physiological parameters, i.e., extractable juice.

2. Determining factors affect chilling requirement for ripening (CRR = days at 30°F to induce ripening capacity).

3. Developing conditioning protocols for early season marketing and 1-MCP treated 'Anjou'.

SIGNIFICANT FINDINGS year-1

1. **Objective 1**
    a. Anjou pear harvested at 15 and 14lb developed a melting (buttery-juicy) texture in 7d at room temperature following 3-5 months in RA (regular air) and 5-8 months in CA (1.5% O_2 + < 0.05% CO_2) at 30°F. They developed a mealy (dry-coarse) texture after 6 months in RA and after 9 months in CA. Anjou pear harvested at 13 and 12lb had a shorter buttery-juicy texture storage life, such as 3-4 months in RA or 5-6 months in CA.
    b. Anjou pear harvested at 15 and 14lb developed better buttery-juicy texture than that harvested at 13 and 12lb after cold storage. Fruit harvested at 17 and 16lb developed good buttery-juicy texture, but inferior flavor and unacceptable shriveling during storage.
    c. Flesh firmness is not always a good indicator for buttery-juicy texture. Softening may occur without development of optimum dessert quality. Water soluble polyuronides (WSP) content was positively correlated with the development of buttery juicy texture, however, measuring WSP is tedious. Extractable juice (EJ) is negatively correlated with WSP and is relatively simple to be measured.
    d. A texture index (TI=1-5) is developed based on EJ and is an objective measurement for buttery-juicy texture: TI=(100-EJ)/10. A critical value of TI being buttery-juicy texture for Anjou pear is >3.5.
    e. Possible factors affecting WSP synthesis: pre-harvest temperature, fruit nutrition, harvest maturity, storage duration, CA, and 1-MCP treatment.

2. **Objective 2**
    The CRR of Anjou pear varied significantly with production locations (Medford, Mid-Columbia, and Wenatchee), production elevations (500, 1000, and 2000ft), harvest maturity, and production years.
    a. Harvest maturity in different production areas and elevations
    Mid-Columbia area: CRR is affected by harvest maturity from 15lb to 11lb
        i. 500ft: $Y = -1.8X^2 + 61.9X - 440.7$

ii. 1000ft: $Y = -2.9X^2 + 85.3X - 577.1$
iii. 2000ft: $Y = -1.1X^2 + 36.4X - 254.4$

For example, harvest maturity at 15lb, it will take 85, 60 and 55 days at 30°F to induce ripening capacity for fruit produced at 500, 1,000, and 2,000 ft, respectively.

Medford area and ***Wenatchee area*** (data under analyzing)

b. Ca content

Anjou pear with low Ca content (i.e. < 500-600ppm dw) requires shorter CRR, but reduces storability significantly. Anjou pear with high Ca content (i.e. ≥ 900ppm) requires ≥ 90 days CRR.

c. Accumulated cold unit (ACU = hours < 50°F during 42d prior to harvest)

A preliminary analysis of the first year data indicated that ACU affects CRR in Anjou pear. A commercially useful model may be developed based on multiple years data.

3. **Objective 3**

a. Early season marketing

To ensure obtaining ripening capacity without over-conditioning (too soften for shipping), ethylene conditioning protocol should be varied according to harvest maturity/production elevation/fruit Ca concentration/cultivar (red or green).

b. 1-MCP treated Anjou pear

A post-storage ethylene conditioning (PSEC) (100ppm for 72h at 68-70°F) improved ripen capacity of the 1-MCP treated Anjou pear after a long-term storage (i.e. > 7-8 months).

METHODS

Objective 1. Lab procedures are developed to quantify cell wall total pectin substances (TPS), WSP, EDTA-soluble pectin (VSP), alkali-soluble pectin (ASP), and insoluble pectin substances (IPS). The key enzymes (PME and PG) regulating pectin degradation process will also be monitored. Anjou pear fruit harvested at commercial maturity will be ripened for 7 d at 68 °F after storing at 30 °F for 0, 1, 2,3,4,5,6,7,8 months in RA and 5, 6, 7, 8, 9 months in RA at 30°F. Tissue samples will be frozen in liquid N_2 and stored at -80 °C until analysis. The effects of harvest maturity (FF = 15, 14, 13, 12 lb), 1-MCP treatment (100ppb), storage temperatures (30, 32 °F), CA storage, and storage duration on cell wall pectin metabolism and buttery-juicy texture development are studied. An industry standard methodology is developed to objectively quantify the buttery-juicy texture.

Objective 2. Factors may affect CRR: maturity/production area/elevation/Ca nutrition/year. ACU and CRR are collected from different orchards at varied elevations (from ~500 to ~2,000ft) in multiple years (2015, 2016, and 2017). Temperature loggers are used to log the temperature profile from full bloom to commercial harvest date in different orchards. ACU is calculated based on the temperature profile in each orchard. Fruit are harvested at FF=15-14lb. CRR is determined after 7d at 68°F following 50, 60, 70, 80, 90, 100 days at 30°F until FF reaches <4lb.

RESULTS

1. Cell wall metabolic mechanism underlying ripening and eating quality

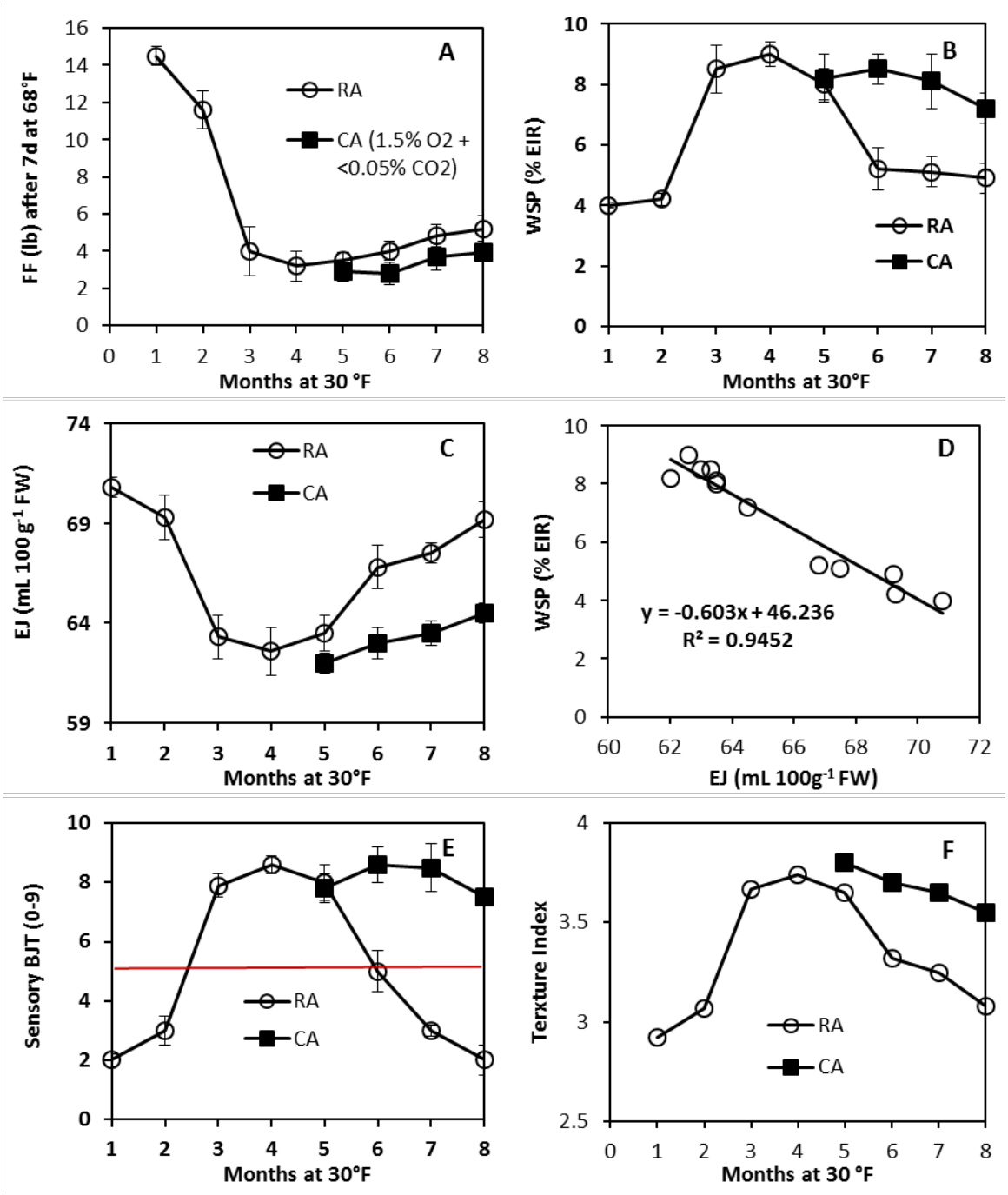


Fig. 1. Fruit flesh firmness (FF) (A), water soluble pectin (WSP) (B), extractable juice (EJ) (C), the relation between EJ and WSP (D), sensory score for buttery-juicy texture (BJT) (E), and texture index in Anjou pear after 7d at 68F following 8 months storage in regular air (RA) or controlled atmosphere (CA = 1.5% O_2 + <0.5% CO_2) at 30°F.

