2008 Northwest Pear Research Review
Wenatchee, Washington

Thursday, February 21

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			Final Reports	
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FINAL PROJECT REPORT

Project Title: New approaches to decay control of pear

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Cooperators: WSU (Chang-Lin Xiao)
SOREC (David Sugar)
Ag Canada (Peter Sholberg, Dan O'Gorman)
New Zealand HortResearch (Trish Virgin, Monika Walter)
Lincoln University (Alison Stewart)

Total project funding: 155,372

Budget: 2005-6: 48,862 2006-7: 51,733 2007-8: 54,777

Significant findings:

- A 4-factor gray mold risk prediction model has been developed for the major Oregon and Washington pear districts.
- A real time PCR method to determine the concentration of decay spores (*Penicillium expansum* and *Mucor piriformis*) in dump tank and flume water has been developed.
- 758 new bull's-eye rot isolates have been identified. *N. perennans* is the most common species in Yakima and Wenatchee and *N. alba* the most common in Hood River and Medford.
- A new unnamed species of bull's-eye rot (Neofabraea sp. nova) was found in all major pear districts
- The benzimidazoles thiophanate methyl (Topsin) and thiabendazole (Mertect) appear to have the most effect on *N. alba* and *N. perennans*
- A qPCR method was developed to determine threshold "residues" of the biocontrol agent CIM required on pear fruit for optimum decay control.
- The most effective preharvest spray for overall decay control in 2005-6 was a tank mix of Topsin M and Nutraphos 24.
- Postharvest fungicides Pristine, Penbotec, and Scholar controlled blue and gray mold
- *Muscodor albus*, a biological control agent, significantly controlled blue mold, gray mold, and mucor rot of d'Anjou pear fruit.
- Protective Chemistries paint formulations 1020, 1024, 3020, and 4020 were very effective for prevention of growth of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* on wood and plastic surfaces.

Results and discussion:

1. New model for decay risk prediction

A 4-factor gray mold risk prediction model is shown below in Table 1. The model was developed using data from pear orchards in Oregon, Washington, and New Zealand in 2004-6 (Table 2). Actual decay aligned extremely well with the predicted risk level for fruit from all districts in all three years (Table 2).

Model validation on a larger scale is necessary before the model can be used by the fruit industry. During the 2007-8 season, the model is being validated in orchards in Wenatchee (Dr. Chang–Lin Xiao), Medford (Dr. David Sugar), and the Mid-Columbia (Dr. Bob Spotts) and will use packinghouse cull analyses when available.

Improvements are continually being made and include new, more specific PCR primers for DNA analysis, use of Millipore membranes for high efficiency capture of spores, and scale-up of laboratory methodology to handle large sample numbers necessary for commercialization.

Table 1. Pear gray mold risk prediction model (version 1.00)

			Orchard rating[4]		
DNA[1]	Fungicide[2]	Rain[3]	1	2	3
L	Yes	No	L	L	M
L	Yes	Yes	L	M	H
L	No	No	L	M	H
L	No	Yes	M	H	E
H	Yes	No	L	M	H
H	Yes	Yes	M	H	E
H	No	No	M	H	E
H	No	Yes	H	E	E

[1]L = *B. cinerea* DNA 0 to 2.2 pg/cm^2; H = over 2.2 pg/cm^2.
[2,3]Yes = fungicide applied within 4 weeks of harvest; 0.02 inches within 2 weeks of harvest.
[4]1 = young to moderate age trees, excellent horticultural and pest/disease practices.
[4]2 = moderate age trees, average horticultural and pest/disease control practices.
[4]3 = old trees, poor horticultural and pest/disease control practices.
[5]Risk levels: L = low, M = moderate, H = high, E = extreme.

Table 2. Pear gray mold predicted risk vs. actual decay in cold stored fruit from Oregon, Washington, and New Zealand

	2004-2005		2005-2006		2006-2007	
Orchard[x]	Predicted risk[y]	Gray mold (%)[z]	Predicted risk[y]	Gray mold (%)[z]	Predicted risk[y]	Gray mold (%)[z]
1	E	14.0	H	8.7	H	7.4
2	H	8.0	H	7.3	H	3.3
3	H	7.0	H	6.6	H	3.0
4	H	5.9	M	5.9	M	3.1
5	H	4.2	M	5.1	M	1.1
6	M	2.6	M	3.8	M	0.9
7	M	2.2	M	3.4	M	0.6
8	M	2.2	L	2.1	---	---
9 Medford					M	1.3
10 Medford					M	1.0
11 Medford					M	0.7
12 Wenatch					M	ND
13 Wenatch					M	ND
14 Wentach					L	ND
15 NZ	M	1.4	L	0.7		
16NZ	L	1.0	L	0.5		
17NZ	L	0.7	L	0.4		
18NZ	L	0.3	L	0.3		
19NZ	L	0.3	L	0.3		
20NZ	L	0.1	L	0.1		

[x]Orchards 1-8 in Mid-Columbia; Orchards 9-11 in Medford; Orchards 12-14 in Wentachee; Orchards 15-20 in Motueka, New Zealand.
[y]Risk levels: L = low, M = moderate, H = high, E = extreme.
[z]Decay after 6 months storage at 30°F.

The average incidence of gray mold at each risk level varied from year-to-year (Table 3). This appears to be related to yearly changes in susceptibility of fruit and matches the annual susceptibility index determined from a standardized lab test (Table 4). For example, the percent gray mold was highest in 2005-6 and lowest in 2006-7 in both actual decay (Table 3) as well as in the standard test (Table 4).

Table 3. Amount of gray mold from decay model risk levels

	Risk level[y]			
	Low	Moderate	High	Extreme
Year	Percent gray mold[z]			
2004-5	0.5	2.1	6.5	14.0
2005-6	0.6	4.5	7.5	---
2006-7	---	1.3	3.7	---

[y]Risk levels from gray mold risk prediction model.
[z]Gray mold is average of all orchards in each risk level.

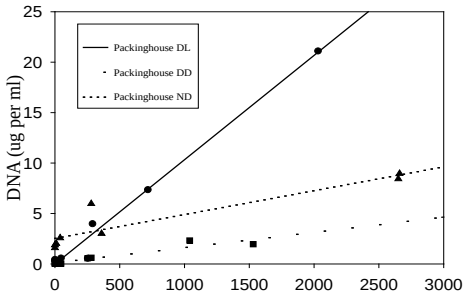
Table 4. Susceptibility of Anjou pear fruit to decay in standardized laboratory conditions

	Infection index[z]		
Year	Gray mold	Blue mold	Mucor rot
2004	22.7b	28.2a	48.4b
2005	28.0b	28.0a	58.2b
2006	13.0a	31.4b	49.2b
2007	32.9c	26.5a	21.6a

[z]Index is calculated as lesion diameter (mm) x proportion of fruit infected. Numbers followed by the same letter within columns are not significantly different at P = 0.05.

2. DNA techniques for rapid, accurate detection of decay spores in packinghouses

Excellent agreement was found between the amount of *P. expansum* DNA in "spiked" dump tank water from three packinghouses and the spore counts from traditional dilution plates. A new method was introduced in 2007 using a Millipore membrane rather than centrifugation to remove spores from the water prior to DNA extraction. The new method is much faster and more accurate than the old method. The experiment needs to be continued with additional water samples from Oregon and Washington packinghouses.

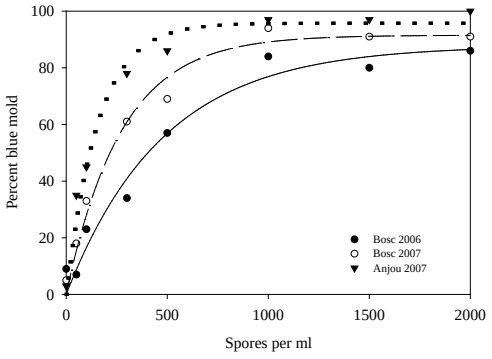


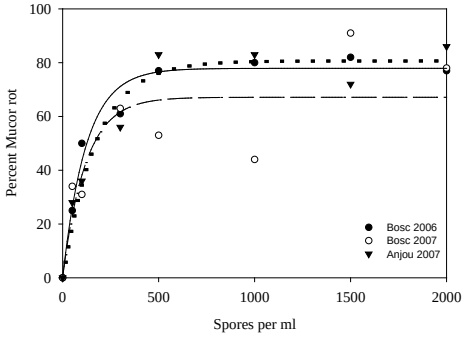
Relationships between decay and spore loads in water

Mucor rot and blue mold decays are closely related to spore loads in packinghouse water systems. We found that the relationship is similar for Bosc pear in 2006(solid line), Bosc in 2007 (dashed line), and Anjou in 2007 (dotted line). The steep curve for Mucor rot between 0 and 500 spores per ml of water indicates that reduction of spore numbers in this part of the curve will result in significant reductions in Mucor rot.

For blue mold, the curve increases more gradually than for Mucor rot, and reductions between 0 and 1,000 spores per ml of water will result in gradual decreases in the amount of decayed fruit.

These results emphasize the importance of good sanitation in the packinghouse. Spore loads in packinghouse water should be reduced as much as possible to reduce decay in storage.





3. Bull's-eye rot species in Washington and Oregon and fungicide sensitivity

We have identified 758 isolates of *Neofabraea* from decayed pear (Table 5) and apple (Table 6) fruit. *N. perennans* is the most common species in Yakima and Wenatchee and *N. alba* the most common in Hood River and Medford. *N. malicorticis* was not found in any Oregon or Washington orchards but is known to occur on the west side of the Cascade Mountains. The new, unnamed species of *Neofabraea* was found in all four districts and was most common on apples from Yakima and Wenatchee (Xiao collection).

Table 5. Summary of *Neofabraea* (bull's-eye) collection in pears

	Percent of total			
Location	*N.alba*	*N.perennans*	New species	Number samples
Yakima	0.0	100.0	0.0	20
Wenatchee	15.8	84.2	0.0	101
Mid Columbia	64.6	34.9	0.6	175
Medford	78.2	16.0	5.8	312

Table 6. Summary of *Neofabraea* (bull's-eye) collection in apples

	Percent of total			
Location	*N.alba*	*N.perennans*	New species	Number samples
Yakima	0.0	81.5	18.5	27
Wenatchee	0.0	88.5	11.5	113
Mid Columbia	0.0	0.0	0.0	0
Medford	90.0	0.0	10.0	10

Table 7. Summary of *Neofabraea* (bull's-eye) entire apple and pear collection

	Percent of total			
Location	*N.alba*	*N.perennans*	New species	Number samples
Yakima	0.0	89.4	10.6	47
Wenatchee	7.5	86.4	6.1	214
Mid Columbia	64.6	34.9	0.6	175
Medford	78.6	15.5	5.9	322

Effect of fungicides on *Neofabraea alba and N. perennans in vitro*

In order to achieve satisfactory control of bull's-eye rot, it is critical to know the *Neofabraea* species profile in each district and use fungicides that have good activity against those species.

We found that the four most effective fungicides for bull's-eye rot control on all four species of *Neofabraea* were the two benzimidazoles, Mertect and Topsin M, Penbotec (pyrimethanil), and Pristine (pyraclostrobin + boscalid) (Table 8). In addition to these four fungicides, other fungicides gave acceptable control of some species of *Neofabraea* but not other species. For example, Scholar controlled bull's-eye rot caused by *N. malicorticis* but not by the other three species.

Copper and ziram have been used routinely for many years in the Pacific Northwest for control of Neofabraea cankers on trees and bull's-eye rot on fruit. We found that ziram was moderately effective, but copper gave poor control of all species except N. malicorticis (Table 8).

Table 8. Control of bull's-eye rot of d'Anjou pear fruit with postharvest dip of fungicides

		Percent bull's-eye rot caused by[x]			
Treatment	Rate[y]	*N. perennans*	*N. alba*	*N. sp. nova*	*N. malicorticis*
Penbotec 400 SC	1000 ppm	0.0a	0.0a	1.7ab	0.0a
Penbotec 400 SC	500 ppm	3.3ab	0.0a	4.2b	0.0a
Topsin M 70WP	1.0 lb/200 gal	0.8ab	1.7ab	0.0a	1.9a
Mertect 340F	16 oz/100 gal	0.8ab	7.4bc	2.6a	0.0a
Pristine 38%	2000 ppm	7.5b	0.0a	0.8a	0.0a
Procure 480SC	12 oz/200 gal	85.8d	13.3c	3.3a	23.4cd
Sovran 50WDG	6.4 oz/200 gal	80.8d	29.0d	18.0b	4.2ab
Ziram 76DF	8.0 lb/200 gal	66.1c	55.8ef	22.4b	18.6cd
Scholar 230 SC[z]	300 ppm	68.3	28.3	58.3	0.0
Scholar 230 SC	150 ppm	91.7ef	44.2de	82.5d	9.4bc
Flint 50WDG	2.5 oz/200 gal	90.6de	67.5f	32.5b	19.3bc
Cuprofix Ultra 40	16 lb/200 gal	96.4fg	98.1g	63.5c	26.9d
Pencozeb 75DF	3.0 lb/200 gal	93.9ef	69.5f	60.8c	45.6e
Water control	---	100.0g	97.4g	79.2d	68.0f

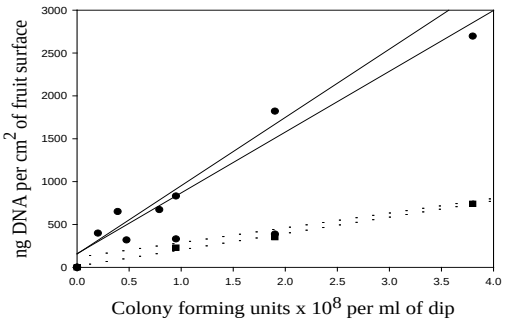
[x]Numbers within columns followed by the same letter are not different according to protected LSD test at P = 0.01.

[y]Rate per 200 gal is the per acre rate.

[z]Single trial results, no statistics.

4. Use of qPCR to determine "residues" of a biocontrol agent on pear fruit

The recommended concentration of the biological control yeast CIM for decay control is 2 x 10^8 cfu/ml. When Anjou (solid lines for 2006 and 2007) and Bosc (dashed lines for 2006 and 2007) fruit were treated with this concentration, the amounts of DNA on the fruit surfaces were about 1700 and 460 ng per cm^2, respectively. This method can be used to assure that CIM is being properly applied to pear fruit on the packing line or in the drench and will result in optimum decay control. Negotiations are underway to license CIM and obtain EPA registration.



5. Preharvest and postharvest fungicides for decay control

Preharvest treatments. For blue mold control, two treatments (Ziram and the foliar nutrient Nutraphos) were ineffective. The most effective preharvest spray for blue mold was Topsin M, either alone or combined with Nutraphos 24 or Ziram.

All fungicides controlled gray mold, but the foliar nutrient Nutraphos was not effective. Pristine was effective when applied twice but not as a single application.

Table 9. Preharvest fungicides to control postharvest decay of d'Anjou pears

Preharvest treatment and rate/A	PHI[z] (wk)	Blue mold (%)[y]			Gray mold (%)[y]		
		2004	2005	2006	2004	2005	2006
Control	--	23b	36d	23b	9c	9b	3ab
Pristine 38 WG 14.5 oz	2	8a	---	---	7bc	---	---
Pristine 38 WG 14.5 oz	1	5a	35d	---	3ab	9b	---
Pristine 38 WG 14.5 oz	2+1	---	23c	10a	---	3a	1a
Topsin M 70WSB 1 lb	2	10a	25c	9a	1a	2a	3ab
Topsin M 70WSB 1 lb	1	8a	11b	10a	2a	2a	2a
Ziram 76DF 8.0 lb	2	19b	45e	22b	2ab	2a	6b
Nutraphos 24 15 lb	2	---	33d	23b	---	8b	5b
Topsin M 70WSB 1 lb + Nutraphos 24 15 lb	2	---	3a	8a	---	2a	1a
Topsin M 70WSB 1 lb + Ziram 76DF 8.0 lb	2	---	---	8a	---	---	2a
Ziram 76DF 8.0 lb + Nutraphos 24 15 lb	2	---	---	24b	---	---	1a

[y]Numbers followed by the same letter within columns are not significantly different at P = 0.05 according to ANOVA and protected LSD of square root transformed data.
[z]PHI = preharvest spray interval in weeks.

Postharvest treatments - Drench application (Table 10). Control fruit (water drench) had 2.7% blue mold. All Pristine and Penbotec drench treatments significantly reduced blue mold and gave 100% control. Ethoxyquin increased blue mold, probably by acting as a wetting agent to increase penetration of spores into wounds. Gray mold in control fruit was 13.1%, and ethoxyquin was not significantly different from the control. Pristine and Penbotec gave 100% control of gray mold. Ethoxyquin caused slight phytotoxicity, and Pristine at 2000 ppm caused very slight phytotoxicity. Phytotoxicity appeared as dark spotting at lenticels where fruit was in contact with the polyliner and was slow to dry.

Table 10. Control of decay of d'Anjou pear fruit with postharvest drenches of Pristine at MCAREC, Hood River, OR in 2006-7

Treatment	Rate product per 100 gal	Percent fruit infected[z]	
		Blue mold	Gray mold
Pristine 2000 ppm	70.0 oz	0.0a	0.0a
Pristine 1000 ppm	35.0 oz	0.0a	0.0a
Pristine 500 ppm	17.5 oz	0.0a	0.0a
Pristine 250 ppm	8.75 oz	0.0a	0.0a
Penbotec 1000 ppm	1.0 quart	0.0a	0.0a
Ethoxyquin 2700 ppm	2.0 quarts	9.5c	14.7b
Water control	---	2.7b	13.1b

[z]Numbers followed by the same letter within columns are not significantly different at P = 0.05.

Postharvest treatments – Line spray application (Table 11). Water and wax control fruit had 27.5 and 5.8% of wounds infected with blue mold, respectively. The wax contains morpholine, which probably contributed to the reduction in decay. All rates of Pristine in both water and wax gave

excellent control of blue mold, and there were no significant differences among rates. Penbotec in water and wax also gave excellent control of blue mold.

Gray mold incidence was 35.8 and 8.3% for the water and wax controls, respectively. All rates of Pristine in both water and wax gave excellent control of gray mold, and there were no significant differences among rates. Penbotec in water and wax also gave excellent control of gray mold. In wax, the Penbotec treatment had significantly less gray mold than the lowest rate (250 ppm) of Pristine. No phytotoxicity was observed with any of the treatments in water or wax.

Table 11. Control of blue mold and gray mold of d'Anjou pear fruit with postharvest line spray application of Pristine at MCAREC, Hood River, OR in 2006-7

		Percent wounds infected[y]	
Treatment[z]	Rate (ppm a.i.)	Blue mold	Gray mold
1. Water control	---	27.5c	35.8d
2. Pristine	250	0.0a	0.2ab
3. Pristine	500	0.2a	0.5ab
4. Pristine	1000	0.0a	0.0a
5. Penbotec	1000	0.2a	0.0a
6. Wax control	---	5.8b	8.3c
7. Pristine	250	0.0a	0.7b
8. Pristine	500	0.0a	0.2ab
9. Pristine	1000	0.2a	0.4ab
10.Penbotec	2000	0.0a	0.0a

[y]Numbers followed by the same letter within columns are not different at P = 0.05.
[z]Treatments 2 to 5 are water suspensions, treatments 7 to10 are wax suspensions.

6. Evaluation of *Muscodor* for decay control

The biological control fungus *M. albus* significantly controlled blue mold, gray mold, and mucor rot of d'Anjou pear fruit at 30° F to 41° F. Phytotoxicity needs to be reduced before *M. albus* can be used commercially on pear fruit. As storage temperature was reduced, phytotoxicity decreased. No phytotoxicity was observed at the 4 gram per box rate at 30° F.

Table 12. Control of pear decays with *Muscodor albus* in 2004-2005

	Percent gray mold[y]			Percent mucor rot[y]			Percent blue mold[y]		
Rate[z]	41° F	32° F	30° F	41° F	32° F	30° F	41° F	32° F	30° F
0	97b	97b	100c	100c	100c	100c	100c	100c	100d
0.5	6a	50a	72b	72b	39a	50b	61b	89bc	78bc
1	6a	47a	31a	70b	58b	45b	19a	84b	53a
2	3a	56a	67b	51b	61b	22a	14a	55a	69ab
4	3a	56a	53ab	26a	53ab	8a	28a	78b	89cd

[y]Numbers followed by the same letter within columns are not significantly different at P = 0.05 according to least significant difference test.
[z]Rate of *M. albus* grams per liter.

7. Evaluation of paint formulations for control of apple and pear postharvest decay fungal pathogens on plastic and wood surfaces

Paint formulations (Protective Chemistries 1020, 1024, 3020, and 4020) were very effective for prevention of growth of *Botrytis cinerea, Mucor piriformis*, and *Penicillium expansum* on wood and plastic surfaces. Mortality of spores of these fungi was 100% in most time/temperature combinations on wood and plastic. Use of these paints on apple and pear bins and on surfaces in

packinghouses and cold storage rooms will significantly reduce or eliminate spores and growth of several important postharvest fungal pathogens and may be a key component in an integrated decay control program.

Table 13. Effect of three paint formulations on sporulation and growth of *Botrytis cinerea* on wood and plastic chips

			0°C			
	1 month @ 20°C		2 months		3 months	
Treatment	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]
			Plastic			
3020	0a[z]	0.0a	0a	0.0a	0a	0.0a
Unpainted	845b	2.0b	198a	1.1b	344a	1.1b
			Wood			
1020	0a	0.0a	0a	0.0a	0a	0.0a
4020	0a	0.0a	0a	0.0a	0a	0.0a
Unpainted	12a	0.4a	115a	0.3a	198a	1.8c

[y]Rating 0 = no fungal visible growth; 1 = some diffuse growth; 2 = moderate to heavy growth.
[z]Each value is the mean of 3 replications with 3 chips per replication. Numbers followed by the same letter are not significantly different at P = 0.05 according to ANOVA and the protected least significant difference test. Initial cfu/chip = 8,000.

Table 14. Effect of three paint formulations on sporulation and growth of *Penicillium expansum* on wood and plastic chips

			0°C			
	1 month @ 20°C		2 months		3 months	
Treatment	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]	Cfu/chip	Rating[y]
			Plastic			
3020	0a[z]	0.0a	0a	0.0a	0a	0.0a
Unpainted	89,246a	0.7a	2,908b	0.3a	25,666b	1.1b
			Wood			
1020	3,595a	0.0a	0a	0.0a	0a	0.0a
4020	0a	0.0a	0a	0.0a	0a	0.0a
Unpainted	56,343a	0.2a	175a	0.9b	6,950a	1.4b

[y]Rating 0 = no fungal visible growth; 1 = some diffuse growth; 2 = moderate to heavy growth.
[z]Each value is the mean of 3 replications with 3 chips per replication. Numbers followed by the same letter are not significantly different at P = 0.05 according to ANOVA and the protected least significant difference test. Initial cfu/chip = 7,000 at 20°C, 10,000 at 0°C.

Table 15. Effect of paint formulations 1020 (New) and 1024 on sporulation and growth on wood of three decay fungi of pear fruit, OSU MCAREC, Hood River, 2007

	1 month at 20°C[x]		0°C[x] 2 months		0°C[x] 3 months	
	CFU/chip	Rating[y]	CFU/chip	Rating[y]	CFU/chip	Rating[y]
			Penicillium expansum			
1020 (New)	nd[z]	0a	0a	0a	328a	0a
1024	nd	0a	0a	0a	297a	0a
Control	nd	2b	355,402b	2b	1,814,302b	2b
			Mucor piriformis			
1020 (New)	0a	0a	8a	0a	0a	0a
1024	0a	0a	8a	0a	0a	0a
Control	2,777,667a	2b	283,333b	2b	257,990b	2b
			Botrytis cinerea			
1020 (New)	nd	0a	0a	0a	nd	0a
1024	nd	0a	0a	0a	nd	0a
Control	nd	1b	19a	0.4b	nd	2b

[x]Each value is the mean of 3 replications with 3 chips per replication. Numbers followed by the same letter are not significantly different at P = 0.05 according to ANOVA and the protected least significant difference test. Initial CFU/chip = 7,261 for P. expansum, 1,845 for M. piriformis, and 5,777 for B. cinerea.

[y]Rating 0 = no fungal visible growth; 1 = some diffuse growth; 2 = moderate to heavy growth.

[z]nd = contamination and/or sporulation not determined.

FINAL PROJECT REPORT
WTFRC Project Number: PR05-502 **(WSU Project # 13L-3661-7366)**

Project Title: Control of postharvest decay in pear

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State/Province/Zip: WA 98801

Cooperators: **N/A**

Total Project Funding: $86,678

Budget History:

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries[1]	13,000	14,803	15,243
Benefits[2]	5,200	5,477	5,335
Wages	3,000	3,000	4,000
Benefits[3]	330	330	460
Equipment	0	0	0
Supplies[4]	4,000	4,000	4,000
Travel[5]	1,000	1,000	2,500
Miscellaneous	0	0	0
Total	26,530	28,610	31,538

Objectives:

1. Develop preharvest programs using new fungicides to control postharvest decay for long-term storage of pears.
2. Evaluate effectiveness of pre- and postharvest fungicides in controlling fruit-to-fruit spread of gray mold and Phacidiopycnis rot during storage.
3. Evaluate effectiveness of preharvest fungicides and postharvest drench with fungicides in controlling Phacidiopycnis rot.

