Northwest Pear Research Review
February 12-13, 2009
Hood River Inn, Oregon

Thursday, February 12

Time	Page	PI	Project Title	Funding period
8:00		Schmitten	Welcome	
8:15		Moffitt	Pear bureau research overview	
8:30		Willett	NHC update	
			Final Reports	
8:45	1	Kupferman	New technologies to control storage scald **final report, early termination**	07-09
9:00	9	Horton	Effect of neem on biology and behavior of pear psylla	08
9:15	16	Dunley	Using degree-days for timing of pear psylla controls	07-08
9:30	17	Hanrahan	Collaborative WTFRC research projects	08
9:45	25	Turner	Field evaluation of new pear rootstocks	06-08
10:00	34	Smith	PNW pear rootstock trials	06-08
Group #		**PI**	**Continuing Reports: Poster Session 11:00 - 12:30**	
1	45	Dhingra	Gene discovery & controlled sport induction (CSI) for pear improvement	07-09
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2		McFerson	SCRI update	
3	81	Kupferman	Comparison of commercial Anjou ripening and conditioning methods **extension**	08
3	89	Mattheis	Factors influencing development of d'Anjou pear scald and speckling	07-09
3	94	Sugar	Pear fruit quality improvement	07-09

FINAL PROJECT REPORT

Project title: New technologies to control storage scald of Anjou pear

PI: Eugene Kupferman
Organization: WSU Tree Fruit Research and Extension Center
Telephone/email: 509-663-8181 x239; kupfer@wsu.edu
Address: 1100 N. Western Avenue
City: Wenatchee
State/Province/Zip: WA 98801

Co-PI: Peter Sanderson
Organization: Pace International
Telephone/email: 509-679-6680 PeterS@PaceInt.com
Address: 25 N. Wenatchee Ave.
City: Wenatchee
State/Province/Zip: WA 98801

Cooperators: Dr. Chang-Lin Xiao, WSU-TFREC; Michael Young, Stemilt Fruit; Bob Gix and Eric Strutzel, Blue Star Growers; Larry Blakeley, Blue Bird Growers.

Total project funding: **Year 1:** $38,633 **Year 2:** 38,060 **Year 3:** Terminated after 2 years

Other funding Sources

Agency Name: Pace International
Amount awarded: Residue analysis, funds for room cleaning and fruit disposal, chemicals and use of thermofogging equipment.

WTFRC Collaborative expenses:

Item	2008-2009	2009-2010
Stemilt RCA room rental	6,368	6,368
Crew labor	0	0
Shipping	0	0
Supplies	0	0
Travel	0	0
Miscellaneous	0	0
Total	6,368	6,368

Budget History

Item	Year 1: 2007	Year 2: 2008
Salaries	13,125	10,920
Benefits (47.3%)	6,208	5,165
Wages	9,000	7,600
Benefits (11.5%)	1,035	875
Supplies	21,643	12,000
Miscellaneous	0	1,000
Travel	500	500
Total	38,633	38,060

RECAP OF OBJECTIVES:

1. **Refine the methodology of thermofogging of the antioxidant ethoxyquin into pear storage rooms by varying the timing, concentration and fan cycling.**
   a. Determine the effect of time between harvest and ethoxyquin application.
   b. Determine the effect of multiple, lower concentration, applications on residue levels.
   c. Evaluate and improve ethoxyquin dispersion in storage room.
2. **Determine whether the phytotoxicity of ethoxyquin can be reduced by rinsing the fruit after it has been drenched ("split-drenching").**
3. **Correlate residue levels with incidence of storage scald.**

SIGNIFICANT FINDINGS—THERMOFOGGING:

a. Determine the effect of time between harvest and ethoxyquin application. Commercial quality fruit was treated 2, 11 and 15 days after harvest.
   - Residue levels were too low to determine if scald was related to application timing.
   - Scald incidence was inversely proportional to ethoxyquin residue level and was controlled at 1.0 ppm.
   - Delay in the initial application of ethoxyquin led to higher phytotoxicity.
   - Fruit on the top of the bins had more severe phytotoxicity than fruit lower in the bin.

b. Determine the effect of multiple, lower concentration applications on residue levels. Commercial quality fruit was treated twice at low concentrations, once 2 days after harvest and again 60 days after harvest.
   - The first application produced low residue levels (< 0.5 ppm). The residue levels after the second application were much higher (3.2 ppm to 5.6 ppm).
   - Despite the higher residue levels there was no phytotoxicity on fruit following long-term CA storage.
   - Residue levels after the initial application were too low to determine if scald was related to multiple applications.

c. Evaluate and improve ethoxyquin dispersion in storage room. Strategies to improve dispersal of ethoxyquin throughout the room included utilization of a free-flowing air manifold fitted with a fan to provide active and passive ventilation of the room to move ethoxyquin through the storage room and different types of bin covers.
   - Manifold: Both active and passive ventilation of the room via the manifold improved dispersion of ethoxyquin throughout the room, within the bins, and the stacks; however, uniformity of residue was not improved.
   - Bin position: Fruit in bins on the top of each stack had higher concentrations than those in bins within the stack.
   - Within bin: The top layer of fruit in each bin had a higher concentration of ethoxyquin than fruit in the middle or bottom of bin.
   - Bin covers: Covers were tested to reduce the concentration on fruit in the top bin. All types of bin covers tested reduced deposition of ethoxyquin on the fruit in the top bin of each stack.
     - In the uncovered bins, many residue levels exceeded the legal limit.
     - In bins covered with wooden palettes, residues exceeded goal levels and sometimes exceeded the legal limit.
     - Plastic sheeting used as bin covers reduced the chemical application to below target levels.

- Pulsing of the overhead coil fans did not improve uniformity of residue.
- Plastic bins: The use of plastic bins did not significantly improve the dispersion of ethoxyquin within the bin or between bins.

RESULTS AND DISCUSSION—THERMOFOGGING

The first goal was to fine tune the operating parameters of the thermofogger, so that the residue of ethoxyquin was appropriate and repeatable. Determination of the optimum method to utilize the thermofogger application machine is complicated since the ethoxyquin molecule is larger and heavier than that of DPA. Thus the ethoxyquin molecule falls out of the air more easily than smaller molecules (DPA) or gases (1-MCP). Pace is actively working to increase the efficiency and uniformity of thermofogging applications. Modifications in equipment and the set-up of the experimental chamber required numerous applications using cull pears before repeatability was accomplished. The results of the tests were settings of air temperature and velocity within the thermofogger that optimized particle size.

Dummy bins (bins wrapped in plastic to prevent through-flow) were used in 2007 to better mimic commercial treatments while minimizing the amount of fruit needed to complete these trials. In 2008, bins of cull fruit were used to fill each room to minimize costs while providing a realistic air flow pattern.

a. Determine the effect of time between harvest and ethoxyquin application. Ethoxyquin was applied to commercial quality fruit 2, 11 and 15 days after harvest. Residue levels and phytotoxicity at the top of each bin were higher than those in the middle or bottom (Table 1). Scald incidence was the lowest at the top of each bin. The target residue for this experiment was between 1 and 3 ppm; which was reached only in the top layer of fruit in the 15-day treatment. This fruit had the lowest incidence of scald (5%). Because most residue levels were too low to effectively control scald, the effect of timing cannot be determined.

   Bins that were fogged 15 days after harvest had unacceptably high levels of phytotoxicity, especially on the top layer of fruit (Table 1).

b. Determine the effect of multiple, lower concentration, applications on residue levels. Low concentrations of ethoxyquin were applied twice to commercial quality fruit: once 2 days after harvest, and again 60 days after harvest. The residues analysis after the application 60 days after harvest resulted in very high ethoxyquin residues compared with that obtained after the application 2 days after harvest (Table 2). Whether uneven residue was due to fruit temperature (45 ºF at 2 days, 32 ºF at 60 days) or room temperature (65 ºF at 2 days, 34 ºF at 60 days) at time of application, or other factors, is unknown. However, when the fruit was removed from long-term CA storage, the residues were below the effective limit.

   Scald was effectively controlled in the fruit at the top of the bin; however, the fruit in the middle of the bin had higher scald levels. This is likely due to the low level of ethoxyquin residue after the first application (0.2 ppm) in the middle of the bin.

   Multiple treatments of ethoxyquin fogging show promise as an effective way to control scald while minimizing phytotoxicity, providing sufficient residue remains on the fruit after the first treatment. From this work, it appears that the target ethoxyquin residue level for this first treatment should be ≥ 0.5 ppm. Additional treatments should increase the total residue to ≥ 1.0 ppm. Delaying the first treatment of pears with ethoxyquin until after they have been in storage does little to control scald and can result in high fruit damage.

c. Evaluate and improve ethoxyquin dispersion in storage room. Our experience showed that fruit at the top of the room retained the highest chemical concentration, at times in excess of maximum legal residue. One approach was to determine whether covering the topmost bin in the stack would help reduce the residue on fruit in those bins. Some bins remained uncovered, some were

covered with shade cloth stapled over the top of the bins, and some were covered with plastic sheeting stapled on pallets to elevate it above the fruit in the top bin, which would allow normal airflow across the top of the bin so as not to restrict cooling. Fruit in the covered bins had lower residue than fruit without covering (Table 3).

In an attempt to reduce this problem, a manifold was constructed from porous flexible plastic pipe and placed on the floor across the back wall and one side of the room then vented out the door. A fan was fitted to the end of the manifold pipe. When the fan was on, the manifold actively ventilated the room. When the fan was off, over-pressuring by the fogger caused the manifold to passively ventilate the room. Full bins of fruit were stacked three-high under the cloth covered bins to determine chemical dispersion throughout the room. Residue levels were higher when the manifold fan was active (Table 4). There was a greater difference in residues on fruit in the middle of the bin as compared with fruit at the top of the bin when the manifold fan was active (Table 5).

Additional treatments were applied to compare bin type, the effect of passive vs. active manifold, the use of the room coil fans, and different cover configurations on the top bin. The treatments are listed below.

Treat	Bin type	Manifold	Coil fan	Covers
1	Plastic	Passive	OFF	Plastic (tight) or wooden pallet
2	Wooden	Passive	OFF	Plastic (tight) or wooden pallet
3	Wooden	Active (fan on)	OFF	Plastic (loose) or wooden pallet
4	Wooden	Active (fan on)	Pulsed	Plastic (loose) or wooden pallet
5	Wooden	Passive	OFF	Plastic (loose) or wooden pallet

Based on residue results from earlier trials, the goal for residual ethoxyquin within each treated bin was 1.5 to 2.0 parts per million (ppm). The upper acceptable limit is 3 ppm. The residue samples taken from the top and middle of each treated bin (without bin covers) are reported in Table 6. The residues for the top-of-stack bins using either plastic sheeting stapled to the bin or elevated wooden pallet covers are shown in Table 7.

Statistical analysis was performed using ANOVA-GLM within SAS v. 9.1, to determine if there were significantly different levels of residue among treatment (1 to 5), bin sampling locations (top or middle) or top-bin cover material (plastic or pallet).

Analysis of this data leads to the following findings:

1. The use of plastic bins did not significantly improve the dispersion of chemical within the bin over wooden bins.
2. No treatment dispersed chemical sufficiently to meet goal residue levels within the bins. Although treatment averages show residues within the target range (1.5 to 2.0 ppm), in only 8% of cases did fruit from both the top and middle of the same bin meet the target level, due to uneven distribution within the bins (data not shown).
3. Plastic sheeting bin covers were effective in reducing high concentrations of residue in the top-of-stack bin but also reduced the chemical application to below effective levels in most cases.
4. Plastic sheeting tightly stapled to the tops of the topmost wooden bins restricted penetration of fog so that insufficient residues were achieved within those bins. This effect was not seen with plastic sheeting on the tops of plastic bins (Table 8).
5. Wooden palette covers reduced excessive deposition on fruit from the topmost bins, but residues exceeded target levels and sometimes exceeded permissible levels.

Table 1. Thermofog ethoxyquin residue levels at each application date, followed by percentage of fruit with scald and phytotoxicity (pink staining) after long-term CA storage.

Location in bin	Ethoxyquin residue, scald and phytotoxicity for each application								
	+2 days			+11 days			+15 days		
	Residue (ppm)	Scald (%)	Phyto (%)	Residue (ppm)	Scald (%)	Phyto (%)	Residue (ppm)	Scald (%)	Phyto (%)
Top	0.7	19%	0%	0.6	9%	7%	**1.0**	**5%**	21%
Middle	0.3	41%	0%	0.5	11%	0%	0.6	8%	9%
Bottom	0.3	42%	0%	0.5	14%	0%	0.6	10%	3%

Table 2. Thermofog residues for low concentrations of ethoxyquin applied 2 and 60 days after harvest, including residues after long-term CA storage and incidence of scald and phytotoxicity.

Location in bin	Ethoxyquin residue (ppm)			Scald (%)	Phyto (%)
	2 days	60 days	Following CA		
Top	0.4	5.6	2.6	1%	0%
Middle	0.2	3.2	2.0	10%	0%

Table 3. Effect of covers on top bins on deposition of ethoxyquin applied by thermofogging.

Cover material	Ethoxyquin (ppm)
None	3.8 a
Cloth	2.0 b
Plastic	2.5 b
	(P = 0.006)
Means separated using Tukey's HSD	

Table 4. Effect of moving air with a free-flowing manifold on deposition of ethoxyquin applied by thermofogging within bins of fruit.

	Ethoxyquin Residue (ppm)
Active manifold	2.2 a
Passive manifold	1.3 b
	(P = 0.021)
Means separated using Tukey's HSD	

Table 5. Effect of moving air with a free-flowing manifold on deposition of ethoxyquin applied by thermofogging within bins of fruit.

	Manifold	
Fruit location	Active	Passive
Top	4.6 a	2.1 b
Middle	2.5 b	1.7 b
	(P = 0.044)	
Means separated using Tukey's HSD		

Table 6. Residue levels for the top and middle of the thermofog treated bins (excluding top-of-stack).

Treat	Bin type	Manifold	Coil fan	Covers	Ethoxyquin residue (ppm)	
					Top of bin	Middle of bin
1	Plastic	Passive	OFF	Plastic (tight) or wooden pallet	2.1	2.1
2	Wooden	Passive	OFF	Plastic (tight) or wooden pallet	3.2	1.7
3	Wooden	Active	OFF	Plastic (loose) or wooden pallet	2.2	1.4
4	Wooden	Active	Pulsed	Plastic (loose) or wooden pallet	1.4	1.5
5	Wooden	Passive	OFF	Plastic (loose) or wooden pallet	2.1	1.7
				Average	***2.2 a***	***1.7 b***

Treatments, *P = 0.1537* (not significant)
Sample position in bin (top or middle), *P = 0.0092*

Table 7. Residue levels for the top and middle of the top-of-stack bins only, by cover type, thermofog trials.

Treat	Bin type	Manifold	Coil fan	Ethoxyquin residue (ppm)			
				Plastic cover		Wooden pallet cover	
				Top of bin	Middle of bin	Top of bin	Middle of bin
1	Plastic	Passive	OFF	0.9	2.0	3.7	2.3
2	Wooden	Passive	OFF	0.4	0.5	3.6	3.1
3	Wooden	Active	OFF	0.5	1.2	4.1	1.9
4	Wooden	Active	Pulsed	0.6	0.9	3.2	2.1
5	Wooden	Passive	OFF	1.1	1.0	5.4	3.3
			Average	***0.9 a***		***3.3 b***	

Treatments, *P = 0.1537* (not significant)
Sample position in bin (top or middle), *P = 0.0092*
Top bin cover type, *P = <0.001*

Table 8. Interaction of bin and cover types on ethoxyquin residue.

Bin type	Cover material	Ethoxyquin (ppm)
Plastic	Wooden pallet	3.0 a
Plastic	Plastic (tight)	1.5 b
Wood	Wooden pallet	3.3 a
Wood	Plastic (tight)	0.5 c
		P = 0.007

SIGNIFICANT FINDINGS—SPLIT DRENCHING

Liquid ethoxyquin was applied as a drench together with a fungicide (control), or as two applications in which the ethoxyquin was applied followed by a second drench (4 hours or 7, 21 or 42 days later) with the fungicide. Residue analysis indicated that there was no significant reduction in ethoxyquin following the second drenches applied over a 42-day period. Correlation of scald, burn and residue data for 2007 crop indicate:

1. Split drenching significantly reduced ethoxyquin burn especially when separated by 21 days or more.

2. Scald was reduced in proportion to the level of residual ethoxyquin with zero scald at levels of 1.0 ppm or greater.
3. Ethoxyquin residue levels were greater the longer the interval between initial and second drenches.

Split drenching was performed on the 2008 crop and residue samples were collected. The fruit will be evaluated for burn and scald in spring 2009. An additional split drench interval of 56 days was added to the 2007 protocol. To date, the ethoxyquin residue has not significantly degraded in the 56 days following initial treatment.

RESULTS AND DISCUSSION—SPLIT DRENCHING

Experiments in 2007 have shown that it is possible to obtain consistent and appropriate residue levels of ethoxyquin without phytotoxicity by drenching first with ethoxyquin and then with a fungicide. This led from the observation in previous years that burn developed over time when 'liquid' ethoxyquin residue remained on the fruit. When this 'liquid' residue was removed by washing or brushing the burn did not develop. Correlation of scald, burn and residue data indicate that split drenching significantly reduced ethoxyquin burn especially when separated by 21 days or more. Scald was reduced in proportion to the level of residual ethoxyquin with zero scald at levels of 1.0 ppm or greater and ethoxyquin residue levels were greater the longer the interval between initial and second drenches (Table 9). Residue levels at the time of drenching for the 2008 crop are shown in Table 10.

RESULTS AND DISCUSSION—CORRELATE RESIDUE LEVELS WITH SCALD

For Anjou pears treated with ethoxyquin via a drench solution (1350 ppm ethoxyquin applied within 2 days of harvest), there appears to be an inverse relationship between ethoxyquin residue (measured after final drench) and scald (Table 9). Fruit with the lowest residue level (0.6 ppm) had the highest incidence of scald (10%), and fruit with the highest residue level (1.0 ppm or higher) had the lowest incidence of scald (0%). Ethoxyquin residue levels were not measured after long-term storage for this drenched fruit.

The research in this project has relied upon analysis of ethoxyquin residue performed by the Pace International laboratory using proprietary methodology developed in that laboratory. Without this information and cooperation, the project would not be possible.

Table 9. Ethoxyquin drench phytotoxicity (burn), and scald after long-term CA storage, and ethoxyquin residue (analyzed after final drench), 2007 crop. For the split drenches, ethoxyquin (1350 ppm) was applied alone, then followed by a drench of TBZ at the time intervals stated.

Treatment	Burn (%)	Scald (%)	Ethoxyquin Residue (ppm)
Control (single drench)	96	5	0.7
Split drenches			
4 hours	30	10	0.6
7 days	22	1	0.8
21 days	3	0	1.1
42 days	5	0	1.0

Table 10. Ethoxyquin and TBZ residue levels at the time of drenching, drench trial 2008 crop. For the split drenches, ethoxyquin (1350 ppm) was applied alone and then followed by a drench of TBZ at the time intervals stated.

	Residues	
Treatment	**TBZ (ppm)**	**Ethoxyquin (ppm)***
Control (single drench)	2.6	1.0
Split drenches		
4 hours	1.2	0.6
7 days	1.6	NA
21 days	1.0	1.0
42 days	1.0	0.6
56 days	1.2	0.8

* Ethoxyquin residue immediately after TBZ application (2nd drench)
NA – Indicates missing data

FINAL PROJECT REPORT

Project Title: Effect of neem on biology and behavior of pear psylla

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

Total Project Funding: $15,000

Budget History (Fresh Pear/Processed Pear Committees):

Item	**Year 1:** (2008)
Salaries	$11,500
Benefits	$ 3,500
Supplies	
Total	$15,000

Restatement of Objectives:
Neem products are being used to manage pear psylla, with emphasis on controlling the summerform generations. These chemicals are thought to have any of several effects on pest psyllids, including growth regulator effects, deterrence, and direct mortality. However, good quantitative data establishing that these effects occur in a biologically meaningful way for pear psylla are limited. Objectives are to assess in laboratory assays the effects of neem on:

- egg and nymphal mortality
- egglaying preferences
- mating success, fecundity, and hatch of eggs deposited by treated females
- post-diapause development in winterforms

Significant Findings and Accomplishments

- Topical applications of Neemix 4.5 caused mortality of nymphs at the field rate and at twice the field rate; mortality rates fell between 20 and 40%.
- Storage of the mixed product led to reduced efficacy against nymphs.
- Topical applications had no effects on egg hatch.
- Topical treatment of virgin females or males did not affect subsequent mating, nor hatch of eggs deposited by mated females.
- Treated pear foliage received as many eggs as untreated foliage in preference tests.
- Treatment of diapausing winterforms with Neemix did not prompt ovarian development or mating.

Results and Discussion
All assays used Neemix 4.5 (Certis). Rate studies were done using the recommended concentration (10 oz/100 gallons water), as well as 2-times that rate (hereafter, 2x) and 0.5-times (0.5x) the recommended rate. Topical treatment of eggs and nymphs were done by misting approximately 0.5 ml of solution on 2-3 inch tall pear seedlings, infested with eggs or nymphs. Solutions were applied using a Nalgene hand-pump aerosol unit. Controls were misted with water.

- **Figure 1. Topical treatment of instar nymphs and eggs**. Early (I-II) instar nymphs, late (IV-V) instar nymphs, and eggs were misted with Neemix at one of three rates. Survival of nymphs and hatch of eggs were assessed at 1 and 2 weeks following treatment. ***Results***. Mortality of early instar nymphs approached 40% at the 2x rate and 20% at the recommended rate, at 2 weeks following treatment. Mortality in late instar nymphs was 20-30% in the 1x and 2x rates; little mortality was seen until the 2 week examination. Dead nymphs often had failed to molt correctly **(Figure 2)**, which is evidence of growth regulator effects.

  Effects of Neemix on egg hatch were modest at best **(Figure 1)**. These results appear to be consistent with studies on other insect species in failing to demonstrate effects on eggs.

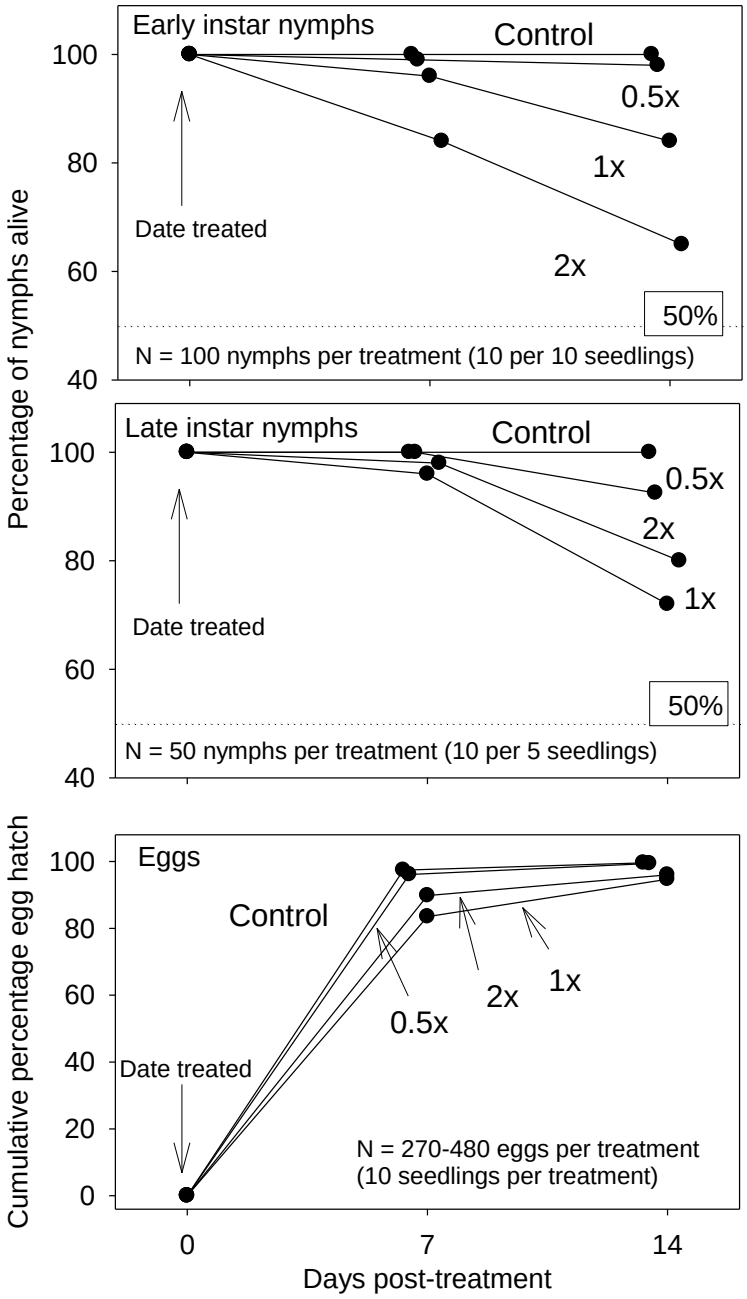


Figure 1. Effects of topical application on immature psylla.

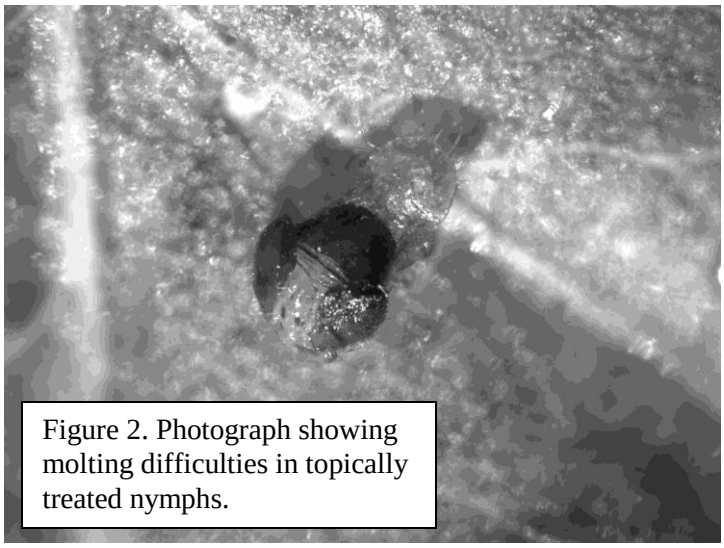


Figure 2. Photograph showing molting difficulties in topically treated nymphs.

- **Figure 3. Comparison of freshly mixed product with stored product**. Late instar nymphs were misted with Neemix at one of 3 rates. The solutions included freshly mixed product and a mixed solution that had been stored for 1 month at room temperature in a darkened cabinet. ***Results.*** Mortality approached 20% at the higher rates for the freshly mixed solution. Storage of the mixed solution for one month appears to have reduced its efficacy (consistent with warning on product label).

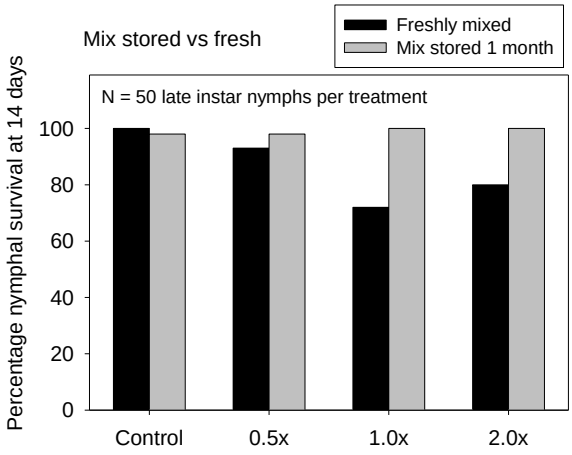


Figure 3. Freshly mixed vs mixed and stored product.

- **Figure 4. Topical treatment of adults and effects on mating and hatch of eggs.** Virgin summerforms were obtained from culture and treated either with Neemix (1x) or left untreated. Males and females were then combined in cages containing clean seedlings as one of 4 treatments: control females + control males; control females + treated males; treated females + control males; treated females + treated males. Females were then pulled from all containers at 4 and 7 days, and dissected to determine mating status. Additional females were moved to clean pear seedlings and allowed to oviposit; egg hatch was monitored. ***Results.*** By seven days, all females had been mated, indicating that treatment by neem did not affect either female attractiveness to males or male ability to inseminate females. Hatch rates of eggs deposited by treated or untreated females, mated with treated or untreated males, approached 90% in all mating combinations.

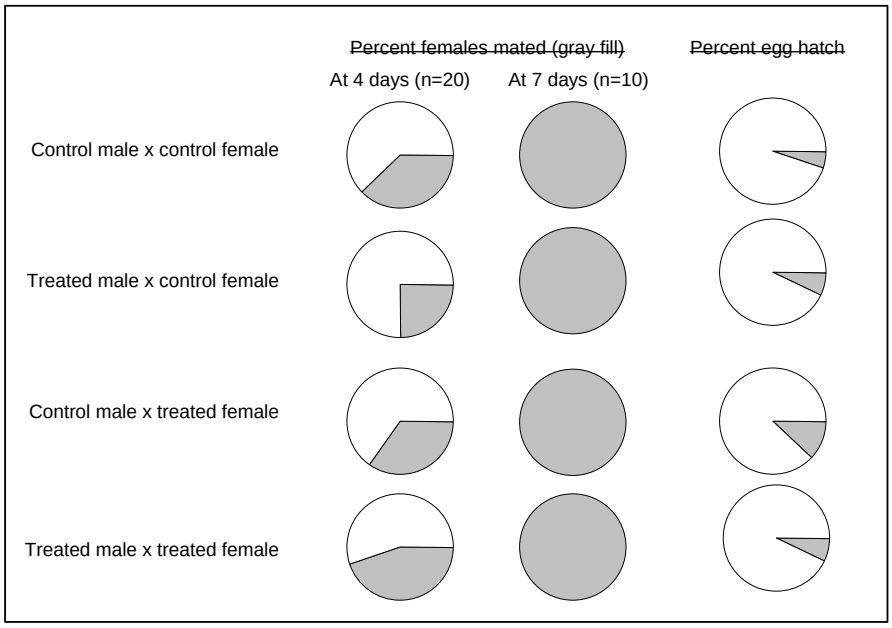


Figure 4. Effects on mating and hatch of eggs deposited by treated females.

- **Figure 5. Topical treatment of adults and effects on fecundity.** Six treated and six untreated females (from previous assay) were collected and set-up individually with males on untreated seedlings. The females were allowed to oviposit for 7 days. After 7 days, eggs were counted. ***Results.*** 7-day egglaying rates were identical by treated and untreated females.

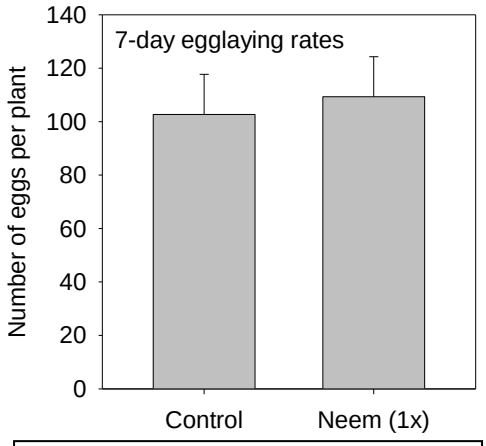
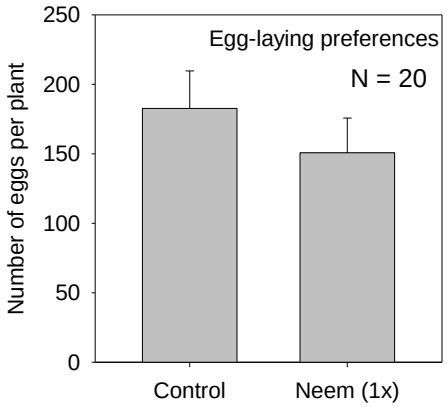


Figure 5. Effects on fecundity of topically treated females.