Sensory evaluation indicated that Anjou pear harvested at 15 and 14lb from MCAREC (500ft, Mid-Columbia area) developed a melting (buttery-juicy) texture in 7d at room temperature following 3-5

months in RA and 5-8 months in CA (1.5% O_2 + < 0.05% CO_2) at 30°F. They developed a mealy (dry-coarse) texture after 6 months in RA and after 9 months in CA. Anjou pear harvested at 13 and 12lb had a shorter buttery-juicy texture storage life, such as 3-4 months in RA or 5-6 months in CA. Anjou pear harvested at 15 and 14lb developed better buttery-juicy texture than that harvested at 13 and 12lb in 7d at room temperature following 2-5 months in RA at 30°F. Fruit harvested at 17 and 16lb developed good buttery-juicy texture, but inferior flavor (taste and aroma) and unacceptable shriveling during storage.

Sensory and physiological parameters on only one harvest maturity (15lb) are presented in Fig. 1. Flesh firmness (FF) is not always a good indicator for buttery-juicy texture (Fig. 1A). FF reduced to below 4lb in 7d at 68°F following 3-8 months in RA at 30°F. After ripening the fruit developed buttery-juicy texture only following 3-5 months; the fruit developed a dry-coarse texture following 6-8 months storage in RA storage. We found that water soluble polyuronides (WSP) content was positively correlated with the development of buttery juicy texture in Anjou pear stored in both RA and CA (Fig. 1B). However, measuring WSP is tedious. Extractable juice (EJ) is negatively correlated with WSP in Anjou pear stored in both RA and CA (Fig. 1C&D) and is relatively simple to be measured. A detailed procedure for measuring EJ is available.

Softening in Anjou pear may occur without development of optimum dessert quality. A texture index (TI) is developed based on EJ and is an objective measurement for buttery-juicy texture: TI=(100-EJ)/10 (Fig. 1F). The TI is positively correlated with sensory buttery-juicy (BJT) score (Fig. 1E). A critical value of TI being buttery-juicy texture for Anjou pear is >3.5.

2. Factors influencing CRR

a. ***Harvest maturity in different production areas and production elevations***
Mid-Columbia area
Production elevation influenced RCR significantly (Fig. 2). Fruit from low elevation (i.e., 500 ft) required longer RCR than that from higher elevation (i.e., 2,000ft) at the same harvest maturity. For examples at harvest maturity of 15lb, it needs 85, 60, and 55 days at 30°F to induce ripening capacity for fruit produced at 500, 1,000, and 2,000 ft, respectively. When harvest at 12lb, it needs 45, 35, and 30 days at 30°F to induce ripening capacity for fruit produced at 500, 1,000, and 2,000 ft, respectively.
Medford and ***Wenatchee*** areas (data under analysis)

b. ***Ca content.*** (data under analysis)
Preliminary analysis indicated that Anjou pear with low Ca content (i.e. < 500-600ppm dw) requires shorter CRR, but reduces storability significantly. Anjou pear with high Ca content (i.e., ≥ 900ppm) requires ≥ 90d CRR.

c. ***ACU*** (data under analysis)
A preliminary analysis of the first year data indicated that the ACU affects CRR in Anjou pear. ACU and CRR will be collected from different orchards at varied elevations (from ~500 to ~2,000ft) in

multiple years (2015, 2016, and 2017). A model may be developed to predict 'Anjou' pear CRR at the time of harvest based on ACU.

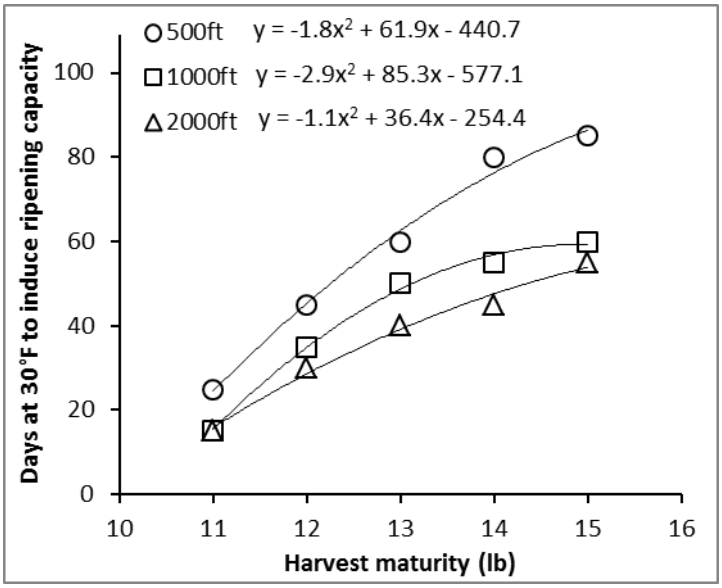


Fig. 2. Relationship of the duration of temperature conditioning at 30°F required to induce ripening capacity to the harvest maturity in Anjou pear from a range of production elevations (~500-2,000 ft).

d. ***Cultivar.*** (data under analysis)
Preliminary analysis indicated that the Columbia red Anjou pear synthesizes higher ethylene and needs shorter CRR compared to green Anjou.

3. Conditioning protocols (data under analysis)

3.1. Early season marketing
To ensure obtaining ripening capacity without over-conditioning (too soften for shipping), ethylene conditioning protocol should be varied according to harvest maturity/production elevation/fruit Ca concentration/cultivar (red or green).

3.2. 1-MCP treated Anjou pear
A post-storage ethylene conditioning (PSEC) (100ppm for 72h at 68-70°F) improved ripening capacity of the 1-MCP treated Anjou pear after a long-term storage (i.e. > 7-8 months).

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

Project Title: Establishing NW-acclimated Pyrus rootstock breeding material

PI: Amit Dhingra
Organization: Washington State University
Telephone: 509 335 3625
Email: adhingra@wsu.edu

Co-PI: Kate Evans
Organization: Washington State University
Telephone: 509-663-8181
Email: kate_evans@wsu.edu

Total Project Request: **Year 1**: 22,000 **Year 2**: 22,185 **Year 3**: **22,992**

Other funding sources

Agency Name: PNW Pear Bureau
Amt. awarded: $273,253 (2015-2018)
Notes: "Pear Rootstock Breeding" PI Evans, Co- PI Dhingra. Synergistic project to advance the selected pear rootstock seedlings via phenotyping and propagation.

Agency Name: WSU CAHNRS Ignite Program
Amt. awarded: $2500
Notes: Support for an undergraduate student to perform phenotyping and tissue culture of selected seedlings and embryo rescue.

Agency Name: Washington State University Graduate school
Amt. awarded: $34,000 (2016)
Notes: Support for Danielle Guzman, Graduate student – she will perform additional crosses with irradiated pollen in 2016.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

Organization Name: WSU **Contract Administrator:** Carrie Johnston
Telephone: 509-335-4564 **Email address:** carriej@wsu.edu

Item	2014	2015	2016
Wages[a]	13,832	14,385	14,960
Benefits	5,577	5,800	6,032
Supplies[b]	1,000	1,000	1,000
Plot Fees[c]	1,000	1,000	1,000
Total	21,409	22,185	**22,992**

Footnotes: a. Technical support for plant handling in greenhouse
b. Greenhouse supplies, pots, soil etc.
c. Greenhouse space fees

OBJECTIVES

1. Screen seedlings germinated in 2012 for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions
2. Germinate and subsequent phenotypic screening of seeds derived from irradiated pollen

This project addresses the long-term need for NW acclimated pear rootstocks in the US and is complementary to larger efforts in this direction. In particular, this project focuses on rapid growth of 149 seedlings derived from crosses between 'Bartlett', 'd'Anjou' and 'Comice' and 49 seedlings derived from crosses using gamma irradiate pollen between 'Bartlett', 'd'Anjou', 'Comice' and 'Abate Fetel' in the greenhouse to perform phenotypic screening.

SIGNIFICANT FINDINGS

- Seedlings from crosses made between 'Bartlett', 'd'Anjou' and 'Comice' are in the fourth dormancy cycle. The juvenile phenotype of thorns is being replaced by spur like structures. Flowering could be observed after the next dormancy cycle.
- A total of 49 seedlings have been established from crosses made with gamma irradiated pollen.
- The ratio of number of nodes to height in the irradiated pollen ranges from 0.53 in a 'Bartlett' × 'Abate Fetel' (irradiated) cross to 1.4 in a 'Bartlett' × 'Comice' (irradiated) cross indicating a great degree of spread between vigor and dwarfing.