Significant findings:

- Experiments were conducted to simulate the worst scenario in which fruit were wounded (punctures, etc.) at harvest and inoculated with pathogens. The purpose was to look at protection of the fruit at harvest by the residues of fungicides that were applied within 2 weeks before harvest. When applied within 2 weeks before harvest, the residues of Pristine and Ziram on the fruit at harvest significantly reduced infections of wounds (punctures) by Phacidiopycnis rot, but the magnitude of reduction in decay incidence was low to moderate, ranging from 54% to 64% in 2005-06 and 29% to 41% in 2006-07, compared with the nontreated control. The residues of Pristine and Ziram on the fruit at harvest did not protect wounds from infections by gray mold. In comparison with the results on Fuji and Red Delicious apples, it appears that residue levels on the fruit at harvest and susceptibility of the fruit both may affect the effectiveness of Pristine in protecting wounds from infection by gray mold. In Fuji and Red Delicious apples, Pristine applied within 2 weeks before harvest was very effective to protect wounds from infection by gray mold. D'Anjou pears may be more susceptible to gray mold than apples. A higher level of fungicide residues on d'Anjou pear fruit at harvest may be needed in order to protect wounds from infections by decay-causing pathogens. However, in addition to protecting wounds from infections by decay-causing pathogens, preharvest fungicides applied near harvest also are beneficial in reducing spore load on the surface of the fruit and eradicating some latent infections. Thus, considering all potential benefits, use of preharvest fungicides such as Pristine and Ziram is recommended for control of postharvest rots.
- In trials conducted in commercial orchards, Pristine by a ground application reduced the amount of decay in the bins by 45-61% in comparison with Pristine by an aerial application; but the aerial application of Pristine was not effective compared with the nontreated control. The results support our recommendations that a ground application to achieve good coverage is essential to the success of a preharvest fungicide program for control of postharvest rots.
- Gray mold and Phacidiopycnis rot were the two major postharvest rots in field bins (the fruit were not drenched prior to storage) in our trials conducted in commercial orchards. Pristine by a ground application was effective to control both gray mold and Phacidiopycnis rot originating from natural infections.
- When applied at 7 days before harvest, Topsin M and Pristine reduced Phacidiopycnis rot by 86% and 41% in 2005-06 and by 77% and 44% in 2006-07, respectively, in comparison with the nontreated control. It appeared that Topsin M was more effective than Pristine for controlling stem- and calyx-end Phacidiopycnis rot.
- When applied as a pre-storage drench treatment, all three postharvest fungicides were very effective in controlling stem-end and calyx-end Phacidiopycnis rot. Over the two-year trials, Mertect and Scholar reduced stem- and calyx-end Phacidiopycnis rot by 94-95% and 88-97%, respectively, in comparison with the nontreated control. Penbotec was highly effective and no decay developed in the fruit treated with Penbotec.
- The residues of Pristine and Topsin M applied at 7 days before harvest on pear fruit were able to suppress the fruit-to-fruit spread of gray mold during storage. Topsin M was more effective than Pristine in suppressing the fruit-to-fruit spread of gray mold. Among the three postharvest

fungicides, when applied as a pre-storage drench treatment, Penbotec was not effective in suppressing fruit-to-fruit spread of gray mold, whereas Mertect and Scholar reduced gray mold resulting from fruit-to-fruit spread by 69% and 73%, respectively, in comparison with the nontreated control.

Methods:

Effectiveness of preharvest applications of Pristine, Topsin M, and Ziram in controlling postharvest gray mold and Phacidiopycnis rot was evaluated on d'Anjou pears. Treatments were arranged in a randomized complete block design with four replicates (1-2 trees in each replicate of each treatment). Fungicides were applied within 2 weeks before harvest. Fruit were harvested from each tree. Fruit from four replicates of each treatment were wounded with a finish nail head and inoculated with spore suspensions of *B. cinerea* and *Phacidiopycnis piri*. Fruit were tray-packed in poly liners, and then stored in RA cold storage. Incidence and severity of gray mold and Phacidiopycnis rot were determined periodically for up to 10 weeks of storage.

Experiment was conducted to determine effectiveness of pre- and postharvest fungicides in controlling fruit-to-fruit spread of gray mold and Phacidiopycnis rot during storage. Selected preharvest fungicides were applied within 2 weeks before harvest. Fruit from the nontreated and fungicide-treated treatments were harvested. Part of the nontreated fruit from the orchard was drenched with each of the three postharvest fungicides (Mertect, Penbotec and Scholar). Fruit were stored in cardboard pear-boxes, and two inoculated fruit (either gray mold or Phacidiopycnis rot) were placed in each box. Fruit were stored in CA for 6 months, at which time the number of decayed fruit resulting from fruit-to-fruit spread in each box was determined.

To evaluate effectiveness of preharvest and postharvest fungicides in controlling Phacidiopycnis rot originating from infections of stem and calyx of the fruit, fruit were inoculated with the fungus during the pear-growing season. Part of inoculated fruit was sprayed with selected fungicides within 14 days before harvest, and a nontreated control also was included. All fruit were harvested. Part of the non-fungicide-treated fruit was treated with one of the three postharvest fungicides. Fruit were then stored in air at 32˚F. Decay development will be evaluated periodically for up to 7 months after harvest, starting 3-4 months after harvest.

Results and discussion:

Preharvest fungicides for control of Phacidiopycnis rot and gray mold originating from infections of wounds.

Experiments were conducted in 2005-06 and 2006-07 seasons to evaluate preharvest fungicides for control of Phacidiopycnis rot and gray mold originating from infections of wounds on the fruit.

Our trials were conducted to simulate the worst scenario in which fruit were wounded (punctures, etc.) at harvest and inoculated with pathogens. The purpose was to look at protection of the fruit at harvest by the residues of fungicides that were applied within 2 weeks before harvest.

In both seasons, we obtained similar results on the performance of Pristine and Topsin M (Fig. 1). In 2005-06, when applied at 7 and 14 days before harvest, Pristine was effective against Phacidiopycnis rot and reduced Phacidiopycnis rot by 54-64% in comparison with the nontreated control (Fig. 1). Surprisingly, Pristine was not effective to protect wounds from infections by gray mold on d'Anjou pears in this trial. Ziram applied at 2 weeks before harvest reduced gray mold by 11% and Phacidiopycnis rot by 24% on wound-inoculated fruit as compared with the nontreated control. Topsin-M applied at 7 days before harvest reduced gray mold by 15% and was not effective to reduce Phacidiopycnis rot on wound-inoculated fruit. In 2006-07, Topsin M did not protect wounds on the fruit from infections by Phacidiopycnis rot and gray mold. Pristine and Ziram significantly

reduced infections of wounds by Phacidiopycnis rot, but the magnitude of reduction in decay incidence was low to moderate, ranging from 29% to 41%, compared with the nontreated control.

In comparison with the results we have done on Fuji and Red Delicious apples, it appears that residue levels on the fruit at harvest and susceptibility of the fruit both may affect the effectiveness of Pristine in protecting wounds from infection by gray mold. In Fuji and Red Delicious apples, Pristine applied within 2 weeks before harvest was very effective to protect wounds from infection by gray mold. D'Anjou pears may be more susceptible to gray mold than apples. A higher level of fungicide residues on d'Anjou pear fruit at harvest may be needed in order to protect wounds from infections by decay-causing pathogens. However, in addition to protecting wounds from infections by decay-causing pathogens, preharvest fungicides applied near harvest also are beneficial in reducing spore load on the surface of the fruit and eradicating some latent infections. Thus, considering all potential benefits, use of preharvest fungicides such as Pristine and Ziram is recommended for control of postharvest rots.

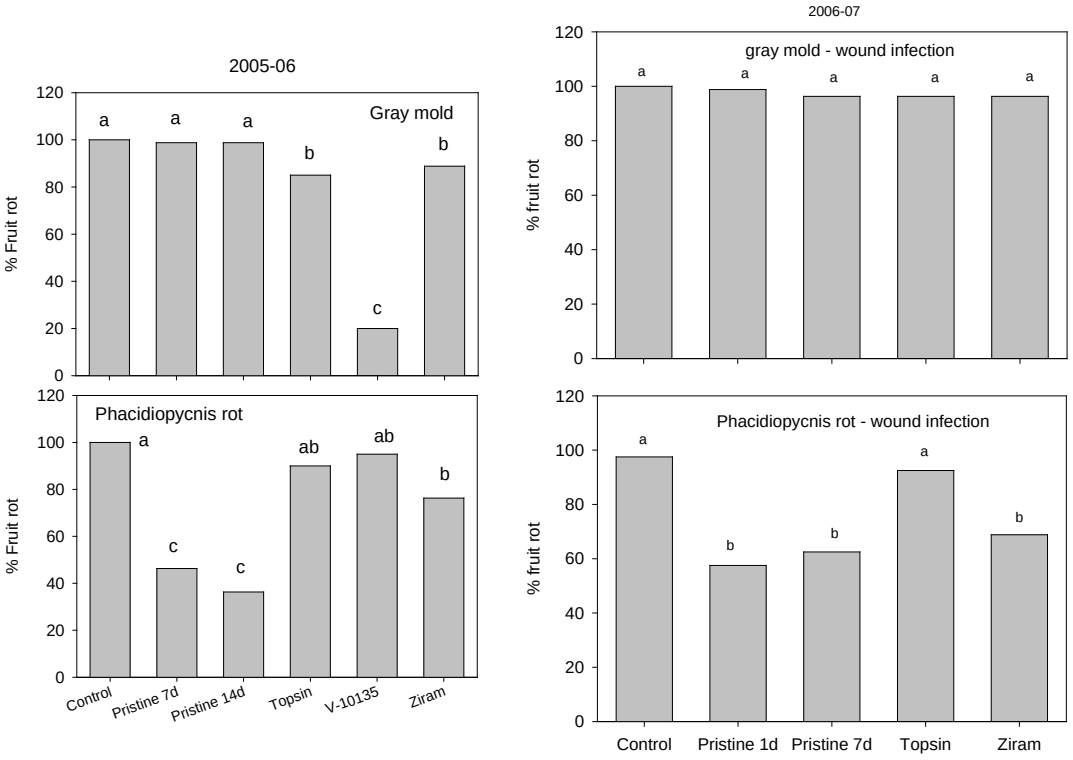

Fig. 1. Effectiveness of preharvest fungicides in controlling postharvest gray mold and Phacidiopycnis rot originating from infections of wounds on d'Anjou pears in 2005-06 and 2006-07 seasons. Pristine was applied at 1, 7 or 14 days before harvest. Topsin M, V-10135, and Ziram were applied at 7 days before harvest.

Preharvest Pristine by air and by ground applications for control of postharvest gray mold and Phacidiopycnis rot conducted in commercial orchards.

The trials were conducted on the 2005 crop in four commercial orchards. Decay assessment was done in the spring of 2006. Incidence of rots in storage bins varied from orchard to orchard. Orchard 1 and Orchard 2 had 7.6% and 5.2% rots in the nontreated fruit, respectively. The other two orchards had approximately 3% rots. Significant differences in decay control between air and ground applications were observed in Orchard 1 and Orchard 2 (Fig. 2). No significant difference in decay control between

the two application methods was seen in Orchard 3 and Orchard 4, likely due to relatively lower levels of rots in these two orchard lots.

Pristine by a ground application (200 gallons per acre) reduced the amount of decay in the bins by 61% in Orchard 1 and by 45% in Orchard 2 in comparison with Pristine by the air application. In these two grower lots, Pristine by air application did not significantly control rots compared with the nontreated control. The results suggest that a high-gallonage spray by a ground application to achieve good coverage is essential to the success of a preharvest fungicide program for control of postharvest rots.

In these four grower lots, gray mold and Phacidiopycnis rot were the two major rots in field bins (the fruit were not drenched prior to storage). This is consistent with our report that gray mold and Phacidiopycnis rot are the primary target diseases in field bins if the fruit are not treated with postharvest fungicides prior to storage. In our trials conducted in commercial orchards, Pristine was effective to control both gray mold and Phacidiopycnis rot originating from natural infections. In Orchard 1 and Orchard 2, Pristine by a ground application significantly reduced gray mold and Phacidiopycnis rot compared with Pristine by an air application (Fig. 3). Blue mold and bull's eye rot were low in these trials.

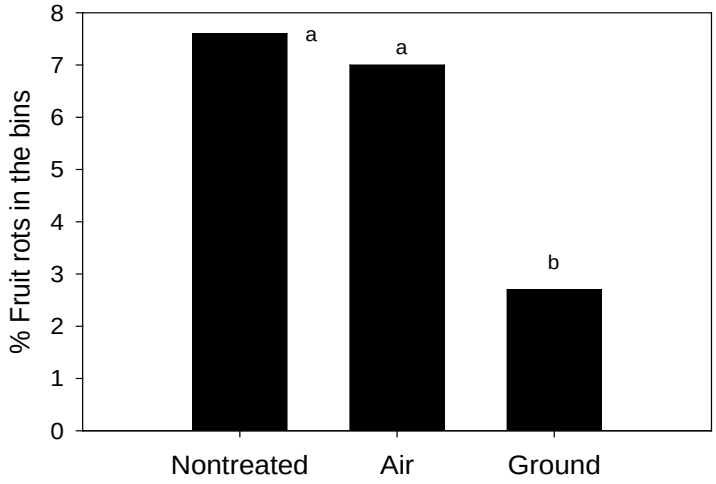

Fig. 2. Comparison of actual losses of d'Anjou pear fruit in field bins between the fruit treated with Pristine applied by a ground application (200 gallons/A) and the fruit treated with Pristine applied by an aerial application. The fruit were not drenched prior to storage. The fruit were stored in CA for 5 months, at which time decay was assessed. Percentage of fruit rots in field bins was expressed as weight of decayed fruit in the total weight of the fruit in a bin.

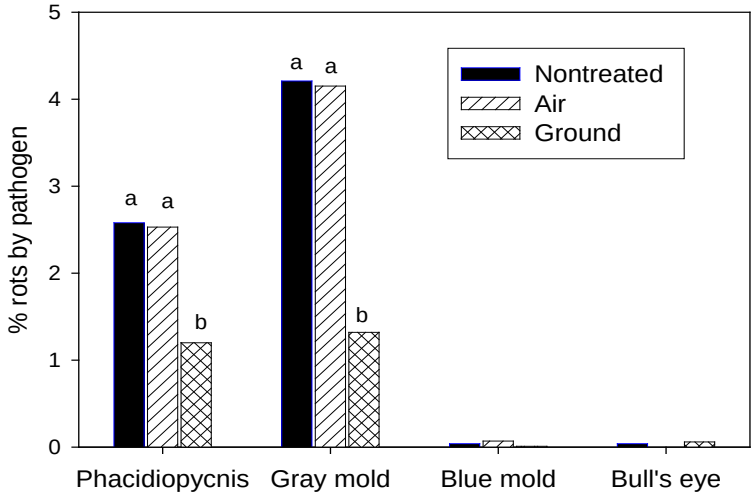

Fig. 3. Comparison of gray mold and Phacidiopycnis rot between the fruit treated with Pristine applied by a ground application (200 gallons/A) and the fruit treated with Pristine applied by an aerial application. The fruit were not drenched prior to storage. The fruit were stored in CA for 5 months, at which time decay was assessed. Percentage of fruit rots in field bins was expressed as weight of decayed fruit in the total weight of the fruit in a bin.

Pre- and postharvest fungicides for control of stem- and calyx-end Phacidiopycnis rot.

Stem-end rot and calyx-end rot are two common types of symptoms of Phacidiopycnis rot in d'Anjou pears. Fruit infected by the fungus at the stem and calyx may not have symptoms at packing, but symptoms develop in the boxes before shipping or after shipping.

Experiments were conducted in 2005-06 and 2006-07 seasons. An experiment also was conducted on 2007 crop to examine the effects of timing of fruit infection on control of Phacidiopycnis rot. The 2007 experiment is still in progress.

Both Pristine and Topsin M applied at 7 days before harvest were effective. In 2005-06, Topsin M and Pristine reduced Phacidiopycnis rot by 86% and 41%, respectively, in comparison with the nontreated control (data has been reported in 2006 report). In 2006-07, Topsin M and Pristine reduced Phacidiopycnis rot by 77% and 44%, respectively (Fig. 4). It appeared that Topsin M was more effective than Pristine for control of stem- and calyx-end Phacidiopycnis rot.

Three postharvest fungicides also were evaluated. When applied as a pre-storage drench treatment, all three postharvest fungicides were effective to control stem-end and calyx-end Phacidiopycnis rot (Fig. 4). Over the two-year trials, Mertect and Scholar reduced stem- and calyx-end Phacidiopycnis rot by 94-95% and 88-97%, respectively, in comparison with the nontreated control. Penbotec was highly effective and no decay developed in the fruit treated with Penbotec.

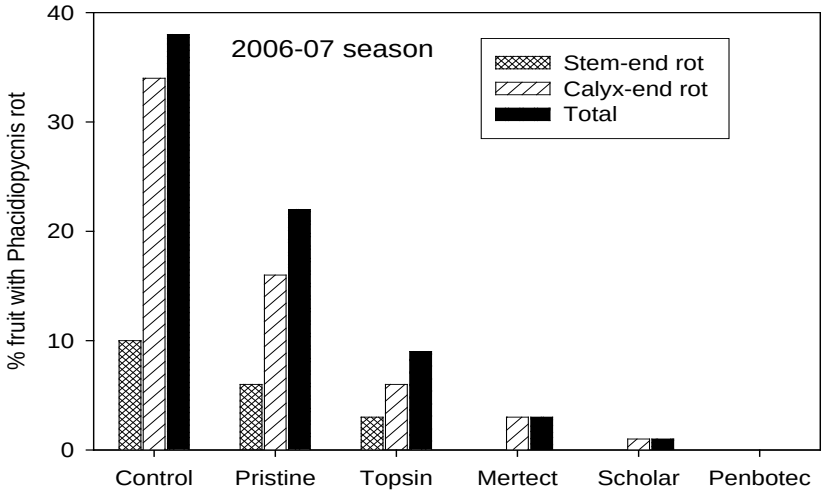

Fig. 4. Control of stem- and calyx-end Phacidiopycnis rot with pre- and postharvest fungicides conducted in 2006-07. The fruit were inoculated with the pathogen in the orchard at 3 weeks before harvest. Pristine and Topsin were applied at 7 days before harvest, and Mertect, Scholar and Penbotec were applied the same day after harvest. Fruit were stored at 32ºF in RA. Decay incidence at 7 months after harvest was presented.

Postharvest fungicides for control of Phacidiopycnis rot originating from wound infections.

The results from the 2006 and 2007 experiments are summarized in Table 1. After 10 weeks in cold storage, 78-99% of the non-treated fruit had Phacidiopycnis rot. No decay developed on the fruit treated with the new formulation of Scholar at 16 fl oz/100 gallon or Penbotec when the treatment was applied one hour after inoculation. Scholar applied 24 h after inoculation reduced Phacidiopycnis rot by over 95% compared with the nontreated control. Penbotec applied 24 h after inoculation reduced Phacidiopycnis rot by 98% in 2006 and 100% in 2007. The results indicate that the three postharvest fungicides have post-infection activities against Phacidiopycnis rot when applied as a pre-storage treatment and that a pre-storage drench treatment applied within 24 h after harvest is effective in controlling Phacidiopycnis rot originating from infection of wounds.

Table 1. Efficacy of Scholar or Penbotec as a drench treatment for control of Phacidiopycnis rot originating from infection of wounds.

	% of Fruit Infected	
Treatment	2006	2007
Nontreated control	77.9	98.8
Mertect 16 fl oz applied 1 hr after inoculation	0	3.8
Mertect 16 fl oz applied 24 hr after inoculation	5	1.3
Scholar 230SC 16 fl oz applied 1 hr after inoculation	0	0.0
Scholar 230SC 16 fl oz applied 24 hr after inoculation	3.3	2.5
Penbotec 16 fl oz applied 1 hr after inoculation	0	0.0
Penbotec 16 fl oz applied 24 hr after inoculation	1.7	0.0

Effectiveness of fungicides in controlling fruit-to-fruit spread.

Experiments were conducted in the 2005-06 and 2007-08 seasons. The 2007 experiment is still in progress. Results will be forthcoming. In the 2005-06 experiment, Phacidiopycnis rot was low. Only the data on gray mold are presented (Fig. 5). When applied at 7 days before harvest, the residues of the two preharvest fungicides (Pristine and Topsin) on pear fruit were able to suppress the fruit-to-fruit spreading of gray mold during storage. Topsin was more effective than Pristine in suppressing the fruit-to-fruit spread of gray mold. Among the three postharvest fungicides, when applied as a pre-storage drench treatment Penbotec was not effective in suppressing fruit-to-fruit spread of gray mold, whereas Mertect and Scholar reduced gray mold resulting from fruit-to-fruit spread by 69% and 73%, respectively, in comparison with the nontreated control (Fig. 5).

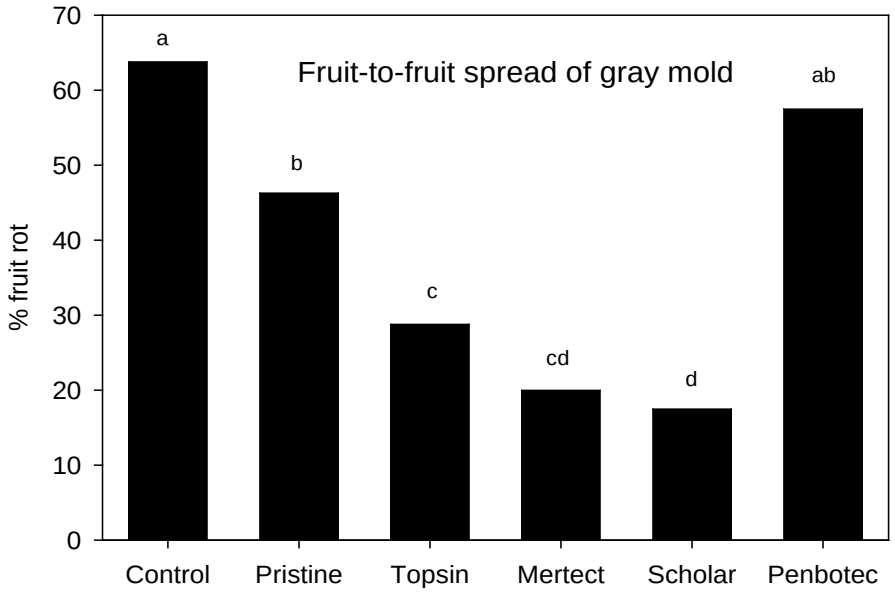

Fig. 5. Effectiveness of preharvest fungicides applied at 7 days before harvest and postharvest fungicides applied as a pre-storage drench treatment in suppressing fruit-to-fruit spread of gray mold in d'Anjou pears during storage.

FINAL PROJECT REPORT
WTFRC Project Number: PR-06-603 **(WSU Project #13L-4164-1207)**

Project Title: Managing storage scald in Anjou pears

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Peter Sanderson, Pace International

Other Funding Sources

Agency Name: Pace International; Cerexagri
Amount awarded:
Notes: Pace International - contribution of chemicals and residue analysis.
Cerexagri - contribution of chemicals**.**

Total Project Funding: Year 1: $34,277 Year 2: $45,301 Year 3: $60,585

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	16,890	13,301	13,634
Benefits	5,067	5,985	6,681
Wages	4,500	6,500	7,000
Benefits	720	715	770
Equipment	700	2,000	0
Supplies	5,400	15,300	31,000
Travel	1,000	1,500	1,500
Miscellaneous	0	0	0
Total	34,277	45,301	60,585

Objectives:
This project was funded by WSU and the Washington Tree Fruit Research Commission for crop years 2004-2006 to initiate new research and integrate previous research on the prevention of storage scald on Anjou pears into a systems approach suitable for use by the industry.

The five specific objectives were:

1. Predict the risk of storage scald through knowledge of preharvest temperatures.
2. Determine the timing of antioxidant application using fruit with different risk levels.
3. Determine the effectiveness of applying antioxidants as a bin drench.
4. Determine the potential for chemical burn from antioxidants and fungicides applied as bin drenches.
5. Evaluate the use of thermofogging to control storage scald and decay (2006 crop).

Significant findings:

OBJECTIVE 1: Predict the risk of scald through knowledge of preharvest temperatures.

Methods: The risk of scald was estimated through knowledge of orchard temperatures by analyzing temperature data using techniques developed by Ma and Chen (2001) for the Hood River Valley in Oregon. To test whether this system would work to predict the risk of scald temperature, data loggers were placed into eight orchards (five in the Wenatchee Valley and three in the Yakima Valley). Data loggers were placed within the canopy 6 weeks prior to anticipated harvest and set to record temperatures on an hourly basis. The incidence of scald was compared to the hourly temperature data for each orchard to correlate cool nighttime temperatures (<50 ºF) with scald development.

Each year, pears from all orchards were harvested at the same firmness level to reduce the effect of maturity on scald development (Table 1). In 2005, fruit were removed from RA (33 ºF) storage at weekly intervals for 12 weeks, starting 30 days after harvest; ripened for 7 days and examined for scald. 2006 crop fruit were stored longer (pullout starting after 60 days in RA), and evaluated for a longer period of time (14 weeks). In addition to the scald evaluation after 7 days ripening, the 2006 fruit was also tested for ripeness (firmness, flesh juiciness and taste) after 7 days and re-evaluated for scald after 14 days.

Dr. Chen's model for Hood River (Ma, et al. 2001) was developed to predict when scald would develop on 10% of the fruit. When this was applied to temperatures in Wenatchee and Yakima the predicted range for 2005 was 62 to 92 days (Table 1). The predicted range for 2006 was 74 to 91 days. This prediction was the reason the first pull-out was increased from 30 days in 2005 to 60 days in 2006.

Results and Discussion: In 2005, scald developed only on fruit from one orchard within the inspection period. The orchard that developed scald had the lowest number of hours below 50 ºF. However, other orchards which also had very low accumulated temperatures (Wenatchee 1, 3 and Yakima 1) did not develop scald within the evaluation period (data not shown).

In 2006, scald did not develop on fruit prior to 100 days in storage (Fig. 1). The same orchard as 2005 (Wenatchee 2) developed the most scalded fruit. Scald was evaluated after both 7 and 14 days ripening, based on the theory that fruit taken out of storage early in the season would take more than 7 days to develop scald symptoms. In all cases, scald symptoms developed on fruit within 7 days, although the severity generally continued to increase up to the 14-day evaluation (data not shown).