- **Figure 6. Egglaying preferences.** Paired clean and Neemix-treated (1x) pear seedlings were placed in small cages. Ten females were added to each cage and allowed to oviposit for 24 hours. At the end of 24 hours, numbers of eggs on each seedling were determined. ***Results.*** There was but a slight (statistically non-significant) preference for the control seedlings, indicating Neemix had no strong oviposition deterrence.

Figure 6. Effects on egglaying preferences.



- **Table 1. Post-diapause development.** Winterforms were collected from the field on 3 dates (Oct. 8, Oct. 28, Nov. 18). Half of the insects were moved to treated (1x Neemix) pear seedlings; the other half were left untreated. Psylla were left on the plants for 48 hours, and then moved onto clean seedlings (long-day conditions, 72^{o} F). Fifteen females were then removed from both treatments on each of 3 days following exposure to Neemix: 3 days, 6 days, and 9 days. The insects were dissected for ovarian scores (0=fully immature, 5=mature) and spermatophore numbers. ***Results***. Neemix had no effects on ovarian development or spermatophore numbers. Both control and treated insects remained in diapause through 6 days. I conclude that Neemix failed to prompt early termination of diapause.

TABLE 1. Effects of product on post-diapause development (ovary maturation and mating).

Date collected from field	Treatment	Ovarian scores				Spermatophore numbers		
		3 days	6 days	9 days		3 days	6 days	9 days
Oct 8	Control	0	0-1	0-1		0	0	0
	Neem	0-1	0-1	0-1		0	0	0
Oct 28	Control	0-1	0-1	0-1		0	0	0
	Neem	0-1	0-1	0-1		0	0	0
Nov 18	Control	0-1	0-1	0-3 (1.2)		0	0	0
	Neem	0-1	0-1	0-3 (1.3)		0	0	0

EXECUTIVE SUMMARY

Project Title: Effect of neem on biology and behavior of pear psylla

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

SUMMARY
Effects of Neem products on different life stages of pear psylla were assessed. The following table summarizes effects seen in these assays:

Eggs	No effects on hatch of topically treated eggs
Nymphs	20-40% mortality of topically treated nymphs after 14 days, apparently due to difficulties in molting by treated insects
Adults	1. Topical treatment of adults had no effects on mating success 2. Eggs deposited by topically treated females hatched successfully 3. Topical treatment of adults had no effects on fecundity 4. Treatment of foliage with Neemix did not deter egglaying 5. Neemix did not cause premature termination of diapause in winterforms

FINAL PROJECT REPORT

Project Title: Using degree-days for timing of pear psylla controls

PI: John Dunley
Organization: WSU-TFREC
Telephone: 509-663-8181
Email: dunleyj@wsu.edu
Address: 1400 N. Western
City: Wenatchee
State/Zip: Washington, 98801

No report submitted.

FINAL REPORT

Project Title: WTFRC internal pear projects

PI: Ines Hanrahan
Organization: WTFRC
Telephone/email: 1 509 669 0267
hanrahan@treefruitresearch.com
Address: 104 N 1st St., Suite 204
City: Yakima
State/Province/Zip WA, 98901

Cooperators: Jim McFerson, Tom Auvil, Felipe Castillo, Tory Schmidt, WTFRC, Wenatchee, WA; Jonathan Toye, Extenday, NZ

Budget:
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: 1 509 665 8271 **Email address:** Kathy@treefruitresearch.com

Item	Year 1: 2008
Salaries	21,176
Benefits (32%)	9,965
Wages	7,914
Benefits (32%)	3,724
Equipment + supplies	1,500
RCA rental	640
Travel	500
Reimbursements	2,000
Total	**43,419**

Salaries: include proportional time spent on pear projects for Hanrahan, Castillo, Schmidt, Auvil
Wages: covers timeslip expenses, based on fiscal year (July 2007-June 2008)
RCA rental: 10% of one room to hold maturity samples (current rate approx. $6,400/room/year)
Travel: fuel costs to travel to and from trial sites
Reimbursements: monetary contribution by Extenday ($1,000 per trial)
Other: all chemicals were donated by industry suppliers

Comment: initial amount approved at 2008 Pear Review was $59,515.

Special thanks to our cooperating growers: Steve Hull, Don Weippert, Paul Strutzel, Hansen Fruit, Don Gibson, Dave Olson, Geoff Thornton, Andrew Sundquist, Rudy Bossart, Ray Schmitten, Jack Anderson, and John Verbrugge.

OBJECTIVES:

1. Investigate the effects of chemical thinners on pear crop load and fruit quality.

2. Determine the effects of Daybright reflective groundcover on horticultural performance of pear orchards.

SIGNIFICANT FINDINGS:

Chemical thinning: The most consistent performers in Washington pear chemical thinning trials are ammonium thiosulfate (ATS) applied during bloom and benzyladenine (BA) applied postbloom. Increases in individual fruit weight are often observed after benzyladenine application, even without significant fruitlet thinning.

Reflective groundcovers: Daybright reflective groundcovers improve yields in Bartlett by increasing fruit set and/or size.

METHODS

Chemical thinning: Within a set of 6 trials in 2008 we evaluated ammonium thiosulfate (ATS), and urea as bloom thinners, as well as BA (Exilis Plus, MaxCel, 6-BA) and NAA as postbloom thinners. ATS (4%) and urea (5%) were applied at 20 and 80% bloom (only 80% at grower-applied sites, both timings at PropTec sites), BA (1%) and NAA (3.6oz/100gal) at 10 mm fruitlet size. All experiments employed randomized complete block designs with 4 replicates. Two of last years trials were small plot trials sprayed with a Proptec tower sprayer operated by WTFRC staff. The remaining four trials were applied by grower-cooperators with their own commercial spray rigs, typically airblast sprayers.

Initial bloom counts were taken prior to treatment and compared to actual fruit set counts taken after June drop. From these data, we calculated the number of fruit set per 100 blossom clusters. We also recorded the quantity of fruit set in each cluster initially counted, allowing us to calculate how many clusters were blanked, thinned to single fruits, two fruits, etc. Return bloom counts of the same experimental units are recorded in the spring following treatment. Standard fruit quality parameters are assessed at commercial harvest, including: fruit size, soluble solids content, titratable acidity and firmness. Sampled fruit was visually graded for defects including: sunburn and russet.

Reflective groundcovers: Trials were conducted in two locations over three seasons (2006 - 2008). The first site (Sunnyside) was a mature Bartlett/seedling block with 4 x 13 ft. spacing, and trained to a v-trellis structure. The second site (Cashmere) was a young 'Bartlett' block, on an OH x F 87 rootstock, with 7 x 14 ft. spacing, and trained to a central leader structure. Daybright was applied from early bloom until harvest in both experiments. The general layout consisted of variable length strips of reflective ground cover applied in four or six plots across several orchard rows alternating with untreated control plots of approximately equal dimensions. Daybright reflective groundcover was placed in orchard alleyways and attached to the tree trunks with elastic bands (ca. 4 inches above ground), covering approximately 80% of intra-row space.

All samples were taken from trees in the middle row. For each experiment, yields and fruit maturity were determined from several individual trees per plot at harvest (4 in Cashmere, 8 in Sunnyside); the Cashmere block was strip-picked at harvest, while the Sunnyside block was picked twice, once for fresh market, and again for cannery pears. Fresh market pears were chosen based on fruit size (minimum 2.55 inches diameter). The remaining pears (cannery) were harvested immediately following the first pick. Fruit maturity parameters were assessed from 10 fruit per tree for each pick, including: fruit weight, firmness, starch, titratable acidity, soluble solids concentration, russet incidence, degree of sunburn.

RESULTS AND DISCUSSION

Chemical thinning: The Mt. Adams, Buena and Tonasket trials were sprayed by grower-cooperators, while those at Sawyer and Cashmere were applied with the WTFRC Proptec tower sprayer (Table 1).

Table 1. Crop load and fruit quality effects of WTFRC pear thinning trials. 2008.

Treatment	Fruitlets/ LCSA cm^2	Fruitlets/ 100 blsm clusters	Fruit diameter (in)	Fruit weight (g)	Box size	Sugars (% brix)	Acids (% m. acid)	Firmness (lbs)
Bartlett / Seedling – Sawyer								
ATS	2.3 ns	52 ns	2.6 ns	200 b	100	10.2 b	0.316 a	17.6 a
6-BA	1.4	48	2.7	212 a	94	10.2 b	0.270 c	16.7 b
NAA	1.8	64	2.6	198 b	101	10.7 ab	0.280 bc	17.1 b
Urea	2.1	49	2.6	203 ab	98	11.4 a	0.307 ab	17.7 a
Control	2.2	61	2.6	202 ab	99	10.6 ab	0.279 bc	17.0 b
Bartlett / OHxF.87 – Cashmere								
ATS	2.3 ab	34 a	2.7 b	211 b	95	13.1 ns	0.354 ns	18.4 ns
6-BA	1.6 b	21 b	2.8 a	232 a	86	13.1	0.352	18.0
NAA	1.8 b	28 ab	2.7 b	206 b	97	13.1	0.359	17.6
Urea	1.8 b	29 ab	2.7 ab	214 ab	93	13.0	0.359	18.1
Control	2.9 a	36 a	2.6 b	201 b	99	13.3	0.356	17.8
Bartlett / OHxF.87 - Mt. Adams								
ATS	6.1 ns	73 ns	2.6 c	160 b	125	11.0 ns	0.243 ns	18.0 a
6-BA	4.6	73	2.6 a	171 a	117	11.1	0.249	17.0 c
Urea	5.0	76	2.6 b	167 ab	120	11.2	0.249	17.2 bc
Control	4.9	80	2.6 b	166 ab	120	10.9	0.237	17.6 ab
Bartlett / OHxF.97 - Mt. Adams								
ATS	3.6 ns	76 ns	2.6 b	171 ab	117	11.2 ns	0.311 a	17.1 ns
6-BA	3.4	67	2.7 a	178 a	112	11.1	0.286 ab	17.0
Urea	3.4	75	2.6 b	170 b	118	10.8	0.259 bc	17.2
Control	4.5	84	2.6 ab	170 b	118	10.7	0.243 c	16.9
Bartlett / Seedling – Buena								
ATS	0.7 ab	44 ns	2.7 ns	214 ns	93	11.4 ns	0.305 ab	17.4 ns
Exilis Plus	0.7 ab	48	2.7	213	94	11.4	0.334 a	17.1
Urea	0.5 b	39	2.7	212	94	11.2	0.265 b	17.3
Control	1.0 a	52	2.7	206	97	11.5	0.292 b	17.3
Bosc / OHxF.97 - Tonasket								
ATS	1.3 ns	85 b	no data	251 a	80	11.3 ns	0.159 ns	14.0 b
MaxCel	1.5	97 a	no data	219 c	91	11.5	0.151	14.8 a
Urea	1.5	79 b	no data	237 b	84	11.0	0.150	14.5 ab
Control	1.7	98 a	no data	211 c	95	12.4	0.142	14.8 a

Fruit set (fruitlets/100 clusters) was significantly reduced once with urea or ATS (Tonasket) and 6-BA (Cashmere), translating into higher individual fruit weight at harvest (Table 1). Although BA did not thin fruitlets effectively, it generally improved final fruit size. Soluble solids, titratable acidity and fruit finish were not affected by any treatment. Chemical thinning effects on fruit firmness are inconsistent: firmness was increased once with ATS and urea, and decreased once with 6-BA and ATS.

Since 2003 we have conducted 25 pear thinning trials, testing an array of bloom (ATS, urea, CFO+LS, LS) and postbloom (BA, NAA) thinners. Table 2 summarizes our results. The overall goals when using crop load adjustment methods are: reduction of fruit set (indicated by fruitlets/100 blossom clusters); increase in mean fruit weight; and consistent annual bearing (indicated by return bloom). ATS and BA products have shown utility in pear blossom and fruitlet thinning. More importantly, BA typically improves final fruit size even in the absence of fruitlet thinning (Table 2).

Table 2. Incidence of statistically significant results for three key crop load parameters. WTFRC pear chemical thinning trials 2003-2008.

THINNING AGENT	*FRUITLETS/100 BLSM CLUSTERS*	*MEAN FRUIT WEIGHT*	*RETURN BLOOM*
NAA	0/6 (0%)	0/6 (0%)	0/2 (0%)
ATS	7/25 (28%)	5/24 (21%)	2/17 (12%)
Urea	1/17 (6%)	3/17 (18%)	0/11 (0%)
CFO + LS	0/3 (0%)	1/13 (8%)	1/2 (50%)
LS	1/13 (8%)	3/13 (23%)	0/13 (0%)
BA	3/12 (25%)	6/10 (60%)	2/5 (40%)

Reflective groundcovers: Daybright, a reflective groundcover manufactured by Extenday, was applied for the third consecutive year in 2008 in an established Bartlett block on a V-trellis (Sunnyside) and a young Bartlett block (Cashmere) from early bloom to harvest. Each year of the trial, fruit was harvested in two picks at Sunnyside and a single pick in Cashmere. Timing and duration of commercial harvest was not affected by Daybright at either site in 2008. Significant results include (Table 3, 4):

- Overall yield was increased by 10.5% in Cashmere, due to increased fruit set.
- Daybright decreased individual fruit size by 1.5% in Cashmere.
- Sugar content was decreased and acidity increased in Cashmere.
- Daybright-treated fruit from the first pick in Sunnyside was larger.
- In Sunnyside, firmness and sugar content increased, while acidity decreased.

Table 3. Yield effects of WTFRC pear reflective groundcover trials. 2008.

Treatment	Total yield kg/tree	Total fruit ct fruit/tree	Yield efficiency fruit/TCSA	Yield efficiency kg/TCSA	1st pick kg % of total	2nd pick kg % of total
Bartlett / Domestic - Sunnyside						

Daybright	25.1 ns	128 ns	1.1 ns	0.21 ns	92 ns	8 ns
Control	25.6	136	1.1	0.21	89	11
Bartlett /OHxF.87 - Cashmere						
Daybright	71.3 a	383 a	4.6 ns	0.85 ns	no data	no data
Control	64.5 b	342 b	4.4	0.83	no data	no data

Table 4. Fruit quality effects of WTFRC pear reflective groundcover trials 2008.

Treatment	Sugars (% brix)	Acids (% malic acid)	Firmness (lbs)	Weight (g)	Diameter (in)
Bartlett / OHxF.87 - Cashmere					
Daybright	11.4 b	0.329 a	17.3 ns	203 b	2.66 b
Control	11.9 a	0.313 b	17.0	217 a	2.70 a
Bartlett / Domestic – Sunnyside (Fresh pick)					
Daybright	10.8 a	0.256 b	17.6 a	199 a	2.64 a
Control	10.2 b	0.279 a	17.4 b	190 b	2.57 b
Bartlett / Domestic – Sunnyside (Cannery pick)					
Daybright	10.5 ns	0.255 b	17.9 ns	123 ns	2.26 ns
Control	10.3	0.273 a	17.7	122	2.25

During the three year trial period, Daybright application resulted in consistently higher yields in two years at both sites (Figure 1). 2008 results suggest that Cashmere fruit set gains were offset by smaller fruit size (10% higher yields with loss of ½ box size). In Sunnyside, yields were comparable, but fruit was ½ box size larger (Table 4).

Reflective groundcovers have shown utility in modern pear plantings and young orchards. Trials with Daybright reflective groundcover demonstrate yield gains in Bartlett due to increased fruit set and/or size. Better light distribution spurred renewed fruiting in lower portions of tree canopies, allowing more of the crop to be managed from the ground.

Figure 1: Percent change in yield (kg/tree) after Daybright application in two pear orchards over a three year period.

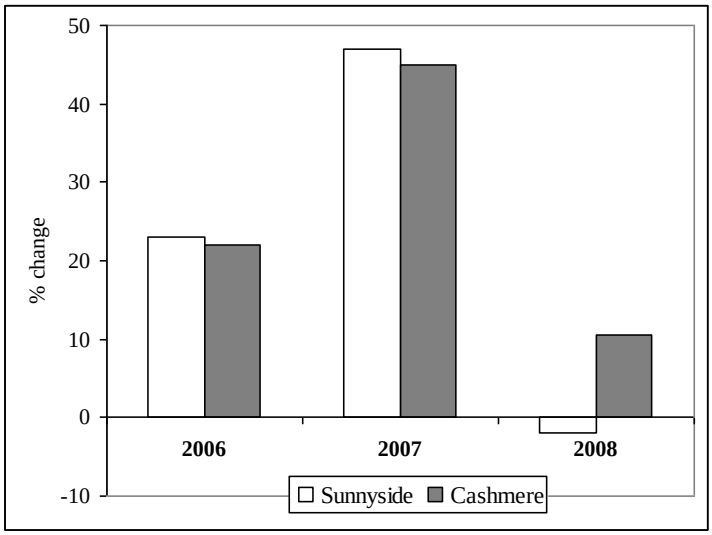
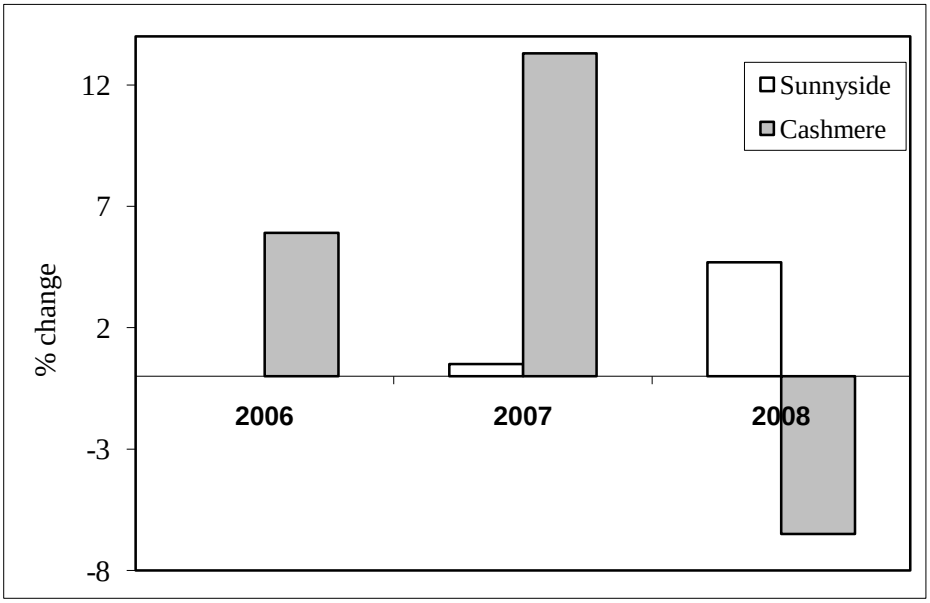


Figure 2: Percent change in individual fruit weight (gram) after Daybright application in two pear orchards over a three year period.



OUTREACH

Posters

Schmidt, T. 2006-2008. Crop load management. Posters at WSHA annual meeting.

Castillo, F. 2006-2008. Update on reflective groundcover evaluation in Washington. Posters at WSHA annual meeting.

Talks at industry meetings

Castillo, F. 2007. Reflective groundcovers in tree fruit. GS Long Grower Meeting.

Castillo, F. 2007-08. Reflective groundcovers. Okanogan Field Day.

Hanrahan, I. 2008. Crop load management in pears. Presentation at Wenatchee Pear Day.

Schmidt, T. 2007. WTFRC Research Programs. Presentation at D & M Growers' Annual Meeting, Yakima, WA.

Schmidt, T. 2008. WTFRC Research Programs. Presentation at D & M Growers' Annual Meeting, Yakima, WA.

Schmidt, T. 2008. Horticultural benefits of reflective materials. Presentation at Cascade Ag Services Growers' Annual Meeting, Chelan, WA.

Scientific publications

Hanrahan, I., Schmidt, T. R., Castillo, F., McFerson, J.R.. 2008. Reflective Ground Covers Increase Yields of Target Fruit. Poster and paper at ISHS in Geneva, NY, August 2008.

Other

Hanrahan, I. 2009. Increasing yields of target fruit with reflective ground covers in pear. Presentation at IFTA conference, Potsdam, Germany.

Schmidt, T. 2007. Improving fruit quality with reflective fabrics and sunburn suppression. Presentation at British Columbia Fruit Growers' Association Hort Forum, Penticton, Canada.

EXECUTIVE SUMMARY

1. Pear crop load management

Since 2003 we have set-up 25 pear thinning trials, testing an array of bloom (ATS, urea, CFO+LS, LS) and postbloom (BA, NAA) thinners. Our overall goals were:

- reduction of fruit set (indicated by fruitlets/100 blossom clusters);
- increase in mean fruit weight;
- consistent annual bearing (indicated by return bloom).

The most consistent performers in Washington pear chemical thinning trials are ammonium thiosulfate (ATS) applied during bloom and benzyladenine (BA) applied postbloom. Increases in individual fruit weight are often observed after benzyladenine application, even without significant fruitlet thinning.

2. Reflective groundcovers in pear

Daybright, a reflective groundcover manufactured by Extenday, was applied for three consecutive years in an established Bartlett block on a V-trellis (Sunnyside) and a young Bartlett block (Cashmere) from early bloom to harvest. Daybright results demonstrate yield gains in Bartlett due to increased fruit set and/or size. Better light distribution spurred renewed fruiting in lower portions of tree canopies, allowing more of the crop to be managed from the ground.

FINAL PROJECT REPORT

Project Title: Field evaluation of new pear rootstocks

PI:	Clark Seavert	**Co-PI(2)**:	Tom Auvil
Organization:	OSU-NWREC	**Organization**:	WTFRC
Telephone:	(503) 678-1264	**Telephone**:	(509) 665-8271
Email:	clark.seavert@orgegonstate.edu	**Email**:	Auvil@treefruitresearch.com
Address:	15210 Miley Road	**Address**:	1719 Springwater Avenue
City:	Aurora	**City**:	Wenatchee
State/Zip:	OR, 97002	**State/Zip**:	WA, 98801

Cooperators: Tim Smith, William Proebsting

Other funding Sources: None

Total Project Funding: $99,144.00

Budget History:

Item	Year 1: 2006	Year 2: 2007	Year 3: 2008
Salaries	15,239	17,508	19,850
Benefits	9,296	10,680	12,109
Wages	1,200	2,000	
Benefits	98	164	
Equipment			
Supplies	4,600	2,000	2,000
Travel	300	1,000	700
Miscellaneous	200	200	
Total	30,933	33,552	34,659

Objectives:

1) Initial screening and evaluation of the Horner rootstock series and evaluate untested rootstocks at OSU-MCAREC.
2) Comprehensive evaluation of the Horner rootstock series and untested rootstocks to be implemented in COS 2015 trials.
3) Identification of new rootstocks for future evaluation.

Significant Findings:

- A range of tree size (based on trunk cross-sectional area) exists in the Horner rootstock series, with a three to seven-fold difference exhibited in relative growth rates.
- Horner 4 produces a very vigorous tree [nearly the largest tree in all plantings, (i.e. both Horner screening and COS)], confirming earlier work.
- Precocious Horner selections have not yet been observed, although this could be due to poor climatic conditions during and immediately following bloom in multiple years of the study.
- DNA anlaysis of Horner 10 has confirmed that material used for propagation did derive from the mother plant (previously killed) at MCAREC.
- COS 'finished trees' reached the top wire in 2nd leaf (13 feet), however 3rd leaf (2008) fruit set was negligible, likely a function of poor climatic conditions during and immediately following bloom in 2008.
- COS 'bench-grafted trees' reached the top wire in 2nd leaf

Methods:

(1) Horner Rootstock Screening:
Three separate plantings (2004, 2005 and 2006) were made for the > 400 Horner selections, randomly planted (two or three single tree replicates), with 'd'Anjou' as the cultivar, and 'Bartlett' trees used as pollenizers. OHF 87 was used as a control in the 2005 and 2006 plantings. Trunk cross-sectional area (TCSA) was recorded in the fall.

(2) COS:
The goal to develop a mature fruiting canopy by the third leaf while allowing adequate light infiltration was accomplished using the following methods;

- Selections of P2535, Bet # 2291, 517-9, 708-13, 96FI11, 96FI12, 96FI14, 96FI15,
- Horner 4, OH 11, OHxF 87, Pyronia, and Q29859 were established.
- 'd'Anjou' (Horner 4, OHF87), 'Bartlett' (Horner 4, OHF 87, 69 and Fox 11), and 'Bosc' (Horner 4, OHF 69), were planted in a 12 ft x 4 ft vertical fruiting wall (907 trees/acre), 8-wire system with wires 18 inches apart, and a trellis height of 13 feet.
- Strategies for efficient shoot initiation and placement on wires were developed and implemented by notching combined with promalin application, at green tip, in years 2 and 3, and pinching of apical region throughout growing seasons to induce bud breaks.
- Labor hours for training and managing shoots were recorded.
- Irrigation was applied to optimize growth at two-2 hour sets per week (or as needed) in the 1st and 2nd leaf.
- Fertigation was applied bi-weekly totaling 16 lbs of actual Calcium Nitrate
- Yield and yield components, (fruitlets/flower bud and individual tree yields taken at harvest) will be used to measure progress (yield data will begin in 2009). TCSA has been collected annually.
- Old Home by Farmingdale 87 used as a control rootstock

Results and Discussion:

(1) Horner Rootstock Screening:

Yield began in the third year, and initial selections by the advisory committee were based on the limited amount of bloom and fruit set, with the hope that the 2007/08 harvests could clarify the choices. Of the entire Horner series, only 6, 2 and 1 of the selections from the 2004, 2005 and 2006 trials, respectively, fruited in 2008 (Tables 1-3). Due to poor fruit set this spring, evaluation of precocity and fruit set and their interactions with vegetative growth was not possible. Trunk cross-sectional area was recorded and expressed in both absolute and relative terms. Relative growth analysis $((TCA_{final} - TCA_{initial}) / TCA_{initial})*100$ was used to reduce the error associated with the large variability in trunk size at planting. There is roughly a three-four fold difference in vegetative growth across the series (Figure 1-3). Cumulative yields are also quite low for the selections, again as a function of severe spring temperatures that likely damaged blossoms and inhibited pollination, in both spring 2007 and 2008 (Tables 4-6). Annual and cumulative yield has been extrapolated to represent yield per acre with spacing of 10x16, (272 trees/acre). It should be noted that several selections had adequate blossom counts, so it appears that there is potential for precocity.

Pre-screening evaluations will continue for the Horner 2004, 2005 and 2006 plantings (funding solicited from Columbia Gorge Fruit Grower's Commission). Bloom density and fruit set data will be compiled in Spring 2009. In addition, the mother block of Horner rootstocks will be assessed to determine the value of re-selecting rootstocks based on expressed characteristics of vegetative growth (limb angle, relative vigor). For example, when viewing the stool bed, Horner 4 is by far the largest plant in the entire 400+ Horner series, consequently, it comes as little surprise that in two of the three plantings in which it has representation (2004, 2005) it transfers this effect to 'Anjou', and is the third largest of 285 selections and the largest of 146 selections in 2004 and 2005 plantings, respectively. Perhaps once fruit set occurs a shift in carbon partitioning will occur, favoring fruit growth. It is difficult to assess currently, in the absence of fruit, if selections such as Horner 4 are leading us in the opposite direction of the original objectives set forth by the committee, which were to advance precocious, size-controlling selections that could not only set adequate fruit numbers but size them as well. Based on a re-evaluation of the stool bed, selections will be moved forward for a more robust planting (i.e. with sufficient replication so that variability within a given selection can be accounted for). To achieve this goal, a minimum of eight replicates per selection will be required.

DNA anlaysis of Horner 10 has been completed. The results confirm that material used for propagation indeed derived from the mother plant at MCAREC. Two clones, Horner 4 and Horner 10, as well as OHxF 87 have been propagated at VanWell Nursery for on-farm trials. Currently, a total of 1,576 plants are available for distribution (comprised of Horner 4, 10 and OHxF 87, each stock worked with 'Bartlett', 'GR Bosc' and 'Anjou'). Dispersal of these materials to growers for on-farm trials beginning in spring 2009 are scheduled among three regions (Yakima, Wenatchee and Hood River).

(2) COS:

There is no yield data to date. Trees were expected to begin production in the 3rd leaf (2008), however severe spring temperatures limited fruit set. Vegetative growth as determined by TCSA, is beginning to show differences, with Horner 4 producing the largest tree for each of the three cultivars that are worked upon it. Horner 4 is roughly 33 % larger than OHF 69 for Bosc, roughly 10 % larger than OHF 69, 87 and Fox 11 (all producing trees of similar size) for Bartlett and approximately of equivalent size to OHF 87 for Anjou (data not shown). The main challenge to overcome in high density pear systems is managing vigor while trying to induce early yields, especially with Anjou. Proper light interception in the canopy is crucial to the success of high density plantings, so it is important to minimize growing points without causing excessive vegetative responses. Trees must be managed to fill only the space allotted to them without encroaching on their neighbor.

When employing techniques to encourage early fruiting, it is necessary to limit pruning in the early years. One of the drawbacks to planting feathered trees comes from the need to remove all limbs at planting time. This is necessary because they are usually 1) already too large and 2) located in the wrong place for training to the wire trellis. This pruning immediately promotes vegetative responses and may ultimately delay fruiting. In consideration of this problem benchgrafts and sleeping eyes were added to the trial for comparison. The advantage to these two types of material is the ability to initiate weak wood at the desired wire height by using notching and Promalin versus pruning.
The goal to grow a mature fruiting canopy was accomplished by the 3rd leaf. Developing a regular, intensive training regime to deal with shoot thinning and positioning before they became unmanageable was critical. It is expected that training intensity in the first three years will be offset by the timeliness of shoot positioning, and the wires will become the guide as the trees progress in later years. In the 2nd leaf, the trees were trained on a weekly basis to position shoots and encourage branching at the wires by pinching the terminal bud when it was 2-4 inches above the wire. The time spent in the 0.8 acre block averaged 64 hours a month during the growing season for the trees in their 2nd leaf. Less time in the 3rd leaf (average 40 hours month) was necessary to maintain the goal. Work was performed with ladders; a mechanical platform could simplify this chore.

Management strategies for managing vigor and encouraging earlier fruiting include;

- Minimal pruning and diligent timing of shoot removal
- Expedited pinching back terminal of buds to encourage branching as shoots grew past the wire.
- Notching above buds and applying Promalin to initiate bud break where shoots were absent.

Conversely, it was necessary to reduce growth by limiting irrigation and fertigation in the 3rd leaf.

- Deficit irrigation (a total of 6 hours of water this summer coupled with monitoring plant moisture stress with the pressure chamber) succeeded in controlling vigor, with OHxF 87 showing significantly better capabilities of withstanding water stress than OHxF69 (Figure 4). This strategy, however, would not be expected to work had a significant crop been present, without reducing fruit size. The severity of water withholding was based on hardening off shoot tips in the absence of fruit.
- Fertigation was limited to one spring application of Calcium Nitrate at the rate of 3.2 lbs actual N per acre.

Parameters such as yield efficiency (yield/unit TCSA) and flower density (flower buds/unit TCSA) will be used to begin analysis of rootstock and cultivar interactions in the 4th leaf.

There were survival issues with one of the three Khazakstan rootstocks, ‘Q 29858’. The rootstocks survived the first winter as sleeping eyes, but died in spring after bud break, possibly damaged by the early spring freeze. The other two, ‘Q29857’ and ‘Q29859’ have been budded and are doing well.