METHODS

Objective 1: Screen seedlings germinated in 2012 for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions

Seeds obtained from crosses made in the 2013 season were stratified and were germinated in 12 inch pots filled with potting soil. Once the seedlings were 6 inches tall, they were moved to larger pots. Previously germinated plants continue to be maintained in 2 gallon pots. Irrigation and fertilization is being performed on an ongoing schedule standardized for greenhouse plants. Seedlings are moved to the cold room to provide 1000 hours of chilling (ecodormancy) at the first sign of phenotypic markers of shoot growth. Plants are completely defoliated prior to being moved back to ambient growth conditions to initiate vigorous growth.

Protocols and approaches developed for accelerating plant growth for apples continues to be used as a model for adaptation to accelerating pear seedling growth in the greenhouse. The plants have been taken through four cycles of dormancy since the project was funded. The activities for the next year would be continue the maintenance of the trees and push the growth of the plants to complete 1-2 additional growth cycles. This will necessitate greenhouse growth and incubation in cold chambers to provide 1000 hours of chilling.

Objective 2. Germinate and subsequent phenotypic screening of seeds derived from irradiated pollen

Over 60% of the seeds obtained with irradiated pollen will require embryo rescue since they are deformed without proper formation of cotyledons. In this technique, the embryo is excised from the seeds and developed using tissue culture procedures. Remaining 40% of the seeds will be germinated conventionally and characterized for desirable phenotypes.

Standard tissue culture protocols have been used for embryo rescue experiments. Briefly, the deformed seeds were surface sterilized in a laminar flow hood using 50% bleach solution. After 10 minutes of treatment, seeds were washed with autoclaved water 5 times. Using a scalpel, the seed coat was excised and the cotyledons were exposed. The embryo area was carefully excised and placed on Murashige and Skoog media for embryo growth.

RESULTS & DISCUSSION

Objective 1: Screen seedlings germinated in 2012 for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions

A total of one hundred and forty nine potted trees representing seedlings obtained from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' are undergoing fourth dormancy cycle and are being maintained in the greenhouse in Pullman. These potted saplings were scored for node count and height in May 2015. Based on the ratio of number of nodes to height and growth habit, preliminary plant selections were made for desirable seedlings for a complementary project being led by Co-PI Evans. Considerable phenotypic variation was observed in plant habit and wide distribution of ratio of number of nodes to height was recorded. Figure 1 provides an example of the variation in habit.

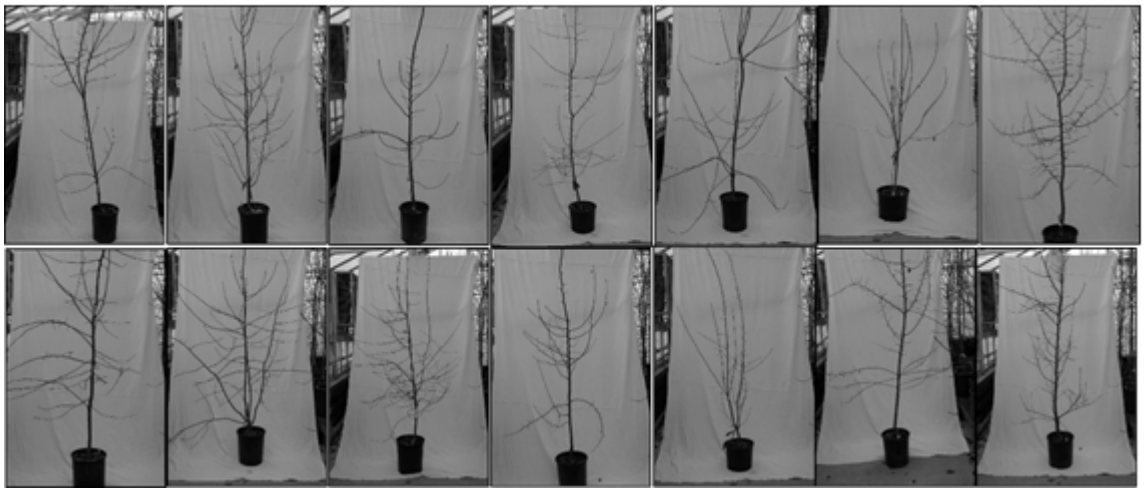


Figure 1: Representative plants from the F1 population demonstrating a wide variation in growth habit.

Objective 2. Germinate and subsequent phenotypic screening of seeds derived from irradiated pollen

A total of 49 seedlings derived from irradiated pollen continue to grow in the greenhouse. This set of plants have been through two sets of rapid cycling. The trees demonstrate a large degree of variation is size and growth characteristics. The plants were phenotyped for height and number of nodes and the ratio between the two parameters was calculated. It is interesting to note that only two crosses yielded a ratio greater than 1. However there were several seedlings where the ratio was closer to 1. Please refer to Table 1. Figure 2 shows a few of the representative seedlings. Note the diversity in growth habit.

Table 1: Ratio of number of nodes/height for seedlings derived from irradiated pollen.

‘Bartlett’ × ‘Bartlett’(irradiated)			
ID	Height(cm)	mber of no	Ratio # nodes/ height
13-6	**71**	**70**	**0.99**
13-4	68	58	0.85
13-1	69	53	0.77
13-2	113	83	0.73
13-5	86	60	0.70
13-7	62	43	0.69
13-3	107	74	0.69

‘Bartlett’ × ‘Comice’ (irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-4	**30**	**42**	**1.40**
13-1	56	42	0.75
13-3	95	71	0.75
13-2	100	67	0.67

‘Comice’ × ‘Comice’(irradiated)			
ID	Height(cm)	Number of nodes	Ratio # nodes/ height
13-5	**61**	**60**	**0.98**
13-6	68	61	0.90
13-1	78	60	0.77
13-7	74	52	0.70
13-2	112	77	0.69
13-4	65	43	0.66

‘Abate Fetel’ × ‘Comice’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-1	88	76	0.86
13-2	83	69	0.83
13-4	81	59	0.73
13-1	81	57	0.70
13-3	96	60	0.63

‘Bartlett’ × ‘d’Anjou’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-4	**41**	**45**	**1.10**
13-1	87	80	0.92
13-8	81	73	0.90
13-9	111	97	0.87
13-6	71	62	0.87
13-7	66	57	0.86
13-3	99	68	0.69
13-2	153	103	0.67
13-5	77	51	0.66

‘Bartlett’ × ‘Abate Fetel’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-2	92	79	0.86
13-3	77	66	0.86
13-9	62	53	0.85
13-14	84	70	0.83
13-8	61	50	0.82
13-11	76	61	0.80
13-5	96	75	0.78
13-13	80	62	0.78
13-6	90	68	0.76
13-15	141	105	0.74
13-10	129	89	0.69
13-4	82	56	0.68
13-1	97	65	0.67
13-16	87	58	0.67
13-7	88	58	0.66
13-17	127	70	0.55
13-12	116	61	0.53

‘Comice’ × ‘d’Anjou’(irradiated)			
ID	Height(cm)	Number of nodes	Ratio # nodes/ height
13-1	55	37	0.67

Figure 2: Representative seedlings derived using gamma irradiated pollen demonstrating a wide variation in growth habit.

OUTREACH

- Good Fruit Grower article focused on the pear rootstock breeding program was published in September.
- Amit Dhingra hosted the Washington AgForestry leadership group at WSU Pullman; pear rootstock breeding was discussed during a visit to the greenhouses to look at the germplasm.
- Amit Dhingra hosted Doug Hemly (CA pear grower); advances in pear rootstocks was the primary discussion point.
- Kate Evans presented the outline of the breeding program at the Washington State Tree Fruit Association meeting in Yakima December, 2015 in a talk entitled 'Developing and implementing new technologies for and from the WSU pome fruit breeding program'.
- Amit Dhingra presented efforts on developing material for pear rootstocks at the Washington State Tree Fruit Association meeting in Yakima December, 2015 in a talk titled, "Smart Plants".

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

Project Title: Evaluation of potential, new pear cultivars for the PNW

PI: Todd Einhorn
Organization: OSU-MCAREC
Telephone: (541) 386-2030 x 216
Email: **todd.einhorn@oregonstate.edu Email:**
Address: 3005 Experiment Station Drive
City: Hood River
State/Zip: OR 97031

Co-PI (1): Tom Auvil
Organization: WTFRC
Telephone: (509) 665-8271
Auvil@treefruitresearch.com
Address: 1719 Springwater Ave
City: Wenatchee
State/Zip: WA 98801

Co-PI (2): Richard Bell
Organization: USDA-ARS
Telephone/email: 304 725 3451 x 353/**Richard.Bell@ars.usda.gov**
Address: 2217 Wiltshire Road
City: Kearneysville
State/Zip: WV 25430

Budget: **Year 1:** $12,578 **Year 2: $17,334** **Year 3:** $19,415
Cooperators: Kate Evans

Other funding sources: None

Budget 1: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Russell Karow
Telephone: 541 737-4866 **Email address:** Russell.Karow@oregonstate.edu

Item	2015	2016	2017
Salaries[1]	2,291	4,720	4,862
Benefits	1,535	3,162	3,257
Wages[2]	0	0	1,040
Benefits	0	0	104
Supplies[3]	500	500	500
Miscellaneous[4]	1,552	1,552	1,552
Total	5,878	**9,934**	11,315

Footnotes: [1]Salaries are calculated as 5% of technician time (2.5 weeks) in year 1 and 10% of technician time in years 2 and 3 (5 weeks). The increase in salary in year 2 reflects a 3% rate increase. Benefits are calculated using OPE rate of 66%. [2]Wages are for part-time employee help harvesting fruit and general maintenance during the season; 80 hours at $13/hr. Part-time employee benefits are calculated at 10%. [3]Supplies are for tree training. [4]Miscellaneous costs account for MCAREC plot fees at a rate of $3,103/acre, prorated to 1/2 acre for field on-site field trials.