Table 1. Harvest maturity and predicted days in storage after which 10% of the fruit will show scald symptoms following 7 days of ripening at 68ºF. Based on (Ma, et al. 2001).

	2005 Crop					2006 Crop					
Orchard	Harvest	Firm (lbf)	Hours <50 ºF*	Scald prediction**		Harvest	Firm (lbf)	Hours <50 ºF*	Scald prediction**		Actual Scald^
Wen1	19-Aug	15.1	9	74d	1-Nov	4-Sep	14.3	28	80d	23-Nov	2-Jan
Wen2	29-Aug	13.6	0	62d	30-Oct	7-Sep	14.3	9	74d	20-Nov	21-Dec
Wen3	29-Aug	13.6	11	75d	12-Nov	7-Sep	15.4	32	81d	27-Nov	4-Jan
Wen4	1-Sep	14.8	55	84d	24-Nov	14-Sep	14.1	76	87d	10-Dec	28-Dec
Wen5	12-Sep	15.0	137	91d	12-Dec	18-Sep	13.5	145	91d	18-Dec	NA
Yak1	23-Aug	15.6	11	75d	6-Nov	31-Aug	14.7	39	82d	21-Nov	18-Jan
Yak2	13-Sep	14.7	181	92d	14-Dec	11-Sep	14.9	141	91d	11-Dec	29-Jan
Yak3	13-Sep	14.5	61	85d	7-Dec	11-Sep	14.9	57	85d	5-Dec	2-Jan

* Accumulated hours below 50 ºF in the 42 days prior to harvest

** Predicted days in storage after which 10% of the fruit will show scald symptoms following 7 days ripening at 68ºF, based on the formula: DIS (10%) = 62.3827 x $ACU^{0.0757}$

DIS = the number of days fruit was held in air storage, ACU = accumulated cold units (hours <50ºF)

^ Actual date by which 10% of fruit developed scald

Summary conclusions. Within the first 100 days of storage there was surprisingly little scald in fruit from the Wenatchee orchards and even less from Yakima. Hood River pears developed scald earlier and at much elevated levels within this first 100 days (Ma et al. 2001). In Washington orchards that had accumulated over 140 hours below 50 ºF there was very little scald. The orchard with the most severe level of scald was that with the lowest number of hours below 50 ºF. However, the relationship between scald and temperature does not appear to be linear for Washington fruit, and more research over additional years is needed before this system can be utilized with confidence.

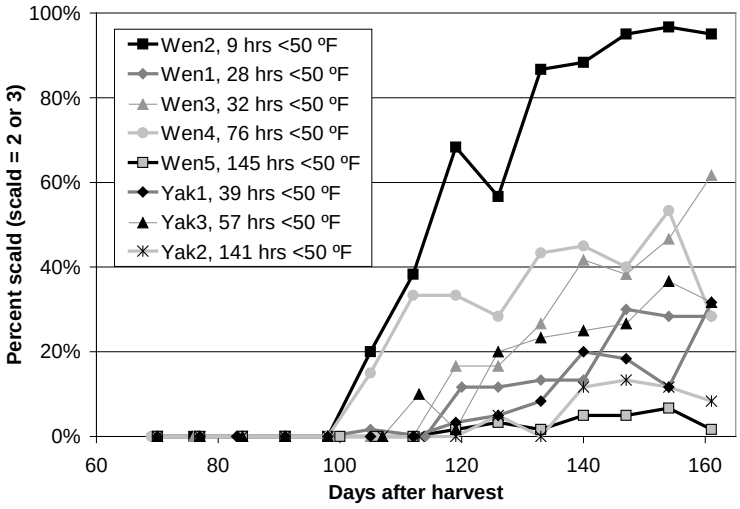

Figure 1. Scald development by orchard, 2006 crop. Rated weekly starting 60 days after harvest, RA storage, followed by 7 days ripening at 70ºF.

Note on the uniformity of ripening: The ripening of the 2006 crop was not uniform. Approximately half way through the 14-week evaluation period (December 28, 2006) firmnesses of "ripened" pears (7 days at 70 ºF) from the eight orchards ranged from less than 1 to over 16 lbf, with an average of 7.2 lbf (Fig. 2). It is not apparent that this wide range of firmness following exposure to time and warm temperatures is typical of what is seen in commerce. It would be valuable to have more information on the uniformity of ripening.

OBJECTIVE 2: Determine the <u>timing</u> of effective antioxidant application on fruit.

Methods: In 2006, two bins of commercially harvested fruit from each of the five Wenatchee orchards were drenched with TBZ only or ethoxyquin (1350 ppm) + TBZ within 1 week of harvest. Bins were stored in RA at 32 ºF for 7, 14 and 42 days prior to packing.

After each storage interval, fruit were passed over the wax section of the USDA ARS-1 packingline using one of three line spray treatments: 1) Penbotec only, 2) 675 ppm ethoxyquin + Penbotec or 3) 1350 ppm ethoxyquin + Penbotec. The fruit were tray packed and placed in CA storage. Fruit were evaluated for phytotoxicity and scald after 7 days ripening in March 2007.

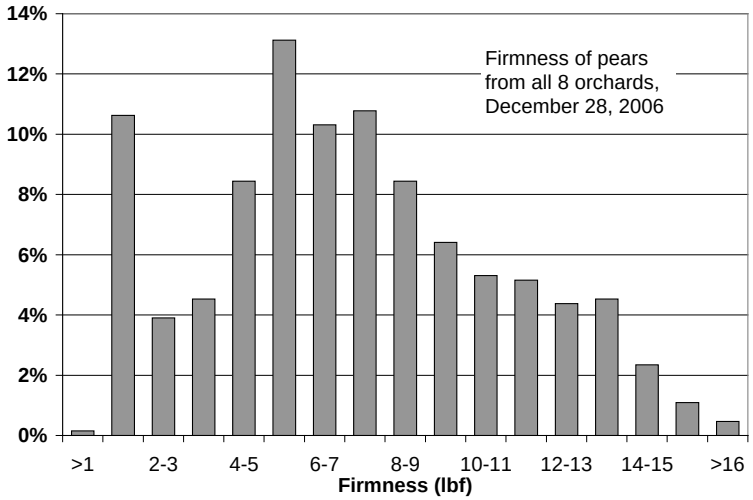

Figure 2. "Ripened" firmness of Anjou from 8 orchards. Data shown is approximately mid-point through 14-week evaluation period (95 to 116 days after harvest, depending on orchard), RA storage, 7 days ripening at 70 ºF, 2006 crop.

Results and discussion: In 2004, superior control of scald was obtained when the antioxidant <u>wrap</u> was applied within 7 days of harvest when compared with delayed application (28, 56 or 112 days after harvest). However, even application at 7 days did not provide effective commercial control. Ethoxyquin wrap was more effective at controlling storage scald than diphenylamine (DPA) wrap (data not shown).

In 2005, to increase scald control, bins were first drenched with either 1350 ppm ethoxyquin or 1350 ppm ethoxyquin +TBZ. Antioxidant <u>wraps</u> were applied 7, 14 or 42 days after harvest. The most effective scald control was an ethoxyquin drench followed by antioxidant wrap applied within 7 days. Ethoxyquin wrap provided superior scald control to DPA wrap (Fig. 3).

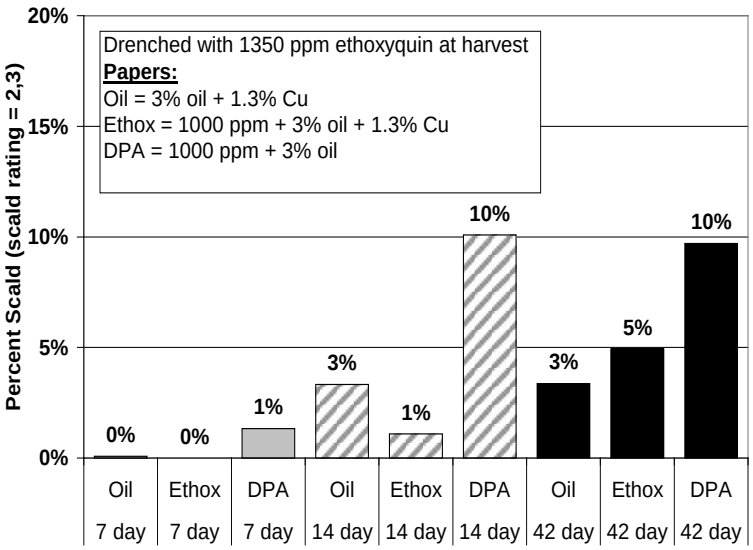

Figure 3. Scald development by antioxidant wrap type and application date. Average of five orchards. 2005 Crop, CA stored, ripened at 70ºF for 7 days, evaluated May 2006.

In 2006, fruit were drenched with either TBZ or ethoxyquin and antioxidant was applied as a <u>line spray</u> rather than a paper wrap. Fruit was evaluated in March 2007 following long-term CA storage. For fruit that was drenched with ethoxyquin at harvest, effective scald control was obtained with a line spray applied within 14 days. A line spray of Penbotec alone was nearly as effective as ethoxyquin in controlling scald on fruit that was previously drenched with ethoxyquin (Fig. 4). For fruit that was drenched with TBZ only at harvest, the most effective scald control was a line spray treatment of 1350 ppm ethoxyquin applied within 7 days (Fig. 4).

Summary conclusions. As other scientists (Chen, Drake) have found previously, in order for the ethoxyquin to be effective at controlling scald it must be applied within 7 days after harvest. Delayed application significantly reduced the effectiveness of the antioxidant. A combination of a half concentration ethoxyquin drench and a paper wrap applied within 7 days of harvest was the most powerful method to control scald. A half-strength drench of ethoxyquin at harvest, followed by a line spray of low concentration ethoxyquin was also very effective in controlling scald.

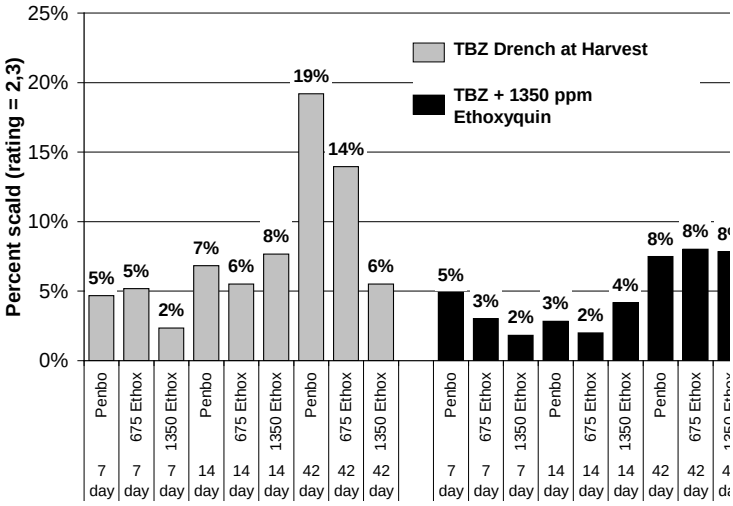

Figure 4. Scald on pears from the 2006 crop after a harvest drench with either TBZ or TBZ + ethoxyquin followed by line sprays at 7, 14 or 42 days after harvest. Fruit were evaluated

OBJECTIVE 3: Determine the effectiveness of applying antioxidants as a bin drench.

Methods: In 2006, three bins of commercially harvested pears from three different Wenatchee orchards were purchased and divided into cherry bins for drenching within 1 week of harvest. Drench solutions included control (TBZ only); 675 ppm ethoxyquin + TBZ; 1350 ppm ethoxyquin + TBZ, Penbotec or Scholar; and 2000 ppm ethoxyquin + TBZ.

Fruit was placed in CA and samples were removed for evaluation in January, March and April 2007. Samples were examined for chemical burn and scald after 7 days ripening. Additional samples were passed over the packingline for additional treatment with either Penbotec alone or ethoxyquin + Penbotec. The packed fruit was held in RA for 30 days and then evaluated for scald and chemical burn and scuffing. Ethoxyquin concentrations on the packingline were 1350 ppm for the 675 and 1350 ppm drenches and 700 ppm for the 2000 ppm drench to stay under the maximum label rate of 2700 ppm.

Results and discussion: In 2004, fruit were drenched with antioxidants and/or fungicides at harvest, stored in CA and evaluated for phytotoxicity and scald. Samples were held in RA for an additional 30, 60 or 90 days following CA storage and evaluated for scald after 7 days of ripening. Following storage for 30 days in RA, only fruit from orchards with few cooling hours developed scald; by 90 days, fruit from all orchards had developed significant scald. The ethoxyquin-treated fruit developed the least amount of scald, but even drenching with a fungicide alone reduced scald by approximately half compared to untreated control fruit.

In 2005 and 2006, fruit were drenched with antioxidants and/or fungicides at harvest and stored in bins in CA. In February, April and May four samples of each treatment were removed from storage and evaluated for scald: 1) after 7 days of ripening; 2) held in RA for 30 days and 7 days of ripening; 3) line spray of Penbotec only, 30 days in RA and 7 days ripening; and 4) line spray of ethoxyquin (not to exceed a total of 2700 ppm for the year) + Penbotec, 30 days in RA and 7 days of ripening.

In both 2005 and 2006, undrenched fruit developed significantly more scald than any of the antioxidant drenches. A 1350 ppm ethoxyquin drench provided equivalent control to the 2000 ppm ethoxyquin and superior control to 675 ppm ethoxyquin or 1000 ppm DPA (DPA included in 2005 trial only). Effective concentration was related to specific orchards. The longer the fruit was stored, the more scald it developed (February vs. April vs. May).

In both years, there was no significant reduction in scald after the application of ethoxyquin as a line spray following storage, even though the ethoxyquin application was maxed out at 2700 ppm. In addition, scuffing was a major problem on the fruit that was run over the packingline after storage (45% scuffed in February, increasing to 70% by May). This is another indication of the importance of applying the antioxidant immediately after harvest, rather than following storage.

Summary conclusion: Application of ethoxyquin as a drench at harvest provided superior control of scald to applying it on the line after storage; however, phytotoxicity was unacceptably high (see Objective 4). Ethoxyquin provided greater suppression of scald than DPA. More scald developed on fruit that was stored longer. Applying a line spray of antioxidant on Anjou after storage did not provide additional scald control.

OBJECTIVE 4: Determine the potential for chemical burn from antioxidants or fungicides applied as bin drenches.

Methods: Fruit used in Objectives 2 and 3 was evaluated for phytotoxicity at time of removal from storage. Fruit from Objective 3 was also evaluated after packing. Because of high phytotoxicity from the antioxidants in the 2004 and 2005 crops, antioxidant application methods in 2006 used lower concentrations applied multiple times.

Results and discussion: The inclusion of the fungicides TBZ, Scholar (fludioxonil) or Penbotec (pyrimethanil) in antioxidant drenches did not increase chemical burn in any years. In 2004, pink-colored permanent chemical burn was found at fruit-to-fruit contact points on a high percentage of fruit treated with ethoxyquin (up to 49% burned fruit). Brown chemical burn was not related to fruit contact and was found on the ethoxyquin-treated fruit (10% burned) and more severely on the DPA-treated fruit (average of 27% burned).

In 2005 and 2006, the pink burn from the Objective 3 ethoxyquin treatments increased with increasing concentration and was unacceptably high in all cases (Figs. 5 and 6). Again, the orchard factor comes into play because pink burn was only a minor problem on fruit from Wenatchee 4 in 2005 and was not present in the 675 ppm ethoxyquin treatment in 2006.

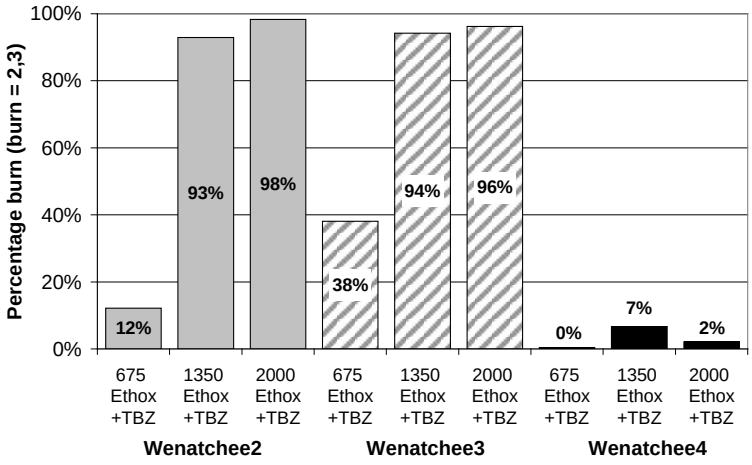

Figure 5. Pink burn on Anjou pears (2005 crop) following ethoxyquin drench and mid-term CA storage (February 13, 2006).

Brown burn in 2005 was associated with DPA treatments and was a relatively minor problem on fruit pulled out of storage in February (data not shown). Brown burn became a serious problem on fruit stored longer and was a more severe problem on fruit from Wenatchee 4, which did not have a problem with pink burn (data not shown).

In contrast with Objective 3, fruit from Objective 2 (2006 crop) that was drenched at harvest with 1350 ppm ethoxyquin sustained little or no burn following long-term CA storage (Fig. 7). This fruit was given a second treatment of fungicide or fungicide + antioxidant mixed with wax and applied as a line spray. It is likely that the ethoxyquin residue from the drench was washed off of the fruit during the waxing process, before the excess ethoxyquin could penetrate the skin and permanently damage the fruit.

Summary conclusion: In drenching experiments conducted over the past 3 years, the presence of pink staining on the ethoxyquin-treated fruit has been a severe problem. Any benefit in scald reduction derived from the application of an ethoxyquin drench has been outweighed by the potential for damage from the treatment. The exception has been a line spray treatment applied within 6 weeks of the drench, which seems to rinse off the excess ethoxyquin residue, which can cause burning, while still providing effective scald control. In future projects, the use of a second rinsing drench will be explored to see if the pink residue from the ethoxyquin can be reduced without the chemical losing its effectiveness.

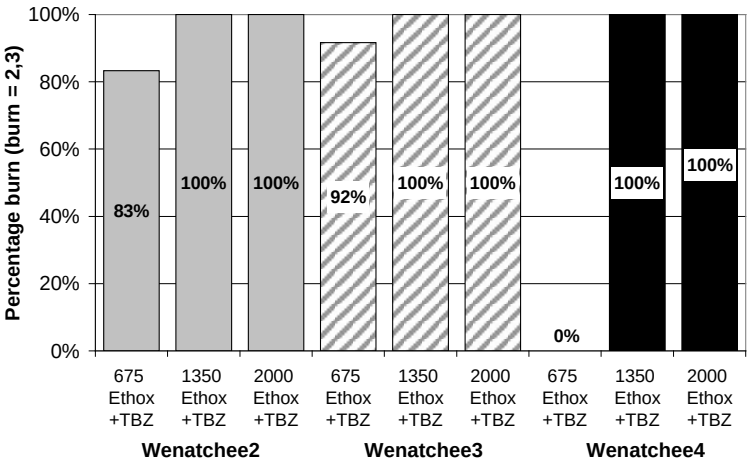

Figure 6. Pink burn on Anjou pears (2006 crop) following ethoxyquin drench and mid-term CA storage (January 15, 2007).

OBJECTIVE 5: Evaluate the use of <u>thermofogging</u> to control storage scald and decay.

Methods: In 2006, we began to study the feasibility of thermofogging with ethoxyquin and/or pyrimethanil on Anjou pears in cooperation with Dr. Peter Sanderson of Pace International. Sixteen bins of pears (4 growers, 4 bins each) were thermofogged in small CA rooms (40 bins capacity) immediately following harvest with one of the following treatments:

- Ethoxyquin alone;
- Pyrimethanil alone;
- Ethoxyquin + Pyrimethanil;
- Not fogged.

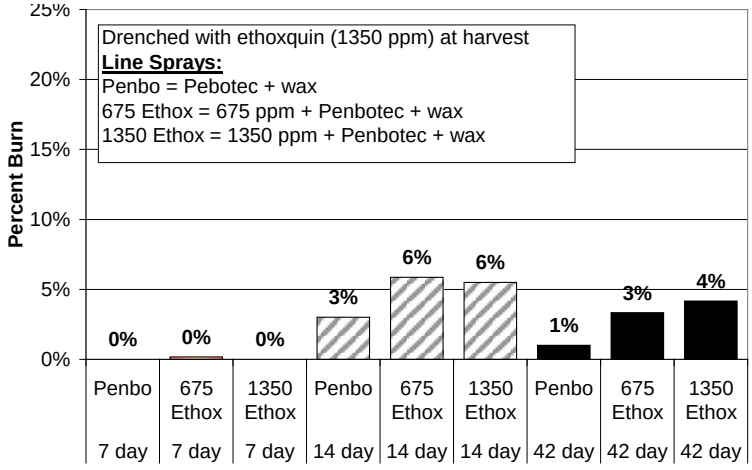

Figure 7. Burn on pears from the 2006 crop after a harvest drench with TBZ + 1350 ppm ethoxyquin followed by line sprays 7, 14 or 42 days after harvest (average of five growers). Fruit were evaluated following long-term storage in March 2007.

This fruit was stored in CA and sampled in January, March and April 2007 for evaluation of phytotoxicity immediately after storage and scald after 7 days.

Results and discussion: Scald control was effective on fruit removed prior to April 2007, 7 months after harvest (Fig. 8). There was a large increase in scald between March and April. Side effects of thermofogging included the development of pink burn on the tops of several bins that had likely been on the top of the stack while treated (Table 2; Fig. 9) and the high level of residue on fruit (Table 3).

The top layer of fruit on all ethoxyquin-treated bins was pink (Fig. 9). Two hundred fruit from the top of each ethoxyquin-treated bin were examined for burn and given a severity rating from 0 to 3. Each fruit was wiped with a glove; superficial marking that wiped off was not considered "burn" and not included in the table. Up to 95% of the top layer of fruit was burned in one bin (Table 2).

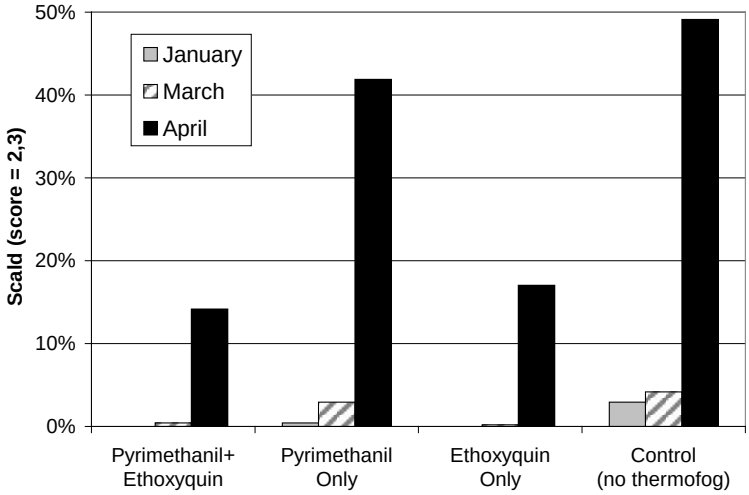

Figure 8. Scald development over three monthly pullouts by thermofog treatment. Average of four orchards. 2006 crop, CA stored, ripened at 70ºF for 7 days.

The thermofogging technique must be refined to promote even distribution of chemical throughout the room. In some bins, fruit had an excessive amount of residue on the top of the pear while the bottom of the same pear developed scald. In some bins, residue levels taken after treatment and again after mid-term CA storage were over the legal limit (3 ppm for both ethoxyquin and pyrimethanil) (Table 3). This procedure might require improved air movement, bin covers or other modifications to disperse the chemicals more evenly.

Table 2. Pink-colored burn from thermofog treatments, rated at packinghouse on January 4, 2007. Percentage of fruit on the top layer of the bin with commercially unacceptable burn (score of 2 or 3).

Treatment	Grower	Pink burn (% of burned fruit)
Pyrimethanil + Ethoxyquin	Grower1	2%
	Grower2	30%
	Grower3	95%
	Grower4	7%
Ethoxyquin Only	Grower1	31%
	Grower2	24%
	Grower3	4%
	Grower4	14%

Commercial experiment with thermofogging is very limited in Washington, especially with ethoxyquin on pears. One packer reported that they have thermofogged ethoxyquin alone on pears for 2 years and plan to expand the trial this season. They said that the burn was limited to the top fruit in the top bins and this was an acceptable amount of damage. Pace International has been working with the European developer of this technology (Xeda) on a larger number of commercial trials on apples with DPA. The same problems appear to exist when DPA is applied to apples—both burn on the top fruit and uneven residues.

Summary conclusion: Additional research is needed to develop a consistent deposition of chemical on all fruit in the room and within the bins so targeted residue concentration are achieved. Chemical burn, although restricted to the uppermost fruit in the top bins, needs to be ameliorated.

Figure 9. Photos of pink (left) and not pink (right) on top layer of pears at packinghouse. Bin A (left) is the ethoxyquin + pyrimethanil treatment and Bin D (right) is the control treatment.

Table 3. Pyrimethanil and ethoxyquin residues taken immediately after thermofogging (18-Sep-06) and after CA room opened (4-Jan-07). (All results in parts per million (ppm).) The legal allowable residue for ethoxyquin or pyrimethanil is 3 ppm in the United States.