Figures

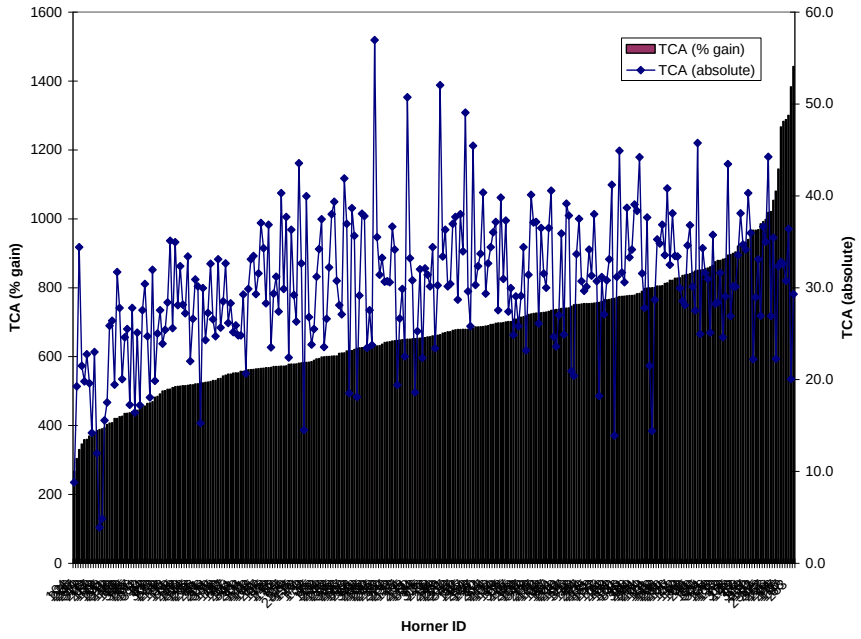


Figure 1. Range of tree size and growth across all selections (285) in Horner 2004 planting. Relative trunk cross-sectional area (TCA) as either % gain (from planting though Fall 2008) or in absolute terms (TCA as of Fall 2008, recorded in cm^2).

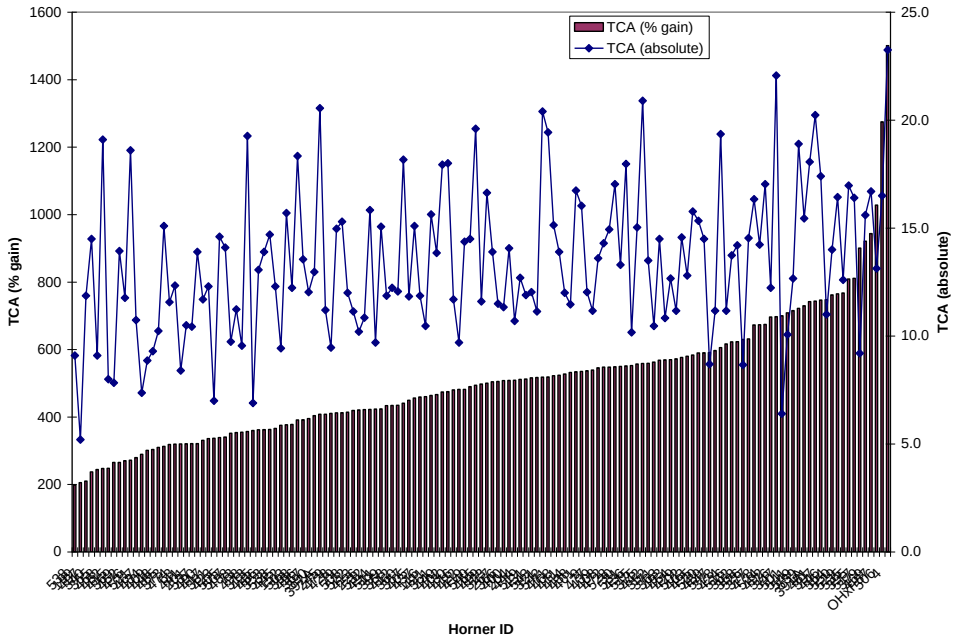


Figure 2. Range of tree size and growth across all selections (146) in Horner 2005 planting. Relative trunk cross-sectional area (TCA) as either % gain (from planting though Fall 2008) or in absolute terms (TCA as of Fall 2008, recorded in cm^2).

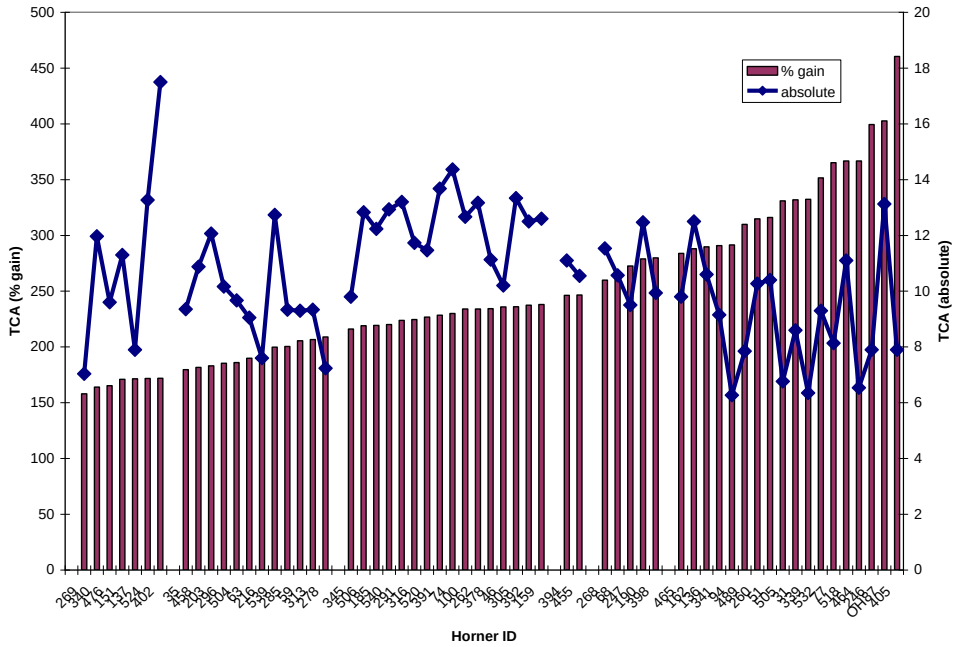


Figure 3. Range of tree size and growth across all selections (65) in Horner 2006 planting. Relative trunk cross-sectional area (TCA) as either % gain (from planting though Fall 2008) or in absolute terms (TCA as of Fall 2008, recorded in cm^2).

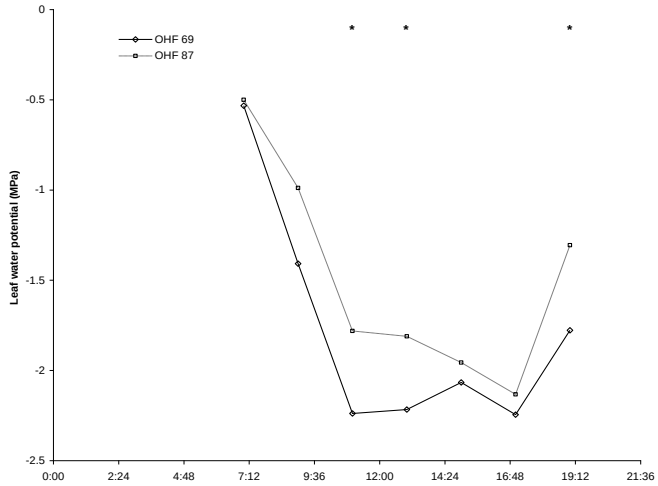


Figure 4. Typical diurnal trend of water potential values recorded on 14-August, 2008 for 'Bartlett' on either OHF 69 or 87. Trees had only received ~ 50 gallons of total irrigation following spring rain events. Asterisks at top indicate significance at $P < 0.05$. Each point is the mean of 9 leaves.

Tables

Table 1. Extrapolated yield for Horner 2004 - 5th leaf harvest.

Per acre extrapolated yield for Horner 2004 block-Harvest 2008[#]							
Horner Rootstock ID	Trees/acre 10x16 spacing	lbs./fruit per acre	44lb.Box/acre 80% packed	2006-08 Yield (lbs)	Box Sz	TCSA cm2	YE lbs fruit/cm2
21	272	245	4	0.9	100	17.2	0.05
21	272	2094	38	7.7	90	24	0.32
38	272	190	3	0.7	120	27.6	0.03
38	272	1877	34	6.9	100	23.2	0.30
45	272	707	13	2.6	100	24.1	0.11
45	272	1224	22	4.5	110	23.1	0.19
119	272	789	14	2.9	110	23.5	0.12
119	272	571	10	2.1	100	25.9	0.08
251	272	789	14	2.9	120	27.7	0.10
251	272	2258	41	8.3	80	19.4	0.43
334	272	1115	20	4.1	100	21.8	0.19
334	272	1360	25	5.0	100	23.2	0.22

[#]data are selections whose replicates have > 10 fruit per tree

Table 2. Extrapolated yield for Horner 2005 - 4th leaf harvest.

Per acre extrapolated yield for Horner 2005 block-Harvest 2008[#]							
Horner Rootstock ID	Trees/acre 10x16 spacing	lbs./fruit per acre	44lb.Box/acre 80% packed	2008 Yield (lbs)	Box Sz	TCSA cm^2	YE lbs fruit/cm^2
498	272	136	2	0.5	90	21.40	0.023
498	272	571	10	2.1	100	35.09	0.060
498	272	381	7	1.4	120	38.17	0.037
352	272	272	5	1	90	13.87	0.072
352	272	571	10	2.1	100	18.87	0.111
352	272	598	11	2.2	100	35.77	0.062

[#]data are selections whose replicates have > 5 fruit per tree

Table 3. Extrapolated yield for Horner 2006 - 3rd leaf harvest.

Per acre extrapolated yield for Horner 2006 block-Harvest 2008[#]							
Horner Rootstock ID	Trees/acre 10x16 spacing	lbs./fruit per acre	44lb.Box/acre 80% packed	2008 Yield (lbs)	Box Sz	TCSA cm^2	YE lbs fruit/cm^2
398	272	435	8	1.6	110	15.15	0.106
398	272	272	5	1.0	90	19.62	0.051

[#] Horner 398 was the only replicated clone that set fruit in 2008

Table 4. Cumulative extrapolated yield for Horner 2004 block, 2006-2008.

Per acre extrapolated yield for Horner 2004 block-Harvest 2006-2008							
Horner Rootstock ID	Trees/acre 10x16 spacing	lbs./fruit per acre	44lb.Box/acre 80% packed	2006-08 Yield (lbs)	Average Box Sz	TCSA cm^2	YE lbs fruit/cm^2
81	272	2584	47	9.5	90	45.84	0.207
81	272	3400	62	12.5	90	52.15	0.240
93	272	1798	33	6.6	110	42.46	0.156
93	272	2176	40	8.0	100	23.00	0.348
119	272	2339	43	8.6	90	23.5	0.366
119	272	1790	33	6.6	90	25.9	0.254
220	272	2258	41	8.3	90	46.60	0.178
220	272	3345	61	12.3	90	62.39	0.197
307	272	2040	37	7.5	110	64.64	0.116
307	272	2040	37	7.5	110	40.64	0.185
232B	272	1605	29	5.9	120	48.55	0.122
232B	272	2339	43	8.6	90	27.24	0.316

[#]data are selections whose replicates have > 15 fruit per tree

Table 5. Cumulative extrapolated yield for Horner 2005 block, 2006-2008[$]

Per acre extrapolated yield for Horner 2005 block-Harvest 2007							
Horner Rootstock ID	Trees/acre 10x16 spacing	lbs./fruit per acre	44lb.Box/acre 80% packed	2007 Yield (lbs)	Box Sz	TCSA cm^2	YE lbs fruit/cm^2
399	272	261	5	0.96	92	6.30	0.152
411	272	134	2	0.49	89	9.28	0.053
403	272	282	5	1.04	85	11.46	0.090
390	272	326	6	1.20	73	9.28	0.129
355	272	219	4	0.81	109	7.03	0.115

[$] data are taken from 2007 harvest (2008 fruit set explained below in Table 6)

Table 6. Horner 2005 flower clusters and fruit set in 2008.

2008 Flower clusters and fruit set for Horner 2005 block						
Horner 2005 ROW	TREE	H-ID#	Number of clusters	#fruit 06/08	Harvest 2008 #fruit	wt (lbs)
2	31	355	0			
8	12	355	9			
12	14	355	8			
2	7	390	2			
6	22	390	0			
10	20	390	29			
2	23	399	19			
8	8	399	6			
11	7	399	43			
1	3	411	98			
8	25	411	89	1	1	0.4
11	22	411	85			
3	20	403	147	8	8	2.8
8	3	403	104	1	1	0.5
10	6	403	29			

Executive Summary

Three pear rootstock trials were initiated at Oregon State University's Mid-Columbia Agricultural Research and Extension Center (MCAREC) to evaluate the influence of the 'Horner' rootstock series on precocity, size control, yield and fruit size of 'd'Anjou' pear trees. The trials were planted over three years (2004-2006) and compared against the standard, 'OHxF 87'. Of the > 400 selections evaluated, there exists a roughly three-four fold difference in vegetative growth across the series. However, individual selections do not consistently stand out for producing 'semi-dwarf' or 'dwarf' trees. 'Horner 4', consistently produced the largest trees in all years that it was trialed. Unfortunately, two years in a row of low temperatures during bloom, limited fruit set, due to direct injury of the flowers. Evaluations will be continued through 2009 to determine if selections have a beneficial impact on fruit set and yield.

A high-density pear trial (907 trees/acre) was planted and trained to an eight-wire system with wires spaced 18 inches apart, and a final trellis height of 13 feet. Several rootstock/scion combinations were planted to include 'Bosc', 'Bartlett' and 'd'Anjou' on 'OHxF 87','OHxF 69', 'Horner 4' and 'Fox 11'. Trees are entering their 4th leaf. The planting has both 'finished' and 'bench grafted' trees. Both tree types reached the top wire by the end of their 2nd leaf, however 3rd leaf (2008) fruit set/yields were negligible, again a function of poor climatic conditions during and immediately following bloom in 2008. Successful strategies were employed to promote shoot initiation and positioning, such as the combination of notching and promalin application at green tip, in years 2 and 3, and pinching of the apical region just above wire tiers throughout growing seasons. Horizontal limb positioning resulted in highly vigorous growth (watersprouts), and required weekly limb removal to contain. Deficit irrigation successfully controlled vegetative growth late in the season however the degree of water withholding was only possible due to the lack of any significant fruit load. The trial will be continued in 2009.

FINAL PROJECT REPORT

Project Title: PNW pear rootstock trial

PI: Timothy J. Smith
Organization: WSU
Telephone: 509-667-6540
Email: smithtj@wsu.edu
Address: 400 Washington Street
City: Wenatchee
State/Zip: Washington, 98801

Assistant: Esteban Gutierrez, WSU Extension (Tonasket & Cashmere Trials)
Cooperators: Janet Turner (Tech.), Clark Seavert, & Steve Castagnoli, OSU Extension (Hood River Trial), Ed and Darrin Kenoyer (Cashmere Trial), Geoff Thornton and Dennis Lorz (Tonasket Trial). Advisors: Fred Valentine, Tom Auvil, Greg Rains, Bob Gix.

Other funding sources
Agency Name: NW Nursery Improvement Inst. supports the Tonasket trellis demonstration, $8,000.

Budget Summary of Total Project: Budget 1, WSU Cashmere & Tonasket Plots:

Item	Year 1: 2006	Year 2: 2007	Year 3: 2008
Salaries	2,667	3,468	**2,884**
Benefits	907	1,179	**981**
Wages	0	400	**0**
Benefits	0	44	**0**
Supplies	2000	400	**400**
Travel	1000	1800	**1575**
Miscellaneous	0	0	**0**
Total	6,574	7,291	**5,840**

Footnotes: 0.0769 FTE (four weeks) Technician (Tonasket & Cashmere sites).Travel is to plots.

Budget 2: Hood River Plot

Item	Year 1 2006	Year 2 2007	Year 3 2008
Salaries[1a]	2,688	2,768	**2,852**
Benefits	1,640	1,688	**1,740**
Wages[2]	514	605	**692**
Benefits	46	54	**62**
Supplies[3]	700	700	**700**
Travel[4]	200	200	**200**
Total	5,788	6,015	**6,246**

Footnotes:[1a] 0.1 FTE (5 weeks) Technician (Hood River site.)

Budget History:

Projects by Site	Year 1: 2006	Year 2: 2007	Year 3: 2008
Cashmere	7,618	7,291	5,840
and Tonasket			
Hood River	5,788	6,015	6,246
Year Total:	13,406	13,306	12,086
3-Year Total:			**$38,398**

Original Objectives:

The pear scions/rootstocks will be evaluated on the following: 1. survival, 2. suckering, 3. vegetative growth potential (trunk size and tree diameter), 4. yield, and 5. fruit size.

Impact of This Work:
There were at least four significant outcomes to this project:

1. A number of potential rootstocks, including some that was being sold commercially in Washington and Oregon, were shown to be inferior due to disease or cold injury susceptibility, yield, fruit size, the production of thorny root suckers, or a combination of these attributes. Early release of this negative data resulted in the cessation of production and sales of a poorly-tested rootstocks that in the Bosc trial presently lag behind the standard OHxF 87 as much as $20,000 per acre in gross receipts. No one will ever know how many acres of thorny roots-suckered, smaller-fruited, low production rootstocks would've been planted in the absence of this trial. Each 50 acres planted would have reduced gross returns by up to $1,000,000 in the first seven years of their production.

2. The OHxF 87 performed well enough in the Golden Russet Bosc trial to become the current industry standard semi-dwarfing rootstock until something better comes along. These data have encouraged the nursery industry to pursue better methods of propagating this rootstock, and they are making it much more available to Pacific Northwest pear growers.

3. Bartlett on Pyro 2-33 appears superior to Bartlett on OHxF 87. The lower fruit set is adequate for good production, but leads to much faster fruit thinning, the fruit is consistently larger, and the compact trees are similar in size. The Pyro 2-33 remains free of diseases, such as pear decline, produces no root suckers, and seems to tolerate cold winter temperatures. This root did not out-perform OHxF 87 in Bosc or D'Anjou trials.

4. Pear horticultural field tours centered on these trials have markedly increased recently, and some "traditional" pear growers have started changing their growing practices as a result.

Extension (Outreach) of the data and horticultural information developed trough this project:

1. Presentations to horticultural meetings: Four, to a total Washington audience of 1150.
2. Web page and trial reports: about 800-900 "unique viewers" per year.
3. Reported to NC-140 North American rootstock working group by Steve Castagnoli. A summary to NC-140 of these rootstock trials is planned by Todd Einhorn.
4. Pear horticulture orchard tours: six, posters at WSHA meetings: two.

Introduction and Justification
Most pear orchards in the USA have rootstocks that induce high vegetative vigor. While many of these orchards are quite old relative to other tree fruit orchards, the well-managed pear orchard continues to produce good yields of high quality fruit. Too many do not, because high tree vigor brings multiple problems, such as inefficient use of labor, difficult insect and post-harvest disease management, and fruit quality problems related to low fruit calcium. Efforts to treat these symptoms of excessive vigor have cost a significant percentage of pear research dollars for decades, but the problems seem to remain. Excessive tree vigor costs growers far more in increased pruning, suckering, thinning and harvest labor costs, additional sprays, and crop loss in the packinghouse. There has been very little obvious economic reason to change existing pear orchard systems, or even

plant significant acreages of new pears. However, over the past two decades, it has become apparent to industry leaders that pear growers may be forced to replace the current 1950's style pear orchard with either another profitable fruit, or, if they decide to stay in pear production, to grow their next pear orchard with smaller, easier to manage trees. In order to make the switch to possible semi-intensive systems, it was obvious that dwarfing or semi-dwarfing rootstocks would be critical to the entire process, as they were to apple producers. While there had been efforts to create or test various pear rootstocks in the Pacific Northwest for several decades, and a few rootstocks in the Old Home x Farmingdale series had gained some recognition and use, there was general dissatisfaction with the speed and direction of the pear rootstock development and evaluation effort.

Overview:
In 2002, after several years of preliminary effort identifying, importing and propagating rootstock candidates from around the world (by Dr. Gene Milke, OSU, retired), a pear rootstock trial was established in four locations in the Pacific Northwest. Grower cooperators provided sites in Tonasket (Bosc) and Cashmere (D'Anjou), one trial was established on the TFRC property in the mid-Yakima Valley (Bartlett), and one was planted in Hood River at the OSU-MCAREC (D'Anjou). The Yakima Valley Bartlett trial suffered serious damage from fire blight in 2004, 2005 and again in 2007, and was suspended as having no value as a rootstock trial.

Seven rootstocks were included the first season, and an additional six were planted on these sites in 2005. In all cases, OHxF 87 was used as the standard "semi-dwarf," and there was hope that the other rootstocks would induce a smaller, more productive tree. The 2002 trees were planted 10 feet apart in the row and were trained as a free-standing central leader. This tree spacing was the standard for rootstock trials at the time, because it allowed each tree to behave relative to the influence of the rootstock, rather than to excessive containment pruning or the competition from the adjacent trees.

Most of the 7th leaf trees currently appear as if they would have been manageable as a tree wall system if planted at 6 – 7 feet in row and 13 – 15 foot row spacing, with support only in the first two seasons on sites with fine textured soils. Starting in the second season, lower scaffold limbs were spread to 45 – 50 degrees from vertical to induce fruitfulness, but to avoid vigorous suckering common with more horizontal or pendant scaffolds. The Boscs and Bartlett pollenizer trees began to produce significant crops long before the scaffolds were able to support the fruit weight, so most of the lower limbs have been supported to the trunk with bailer twine to prevent breakage. Pruning to stiffen lower scaffolds would reduce the need to support limbs, as the vegetative scaffolds would have less fruit to support. Early fruiting has a pronounced effect on tree vigor and size, so the twine stays.

In order to reduce limb spreading, followed by limb tying, the 2005 trees were planted at 6 foot row spacing, and are trained on a 4 wire upright trellis in the D'Anjou trial at Cashmere and the Bosc trial in Tonasket. The 2005 D'Anjou rootstock trial in Hood River was planted free-standing at the ten foot spacing standard of this trial, and may serve as a contrast of rootstock behavior on intensive vs. semi-intensive systems. Pruning and training has been directed or carried out by local experts, with the intention of bringing the trees into early production, while building a proper framework for the free-standing system.

Also in 2005, the author, who is not a horticultural person, became the default P.I. of this project, because the other original principle cooperating university faculty had retired or changed employers.

Summary of Results and Discussion:

Of the four trial sites, only the Tonasket Golden Russet Boscs and Cashmere and Tonasket Bartlett pollenizers have produced consistent yields leading to consistent data, sometimes with better early

production than would be expected with standard rootstocks. While data was taken at other sites, and will be provided to anyone if deemed necessary, yields were generally disappointing, and will not be reported here. The best data, unhindered by frost, cold bloom times , poor pollenization, fire blight or herbicide damage, has been from the 2002 and 2005 Bosc Trials in Tonasket. There is somewhat limited, but complete data that was taken from the numerous Bartletts interplanted as pollenizers in the Cashmere and Tonasket trial sites. Fortunately, the rootstocks that were most interesting in the Bosc trial, good and poor performers, are also included in the Bartlett pollenizer results.

Survival of the tree:
Other than in the Yakima trial, most of the trees survived and are healthy to date. However, there are some significant exceptions. Having Asian pear in the heritage of the rootstock usually leads to a high chance of phytoplasma-induced "pear decline" disease. Although the percentage of the plot trees that died was variable, the 708-36 rootstock appears unacceptably prone to this disease. The BU-2 and BU-3 in the 2005 trial also appear to be affected by pear decline at the Cashmere D'Anjou site. Temperatures of -10F or lower in the second winter at the Tonasket Bosc trial killed three of ten rootstocks in both Fox 11 and Fox 16. No winter damage has been observed on these or any other rootstock since that incidence, but the temperatures have been no colder than about -4F since then.

Trunk and vegetative growth:
The 2002 plot tree vigor and resulting size occurred in the following descending order, reported as a percentage comparison of the cross sectional area of the trunk, with the OHxF 87 trunk as 100:

Bosc- OHxF 87 (100), Pyrodwarf (91), OHxF 40 (82), Pyro 2-33 (73), Fox 11 (73), Fox 16 (64), and 708-36 (57). For data, see Tables 1 and 3-1.

After the first three seasons, when production increased significantly in the Bosc trial, foliage on the 708-36 rooted trees was inadequate to support the fruit load. The other rootstocks had adequate vigor, and none have produced an excess of "sucker" growth on the upper surfaces of scaffolds, especially after significant fruit production started. These rootstocks may induce trees with far more vegetative vigor and greater ultimate size than the trees in these plots when planted on sites with deep, high quality soils.

While trees on Pyro 2-33 produced 58% more fruit than those on Pyrodwarf, the trunk and tree size were almost identical.

Data supports the previously reported concept that yield and tree size are not closely correlated with currently available pear rootstocks.

	OHxF 87	Pyro-dwarf	OHxF 40	Pyro 2-33	Fox 11	Fox 16	708-36	Winter Nellis
Tonasket Bosc	94	86	77	73	69	60	54	-
Cashmere D'Anjou	117	99	115	97	89	80	83	-
Hood River D'Anjou	89	91	89	85	85	-	85	89
Tonasket Bartlett	-	62	-	63	-	-	-	-
Cashmere Bartlett	69	68	-	68	-	-	-	-

Table 1. Seventh season tree trunk size expressed as square centimeters, cross sectional area. Example: A 90 sq. cm. tree trunk has a diameter of 4.36 inches. Note: Growth of Boscs and Bartletts occurred with significant fruit yields in year 5, 6 and 7. Growth of Hood River D'Anjou occurred with low to modest yields in years 5 and 6. The growth of the Cashmere D'Anjou trees has not been restrained by significant fruit production.

Root suckering:
No significant suckering was observed on any rootstock other than Pyrodwarf. Pyrodwarf has developed numerous, large and seriously thorny suckers, obvious by their third season of growth.

Yields and Efficiency:
Relative total yields, adjusted to tree size, reported as a percentage of the standard OHxF 87:
Bosc: OHxF 87 (100), Pyro 2-33 (70), OHxF 40 (70), 708-36 (55), Fox 11 (54), Fox 16 (44), and Pyrodwarf (43). See Tables 3-1 and 3-2 for actual yield per tree and extrapolated yields per acre.
Cashmere Bartletts: OHxF 87 (100), Pyro 2-33 (124), Pyrodwarf (80). See Table 1-2.

Bartlett 2002 Planting Tonasket	2008 Pounds Fruit/ Acre, 7th Year	2005-08 Average Box Size 44 / Avr. Fr. Wt	2008 Average Box Size 44 / Avr. Fr. Wt.	2008 Average Fruit Weight (Grams)	Total Bins Fruit per A 04 - 08	2008 Lbs. Fruit per Tree	Trunk Cross Sec Area cm^2	2008 lbs. Fruit per cm^2 of Trunk (Efficiency)
Pyro 2-33	52,081	82	68	294	121	117	62	1.89
Pyrodwarf	32,767	100	80	250	77	74	60.2	1.23

Table 2-1. 2002 planting of Bartlett pear, Tonasket, (7^{th} season), yield per tree, extrapolated yield, fruit size, trunk size and fruiting efficiency. Yield estimates based on 444 trees per acre (7 x 14 ft). Note: the higher the box size number, the smaller the fruit.

Yield efficiency, which relates the amount of total fruit produced to the size of the trunk, reported as a percentage of the standard OHxF 87:
Bosc: OHxF 87 (100), Pyro 2-33 (94), 708-36 (88), OHxF 40 (84), Fox 11 (62), Fox 16 (59), and Pyrodwarf (48). See Table 3-1.

Cashmere Bartletts: OHxF 87 (100), Pyro 2-33 (126), Pyrodwarf (81). See Table 2-2.

Bartlett 2002 Planting Cashmere	**2008 Pounds Fruit/ Acre, 7th Year**	**2008 44 lb. Box/ Acre, 80% Packed**	**2008 Average Box Size**	**2008 % 100's and Larger**	**Total Bins Fruit /A 2004 - 2008**	**2008 Lbs. Fruit per Tree**	**Trunk Cross Sect. Area CM^2**	**2008 lbs. Fruit / CM^2 Trunk *Efficiency***
Pyro 2-33	32,614	593	80	94	67	84	65.8	1.28
Pyrodwarf	18,872	343	97	69	43	48	66.1	0.73
OHxF 87	27,690	503	79	96	54	71	66.8	1.06

Table 2-2. 2002 planting of Bartlett pear, Cashmere, (7th season), yield, extrapolated yield, fruit size, trunk size and efficiency. Yields based on 390 trees per acre (7.5 x 15 ft.).

Bosc- 2002 Planting Tonasket	**2008 Pounds Fruit/ Acre, 7th Year**	**Calc. Trees Per Acre**	**2008 44 lb. Box/ Acre, 90% Packed**	**2008 Avr. Box Size**	**Avr. Box Size 06-08**	**Total Bins Fruit / A 04 -08**	**2008 Lbs. Fruit Tree**	**2008 Trunk Cross Sect. Area CM^2**	**2008 lbs. Fruit / CM^2 of Trunk**	**Total 04-08 lbs. Fruit / CM^2 of Trunk**
OHxF 87	47,952	444	981	68	71	**162**	108	94	1.15	4.10
Pyro 2-33	36,852	444	754	71	74	**113**	83	73	1.14	3.83
OHxF 40	27,528	444	563	74	76	**113**	62	77	0.81	3.45
708 - 36	22,145	515	453	69	80	**87**	43	54	0.80	3.62
Fox 11	31,415	515	643	68	72	**86**	61	69	0.88	2.56
Pyro-dwarf	26,196	444	536	72	79	**70**	59	86	0.69	1.97
Fox 16	25,750	515	527	63	67	**69**	50	60	0.83	2.43

Table 3-1. 2008 Data from 2002 planting of Golden Russet Bosc, (7th season), yield, extrapolated yield, fruit size, trunk size and efficiency, in descending order of total yield. Planting space was calculated at 7 x 14 for the 444 trees / A, and 6.5 x 13 for the 515 trees / acre.

Fruit size:

Average box size 2006 – 2008, in descending order:

Bosc: Fox 16 (67), OHxF 87 (71), Fox 11 (72), Pyro 2-33 (74), OHxF 40 (76), Pyrodwarf (78), and 708-36 (80).

Tonasket Bartletts: Pyro 2-33 (79), Pyrodwarf (94).

Cashmere Bartletts: Pyro 2-33 (86), OHxF 87 (94), Pyrodwarf (107).

While average fruit size is reported here, at least 400 individual fruit were weighed per rootstock each season to create a "box size" curve and to better calculate potential fruit economic value. The Bosc and Bartlett data support the following summary statements:

Fruit size summary: Average fruit size varied from one season to another, but the ranking of the various cultivars/rootstocks remained relatively consistent. (The Boscs in 2008 were picked relatively late in their harvest season, and were abnormally large.) The trees on Pyrodwarf bore fruit that was of acceptable commercial size on some seasons, but the fruit averaged significantly smaller than fruit produced on trees with other rootstocks in the more representative productive trials. The only exception to this was in comparison to the 708-36, which had Bosc fruit that was unusually small in the 6th leaf due to excessive fruit load. Pyrodwarf's fruit load, being consistently light, was probably not the cause of its relatively smaller fruit size. The Tonasket Bosc fruit was generally large by industry standards, and the Bartlett pollenizer fruit at that site tended to be moderate to large size. The Cashmere Bartletts trended small to medium, always smaller fruit in comparison to fruit from the same rootstock in Tonasket.

The fruit size did not correlate to tree size or fruit load. The only obvious situation where fruit load affected fruit size was with 708-36 in the sixth leaf, when a very heavy fruit set limited vegetative growth, leaf:fruit ratio was about 3:1, and the fruit was small and sunburned.

In the Bartletts, the Pyro 2-33 scattered fruit evenly throughout the tree, and required only light hand thinning. The Bartletts on OHxF 87 set much more fruit and required twice as many fruit to be removed. If left unthinned, the Bartletts on OHxF 87 would likely produce much higher yields, but of fruit of smaller average size.

Bosc-2002 Planting, Tonasket	**2004-05 Yield in Pounds per Acre 3rd+4th**	**2006 Yield In lbs. per Acre 5th Leaf**	**2007 Yield In lbs. per Acre 6th Leaf**	**2008 Yield In lbs. per Acre 7th Leaf**	**2006 Fruit Box Size (# Fruit / 44 lb. Box)**	**2007 Fruit Box Size**	**2008 Fruit Box Size**
OHxF 87	20,525	44,849	64,536	47,952	70	75	71
Pyro 2-33	8,636	29,002	49,253	36,852	76	75	74
OHxF 40	13,579	32,875	50,229	27,528	74	80	76
708 - 36	14,590	20,640	38,299	22,145	82	88	80
Fox 11	6,014	16,028	41,267	31,415	74	75	72
Fox 16	689	14,466	34,202	26,196	69	70	67
Pyrodwarf	4,631	12,598	33,575	25,750	86	75	78

Table 3-2. History of yearly extrapolated yield and average fruit size in 2002 planting of Golden Russet Bosc, in descending order of total yield.