Budget 2: Tom Auvil
Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
Telephone: 509-665-8271 **Email address:** Kathy@treefruitresearch.com

Item	2015	2016	2017
Salaries	3,000	3,500	4,000
Benefits	1,200	1,400	1,600
Supplies	1,000	1,000	1,000
Travel[1]	500	500	500
Miscellaneous[2]	1,000	1,000	1,000
Total	6,700	7,400	8,100

Footnotes: [1]Ten trips to Wapato/Dryden from mid-August through mid-Oct. [2]RCA cold storage room charges.

Objectives:

1. To test five new scion selections from the USDA-ARS pear breeding program in small-scale plantings in WA and OR.

2. To test two new pear cultivars from Prevar, Australia, in medium-scale plantings in WA and OR.

Significant Findings:

Objective 1

- 2015 was the first cropping year for third-leaf USDA-ARS scions. All scions fruited at both locations (WA and OR) but not all scions had a sufficient number of fruit to evaluate.
- Scion 84907-166 flowered profusely and produced attractive, blushed fruit. Yields were similar to Bartlett.
- Fruit size was on the slightly smaller end of the commercial scale (100s and 90s) in Hood River, but did not differ from Bartlett, with the exception of scion 078 which was small. In WA, all cultivars (including Bartlett) had relatively small fruit size. Given the precocity of scion 166, hand thinning was required to achieve balanced crop loads.

Objective 2

- Tree growth in Hood River was strong in 2015 (second year) despite poor growth in year 1 due to weak trees at planting.
- Tree growth at both WA sites was weak but expected to recover with increased management oversight in 2016.
- Minimal fruiting is expected in 2016.

Plant material:

1. USDA-ARS cultivars. Five European pear scion selections from USDA-ARS were established in 2013 at two sites in Washington (Wapato, Chuck Peters; and, Wenatchee, Josh Koempel) and one site in Oregon (Hood River, MCAREC) via a 3-year project entitled, 'Pear scion trials in the Pacific Northwest' (see Evans et al. 2015 Final Report). At all sites, 5 single-tree replicates were randomized in high-density, modern training systems with 'd'Anjou', 'Bartlett', and 'Bosc' trees as controls. At Wenatchee, trees were planted 3 ft. in-row x 12 ft. between rows (1,210 trees per acre) without a trellis. Trees will be positioned ~70° from the vertical in year 4. At Wapato, trees were spaced 4 ft. in-row x 12 ft. between rows (908 trees per acre); each tree was tipped opposite its neighbor in a narrow V trellis. At MCAREC, spacing is 5 ft. in-row x 12 ft. between rows (726 trees per acre) and trained to a V, similar to Wapato.

2. Australian (Prevar) cultivars. Two bi-colored, Australian cultivars were to be established in medium-scale plantings in WA and OR in 2014. 'Lanya' (ANP-0118) was planted at two Washington sites (Dryden, Josh Koempel; and, Wapato, Chuck Peters) and at one site in Oregon (Hood River, MCAREC). Each site had a minimum of ~80 trees. At Dryden, trees were planted in a double-row design spaced 3 ft. x 12 ft. (1,210 trees per acre). At Wapato, trees are trained to a tall spindle and spaced 4 ft. x 12 ft. (908 trees per acre). In Hood River, trees were planted and trained identical to the USDA-ARS selections described above. The second cultivar, 'Deliza' (ANP-0131), however, was only established at MCAREC (40 trees) due to a shortage of nursery material.

Additional trees were budded and cultured by a nursery collaborator for 2016 delivery (funding provided from the previous grant).

Results:

1. USDA-ARS cultivars. Tree size was fairly similar to Bartlett and Anjou for all scions except 069, which appears to be a weak tree (~50% the size of Anjou). In Hood River, we observed a wide range of precocity among the four scions evaluated; 166 >> 038 = 069 > 078. All scions bloomed with Anjou, with the exception of 166 which bloomed with Bartlett. Fruit set was highest for 166, followed by Bartlett. A significant number of fruit were hand thinned for Bartlett and 166; all other scions had only a few fruit thinned if individual clusters were overset. Fruit maturity was monitored weekly via firmness measurements beginning with the last week of July based on preliminary data from Dr. Richard Bell. There wasn't enough fruit of 078 to evaluate. For the other three scions, we only pressured a couple of fruit each week in order to preserve as many fruit as possible for harvest and postharvest evaluations. On August 3, Bartlett entered the harvest window (~19 lbf); 166 was ~1.5 lbs firmness softer than Bartlett but markedly firmer than 038 or 069. We had enough fruit of 166 to evaluate fruit size and firmness over 3 harvest dates, but only a sufficient volume of fruit to store and ripen at the Aug 4 harvest date.

2015, 3rd leaf bloom, fruit set, harvest data, and tree size of 4 USDA-ARS advanced selections in Hood River, OR compared to commercial standards.

Cultivar	Full Bloom (date)	Flower clusters (no. per tree)	Fruit set (fruit per cluster)	Fruit after thinning (no. tree)	Harvest (date)	Fruit weight (g)	Fruit shape (length:width)	Firmness (lbf)	Trunk cross-sectional area (cm^2)
69426-038	2-Apr	33	0.46	10.2	3-Aug	144.8	1.44	12.4	17.2
84907-069	1-Apr	35	0.11	4.6	3-Aug	226.7	1.29	14.4	11.2
84907-078	2-Apr	8	0.55	n.d.	n.d.	n.d.	n.d.	n.d.	17.1
84907-166	5-Apr	91	1.43	35	4-Aug	192.3	1.23	17.2	15.5
					19-Aug	249.2	1.18	16.4	
					29-Aug	249.7	1.21	15.3	
Anjou	1-Apr	5	0.1	0.8	n.d.	n.d.	n.d.	n.d.	23.1
Bartlett	5-Apr	122	0.83	40.2	3-Aug	222.4	1.29	18.9	20
Bosc*	n.d.	0	n.d.	0	n.d.	n.d.	n.d.	n.d.	2.8

n.d., no data

* Bosc trees were planted from small containers in 2015

In WA, fruit size was small for all scions, including Bartlett. While it is difficult to estimate maturity for new scion cultivars, fruit size was likely not due to pre-mature harvests since Bartlett firmness was below the lower threshold of the commercial range (19 lbf to 17 lbf) when harvested (i.e., 16.2 lbf). Titratable acidity was quite low for 038 and 078. This could have been due to high temperatures or, perhaps, they are low-acid types.

2015, 3rd leaf harvest fruit weight and quality of 5 USDA-ARS advanced selections planted in WA and compared to commercial standards.

Cultivar	Harvest (date)	Fruit weight (g)	Firmness (lbf)	SSC (%)	TA (%)
69426-038	21-Aug	127	10.2	12.4	0.16
84907-069	21-Aug	182	11.8	12.6	0.24
84907-078	21-Aug	118	14	12.7	0.14
84907-166	21-Aug	138	14.3	11.3	0.33
71655-014	21-Aug	169	10.9	14.7	0.31
Bartlett	21-Aug	186	16.2	12.4	0.35

2015, 3rd leaf post-harvest fruit quality of 4 USDA-ARS advanced selections in Hood River, OR compared to commercial standards following 3 months cold storage (31°F, >95% RH, Regualr Air storage).

Cultivar	Firmness (lbf)	SSC (%)	TA (%)	Harvest (date)
69426-038	3.1	13.1	0.19	3-Aug
84907-069	2.7	13.5	0.28	3-Aug
84907-078	n.d.	n.d.	n.d.	n.d.
84907-166	3.4	12.6	0.34	4-Aug
Anjou	n.d.	n.d.	n.d.	n.d.
Bartlett	1.9	11.7	0.45	3-Aug
Bosc*	n.d.	n.d.	n.d.	n.d.

SSC, soluble solids concentration

TA, titratable acidity

Given that all scions are considered summer pears (R. Bell, personal communication), we expected that they would ripen to a soft juicy texture after several months of cold storage (i.e., below 4 lbs flesh pressure). In Hood River, most of the selections attained acceptable SSC (range of 12% to 13%) but none would be considered to have high sugar content. Titratable acidity was fairly low for all scions, especially for 038, as similarly observed in WA.