			18- Sep Residues		4-Jan Residues*	
Grower	**Pyrimethanil**	**Ethoxyquin**	**Pyrimethanil**	**Ethoxyquin**	**Pyrimethanil**	**Ethoxyquin**
1**	Yes	Yes	5.6	4.3	1.3	nd
1	Yes	No	2.2	--	2.7	nd
1	No	Yes	--	7.3	0.1	nd
1	No	No	--	--	nd	nd
2	Yes	Yes	1.7	2.1	3.1	1.0
2	Yes	No	0.4	--	0.8	nd
2	No	Yes	--	5.0	nd	4.0
2	No	No	--	--	0.2	nd
3	Yes	Yes	4.2	2.5	1.3	nd
3	Yes	No	4.9	--	1.0	nd
3	No	Yes	--	1.4	0.2	nd
3	No	No	--	--	nd	nd
4	Yes	Yes	4.2	1.7	3.0	1.7
4	Yes	No	5.1	--	0.2	nd
4	No	Yes	--	1.9	0.2	2.3
4	No	No	--	--	nd	nd

* nd = not detectable

** Additional samples from the top middle and bottom layers of this bin were analyzed on 4-Jan:

- Top = 5.7 pyrimethanil, 0.9 ethoxyquin
- Middle = 1.8 pyrimethanil, nd ethoxyquin
- Bottom = 1.7 pyrimethanil, 0.5 ethoxyquin

Literature cited:

Ma, S., D.M. Varga and P.M. Chen. 2001. Using accumulated cold units to predict the development of superficial scald disorder on Anjou pears during cold storage. J. Hort. Sci. & Biotechnology

76(3):305-310.

FINAL PROJECT REPORT
WTFRC Project Number:

Project Title: Protocols for conditioning Anjou pears with ethylene release capsules

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Cooperators:

Other funding Sources

Agency Name:
Amount awarded:
Notes:

Total Project Funding: US$19,500

Budget History:

Item	2006	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment	900		
Supplies	2,145		
Travel	16,455		
Miscellaneous			
Total	**19,500**		

Objective To establish protocols for conditioning pre-packed loads of Anjou pears using Ethylene Release Capsules (ERCs) at three different times during the pear season (fall, winter, spring*). *N.B. The spring trial was dropped, with agreement from the Fresh Pear Committee, based on advice from our collaborating packer/shipper that it was unlikely that conditioned fruit in spring would survive delivery in a saleable state.

Pallet scale conditioning using Ethylene Release Capsules

The usefulness of smaller prototype ERCs for conditioning pears on an individual clamshell or box scale has been demonstrated previously (Sharrock and Henzell, 2007). The ERC tested in the trials reported here to condition whole pallets was a much larger device, which is basically contained within an aerosol can (Fig. 1). A key feature is its unique internal valve (patent pending) that releases compressed ethylene at a constant rate for an adjustable period of up to seven days. This is achieved without electronics or moving parts, thus maximizing its reliability and cost-effectiveness.

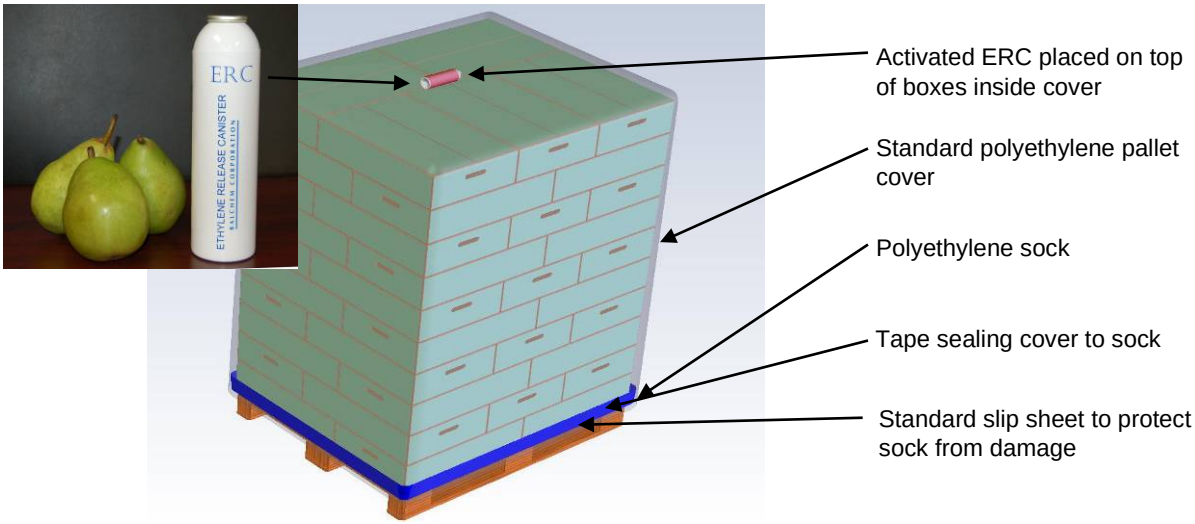

Figure 1. Covered pallet conditioning using an ERC (enlarged in insert)

Significant findings

- ERCs provide an effective means of conditioning early season Anjou pears within covered pallets of either double-layer Euro-boxes or standard cartons of individually wrapped fruit.
- A single ERC at the top of a covered pallet resulted in >100 ppm ethylene throughout the pallet within 17 hours and maintained this for a pre-defined period of up to 7 days.
- Early in the Fall, Anjou conditioned by ERC for 5-7 days were **distinctly more aromatic and flavorsome**, once fully ripe, than those conditioned conventionally at higher temperatures for a shorter period. Fruit that were simply warmed without ethylene failed to ripen acceptably.
- In February, using CA-stored fruit, ethylene conditioning had no effect on subsequent rates of softening but still resulted in detectable aroma and flavor enhancement.
- Covering alone had little impact on rates of warming and softening of fruit, and no detrimental effects became evident during subsequent ripening in either Winter or Fall.

Results and discussion
Winter Conditioning Trial (February 07)

This trial involved five pallets each containing 12 layers of double layer Euro-boxes of 70 ct Anjou (42 per box). The fruit had been picked on 13 Sept. 07, sealed in CA at Underwood Fruit Packers,

White Salmon on 16 Sept, removed from CA on 2 February, packed on 8 February, and held at 30°F in RA storage until the trial commenced on 14 February. Each pallet except the uncovered control C3 was rebuilt on top of a sock created by cutting down a polyethylene pallet cover, which in turn was seated on a slip sheet to protect it from damage by the slats of the pallet base. Miniature "iButton®" temperature loggers (Maxim Integrated Products Inc.) were attached directly to fruit with rubber bands, within five labeled representative boxes located on layers 1 (top), 3, 6, 10, 12 (base) within each pallet. Each pallet was then subjected to one of the following five treatments in a disused store room with minimal electric heating sufficient to achieve and maintain an air temperature of 60-65°F.

Treatments:

T1. **3 days conditioning with ERC** inside a sealed pallet cover; after 2 days uncovered;
T2. **5 days conditioning with ERC** inside a sealed pallet cover, with no pre-warming period.

Controls

C1. As for T1 but without an ERC;
C2. As for T2 but without an ERC;
C3. Simply left uncovered for 5 days.

Temperatures of fruit and the surrounding room, and concentrations of CO_2, O_2 and ethylene inside the boxes at various levels within the pallets were monitored throughout conditioning using portable meters (PBI Dansensor and Dräger). Typical examples of the data obtained are shown in Figs 2 & 3.

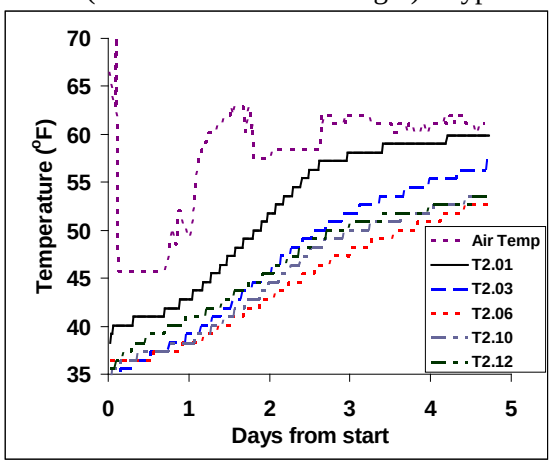

Figure 2: Temperatures of fruit in selected layers of the T2 covered pallet, and of the surrounding room air during conditioning in February 07.

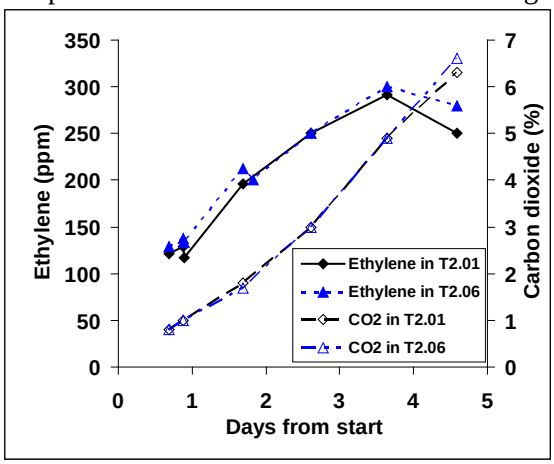

Figure 3: Carbon dioxide and ethylene concentrations in layers 1 and 6 of the T2 covered pallet in February 07.

Effective warming of the pallets only began at the end of the first day, when electric heaters were introduced to counter the effects of cold weather. The fruit in the top layer warmed up much more rapidly than those further down the pallet. The middle (layer 6) was the slowest to warm up, remaining 10°F and 5°F colder than the top (layer 1) and bottom (layer 12) respectively, after 3 days of warming (Fig. 2). Layers 3, 10 and 12 all warmed at similar rates. In contrast, concentrations of ethylene and CO_2 showed no significant difference between the middle and top layers Fig. 3). This knowledge of relative temperatures and gas distribution within the pallets is pertinent to interpretation of positional effects on responses to ethylene.

Ethylene concentrations within the covered pallets T1 and T2, which each contained an ERC, exceeded 100 ppm within 17 hours of covering (possibly sooner, but no earlier assessment was done). Levels of >100 ppm ethylene, generally accepted as sufficient for conditioning pears, were maintained by the ERCs in covered pallets T1 and T2 for three and five days, respectively. The

covered control pallets, with no ERCs, were found to also contain ethylene, albeit at a much lower concentration of around 10 ppm, from the first assessment at 17 h after covering until the end of the conditioning period. This was due to production of ethylene by the pears (that had been in storage for 5 months), since regular monitoring of the store room atmosphere revealed < 0.5 ppm ethylene.

After conditioning, the pallets were dismantled so that sample boxes of fruit from layers 1, 3, 6, 10 and 12 of each pallet could be removed and ripened at 68°F at MCAREC. The remaining bulk of each pallet was returned to 30°F storage and sold. During ripening, samples of each batch of fruit were assessed daily in terms of firmness and aroma production (using ripeSense® sensors).

Sealing the pallets inside a polyethylene cover during the conditioning period had no detectable effect on subsequent softening (Fig. 4B & C). Exposure to ethylene within the pallet cover also had little effect on the rates of softening (Fig. 4A & B). Fruit of treatments T1 and C1 (not shown) exhibited almost identical softening curves to the above. In all treatments, fruit of the top layer of the pallet began softening sooner than those of lower layers (Fig. 4), which is probably a reflection of their different rates of warming during the conditioning period, as revealed in Fig. 2.

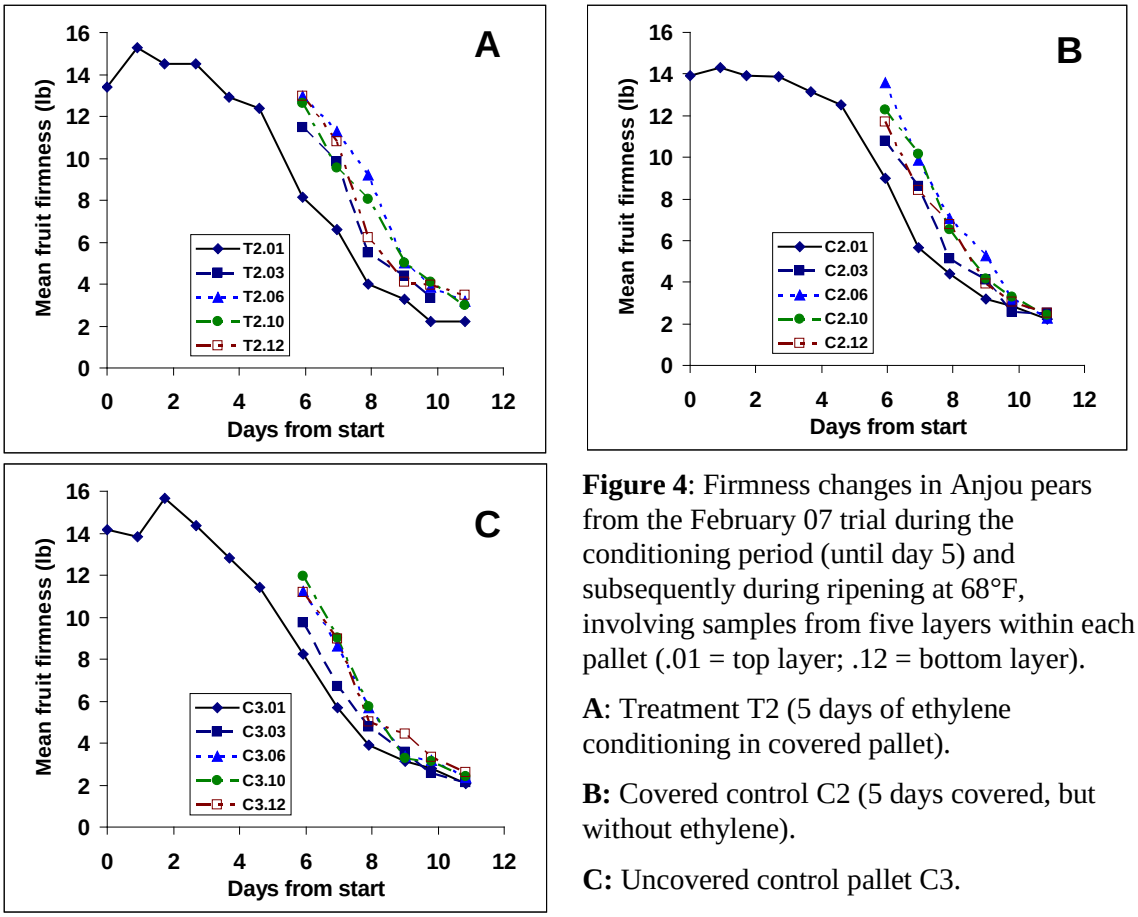

Figure 4: Firmness changes in Anjou pears from the February 07 trial during the conditioning period (until day 5) and subsequently during ripening at 68°F, involving samples from five layers within each pallet (.01 = top layer; .12 = bottom layer).

A: Treatment T2 (5 days of ethylene conditioning in covered pallet).

B: Covered control C2 (5 days covered, but without ethylene).

C: Uncovered control pallet C3.

Ethylene production by fruit from all five pallets, including the controls, was evident when the fruit were placed in ripeSense® clamshells during the final 20 h of ripening. There appeared to be no relationship between the rate of ethylene production during ripening and whether fruit had earlier been artificially exposed to >100 ppm ethylene during conditioning. The capacity of these fruit, following 5 months storage, to produce their own ethylene is a key point of difference between this trial and the subsequent investigation in September 07 using freshly harvested fruit (see below).

Aroma development by the ripening fruit proved difficult to assess reliably by the method employed

during the February trial, in which fruit were sealed in clamshells containing ripeSense® sensors for just 20 h before colors of the sensors were assessed and the fruit were subjected to destructive firmness testing. This incubation period proved too short to obtain reliable and meaningful sensor responses, and consequently this was increased substantially during the following trial in Fall 07 (below). Based on sense of smell alone, the two researchers involved were usually able to distinguish between ethylene-treated and control fruit in blind testing, but the differences in aroma were subtle. In contrast with controls in the Fall trial (which remained virtually inedible), the February control fruit produced appreciably more aroma, and could be enjoyably eaten.

Taste comparisons performed "blind" by the two researchers on February trial fruit, fully ripened to around 2 lb firmness, revealed definite flavor differences between ethylene conditioned and control fruit. Ethylene treatment resulted in a more intense and attractive flavor. However, the flavor difference was far less marked than in the September trials (see below), probably because the February fruit, including the controls, were capable of producing their own ethylene.

Fall Conditioning Trial (September 07)

Green Anjou pears, all from the same lot, were harvested at commercial maturity on September 13th 2007, and kept in cold storage until grading and packing 12 days later. Packed 90 ct trial fruit (seven pallets of standard cartons and six pallets of double-layer Euros) were returned to 30°F storage overnight.

On September 26th, all thirteen pallets were rebuilt to permit gas sampling tubes, thermocouples and iButton® temperature loggers to be attached to fruit within the bottom, middle and top layers of each and then either sealed within pallet covers, as in February (see above), or left open, either as controls, or to be treated with ethylene in the conventional manner. Pallet labels denoted the treatments to be employed during conditioning (listed below), preceded by either an "S" (for standard carton) or an E (for Euro). Standard carton pallets received all seven treatments below. Euro-boxes received only six treatments (not T4). Ten of the pallets spent the conditioning period in an unheated dry-store warehouse, with an air temperature that fluctuated around 60°F. Exceptions were ST3 & ET3, treated in a conventional heated conditioning room at Underwood Fruit, and ST4, which was conditioned in the Underwood apple packing room (warmer than the warehouse but containing variable, and sometimes significant amounts of ethylene from the apples).

Ethylene treatments

T1: Two days uncovered, then **five days covered with ERC** in dry goods warehouse
T2: **Seven days covered with ERC** in dry goods warehouse
T3: Conventional conditioning (one day warming, **two days ethylene from catalytic generator** in Underwood's normal conditioning room)
T4: Two days uncovered, then **four days covered with ERC** in apple packing room throughout

Controls

C1: Two days uncovered, then five days covered **without ERC** in dry goods warehouse
C2: Seven days covered **without ERC** in dry goods warehouse
C3: Seven days uncovered **without ERC** in dry goods warehouse

Ethylene in the room atmosphere in the warehouse was generally <0.5 ppm, but in the apple packing room sometimes reached 20 ppm. Temperatures of fruit and the surrounding room, and concentrations of CO_2, O_2 and ethylene inside the boxes at various levels within the pallets were monitored throughout conditioning. Gas samples were collected via tubes to the interiors of boxes in the top, middle and bottom layers. Examples typical of the data obtained are shown in Figs 5, 6 &7.

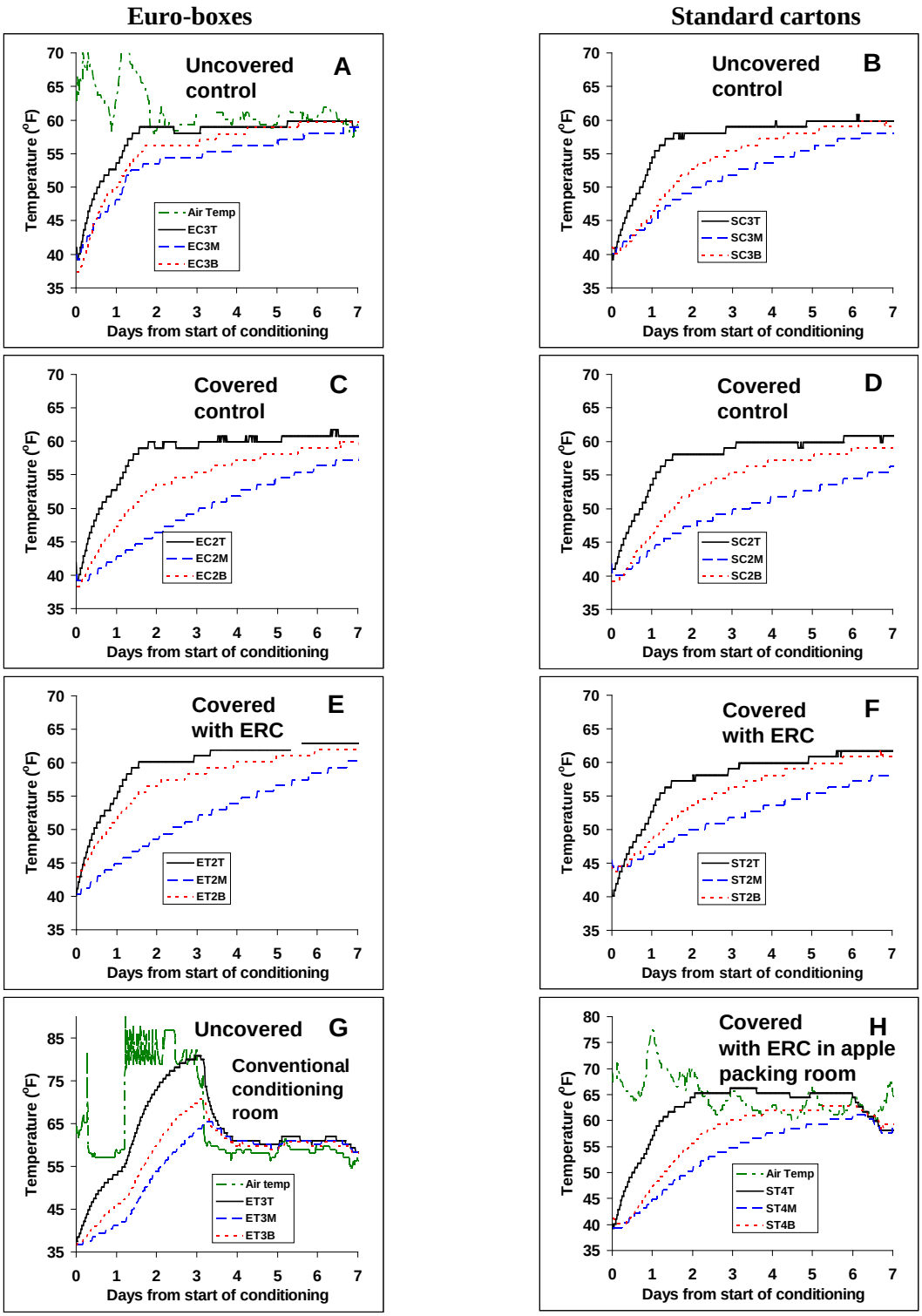

Figure 5: Temperatures of fruit within pallets in the top (T) middle (M) and bottom (B) layers. Graphs **A & B** relate to uncovered control pallets; **C & D** to covered control pallets; **E & F** to covered ERC-treated pallets; **G** to conventional conditioning of an uncovered pallet of pears in Euro-boxes (ET3) in a commercial ethylene room; **H** to a covered ERC-treated pallet of pears in standard cartons in the Apple Packing Room at Underwood Fruit. Air temperatures of the surrounding room (dry goods warehouse) shown in A also apply to B-F.

iButton® temperature loggers attached to fruit at various levels within the pallets clearly demonstrated the very significant thermal inertia of full, tightly packed pallets of pears in both box types (Fig. 5). The middle layer warmed up much more slowly than the top layer, resulting in temperature differentials of up to 15°F within the same pallet. This temperature gradient was less apparent in the uncovered (Fig 5 A & B) than in the covered pallets (C & D), but still existed even in the pallet of Euro-boxes subjected to forced air warming and ethylene conditioning in a commercial conditioning room (Fig. 5G). Metabolic warming of the middle layers due to ethylene-induced acceleration of fruit respiration was barely detectable, based on comparison of rates of internal warming of pallets ET2 and ST2 (Fig 5 E & F) vs the covered controls without ethylene (C & D).

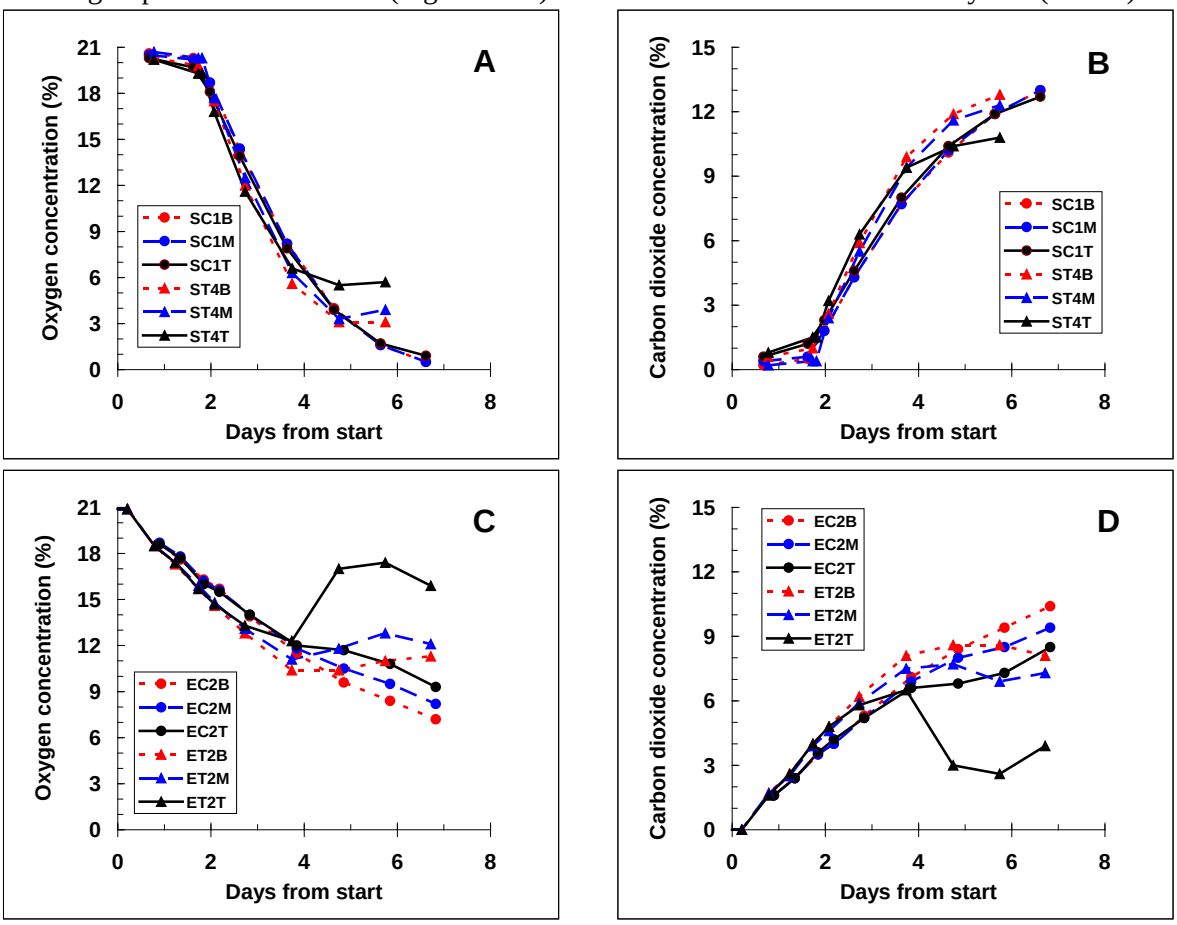

Figure 6. Oxygen and carbon dioxide concentrations within covered pallets during conditioning. **A & B** compare ethylene treatment ST4, involving a pallet of standard boxes, with the most relevant control, SC1, covered for a similar period but without an ERC included. **C & D** compare the longest (7 day) conditioning period (ET2) with its corresponding no-ethylene control (EC2). T = top; M = middle; B = bottom layer.