Tonasket 2002 GR Bosc	OHxF87	Pyro 2-33	708-36	Fox 11	Fox 16	OHxF40	Pyro-dwarf
120 & -	0	0	0	0	0	1.0	0
110	0	0	0.9	0	0	2.8	0.7
100	0.8	1.1	2.8	2.0	0.7	3.4	2.7
90	3.4	11.3	7.6	7.8	2.1	7.9	10.8
80	15.8	19.1	15	12.9	7.9	18	21.3
70	29.0	31.7	37.1	28.1	20.9	26.4	33.1
60	33.9	20.9	26.4	34	38.7	25.6	26
50	10.7	11.7	10.3	15.3	21.4	10.7	11.7
40	3.0	0	0	0	3.6	2.5	0

Table 3-3. Tonasket 7th Year Bosc, percent of fruit by weight in each box size.

Tonasket 2002 GR Bosc -08	OHxF87	Pyro 2-33	OHxF40	Fox 11	708-36	Fox 16	Pyro-dwarf
120 & -	0	0	$39	0	0	0	0
110	0	0	138	0	$36	0	$32
100	$90	$95	220	$148	146	$43	163
90	461	1,177	615	693	476	156	786
80	2,250	2,091	1,472	1,204	987	615	1,629
70	4,173	3,505	2,181	2,649	2,465	1,643	2,558
60	4,678	2,217	2,028	3,074	1,683	2,918	1,927
50	1,771	1,162	980	1,296	615	1,766	812
$ / Acre	**$13,423**	**$10,247**	**$7,671**	**$9,062**	**$6,406**	**$7,139**	**$7,907**
$ / Acre 06+07+08	**$37,120**	**$27,700**	**$25,800**	**$23,270**	**$23,550**	**$18,790**	**$16,390**
Gross Re: OHxF 87	**(same)**	**- $9,420**	**- $11,320**	**- $13,850**	**- $13,570**	**-$18,330**	**- $20,730**

Table 3-4. Bosc plot, estimated yearly gross returns per acre: extrapolated yield per acre was assumed to be 90% packable. Fruit size data was used to estimate the number of boxes of each size fruit would be produced per acre. Those box numbers were multiplied by the average returns by box size reported each year (minus $9.70 per box packing charge), 2008 crop data current to December.

2005 Planted Section of the Rootstock Trial:
The 2005 planted trials have some rootstocks that were not included in 2000, such as BU-3, BU-6, BM 2000, and Horner 4. In Hood River, the D'Anjou scion is trained as a free-standing central leader, 10 feet apart in the row. In Cashmere, the D'Anjou trial is trained on an upright trellis, 6 feet apart in the row. In Tonasket, the Golden Russet Boscs are on a similar trellis, and had significant production in their 4th season (see Table 4).

Bosc-2005 Planting Tonasket (on a trellis)	**2008 Pounds Fruit/ Acre, Fourth Year**	**2008 44 lb. Box/ Acre, 95% Packed**	**2008 Average Box Size 44 / Avr. Fr. Wt.**	**2008 Total 1100 lb. Bins Fruit / Acre**	**2007+ 2008 Total Bins Fruit / Acre**	**2008 Trunk Cross Sectional Area in CM^2**	**2008 Lbs. Fruit / Tree**	**2008 lbs. Fruit per CM^2 of Trunk (Efficiency)**
OHxF 87	14,780	319	68	13.4	20.1	30.1	24.4	0.81
Pyro 2-33	9,060	196	67	8.2	10.3	20.1	15.0	0.75
Pyrodwarf	9,238	199	78	8.4	12.7	29.3	11.3	0.39
BM 2000	9,937	215	87	9.0	12.1	29.7	16.4	0.55
Horner 4a	6,844	148	70	6.2	2.9	28.1	15.3	0.54
BU-3	2,334	50	62	2.1	3.4	11.0	3.9	0.35
Bartlett Horner 4a	10,231	209	75	9.3	12.6	20.2	16.8	0.83
2002 Bosc in 4th Leaf OHxF 87	*10,123*	*218*	*74*	*9.2*	*11.3*	*31.9*	*22.8*	*0.71*

Table 2-1. 2005 planting of Golden Russet Bosc pear, Tonasket, (4th season), 6 x 12 ft. on 4-wire upright trellis, ineptly trained. Yield, extrapolated yield, fruit size, trunk size and efficiency. Bartletts are pollenizers. Note comparison of 4th leaf results in the 2002 planted trial, lower row of table.

Next Steps:
There is currently a flower bud set that may lead to great differences next year amongst the trellised trees in the 2005 planting. Some rootstocks in the 2005 trial are duplicates of those that performed well in the 2002 trial, so comparisons will be made between their production on wire vs. a free standing central leader training system. If carried to completion, the 2005 planting may be contrasted economically to the 2002 free-standing plot. It is possible that the trellis system, in this case, may be less profitable than the free-standing tight planted tree wall.

If no new 2005 trial rootstock stands out by the end of the 6th leaf (fall 2010), this trial may be terminated or greatly scaled back a year earlier than planned, with data taken in the 7th year only from the best performing two or three root/scion candidates. These data would be used to compare the economics of free standing vs. simple trellis systems for production of Bosc pears on this site.

Executive Summary:
In 2002, a pear rootstock trial was set up in four locations in Washington and Oregon to look at the effect of various semi-dwarfing pair rootstocks produced by breeding programs from around the world. There were two compelling reasons for these trials: 1. It was apparent to many leaders of the industry that many of the problems faced by pear growers were due to large tree size and vigor. Insect management, fruit rot management, reduction of fruit calcium disorders, pruning thinning, and harvest; all were made much more difficult by large tree size induced by vigorous rootstocks. 2. Essentially all previous rootstock work had been done in Hood River, and there were some concerns that Hood River growing conditions did not reflect those of the Yakima Valley or the Wenatchee District. D'Anjou, Bartlett and Golden Russet Bosc were chosen as scion varieties due to their predominance in the industry. OHxF 87 rootstock was chosen as the trial standard, as it was the most common semi-dwarfing pear rootstock being used by the industry at that time. The other rootstocks came from German, English, USA and Italian rootstock breeding programs. For representative trial sites were selected: Hood River – D'Anjou, Yakima Valley – Bartlett, Cashmere – D'Anjou, and Tonasket – Bosc. Ten of each rootstocks/scion were planted at each trial site, in ten blocks of seven trees. Bartletts on two test semi-dwarfing rootstocks were used as pollenizers in all except the Yakima site, Bartlett on OHxF 87 were added to the Cashmere site.

The trees have grown well on all sites except for the Bartletts in Yakima, which were so affected by the fire blight during their first five seasons that horticultural data was meaningless. Fortunately, the pollenizer Bartlett trees in Cashmere and Tonasket have produced interesting results on what are likely the two most promising rootstocks, OHxF 87 and Pyro 2-33. D'Anjou yields have been disappointingly low at the Hood River site, and have been almost nonexistent at the Cashmere site. Had it not been for the very good production and high-quality data generated by the Tonasket Golden Russet Boscs, this trial would have been discontinued in 2006 or 2007. We continue to be encouraged by the results in Tonasket, but after seven seasons, we will discontinue taking yield and fruit size data from all but two of the 2002 planted rootstocks, OHxF 87 and Pyro 2-33.

Due to difficulties encountered in propagating the trees for the 2002 planting, four rootstocks were not placed in these trials until 2005. This portion of the project will be described more thoroughly in the proposal for the continuation of this project. Much more data was gathered than can fit into this report. See the author for more details.

Impact of This Work:
There were at least four significant outcomes to this project:

A number of potential rootstocks, including one that was being sold commercially in Washington and Oregon, were shown to be inferior due to disease or cold injury susceptibility, comparative yield, fruit size, the production of thorny root suckers, or a combination of these attributes.

The OHxF 87 performed well enough in the Golden Russet Bosc trial to become the industry standard semi-dwarfing rootstock until something better comes along. Nurseries responded by growing more.

Bartlett on Pyro 2-33 appears superior to Bartlett on OHxF 87, and especially to those on Pyrodwarf.

Some "traditional" pear growers have changed to semi-intensive planting systems due to horticultural field tours centered on these trials, others may soon follow.

EXECUTIVE SUMMARY

Thermofogging

This has been a joint project with Pace International to determine how best to apply the antioxidant ethoxyquin to Anjou pears in storage using thermofogging. This partnership has been funded for 2 years by the Fresh Pear Committee and Pace International. This initially was planned as a 3 year project, but in the view of the researchers, there is little additional research that can be done on a small scale due to facilities and engineering considerations.

Each thermofogging machine must be calibrated to the size of the storage room in order to determine the correct dosage. Therefore, the first goal was to tune the operating parameters of the thermofogger, so that the residue of ethoxyquin was appropriate and repeatable. This was difficult due the small size of the 40-bin CA rooms at Stemilt. Modifications in equipment and setting up the experimental chamber required numerous experiments using cull pears before the dosing was repeatable.

We determined that location of the bin in the stack, and fruit location in the bin affected residue. Residue on fruit in the topmost bins in the stack often exceeded legal residue limits, which was alleviated when those bins were covered. In initial applications in which relatively low residues were detected, fruit at the top of the bin had minimal scald, while fruit within the bin developed more scald due to insufficient residue. This led to awareness about the lack of information on the appropriate residue level necessary for scald control and the rate of ethoxyquin degradation over time.

In a series of experiments, we determined: 1) ethoxyquin residue levels must be 1.0 ppm or greater to control scald, 2) a delay of more than 2 weeks in the initial application after harvest resulted in serious skin burn, 3) a light initial application close to harvest followed by a second application 60 days later reduced skin burn, and 4) high residues measured after the second application dissipated to acceptable levels after long-term CA storage.

In an effort to reduce the excessive residue on the fruit in the topmost bins, we tried various covers including porous fabric stapled to the bins, sheet plastic stapled to the bins, plastic sheets elevated over the bins, and wooden pallet bottoms covered in plastic. All cover types prevented excessive ethoxyquin residues in top bins. However, plastic sheeting tightly stapled to the tops of wooden bins restricted penetration of fog so that insufficient residues were achieved. This effect was not seen when plastic bins were used. Open structured covers (bin bottoms, pallets covered with plastic and shade cloth) were each acceptable. Bin type (wooden or plastic), room ventilation and room fans did not improve chemical dispersion into the topmost bins.

Distribution of ethoxyquin residues was not affected by bin position in the stack except when the topmost wooden bins were tightly covered with plastic sheeting, which significantly reduced residues. Pressure venting systems, manifolds and pulsed fans did not affect residue distribution among or within bins.

The small room size with high power fans presented challenges that may not be present in large commercial rooms. The thermofogging unit adapted for the research rooms also presented a situation that might not represent a commercial operation. Thus this research cannot proceed beyond what we have accomplished so additional funding is not being requested.

Split Drench

Liquid ethoxyquin was applied as a drench together with a fungicide, or as two applications in which the ethoxyquin was applied followed by a second drench with the fungicide up to 56 days later. This split application may prove to be a useful method of controlling scald while reducing levels of phytotoxicity. Data thus far are positive with regard to fungicide and ethoxyquin residue levels, effective control of scald and reduced phytotoxicity. Fruit from the 2008 crop will be examined in the spring of 2009.

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

Project Title: Gene discovery & controlled sport induction (CSI) for pear improvement

PI: Amit Dhingra
Organization: Washington State University
Telephone/email: 509 335 3625, adhingra@wsu.edu
Address: PO Box 646414
City: Pullman
State/Province/Zip WA 99164

Cooperators: Fred Bliss, Bruce Barritt, Herb Aldwinckle and Mickael Malnoy

Total project funding request:	Year 1:	Year 2:	**Year 3:**
	54,300	59,492	**63,252**

Budget 1:
Organization Name: Washington State University **Contract Administrator:** Mary Lou Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	Year 1: 2007/08	Year 2: 2008/09	**Year 3: 2009/10**
Salaries[1]	30,000	31,200	**32,448**
Benefits[2]	12,300	12,792	**13,304**
Wages			
Benefits			
Equipment			
Supplies[3]	10,000	11,000	**13,000**
Travel[4]	2,000	2,000	**2,000**
Sequencing		2,500	**2,500**
Miscellaneous			
Total	54,300	59,492	**63,252**

Footnotes:
[1]Post Doc for 12 months at 1.0 FTE.
[2]Benefits are calculated at 41%.
[3]Supplies: RNA kits and mutation reagents
[4]Travel: sample collection

There is an urgent need to develop a *dwarfing pear rootstock* and improve *post-harvest quality* and storage abilities of pear varieties grown in the Pacific Northwest. However, narrow germplasm diversity in pears precludes the use of existing varieties from pear improvement programs. Only source of new and successful commercial varieties have been the random sports such as Red Anjou and Red Bartlett. In order to overcome this bottleneck, we had proposed integrating two novel techniques, *Controlled Sport Induction and Gene Discovery*, to aid in the improvement of pear focusing on post-harvest issues and plant architecture. The following report discusses the progress that has been made with these technologies to facilitate improvement of pears.

OBJECTIVES: Proposed objectives of the project were:

1. *Prioritization of economically important pear traits*.

   Progress: The Northwest pear industry is in urgent need of a dwarfing, precocious rootstock similar to the ones that revolutionized the apple industry. Furthermore, producing pears with long post-harvest storage abilities is of utmost importance. The priority areas were selected based on discussions with members of the pear industry and these feature as the high priority areas in research concerns for 2008 (http://www.treefruitresearch.com/nw-pear-review).

2. *Gene discovery for establishing trait-gene relationships using an economical yet high-throughput methodology called Differential Display*

   Progress: In order to enable gene discovery, experiments are performed to analyze the message produced by the genetic material in individual pears. This message called RNA (derived from the genetic material DNA) impacts the physiology of the fruit. The peel and the cortex represent two contrasting sites of physiological activity that determines a fruit's pre and post-harvest condition. Thus core and peel samples from Bartlett and D'Anjou pear were collected over the developmental continuum starting from 30 days after pollination to maturity. These samples have been processed to analyze the RNA. Fruit samples are not amenable to RNA extraction due to the presence of sugars and other complex compounds. With the help of an equipment grant from WSU, we have acquired a freezer mill that overcomes this problem. Thus RNA extraction from fruits has been standardized representing the first successful step towards performing gene discovery experiments using differential display. We have acquired next generation sequencing technology in the lab that is worth $650,000. It enables performing differential display very efficiently and in very less time.

3. *Controlled Sport Induction using tissue culture derived propagules combined with high-throughput screening of allelic diversity for genes responsible for desirable trait.*
   Progress: We have used D'Anjou and Bartlett tissue to produce propagules that will form the starting material for both controlled and random sport induction. The tissue culture material has been established and suspension cultures are being continually grown to reach quantities where we can begin sport induction. In order to perform the CSI experiment, we have obtained the equipment vital to carry out these procedures. This was made possible due to the equipment grant from WSU to the PI. This experiment will provide for a proof of concept of controlled sport induction in pear and provide for a quicker way to evaluate genetic factors affecting flowering and fruit development. We have also established leaf based regeneration from pear leaves that will be utilized for targeted sports induction.

   Proposed goals and objectives for 2009:

- Create full-length cDNA libraries for selected pear peel and core samples.

- Discover genes expressed during pear fruit development using next-generation sequencing techniques in D'Anjou and Bartlett cultivars.
- Optimize pear bud, leaf and rootstock tissue culture regeneration systems for D'Anjou and Bartlett cultivars through the use of solid and liquid media
- Perform random mutation experiments and CSI associated with desirable pear traits like juvenility and non-browning

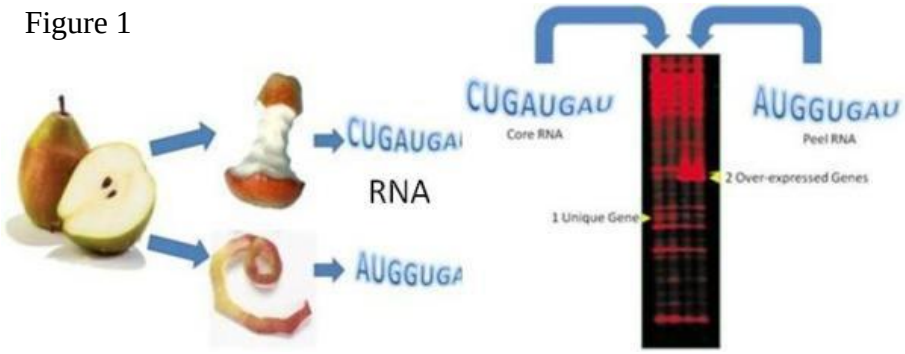
SIGNIFICANT FINDINGS

- RNA extraction from peel and core tissue of pear optimized using SPEX SamplePrep Freezer mill.
- Next generation differential display with the 454 standardized.
- Optimal regeneration of pear shoots from bud samples and leaf occurred on a modified N6MS medium. Regeneration from fruit tissue is sluggish that can increase the chances of somaclonal variations.
- The RITA system (temporary immersion) shows a potential to significantly increase the amount of pear tissue on hand for performing CSI experiments.

METHODS

The methods employed in gene discovery in pear are depicted in Figure 1. Peel and core samples were taken from fruit sterilized with ethanol.

Figure 1



RNA was isolated from the ground tissue using a Qiagen RNA extraction kit or other improved protocols. This season's material has been processed with the Qiagen kit. Differential display has been performed using the isolated and quality tested RNA**.** Besides the gel based differential display, we have performed comparative RNA profiling using the 454 next-generation sequencer. It provides sequence based information on genes and represents the entire transcriptome at the same time. In short we can capture the response of the entire transcriptome in one shot. Figure 2 below (graph) shows such a snapshot where a cluster represents a single gene and read frequency indicates its abundance. The bars in blue and red represent two different genotypes.

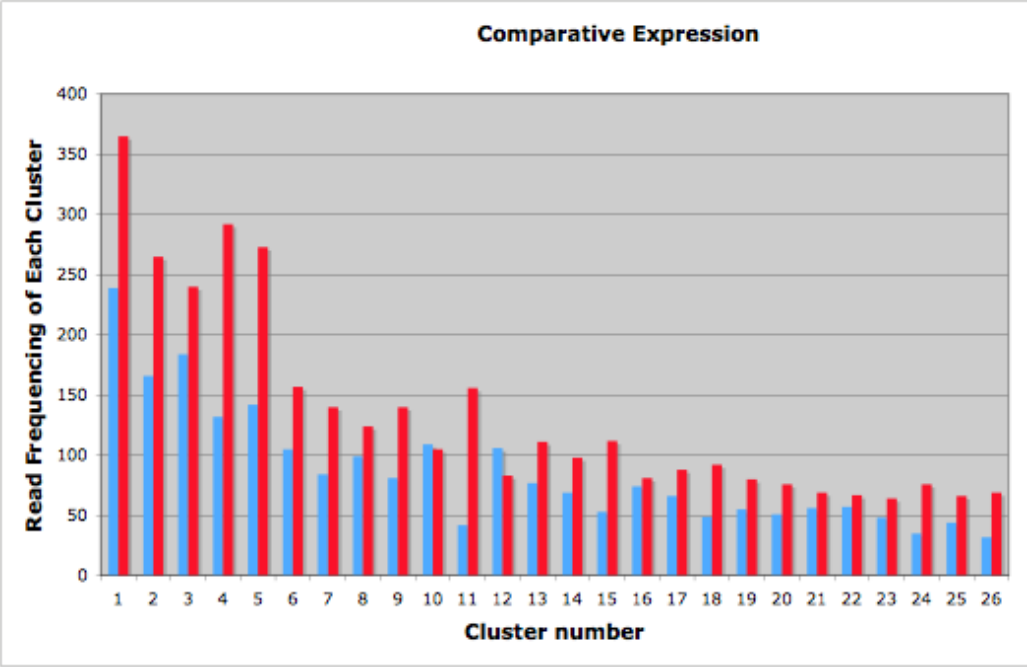


Figure 2: Quantitative transcriptome profiling using 454 technology

CSI experiments: Although suspension cultures have been established for Bartlett and D'Anjou we have incorporated a unique concept of targeted mutation induction using leaf material. This will be performed with the gene gun and is going to be more rapid than the radiation process. We have two initial targets - reduction of juvenility and non-browning. Some of these mutants can be directly utilized as new varieties or in the breeding program. The mutations are induced by transiently introduction of DNA-RNA hybrid molecules as shown in Figure 3 above. Bases in dark have been modified to induce mutations.

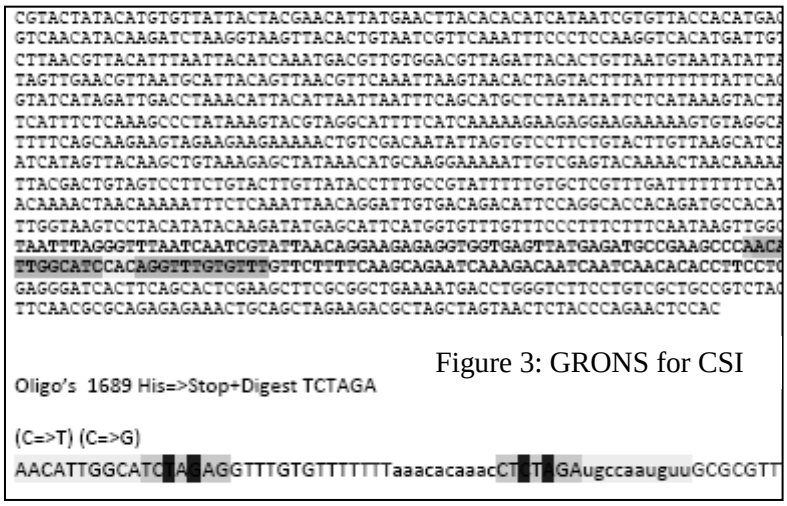


Figure 3: GRONS for CSI

RESULTS AND DISCUSSION

The differential display experiments performed on the gel and the next generation sequencer have provided enormous amount of information that is currently being distilled and modeled into fruit-specific pathways. This exercise will enable us to identify the genes that are pertinent to traits related to fruit development and quality. Samples of Bartlett and D'Anjou pear have been utilized for the gene identification project. Development of callus and suspension cultures and regeneration from leaf

and meristem tissue has been optimized for both Bartlett and D'Anjou. We have initiated the CSI experiments in pear.

New varieties of pear can be tested commercially after the complete procedures of this technology are worked out. As this approach involves no transgenic modification, there will not be any issues with implementing this technology. During mutagenesis (sport induction) some deleterious mutations may also be generated, but can be eliminated in the segregating population. The clonal variants will also serve as defined donors or parents of desirable traits for Marker Assisted Breeding. Materials developed using this technology may offer opportunities for new intellectual property in the form of novel clonal variants. We plan to attract long-term federal funding for continued pear improvement after generating the initial controlled sport induction.

Outreach:

1. The work and the ideas underlying this project were featured in the invited presentation at the USApple annual convention in August 2007 to communicate the concepts to the stake holders.
2. The work was presented in an invited talk at the AEMP 2007 meeting in Portugal in September 2007 and AEMP 2008 meeting in Bangalore, India in December 2008.
3. The concepts and progress on the project was presented at the WSHA meeting in 2007 and 2008 by Scott Schaeffer and Laura Burke, graduate students in the Dhingra lab.
4. This work was presented at the Annual Rosaceae Genomics Conference in Chile in March 2008 and American Society of Plant Biologist annual meeting in July 2008.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-07-702

Project Title: Quantifying biological control of pear psylla in a cover crop system

PI:	David Horton	**Co-PI(2):**	Tom Unruh
Organization:	USDA-ARS	**Organization:**	USDA-ARS
email:	david.horton@ars.usda.gov	**email:**	thomas.unruh@ars.usda.gov
Address:	5230 Konnowac Pass Rd.	**Address:**	5230 Konnowac Pass Rd.
City:	Wapato	**City:**	Wapato
State/Zip	WA 98951	**State/Zip:**	WA 98951

Co-PI(3): Vince Jones
Organization: Washington State University
email: vpjones@wsu.edu
Address: TFREC
City: Wenatchee
State/Province/Zip WA 98801

Total funding request: **Year 1:** $25,000 **Year 2:** $20,000 (revised) **Year 3:** $0 (revised)

Other funding Sources

Agency Name: Western SARE
Amount funded: $121,092 (2008-2009)
Notes: Expands the current project to include 3 commercial organic pear orchards.

Agency Name: WSU (CSANR): Organic Cropping Research for the Northwest
Amount funded: $34,178 (2009)
Notes: Expands the current project to include 3 commercial organic pear orchards.

Budget 1:
Organization Name: USDA-ARS **Contract Administrator:** Bobbie Bobango
Telephone: 509-454-6575 **Email address:** bobbie.bobango@ars.usda.gov

Item	2007	2008 (revised)	2009 (revised)
Salaries	11,750	12,500	
Benefits	2,180	1,000	
Wages	4,500		
Benefits	500		
Supplies	1,070	1,500	
Total	20,000	15,000	**0**

Budget 2:
Organization Name: WSU-TFREC **Contract Administrator:** Mary Lou Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	2007	2008	2009 (revised)
Salaries[1]	3,148	3,273	
Benefits	1,133	1,178	
Supplies[2]	719	549	
Total	5,000	5,000	0

COMMENT ON FUNDING: We have been funded by Western SARE and CSANR-WSU (see above), and consequently have eliminated our 2009 funding request to FPC/PPC.

OBJECTIVES:

1. Estimate levels of psylla biological control in large plots of an alfalfa cover crop vs control (grass understory) plots;
2. Estimate movement rates of predators from orchard floor to tree and determine whether colonizing predators will then attack pear psylla (by simultaneous use of protein markers [Jones] and gut contents analysis [Unruh]).
3. Test whether alfalfa cover crop leads to increased nitrogen in trees having the alfalfa understory.
4. Expand project into 3 commercial organic orchards (funding by SARE and CSANR).

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS:

- Densities of generalist predators were 8-fold higher in understory of alfalfa plots than grass plots.
- Despite the high densities of predators in the alfalfa cover crop, we found no statistical increase in densities of predators in the canopy of trees having the alfalfa understory, and no effects on psylla densities.
- Over 3000 specimens were collected from orchard floor and tree canopy for assessment of marker presence. The specimens are currently being assayed (with ELISA). The data will tell us whether there is evidence for predator movement from cover crop into tree.
- Specimens are currently being analyzed with ELISA for gut contents (to assess presence of pear psylla remains). We observed a trend towards increased nitrogen levels in trees having the alfalfa understory.
- Funding was obtained from Western SARE ($121,092) and CSANR-WSU ($34,178) to expand project into 3 commercial organic orchards; plots were set out and planted in spring 2008.

METHODS:

Plot design. The studies are being done at the Moxee farm (5-8 year old Bartlett trees). We have 4 blocks, each composed of an alfalfa cover crop plot and a control grass plot (Figure 1).

Psylla and predator densities. We monitor densities of prey and predators in trees and orchard understory. Pear psylla numbers are monitored with beat trays and leaf samples (eggs and nymphs). Predator numbers are assessed using beat trays (trees), sweep nets (understory), and sticky traps; the sticky traps are placed at two heights: 1 foot and mid-tree canopy. Tree samples are limited to the 21 interior trees in each plot (shown by shading for two of the 8 plots in Figure 1). All 4 aisles in each plot are swept for understory samples.

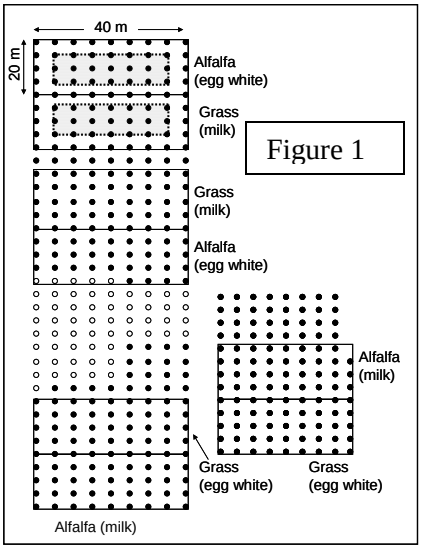


Figure 1

Protein marker methods. The cover crop and grass control plots are sprayed with a 10% liquid egg white solution or 20% whole milk solution, splitting the two markers so that both cover crop and grass control plots receive both types of marker (see Figure 1); this design was chosen to overcome

differences in marking efficiency of the egg and milk markers. The solutions are sprayed using a 25 gallon weed sprayer attached to an ATV, fitted with a 3 meter long boom having 7 flat fan tip nozzles.

Predators are collected from the tree by jarring limbs with a rubber hose, and trapping the dislodged insects on a section of cardboard that has been coated with a thin layer of tanglefoot. The predators are removed from the adhesive in the field using wooden toothpicks, and transferred singly into 1.5 ml microtubes. Similar methods are used to obtain arthropods from the ground covers, except that the vegetation is shaken over the top of the cardboard sheet.

Microtubes containing the insects and spiders are washed in 1 ml of TBS buffer solution. The buffer is then aspirated from the tube, placed into a second microtube, and shipped frozen to Vince Jones to assay for presence of marker proteins using ELISA. The insect specimen are transferred to a new tube and given to Tom Unruh for gut contents assessment. Both tubes (insect and associated buffer wash) receive identical labels, so that presence of a particular marker protein can be linked to results of the gut contents analyses.

Leaf nitrogen. Pear leaves were collected from control and cover crop plots for N-analysis using sampling methods recommended by Michigan State University Extension. Leaf nitrogen was determined using the Bradford assay for soluble nitrogen, modified for analysis of plant tissues. Sampling was limited to a single date in July, to develop methods. More rigorous sampling will be done in summer 2009 to include additional dates.

Expand project into 3 commercial organic orchards. We are collaborating with 3 commercial organic growers in the Yakima valley. In spring 2008, we planted 0.5 meter wide strips of alfalfa in each of 3 plots at each orchard. Replicated control (grass) and alfalfa plots were established within each orchard. In 2009, we will monitor pest and predator densities, predator movement (using sticky cards), and leaf nitrogen in control and alfalfa plots.

RESULTS AND DISCUSSION:

Psylla and predator densities. Generalist predators in the tree canopy and orchard understory were dominated by true bugs, ladybird beetles, green lacewings, and spiders. Densities of predators in the orchard floor vegetation were over 8-fold larger in the alfalfa plots than the grass plots (Fig. 2). There was a significant presence all season of predators in the alfalfa cover crop, except immediately following mowing (Fig. 2).