An informal evaluation of flavor was performed after ripening. In Hood River, fruits of all scions were generally considered acceptable and possessed a relatively similar flavor profile as Bartlett. WA evaluated fruit using a 3-point scale where a value of 1 represented good flavor, a 2 represented no flavor, and a 3 represented off-flavor. Generally, fruit ranked similar to Bartlett, with the exception of 166. In 2016, all fruit wedges following ripening not used for analytical analyses will be consumed and subjectively rated for taste attributes using a sensory evaluation form provided by the Oregon State University Food Innovation Center. We did not pursue this in 2015 given the limited amount of fruit to evaluate. Additionally, industry tastings will be scheduled in each of the three production regions (Hood River, OR, Yakima, WA and Wenatchee, WA) once a sufficient volume of fruit is produced (likely in 2017).

2015, 3rd leaf post-harvest fruit quality of 5 USDA-ARS advanced selections planted in WA and compared to commercial standards following ~1.5 months cold storage (31°F, >95% RH, Regualr Air storage) plus a ripening treatment at room temp.

Cultivar	Firmness (lbf)	SSC (%)	TA (%)	Flavor (1-3 scale)*
69426-038	2.1	11.9	0.16	1.6
84907-069	2.9	11.5	0.24	2
84907-078	1.9	9.4	0.14	2.2
84907-166	3.7	10.3	0.33	2.5
71655-014	2.1	13.9	0.31	1.5
Bartlett	2	11.1	0.34	1.3

SSC, soluble solids concentration

TA, titratable acidity

* flavor was subjectively scored as Good-Flavor, 1; No-Flavor, 2; and, Off-Flavor, 3

Proposed 2016 and 2017 activities. Once fruit enter the harvest window, a 25-fruit sample per rep will be harvested weekly for each scion for three to four weeks. Five fruits will be tested immediately at harvest (individual fruits will be weighed and pressure tested [FF, 2 punches on opposite sides of the fruit], and a composite juice sample per rep will be tested for soluble solids concentration (SSC) and titratable acidity (TA)). The other 20 fruits will be placed into RA storage. Ten fruits will be removed at 30 d RA and ripened for 7 d at 68-70°F then assessed for quality as described above. The

remaining 10 fruits will be removed from RA at 60 d, ripened, and assessed. This information will provide a general understanding of the necessary chill required to develop ripening competency (< 4 lbf after 7 d ripening), as a function of the harvest pressure.

Long-term storage will be accomplished in the third year of the project when sufficient fruit are available and, ideally, we have narrowed the harvest timing to a minimum of two picks. For long-term storage, fruit will be stored in RA and evaluated at monthly intervals (depending upon the volume of fruit) for up to 6 months. Superficial scald will be evaluated using previously published 4-point scale (Y. Wang).

2. Australian (Prevar) cultivars.

We propose to continue with training and development of these plantings and to monitor early production. For WA sites, we expect to receive the remaining 'Deliza' trees spring of 2016 from the nursery. Trees were exceptionally small when planted in 2014. Despite limited growth the year of planting (at all sites), good growth was observed in 2015 in Hood River, OR. In WA, a change in management of one site and a shift to organic production at the other site both reduced annual tree growth in 2015. No mortality was reported and we expect that trees will be managed appropriately to optimize growth in 2016.

Bloom density and fruit set will be monitored and all harvested fruit will be evaluated as described above. Fewer harvest dates will be necessary to determine physiological maturity of these cultivars because published information already exists; however, we still intend to pursue multiple picks during 2016 and 2017 to identify optimal harvest timings under PNW conditions. Postharvest storability and fruit quality will be evaluated as described above for the USDA-ARS selections. Because 'Lanya' is considered crisp and ready-to-eat, we will evaluate fruits of this cultivar immediately following harvest and monthly when removed from RA (prior to and after ripening).

Fire blight incidence will be monitored in the plantings and all strikes recorded. Depending on the availability of resources and time, controlled *Erwinia* inoculations will be performed.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3 (No cost extension)
WTFRC Project Number: PR-14-111

Project Title: Development of marker-based breeding technologies for pear improvement

PI: David Neale
Organization: UC Davis
Telephone: 530-754-8431
Email: dbneale@ucdavis.edu
Address: One Shields Avenue
Address 2: 262 Robbins Hall
City/State/Zip: Davis, CA 95616

Co-PI (2): Amit Dhingra
Organization: Washington State University
Telephone: 509-335-3625
Email: adhingra@wsu.edu
Address: Dept. of Horticulture
Address 2: 155 Johnson Hall
City/State/Zip: Pullman, WA 99164

Co-PI (3): Sara Montanari
Organization: UC Davis
Telephone: -
Email: smontanari@ucdavis.edu
Address: One Shields Avenue
Address 2: 262 Robbins Hall
City/State/Zip: Davis, CA 95616

Cooperators: Richard Bell (USDA/ARS Kearneysville, WV), Joseph Postman and Nahla Bassil (USDA/ARS Corvallis, OR), Kate Evans (Washington State University), Sara Montanari (UC Davis), Rachel Elkins (UC Cooperative Extension)

Total Project Request: **Year 1:** $50,000 **Year 2: $50,000** **Year 3:** $0

Other funding sources: None

Budget
Organization Name: Regents of the University of California
Contract Administrator: Kevin Waterson
Telephone: 530-752-1895 **Email address: kbwaterson@ucdavis.edu**

Item	2015-2016	Balance (Jan. 21, 2016)
Salaries		
Benefits		
Wages		
Benefits		
Equipment		
Supplies	$50,000	$50,000
Travel		
Plot Fees		
Miscellaneous		
Total	$50,000	**$50,000**

Footnotes: The supplies funding will be spent on design of the SNP array and the genotyping of the ~2000 samples over the coming calendar year.

OBJECTIVES

Pear production can be increased by developing new varieties with improved agronomic characteristics, such as disease/insect resistances and dwarfing stature, which can be combined with high fruit quality and many other traits. In traditional breeding the selection of such elite cultivars is based on visual evaluation of the phenotype, and in woody perennial crops, including pear, this process is time consuming and expensive, because of the trees' long juvenile phase, laborious trait assessment, and large land requirement. Marker-assisted selection (MAS) technologies are currently routinely and successfully applied for several plant crops, and they can potentially increase pear breeding efficacy. In this project, we aimed at developing a high number of molecular markers (single nucleotide polymorphisms or SNPs) to be used to screen the entire germplasm collection held at the USDA Clonal Germplasm Repository in Corvallis, OR. The large set of genotypic data produced will be useful to find marker-trait associations to be applied for MAS in pear.

Activities:

1. Design a re-sequencing project and a SNP genotyping assay (accomplished).
2. Collect leaf samples from *Pyrus* spp. accessions from the National Clonal Germplasm Repository (NGCR) in Corvallis, OR (accomplished).
3. Conduct bioinformatics analysis of the re-sequencing data and design a SNP array (in progress).
4. Genotype all the collected samples.
5. Submit the re-sequencing and genotypic data to the Genome Database of Rosaceae (https://www.rosaceae.org/).

SIGNIFICANT FINDINGS

1. We collected leaf samples from ~2000 *Pyrus* spp. accessions from the National Clonal Germplasm Repository (NGCR) in Corvallis, OR.
2. We selected 55 accessions to represent the SNP discovery panel and we extracted high quality DNA from them.
3. We processed the 55 selected accessions for whole-genome, low-coverage sequencing (re-sequencing).
4. We performed bioinformatics analysis of the re-sequencing data and SNP calling.

METHODS

Design a re-sequencing project and a SNP genotyping assay for pear

Researchers working on pear breeding and genomics in the U.S., their extension collaborators, and the pear marketing boards created the Pear Genomics Research Network (PGRN), with the aim of bringing together their efforts for the enhancement of the pear-growing industry in the U.S. Within this collaboration, we started a re-sequencing project for the evaluation of *Pyrus* genetic diversity. We selected 55 pear accessions, representing founding cultivars and a total of 29 species and hybrids, within the NGCR in Corvallis, OR, and the Appalachian Fruit Research Station (AFRS) in Kearneysville, WV, to constitute the polymorphism discovery panel in this project (Table 1). These accessions were processed for whole-genome, low-coverage sequencing.

Sample collections and DNA extraction

During the summer 2014 we collected leaves from 1870 different *Pyrus* spp. cultivars and hybrids maintained at NGCR and AFRS. For the 55 samples included in the discovery panel, we extracted DNA from freeze-dried leaves using the DNeasy Plant Mini Kit (Qiagen®). For each sample, paired-end libraries were constructed using the Nextera DNA Sample Preparation kit (Illumina®) at the UC

Davis Dept. of Evolution and Ecology. Libraries were sent to the Institute for Genomic Medicine at UC San Diego for sequencing on an Illumina® HiSeq2500 in high output mode with v4 chemistry and 2x100 bps runs.

The remaining collected leaf samples were lyophilized for long-term preservation in the Neale Lab at UC Davis.