Substantially modified atmospheres developed within the pallets sealed inside polyethylene covers. Examples shown in Fig. 6 A & B displayed the greatest atmospheric modification, with O_2 falling below 1% and CO_2 rising to 13% by the end of the period of enclosure (5 days). No detrimental effects of these short exposures to such modified atmosphere were detected during subsequent cold storage by Underwood's quality inspectors, and excess fruit from covered pallets were deemed fit-for-sale. Inclusion of an ERC had no obvious impact on the rate of accumulation of CO_2 and depletion of O_2 within the covered pallets. There was considerable variation between pallets in the final levels of CO_2 and O_2, almost certainly attributable to variable losses of accumulated gases

during daily sampling of fruit from the top of each sealed pallet, followed by an attempt to completely reseal the damaged polyethylene. In commercial use, internal sampling and resealing during the course of conditioning of a covered pallet would of course not normally be necessary.

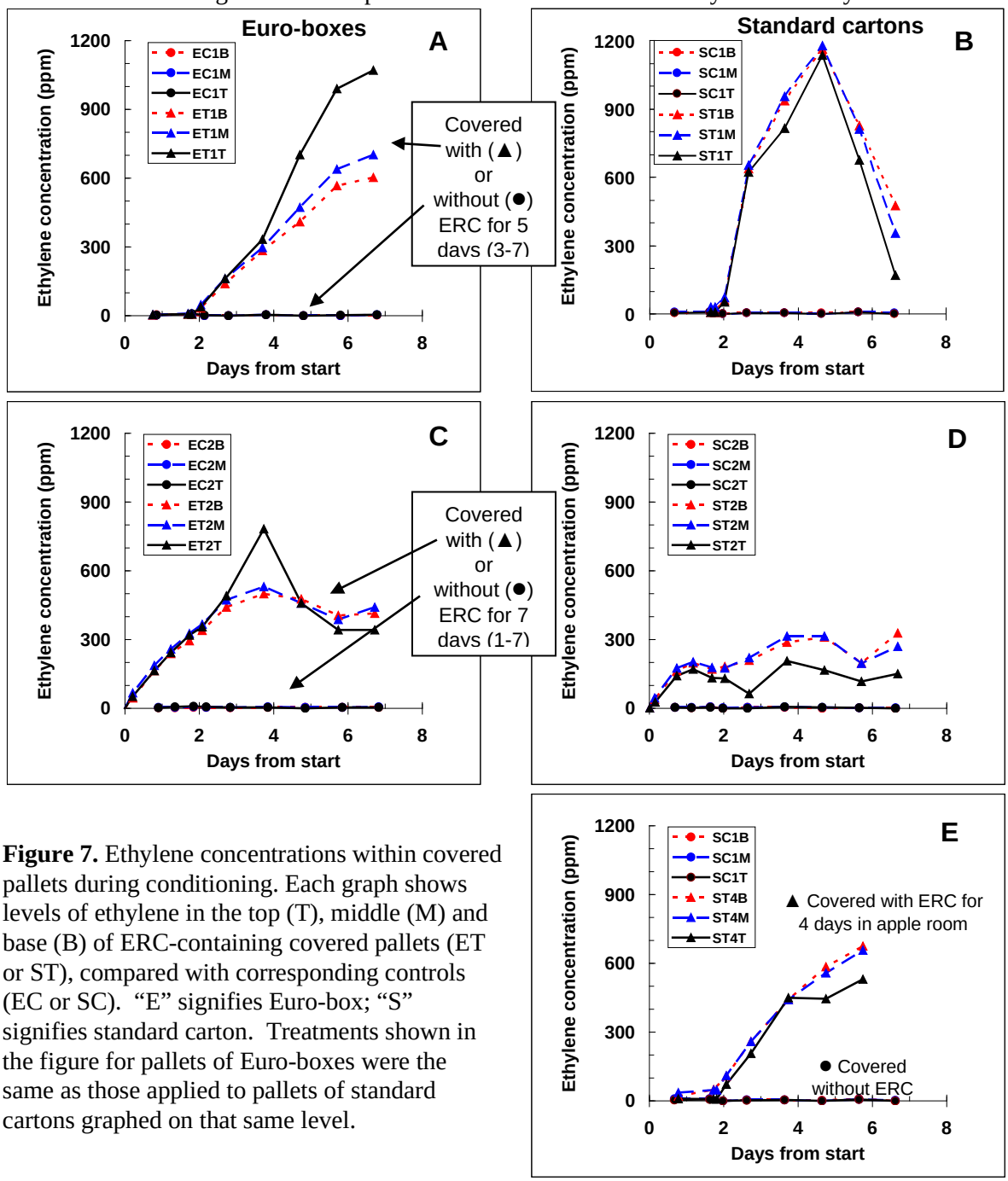

Figure 7. Ethylene concentrations within covered pallets during conditioning. Each graph shows levels of ethylene in the top (T), middle (M) and base (B) of ERC-containing covered pallets (ET or ST), compared with corresponding controls (EC or SC). "E" signifies Euro-box; "S" signifies standard carton. Treatments shown in the figure for pallets of Euro-boxes were the same as those applied to pallets of standard cartons graphed on that same level.

Ethylene within the five covered pallets each containing an ERC rose rapidly to around 50 ppm by 3.5 h after sealing. The ethylene concentrations in each of these pallets continued to climb, exceeding 100 ppm by one day after sealing. The levels fluctuated and varied between pallets during the period of conditioning (Fig. 7), due primarily to loss of ethylene during daily opening and closing of the top of the pallet cover to remove fruit, and, in some cases, to inadequate resealing. Despite these difficulties, the rate of continued ethylene release from the ERCs was in all cases sufficient to maintain at least 100 ppm ethylene around the fruit in the covered pallets for the entire conditioning

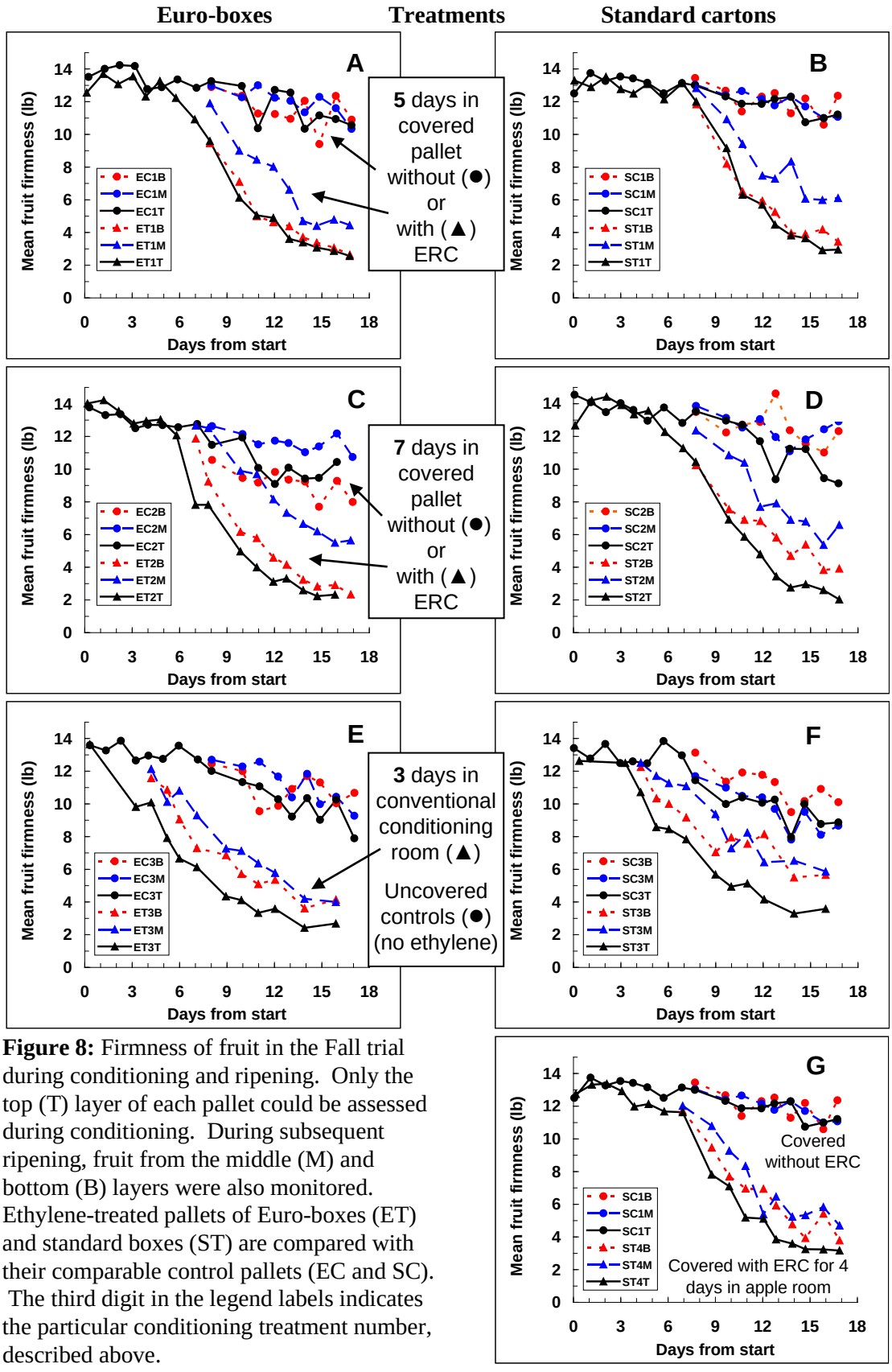

Figure 8: Firmness of fruit in the Fall trial during conditioning and ripening. Only the top (T) layer of each pallet could be assessed during conditioning. During subsequent ripening, fruit from the middle (M) and bottom (B) layers were also monitored. Ethylene-treated pallets of Euro-boxes (ET) and standard boxes (ST) are compared with their comparable control pallets (EC and SC). The third digit in the legend labels indicates the particular conditioning treatment number, described above.

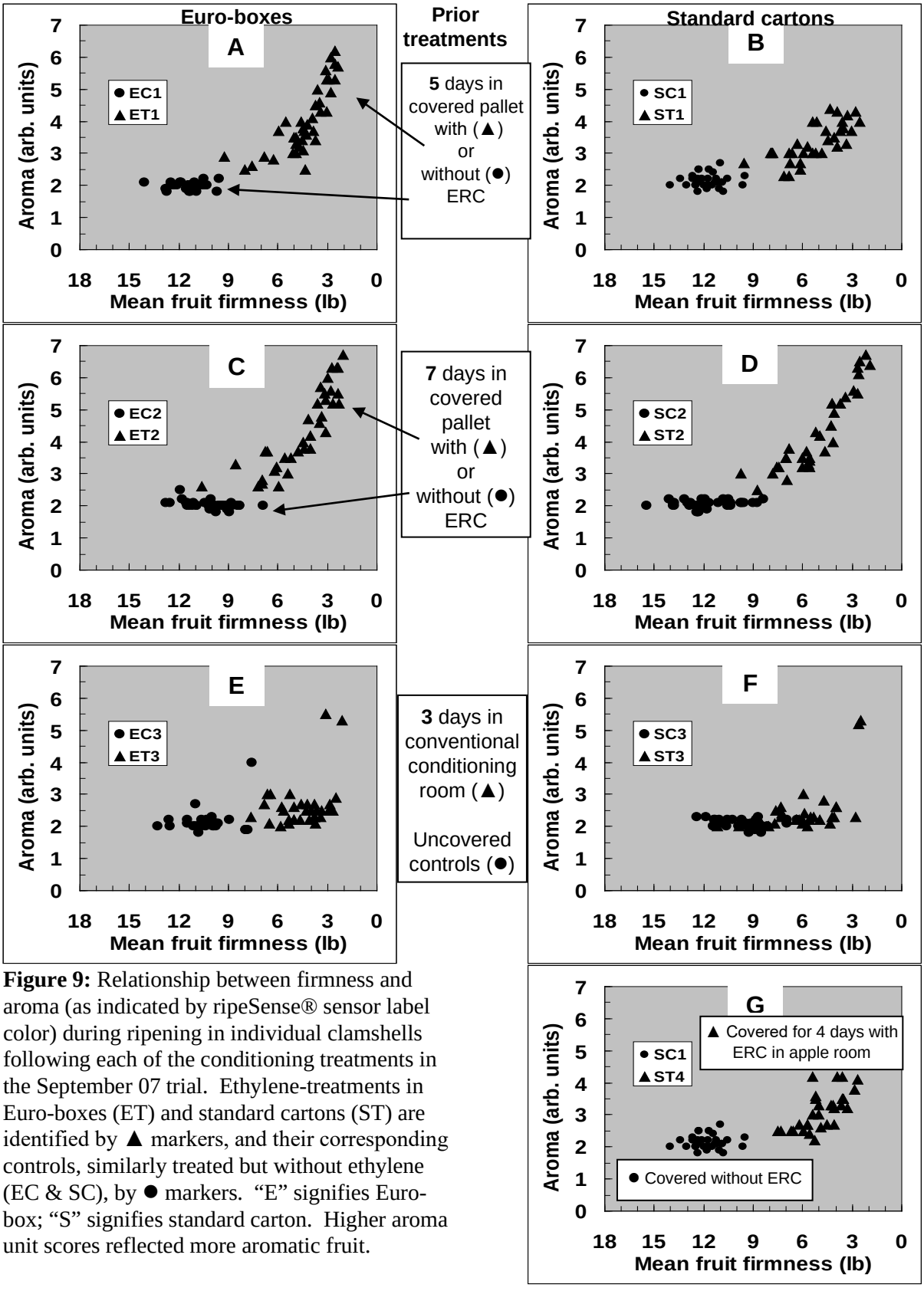

Figure 9: Relationship between firmness and aroma (as indicated by ripeSense® sensor label color) during ripening in individual clamshells following each of the conditioning treatments in the September 07 trial. Ethylene-treatments in Euro-boxes (ET) and standard cartons (ST) are identified by ▲ markers, and their corresponding controls, similarly treated but without ethylene (EC & SC), by ● markers. "E" signifies Euro-box; "S" signifies standard carton. Higher aroma unit scores reflected more aromatic fruit.

period of up to 7 days (Fig. 7). The control pallets that were covered but contained no ERCs did not accumulate any detectable ethylene (in contrast with the February trial, when the control fruit was capable of producing its own ethylene). Ethylene concentrations in the conventional conditioning room during conditioning of pallets ET3 and ST3 were 155 and 175 ppm during the first 24 h of ethylene exposure, but had fallen to 40 ppm by the end of the 48 h ethylene treatment.

Ripening following conditioning was carried out using fruit from sample boxes from the top, middle and bottom of each pallet. The remaining boxes were returned to 30°F after conditioning, inspected and deemed fit-for-sale. The sampled fruit were ripened in ripeSense® clamshells in the same dry goods warehouse in which most had been conditioned, at an uncontrolled temperature that averaged around 60°F. Ethylene, CO_2 and O_2 levels (not shown) and fruit firmness (Figure 8) within representative clamshells were measured daily. Aroma development (Figure 9) was monitored during ripening using ripeSense® sensor labels.

Ethylene production during ripening of these early season fruit, in marked contrast with the February trial, was restricted to only those fruit that had been conditioned with ethylene. Those that had been conditioned for the longest period (pallets ET2 and ST2) produced the most ethylene, which accumulated in their clamshells to almost 10 ppm by the end of ripening (data not shown).

Softening rates during ripening were also clearly dependent upon conditioning with external ethylene (Fig. 8). Controls (C1, C2 and C3), which were warmed and, in some cases, covered but not ethylene treated during conditioning, were invariably far slower to soften than the corresponding ethylene treatments (Fig. 8). Fastest softening occurred in the top layer of each ethylene-treated pallet. The T3 treatments (conventional conditioning room, Fig. 8 E&F) produced the fastest mean rates of softening across the whole pallets. This probably reflects the faster rates of warming of the T3 and top layer fruit during conditioning (Fig. 5), which meant that they more rapidly reached temperatures at which they were able to respond to ethylene.

Aroma production during softening was distinctly greater in fruit that had received the longer exposures to ethylene during conditioning (Fig. 9). These data are based on ripeSense® sensor responses, but corresponded with what our noses and taste buds told us. T1 & T2 fruit, conditioned with ethylene from ERCs for 5 and 7 days respectively in covered pallets, were clearly preferable in aroma and flavor criteria over T3 fruit, which had received the standard commercial conditioning involving one day of warming and two days of ethylene exposure. The ripened T1 and T2 fruit were generally acknowledged as excellent eating quality, which for Anjou is remarkable in view of the fact that they had been harvested just 10 days prior to commencement of the September conditioning trial. The conventionally conditioned T3 fruit softened to the same extent, but developed very little aroma as they softened (Fig 9 E&F), in comparison with fruit of T1 (A&B) and T2 (C&D). T4 fruit (Fig. 9G), which received a shorter, 4 day conditioning at a slightly higher mean temperature than T1 and T2, did not produce as much aroma as fruit of the latter two treatments, but were still more aromatic than the conventionally conditioned fruit.

These observations of the aroma and flavor benefits resulting from prolonged (5-7 days) ethylene exposure of Anjou pears during the first month after harvest are in keeping with the results of our previous project, obtained over several seasons (Sharrock and Henzell, 2007). The ERC, expected to be commercially available soon, offers a practical means of achieving such long conditioning periods at minimal extra cost, and with improved logistical flexibility.

Reference: Sharrock, K. R. and Henzell, R. F. (2007) Ethylene ripening of pears by unconventional means. Final Project Report. NW Pear Research Review 2007, 10 pp. (N.B. Complete report, including last 4 pages omitted from Review Proceedings and CD, is obtainable from the authors).

Acknowledgements: Thanks to Underwood Fruit for the free use of their facilities, and supply of top

quality fruit and packaging, and to MCAREC for the use of their ethylene, ripening rooms and house.

EXECUTIVE SUMMARY OF FINAL PROJECT REPORT

Project Title: Protocols for conditioning Anjou pears with ethylene release capsules

Pallet scale conditioning using ethylene release capsules (ERCs)

Covered pallet conditioning trials were conducted on Green Anjou in Winter (February 2007) and Fall (September 2007) at Underwood Fruit, White Salmon WA. The ERC device used is basically contained within an aerosol can (Fig. 1). A key feature is its unique internal valve (invented by HortResearch, patent pending) that releases compressed ethylene at a constant rate for an adjustable period of up to seven days. This is achieved without electronics or moving parts, maximizing its reliability and cost-effectiveness.

A standard polyethylene pallet cover was used to confine the ethylene released by a single ERC placed on top of the boxes inside the cover. Loss of ethylene through the polyethylene was compensated for by continual release from the ERC throughout conditioning.

Figure 1. Commercial prototype ERC

Significant findings

- ERCs provide an effective means of conditioning early season Anjou pears within covered pallets of either double-layer Euro-boxes or standard cartons of individually wrapped fruit.
- A single ERC at the top of a covered pallet resulted in >100 ppm ethylene throughout the pallet within 17 hours and maintained this for a pre-defined period of up to 7 days.
- Early in the Fall, Anjou conditioned by ERC for 5-7 days were **distinctly more aromatic and flavorsome**, once fully ripe, than those conditioned conventionally at higher temperatures for a shorter period. Fruit that were simply warmed without ethylene failed to ripen acceptably.
- In February, using CA-stored fruit, ethylene conditioning had no effect on subsequent rates of softening but still resulted in detectable aroma and flavor enhancement.
- Covering alone had little impact on rates of warming and softening of fruit, and no detrimental effects became evident during subsequent ripening in either Winter or Fall.

Practical implications and benefits

Pallet-scale conditioning using ERCs offers a practical and more flexible alternative to conventional conditioning in a purpose-built room or trailer. Each individual pallet shrouded in polyethylene becomes a ripening room, so any available corner of a warehouse or packing room can become temporary conditioning space, offering several real advantages.

1. For those companies that utilize the ERC exclusively, there are no upfront expenditures, which can range from thousands to hundreds of thousands of dollars to purchase conditioning rooms or trailers, and associated ethylene generators, chemicals or dangerous cylinders of ethylene.
2. Small orders can be more cost-effectively conditioned on demand.
3. Extended conditioning periods of up to 7 days, shown to trigger enhanced aroma production by early season fruit in particular, become logistically feasible.
4. For smaller companies that outsource their conditioning requirements, utilizing the ERC in-house gives them greater control of the conditioning process which should result in less damaged fruit versus their experiences with outside conditioning contractors.
5. The ERC conditioning method can be used at any point in the fruit's journey to the end user, including some in-transit situations.
6. The individual dose ERC permits application of a controlled amount of ethylene at a designed dwell. The efficiency of the process which yields a better-tasting fruit with a pleasing aroma is the defining result.

FINAL PROJECT REPORT

Project Title: Winter pear quality maintenance

PI:	Jinhe Bai	**Co-PI(2):**	Kristi Deschuytter
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City:	Hood River	**City:**	Hood River
State/Province/Zip	OR 97031	**State/Province/Zip:**	OR 97031

Cooperators:

Total Project Funding: $33,693

Budget History:

Item	**Year 1:** 2007		
Salaries	19,760		
Benefits	9,880		
Wages			
Benefits			
Equipment			
Supplies	3,500		
Travel	500		
Miscellaneous			
Total	**33,693**		

Significant Findings:
2006-2007 season

- Pre-harvest spray with 1-MCP did not control scald
- Pre-harvest 1-MCP spray combined with delayed harvests of two and four weeks resulted in decreased fruit firmness and increased fruit yellowing
- Low-dose application of 1-MCP (65ppb) after 5months of RA storage, combined with five or ten days preconditioning at 50°F, allowed fruit to soften with moderate scald control.
- 65ppb of 1-MCP on 9mth CA stored pears, combined with five days preconditioning at 50°F allowed fruit to soften almost to eating quality (6lbs) with excellent scald control.
- 'd'Anjou' pears from an elevation of ~2000ft, applied with 100ppb 1-MCP, when combined with five or ten days preconditioning at 50°F for 3, 5, 6, and 9 months, completely controlled scald while allowing the fruit to soften.

2007-2008 season (in progress)

- Fruit stored for 3 months RA storage when combined with ten days preconditioning at 50°F softened to 7lbs with excellent scald control.
- Fruit from a higher elevation (~2000 ft), applied with 100ppm of 1-MCP, when combined with five or ten days preconditioning at 50°F softened to eating quality with good scald control.
- Fruit harvested from ~500 ft. in elevation, applied with 65ppb 1-MCP, stored for 3 months RA, and applied with ethylene for 48 hrs. at 70°F softened to eating quality with 6% scald.

Results and Discussion:

Pre-harvest 1-MCP application

FF is the pole indicator for Anjou and other pear harvest maturity judgment. Current harvest maturity of Anjou fruit is about 15 lb where soluble solids accumulate to 12-13%, and fruit will have a juicy and buttery texture upon long term storage and a proper ripening. When FF is greater than 15 lb, fruit does not accumulate enough soluble solids, and has a poor taste. In contrast, when FF is less than 15 lb, fruit is susceptible to decay, and tends to have a mealy texture upon long storage and ripening.

FF at commercial maturity was 15 lb, and decreased to 13.5 and 12 lb by 14 and 28 days delayed harvest, respectively. FF of 75 g 1-MCP treated fruit was slightly greater although there was no significant difference (Fig. 2). Because 1-MCP application often inhibits/delays softening of fruit during storage and subsequent ripening, we proposed that a 1-MCP + delayed harvest will decrease scald (1-MCP), and increase softening ability (delayed harvest) without mealiness (1-MCP).

In fruit treated with a 75 g dose of 1-MCP, SSC was about 13.5%, 1% higher than the control and the treatment of 150 g 1-MCP at commercial harvest maturity and 14 days delayed harvest. However SSC increased in the later two treatments to the same level in the 28 days delayed harvested fruit (Fig. 2). Titratable acidity content was 0.32% at commercial harvest maturity in all treatments, but decreased to 0.30% and 0.27% by 14 and 28 days delayed harvests, respectively, in the control. TA contents in the 1-MCP treatments remained constant by 14 days delayed harvest but decreased to 0.30% by 28 days delayed harvest (Fig. 2). SSC in Anjou pears is 12-14% at commercial harvest maturity depending on the region and year, and SSC usually does not change by delaying harvest. Higher SSC and TA indicate a better taste. Therefore 1-MCP application, especially 75 g dose, was the best treatment (Fig. 2).

Fruit surface color yellowed continually during the delayed harvests as indicated by an increased a* (degreening), b* (yellowing), C* (higher chroma) and L* (higher lightness) values, and a decreased h° (hue angle to lower green and higher yellow color) value. However, 1-MCP did not consistently influence the changes.

Average fruit size was 246 g at commercial maturity, and increased to 280 and 205 g by 14 and 28

days delayed harvests, respectively. The final fruit weight after 28 days delayed harvest was similar in all three treatments.