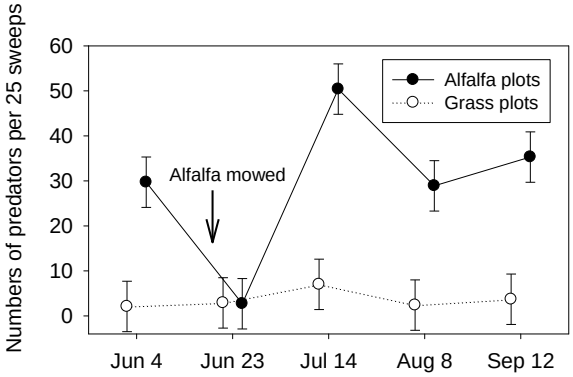
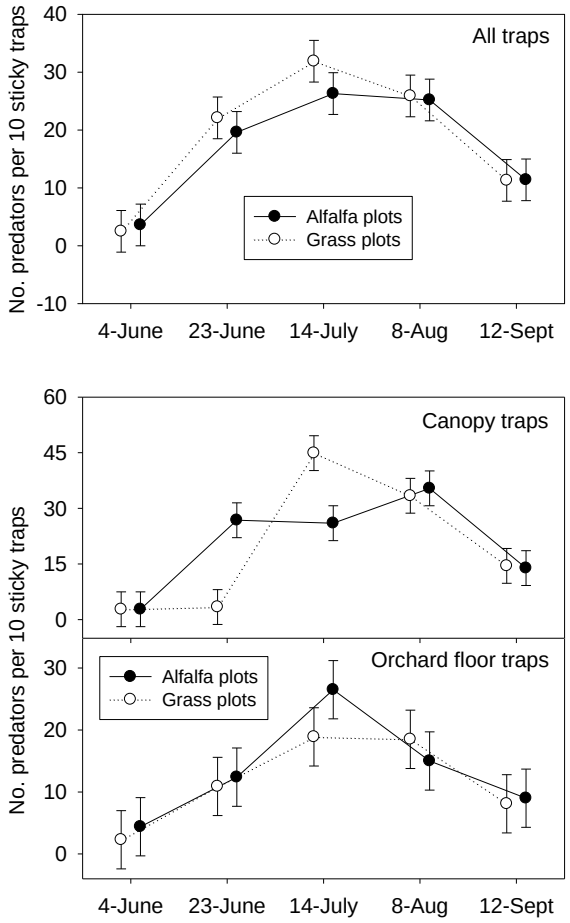


Figure 2. Sweep net samples

Sticky trap catches of generalist predators are shown for canopy-height traps (Fig. 3, upper panel), ground-level traps (Fig. 3, middle panel), and combined heights (Fig. 3, lower panel). Numbers of predators on traps were similar in the alfalfa and grass plots (Fig. 3). Trap catch was dominated by the true bugs and lacewings.

Figure 3. Sticky trap samples



Tray counts of predators were, if anything, larger in the grass plots than the alfalfa plots (Fig. 4), results that are similar to those in 2007. Because predator counts in the floor vegetation were so much larger in the alfalfa than grass plots (Fig. 2), the beat tray results suggest that there was not much movement by predators from alfalfa into the tree canopy.

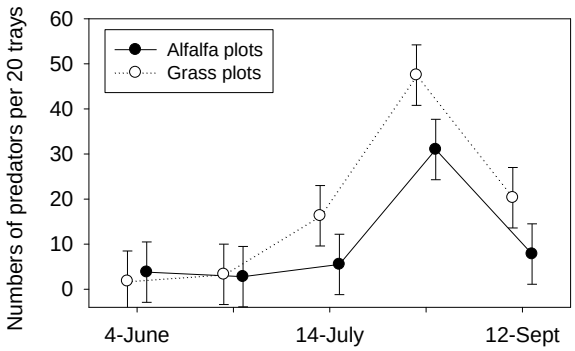


Figure 4. Predator beat tray counts

Densities of immature psylla were statistically similar in grass and alfalfa plots (Fig. 5). Tray counts of adults were also similar in grass and alfalfa plots (Fig. 6).

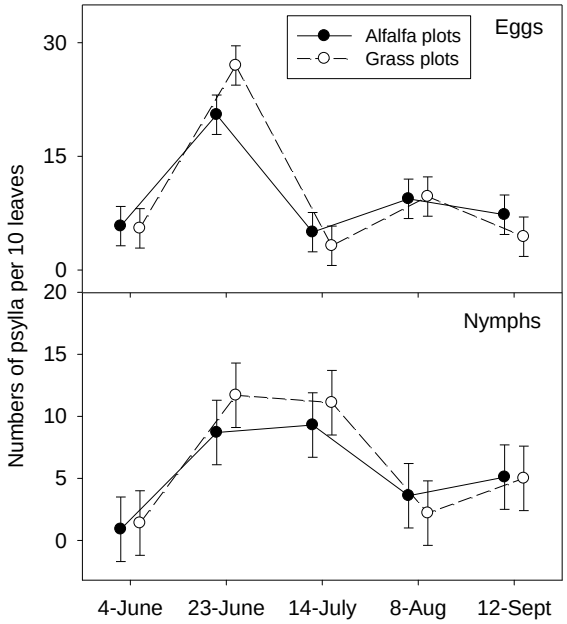


Figure 5. Immature psylla: leaf counts

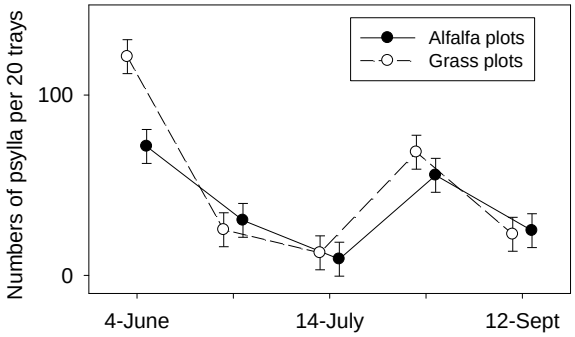


Figure 6. Adults psylla: beat tray counts

Marker results. Almost 4,000 specimens were collected from tree and ground cover to assay for marker presence. The specimens are still being assayed.

Gut contents results. Specimens are still being assayed.

Leaf nitrogen. Pear leaves were collected from each plot on July 30, and assayed for nitrogen content. Our objectives this year were to develop and test our methods, rather than to assess seasonal trends in nitrogen, thus our collection was limited to a single date. In 2009, we plan to sample on several occasions over the growing season, and to sample also in the commercial orchards. Results suggest strongly that an alfalfa understory led to increased levels of nitrogen in the pear tree canopy (Fig. 7).

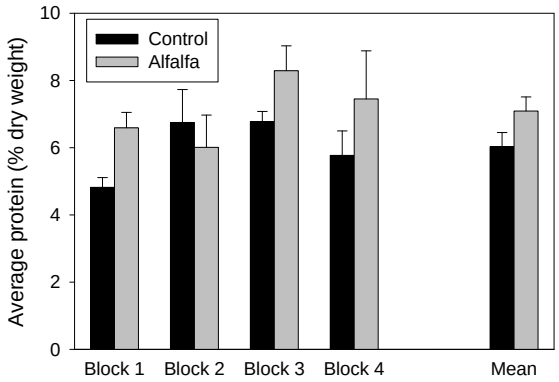
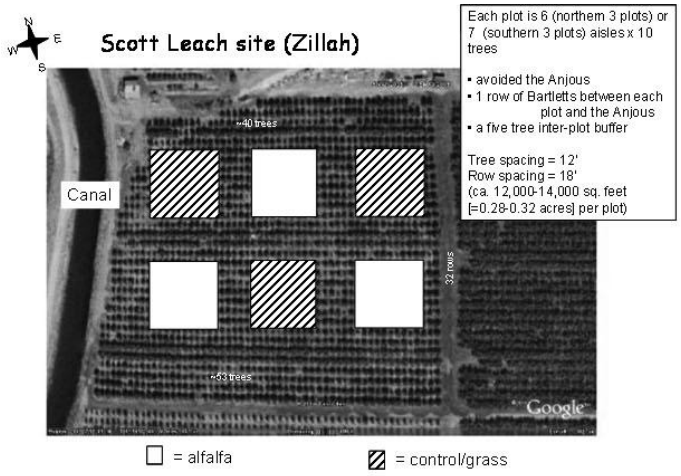
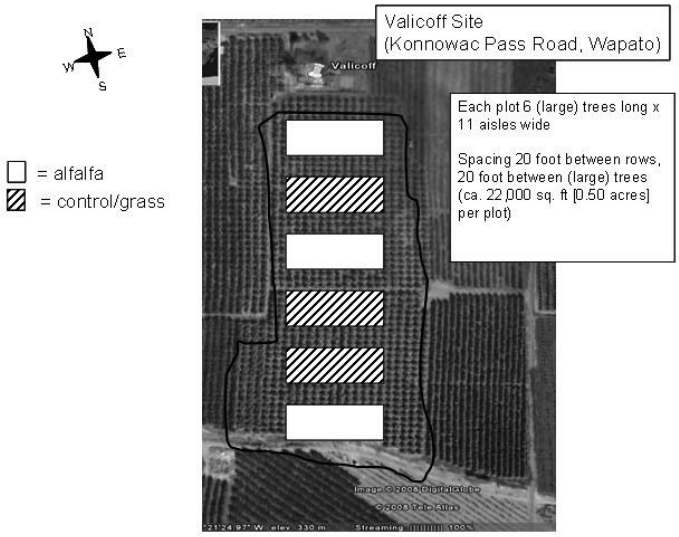
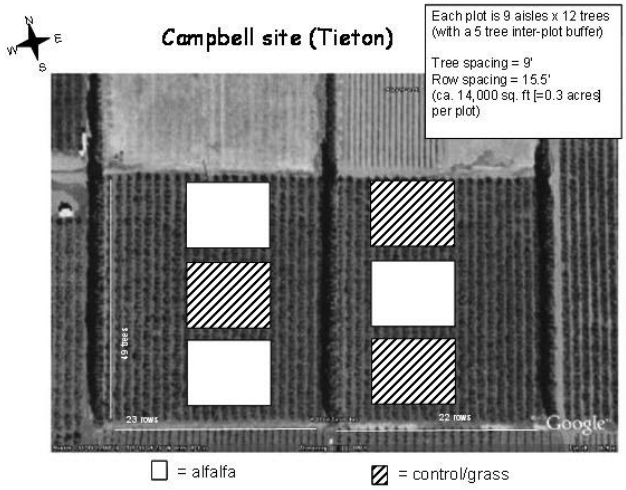


Figure 7. Foliar nitrogen (by % dry weight) in pear leaves from control and alfalfa plots.

Commercial orchards. Replicated control and alfalfa plots were established in 3 commercial organic orchards (Fig. 8). Plots were each 0.3 to 0.5 acres in size. The alfalfa was planted in April 2008 as ½ meter wide strips down the centers of aisles.







CONTINUING PROJECT REPORT **YEAR**: 1 of 2

Project Title: Volatile sex attractants in pear psylla

PI: David Horton
Organization: USDA-ARS
email: david.horton@ars.usda.gov
Address: 5230 Konnowac Pass Rd.
City: Wapato
State/Zip WA 98951

Co-PI(2): Pete Landolt
Organization: USDA-ARS
email: peter.landolt@ars.usda.gov
Address: 5230 Konnowac Pass Rd.
City: Wapato
State/Zip: WA 98951

Co-PI(3): Christelle Guédot
Organization: USDA-ARS
email: christelle.guedot@ars.usda.gov
Address: 5230 Konnowac Pass Rd.
City: Wapato
State/Province/Zip WA 98951

Total funding request: **Year 1:** $15,000 **Year 2:** $15,000

Other funding Sources

Agency Name: USDA-CSREES-NRI
Amount funded: $233,473 (FY 2006-2008)
Cooperator: Jocelyn Millar (University of California, Riverside)

Agency Name: Binational Agricultural Research and Development Fund (BARD)
Amount funded: $273,000 (FY 2008-2010)
Cooperators: Vicky Soroker and Anat Zada (Volcani Center, Israel)

Budget
Organization Name: USDA-ARS **Contract Administrator:** Bobbie Bobango
Telephone: 509-454-6575 **Email address:** bobbie.bobango@ars.usda.gov

Item	2008	2009
Salaries[1]	$11,500	$11,500
Benefits	$ 3,500	$ 3,500
Total	$15,000	$15,000

[1]**Partial support for GS-6 technician; benefits at 30%**

OBJECTIVE:

- Test for attractiveness to male pear psylla compounds extracted and identified from surface washes of psylla or from collections of headspace volatiles.

SIGNIFICANT FINDINGS:

- Demonstrated that surface extracts from females are as attractive to males in an olfactometer as an equivalent number of live females.
- Demonstrated attraction of males to volatiles collected from headspace of females.
- Demonstrated repellency to males of live males, surface washes of males, and headspace volatiles of males.
- Used GC-MS to identify several compounds abundantly present in washes of females but either absent from males or present in substantially lower concentrations in males. Trials are ongoing in the olfactometer with these sex-specific products. One compound has been assayed, and shown to attract male summerforms.
- Made progress in developing electroantennogram methods.

METHODS:

Whole body cuticular extracts are obtained by washing known numbers of psylla in pentane. Headspace volatiles are collected by placing known numbers of psylla in a gas collecting jar, and drawing purified air through the jar. The volatiles are collected on Super Q traps, and extracted in solvent. Both types of extracts are assayed for biological activity by applying the extract to filter paper disks, and assaying disks in the olfactometer against solvent controls. We use GC-MS to compare chemical profiles of extracts obtained from males and females; compounds shown on a chromatogram to be common in one sex but not the other sex are of interest. Compounds of interest are identified and synthesized, and then tested for biological activity in the olfactometer. Electroantennogram response to psylla-infested pear foliage was successfully demonstrated; the methods will be used eventually to assay headspace volatiles collected from psylla.

RESULTS AND DISCUSSION:

Olfactometer trials with cuticular extracts. Pentane extracts of female psylla were attractive to male psylla in olfactometer assays (**Figure 1: top chart**). Conversely, males were repelled by extracts of other males (**Figure 1: middle chart**), which appears to be the first example of male-male repellency in any psyllid species. The female extract was statistically as attractive to males as an equivalent number of live females (**Figure 1: bottom chart**).

GC-MS. A GC-MS analysis of extract from female and male psylla identified 6 compounds present in washes from females (labeled F1-F8 in **Figure 2**) but not present in males or present only in very low concentrations, and 3 compounds (labeled M1-M3 in **Figure 2**) specific to males.

Olfactometer trials with compound F2. The compound labeled "F2" in **Figure 2** was identified and synthesized, and applied in solvent to filter paper disks. The compound was attractive to male psylla in the olfactometer (**Figure 3**).

Olfactometer trials with extracts from headspace volatiles. Headspace volatiles were attractive to males in olfactometer assays (**Figure 4: top chart**). As with the cuticular extracts, volatiles from males repelled males (**Figure 4: bottom chart**).

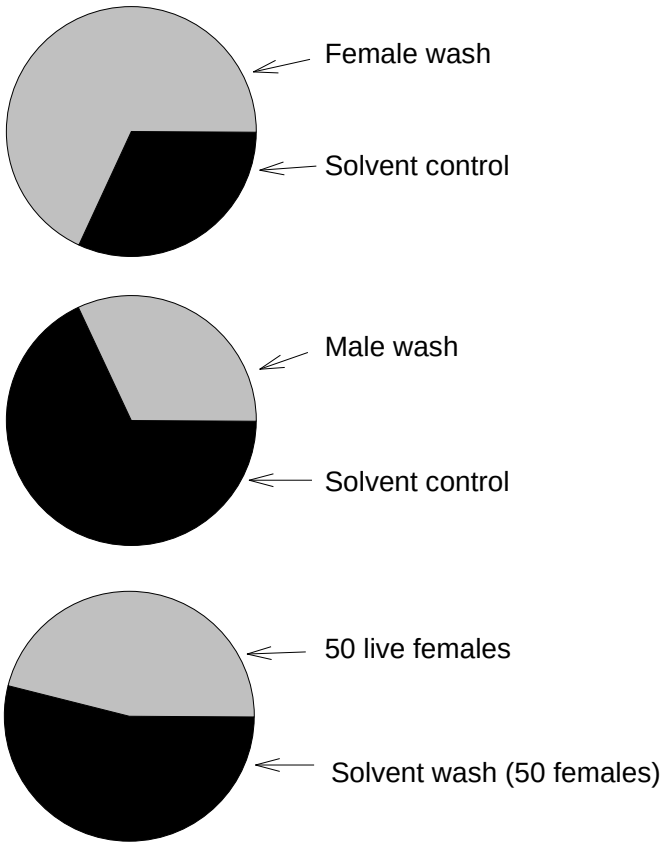
EAG methods. We have successfully developed methods for attaching antennae of pear psylla to an electroantennograph (**Figure 5**), and succesfully demonstrated physiological response by antennae to volatiles from psylla-infested pear foliage (**Figure 6**). Future work will include an assessment of antennal response to extracts or to synthesized chemicals.

PUBLICATIONS

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

Guédot, C., D.R. Horton, and P.J. Landolt. 2009. Attraction of male winterform pear psylla to female-produced volatiles and to female cuticular extracts with evidence of male-male avoidance. *Entomologia Experimentalis et Applicata* (in press).

Figure 1. Results of olfactometer trials testing attraction of male psylla to surface washes of females, surface washes of males, or live females. N is 100-120 males assayed per pie chart.



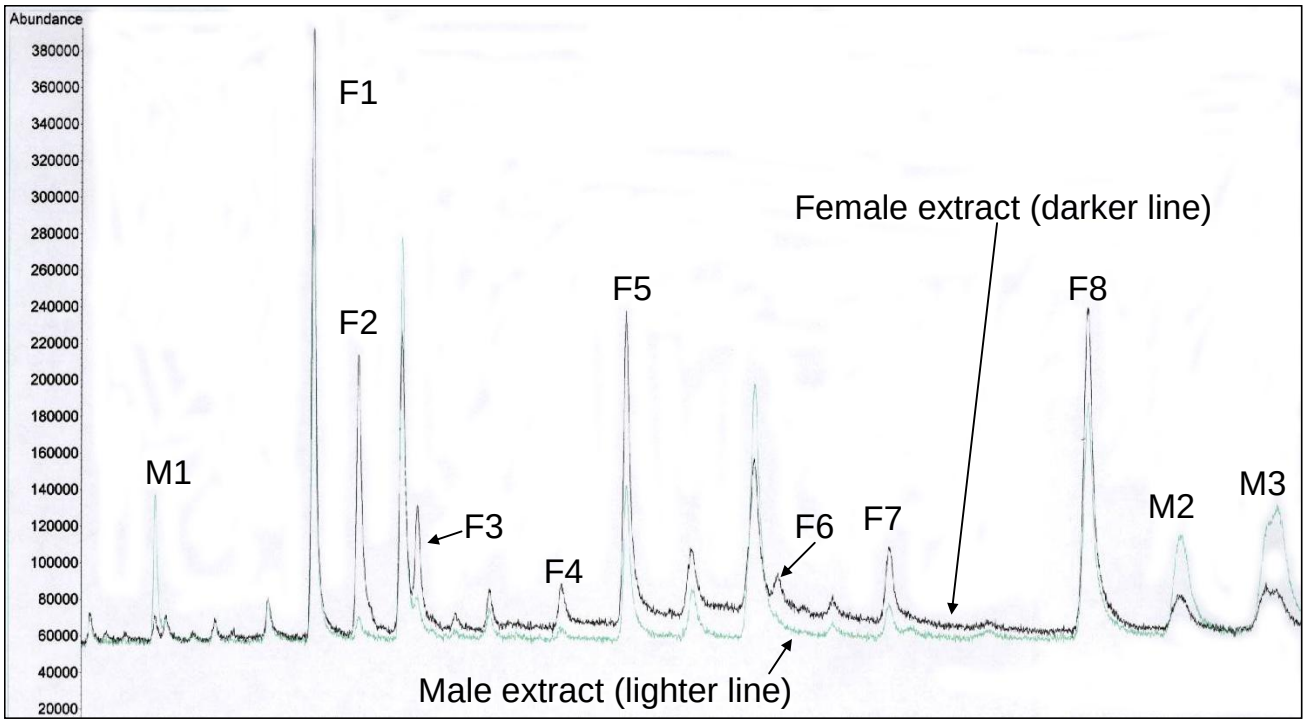


Figure 2. Chromatograms from GC-MS comparing cuticular extracts from female (dark trace) and male (light trace) psylla. M1-M3 are male-specific peaks; F1-F8 are female-specific peaks.

Figure 3. Results of olfactometer trials testing attractiveness of female-specific compound "F2" (see Figure 2) to male psylla. N is 100 males assayed.

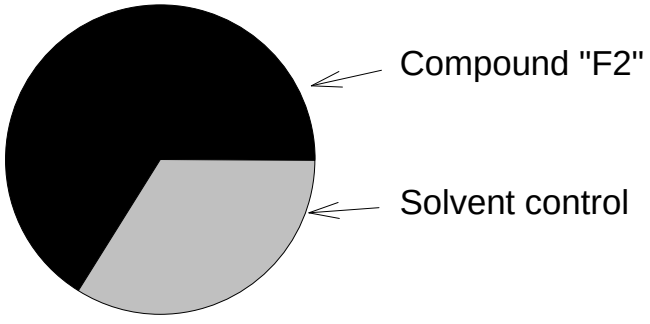
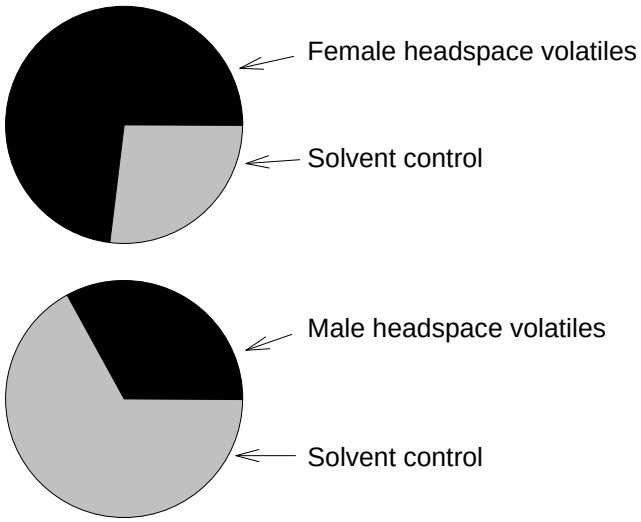


Figure 4. Results of olfactometer trials testing attractiveness of headspace volatiles to male psylla. N is 100 males assayed per pie chart.



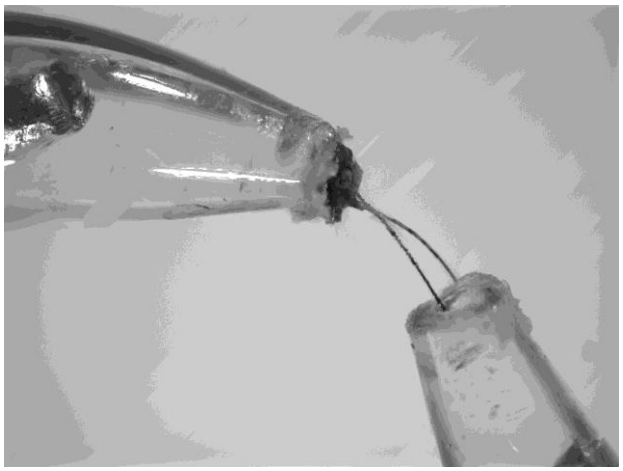


Figure 5. Head and antennae of a pear psylla positioned for EAG assay.

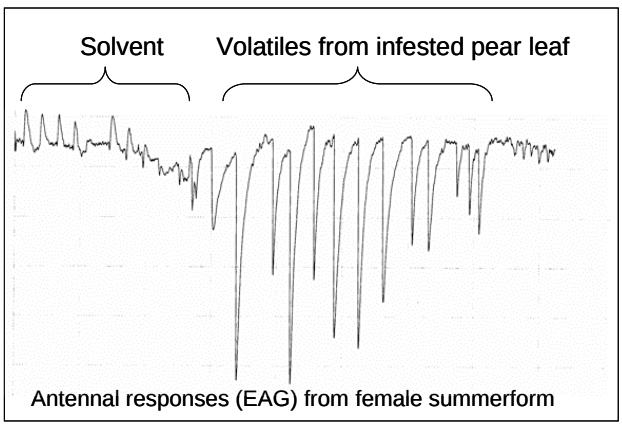


Figure 6. Antennal responses (EAG) by female psylla challenged with volatiles from psylla-infested pear foliage.

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

Project Title: Rapid detection of fire blight pathogen

PI: Ken Johnson
Organization: Oregon State University
Telephone/email: 541 737-5249
Address: Department of Botany and Plant Pathology
Address 2: 2082 Cordley Hall
City: Corvallis
State/Province/Zip OR 97331-2902

Cooperators: Todd Temple, Virginia Stockwell, 2nd years: David Sugar, Steve Castagnoli; additions in 3rd year: Bob Spotts, Clive Kaiser, Kent Evans (Utah), others.

Total project funding request: Year 1: $31,369 **Year 2:** $32,310 **Year 3: (this year) No Cost**

Other Funding Sources: Yes
Agency Name: UDSA Western Region Integrated Pest Management Competitive Grants Program
Amount awarded: $60K
Notes: Awarded for implementation research in ’09 and ’10.

WTFRC Collaborative expenses: None

Budget 1:
Organization Name: OSU Agric Research Foundation **Contract Administrator:** Dorothy Beaton
Telephone: 541 737-3228 **Email address:** dorothy.beaton@oregonstate.edu

Item			
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Total	**No Cost***		

Footnotes: *See information under ‘Other Funding Sources’

Objectives:

In 2008:

1. Design a LAMP reaction to detect small quantities of the fire blight pathogen based on primers from *E. amylovora*-specific DNA sequences.
2. Determine specificity and sensitivity of the designed LAMP reaction against a diverse selection of microorganisms commonly found in pear and apple orchards.
3. Determine the sensitivity of LAMP reaction when one flower with a natural infection of *E. amylovora* is added to 100 flower clusters.
4. Use the LAMP reaction to detect *E. amylovora* in flower samples from inoculated and non-inoculated orchard trees.
5. Use the LAMP reaction to detect *E. amylovora* in flower samples from commercial orchards.

In 2009:

1. Determine the sensitivity of LAMP reaction when one flower with a natural infection of *E. amylovora* is added to 100 flower clusters.
2. Use LAMP to detect *E. amylovora* in flower samples from inoculated orchard trees, and from surveyed commercial orchards in the Pacific Northwest.
3. Optimize sampling protocols for implementation by growers or farm service providers.

Significant findings:

- **We have developed two LAMP primer sets with high specificity to *E. amylovora*.** Two DNA primer sets are being used with field samples (from 40 that we evaluated). One set is targeted to plasmid pEA29 and the other to a chromosomal gene. LAMP reactions are highly specific for *E. amylovora*, and test negative with other bacteria recovered from floral washes.

- **Positive LAMP reactions were attained using a gradient of pathogen mixed with a gradient of flowers.** *E. amylovora* was spiked into flower suspensions at 0, 500 and 5000 CFU per ml resulting in positive LAMP reactions if the pathogen was present. LAMP reactions were negative in the zero pathogen suspensions. Floral density in the wash had no effect on pathogen detection.

- **Mixed LAMP results were attained after adding a single flower infested with 10^5-10^7 CFU of *E. amylovora* to 100 floral clusters.** Single, pathogen-infested flowers when mixed in 1.5 L water yielded concentrations of 1×10^2 to 5×10^4 CFU per ml. LAMP reactions were positive when *E. amylovora* populations were $\geq 1 \times 10^4$ CFU per ml. Concentrating the wash with a filter improved detection. A reduced volume of wash will be evaluated in 2009.

- **Positive LAMP reactions were attained from 100 flower cluster samples taken from experimental orchards inoculated with *E. amylovora*.** Moreover, LAMP reactions were negative for samples from non-pathogen-inoculated apple and pear orchards. Populations of indigenous bacteria in the washes ranged from 10^5 to 10^7 CFU/ml.

- **LAMP reactions accurately predicted fire blight infections in commercial orchards.** Flower samples were taken from 8 commercial orchards in the Rogue and Hood River valleys. LAMP reactions were negative in the four orchards in which the disease was not observed. Positive reactions were obtained in the four orchards with fire blight, although in 3 of the 4 orchards, the floral wash needed to be concentrated to achieve a positive test result. Optimizing the sampling of commercial orchards will be a focus in 2009 and 2010.

Justification: The goal of this project is to develop a rapid detection protocol for the fire blight pathogen, *Erwinia amylovora,* in pear and apple orchards. For this destructive disease, early detection of pathogen cells growing as epiphytes on flowers would improve the prediction of significant infection events, and correspondingly, increase the efficiency of protective sprays. Current methods for detection of epiphytic *E. amylovora* (stigma prints and PCR) are not used routinely due to time delays and costs required for laboratory processing. Our protocol will employ a new type of DNA amplification called loop-mediated isothermal amplification (termed 'LAMP'). Similar to PCR, LAMP utilizes specific primers to amplify DNA from a target organism. Unlike PCR, LAMP can be done under field conditions with a 12-volt power supply, and can detect as few as 25 copies of target DNA with a 60 minute reaction time. The epiphytic phase of *E. amylovora* is an ideal candidate for an early detection system, as pathogen must grow to a population size of ~10^5 cells per flower on a substantial number of flowers to cause a significant infection event. Literature reports on LAMP reactions show its application for rapid detection of protozoan parasites of amphibians, bacterial pathogens of fish, and the viruses that cause severe acute respiratory syndrome and west Nile encephalitis. Our objectives are: 1) to quantify the sensitivity of the LAMP method for detection of epiphytic *E. amylovora* in flower samples of various sizes, and 2) to develop an efficient orchard sampling scheme that will detect pre-infection populations of *E. amylovora* at levels expected to cause a significant infection event.

Methods:

Objective 1. Design a LAMP reaction to detect small quantities of the fire blight pathogen based on primers from *E. amylovora*-specific DNA sequences.

The *E. amylovora* specific pEA29 (accession AF264948) DNA sequence obtained from NCBI (http://www.ncbi.nlm.nih.gov/) and chromosomal sequence obtained from the Sanger Institute (http://www.sanger.ac.uk/) were used for generating primers by entering the sequence into the PrimerExplorer V4 software program (http://primerexplorer.jp/elamp4.0.0/index.html) which designs several sets of primers meeting the specifications for LAMP amplification.

A standard LAMP reaction in a 50 µl volume contained 2.4 µM (each) FIP (Forward Inner Primer) and BIP (Back Inner Primer), 0.2 µM (each) F3 (Forward outer primer) and B3 (Back outer primer), 1.4 mM dNTP's, 4 mM $MgSO_4$, 0.8 M betaine, 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 3 U of *Bst* DNA polymerase, and the template DNA (Notomi, et al., 2000).

Primer sets were tested for their ability to amplify *E. amylovora* DNA in LAMP reactions. Positive LAMP reactions produced a white precipitate indicative of amplification of target DNA (Fig. 1, below). In positive reactions, amplified products were sequenced and compared to pEA29 and chromosomal sequence used for LAMP primer design. LAMP primer sets targeted to pEA29 and chromosomal DNA of *E. amylovora* were chosen for continued evaluation based on specificity and sensitivity reactions.

Fig. 1. LAMP reaction tubes representing a positive (left) and a negative reaction (right) as indicated by turbidity of the magnesium pyrophosphate by-product when the target DNA of *E. amylovora* is amplified.

Methods (continued):

Objective 2. Determine specificity and sensitivity of the designed LAMP reaction against a diverse selection of microorganisms commonly found in pear and apple orchards.

E. amylovora at 0, 500 or 5000 CFU per ml concentrations were mixed with either pear or apple flowers at densities of 0, 10, 100 or 1000 in 3 L of water. A 1 ml sample of the cell/flower suspension was used for serial dilution plating onto CCT medium for enumeration of Ea153N and on Pseudomonas agar F (PAF, Difco laboratories) for enumeration of total bacterial populations. 30 ml of the flower suspension was filtered through first 2 layers of cheesecloth and then passed through a 35 µm microsieve screen. Filtrates were further concentrated by filtering cells and embedding into a 0.2 micron membrane; embedded bacteria were re-suspended in 1 ml sterile water. DNA was extracted from the 1 ml samples (pre and post-filter-concentration) using InstaGene^{TM} matrix. Samples (5 µl) of the cell/flower suspension and concentrated suspensions were used for LAMP reactions (described above). Representative populations of indigenous bacteria present on dilution plates were collected and stored for further testing.

Objective 3. Determine the sensitivity of LAMP reaction when one flower with a natural infection of *E. amylovora* is added to 100 flower clusters. *E. amylovora* was inoculated onto the stigma of pear or apple flowers and incubated at room temperature to attain natural populations on stigmas. Infested and non-inoculated control flowers were first washed individually in 1 ml sterile water and dilution plated (as described above). Each flower and wash was then transferred to a plastic bag containing 1.5 L water with 100 flower clusters (600 to 700 flowers) obtained from a non-inoculated orchard. Additional controls were 100 flower clusters only in 1.5 L in water and water-only. Bags containing flowers were hand massaged and sonicated for 2 minutes. Samples were processed, filter-concentrated, and run in LAMP reactions as in objective 2 with the modification that only 15 ml (not 30 ml) of floral wash was embedded onto 0.2 micron membranes.