Bioinformatics analyses of re-sequencing data
We verified the quality of the sequences with FastQC and we calculated the sequencing depth for each sample. We then aligned the sequences to the published *P. communis* 'Bartlett' v1.0 reference genome (Chagné et al., 2014) using the Burrows-Wheeler Alignment (BWA) software. Finally we used the software SAMtools to identify the polymorphic sites (variants) in each of the 55 samples and we reported all the discovered variants in a unique file (VCF file format). We are now processing the VCF file through a three-stage filtration pipeline (Fig. 1), in order to remove artifacts and guarantee a final set of high-quality SNPs. Afterwards, we will submit the filtered SNPs to Affymetrix® for the construction of a custom genotyping array, according to the Axiom myDesign™ protocol, which will include between 50k and 675k molecular markers (depending on the success of the filtration process).

SNP validation and genotyping of the whole NGCR collection
We will select a subset of ~200 samples to be genotyped with the newly developed Axiom array, with the objective of validating the chosen molecular markers and finally use them to genotype the entire set of 1870 samples.

RESULTS & DISCUSSION

The Pear Genomics Research Network
The University of California (UC) Davis, UC Cooperative Extension, the NGCR in Corvallis, OR, the AFRS in Kearneysville, WV, Washington State University (WSU) and Oregon State University (OSU), have teamed up under the new Pear Genomics Research Network (PGRN), which also involves the industry organizations California Pear Advisory Board (CPAB), Pear Pest Management Research Fund (PPMRF), Pear Bureau Northwest (USA Pears), and Washington Tree Fruit Research Commission (WTFRC). A website for the PGRN (http://ucanr.edu/sites/peargenomics/) was developed in March 2015, and since then there have been 3596 unique visits with 4593 page views.

Re-sequencing of the SNP discovery panel
The SNPs discovery panel included 55 accessions, of which 19 were *P. communis* and *P. communis* subspecies cultivars, 6 were samples from *P. communis* ancestors and close relatives, 8 were varieties from the most widely cultivated Asian species (*P.* x *bretschneideri*, *P. pyrifolia,* and *P. ussuriensis*), 14 were samples from wild East Asian species, and 8 were interspecific hybrids (Table 1). Most of these accessions are founders in the breeding programs at WSU and AFRS, as well as in pear breeding programs carried out in other countries. The wild species were included because of traits of particular interest to breeding programs.

We extracted high quality DNA from these 55 samples. Sequencing resulted in a total of 731.2 Million read pairs, with a per sample coverage of 3.3x to 5.4x. The quality of the sequences was high; hence no manipulation of the reads was necessary before the alignment to the reference genome.

Variants discovery and filtration pipeline
We discovered a total of 66,787,567 unique variants, including 62,176,050 SNPs and 4,611,517 insertions and deletions (indels). Variants passing the Stage 1 filtration will be used to evaluate the genetic diversity among the 55 accessions of the discovery panel. This is expected to give us

information about relatedness among the different re-sequenced species and about *Pyrus* domestication.
Afterwards, we will subject variants to Stage 2 and Affymetrix filters, which is expected to drastically reduce their number to a set of high-quality SNPs useful for large-scale genotyping.

Discussion

All partners in the PGRN will take advantage from this new collaboration, as they will share old and new data produced from their individual research projects, as well as their expertise and established resources. The highly-dense SNP array we want to produce for pear will represent a fundamental tool for the enhancement of MAS in this crop. Currently, a SNP array including about 1000 European pear SNPs is available (Montanari et al., 2013), and it has been proved useful for the construction of dense genetic maps and application in quantitative trait locus (QTL) mapping projects. However, sequencing technologies have progressed at a very fast pace in the last few years, and it is now possible to design arrays with a much greater number of SNPs at a relatively small cost. Such a high-throughput genotyping tool will enhance genome wide association studies, pedigree-based analysis, and MAS in pear.

The 55 accessions included in the discovery panel are extremely diverse. By detecting species-specific variants, we may be able to identify subgroups of closely related species, thus elucidating their ancestry and natural distribution area, which in some cases is poorly understood. More interestingly, we may identify genomic regions highlighting diversity between cultivated and wild pears; these regions could have been selected during domestication, and thus are associated with important agronomic features.

REFERENCES

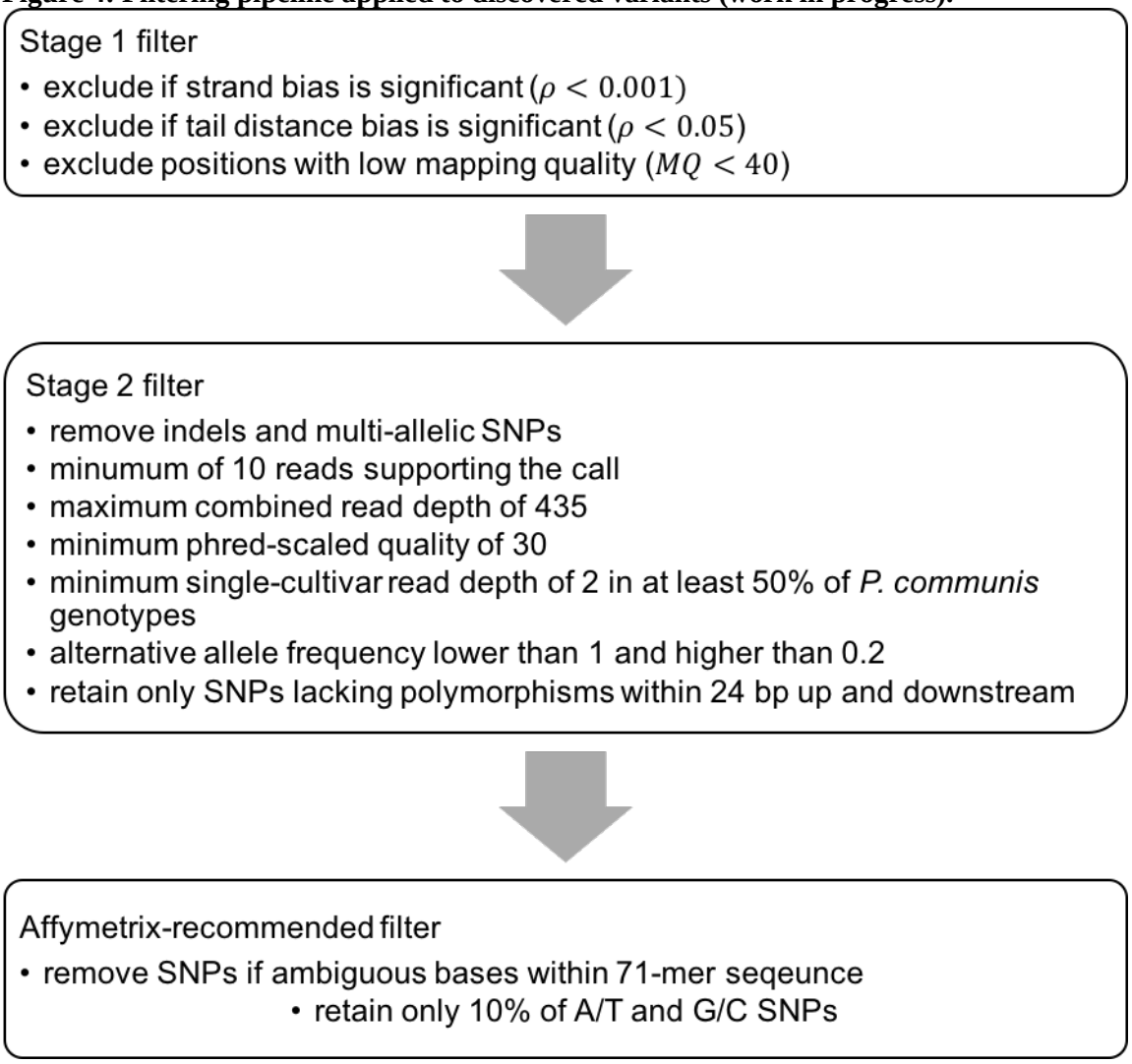
Chagné, D., Crowhurst, R. N., Pindo, M., Thrimawithana, A., Deng, C., Ireland, H., … Velasco, R. (2014). The draft genome sequence of European pear (Pyrus communis L. “Bartlett”). *PLOS ONE*, *9*(4), 1–12. http://doi.org/10.1371/journal.pone.0092644

Montanari, S., Saeed, M., Knäbel, M., Kim, Y., Troggio, M., Malnoy, M., … Chagné, D. (2013). Identification of Pyrus Single Nucleotide Polymorphisms (SNPs) and evaluation for genetic mapping in European pear and interspecific Pyrus hybrids. *PLOS ONE*, *8*(10), 1–11. http://doi.org/10.1371/journal.pone.0077022

Table 1: List of the pear cultivars and hybrids included in the polymorphism discovery panel.