Fruit treated with 1-MCP and harvested at commercial maturity did not ripen to eating softness (<6 lb) when stored up to 9 months, regardless of 1-MCP dose and storage time. However, fruit ripened when harvest was delayed for 14 or 28 days. 1-MCP field application at doses of 132 ppm decreased scald incidence. However, the scald incidence was still unacceptable (more than 10%) in most of the cases. The only successful combination was 132 ppm of 1-MCP + 14 days delayed harvest + 6 months of CA storage where fruit ripened with an acceptable scald incidence. Fruit treated with 66 ppm of 1-MCP did not decrease scald. Delayed harvested fruit had higher scald incidence compared with commercial harvested fruit. Additionally, delayed harvested fruit had higher decay rates during storage and without 1-MCP treatment; delayed harvested fruit became mealy after 5 months of RA and 9 months of CA storage. However, with 1-MCP treatment, fruit maintained a juicy and buttery texture during the entire storage period regardless of harvest time (data not shown).

Post-Harvest 1-MCP application

Superficial scald is one of the major postharvest issues for 'd'Anjou' pears. It appears after the fruit has ripened, making it difficult to detect in the packinghouse. Currently, postharvest line sprays or drenching with ethoxyquin is being used to control scald. In some cases, however, these treatments give rise to phytotoxicity, which is also a cause for fruit rejection. 1-MCP, in pears, could be become a very good alternative to ethoxyqin because of the ease of application, efficacy, and low residues. It has been used very successfully on apples, and we have shown that there is great promise for its use on pears.

In the 2006-2007 season (Table 2), fruit treated were treated with 65 or 100ppb 1-MCP and stored for 3- and 5-months RA and 6 and 9- months CA. Overall, fruit firmness for fruit treated with 65ppb 1-MCP softened more than fruit treated with 100ppb. However, only one combination, fruit treated with 65ppb, stored for 5 months with 10 days preconditioning, ripened to eating quality. Although the 65ppb was able to soften, it also did not control scald as well as the 100ppb treatment. Both 1-MCP treatments were applied 1-2 days after harvest at 30°C for 24hrs. It is possible that applying the 1-MCP at a warmer temperature may increase it's efficacy for scald control.

In the same harvest season, fruit from Parkdale, Oregon (~2000 ft in elevation) were also treated with 65 and 100ppb 1-MCP and stored for 3 and 5 months RA and 6 and 9 months CA (Table 2). Fruit firmness, regardless of length of storage, softened to eating quality with either 5 or a 10 day preconditioning period at 50°F. In all cases, scald was significantly decreased with 100ppb 1-MCP showing very little scald symptoms.

Fruit in the 2207-2008 season were harvested from both Hood River and Parkdale, Oregon (Table 3). Fruit from Hood River (elevation ~500 ft) was treated with 65ppb 1-MCP at 50°F for 24 hours and stored for 3-9 months in RA and CA. After three months of storage, fruit subjected to a preconditioning at 50° F for 10 days softened to approximately 7lbs with excellent scald control.

Additional fruit applied with 65ppb 1-MCP underwent exposure to ethylene for 24 or 48 hour at either 60° or 70° F (Table 4). No temperature preconditioning was applied for this experiment. After three months of RA storage, fruit stored at 70°F for 24hrs or ethylene exposure had the most scald control. However, the fruit failed to reach eating quality. However, fruit treated with 1-MCP and exposed to 48 hours of ethylene at 70°F did soften to eating quality with good scald control. However, 48 hours of ethylene exposure, did increase yellowing in the fruit, but those fruit treated with 1-MCP did appear greener than those that were not treated.

Fruit from Parkdale, Oregon was treated with 100ppb 1-MCP for 24hours at 30°F and then stored for 3-9 months in RA and CA (Table 3). After three months of storage, fruit that were subjected to a five or ten day preconditioning period at 50°F softened to eating quality. However, scald control was not as good as expected. Although the conditions remained the same from the previous year, it is possible that an increase in temperature when applying the 1-MCP could impact its efficacy.

Evaluations for 5, 6, and 9 months are in progress and will be completed in June 2008.

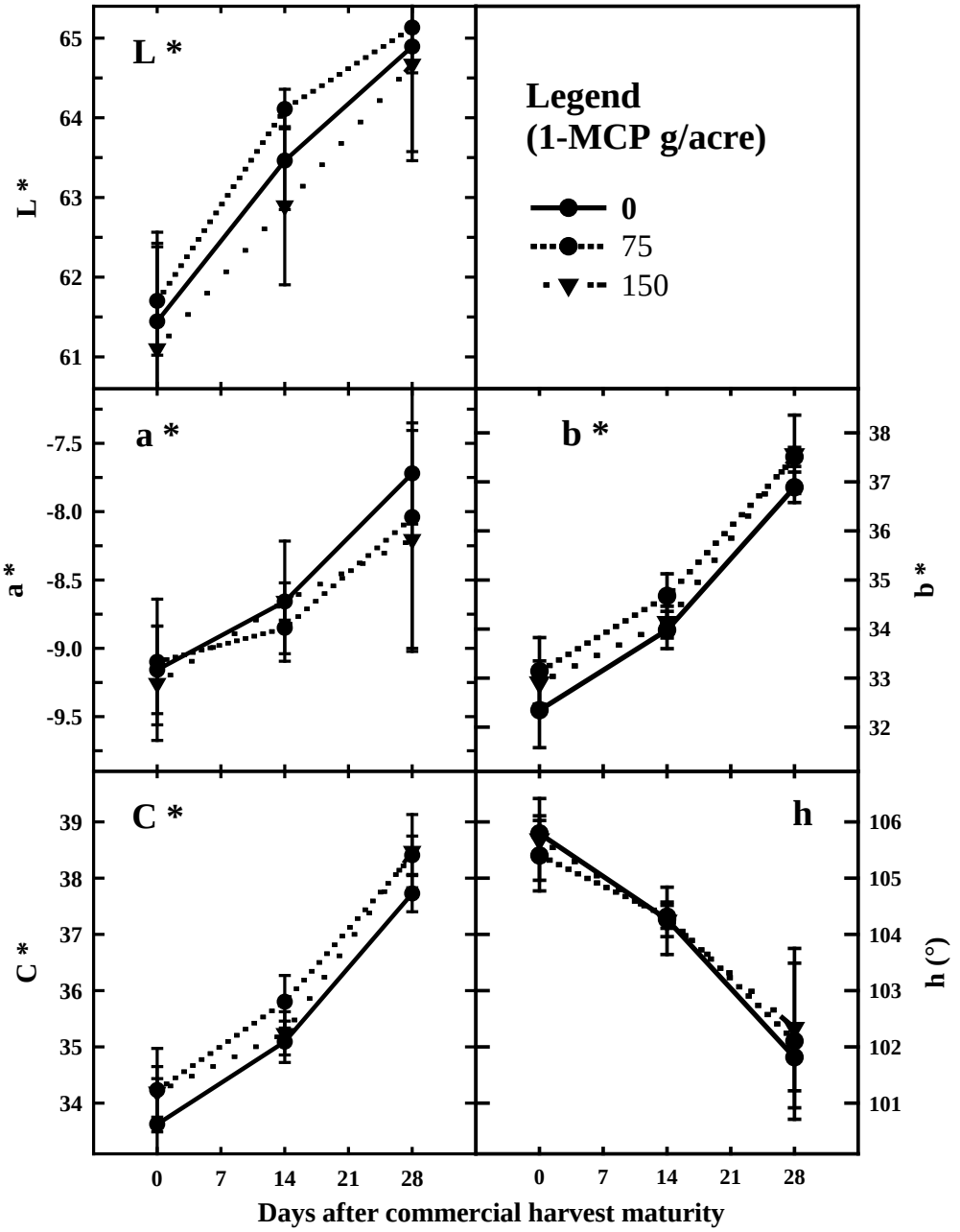

Fig. 1. Changes of surface color factors of 'Anjou' pear fruit during 28 days of delayed harvest period. Application of 1-MCP (0, 75 or 100 g/acre) was carried out 7 days before commercial harvest maturity.

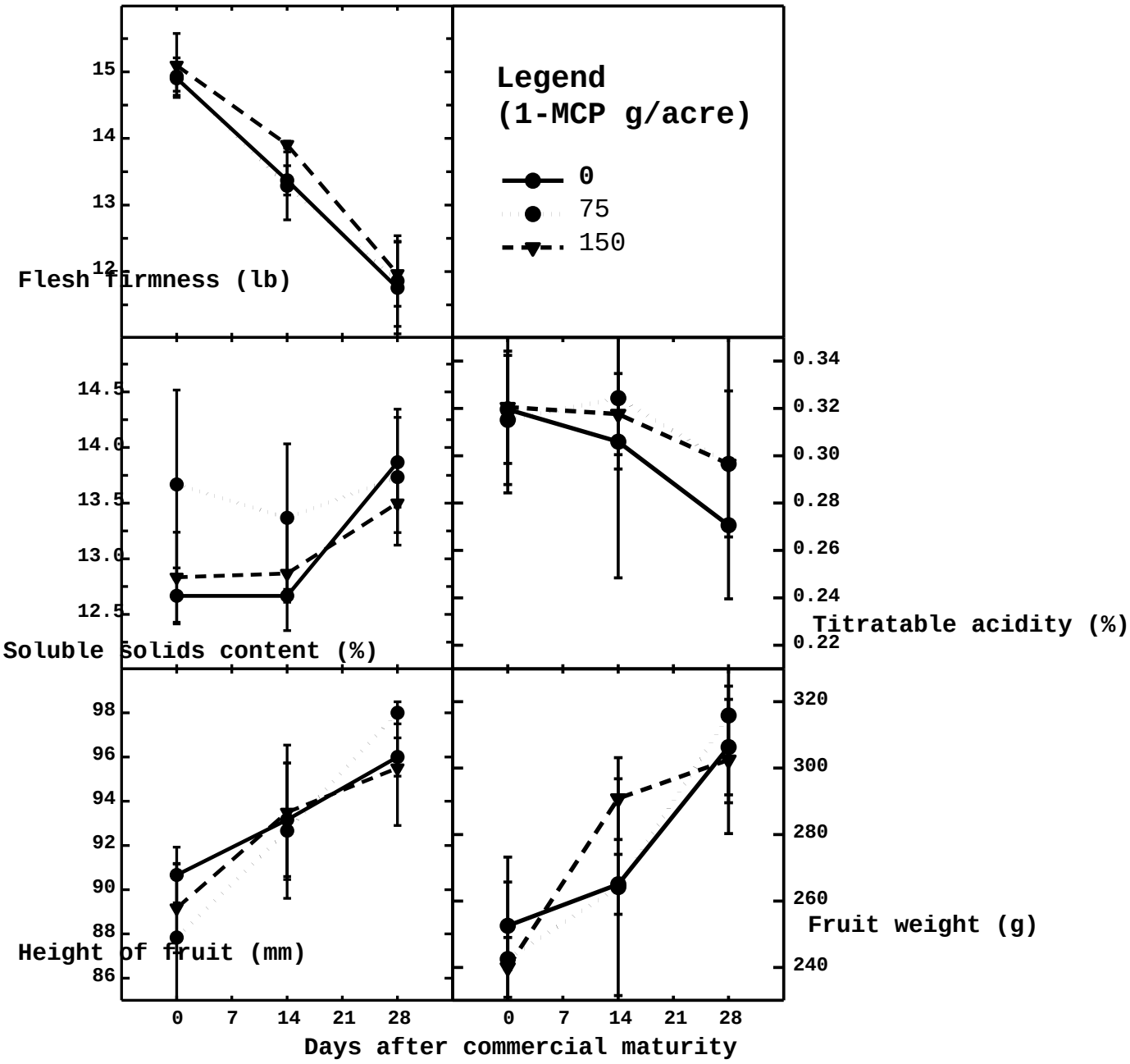

Fig. 2. Changes of quality parameters of 'Anjou' pear fruit during harvest period. Application of 1-MCP (0, 75 or 100 g/acre) was carried out 7 days before commercial harvest maturity.

28 days of delayed

Table 1. Effect of MCP dose and harvest maturity on flesh firmness and the incidence of superficial scald of Anjou pears. MCP was field-sprayed 7-days before commercial harvest time. Fruit were harvested at commercial maturity or 14 or 28-days delayed. After 3-9 months of storage in RA or CA at -1°C, fruit were transferred to 20°C for 7 days to simulate ripening condition.

MCP dosage (ppm)	Harvest delay (day)	Storage atmosphere and time							
		RA 3 months		RA 5 months		CA 6 months		CA 9 months	
		FF (lb)	Scald (%)	FF (lb)	Scald (%)	FF (lb)	Scald (%)	FF (lb)	Scald (%)
0	0	4.0 de	0.0 a	3.2 c	92.9 a	2.7 b	8.9 b	3.0 b	33.5 bc
	14	2.5 e	0.0 a	2.8 c	85.5 a	2.4 b	22.4 ab	2.8 b	66.7 ab
	28	3.4 e	0.0 a	2.7 c	89.1 a	2.0 b	40.4 ab	2.1 b	89.0 a
66	0	10.7 b	0.0 a	5.6 b	56.4 b	9.5 a	8.1 b	7.0 a	48.0 a-c
	14	5.7 dc	0.0 a	3.5 bc	89.4 a	3.1 b	33.1 ab	3.4 b	84.2 a
	28	3.1 e	0.0 a	2.5 c	94.0 a	1.9 b	50.6 a	2.2 b	65.3 a-c
132	0	14.0 a	0.0 a	10.9 a	22.1 c	9.7 a	8.2 b	8.3 a	21.9 c
	14	6.2 c	0.0 a	3.8 bc	81.9 a	3.0 b	8.8 b	3.5 b	37.9 bc
	28	3.5 de	0.0 a	2.7 c	95.4 a	2.3 b	29.4 ab	2.3 b	81.9 a
Source	df	F value and significance							
MCP dose (M)	2	62.3 ***	0 NS	28.8 ***	17.8 ***	57.8 ***	6.3 **	34.9 ***	8.6 **
Harvest (H)	2	33.1 ***	0 NS	14.9 ***	6.5 **	15.7 ***	1.4 NS	9.8 **	1.7 NS
M x H	4	13.2 ***	0 NS	10.4 ***	7.4 **	11.8 ***	0.4 NS	6.1 **	1.6 NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS: not significant

Table 2. Effect of orchard location, MCP dose and pre-conditioning time on flesh firmness and the incidence of superficial scald of Anjou pears. Fruit were treated with MCP after harvest, stored in RA or CA at -1°C for 3-9 months, and then pre-conditioned at 10°C for 0-10 days before transfer to 20°C for 7 days.

Location	MCP dosage (ppb)	Pre-conditioning time (days)	Storage atmosphere and time							
			RA 3 months		RA 5 months		CA 6 months		CA 9 months	
			FF (lb)	Scald (%)	FF (lb)	Scald (%)	FF (lbs)	Scald (%)	FF (lbs)	Scald (%)
Hood River	0	0	6.5 cd	20.3 a	3.6 e	100.0 a	2.3 de	60.0 a	3.0 ef	70.6 a
	65	0	13.1 a	0.0 b	10.0 b	40.0 bc	13.8 a	0.0 b	9.9 b	3.3 d
		5	10.6 ab	0.0 b	6.2 d	30.0 bc	10.1 b	0.0 b	8.4 c	0.0 d
		10	6.5 cd	0.0 b	2.1 ef	37.6 b	7.1 c	1.4 b	6.5 d	19.1 cd
	100	0	14.4 a	0.0 b	13.9 a	0.0 c	14.6 a	0.0 b	13.1 a	0.0 d
		5	14.3 a	0.0 b	13.5 a	0.0 c	14.1 a	0.0 b	12.7 a	0.0 d
		10	13.1 a	0.0 b	9.3 bc	0.0 c	11.8 ab	0.0 b	11.9 a	4.8 d
Parkdale	0	0	4.2 de	12.4 a	2.6 ef	36.7 b	3.1 de	1.6 b	3.1 ef	30.9 bc
	65	0	3.8 de	0.0 b	2.1 ef	26.7 bc	3.2 de	0.0 b	3.0 ef	37.7 bc
		5	1.7 e	0.0 b	1.6 f	46.7 b	1.8 e	0.0 b	2.2 ef	33.3 bc
		10	5.2 c-e	0.0 b	1.9 f	39.7 b	1.8 e	14.8 b	2.1 f	46.7 b
	100	0	12.4 a	0.0 b	8.0 c	0.0 c	10.2 b	0.0 b	6.8 d	0.0 d
		5	8.5 bc	0.0 b	2.5 ef	0.0 c	5.0 cd	6.7 b	3.5 e	6.7 d
		10	4.7 c-e	0.0 b	1.8 f	0.0 c	2.6 de	0.0 b	3.1 ef	0.0 d
Source		df	F value and significance							
Location (L)		1	53.88 ***	1.24 NS	288.1 ***	4.55 *	136.5 ***	14.89 ***	573.3 ***	0.54 NS
MCP dose (M)		2	32.52 ***	22.23 ***	204.2 ***	48.21 ***	72.12 ***	28.02 ***	200.4 ***	29.9 ***
Pre-conditioning (P)		2	8.18 **	0 NS	87.83 **	1.07 NS	26.74 ***	0.73 NS	29.41 ***	1.17 NS
L x M		2	1.41 NS	1.24 NS	50.19 ***	10.21 ***	14.69 ***	25.12 ***	58.81 ***	15.11 ***
L x P		2	1.05 NS	0 NS	13.01 ***	0.17 NS	0.73 NS	0.49 NS	2.62 NS	0.31 NS
M x P		2	0.94 NS	0 NS	1.87 NS	1.07 NS	0.44 NS	1.52 NS	0.77 NS	1.22 NS
L x M x P		2	8.98 ***	0 NS	27.1 ***	0.17 NS	8.39 **	1.15 NS	9.7 ***	0.07 NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS: not significant

Table 3: The effects of preconditioning on fruit firmness, soluble solid content, titratable acidity and % scald incidence for fruit from Hood River and Parkdale, Oregon. Fruit were treated one day after harvest with 1-MCP and stored for 3-9 months at -1°C (30°F). Prior to evaluation, fruit were stored at 10°C (50°F) for 0, 5, or 10 days and ripened for seven days at 20°C (70°F).

Location	Preconditioning	MCP dosage	FF (lbs)	SS	TA	% scald
Hood River	0 days	65	11.78667	12.6	0.40803	0.775194
	5 days		11.34333	12.63333	0.364033	0
	10 days		7.57	13.06667	0.37989	1.646341
	p-value		0.259	0.393	0.122	0.279
Parkdale	0 days	100	12.82	11.93333	0.29145	7.004831
	5 days		4.993333	12.13333	0.265767	7.786358
	10 days		2.653333	12.16667	0.247453	9.982882
	p-value		0*	0.134	0.147	0.898

Table 5: Analysis on the effects of temperature and length of exposure to ethylene on fruit control and fruit treated with 65ppb 1-MCP. Fruit were treated one day after harvest with 65ppb 1-MCP at 20°C (50°F) for 24 hours and stored for 3-9 months in RA or CA storage. After storage, boxes were treated with ethylene (100-1000ppm) for either 24 or 48 hours at 60° of 70° F. Fruit was then placed back in -1°C (30°F) RA for five days before subjecting the fruit to a seven day ripening period. The following measurements were taken after the seven day ripening period.

			L*	a*	b*	C*	h°	FF (lbs)	SS	TA	%SI
24 hrs	**60°F**	**no-MCP**	64.086	-6.8197	38.563	39.198	100.052	9.7	12.67	0.391	31.92
		65ppb MCP	63.395	-7.274	38.352	39.062	100.749	8.04	12.87	0.427	9.77
	70°F	**no-MCP**	62.813	-6.633	38.498	39.084	99.795	6.42	12.73	0.421	55.94
		65ppb MCP	64.0198	-7.098	37.948	38.634	100.568	10.92	12.57	0.398	1.85
two-way anova		MCP (M)	0.739	0.156	0.471	0.557	0.16	0.21	0.937	0.437	0.001*
		Temp (T)	0.676	0.553	0.652	0.585	0.657	0.953	0.581	0.916	0.335
		M x T	0.24	0.987	0.744	0.75	0.939	0.032*	0.392	0.007*	0.077
48 hrs	**60°F**	**no-MCP**	65.412	-5.132	41.06	41.437	97.157	3.533	12.6	0.339	28.9
		65ppb MCP	64.514	-6.531	39.456	40.036	99.432	7.51	12.8	0.408	8.26
	70°F	**no-MCP**	66.892	-3.62	42.913	43.114	94.807	2.066	13.3	0.345	26.45
		65ppb MCP	65.462	-4.72	40.622	40.975	96.77	4.316	12.97	0.39	6.34
two-way anova p-values		MCP (M)	0.172	0.135	0.08	0.082	0.115	0.034*	0.772	0.014*	0.034*
		Temp (T)	0.156	0.058	0.159	0.18	0.07	0.093	0.087	0.756	0.79
		M x T	0.741	0.847	0.733	0.689	0.9	0.5	0.265	0.542	0.974
	Source		**p-value and significance**								
	MCP (M)		0.412	0.05*	0.049*	0.058	0.042*	0.013*	0.87	0.006*	0*
	Temperature (T)		0.421	0.036*	0.261	0.319	0.051	0.17	0.308	0.806	0.607
	Hrs of Ethylene (E)		0.002*	0*	0*	0*	0*	0*	0.185	0.001*	0.206
	M x T		0.535	0.859	0.645	0.61	0.928	0.23	0.154	0.058	0.18
	M x E		0.205	0.342	0.172	0.162	0.299	0.366	0.785	0.023*	0.132
	T x E		0.172	0.085	0.13	0.137	0.095	0.195	0.086	0.74	0.373
	M x T x E		0.276	0.85	0.76	0.836	0.883	0.037*	0.785	0.392	0.166

* <0.05

FINAL PROJECT REPORT
WTFRC Project Number: #PR-05-504

Project Title: Chemical ecology of pear psylla

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Cooperators: Dr. Jocelyn Millar (UC Riverside) – funded by NRI
Dr. Victoria Soroker (Volcani Institute) – funded by BARD
Dr. Anat Zada (Volcani Institute) – funded by BARD
Bob Brown (WSU Master's candidate) – funded by USDA-ARS

Other funding Sources

Agency Name: USDA-CSREES-NRI
Amount awarded: $230,000 for FY 2006-2008; $120,000 to Wapato lab
Notes: Funding for GS-6 term technician (Horton's laboratory) and Dr. Jocelyn Millar (pheromone chemist at U.C. Riverside)

Agency Name: Binational Agricultural Research and Development (BARD)
Amount awarded: $273,000 for FY 2008-2010; $103,000 to Wapato lab
Notes: Partial funding for postdoctoral scientist Dr. Christelle Guédot (Landolt's laboratory), Dr. Victoria Soroker (Volcani Center), and Dr. Anat Zada (Volcani Center)

Budget History (WTFRC):

Item	**Year 1:** (2005)	**Year 2:** (2006)	**Year 3:** (2007)
Salaries	15,000	27,500	20,000
Benefits	4,500	8,250	6,000
Supplies		4,000	
Total	19,500	39,750	26,000

Significant Findings and Accomplishments

- Data obtained using WTFRC funding allowed us to apply for grants elsewhere. Outside funding has been obtained from two granting agencies, to supplement WTFRC funding:
    - $230,000 from USDA-CSREES-NRI used to fund technician (Horton's lab) and Dr. Jocelyn Millar, a pheromone chemist at University California, Riverside
    - $273,000 from Binational Agricultural Research and Development (BARD) used to partially fund a post-doctoral scientist (Dr. Christelle Guédot) in Landolt's lab, and two scientists at the Volcani Center in Israel. The Israeli scientists will work on the pheromone of a closely related pear psyllid.
- We brought in a Master's student candidate (Bob Brown) on USDA funds, who has developed a trap suitable for field-testing psylla attractants.
- Olfactometer assays were developed and used to define the life history traits in pear psylla that lead to maximal female attractiveness and optimal behavioral responses in males. It is necessary to have these behavioral data before we attempt to move into the chemistry portion of the project.
- We began attempts to isolate and identify chemical attractants from known attractive females. Two types of products were collected: surface extracts and headspace volatiles. One volatile apparently associated with female psylla but not male psylla was identified, and preliminary trials in the olfactometer were completed showing that males were indeed attracted to the compound.

Results and Discussion

OBJECTIVE: DEFINE LIFE HISTORY CHARACTERISTICS LEADING TO FEMALE ATTRACTIVENESS AND OPTIMAL MALE RESPONSE (HORTON, LANDOLT, GUÉDOT)

These trials were done using a Y-tube olfactometer (Fig. 1). Each comparison included a minimum of 10 replicates, with each replicate consisting of 10 males assayed one-at-a-time (i.e., a minimum of 100 males per test). Figure 2 shows typical results, in this case for an assay with summerforms in which we tested whether the host plant must be present to obtain attraction. Each bar is a single replicate of 10 males (assayed one-at-a-time). Figure 2A shows that most males chose the arm of the olfactometer connected to a female-infested seedling over an uninfested seedling. Figure 2B shows that females even in the absence of a seedling were attractive to males. Finally, Figure 2C shows that a female-infested seedling was no more attractive to males than an equivalent number of females in the absence of a seedling.