Objective 4. Use the LAMP reaction to detect *E. amylovora* in flower samples from inoculated and non-inoculated orchard trees. One hundred floral clusters per walk (3 walks total) were sampled from each of three inoculated orchards (Bartlett pear, Gala apple or Golden Delicious apple). Samples were taken at full bloom 2 days after pathogen inoculation (inoculum ~1 x 10^6 CFU per ml). Similarly, 100 floral clusters per walk (3 walks total) were sampled from three non-inoculated orchards (Bartlett pear, Jonathon apple, or Fuji apple). After processing the floral clusters, dilution plating and LAMP reactions were performed as in objective 3.

Objective 5. Use the LAMP reaction to detect *E. amylovora* in flower samples from commercial orchards. With the help of OSU staff in Medford and Hood, eight commercial orchard blocks were selected for sampling. These orchards ranged in size from 2.5 to 7.5 ha, and were planted to important cultivars susceptible to fire blight: Bartlett, Bosc and Red d'Anjou pear in the Rogue valley and Bartlett, Bosc, Red d'Anjou pear and Gala and Jonagold apple in Hood River valley. The sampling scheme evaluated in each orchard was 'thorough but efficient', which is a classic IPM sampling scheme where the scout makes five transects through the orchard walking on a 'W- pattern'. A bulk flower sample (100 flower cluster/walk) was made on each sampling transect. Bulk floral samples were taken at 1-3 times during bloom: 30% bloom, 70% bloom and full bloom in pear orchards, and once in apple orchards (30 % bloom). Dilution plating was performed as described above with the exception that Miller-Schroth medium was used to enumerate *E. amylovora*. Suspect

colonies that were *E. amylovora*-like were transferred to CCT for positive identification. The LAMP reaction was performed after processing the floral clusters as in objective 3.

Results and Discussion:

Objective 1. Design a LAMP reaction to detect small quantities of the fire blight pathogen based on primers from *E. amylovora*-specific DNA sequences.
A positive LAMP reaction resulting in a white magnesium pyrophosphate precipitate (Fig. 1) in the PCR tube corresponded to dilution plate enumeration of $\geq$ 25 CFU of the pathogen. Pathogen cell concentrations below this level resulted in inconsistent precipitate formation in the PCR tube. Laboratory strains *P. fluorescens*, *P. syringae*, and *P. agglomerans* were negative for precipitate formation in the LAMP reaction (data not shown). In addition, whole pear flowers, pear flower petals or pear flowers minus petals were negative for the LAMP reaction.

Objective 2. Determine specificity and sensitivity of the designed LAMP reaction against a diverse selection of microorganisms commonly found in pear and apple orchards. As designed, **p**athogen populations recovered from the spiked cell/flower suspensions were 5×10^2 and 5×10^3 CFU per ml. Populations of indigenous bacteria in floral suspensions averaged 10^2, 10^4, and 10^6 CFU per ml for suspensions with 10, 100 or 1000 flowers per 3 L, respectively. Indigenous bacteria were not recovered from wash suspensions with no flowers. All LAMP reactions for wash suspensions containing no pathogen cells were negative.

For both Bartlett pear and Gala apple, when *E. amylovora* was spiked into flower suspensions at 500 and 5000 CFU per ml, 100% of LAMP reactions were positive (Table 1). The number of pear flowers in the suspension had no effect on the incidence of positive LAMP reactions.

Importantly, concentrating 30 ml of the wash suspension by embedding on 0.2 micron membrane increased pathogen cell densities by one log unit (as determined by dilution plating). Also, DNA extraction with the InstaGene™ Matrix increased the incidence of positive LAMP reactions from similar reaction without concentration and DNA extraction in 2007.

Table 1. Incidence of positive LAMP reactions in buckets of water/floral tissue[a] spiked with *E. amylovora* averaged over all methods of sample preparation.

	Concentration of *E. amylovora* cells per ml		
Cultivar	**0**	**500**	**5000**
Bartlett pear	**0%[b]**	**100%**	**100%**
Gala apple	**0%**	**100%**	**100%**

[a] 0, 10, 100 or 1000 pear or apple flowers washed in 3 L water.
[b] Incidence of positive LAMP reaction is the average of 3 (pear) or (apple) bucket experiments where samples were prepared by filtration, concentration and DNA extraction.

Objective 3. Determine the sensitivity of LAMP reaction when one flower with a natural infection of *E. amylovora* is added to 100 flower clusters. The wash from single infested apple or pear flower suspended in 1.5 L of water resulted in populations of *E. amylovora* ranging from 1×10^2 to 5×10^4 CFU per ml, which yielded a mix of positive and false negative LAMP results (Table 2). After mixing in water, a minimum of 1×10^3 CFU per ml of pathogen cells (or $> 10^6$ cells on a single flower prior to the mixing) was required for obtain a positive LAMP. Wash concentrations with the pathogen at $\geq 10^4$ CFU per ml were consistently positive.

Concentration of the pathogen by embedding subsamples of floral washes onto filters increased incidence of detection to 100% (Table 2). Pathogen populations after concentration ranged from 9 x 10^3 to 4.8 x 10^5 CFU per ml. Indigenous bacteria were recovered in all experiments at populations averaging 2.6 x 10^4 CFU per ml.

Table 2. Summary of LAMP reactions for single-infested flower added to non-infested, 100 flower cluster samples[a].

	F lower with 10^5-10^7 CFU[b] added to 1.5 L wash - 1 ml sampled for DNA extraction			
Cultivar	**0**	**100 clusters**	**1 flower (*Ea*)**	**1 flower (*Ea*) + 100 clusters**
Bartlett pear	0%	0%	0% (0 of 3)	0% (0 of 3)
Gala apple	0%	0%	50% (3 of 6)	50% (3 of 6)
	Flower with 10^5-10^7 CFU[b] added to 1.5 L wash --- 15 ml filter concentrated and resuspended in 1 ml for DNA extraction			
Cultivar	**0**	**100 clusters**	**1 flower (*Ea*)**	**1 flower (*Ea*) + 100 clusters**
Bartlett pear	0%	0%	100% (3 of 3)	100% (3 of 3)
Gala apple	0%	0%	100% (6 of 6)	100% (6 of 6)

[a] Pear or apple flower clusters (~600 to 700 flowers) washed in 1.5 L water in a re-sealable, plastic freezer bag.
[b] Concentrations of *E. amylovora* on a single flower prior to adding to 1.5 L water with 100 flower clusters.

Objective 4. Use the LAMP reaction to detect *E. amylovora* in flower samples from inoculated and non-inoculated orchard trees.
Floral clusters (100 clusters per walk) sampled from non-pathogen-inoculated apple and pear orchards yielded no positive LAMP reactions. Moreover, *E. amylovora* was not detected on dilution plates, but population of other bacterial epiphytes ranged from 2.7 x 10^5 to 6.0 x 10^6 CFU per ml.

All washes of apple and pear flowers from orchards inoculated with *E. amylovora* 153N had positive LAMP reactions. In addition to the other bacterial epiphytes, the pathogen was recovered at populations ranging from 1.2 x 10^3 to 4.7 x 10^5 CFU per ml.

Objective 5. Use the LAMP reaction to detect *E. amylovora* in flower samples from commercial orchards.

Rogue Valley. The three commercial orchards sampled in the Rogue Valley of Oregon were all negative for detection of *E. amylovora* by LAMP or dilution plate, and for development of fire blight. Fire blight risk, as modeled by COUGARBLIGHT, was low during the mid-April sampling period.

Hood River Valley (Parkdale). Bloom at higher elevations in the Hood River Valley coincided with a period of extreme fire blight risk (Fig. 2). The first samples (30% bloom in pear) occurred at low risk, and *E. amylovora* was not detected. For the 3rd sample time (May 19), which occurred during the risk period, *E. amylovora* was detected by LAMP in 4 of 5 orchards, all of which developed some fire blight (Table 3). Positive pathogen detection by LAMP in 3 of 4 orchards, however, required concentration of the extracted DNA by high speed, low temperature evaporation.

Fig. 2. Fire blight risk in spring 2008 based on temperatures measured at Parkdale, Oregon. Arrows indicate samples dates.

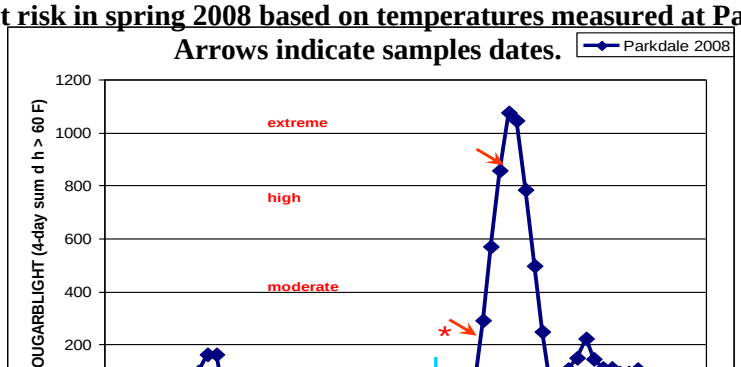


Table 3. Detection of epiphytic *E. amylovora* on pear or apple flowers and observation of fire blight in commercial orchards located in the Rogue and Hood River valleys in 2008.

Rogue Valley	**LAMP**	**Media isolation**	**Disease**
Bartlett pear	N	N	N
Bosc & Red d'Anjou pear	N	N	N
Bartlett pear	N	N	N
Hood River Valley			
Red d'Anjou pear	N	N	N
Bartlett, d'Anjou, Bosc pear	N/Y* (2 of 5)	Y (5.6×10^2 CFU/ml)	Y light
Jonagold Apple (30% bloom only)	N/Y* (2 of 5)	N	Y light
Bartlett & Bosc pear	Y (4 of 5)	Y (3.9×10^3 CFU/ml)	Y mod
Gala apple (30%) bloom only)	N/Y* (5 of 5)	N	Y mod

*After concentration of extracted DNA by a high speed, low temperature evaporation.

Results summary:

- Developed LAMP primer sets for *E. amylovora* detection.
- Detection limit with pure cultures is ~25 pathogen cells per ml. Practical detection limit with field samples is ~10,000 cells per ml.
- We consistently detect *E. amylovora* in spiked washes, and inoculated field trials.
- We have a field sample size: 100 clusters, ~ one sample per hectare (minimum 5 samples per orchard).
- Our sample volume of 1.5 L of water is too large. Concentration of samples improved detection in single-infested flower experiment, and commercial orchards.
- Best detection in commercial orchards coincided with full bloom at high COUGARBLIGHT risk.

Plans for 2009: We will continue to refine and evaluate a LAMP-based early detection protocol for the fire blight pathogen. We will increase the number of commercial orchards that are sampled, and continue to target orchards with a range of fire blight risk profiles. We will compare the incidence of pathogen detection to the development of disease, and calculate and evaluate the costs (time and expenses) of an early fire blight pathogen detection protocol.

Given the known sensitivity of LAMP and our preliminary results, we expect that our sampling scheme will readily detect *E. amylovora* at high levels of infestation. Through refinement of the method used wash bulk flower samples, we expect to improve on detection of *E. amylovora* at lower levels of infestation. By sampling multiple orchards from several districts, we expect to begin to understand the utility of an early detection protocol for *E. amylovora*.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number:

Project Title: Decay risk prediction models and novel decay control methodology

PI: Robert A. Spotts
Organization: OSU Mid-Columbia Ag Research and Ext. Ctr.
Telephone: 541-386-6534 ext. 15
Email: Robert.spotts@oregonstate.edu
Address: 3005 Experiment Station Drive
City: Hood River
State/Zip: OR 97031

Co-PI(2): Chang-Lin Xiao
Organization: WSU-TFREC, Wenatchee
Telephone: 509-663-8181 X229
Email: CLXIAO@WSU.EDU
Address: 1100 N. Western Ave.
City: Wenatchee
State/Zip: WA 98801

Co-PI(3): David Sugar
Organization: OSU Southern Oregon Ag Research and Extension Center
Telephone: 541-772-5165
Email: david.sugar@oregonstate.edu
Address: 569 Hanley Rd.
City: Central Point
State/Zip: OR 97502

Cooperators: Peter Sholberg and Dan O'Gorman (Ag Canada)

Total project funding request: **Year 1**: $43,822 **Year 2**:$0 **Year 3**:N/A

Other funding Sources: None

WTFRC Collaborative expenses: None

Budget 1
Organization Name: Ag Res. Foundation **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-3228 **Email address:** beaton@oregonstate.edu

Item	2008	2009	2010 (N/A)
Salaries	26,557	0	
Benefits	16,465	0	
Wages			
Benefits			
Supplies	800	0	
Miscellaneous			
Total	43,822	0	

Objectives:

1. Validate gray mold decay risk prediction model
2. Develop blue mold decay risk prediction model
3. Implement real time DNA techniques for detection of decay spores in packinghouse water systems
4. Evaluate new fungicides and biological control agents in preharvest and postharvest integrated systems
5. Develop pre- and post-storage integrated programs for decay control (Xiao: coordinator and d'Anjou pears in WA; Spotts: d'Anjou pears in Hood River; Sugar: Bosc pears in Medford)

Significant findings:

- The gray mold incidence of fruit predicted to be in the Low risk category was significantly less than that in the higher risk levels, and all fruit lots considered as Low risk had less decay than any of the fruit lots in the High risk category.
- The factors that are important for gray mold prediction did not affect blue mold similarly. Blue mold is more closely related to contamination of packinghouse surfaces and water.
- Resistance of pear fruit to decay changes yearly and can be quantified.
- The relationship between decay and spore load is important for establishing action thresholds for packinghouse water systems. Results emphasize the importance of good sanitation.
- All tested preharvest fungicides reduced gray mold but only Topsin reduced blue mold.

Methods:

1. Validate gray mold decay risk prediction model

A model to predict the risk of gray mold decay of pears in long term storage was developed from data collected in Oregon, Washington, and New Zealand. Model validation will involve a coordinated effort among researchers in Hood River (Spotts), Washington (Xiao), and Medford (Sugar).

Validation of the model on a large scale in major pear districts is essential before commercial implementation can occur. Validation will involve sampling fruit from 36 orchards in Oregon (Hood River and Medford) and Washington and developing scaled-up laboratory procedures for real time PCR analysis of *Botrytis cinerea* DNA on the fruit surface. Other information required to make predictions includes preharvest rainfall, preharvest fungicide application, and orchard rating. Fieldmen will supply this information.

The risk level of each sample will be determined and made available shortly after harvest. Actual validation decay data will come from packinghouse cull analyses. The entire DNA and risk prediction protocol will ultimately be turned over to commercial analytical service laboratories.

2. Develop blue mold decay risk prediction model

During development of the gray mold model, *Penicillium expansum* DNA on fruit surfaces also was determined. This information, along with the other factors used in the gray mold model, will be used and importance of the factors will be determined for successful model development. Also, it may be necessary to include packinghouse spore load information in the blue mold model.

3. Implement real time DNA techniques for rapid detection of decay spores in packinghouse water systems

We are in the process of improving our DNA technology in several ways such as evaluation of new, more specific primers and use of new spore capture methods (collection on membrane filters). These new methods will be tested, optimized, and used to replace our original, less sensitive methods. This technology will be implemented in the risk models and in risk determination in packinghouse water systems. A range of threshold values of spore numbers will be established for Anjou and Bosc pears. Weekly sampling will be done in packinghouses using various water handling systems to compare systems and determine how long water can be used before waste disposal is necessary. Determination of decay risk from contaminated packinghouse water systems requires a multi-year analysis.

4. Evaluate new fungicides and biological control agents in preharvest and postharvest integrated systems

An integrated approach to evaluation of pre- and postharvest fungicides for decay control will be undertaken in cooperation with Dr. Chang Lin-Xiao and Dr. David Sugar. New products will be tested as part of an integrated system. Testing will be coordinated so results can be compared among researchers in the various growing districts.

Evaluation of new fungicides and biological control agents for pre- and postharvest decay control is an ongoing process as new products become available. Additional financial support for project objective 4 will be obtained from companies in the agricultural chemical and biological control industries.

5. Develop pre- and post-storage integrated programs for decay control (Xiao: coordinator and d'Anjou pears in WA; Spotts: d'Anjou pears in Hood River; Sugar: Bosc pears in Medford)

Experiments will be conducted to evaluate various postharvest fungicide drench treatments in combination with online fungicides or biocontrol treatments at packing for control of decay. This experiment is to simulate commercial operations in which fruit are treated with fungicides prior to storage and then treated again on the packing line. Fruit will be stored in CA up to 6 months. Fruit will be subjected to the packing process and then inoculated with *P. expansum*. For each fungicide-drench treatment, part of the inoculated fruit will be treated with either biocontrol agents (BioSave and CIM) or one of the two other postharvest fungicides after inoculation. Nontreated fruit will be used as controls.

Results and discussion:

1. Validate gray mold decay risk prediction model

The first complete year of validation was in 2007-2008 and included pear fruit from 34 orchards in OR and WA (Table 1).

Pear fruit from 9 orchards were stored field-run at MCAREC (Hood River) and SOREC (Medford). Gray mold in this fruit ranged from 0.4 to 8.9% (Table 2). There was no difference in the incidence of gray mold in fruit from orchards in the Low and Moderate risk categories, but fruit predicted to be at High or Extreme risk had significantly more gray mold than fruit from lower risk orchards and were different from each other.

Fruit from commercial storages had 0.07 to 0.32% gray mold (Table 3), which is a reduction in the Low, Moderate, and High risk categories of 95, 40, and 90% compared to percent gray mold in the same categories stored field run without any postharvest treatments. The gray mold incidence of fruit predicted as Low risk was significantly less than that in the higher risk levels. Although postharvest

treatments and cold storage conditions varied considerably among packinghouses, the model still gave accurate predictions of gray mold risk. Among the 17 orchards represented in this group, all of the fruit lots considered as Low risk had less decay than any of the fruit lots in the High risk category.

Fruit from eight orchards was shipped before decay data could be collected and was not included in the analysis. Fruit for the second year of validation representing 47 orchards currently is in storage until spring 2009.

It is important to note that **orchard rating** was the most significant predictor of gray mold risk. Problem orchards often had old trees with dead limbs and poor weed control. Fruit on lower limbs often were intermingled with various weeds and grasses. **Preharvest fungicide** application was the second most important predictor of gray mold risk.

Table 1. Gray mold risk model validation orchards 2007-8

Packer	Orchard	2007 Harvest Date	DNA	Preharvest fungicide	Rain	Orchard Rating	Time stored (mo)	Predicted Risk	Total %Bot
A	1	9/15	L	Yes	Yes	1	ND	L	ND
A	2	9/12	L	Yes	Yes	2	ND	M	ND
A	3	9/24	L	Yes	No	1	ND	L	ND
A	4	9/6	L	Yes	Yes	2	ND	M	ND
A	5	9/4	L	No	Yes	2	6*	H	2.85*
A	6	9/7	L	No	Yes	2	6*	H	3.29*
A	7	9/18	L	Yes	Yes	1	ND	L	ND
B	1	9/20	L	Ziram	Yes	2	7	M	0.33
B	2	9/24	L	Ziram	No	2	6	L	0.51
B	3	9/8	L	Ziram	Yes	2	8	M	0.29
B	4	9/8	L	Ziram	Yes	2	8	M	1.39
B	5	9/8	L	Ziram	Yes	2	7.75	M	0.22
B	6	9/19	L	Ziram	Yes	2	6*	M	0.88*
B	7	9/20	L	Ziram	Yes	2	7.75	M	0.37
C	1	9/8	L	Yes	Yes	2	4.5	M	0.22
C	2	9/10	L	Yes	Yes	3	5.25	H	0.39
C	3	9/8	L	Yes	Yes	2	4.5	M	0.08
C	4	9/15	L	Yes	Yes	1	7.5	L	0.04
C	5	ND	L	Yes	Yes	2	ND	M	ND
C	6	ND	L	Yes	Yes	3	4.5	H	0
C	7	9/11	L	Yes	Yes	1	4.5	L	0
C	8	9/13	L	Yes	Yes	3	4.5	H	0.28
D	1	9/17	L	Topsin	Yes	2	ND	M	ND
D	2	9/17	L	Topsin	Yes	2	6.25	M	0.01
D	3	9/21	L	Topsin	Yes	2	ND	M	ND
D	4	9/10	L	Topsin	Yes	2	6.5	M	0.24
D	5	9/14	L	Topsin	Yes	3	6*	H	3.48*
D	6	9/19	L	Yes	Yes	2	6.5	M	0.21
D	7	9/14	L	No	Yes	3	6*	E	9.9*
D	8	9/14	L	No	Yes	3	6*	E	7.8*

Table 1 (Continued) . Gray mold risk model validation orchards 2007-8

E	1	9/6	L	No	No	2	4	M	0.54
E	2	9/6	L	Ziram	No	2	4	L	0.18
E	3	9/6	L	No	No	2	4	M	0.18
F	1	8/30	L	Yes	Yes	2	6*	M	0.15*
F	2	9/17	L	No	No	2	6*	M	0.07*
F	3	9/17	L	Yes	No	2	6*	L	1.3*

*=fruit not in commercial storage but field run in MCAREC or SOREC room. ND=Not determined.

Table 2. Anjou pears stored field-run at MCAREC and SOREC for gray mold risk model validation 2007-8

Predicted risk level	Avg. gray mold (%)[z]	No. orchards
Low	1.3a	1
Moderate	0.4a	3
High	3.2b	3
Extreme	8.9c	2

[z]Fruit stored six months; different letters indicate statistical differences at $P = 0.05$.

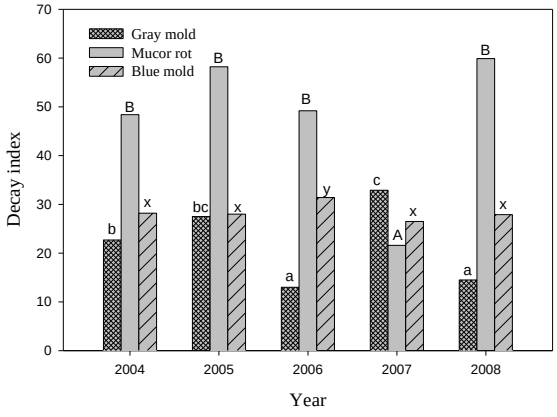
Table 3. Pears run over commercial packinglines and stored in commercial cold rooms for gray mold risk model validation 2007-8

Risk level	Avg. gray mold (%)[z]	No. orchards
Low	0.07a	3
Moderate	0.24b	11
High	0.32b	3

[z]Fruit stored 4 to 7.75 months; different letters indicate statistical differences at $P = 0.05$.

2. Develop blue mold decay risk prediction model

Blue mold decay levels were low in fruit from orchards used for the gray mold model. The factors that are important for gray mold prediction did not affect blue mold similarly. It appears that blue mold is more closely related to contamination of packinghouse surfaces and water systems (drenchers, dump tanks, flumes) than to orchard factors.



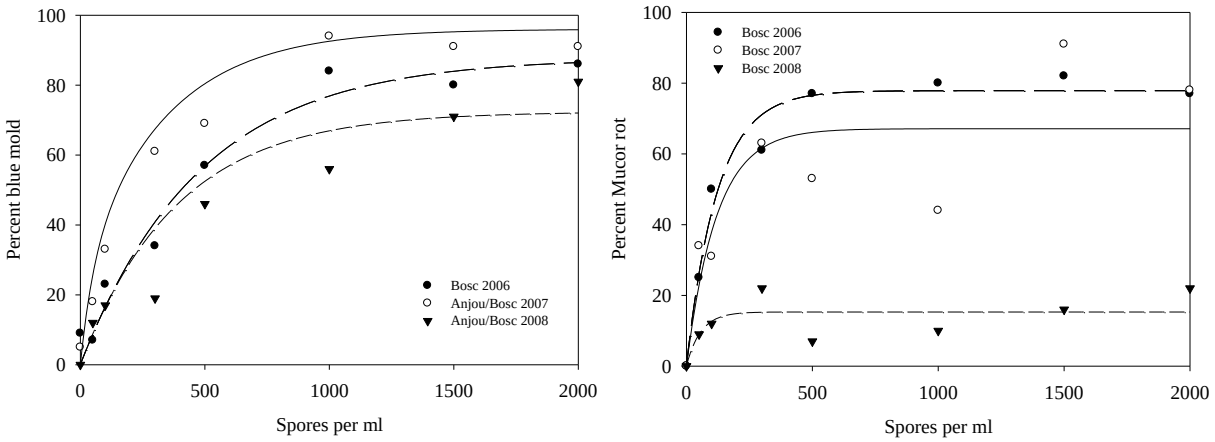
Resistance of pear fruit to decay changes yearly. We developed a test to measure this at the beginning of each packing season. Fruit resistance eventually needs to be incorporated into gray mold and blue mold risk prediction models.

3. Implement real time DNA techniques for rapid detection of decay spores in packinghouse water systems

Spore concentration, DNA extraction, and real time PCR protocols that have been successful for

Botrytis are inadequate for *Penicillium*. Considerable effort has been focused on developing a protocol for detection of *Penicillium* spore numbers considered in the threshold range (100 to 300 per ml or less) for blue mold problems in packinghouses. Because of the lack of efficient and specific primers for *P. expansum*, the protocol remains under development.

The relationship between decay and spore load is important for establishing action thresholds for packinghouse water systems. For blue mold and Mucor rot, the steep curve between 0 and 500 spores per ml indicates that reduction of spore numbers in this part of the curve will result in significant reductions in decay. These results emphasize the importance of good sanitation.



4. Evaluate new fungicides and biological control agents in preharvest and postharvest integrated systems

In 2007-8, all tested preharvest fungicides reduced gray mold but only Topsin reduced blue mold (Table 4). Preliminary data are available for 2008-9 and show that Topsin again was the most effective preharvest fungicide for control of blue mold.

Table 4. Preharvest fungicides for control of postharvest decay of d'Anjou pear fruit

	2007-8		2008-9 (3 mo.)
Fungicide and rate/A	Gray mold (%)	Blue mold (%)	Blue mold (#/box)
Topsin 70WP 1/0 lb	2.2a	6.2a	5.0a
Pristine 38WG 14.5 oz	3.1a	21.3b	6.5ab
Ziram 76DF 8.0 lb	2.9a	19.4b	---
Yucca Ag Aide 2%	---	---	7.7abc
Silmatrix 2%	---	---	12.5c
Unsprayed	7.5b	26.0b	12.3bc

In 2007, all fungicides contained Nutraphos 24. In 2008, Pristine used at 18.5 oz with Silgard 4.0 oz. Fungicides applied 2 wks before harvest and evaluated after 3, 6, and 8 months at 30ºF.

5. Develop pre- and post-storage integrated programs for decay control (Xiao: coordinator and d'Anjou pears in WA; Spotts: d'Anjou pears in Hood River; Sugar: Bosc pears in Medford)

Dr. Xiao will report the results for this objective.

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

Project Title: Control of postharvest fruit rots in pears

PI: Chang-Lin Xiao
Organization: WSU-TFREC, Wenatchee
Telephone: 509-663-8181 X229
Email: clxiao@wsu.edu
Address: 1100 N. Western Ave.
City: Wenatchee
State/Zip: WA/98801

Cooperators: Robert Spotts, Oregon State Univ. (Hood River); David Sugar, Oregon State Univ. (Medford); Selected packinghouses across the state

Total Budget Request: **Year 1:** $29,719 **Year 2: $32,165** **Year 3:** $33,150

Other funding sources

Agency Name: WSCPR
Amount requested: $13,056
Notes: A grant proposal has been submitted to the Washington State Commission on Pesticide Registration (WSCPR) for consideration for funding at its research review meeting to be held on 1/14/09. The proposed research is on the control of Phacidiopycnis rot on the 2009 pear crops. The WSCPR project is part of this proposal submitted to the Fresh Pear Committee. The WSCPR funding is contingent upon the provision of funds as co-funding from the Fresh Pear Committee.

WTFRC Collaborative expenses:

Item	2008	2009	2010
Stemilt RCA room rental	3,184.21	**3,184.21**	3,184.21
Crew labor	0	**0**	0
Shipping	0	**0**	0
Supplies	0	**0**	0
Travel	0	**0**	0
Miscellaneous	0	**0**	0
Total	3,184.21	**3,184.21**	3,184.21

Footnotes: The estimate of the RCA room rental cost was based on a projection of 20-bin space needed for this research project.

Budget 1:
Organization: Washington State University **Contract Administrator:** M L Bricker; Kevin Larson
Telephone: 509-335-7667; 509-663-8181 x221 **Email:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	2008	2009	2010
Salaries[1]	19,778	**17,844**	18,558
Benefits	8,900	**6,781**	7,052
Wages (time slip)	3,000	**3,000**	3,000
Benefits	471	**540**	540
Equipment	0	**0**	0
Supplies[2]	4,000	**3,000**	3,000
Travel[3]	1,000	**1,000**	1,000
Miscellaneous	0	**0**	0
Total	37,149 (approved 29,719)	**32,165**	33,150

Footnotes:
[1] Salary for 2009 and 2010 is for Robin Boal (0.33 FTE) at 38% benefit rate.
[2] Supplies include cost of fruit purchased from commercial orchards or packers and lab supplies.
[3] We will be using a leased vehicle.

Objectives:

1. Develop preharvest fungicides and postharvest fungicides or biocontrol integrated programs for decay control.
2. Develop pre- and post-storage integrated programs for decay control.
3. Develop molecular-based assays for diagnosis and detection of pear fruit infection by the Phacidiopycnis fungus leading to Phacidiopycnis rot in storage.

In addition, we will assist Bob Spotts with validation of gray mold decay risk model in Washington. The objectives were slightly modified because only 80% of the original budget request was approved.

Significant Findings:

- Residual activity of Pristine in pear fruit in combination with biocontrol BioSave or fungicides for blue mold control was evaluated. For the fruit that were sprayed with Pristine before harvest and packed 1 week after harvest, Pristine alone without any postharvest treatments reduced blue mold incidence by 56.4% compared with the control 8 weeks after packing, indicating the existence of residual activity of Pristine in pear fruit.

- When BioSave was applied to the fruit at packing, preharvest Pristine plus postharvest BioSave was more effective than Pristine alone and reduced blue mold incidence by 97.4% and 94.1% compared with the nontreated control and Pristine alone, respectively. However, the effectiveness of preharvest Pristine in combination with postharvest BioSave was reduced after the fruit had been stored at room temperature for one additional week. Our results indicate that preharvest Pristine plus postharvest BioSave could be a promising program for blue mold control.

- A PCR assay was developed based on specific primers of ITS region for three target pathogens, *Potebniamyces pyri* (*Phacidiopycnis piri)*, *Botrytis cinerea*, and *Sphaeropsis pyriputrescens*. Validation with stem-end rot and calyx-end rot samples collected from a packinghouse indicated that ITS-based PCR assay and isolation-based assay yielded consistent results in the identification of causal agents of decayed fruit.

- A second PCR assay also was developed based on the primer set of the partial sequence of elongation factor-1α. The size of EF-1α amplicon of *Potebniamyces pyri* was found unique among various fungi tested. For all successful amplifications from decayed fruit, causal agents inferred with EF-1α amplicons were consistent with those inferred with ITS-based PCR assay as well as those from isolation-based method.

- Experiments for objective 2 are in progress and will be finished in late spring 2009.

Methods:

Preharvest Pristine in combination with postharvest biocontrol BioSave or fungicides for blue mold control was evaluated on d'Anjou pears. Pristine was applied 7 days before harvest. Fruit were harvested and stored in RA. Part of the fruit was removed from RA at 1 week and 2 months after harvest. Fruit were run through a research packingline and inoculated with *P. expansum*. Part of the inoculated fruit was treated with each of the three postharvest fungicides (TBZ, Penbotec and Scholar) or the biocontrol agent BioSave after inoculation. Untreated fruit were used as controls. All fruit were stored in cold storage for 8 weeks and then for 7 days at room temperature.