List of re-sequenced accessions		
EUROPEAN SPECIES	**ASIAN SPECIES**	**INTERSPECIFIC HYBRIDS**
Pyrus communis	*Pyrus* × *bretschneideri*	(*Pyrus ussuriensis* x *P. pyrifolia*) x *P. communis* (probably)
'Anjou'	'Ta-Shian-Sui Li'	NJ487601193
'Bartlett'	'Xuehuali' (Snowflake)	NJA2R59T69
'Bosc'	'Ya li'	*Pyrus communis* x *P. ussuriensis*
'Coscia'	*Pyrus pyrifolia*	NJB9R1T117
'Gem'	'Dan bae' (Olympic)	NY 10262
'Gin'	'Nijisseiki'	NY 10353
'Harrow Delight'	'Zao su'	'Takisha'
'Harrow Sweet'	*Pyrus ussuriensis*	*Pyrus ussuriensis* x *P. pyrifolia*
'Old Home'	'Pai Li' (Beijing White Pear)	Illinois 76
'Para de Zahar de Bihor'	*P. ussuriensis* No. 2 (Korea)	
'Roi Charles de Würtemburg'	*Pyrus pashia* 'Naspati'	
'Seckel'	*Pyrus elaeagrifolia* MSU6768	
US 309	*Pyrus glabra*	
US76128-009	*Pyrus regelii*	
US82720-002	*Pyrus sachokiana* GE-2006-115	
Pyrus communis subsp. *pyraster*	*Pyrus salicifolia* GE-2004-141	
'Erabasma'	*Pyrus spinosa* (*amygdaliformis*)	
'Mednik'	*Pyrus syriaca*	
ALB-2011-024	*Pyrus betulifolia* 2291.002	
Pyrus communis subsp. *caucasica*	*Pyrus betulifolia* 2291.006	
Pyrus cordata pure	*Pyrus fauriei*	
Pyrus cordata (Turkey)	*Pyrus koehnei*	
Pyrus cossonii (Russia)	*Pyrus hondoensis*	
Pyrus gharbiana No. 1	*Pyrus pseudopashia*	
Pyrus mamorensis	*Pyrus* × *sinkiangensis* 'Ho mon'	
Pyrus nivalis		

Figure 4: Filtering pipeline applied to discovered variants (work in progress).



CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-15-105

Project Title: Pear rootstock breeding

PI:	Kate Evans	**Co-PI (2):**	Amit Dhingra
Organization:	Washington State University	**Organization**:	Washington State University
Telephone:	509 663 8181 x245	**Telephone**:	509 335 3625
Email:	kate_evans@wsu.edu	**Email**:	adhingra@wsu.edu
Address:	1100 N. Western Ave	**Address**:	P O Box 616414
City/State/Zip:	Wenatchee 98801	**City/State/Zip**:	Pullman WA 99164

Cooperators: David Neale (UC-Davis); Stefano Musacchi (WSU-TFREC); Richard Bell (USDA-ARS WV); Joseph Postman (USDA-ARS Corvallis).

Total Project Request: **Year 1:** $63,499 **Year 2: $112,138** **Year 3:** $97,616

Other funding sources

Agency Name: PNW Pear Bureau
Amt. awarded: $66,586 (2014-2017)
Notes: "Establishing NW-acclimated *Pyrus* rootstock breeding material" PI Dhingra, Co-PI Evans. Synergistic project to develop and establish pear rootstock seedlings.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

WTFRC Collaborative Expenses: None

Budget
Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509 335 4564/509 663 8181 **Email address:** carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2015	2016	2017
Salaries[1]	29,064	67,666	58,406
Benefits[1]	10,501	22,116	17,463
Wages[2]	5,760	5,990	6,230
Benefits[2]	1,094	3,786	3,937
Equipment & Supplies Pullman	6,500	6,500	6,500
Equipment & Supplies TFREC	6,000	2,500	1,500
Travel[3]	4,580	3,080	3,080
Plot Fees	0	500	500
Total	63,499	**112,138**	97,616

Footnotes:
[1]Salaries for Nathan Tarlyn (Research intern, Dhingra lab) and researcher to be appointed (Evans lab);
[2]Wages for time-slip labor for orchard management and trait phenotyping;
[3]In-state travel between collaborators and year 1 trip to Corvallis, OR for collection of propagating wood.

OBJECTIVES

1. Phenotyping USDA-ARS Corvallis accessions for dwarfing and rooting.
2. Phenotyping established seedling populations for dwarfing.
3. Establish the Pear Rootstock Breeding Program.

This proposal aims to build on recent (and concurrent) research to develop a long-term, dedicated pear rootstock breeding program at the Tree Fruit Research and Extension Center, Wenatchee. Germplasm currently growing in Pullman will be transferred to Wenatchee for establishment in the orchard and development of high quality phenotypic data essential to exploit the genomic data being generated in the Neale project (*PR-14-111*) and others. New germplasm will be produced using the traditional breeding method of crossing and selection. Parents for crossing within this 3-year proposal will focus on *Pyrus;* however, it is expected that should the breeding program continue, parents will also be sourced from other species, for example *Amelanchier* and Quince (*Cydonia oblongata*).

SIGNIFICANT FINDINGS

- Hardwood cuttings of 78 accessions from the Corvallis collection were collected for rooting potential tests
- Established seedling populations phenotyped for height and number of nodes. Propagation has begun of a subset with a range of habit types.
- Seedlings from irradiated pollen have been phenotyped for height and number of nodes.

METHODS

Objective 1: Phenotyping USDA-ARS Corvallis accessions for dwarfing and rooting.

a. Greenhouse phenotyping of rooting potential.

A diverse subset of accessions from the US pear germplasm repository (Corvallis, OR) has already been selected for genotypic analysis in the Neale project (*PR-14-111*). Hardwood cuttings of this set (plus commercial controls and as many other accessions as possible) will be collected straight after leaf fall of the germplasm to be tested. The absolute number of accessions tested will depend on the availability of sufficient propagating wood and on the size and number of wooden bins that we are able to obtain. Following removal of spines, the cuttings will be bundled into 50's and the ends cut flat and dipped into rooting hormone. Tops of the cuttings will also be sealed to stop dehydration. The bundles will be placed upside down in wooden bins lined with black plastic liners and filled with peat moss and maintained at temperatures around 15ºC (59F) until root callus starts to form (usually by the following January). Appearance of callus will be scored as an indication of rooting potential. Callused cuttings can be potted into soil-less media or stored at 4ºC (39F) until ready to plant. After 3 months of growth, plants will be uprooted, medium removed and extent of rooting and architecture documented.

Accessions that fail to produce roots as hardwood cuttings will be micropropagated to provide rooted shoots for (Objective 1b, below). Although typically in the breeding program these would be selected against, this germplasm may provide valuable parental alleles for size control of the scion. Although new micropropagation facilities are available at the TFREC (Musacchi lab), making use of the considerable expertise of the Dhingra lab with micropropagation of *Pyrus* should expedite this process.

b. Phenotyping of dwarfing potential.

Ten rooted cuttings from each of the accessions rooted in Objective 1a (above) will be budded with a standard scion variety (to be determined, but most likely d'Anjou) and grown in pots in the

greenhouse prior to planting in the field in a randomized block design. It is expected that this will be in two waves of planting, the accessions that root from hardwood cuttings would be the first wave followed by those that require micropropagation.

Trees will be grown in the field for the remainder of the project and shoot length and trunk diameter (and precocity if relevant) will be assessed as a measure of vigor. One problem that may be encountered is incompatibility of the scion to the rootstock. If this is the case, an alternative scion variety will be considered.

Depending on how fast we can determine a good dwarfing phenotype (which may be beyond the time frame of this project), we will also test the genomic loci previously reported to be involved in dwarfing (pear - *PcDw* locus [Wang et al., 2011]; apple - *Dw1* and *Dw2* loci [Celton et al., 2009, Rusholme Pilcher et al., 2008, Fazio et al., 2014]) to determine whether or not there is a good correlation in this germplasm. If well-correlated, these DNA-based tools will be a useful indication of dwarfing in new populations of seedlings. Should new DNA-based tools be developed from other projects within the timeframe of this project, we will also attempt to incorporate them where relevant.

Objective 2: Phenotyping established seedling populations for dwarfing.
Seedlings will be selected using the growth habit, precocity and floriferousness data generated in the Dhingra/Evans project and will be propagated *in vitro* and budded with a standard scion cultivar (most likely 'd'Anjou'). These seedlings are predominantly derived from the crosses 'Barlett' × 'd'Anjou' and 'Bartlett' × 'Comice' (reminder: the true parentage of OH×F 87 was recently identified as 'Old Home' × 'Bartlett'). The most dwarf individuals (short inter-noded) will form the bulk of those selected but some individuals from medium and high vigor groups will also be selected (up to a maximum of 50 individuals). Budded trees will be planted in the field; shoot length and trunk diameter (and precocity if relevant) will be assessed as a measure of vigor. Seedlings derived from the irradiated pollen that can be rescued in the Dhingra/Evans project will also feed into this phenotyping when available.