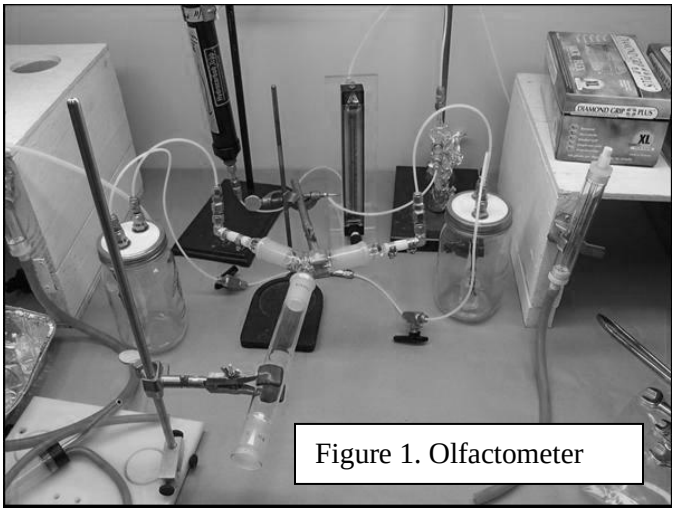

Figure 1. Olfactometer

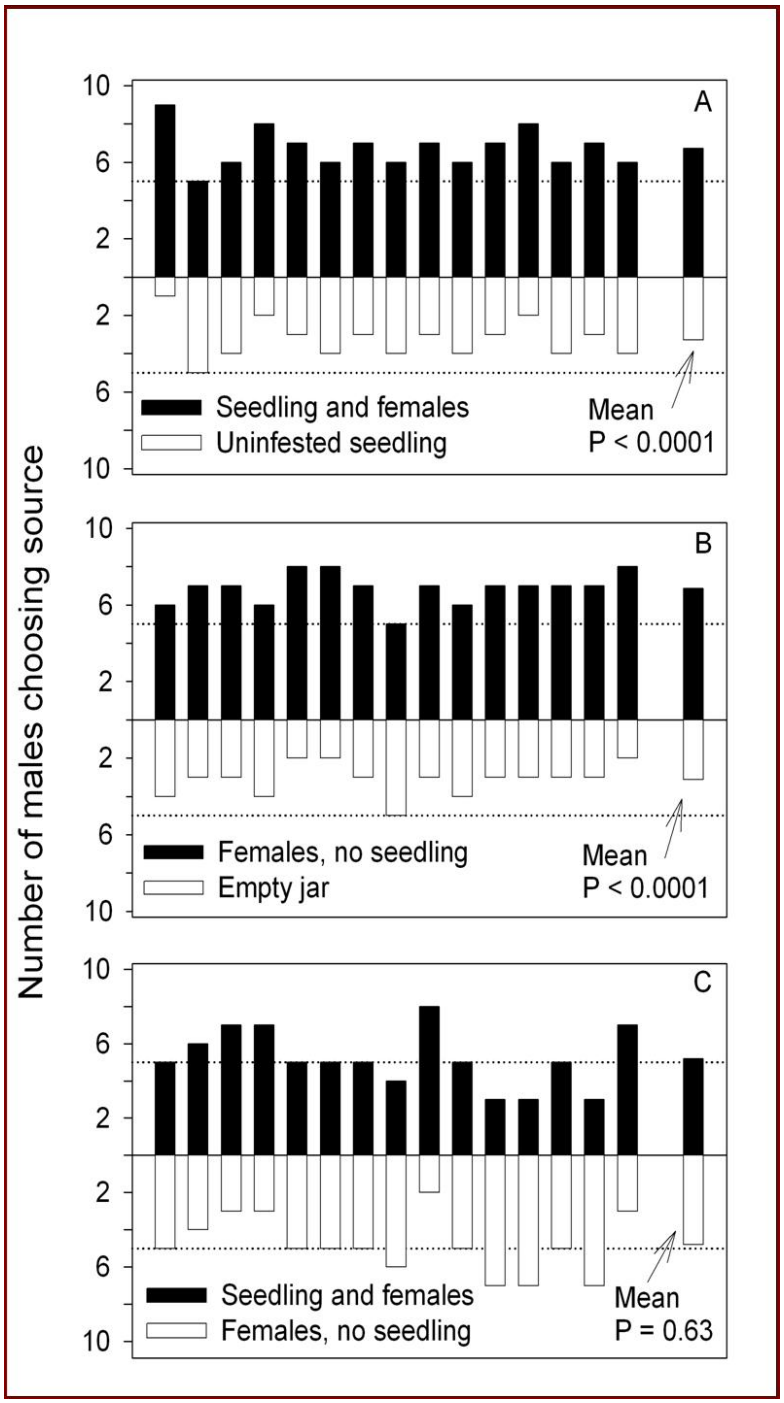

Figure 2. Numbers of males choosing arm of olfactometer attached to treatment or control odor sources. These assays tested whether presence of the host plant was necessary to prompt volatile production by females.

Rather than present a series of figures similar to Figure 2 to show the results of our olfactometer work, we summarize the results in tabular form (shown in Table 1).

Table 1. Summary of olfactometer results assessing the role of various life history factors affecting female attractiveness and male response. Underline indicates significant preference for that choice.

CHOICE 1 IN OLFACTOMETER	CHOICE 2 IN OLFACTOMETER
Summerforms	
Female-infested seedling	Uninfested seedling
8-10 day old females	2-5 day old females
Virgin females on seedling	Uninfested seedling
Mated females on seedling	Uninfested seedling
Virgin females on seedling	Mated females on seedling
Female-infested seedling	Uninfested seedling
Females, no seedling	Blank
Females, no seedling	Female-infested seedling
30 dead females	Blank
30 dead females	30 dead males
30 dead males	Blank
Winterforms	
Female-infested shoots	Uninfested shoots
Shoots previously infested (females)	Uninfested shoots
Female-infested shoots	Shoots previously infested (females)
Females exposed to long-days	Females kept at short-days
Females exposed to fenoxycarb	Control females
Shoots and diapause females	Uninfested shoots
Shoots and post-diapause females	Uninfested shoots

A final assay was done to determine when, seasonally, female winterforms begin to attract males in the olfactometer. Male and female winterforms were collected from the field at intervals between October and February, and assayed in the olfactometer. Females were not attractive to males until ovarian development and mating was seen in the field, beginning in early- to mid-February (Fig. 3). These results suggest that volatile production by female winterforms is closely associated with diapause status.

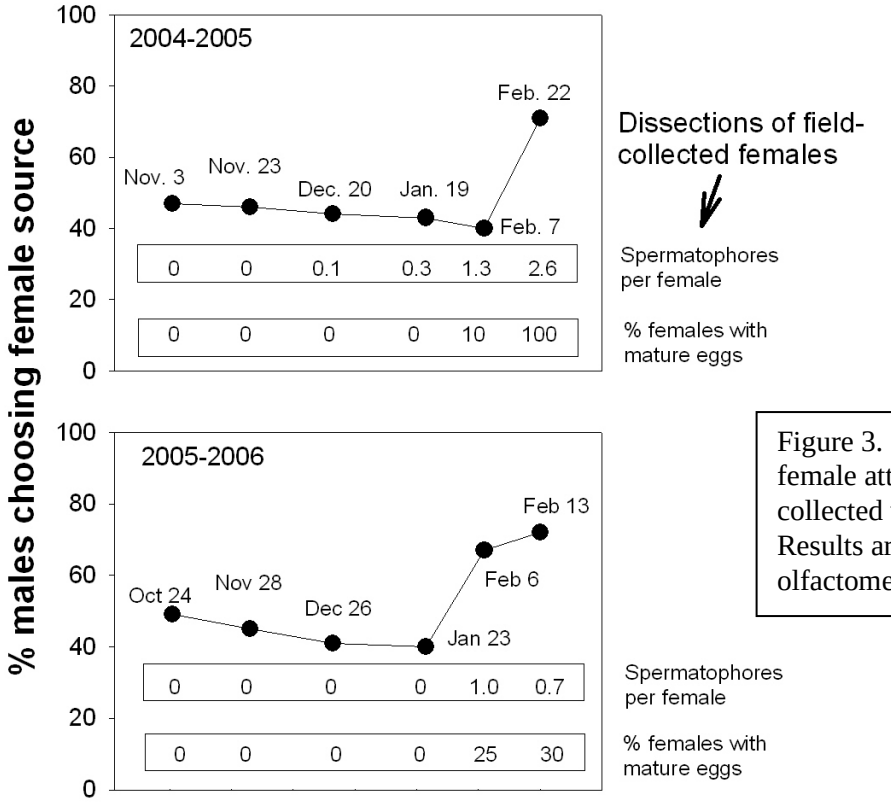

Figure 3. Seasonality of female attractiveness in field-collected winterforms. Results are from olfactometer trials.

Summary of olfactometer trials

- The results of the olfactometer trials indicated the following for summerforms:
  - ❖ both virgin and mated females attract males
  - ❖ older females are more attractive than very young females
  - ❖ the host plant does not have to be present for females to be attractive
  - ❖ even freshly killed females attract males

- The results for winterforms indicated the following:
  - ❖ shoots that had previously been occupied by females attract males in the olfactometer, suggesting residues left by females are both volatile and attractive
  - ❖ post-diapause females attract males, whereas diapausing females do not
  - ❖ onset of attractiveness (volatile production) coincides approximately with mating and ovarian development in the field

OBJECTIVE: DEVELOP FIELD-TRAPPING METHODS (BROWN, LANDOLT, HORTON)

Ultimate objectives of this project are to test candidate products in the field for their long-distance attractiveness to male pear psylla. This objective requires that we first develop a suitable trap. The trap shown in Figure 4 (developed by Bob Brown, WSU Master's candidate) is composed of tanglefoot-covered mesh, which is used to envelope a small organdy bag holding the source attractant. In testing this trap, we used live insects as our source of attractants. For both summerforms and winterforms, males were attracted to female-baited traps compared to male-baited or unbaited traps (Fig. 5). Conversely, females were distributed evenly among the three types of traps

(Fig. 5). We conclude that this trap design will be suitable for testing synthetic attractants, once those products have been identified and synthesized.

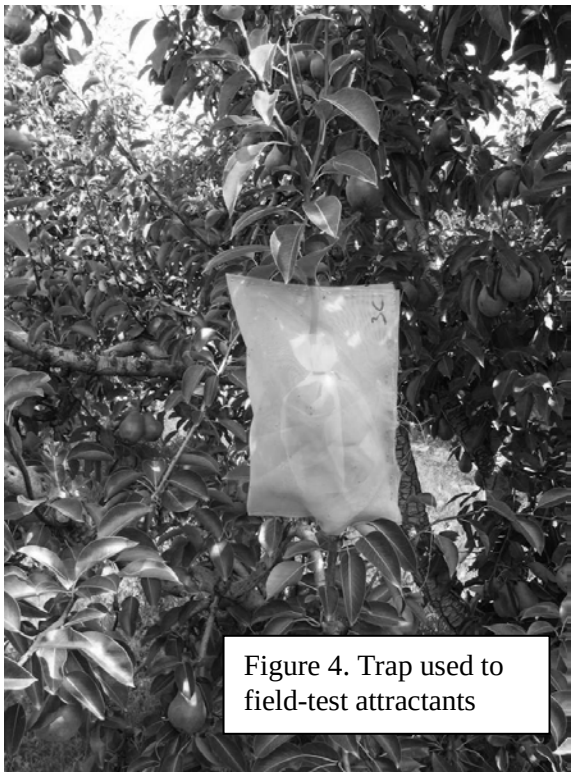

Figure 4. Trap used to field-test attractants

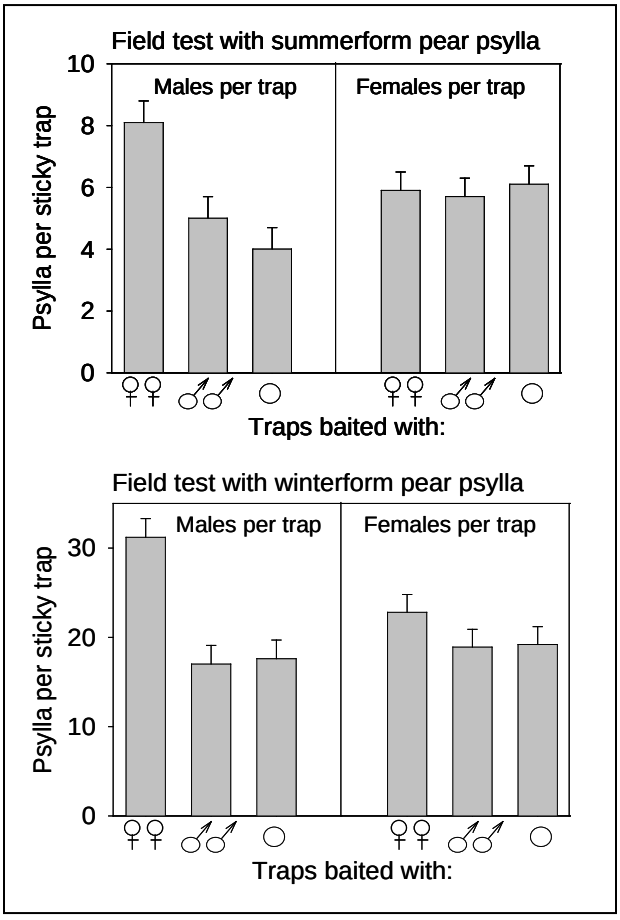

Figure 5. Field-test of trap, using live insects as attractants

OBJECTIVE: ISOLATE AND IDENTIFY THE ATTRACTANT (MILLAR, GUÉDOT, LANDOLT, HORTON)

Surface extracts of post-diapause winterforms were obtained by washing 500 live females in pentane. Fifty-female aliquots of the extract were then applied to filter paper disks, which were paired in the olfactometer against solvent-treated disks. The extract was attractive to post-diapause male winterforms (Fig. 6). Identification of the attractant has not yet been done.

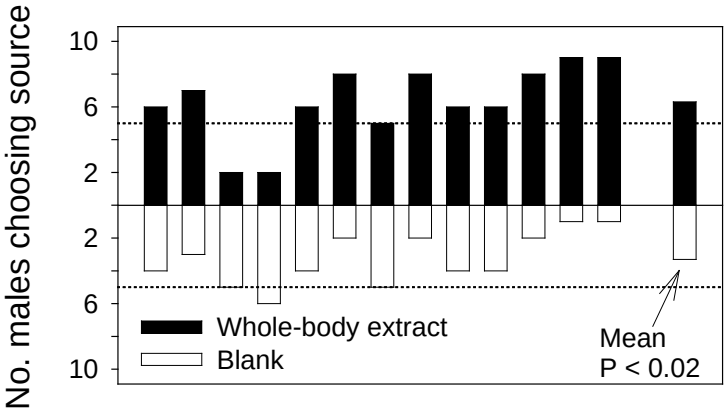

Figure 6. Attraction by males in olfactometer to filter paper disks treated with pentane (surface) extract of 50 female winterforms if paired with solvent control.

Head-space volatiles were collected by J. Millar from post-diapause female and male winterforms. The volatiles were adsorbed on an SPME fiber then desorbed directly into a gas chromatograph. GC-traces for female and male extracts were then compared (Fig. 7). Millar identified one peak in the trace that was found in the female-produced volatiles but not in the male-produced volatiles (shown by the arrow in Fig. 7). A synthetic formulation of the chemical was obtained. The formulation was then applied in solvent to filter paper disks, and compared in an olfactometer to solvent-treated disks. Summerform males were attracted to the compound (Fig. 8); winterform males, but not females, were also attracted to the compound (Fig. 9).

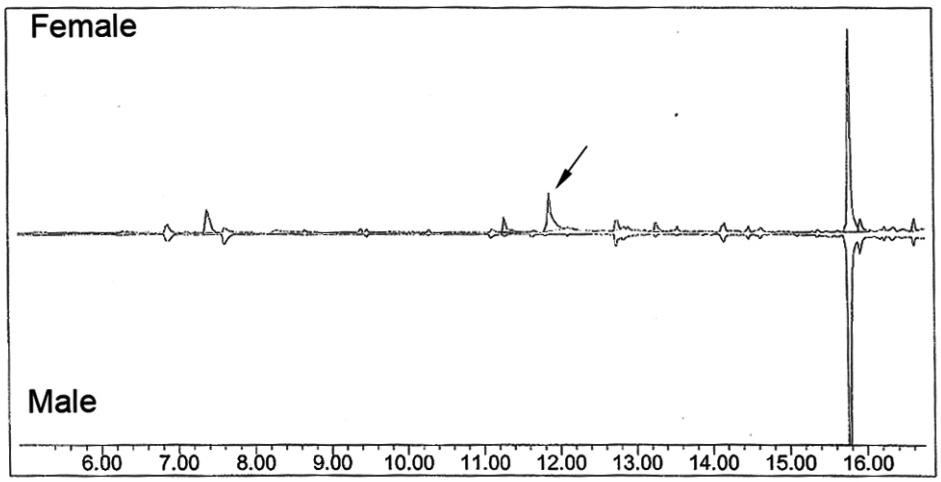

Figure 7. GC-traces from headspace volatiles

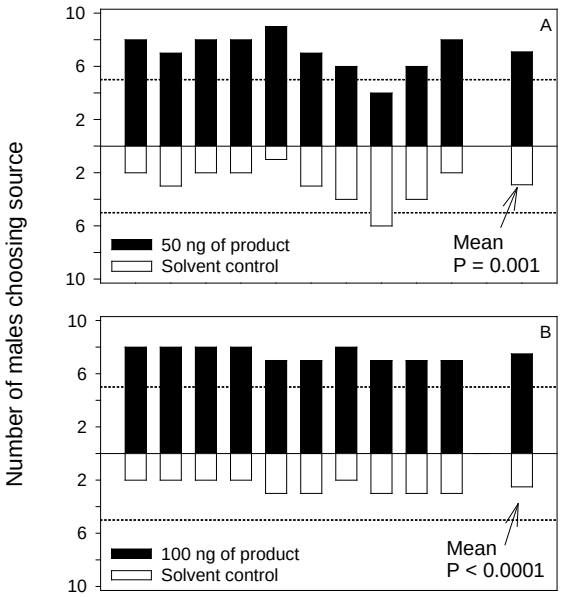

Figure 8. Preference by male summerforms for 50 or 100 ng of Millar product if paired against a solvent control

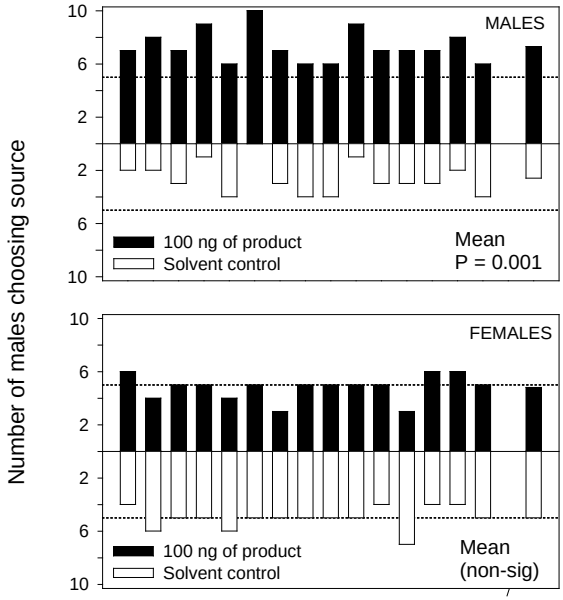

Figure 9. Preference by male or female winterforms for Millar product if product paired against solvent control

Presentations

Horton, D.R., C. Guédot and P. Landolt. 2006. Diapause affects attraction of male pear psylla to volatiles from females. 102nd Annual Meeting, Wash. State Hort. Assoc., Yakima, WA.

Guédot, C., D.R. Horton and P.J. Landolt. 2006. Chemical ecology of the sexual attractants in pear psylla, *Cacopsylla pyricola*. Entomological Society of America, Indianapolis, IN.

Brown, R., P.J. Landolt, D.R. Horton and R. Zack. 2007. Field demonstration of sex attraction in *Cacopsylla pyricola*. Pacific Branch, Entomological Society of America, Portland, OR.

Brown, R.L., P.J. Landolt, D.R. Horton and R.S. Zack. 2007. Sex attraction of the pear psylla, *Cacopsylla pyricola* (Hemiptera: Psyllidae) with a description of the diel periodicity of the attraction. Entomological Society of America. San Diego, CA.

Landolt, P., J. Millar, D. Horton and C. Guédot. 2007. Characterization and chemistry of sexual communication in pear psylla. Entomological Society of America, San Diego, CA.

Guédot, C., D.R. Horton, and P.J. Landolt. 2007. Attraction of male summerform pear psylla to volatiles from females: effects of female age, mating status, and presence of host plant. 103rd Annual Meeting, Washington State Horticultural Assoc., Wenatchee, WA.

Publications

Horton, D.R. and P.J. Landolt. 2007. Attraction of male pear psylla, *Cacopsylla pyricola*, to female-infested pear shoots. *Entomologia Experimentalis et Applicata* 123: 177-183.

Horton, D.R., C. Guédot, and P.J. Landolt. 2007. Diapause status of females affects attraction of male pear psylla, *Cacopsylla pyricola*, to volatiles from female-infested pear shoots. *Entomologia*

Experimentalis et Applicata 123: 185-192.
Horton, D.R., C. Guédot, and P.J. Landolt. 2008. Attraction of male summerform pear psylla to volatiles from female psylla: effects of female age, mating status, and presence of host plant. *Canadian Entomologist* (in press).

CONTINUTING PROJECT REPORT **YEAR: 1** OF 2
WTFRC Project Number: PR-07-701 (WSU Project #13L-3643-5431)

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

PI: John E. Dunley, Associate Entomologist
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Cooperators: Vincent P. Jones, Jay F. Brunner

Total project funding request: **Year 1:** $31,126 **Year 2: $39,820**

Other Funding Sources: None

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

Item	2007	2008
Salaries[1]	22,100	**22,100**
Benefits (38%)	7,956	**8,398**
Wages[2]	6,000	**6,000**
Benefits (15.7%)	690	**942**
Supplies[3]	1,200	**1,200**
Travel[4]	1,180	**1,180**
Total	39,126*	**39,820**

***actual funding amount = $31,300**

[1] A portion of the salary for Agricultural Research Technologist position.
[2] Time-slip wages.
[3] Supplies: items including cages, screening, sewing services, Tanglefoot, beating trays, opti-visors. Cell phone charges are allowed under this grant.
[4] Travel: local travel to research plots only.

Objectives:

1. ***Develop a temperature-based model for pear psylla development.***
    a. Effort is currently underway to complete this objective. The lower threshold for eggs has been estimated from laboratory data, and field data has been collected. A development model is being developed.
2. ***Validate the degree-day model and determine the appropriateness for timing applications throughout the growing season.***
    a. Data have been collected for 2007, and will again be collected in 2008. These data will be used to validate the developed model over the growing season.
3. ***Determine the effects of control tactics targeting the second generation of pear psylla.***
    a. (This objective was dropped at the request of the research commission)

Significant Findings:

- Developmental rates of pear psylla eggs under controlled temperatures were determined. Egg development threshold was determined to be 4.81ºC (40.65ºF)
- Development of pear psylla eggs and nymphal instars were measured in the field.

Methods:

To determine developmental rates of pear psylla eggs, adult pear psylla were collected from a pear orchard located at Smith Tract, WSU-TFREC, near Orondo, Washington on May 22, 2007. Adults were collected by beating tray and aspirator and transported to the laboratory. Adults were then exposed to CO2 to immobilize and separate them by sex. Four separate cages containing pear shoots were then loaded with approximately 200 females each and oviposition was allowed for sixteen hours. Shoots were then removed from the cages and the numbers of eggs laid on the shoots were determined. Of those laid in each cage, 98-107 eggs were identified on the leaves and shoot bark and circled with a marking pen. Shoots were then placed in temperature-controlled chambers at 10, 15, 20, and 25ºC. Eggs were then checked daily, and the number hatched each day recorded. Nymphs were removed after counting. The eggs were monitored for 30 days.

Regression analysis was performed on the rate of eggs hatching at the four temperatures. The resulting regression model was then used to estimate the temperature threshold for egg development, where the rate of development is equal to zero (the x-intercept).

Pear psylla populations were sampled weekly from four pear orchards in the Wenatchee Valley (Wenatchee, Orondo, Monitor, and Dryden). Adult pear psylla densities were sampled by beating tray, while nymph and egg densities were sampled by collecting leaves then dislodging the insects onto glass plates using a leaf-brushing machine. Eggs and nymphs were then counted and developmental stage noted by examination under a dissecting microscope.

Additionally, individual eggs were identified and marked on foliage in a pear orchard at WSU-TFREC. Leaves, or areas of leaves, were identified that were free of pear psylla eggs and nymphs and then labeled using a marking pen. Oviposition was then noted on subsequent days, and individual eggs marked for monitoring. Daily observations of the status of the eggs were made. Observations were also made of nymphs following eclosion; however, the movement of the insects, further oviposition, and weather made these data of little utility.

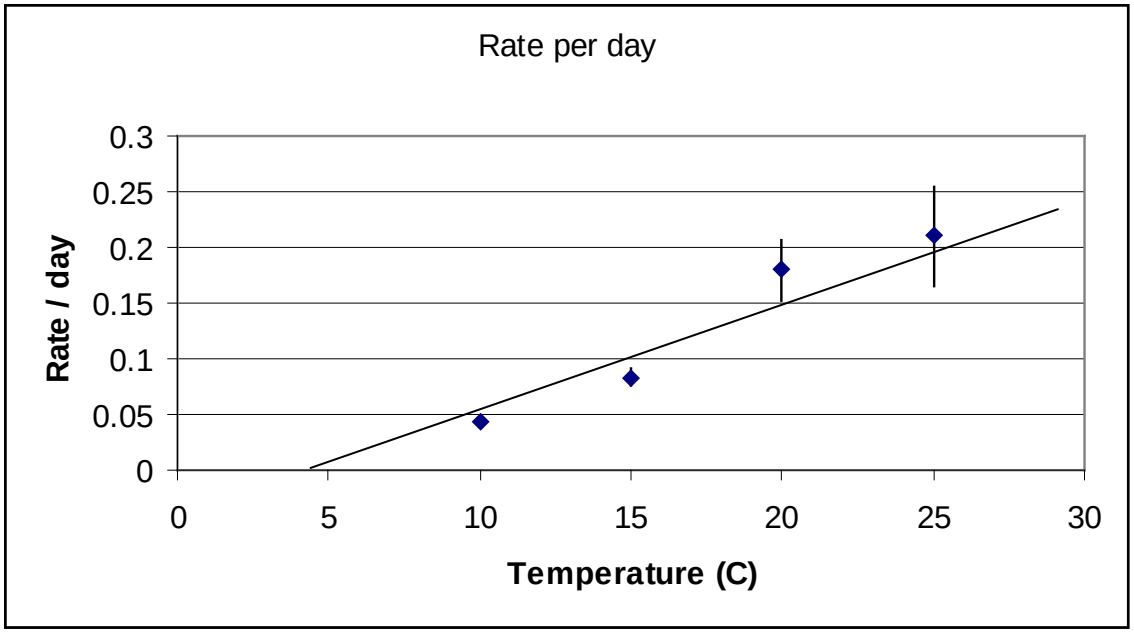
Results and Discussion:

Pear psylla egg developmental rate demonstrated a linear relationship among the four constant temperatures examined (Figure 1, Table 1). There was variation in mortality among the four temperature treatments, in that egg mortality was lowest at 25ºC (survivorship at 10, 15, 20, and 25ºC: 77%, 70%, 87%, and 95%, respectively). The linear model was a good fit for the data ($p < 0.0001$), and regression coefficient was also significant ($r^2 = 0.705$).