Experiment was conducted in a research orchard of d'Anjou pear near Wenatchee. To ensure a necessary disease level, fruit were inoculated with spore suspensions of the Phacidiopycnis fungus at

5 weeks before harvest. For preharvest fungicide treatments, fungicides Pristine and Topsin M were applied within 2 weeks before harvest, and a nontreated control was included. For postharvest fungicide treatments, fruit were not sprayed with preharvest fungicides. Treatments were arranged as a randomized complete block design with four replicates, with 1-2 trees per replicate. Fruit were harvested in mid-September. Fruit for postharvest fungicide treatments were treated with one of the three postharvest fungicides. All fruit were packed on fruit trays in cardboard boxes and stored in air at 32ºF. All fruit were visually examined for decay development (calyx-end rot and stem-end rot, etc.) every 2 weeks for 5 months, starting in December.

Experiment has been set up to evaluate various postharvest fungicide drench treatments in combination with online fungicides or biocontrol treatments at packing for control of decay. This experiment is to simulate commercial operations in which fruit are treated with fungicides (for example, drench) prior to storage and then treated again on the packing line. Commercially harvested fruit without use of preharvest fungicides were used for this study. Fruit were either not drenched or drenched with one of the postharvest fungicides. Fruit are currently stored in CA. Part of the fruit will be removed from CA 4 and 6 months after harvest. Fruit will be subjected to packing process and then inoculated with *P. expansum*. For each fungicide-drench treatment, part of the inoculated fruit will be treated with either the biocontrol BioSave or one of the two other postharvest fungicides after inoculation. Nontreated fruit will be used as controls. All fruit will then be stored in cold storage for 8 weeks and then for 7 days at 68ºF at which time decay development will be evaluated.

Molecular-based assays for diagnosis of Phacidiopycnis rot, gray mold and Sphaeropsis rot were developed. The ITS region and partial region of the translation elongation factor 1α (EF-1α, nucleotides 526 to 986) were sequenced to design specific primers for three target pathogens, *Potebniamyces pyri* (*Phacidiopycnis piri)*, *Botrytis cinerea*, and *Sphaeropsis pyriputrescens*. Because some other pathogenic and nonpathogenic fungi also are associated with the stem and calyx of pear fruit, the specificity of primer/probe sets designed were tested against these fungi. PCR based assays were validated using naturally infected fruit collected from packinghouses.

Results and Discussion

Preharvest Pristine in combination with postharvest biocontrol BioSave or fungicides for blue mold control

This experiment was to evaluate residual activity of Pristine in pear fruit in combination with biocontrol BioSave or fungicides applied at packing for control of blue mold.

For the fruit that were not sprayed with any preharvest fungicide and packed 1 week after harvest, Scholar and Penbotec applied at packing were highly effective for control. TBZ was not effective as the strain was resistant to TBZ. BioSave alone reduced blue mold incidence by 29.1-37.1% compared with the nontreated control (Table 1).

For the fruit that were sprayed with Pristine before harvest, Pristine alone, without any postharvest treatments, reduced blue mold incidence by 56.4% compared with the control 8 weeks after packing, indicating the existence of residual activity of Pristine in pear fruit. When BioSave was applied to the fruit at packing, preharvest Pristine plus postharvest BioSave was more effective than Pristine alone and reduced blue mold incidence by 97.4% and 94.1% compared with the nontreated control and Pristine alone, respectively. However, the effectiveness of preharvest Pristine in combination with postharvest BioSave was reduced after the fruit had been stored at room temperature for one additional week. Our results indicate that preharvest Pristine plus postharvest BioSave could be a promising program for blue mold control.

For the experiment with fruit that were stored for 2 months before packing, the fruit have been run and inoculated with the pathogen and are currently in storage for decay development. Results will be forthcoming.

Table 1. Preharvest Pristine in combination with postharvest fungicide and biocontrol agent for control of blue mold in d'Anjou pears

Preharvest Treatment	Fungicide applied 1 week after harvest	8 weeks at 32F after packing		1 week at room temp after cold storage	
		% decay	Lesion (mm)	% decay	Lesion (mm)
Nontreated	No fungicide	97.5 a	22.7 a	98.8 a	60.3 a
	Scholar	0.0 d	0.0 d	0.0 d	0.0 f
	Penbotec	0.0 d	0.0 d	0.0 d	0.0 f
	TBZ	100.0 a	24.9 a	100.0 a	62.4 a
	BioSave	61.3 b	18.1 b	70.0 b	51.6 b
Pristine	No fungicide	42.5 c	10.6 c	70.0 b	36.0 d
	TBZ	67.5 b	17.5 b	96.3 a	42.2 c
	Scholar	0.0 d	0.0 d	0.0 d	0.0 f
	Penbotec	0.0 d	0.0 d	0.0 d	0.0 f
	Biosave	2.5 d	1.9 d	17.5 c	22.6 e

Pre- and postharvest fungicides for control of Phacidiopycnis rot originating from infections during the fruit-growing season

An experiment was conducted on the 2008 d'Anjou pear crops to evaluate whether preharvest fungicides applied within 2 weeks before harvest or postharvest fungicide drenches are effective for control of Phacidiopycnis rot originating from infections during the fruit-growing season. In this study, the fruit were inoculated with the fungus 5 weeks before harvest. The fruit are currently in storage and are being evaluated for decay development. The evaluation for decay will last until late spring 2009. Results will be forthcoming.

Pre- and post-storage integrated programs for blue mold control

An experiment has been set up on the 2008 d'Anjou pear crops to evaluate pre-storage fungicide drench treatments in combination with biocontrol BioSave or fungicides for blue mold control. Commercially harvested fruit were drenched with one of the three postharvest fungicides. Non-drenched fruit were used as a control. The fruit are currently in CA. Part of the fruit will be removed from CA 4 or 6 months after harvest. The fruit will be run through a research packing line and inoculated with *Penicillium expansum*. The experiment will end in spring 2009. Results will be forthcoming.

PCR-based assays for diagnosis and detection of Phacidiopycnis rot, gray mold, and Sphaeropsis rot in pears

Phacidiopycnis rot, gray mold, and Sphaeropsis rot all can cause stem-end rot and calyx-end rot on pears. The symptoms of these three diseases are very similar, particularly in the early stage of symptom development. In this project, two PCR-based assays were developed and compared with the isolation-based method for diagnosis and detection of these three diseases.

The first PCR assay was based on specific primer sets designed based on the sequences of ITS region. One primer set per target pathogen was selected and applied in both conventional and real-time PCR

in this study. Specificity of the three primer sets against target pathogens and non-target pathogens or fungi was tested (Table 2). At specified annealing temperatures, amplification for each pathogen was strong and detected only with its own isolates, while no amplifications were detected with other fungi and the fruit DNA. Validation with stem-end rot and calyx-end rot samples collected from a packinghouse indicated that ITS-based PCR assay and isolation-based assay yielded consistent results in the identification of causal agents of decayed fruit (Table 3).

The second PCR assay was based on the primer set based on the partial sequence of elongation factor-1α. Size variation was observed among amplicons of *Potebniamyces pyri*, *B. cinerea*, and *S. pyriputrescens* amplified with primer set EF-1α 526F and 986R (Fig. 1A). The amplicon sizes were around 520 bp, 480 bp, and 236 bp for *B. cinerea*, *S. pyriputrescens*, and *Potebniamyces pyri*, respectively. The size of EF-1α amplicon of *Potebniamyces pyri* was found unique among various fungi tested (Fig. 1A). Only one product was amplified for all successful amplifications from decayed fruit (Fig. 1B). For all successful amplifications from decayed fruit, causal agents inferred with EF-1α amplicons were consistent with those inferred with ITS-based PCR assay as well as those from isolation-based method (Table 3).

Table 2. Fungal and plant species used to test specificity of primer sets designed for PCR-based assays for diagnosis and detection of Phacidiopycnis rot, Sphaeropsis rot, and gray mold in d'Anjou pears

Species [a]	# of isolates	host	Specificity of primer set[b]		
			Set1 for *S. pyriputrescens*	Set2 for *B. cinerea*	Set3 for *P. pyri*
Potebniamyces pyri	25	Pear	-	-	+
Botrytis cinerea	10	Pear	-	+	-
Sphaeropsis pyriputrescens	10	Pear	+	-	-
Alternaria spp.	2	Pear	-	-	-
Aureobasidium spp.	2	Pear	-	-	-
Cladosporium spp.	2	Pear	-	-	-
Mucor spp.	1	Pear	-	-	-
Neofabarea alba	1	Pear	-	-	-
N. perennans	1	Pear	-	-	-
N. n.sp.nov	3	Pear	-	-	-
Penicillium expansum	1	Apple	-	-	-
Penicillium spp.	1	Pear	-	-	-
S. malorum	1	Pear	-	-	-
unidentified fungi	2	pear	-	-	-
Pyrus communis	1	-	-	-	-

[a] Fungal species of *Neofabarea alba* and one of *N*. n.sp. nov. were from R. A. Spotts, Oregon State University Mid-Columbia Agricultural Research and Extension Center. Other fungal species were lab collections isolated from either diseased or apparently healthy fruit.

[b] All three primer sets were developed from internal transcribed spacer (ITS) region of nuclear ribosomal DNA.

"+" means positive amplification; "-" means no amplification.

Table 3. Identification of causal agents of naturally infected pear fruit using three different approaches

Sample collection date [a]	Symptoms (# of samples)	Causal agent	Approaches		
			Isolation	PCR-based assays	
				EF-1α-based PCR	ITS-based PCR
01/16/ 2008	Stem-end rot (20)	*Potebniamyces pyri*	9[b]	9	9
		B. cinerea	10	10	10
		S. pyriputrescens	1	1	1
	Calyx-end rot (20)	*Potebniamyces pyri*	16	16	16
		B. cinerea	4	4	4
		S. pyriputrescens	0	0	0
01/22/ 2008	Stem-end rot (20)	*Potebniamyces pyri*	9	9	9
		B. cinerea	11	9	11
		S. pyriputrescens	0	0	0
	Calyx-end rot (20)	*Potebniamyces pyri*	14	14	14
		B. cinerea	2	2	2
		S. pyriputrescens	4	4	4
04/14/ 2008	Stem-end rot (20)	*Potebniamyces pyri*	9	9	9
		B. cinerea	11	11	11
		S. pyriputrescens	0	0	0
	Calyx-end rot (14)	*Potebniamyces pyri*	9	9	9
		B. cinerea	3	2	3
		S. pyriputrescens	1	1	1

[a] Samples were collected from a commercial packinghouse. At least 20 stem-end rot and calyx-end rot samples were included in each collection if available.
[b] Number of samples in which the pathogen was inferred as the causal agent.

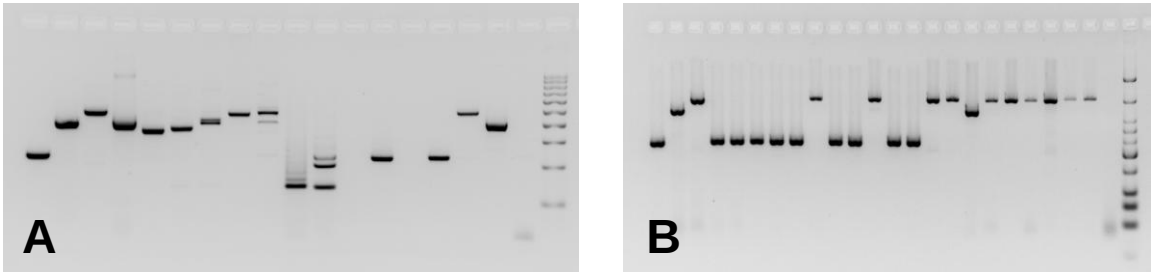


Fig. 1. Sizes variation of PCR products amplified with primer set EF-1α 526F and 986R from DNA of various fungi and biological samples. (**A**) From left to right, DNA samples were: 1, *Potebniamyces pyri*; 2, *S. pyriputrescens*; 3, *B. cinerea*; 4, *Alternaria spp*.; 5, *Aureobasidium* spp.; 6, *Cladosporium* spp.; 7, *S. malorum*; 8, *Neofabarea* spp.; 9, *N.* sp.nov.; 10, *P. expansum*; 11, *Mucor* spp.; 12, W210-2 (no dilution); 13, W210-3 (no dilution); 14, W1530-2 (no dilution); 15, W210-2 (1:5 dilution); 16, W1530-2 (1:5 dilution); 17, W1022-2 (1:5 dilution); 18, water control; 19, ladder. CLX210, *Potebniamyces pyri*; CLX1530, *B. cinerea*; CLX1022, *S. pyriputrescens*. W210-2 = wound inoculated fruit by isolate CLX210, sample #2; other samples from lane12-17 were coded in the same manner. (**B**) From left to right, DNA samples were: 1, *Potebniamyces pyri*; 2, *S. pyriputrescens*; 3, *B. cinerea*; 4 to 23, stem-end rot samples collected on 1/16/2008 (Table 3); 24, water control; 25, DNA ladder.

CONTINUING PROJECT REPORT **YEAR**: 1 of 1 **(Extension)**

Project Title: Comparison of commercial Anjou ripening and conditioning methods

PI:	Eugene Kupferman	**Co PI:**	Ann Colonna
Organization:	WSU – TFREC	**Organization:**	OSU Food Innovation Center
Phone:	509-663-8181 ext 239	**Phone:**	(503) 872-6677
E-mail:	Kupfer@wsu.edu	**E-mail:**	Ann.Colonna@oregonstate.edu
Address:	1100 N. Western Ave.	**Address:**	1207 NW Naito Parkway
City:	Wenatchee	**City:**	Portland
State/Zip:	WA/98801	**State/Zip:**	OR/97209
Co PI:	Keith Sharrock	**Co-PI:**	Kevin Moffitt
Organization:	HortResearch Ruakura	**Organization:**	Pear Bureau Northwest
Phone:	+64 7 858 4650	**Phone:**	(509) 335-3243
E-mail:	KSharrock@hortresearch.co.nz	**Email:**	KMoffitt@usapears.com
Address:	Private Bag 3123 Waikato Mail Centre	**Address:**	4382 SE International Way Ste A
City:	Hamilton 3240	**City:**	Milwaukie
State/Zip:	New Zealand	**State/Zip:**	OR/97222

Co-PI: Dennis Kihlstadius, Ripening Consultant
Organization: Pear Bureau Northwest
Phone: (218) 759-0268
E-mail: dkxy2@paulbunyan.net
Address: 4382 SE International Way Ste A
City: Milwaukie
State/Zip: OR/97222

Cooperators: Select packinghouses

Total Project Funding: **Year 1:** $24,227

Other funding Sources

Agency Name: Pear Bureau Northwest
Amount awarded: $5,000

WTFRC Collaborative Expenses: None

Budget:

Item	2008	2009
Salaries[1]	4,188	
Benefits	1,508	
Wages	5,040	
Benefits	791	
Supplies[2]	1,200	
Travel[3]	1,500	
Miscellaneous[4]	10,000	
Total	24,227	**0**

Footnotes: [1] Chris Sater, Associate in Research, [2] Fruit, laboratory supplies, [3] Travel to warehouses and to Portland for the consumer tests, [4] Fee for two consumer tests done at the Food Innovation Center, Portland.

GOAL

Provide methods by which Anjou pears in retail markets are just 2 to 3 days away from being of excellent eating quality (EEQ). The premise is that pears must be 'conditioned' by the shipper or wholesaler prior to being shipped to retail stores where they will be kept cold then 'ripened' by the consumer at room temp for 2 to 3 days depending on how soft the consumer wants them.

OBJECTIVES

1) **Consumers:** Define EEQ for Anjou pears by addressing the following questions:
   a) What is the ideal firmness of an EEQ pear?
      1) Is there a difference in acceptability between pears of the same firmness from different conditioning regimes?
   b) What is the ideal soluble solids level?
   c) What is the ideal juiciness?
   d) How long are consumers willing to wait for a pear to ripen?

2) **Conditioning:** Determine the best methods to condition pears that lead to EEQ upon ripening by addressing the following questions:
   a) What is the most economical method of conditioning?
      1) How long do pears need to be conditioned?
      2) What is the best method of conditioning?
      3) Do pears soften during conditioning?
      4) How does time in storage (length of chilling) affect method?
      5) How do quality attributes (firmness, acidity, soluble solids) after conditioning (with and w/o ethylene) compare with pears that have been ripened but not conditioned?
   b) Are pears conditioned with ethylene superior?
      1) Does ethylene reduce variability in pear quality?
      2) Is conditioning with the Ethylene Release Canister (ERC) realistic?
         i. Effect of high (13%) CO_2 levels on internal quality.
      3) Will the same ethylene conditioning protocol produce EEQ pears throughout the packing season?
   c) How does the conditioning of pears in standard hand-wrapped poly-lined boxes compare to pears in vented boxes?
      1) What is the temperature profile within the box?
      2) Will ethylene penetrate the poly-lined carton?

3) **Determine the difference in the quality of Anjou pears ripened in commercial chambers that use different systems to condition fruit (**planned for February 2009 and will be reported next year).

SIGNIFICANT FINDINGS

Consumer Experiment 1—Anjou Pears That Had <u>Not Met</u> Their Chilling Requirement

Conditioning treatments were 2, 4 or 6 days in ethylene or 7 days in air followed by 48 hours cooling then ripening for 3 days in warm. Consumers overwhelmingly preferred the 6-day ethylene-conditioned pears to other conditioning treatments.

- The 6-day ethylene pears scored highest in every preference category (overall, pear flavor, sweetness, juiciness, firmness and texture liking).
- The 6-day ethylene pears were ranked first ("best") by 74% of consumers.
- The 4-day ethylene pears were ranked first by 17% of consumers, and scored the second highest in every preference category.

- The 2-day ethylene and 7-day air pears scored lowest in the preference categories and were ranked first by 2% and 7% of consumers, respectively.

Consumer Experiment 2—Anjou Pears That Had Met Their Chilling Requirement
Conditioning treatments were 1, 2 or 4 days in ethylene or 5 days in air followed by 72 hours cooling then ripening for two days in warm. Consumers overwhelmingly preferred the 4-day ethylene-conditioned pears to other conditioning treatments.

- The 4-day ethylene pears scored highest in every preference category (overall, pear flavor, sweetness, juiciness, firmness and texture liking).
- The 4-day ethylene pears were ranked first ("best") by 50% of consumers.
- The 5-day air and 2-day ethylene pears scored in the middle of the preference categories and were ranked first by 23% and 16% of consumers, respectively.
- The 1-day ethylene pears scored the lowest in the preference categories and were ranked first by 11% of consumers.
- More consumers liked air conditioned pears once the chilling requirement had been met than in the previous experiment (23% vs 7%), but less than the ethylene conditioned pears.

MATERIALS AND METHODS

To address objectives 1 and 2 above, the consumer part of this project has been done twice to date. Once with Anjou pears that had not met their chilling requirement (October) and once with Anjou pears that had met their chilling requirement (December) in air storage. We have scheduled an additional trial for March 2009 to examine the conditioning and ripening of Anjou pears that have been stored in controlled atmosphere.

Research on objective 3 is scheduled for February 2009 and will be reported at a later date.

The general procedure to address objectives 1 and 2 utilized four conditioning treatments followed by consumer trials. Conditioning included three ethylene treatments for up to 6 days, and one warm air conditioning for 5 or 7 days. The actual treatment for each testing date (Oct or Dec) is described below. Prior to conditioning, all fruit was stored in the cold (33 ºF). Twenty-four hours prior to conditioning the fruit was placed into a warm room (72 ºF) prior to conditioning. Fruit was then moved to a conditioning room held at 65 ºF or 74 ºF for treatments with or without ethylene. Following conditioning, all fruit was returned to cold storage (33 ºF) for 48 or 72 hours to simulate transit to retail market. Because a goal of the project is to determine quality after ripening, all fruit was removed from cold storage and held at 70 ºF for 2 or 3 days prior to consumer evaluation.

Consumer evaluation occurred at the Food Innovation Center, Oregon State University, Portland Oregon. Qualification criteria for consumer participation were: the consumers fall between the ages of 24 to 65 yrs, they have purchased fresh pears in season at least twice in the past month, 75 to 80% females, 20 to 25% males, at least 70% Caucasian, annual household income of 25K or over, and at least a college degree. A panelist incentive of $25 was paid to participants of the consumer taste test.

Each consumer was served one-third of a pear; the rest of the pear was used for firmness and soluble solids testing on the same day of the consumer evaluation. Consumers rated the pears for overall liking, pear flavor, sweetness, juiciness, firmness, texture and purchase intent. Consumers then ranked the pears for overall preference and were asked a series of marketing and demographic questions. Consumers also answered a number of comment questions.

Consumer Experiment 1—Anjou Pears That Had Not Met Their Chilling Requirement
Anjou pears from a single grower lot harvested between Sept 15th and Sept 21st were packed into Euro boxes with plastic trays by a commercial packer. The pears were obtained on Sept 29th so they had not received sufficient time in storage to have completed their chilling requirement. Fruit quality was

evaluated at time of receipt, with the pears averaging 13.4 lbf with a color rating of 4.8 on a 1 to 10 scale (1 = dark green to 10 = yellow).

There were four conditioning treatments for the consumer trials in Portland; ethylene conditioning for 2, 4, or 6 days, or warm air conditioning for 7 days. Conditioning was done at 65 ºF using an Ethy-Gen catalytic ethylene generator and Ethy-Gen II concentrate (generator and concentrate from Catalytic Generators LLC, Norfolk, VA). The conditioning room averaged 131 ppm ethylene over the 6-day conditioning period.

Following conditioning, all fruit was returned to cold storage (33 ºF) for 48 hours to simulate transit to retail market. Three days prior to consumer evaluation all fruit was removed from cold storage and held at 70 ºF until testing.

Consumer Experiment 2—Anjou Pears That Had Met Their Chilling Requirement

Anjou pears from a single grower were packed into Euro boxes with plastic trays by a commercial packer. Pears were obtained on November 13th and fruit quality was evaluated at time of receipt. The pears averaged 12.9 lbf with a color rating of 4.9 on a 1 to 10 scale (1 = dark green to 10 = yellow).

There were four conditioning treatments for the consumer trials in Portland; ethylene conditioning for 1, 2, or 4 days, or warm air conditioning for 5 days. Conditioning was done at 74 ºF in shroud covered box pallets using Ethylene Release Capsules (Balchem Corporation, New Hampton, NY). The conditioning atmospheres for the ethylene treatments are listed in Table 1. For the fruit conditioned in air, the natural ethylene (C_2H_4) levels in the boxes averaged less than 1 ppm, the oxygen (O_2) levels averaged above 20%, and the carbon dioxide (CO_2) levels averaged less than 1% during each treatment.

Following conditioning, all fruit was returned to cold storage (33 ºF) for 72 hours to simulate transit to retail market. Two days prior to consumer evaluation all fruit was removed from cold storage and held at 70 ºF until testing.

Table 1. Experiment 2 (December 2008) atmospheres in the pallet shroud and boxes during ethylene treatment using ERCs.

	Pallet shroud				Boxes			
	24 hrs	End of treatment			24 hrs	End of treatment		
Treatment (days)	C2H4 (ppm)	C2H4 (ppm)	O_2 (%)	CO_2 (%)	C2H4 (ppm)	C2H4 (ppm)	O_2 (%)	CO_2 (%)
1	252	252	19.5	1.4	179	179	18.4	2.5
2	255	490	18.2	2.5	241	411	16.9	3.9
4	262	988	15.4	4.8	270	816	14.5	5.8

Table 2. Consumer liking scores for six Anjou pear attributes and pear quality measurements for each treatment; consumer sensory trials at the OSU FIC, Portland Oregon, **October 15-16, 2008**.

	Consumer Liking Scores*							Pear Quality	
Treatment	**Overall**	**Pear flavor**	**Sweetness**	**Juiciness**	**Firmness**	**Texture**	**Ranked First****	**Soluble solids (%)**	**Firmness (lbf)**
6 day ethylene	7.48 a	7.46 a	7.11 a	7.95 a	6.97 a	7.26 a	74%	14.5 b	2.23 d
4 day ethylene	6.33 b	6.43 b	5.71 b	5.82 b	6.38 a	6.03 b	17%	14.6 b	3.46 c
2 day ethylene	4.49 c	4.82 c	3.93 c	3.17 c	4.96 b	4.13 c	2%	14.6 b	6.11 b
7 day air	4.33 c	4.74 c	3.73 c	2.47 d	4.24 c	4.08 c	7%	14.9 a	11.13 a

Scale for liking is 1 =dislike extremely to 9 = like extremely
* Scale for sweetness/juiciness is 1 =not sweet/juicy to 9 = ideally sweet/juicy
** Percentage of fruit in each treatment ranked first ("best")

RESULTS AND DISCUSSION

Consumer Experiment 1—Anjou Pears That Had Not Met Their Chilling Requirement

The 6-day ethylene pears scored highest in every preference category and were ranked first ("best") by 74% of consumers (Table 2). Reasons for liking and disliking are listed in Table 6. Overall, 32 out of 480 pears were given an overall liking score of 9 (highest possible score) and 26 pears were given an overall liking score of 1 (lowest possible score). The average scores for all the preference categories, along with the average firmness and soluble solids values for these fruit are shown in Table 3.

Table 3. Average preference scores, firmness values, and soluble solids levels for the highest (9) and lowest (1) scored fruit over 2 days of sensory testing at the OSU FIC, Portland Oregon, **October 15-16, 2008.**

Overall Liking	**Pear Flavor**	**Sweetness**	**Juiciness**	**Firmness**	**Texture**	**Firmness (lbf)**	**Soluble solids (%)**
9.0	8.6	8.4	8.5	8.1	8.3	2.6	14.6
1.0	2.4	2.2	1.5	1.6	1.4	9.2	14.7

Scale for liking is 1 =dislike extremely to 9 = like extremely
Scale for sweetness/juiciness is 1 =not sweet/juicy to 9 = ideally sweet/juicy

Fruit with an overall liking score of 9 (liked extremely) consisted mostly of 6-day ethylene-treated fruit (81%) and was most often described as "sweet" (53%). Other words consumers used to describe the characteristics of this fruit included, "perfect pear," "sweet and juicy," and texture that "melts." The soluble solids levels of the highest scored fruit, was actually slightly lower than that of the lowest scored fruit, so the characteristic described as "sweet" by consumers is not necessarily related to the measurable solids content of the fruit.

Fruit with an overall liking score of 1 (disliked extremely) consisted mostly of 7-day air -treated fruit (62%). The most common reason given for disliking the fruit was "flavor" (58%). Other words consumers used to describe the characteristics of this fruit included, "bland," "no pear flavor," and "mealy."

Consumer Experiment 2—Anjou Pears That Had Met Their Chilling Requirement

The 4-day ethylene pears scored highest in every preference category and were ranked first ("best") by 50% of consumers (Table 4). Reasons for liking and disliking are listed in Table 6.

Table 4. Consumer liking scores for six Anjou pear attributes and pear quality measurements for each treatment; consumer sensory trials at the OSU FIC, Portland Oregon, **December 9-10, 2008**.

	Consumer Liking Scores*							Pear Quality	
Treatment	**Overall**	**Pear flavor**	**Sweetness**	**Juiciness**	**Firmness**	**Texture**	**Ranked First****	**Soluble solids (%)**	**Firmness (lbf)**
4 day ethylene	7.46 a	7.47 a	6.83 a	7.57 a	6.62 a	6.88 a	50%	15.1 a	2.47 c
2 day ethylene	6.13 bc	6.03 bc	5.06 c	4.97 c	6.17 ab	5.94 b	16%	14.7 bc	4.56 b
1 day ethylene	5.58 c	5.73 c	4.34 d	3.67 d	5.65 b	5.23 c	11%	14.5 c	6.71 a
5 day air	6.42 b	6.45 b	5.92 b	6.43 b	5.89 b	5.82 bc	23%	14.8 b	2.75 c

Scale for liking is 1 =dislike extremely to 9 = like extremely
* Scale for sweetness/juiciness is 1 =not sweet/juicy to 9 = ideally sweet/juicy
** Percentage of fruit in each treatment ranked first ("best")

Overall, 37 out of 448 pears were given an overall liking score of 9 (highest possible score) and 20 pears were given an overall liking score of 1 or 2 (lowest scores). The average scores for all the preference categories, along with the average firmness and soluble solids values for these fruit, are shown in Table 5.

Fruit with an overall liking score of 9 (liked extremely) consisted mostly of 4-day ethylene-treated fruit (60%) and was most often described as having good "texture" (54%), followed closely by "sweetness" (51%). Other words consumers used to describe the characteristics of this fruit included, "smooth and buttery," "just right," and "very good."

Fruit with an overall liking score of 1 or 2 (disliked extremely and disliked very much) consisted mostly of 1-day ethylene -treated fruit (60%). The most common reason given for disliking the fruit was "texture" (60%). Other words consumers used to describe the characteristics of this fruit included, "mealy," "too firm," and "grainy."

Table 5. Average preference scores, firmness values, and soluble solids levels for the highest (9) and lowest (1 and 2) scored fruit over two days of sensory testing at the OSU FIC, Portland Oregon, **December 9-10, 2008**.

Overall Liking	**Pear Flavor**	**Sweetness**	**Juiciness**	**Firmness**	**Texture**	**Firmness (lbf)**	**Soluble solids (%)**
9.0	8.5	7.8	8.2	8.1	8.3	2.8	15.2
1.9	2.7	2.1	2.4	3.0	2.6	6.0	14.6

Scale for liking is 1 =dislike extremely to 9 = like extremely
* Scale for sweetness/juiciness is 1 =not sweet/juicy to 9 = ideally sweet/juicy

Consumer Comments

In Experiment 1 (October), the most common reason for liking was juiciness (42%), followed by sweetness (35%). In Experiment 2 (December), these attributes were reversed, with sweetness the most common (42%) followed by juiciness (23%). It is interesting that firmness is only the third most common reason for liking, below sweetness and juiciness. In both experiments, lack of flavor was the most common reason for disliking (33% and 35%, respectively) (Table 6).

Table 6. Reasons for liking and disliking pears, Experiments 1 and 2

	Reasons for Liking/Disliking Fruit	
	Experiment 1, Oct. 2008	**Experiment 2, Dec. 2008**
Reasons for Liking		
Juiciness	**42%**	23%
Sweetness	35%	**42%**
Firmness	17%	21%
Tartness/sourness	3%	5%
Other	3%	8%
Smell/aroma	1%	0%
Reasons for Disliking		
Lack of flavor	**33%**	**35%**
Too hard	29%	18%
Gritty texture	16%	13%
Too soft	7%	14%
Lack of sweetness	5%	2%
Too tart or sour	4%	7%
Lack of juiciness	4%	6%
Other	2%	3%
Not tart or sour enough	0%	1%
Skin color	0%	1%

Ripening Expectation

To further define the target consumer's expectations they were asked how long they would be willing to wait for pear to ripen after purchase. Their response was resoundingly 4 days or less.

- 1 to 2 days - 36%
- 3 to 4 days - 54%
- 5 to 6 days – 10%

Response to Ethylene as a Conditioning Agent

Ethylene used during conditioning speeds the ripening of Anjou pears as compared to warm room conditioning as shown in Tables 2 and 4. An additional contribution of ethylene conditioning is the promotion of uniformity of ripening. Thus a box, pallet or truckload of Anjou pears conditioned with ethylene can be expected to ripen more uniformly. This can be seen by comparing the standard deviations in both the tests performed with the fruit conditioned for the consumer trials, but also in more detailed laboratory studies that were run concurrently (Tables 7 and 8).

Table 7. Comparisons of firmness and standard deviations between the most acceptable ethylene and the most comparable air conditioned fruit used in the consumer trials.

	Experiment 1		**Experiment 2**	
Firmness	**6-day ethylene**	**7-day air**	**4-day ethylene**	**5-day air**
Minimum	1.4	6.4	1.4	1.3
Maximum	4.8	19.3	4.1	7.0
Average	**2.4**	**11.6**	**2.5**	**2.7**
Standard Deviation	***0.6***	***1.9***	***0.5***	***1.3***

Table 8. Standard deviations after various conditioning treatments followed by ripening to ideal eating firmness of approximately 2 lbf.