Objective 3: Establish the Rootstock Breeding Program.
A crossing program will be initiated to generate seedlings focused on the principal targets determined in the earlier PNW-funded project of size-controlling, precocity, good fruit size and finish, resistance to fire blight and pear decline, ease of propagation and winter hardiness.

Crosses will be made in year 1, fruit harvested and seeds collected in the fall. Those seeds will be vernalized and then germinated in the TFREC greenhouse in spring of year 2. Seedlings will be planted at close spacing in the orchard in Wenatchee (year 2) and budded with a popular scion cultivar (most likely 'd'Anjou') in year 3. Crosses will also be made in year 2 and year 3.

These seedlings would form the basis for an on-going, long-term breeding program. They will be grown using standard orchard practices and assessed annually (beyond the scope of this project) for vigor by measuring shoot length and trunk diameter. Bloom date and amount will be recorded annually to determine the precocity of the seedling rootstock. Fruit data recorded will include harvest date, yield, size, skin finish, firmness, titratable acidity and ^{o}Brix. Seedlings that are selected as dwarfing and precocious will be cut back to remove the scion and earthed up to promote the production of rooted suckers. This method has been successfully used by PI Evans in her previous rootstock breeding program at East Malling Research, UK.

RESULTS AND DISCUSSION

Objective 1a: Greenhouse phenotyping of rooting potential.

Hardwood cuttings of 78 accessions from the Corvallis collection were collected for rooting potential tests (Fig 1). These tests are on-going.

Figure 1: Hardwood cuttings rooting at WSU-Wenatchee

Objective 2: Phenotyping established seedling populations for dwarfing

One hundred and forty-nine potted trees derived from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' are currently in the fourth dormancy cycle being maintained in the greenhouse in Pullman. Node count and tree height were measured in May 2015 which was used as the basis for making preliminary plant selections for desirable plant habit (Figure 2). Considerable variation was seen between internode lengths of the seedlings.

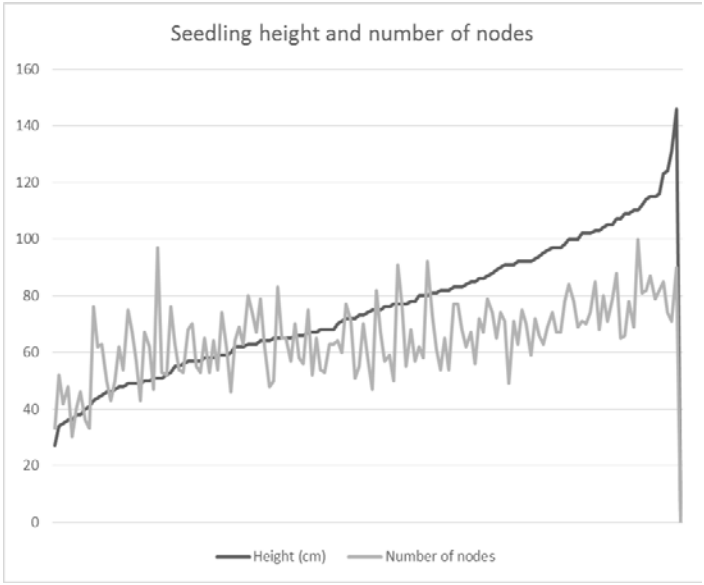


Figure 2: Seedling height (cm) vs number of nodes of F1 hybrids obtained from 'Bartlett' × 'd'Anjou' and 'Bartlett' × 'Comice' crosses.

In spring 2015, a subset of individuals was selected for propagation by conventional methods of rooted cuttings. Figure 3 shows some of these selections and their respective growth habit. The cuttings were rooted and transitioned into dormancy in August 2015. In December 2015, the cuttings

were transitioned back into the greenhouse to reinitiate plant growth. As of January 2016, the cuttings are either breaking bud or have newly opened buds with expanding leaves.

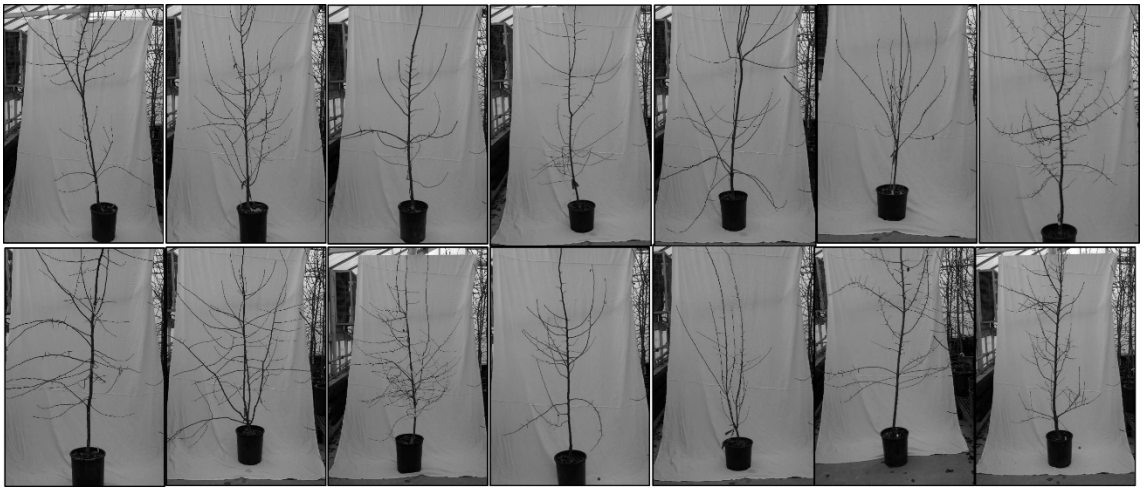


Figure 3: Growth habit of a subset of F1 hybrids selected for further propagation.

In addition to the regular crosses, 49 individuals were derived using gamma irradiated pollen (Figure 4). Measurements of node count and height were made in August 2015 and were highly variable between seedlings as shown in Table 1. These trees are currently transitioning between paradormancy (stage 1 dormancy) and endodormancy (stage 2 dormancy) in the cold.

Figure 4: Seedlings derived from irradiated pollen demonstrate a large variability in size and growth habit.

Table 1: Height (cm) and number of nodes of seedlings derived from irradiated pollen.

'Bartlett' × 'd'Anjou'(irradiated)

ID	Height(cm)	Number of nodes
13-4	41	45
13-7	66	57
13-6	71	62
13-5	77	51
13-8	81	73
13-1	87	80
13-3	99	68
13-9	111	97
13-2	153	103
Mean	*87.3*	*70.7*
S.D.	*31.7*	*19.8*

'Bartlett' × 'Bartlett'(irradiated)

ID	Height(cm)	Number of nodes
13-7	62	43
13-4	68	58
13-1	69	53
13-6	71	70
13-5	86	60
13-3	107	74
13-2	113	83
Mean	*82.3*	*63.0*
S.D.	*20.4*	*13.6*

'Comice' × 'Comice'(irradiated)

ID	Height(cm)	Number of nodes
13-5	61	60
13-4	65	43
13-6	68	61
13-7	74	52
13-1	78	60
13-2	112	77
Mean	*76.3*	*58.8*
S.D.	*18.5*	*11.3*

'Comice' × 'd'Anjou'(irradiated)

ID	Height(cm)	Number of nodes
13-1	55	37

'Abate Fetel' × 'Comice'(irradiated)

ID	Height(cm)	Number of nodes
13-1	81	57
13-4	81	59
13-2	83	69
13-1	88	76
13-3	96	60
Mean	*85.8*	*64.2*
S.D.	*6.4*	*8.0*

'Bartlett' × 'Comice'(irradiated)

ID	Height(cm)	Number of nodes
13-4	30	42
13-1	56	42
13-3	95	71
13-2	100	67
Mean	*70.3*	*55.5*
S.D.	*33.3*	*15.7*

'Bartlett' × 'Abate Fetel'(irradiated)

ID	Height(cm)	Number of nodes
13-8	61	50
13-9	62	53
13-11	76	61
13-3	77	66
13-13	80	62
13-4	82	56
13-14	84	70
13-16	87	58
13-7	88	58
13-6	90	68
13-2	92	79
13-5	96	75
13-1	97	65
13-12	116	61
13-17	127	70
13-10	129	89
13-15	141	105
Mean	*93.2*	*67.4*
S.D.	*22.8*	*13.7*

Objective 3: Establish the Rootstock Breeding Program.
Crosses will begin in spring 2016 (year 1 of this project).

Outreach
Good Fruit Grower article focused on the pear rootstock breeding program was published in September.
Amit Dhingra hosted the Washington AgForestry leadership group at WSU Pullman; pear rootstock breeding was discussed during a visit to the greenhouses to look at the germplasm.
Amit Dhingra hosted Doug Hemly (CA pear grower);advances in pear rootstocks was the primary discussion point.
Kate Evans presented the outline of the breeding program at the Washington State Horticultural Association Show, Yakima in a talk entitled 'Developing and implementing new technologies for and from the WSU pome fruit breeding program'.