Treatment	Days			Firmness (lbf)			Std Dev
	Conditioned	Cooling	Ripening	Minimum	Maximum	Average	
Air	7	3	1	1.54	6.07	**2.58**	***1.30***
Air	7	3	3	1.01	1.72	**1.36**	***0.23***
Air	5	3	3	1.17	6.54	**2.55**	***1.73***
Air	5	3	5	1.38	4.10	**2.45**	***0.86***
Ethylene	4	0	0	1.78	4.88	**2.83**	***0.90***
Ethylene	4	3	1	1.70	2.99	**2.13**	***0.39***
Ethylene	2	3	3	2.44	3.09	**2.85**	***0.23***
Ethylene	2	3	5	1.36	2.68	**1.83**	***0.38***
Ethylene	2	3	3	2.44	3.09	**2.85**	***0.23***
Ethylene	2	3	5	1.36	2.68	**1.83**	***0.38***

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

Project Title: Factors influencing development of d'Anjou pear scald and speckling

PI: Jim Mattheis
Organization: USDA, ARS
Telephone/email: 509-664-2280
James.Mattheis@ARS.USDA.GOV
Address: 1104 N. Western Ave.
City: Wenatchee
State/Province/Zip WA 98801

Co-PI(2): Dave Rudell
Organization: USDA, ARS
Telephone/email: 509-664-2280
Dave.Rudell@ARS.USDA.GOV
Address: 1104 N. Western Ave
City: Wenatchee
State/Province/Zip: WA 98801

Total project funding request: **Year 1:**$25,875 **Year 2**:$27,200 **Year 3: $27,990**

Other funding Sources: none
WTFRC Collaborative expenses: none

Budget 1:
Organization Name: USDA, ARS **Contract Administrator:** Charles Myers
Telephone: 510-559-6019 **Email address:** Charles.Myers@ARS.USDA.GOV

Item	Year 1: 2007	Year 2: 2008	Year 3: 2009
Salaries	25,375*	26,690*	27,490*
Benefits	0	0	0
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	500	500	500
Travel	0	0	0
Miscellaneous	0	0	0
Total	25,875	27,190	**27,990**

Footnotes: *0.5 salary for GS11 postdoctoral research associate

Objectives:

1. Characterize pear peel metabolic profiles during air and CA storage under conditions known to enhance or suppress development of superficial scald and speckling.

2. Identify lot specific metabolic profiles that are indicative of susceptibility to development of superficial scald and speckling.

Significant Findings:

- No speckling developed and scald developed only on fruit stored in air.
- Core browning (limited to seed cavity walls) developed in all lots stored at 0.4 or 0.5% O_2 (UA) and 3 or 5 lots stored at 1.5% O_2 (CA).
- Differences in peel metabolic profiles for fruit stored in 0.5 or 1.5% O_2 and air were detected at one month and continued through 6 months.
- Some lots stored in UA through 4 months did not soften to eating ripe during 7 days at 68F.

Methods

Fruit obtained at commercial harvest in 2007 from 5 orchards in the Wenatchee area was stored at 33 ^{o}F in air or CA (1.5% O_2/ 0.5% CO_2) established by day 3 after harvest. Another low O_2 environment (ultra-low atmosphere, UA) was established with the same CO_2 concentration but a low O_2 setpoint (the O_2 concentration at which a change in chlorophyll fluorescence occurred for each lot plus 0.2% O_2). This value was determined on day 2 after harvest, and the low O_2 setpoint was established by day 3. Peel samples were collected at harvest and after 1,2,3, and 4 weeks (CA, UA), and 2,4 and 6 months. Peel tissue was extracted and analyzed by GC/MS and LC/MS for water and lipid soluble compounds, particularly compounds related to respiration, anaerobiosis, as well as pigments and other secondary metabolites. Ethanol analyses were conducted by GC/FID on additional peel samples at each pull date. Fruit quality (color, firmness, acidity, soluble solids content, disorders), ethylene, and CO_2 production was evaluated at harvest and after storage. A similar study is in progress with fruit harvested in 2008.

Results and Discussion

The five orchard lots used in the 2007 study differed slightly in the O_2 concentration at which a change in chlorophyll fluorescence was detected. The change occurred in 3 lots at 0.2% and in 2 lots at 0.3%. The fluorescence signal returned to a base level following an increase in O_2 to the final setpoints (0.4 and 0.5% O_2). These values are in the same range as those observed in our previous work.

Scald did not develop on fruit stored in CA or UA although based on fruit stored in air, scald susceptibility was high in only one orchard. No speckling was observed on any fruit indicating a possible impact from season or orchard at least for fruit produced in the Wenatchee river valley. Fruit from orchards 1-4 in 2007 and from one orchard in 2008 stored in UA softened slower relative to fruit stored in air or CA indicating the rate of ripening after storage may be slower in fruit stored at lower O_2 concentrations. We have observed this residual impact of low O_2 CA in previous years.
The incidence of seed cavity browning (Figure 1) in fruit from all lots stored in UA has not been observed previously. At 2 and 4 months, ethanol content was highest in fruit stored at the low O_2

setpoint, however, further work is needed before a cause and effect relationship between fruit ethanol content and seed cavity browning development can be established.

The metabolic data indicated fruit could be chemically differentiated by storage treatment after one month (Figure 1). The differences in these profiles were due to a number of individual compounds including but not limited to primary (amino and organic acids, sugars) metabolites, vitamins, antioxidants, and pigments. Patterns of some individual compounds included a large reduction in vitamin C during the first 4 weeks after harvest regardless of storage regime and a more moderate reduction in vitamin E throughout the storage period. A number of peel chemicals including ursolic acid and β-sitosterol with putative cholesterol-lowering and antioxidant capacity were found in the peel and were differentially impacted by both storage atmosphere and storage duration (Figure 3). More work is needed to identify some of these sterols and to determine what if any relationship they have to peel disorders or the lack thereof.

Orchard 1

Storage	1.5% O_2, 0.5% CO_2			0.5% O_2, 0.5% CO_2			air	
Months	2	4	6	2	4	6	2	4
Titr.Acidity %	0.201	0.160	0.174	0.244	0.185	0.211	0.184	0.165
Peel Color[1]	1	1	1.6	1	1.3	1.1	1.7	1.7
core browning %[2]	0	0	0	39	6	28	0	0
scald %[2]	0	0	0	0	0	0	0	0
lbs	4.8	3.0	1.8	5.4	5.3	3.2	2.5	2.2
CO_2 umol	410	540	530	460	550	650	530	630
ethylene umol	0.10	0.30	1.05	0.13	0.37	1.87	0.30	1.0
ethanol umol	9.9	35	198	43	157	288	11	39

Orchard 2

Storage	1.5% O_2, 0.5% CO_2			0.4% O_2, 0.5% CO_2			air	
Months	2	4	6	2	4	6	2	4
Titr.Acidity %	0.241	0.224	0.211	0.272	0.281	0.247	0.237	0.236
Peel Color[1]	1.1	1	1.3	1	1	1.3	1.6	1.4
core browning %[2]	0	0	0	11	89	67	0	0
scald %[2]	0	0	0	0	0	0	0	6
lbs	8.1	2.5	2.4	7.0	7.5	2.6	2.8	2.9
CO_2 umol	290	390	420	340	300	610	420	580
ethylene umol	nd[3]	0.05	0.18	0.01	0.01	0.52	0.01	80
ethanol umol	13	15	50	51	86	243	27	19

Orchard 3

Storage	1.5% O_2, 0.5% CO_2			0.4% O_2, 0.5% CO_2			air	
Months	2	4	6	2	4	6	2	4
Titr.Acidity %	0.244	---	0.181	0.242	---	0.188	0.213	---
Peel Color[1]	1.1	1	1.5	1	1.33	1.1	1.4	1.4
core browning %[2]	0	0	0	33	39	0	0	0
scald %[2]	0	0	0	0	0	0	0	6
lbs	6.7	3.2	2.4	6.1	7.2	2.3	2.3	2.7
CO_2 umol	340	360	560	340	320	340	560	450
ethylene umol	nd[3]	0.12	0.64	0.03	0.03	0.26	0.04	0.35
ethanol umol	22.7	21.4	99	21.2	45.3	156	10.5	31.5

Orchard 4

Storage	1.5% O_2, 0.5% CO_2			0.5% O_2, 0.5% CO_2			air	
Months	2	4	6	2	4	6	2	4
Titr.Acidity %	0.264	0.183	0.199	0.226	0.277	0.206	0.231	0.154
Peel Color[1]	1	1.2	1.9	1	1.1	1.7	1.8	2.2
core browning %[2]	6	11	6	83	56	67	0	0
scald %[2]	0	0	0	0	0	0	0	0
lbs	5.5	2.9	2.1	5.6	4.8	2.8	2.4	1.78
CO_2 umol	375	500	510	470	430	435	530	690
ethylene umol	0.04	0.53	0.99	0.19	0.1	0.68	0.26	1.3
ethanol umol	2.7	23.9	197	32.1	79	319	17.4	40.6

Orchard 5

Storage	1.5% O_2, 0.5% CO_2			0.4% O_2, 0.5% CO_2			air		
Months	2	4	6	2	4	6	2	4	6
Titr.Acidity %	0.207	0.197	0.203	0.197	0.174	0.157	0.180	0.161	0.168
Peel color[1]	1.1	2.1	1.6	1	1.6	1.3	2.1	3.2	5
core browning %[2]	17	28	33	61	63	67	0	0	25
scald %[2]	0	0	0	0	0	0	0	47	5
lbs	4.6	2.0	2.0	5.4	2.9	2.0	2.3	2.2	3.4
CO_2 umol	330	444	545	330	364	430	460	495	600
ethylene umol	nd[3]	1.2	1.6	nd	0.85	0.85	nd	2.0	1.05
ethanol umol	21.3	64	489	62	270	814	15.5	71	858

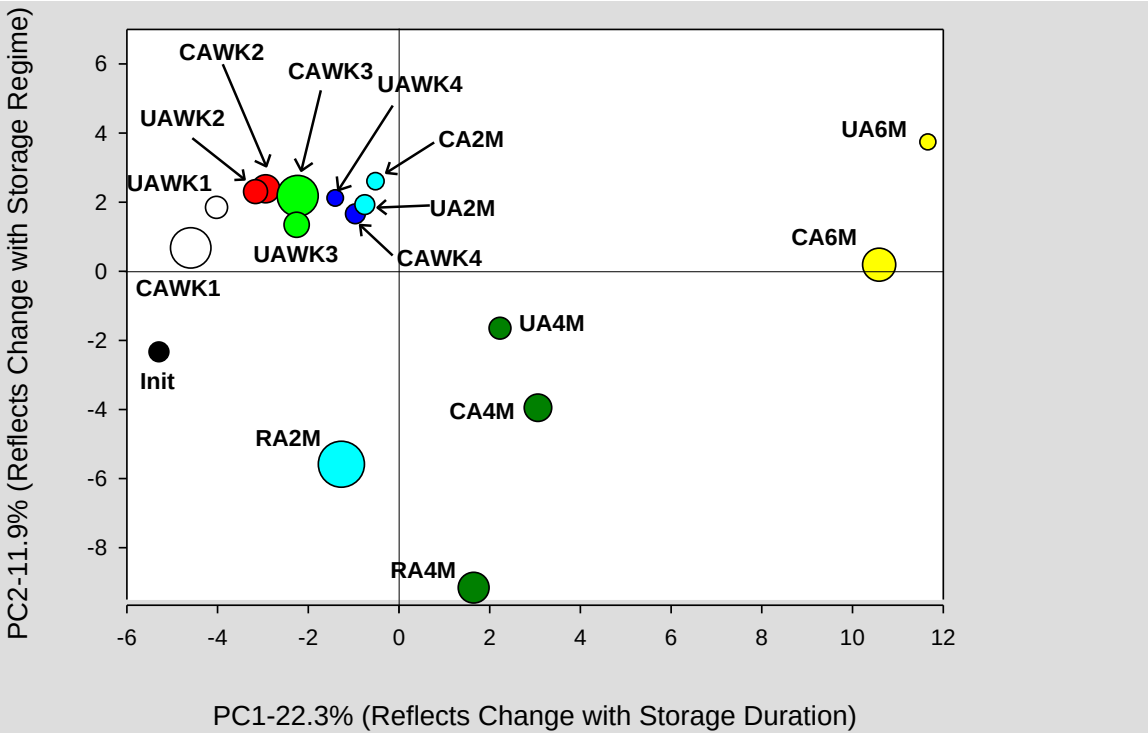
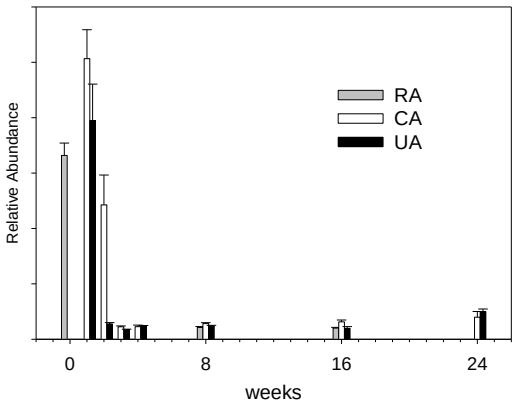


Figure 1. Separation of Anjou peel metabolic profiles based on storage duration and storage atmosphere. Fruit were stored up to 6 months in air (RA) or 0.5% CO_2 with 1.5 (CA) or 0.5 (UA) % O_2 at 33F.



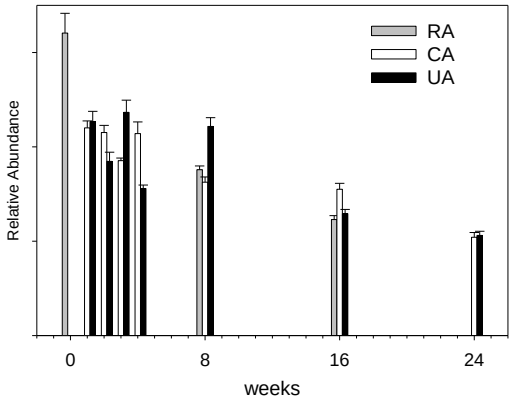
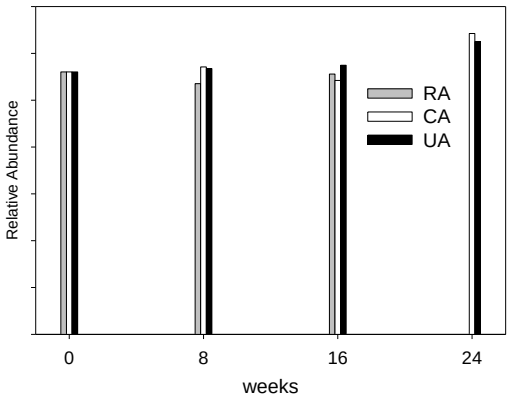


Figure 2. Vitamin C in Anjou pear peel. Vitamin E in Anjou pear peel.
Fruit stored in air (RA) or 0.5% CO_2 with 1.5 (CA) or 0.5 (UA)% O_2 at 33F. Samples collected immediately after removal from storage.



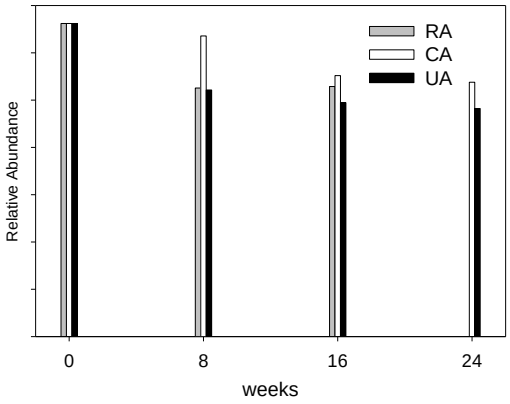


Figure 3. Ursolic acid in Anjou pear peel. B-sitosterol in Anjou pear peel.
Fruit stored in air (RA) or 0.5% CO_2 with 1.5 (CA) or 0.5 (UA)% O_2 at 33F. Samples collected immediately after removal from storage.

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

Project Title: Pear fruit quality improvement

PI: David Sugar
Organization: Oregon State University
Telephone/email: 541-772-5165
Address: Southern Oregon Research and Extension Center
Address 2: 569 Hanley Rd.
City: Medford
State/Province/Zip Oregon 97502

Total project funding request: **Year 1:** 28,997 **Year 2:** 28,997 **Year 3: 28,997**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget:

Organization: Oregon Agricultural Research Foundation	**Contract Administrator:** Dorothy Beaton
Telephone: 541-737-3228	**Email:** dorothy.beaton@oregonstate.edu

Item	**Year 1: 2007**	**Year 2: 2008**	**Year 3: 2009**
Salaries	18,238	18,238	18,238
Benefits	10,759	10,759	10,759
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Total	28,997	28,997	**28,997**

Objective: Develop methods to improve pear fruit quality, with respect to managing fruit ripening capacity and postharvest decay, enhancing fruit size, and reducing or enhancing fruit russet.

Significant Findings:

1. **Developing early ripening capacity in Comice, Bosc, and Anjou pears**. The relationship between harvest maturity and the duration of "chill" or "temperature conditioning" required to induce ripening, and the temperature at which chill is accomplished were studied in Comice, Bosc, and Anjou. The number of days of chill required decreased in a linear, predictable fashion with the number of days after the orchard block reached the top of the maturity range. A potentially useful technique was developed to induce ripening capacity in a shorter period of time by conditioning at 41-50°F rather than at 31 °F. In Anjou pears exposed to ethylene for 24-48 hours, less than 10 days of further conditioning at 50°F were needed to induce the capacity to ripen to excellent eating quality.

2. **Postharvest decay control programs**. The focus of this year's study has been dealing with the facts that (1) postharvest fungicide and biocontrol treatments lose effectiveness if applied more than 3 weeks after harvest, and (2) there is a trend in the pear industry to delay postharvest line-spray fungicide treatments due to prolonged storage in field bins prior to packing. The strategy suggested by this research emphasizes summer calcium treatments followed by application of appropriate fungicides approximately one week before harvest.

3. **Fruit size enhancement**. MaxCel applied at 125 ppm appears to enhance fruit size in Bartlett pears when applied at the 10-12 mm fruit diameter stage, but slightly earlier may be optimum for other varieties. There are indications that sequential treatments of urea and MaxCel can provide greater size enhancement to either material alone.

4. **Russet management**. Russet development in Bosc was enhanced by lime sulfur application at late petal-fall. Comice russet was reduced by Pristine, Mancozeb, and Surround during the four weeks following petal-fall. Combinations of these materials were the most effective. Comice and Bosc susceptibility to russet was greatest when fruit were kept wet during the second week after petal-fall. Long-term correlations between russet and weather factors also point to this period of susceptibility.

Methods:

A variety of orchard and postharvest treatments were applied in a wide range of experiments.

Results and Discussion:

1. **Developing early ripening capacity in Comice, Bosc, and Anjou pears**. Important discoveries have been made regarding the relationship of maturity at harvest to the length of "chill" time needed to induce ripening capacity in Comice, Bosc, and Anjou pears. The number of days of chill (temperature conditioning) at 31°F reduces in a predictable fashion with later harvest. Furthermore, across all harvest dates and in all varieties, conditioning is substantially faster if provided at 41-50°F. The optimum conditioning temperature appears to be 50°F. When ethylene 100 ppm is provided for 24-48 hours prior to conditioning at these "intermediate" temperatures, the process of inducing ripening capacity for early marketing can be quite brief. The approximate number of days of conditioning necessary at each temperature is summarized in Table 1.

2. **Postharvest decay control programs**. The focus of this year's study has been dealing with the facts that (1) postharvest fungicide and biocontrol treatments lose effectiveness if applied more than 3

weeks after harvest, and (2) there is a trend in the pear industry to delay postharvest line-spray fungicide treatments due to prolonged storage in field bins prior to packing. A large experiment this year tested a "calcium backbone" followed by pre-harvest fungicide sprays of Pristine, Topsin M, or Flint for how well the orchard program could protect wounds at harvest from decay until postharvest fungicides were applied. Timing of postharvest application ranged from 0 to 8 weeks after harvest. Results are expected in early February 2009. In addition to factors testing in this research, decay control programs should always include minimizing fruit nitrogen content through controlling the amount and timing of fertilizer application, and prompt harvest after the onset of fruit maturity.

3. **Fruit size enhancement**. The timing of application of MaxCel at 125 ppm was tested in Bartlett, Red Anjou, Bosc, and Comice pears. It appears that the 10-12 mm fruit diameter application timing recommended by the manufacturer is optimum for Bartlett, but may be somewhat earlier for the other varieties (Tables 2-5). The effectiveness of urea applications increased with increasing dosage of urea in the solution from 5% through 9%. In several cases, it appears that urea treatment followed by MaxCel at 125 ppm can enhance fruit size to a greater extent than either treatment alone. This sequential treatment program will be the focus of the next phase of this project. In many cases, MaxCel treatment reduced the overall tonnage of production, but either did not affect or enhanced yield of fruit of greater than or equal to size 90 (Tables 2-5).

4. **Russet management**. While copper treatment has enhance Bosc russet in most years, in 2008 russet was not enhanced by copper at petal-fall, but lime sulfur treatment was effective (Table 6). Some fruit treated with lime sulfur showed "splotchy" russet patches. In analyzing 10 years of russet data and weather factors in Comice, the period 15-21 days after full bloom shows the greatest correlation between weather factors and russet, evapotranspiration (ET) being the most predictive (Table 7). The period 8-14 days before full bloom appears to being important for ET to influence the susceptibility of Bosc to russet development, along with wind in the period 22-28 days after full bloom. These are factors that are believed to stimulate cuticle formation, which protects against russet development. Similarly, the effectiveness of copper treatment for enhancing Bosc russet over 7 years indicates that copper treatment is most effective when weather conditions that suppress natural cuticle formation occur (Table 7). Keeping fruit wet in plastic bags for various one-week periods after petal-fall showed that Comice and Bosc pears were most sensitive to develop russet in response to wetting during the second week after petal-fall (Table 8). Pristine, mancozeb, and Surround applied during the four weeks following petal-fall reduced russet in Comice, and combinations of two or more of these materials appeared to provide greater russet control (Table 9). These results indicate that targeting russet control treatments with these materials to the period of greatest susceptibility should provide the greatest benefit.

Table 1. Approximate number of days of temperature conditioning ("chill") needed to induce ripening capacity in Bosc, Comice, and Anjou pears when conditioning is provided at various temperatures, and with or without prior exposure to 100 ppm ethylene.

	Days of conditioning needed to induce ripening capacity (Fruit harvested at beginning of maturity range)								
	No ethylene			**24 h ethylene at 68°F**			**48 h ethylene at 68°F**		
	31°F	**41°F**	**50°F**	**31°F**	**41°F**	**50°F**	**31°F**	**41°F**	**50°F**
Bosc	15	9	5	0	0	0	0	0	0
Comice	30	18	12	15	7	3	7	2	2
Anjou	>60	35	17	44	18	<10	38	12	<10

Table 2. Bartlett: Effect on fruit quality and production parameters of various treatments with MaxCel plant growth regulator applied at 125 ppm, urea applied as a 5% solution, or sequential combinations of the two treatments.

Bartlett	**Timing**	**Av.fruit diameter**	**Ave. fruit weight (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water			205 c	71.7 a	16.5 a	40.3 c	6.7 b
MaxCel 125	Petal-fall	5 mm	202 c	61.7 ab	16.3 ab	39.0 c	6.3 b
MaxCel 125	PF+ 5 d	7 mm	212 c	56.0 ab	15.1 abc	47.9 c	7.2 ab
MaxCel 125	PF+10 d	10 mm	245 ab	48.4 ab	11.7 c	72.8 ab	8.7 ab
MaxCel 125	PF+15 d	12 mm	252 a	52.5 ab	12.6 bc	78.7 a	9.9 a
MaxCel 125	PF+18 d	17 mm	233 b	39.9 b	13.6 abc	66.7 b	9.0 ab

Bartlett	**Timing**	**Ave. fruit weight (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit > size 90**	**Tons / acre > size 90**
Water		205 c	71.7 a	16.5 a	40.3 b	6.7 a
Urea 5%	80% bloom	213 bc	62.4 a	16.7 a	47.9 ab	7.8 a
Urea 7%	80% bloom	227 ab	35.2 b	14.6 a	59.5 a	8.5 a
Urea 9%	80% bloom	239 a	37.7 b	12.0 a	65.1 a	7.7 a

Bartlett	**Timing**	**Ave. fruit weight (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water		205 b	71.7 a	16.5 a	40.3 b	6.7 b
Urea 5%	80% bloom	213 b	62.4 a	16.7 a	47.9 b	7.8 b
MaxCel 125	Petal-fall	202 b	61.7 a	16.3 a	39.0 b	6.3 b
Urea 5%, then MaxCel 125	80% bloom Petal-fall	252 a	57.3 a	14.8 a	76.9 a	11.4 a

Table 3. Red Anjou: Effect on fruit quality and production parameters of various treatments with MaxCel plant growth regulator applied at 125 ppm, urea applied as a 5% solution, or sequential combinations of the two treatments.

Red Anjou	**Timing / fruit diameter**	**Ave. fruit wt (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water		187 c	65.1 a	12.0 a	24.9 b	3.5 a
MaxCel 125	Petal-fall	220 ab	36.8 ab	8.4 ab	56.2 a	4.6 a
MaxCel 125	PF+ 5 d/6-8 mm	221 ab	26.5 b	6.3 b	58.4 a	4.3 a
MaxCel 125	PF+10 d/8-11mm	239 a	21.2 b	4.3 b	71.9 a	3.2 a
MaxCel 125	PF+15 d/11-13 mm	211 bc	33.1 b	6.3 b	48.0 ab	3.1 a

Red Anjou	**Timing**	**Ave. fruit wt (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water		187 b	65.1 a	12.0 a	24.9 b	3.5 a
Urea 5%	80% bloom	218 ab	43.1 a	9.1 a	53.3 a	5.4 a
MaxCel 125	Petal-fall	220 a	36.8 a	8.4 a	56.2 a	4.6 a
Urea 5%, then MaxCel 125	80% bloom Petal-fall	248 a	49.6 a	9.3 a	78.4 a	7.1 a

Table 4. Bosc: Effect on fruit quality and production parameters of various treatments with MaxCel plant growth regulator applied at 125 ppm, urea applied as a 5% solution, or sequential combinations of the two treatments.

Bosc	**Timing / fruit diameter**	**Ave. fruit weight (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water		234 b	111.9 a	15.1 a	70.1 a	10.5 a
MaxCel 125	Petal-fall	241 b	99.3 a	13.7 a	74.4 a	10.1 a
MaxCel 125	PF + 5 d/5-7 mm	270 a	94.7 a	11.8 a	83.9 a	9.8 a
MaxCel 125	PF+10 d/8-10 mm	251 ab	95.1 a	15.9 a	80.0 a	12.7 a
MaxCel 125	PF+15 d/10-12 mm	251 ab	96.2 a	15.4 a	83.0 a	12.8 a

Bosc	**Timing**	**Ave. fruit weight (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water		234 b	111.9 a	15.1 a	70.1 a	10.5 a
Urea 5%	80% bloom	238 ab	98.0 a	12.9 a	75.1 a	9.5 a
MaxCel 125	Petal-fall	241 ab	99.3 a	13.7 a	74.4 a	10.1 a
Urea 5%, then MaxCel 125	80% bloom Petal-fall	266 a	77.7 a	11.7 a	83.0 a	9.6 a

Table 5. Comice: Effect on fruit quality and production parameters of various treatments with MaxCel plant growth regulator applied at 125 ppm, urea applied as a 5% solution, or sequential combinations of the two treatments.

Comice	**Timing / fruit diameter**	**Ave. fruit weight (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water		237 b	65.0 a	13.3 a	65.2 c	8.5 a
MaxCel 125	Petal-fall	244 ab	33.5 b	10.2 ab	68.3 bc	7.1 a
MaxCel 125	PF+5 days / 5-7 mm	261 ab	32.8 b	11.2 ab	73.6 abc	8.1 a
MaxCel 125	PF+10 d / 7-9 mm	278 a	27.8 b	9.3 ab	84.5 a	7.8 a
MaxCel 125	PF+15 d/10-12 mm	271 ab	23.0 b	7.4 b	80.3 ab	6.0 a

Comice	**Timing**	**Ave. fruit weight (g)**	**Fruit set / 100 clust.**	**Tons / acre**	**% of fruit ≥ size 90**	**Tons / acre ≥ size 90**
Water		237 a	65.0 a	13.3 a	65.2 a	8.5 a
Urea 5%	80% bloom	251 a	33.3 b	11.5 a	69.0 a	7.8 a
MaxCel 125 ppm	Petal-fall	244 a	33.5 ab	10.2 a	68.3 a	7.1 a
Urea 5%, then MaxCel 125	80% bloom Petal-fall	263 a	31.3 b	9.3 a	78.3 a	7.5 a

Table 6. Effects of treatments with Kocide 2000 and lime-sulfur on incidence of high-russet fruit in standard Bosc pears.

Material	**Timing**	**Rate basis**	**% of fruit with ≥ 97% russet**
Untreated	-		32.3 a
Kocide 2000	Early petal-fall	0.25 lb/100 gallons	38.9 ab
Kocide 2000	Early PF	0.50 lb/100	31.4 a
Lime-Sulfur	Late PF	3 gal/acre	52.2 bc
Kocide 2000 Lime-Sulfur	Early PF Late PF	0.25 lb/100 3 gal/acre	65.5 c
Kocide 2000 Lime-Sulfur	Early PF Late PF	0.50 lb/100 3 gal/acre	64.7 c

Table 7. Correlations between russet coverage of Comice and Bosc pears and environmental measures during periods before and after bloom.

Variety	**Years of data**	**Period of greatest effect**	**Significant factors**	**Correlation measure**	**Statistical significance**
Comice	10	15-21 d after full bloom (FB)	Temperature Rainfall EvapoTransp.	-0.809 +0.723 -0.857	0.005 0.018 0.002
Bosc	7	8-14 d before FB	EvapoTransp. Solar radiation	-0.932 -0.855	0.002 0.014
		22-28 d after FB	Wind run	-0.781	0.038
Bosc + copper at petal-fall	7	15-21 d after FB	Wind run Rainfall	-0.957 +0.805	0.001 0.029
		22-28 d after FB	Rainfall EvapoTransp. Wind run	+0.872 +0.760 -0.908	0.011 0.047 0.005

Table 8. Development of russet in Comice and Bosc pears kept constantly wet for different 1-week periods beginning at petal-fall.

	Comice	**Bosc**
Timing of fruit wet period	**% of fruit with ≥ 6% russet**	**% of fruit with ≥ 87% russet**
No wet period	5.0 c	0.0 c
1st week after PF	54.3 b	46.9 b
2nd week after PF	92.3 a	73.1 a
3rd week after PF	51.5 b	48.9 b
4th week after PF	18.6 c	54.7 ab

Table 9. Control of russet in Comice pears with treatment programs applied at petal-fall and 2 and 4 weeks after petal-fall (Mancozeb is active ingredient in products Dithane and Manzate).

Treatment	**Rate**	**% of fruit with ≥ 6 % surface russet**
Untreated		11.6 a
Pristine	14.5 oz/acre	5.5 b
Pristine + Mancozeb	14.5 oz + 3 lb/acre	2.2 b
Pristine + Mancozeb + Surround	14.5 oz + 3 lb + 25 lb/acre	1.5 b

Untreated		11.6 a
Mancozeb	3 lb/acre	9.8 ab
Mancozeb + Procure	3 lb + 12 fl oz /acre	8.6 ab
Mancozeb + Pristine	3 lb + 14.5 oz /acre	2.2 bc
Mancozeb + Surround	3 lb + 25 lb/acre	1.3 c
Mancozeb + Pristine + Surround	3 lb + 14.5 oz + 25 lb/acre	1.5 c

Untreated		11.6 a
Surround	25 lb/acre	6.5 b
Surround + Mancozeb	25 lb + 3 lb/acre	1.3 c
Surround + Mancozeb + Pristine	25 lb + 3 lb/acre + 14.5 oz	1.5 c