Table 1. Mean developmental rates of pear psylla eggs maintained at constant temperature.

Temperature (ºC)	Developmental Rate / Day	Standard Deviation
10	0.043921	0.004208
15	0.083426	0.006846
20	0.180136	0.027039
25	0.21	0.043995

Figure 1. Mean developmental rate of pear psylla eggs (±SD) and the linear regression of rate on temperature treatment. $y=0.0122 \cdot T - 0.0589$, where y is developmental rate and T is temperature.



Using the regression model, the temperature threshold for egg development of *Cacopsylla pyricola* was estimated to be 4.81ºC (40.65ºF). This is slightly higher than the estimates for *Cacopsylla pyri* of 3.46ºC (38.22ºF) from Schaub et al. (2005) and 3.70 º C (38.66ºF) from Sonnemaison and Missionier (1956; calculated in Schaub et al. 2005). The effects of these slight differences (sensitivity) in temperature thresholds can be seen in Table 2, where 2007 weather data from Wenatchee TFREC were used in a hypothetical single sine phenology model to estimate degree days from January 1 to May 1, 2007.

Table 2. Estimates of degree day accumulations from January 1 to May 1, 2007, at WSU-TFREC, using three different estimates of lower temperature thresholds in a single sine model.

Lower threshold (º C)	DD Accumulation (1/1/07-5/1/07)
3.46	720.4
3.70	693.2
4.81	580.1

Data collected from the field, both from field populations and examinations of individual data, will be used to calibrate and validate a phenology model once developmental rates of nymphal instars has been determined.

Development and validation of a predictive degree-day model will not only allow for better monitoring of pear psylla populations, it will improve timing of the newer insecticides. This is critical in that newer insecticides typically have smaller windows of opportunity to control the pest, and target only one or two life stages (particularly more environmentally-benign tactics such as insect growth regulators).
The resulting predictive degree-day model will also be included in the WSU-TFREC Decision Aid System implemented by Jones et al. This will help pear growers monitor pear psylla development and improve timing, as they currently can for codling moth, leafrollers, and other pests.

Literature Cited:

Bonnemaison, L. and J. Missonnier. 1956. Le psylle du poirier (*Psylla pyri* L.): morphologie et biologie. Méthode de Lutte. Annales Epiphyties 7: 263–231.

Schaub, L., B. Graf, and A. Butturini. 2005. Phenological model of *Cacopsylla pyri*. Entomol. Experiment. et Applic. 117: 105-111.

CONTINUING PROJECT REPORT **YEAR**: 1 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
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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:** $15,000 (revised)

Other funding Sources

Agency Name: Western SARE
Amount requested: $121,092 (2008-2009)
Notes: The grant was submitted in November, with a funding decision to be made in April-May 2008. We propose to expand the current project to include 4 commercial organic pear orchards.

Agency Name: WSU Center for Sustaining Agriculture and Natural Resources (CSANR): Organic Cropping Research for the Northwest
Amount requested: $69,530 (2008-2009)
Notes: The grant pre-proposal was submitted in January 2008. We propose to expand the current project to include 4 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[1]	8,000
Benefits	2,180	1,000	600
Wages	4,500		
Benefits	500		
Supplies	1,070	1,500[2]	1,400
Total	20,000	15,000	10,000

[1] Two GS-4 technicians ($12.50 per hour) for 25 weeks total; benefits at 7.65%. Split between Horton and Unruh.
[2] Orchard upkeep (fertilizer, herbicide, fireblight control), microtubes, tangle trap, ELISA supplies

Budget 2:

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

Item	2007	2008	2009
Salaries[1]	3,148	3,273	3,404
Benefits	1,133	1,178	1,225
Equipment			
Supplies[2]	719	549	371
Travel			
Total	5,000	5,000	5,000

[1]Partial support for Associate in Research. Benefits calculated at 36%

[2]ELISA supplies

COMMENT ON FUNDING: We are hoping to obtain funding from other sources (see above), that would allow us to expand the full project into 4 commercial organic orchards. Our funding request to WTFRC has been lowered with the expectation that some supplemental funding will indeed materialize.

OBJECTIVES:

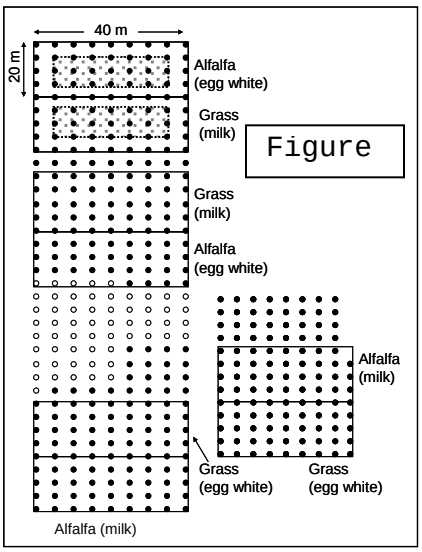
1. Estimate levels of psylla biological control in large plots of an alfalfa cover crop vs control (grass understory) plots;
2. Estimate movement rates of predators from orchard floor to tree and determine whether colonizing predators will then attack pear psylla (by simultaneous use of protein markers [Jones] and gut contents analysis [Unruh]).
3. Manipulate cover crop habitat (by mowing) or tree habitat (by use of volatile predator attractants) to change relative attractiveness of those two habitats, in efforts to prompt higher rates of movement from cover crop into tree at specific times in the growing season.
4. (NEW) Expand project into 4 commercial organic orchards (contingent upon SARE or CSANR funding).

SIGNIFICANT FINDINGS:

- Densities of generalist predators were 6-fold higher in understory of alfalfa plots than grass plots.
- Sticky trap catch of predators consequently was higher in the alfalfa plots than the grass plots, particularly early in the growing season. Higher counts in the alfalfa plots were observed even in traps placed in the tree canopy, which suggests that the predator community in trees included specimens that had originated in the alfalfa.
- However, despite the sticky trap data, predator densities in trees (from beat trays) were, if anything, higher in the grass plots. This result may indicate that predators which colonized the tree canopy from the alfalfa failed to remain there, possibly returning to the alfalfa. If so, it is possible that the alfalfa cover crop was attractive enough to predators that it led to a net loss of natural enemies establishing in the tree canopy.
- As a consequence of these treatment differences in predator counts, densities of immature psylla were not lower in the alfalfa plots than the grass plots; if anything, densities were actually lower in the grass plots.
- Densities of summerform adults early in the season were higher in the alfalfa plots than the grass plots. Because early-season densities of predators were relatively low in all plots, it is difficult to ascribe these treatment differences to biological control. We are considering the hypothesis that nitrogen produced in association with the alfalfa cover crop was taken up by the pear trees, leading to higher densities of adult psylla in those plots (nitrogen and tree vigor to be monitored in 2008).
- Over 4000 specimens were collected from orchard floor and tree canopy for assessment of marker presence. The specimens are currently being assayed (with ELISA). We have finished assays for two predators: *Anthocoris* spp. and *Deraeocoris brevis*. Marker presence on tree-collected specimens was statistically higher in the alfalfa plots than the grass plots, which is evidence that the alfalfa was a source of these two predator species moving into trees. There was a seasonal decline in marker frequency for the tree-collected insects, results which parallel our sticky trap data.
- Specimens are currently being analyzed with ELISA for gut contents (to assess presence of pear psylla remains). A subsample of the *Deraeocoris* specimens has been processed, and confirms that this species feeds extensively on pear psylla. The remaining samples for this species and the other predator species will be processed over the next few months.

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.

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 and tree vigor (added for 2008). Pear leaves will be collected from control and cover crop plots for N-analysis using sampling methods recommended by Michigan State University Extension (MSU 1994). Leaf nitrogen will be determined using the Bradford assay (Bradford 1976) for soluble nitrogen, modified for analysis of plant tissues (Jones et al. 1989). Three replicate leaf nitrogen assessments will be taken on each of three dates through the season. Tree vigor will be assessed from measurements of shoot growth. Thirty fruit and thirty vegetative shoots in each plot will be flagged in spring. Shoot length and leaf numbers per shoot will be determined for each flagged shoot in late August.

Expand project into 4 commercial organic orchards (contingent upon funding from SARE or CSANR). We have an arrangement with 4 commercial organic growers in the Yakima valley to plant 0.5 meter wide strips of alfalfa directly through existing grass understory. Replicated control (grass)

and alfalfa plots will be established within each orchard. We will monitor pest and predator densities, predator movement (using sticky cards), leaf nitrogen, and tree vigor in control and alfalfa plots. If funding is obtained from both SARE and CSANR, we will add the marking trials and gut contents analyses to the commercial orchard sites (to complement the same work at the Moxee farm).

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 6-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 (Fig. 2). The top panel of Figure 2 shows relative contribution of each predator group to the overall predator community (for each date, size of a circle is proportional to relative abundance of that predator taxon). Data are shown for alfalfa (black circles) or grass understory (white circles). The true bugs dominated the early-season net samples, while later-season samples were dominated by spiders. Ladybird beetles were relatively common in the alfalfa plots in early July, but were very uncommon in grass plots all season. Green lacewings were at relatively low densities in both types of plots all season.

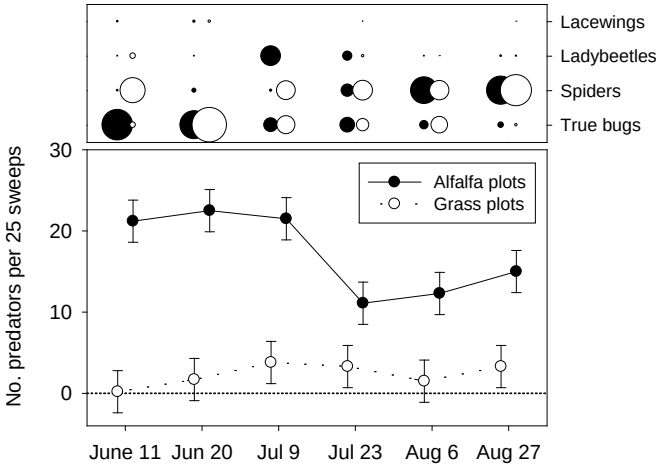


Figure 2. Sweep net

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 higher in the alfalfa plots than the grass plots early in the season, but not later in the season (Fig. 3). The sharp decline in trap catch later in the season is surprising, given that sweep net samples (Fig. 2) showed that the alfalfa plots especially supported substantial numbers of predators even late in the season. Trap catch was dominated by the true bugs early in the season and by lacewings (canopy traps) or ladybird beetles (ground traps) later in the season; ground traps in the alfalfa plots captured relatively large numbers of ladybird beetles.

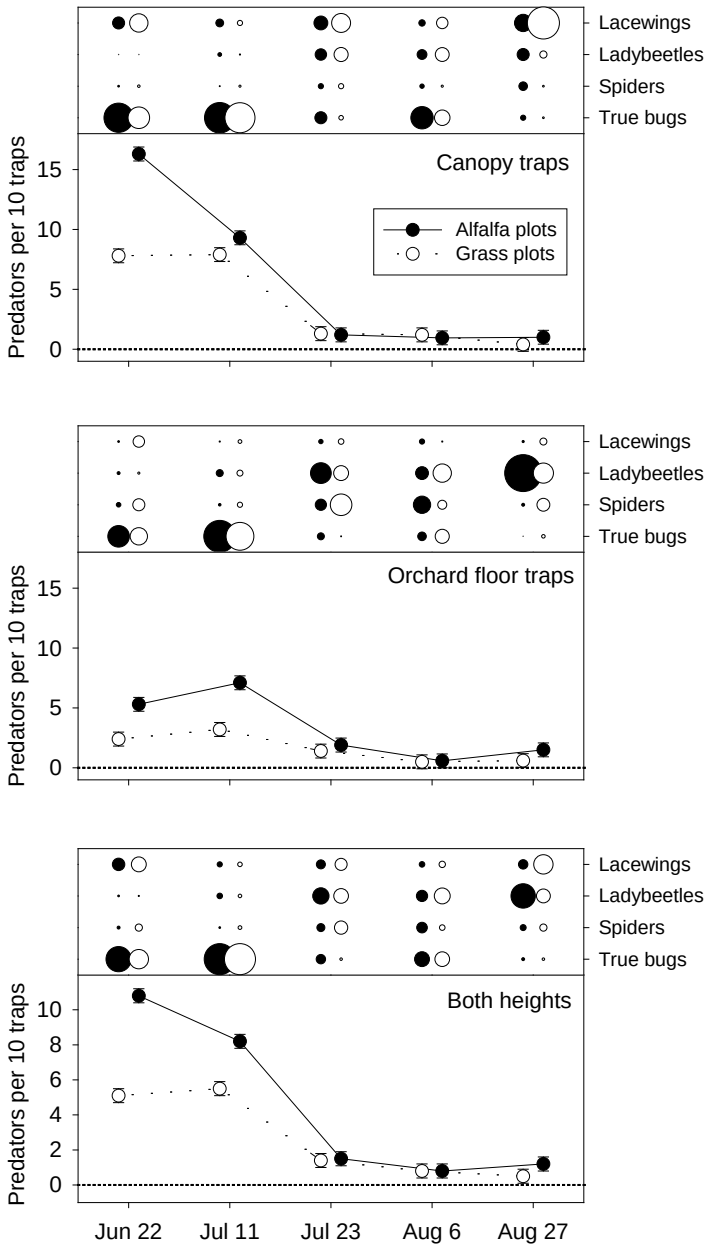
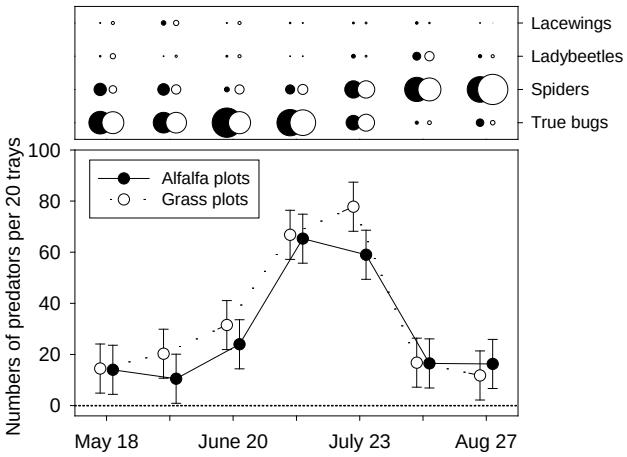


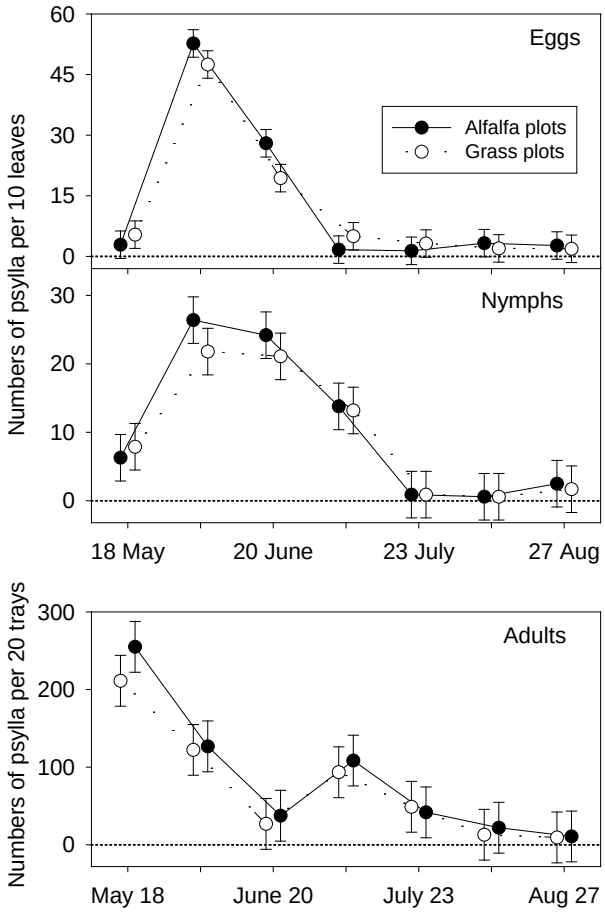
Figure 3. Sticky `trap`

Figure 4. Predator beat tray



Tray counts of predators were, if anything, larger in the grass plots than the alfalfa plots (Fig. 4). 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. This result seems to contradict the data for the canopy-height sticky traps (Fig. 3 upper panel) as well as the marker data (see below), which suggested that the alfalfa did indeed act as a source of predators colonizing the tree canopy; possibly predators moved from alfalfa into the tree canopy, but failed to establish. Taxonomic composition of predators on beat trays were similar in alfalfa and grass plots, as shown by similarities in size of paired black and white circles (Fig. 4 upper panel).

Figure 5. Pear psylla

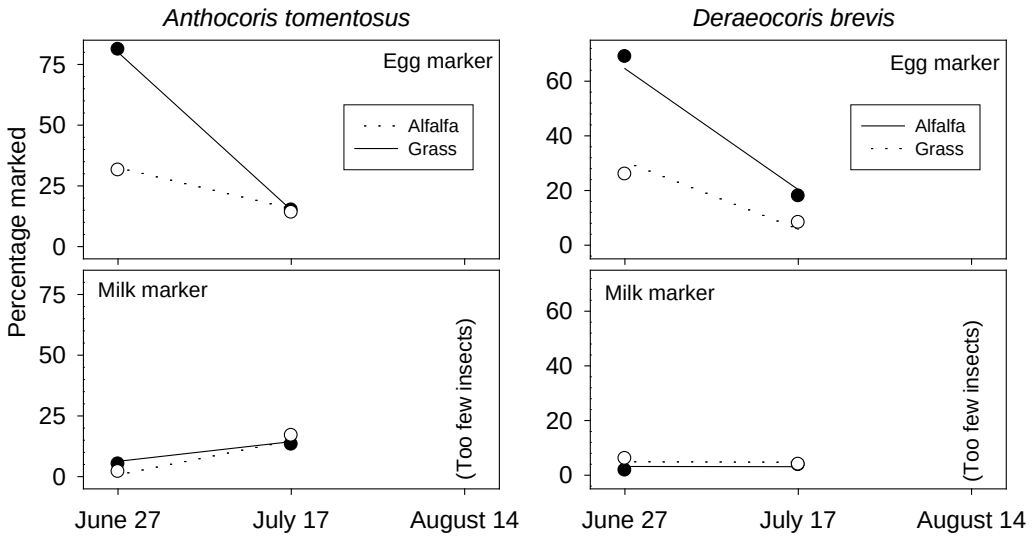


Densities of immature psylla were statistically similar in grass and alfalfa plots, although there was a suggestion that densities early in the season were actually slightly higher in the alfalfa plots (Fig. 5). Early-season tray counts of adults also showed a trend towards being larger in the alfalfa plots than the grass plots (Fig. 5 bottom panel). Because early-season densities of predators were relatively low in all plots, it is difficult to ascribe these treatment differences to biological control. It may be that nitrogen produced in association with the alfalfa cover crop was taken up by the pear trees, and that this led to higher densities of adult psylla in alfalfa plots. Effect of the alfalfa cover crop on tree nitrogen is to be assessed in 2008 (see above).

Marker results. Egg-marked specimens of *Anthocoris* spp. (mostly *A. tomentosus*) and *Deraeocoris brevis* were substantially more common than milk-marked specimens in our tree samples, suggesting we had poor marking

with the milk solution (Fig. 6). Very few specimens of either species were collected during our August trial, so results are presented only for the June and July trials (Fig. 6). For both species, there was a statistically significant drop in percentage marked in the July samples (Fig. 6), suggesting that movement from orchard floor to tree canopy was higher in June than July (similar to what was shown by the sticky trap data; Fig. 3); in Figure 6, the lines show predicted results from loglinear models, while symbols show observed results. For both species, percentage marked in the tree-collected specimens was higher in the alfalfa plots than the grass plots (Fig. 6), again suggesting again that the alfalfa was a source of predators moving into the tree canopy. Results for the remaining specimens are still being analyzed.

Figure 6. Results of marker trials for tree-collected *Anthocoris* and *Deraeocoris*



Gut contents results. The majority of specimens are still being processed. We have finished a subsample of the *Deraeocoris* specimens. Virtually all specimens of this predator were found to contain psylla remains (Fig. 7). The remaining specimens will be processed over the next several months.

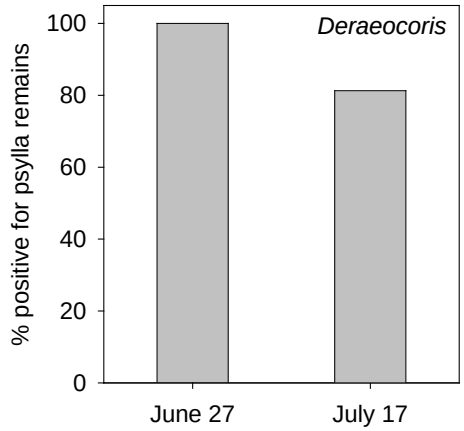


Figure 7. Percentage of *Deraeocoris* collected from the tree canopy showing evidence of having fed on pear psylla.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number:

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 and 3rd years: David Sugar, Steve Castagnoli

Total project funding request: Year 1: $31369 **Year 2:** $32310 (this year) **Year 3:** $33279**(?)***

Other Funding Sources: *potentially
Agency Name: UDSA Western Region Integrated Pest Management Competitive Grants Program
Amount requested or awarded: requested $60K (total) for implementation research in '09 and '10.

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

Item	2007	2008	
Salaries	16995	17505	
Benefits	11387	11728	
Wages			
Benefits			
Equipment			
Supplies	1928	1346	
Travel	1250	2700	
Miscellaneous	750		
Total	$32,310	**$33,279**	

Footnotes: *See information under 'Other Funding Sources'

Objectives:

In 2007:

1. Design a LAMP reaction to detect small quantities of the fire blight pathogen based on primers designed for amplification from the pEA29 plasmid of *Erwinia amylovora*.
2. Evaluate fluorescent DNA probes to be used with LAMP primers from Objective 1 to simplify scoring of positive reactions.
3. Determine specificity and sensitivity of the designed LAMP reaction against a diverse selection of microorganisms commonly found in pear and apple orchards.
4. Use the LAMP reaction to detect *E. amylovora* in flower samples from inoculated orchard trees.
5. Optimize sampling protocols for implementation by growers or farm service providers.

In 2008:

3. Determine specificity and sensitivity of the designed LAMP reaction against a diverse selection of microorganisms commonly found in pear and apple orchards..
4. Use LAMP to detect *E. amylovora* in flower samples from inoculated orchard trees, and from surveyed commercial orchards in the Pacific Northwest .
5. Optimize sampling protocols for implementation by growers or farm service providers (*2008 and 2009*).

Significant findings:

- **We have developed two LAMP primer sets with high specificity to *E. amylovora* DNA sequences.** To date, we have designed, screened and intensively evaluated over 30 LAMP primer sets resulting in two primer set candidates ready for field evaluation. One set is targeted to the plasmid pEA29 and the other is targeted to a chromosomal gene of *E. amylovora*.
- **A positive LAMP reaction resulting in a white magnesium pyrophosphate precipitate in the PCR tube corresponded to a dilution plate enumeration of ~25 cells 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. In addition, whole pear flowers, pear flower petals or pear flowers minus petals were negative for the LAMP reaction.
- **Positive LAMP reactions were attained using gradients of pathogen cells mixed with differing levels of floral tissue.** Pathogen populations were 5×10^2 to 5×10^3 CFU per ml and populations of indigenous bacteria in floral suspensions averaged 10^2 to 10^5 CFU per ml. For Bartlett pear, when *E. amylovora* was spiked into flower suspensions at 500 and 5000 CFU per ml, 63 and 96% of LAMP reactions were positive, respectively. Similarly for Gala apple, treatments of 500 and 5000 pathogen cells resulted in 61% and 89% positive LAMP reactions, respectively. LAMP reactions for treatments containing no pathogen cells had 0% precipitate formation for apple and 8% precipitate formation for pear experiments.
- **The LAMP reaction detected cells of the pathogen that corresponded to dilution plating on orchard trees inoculated with *E. amylovora*.** On pear flowers, pathogen populations averaged 1.6×10^5 and 6.3×10^4 CFU per ml on 6 and 9 days after pathogen inoculation, respectively. At 6 and 9 days after inoculation, positive LAMP reactions were 97 and 100%, respectively, and false negative reactions (i.e., no LAMP precipitate when *E. amylovora* was recovered on culture media) were 13% and 0%, respectively. Pathogen populations on apple flowers averaged 1.0×10^2, 3.5×10^2, and 9.6×10^4 CFU per ml on -5, 0 and 7 days after pathogen inoculation, respectively. Positive LAMP reactions were 97, 84 and 97%, false negatives were 3, 6 and 0% for days -5, 0, and 7, respectively. Overall, when *E. amylovora* was present on flowers at moderate to high populations, the pathogen was consistently detected with the LAMP reaction.

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 greatly 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 designed for amplification from the pEA29 plasmid of *E. amylovora*. 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.

Objective 2. Evaluate fluorescent DNA probes to be used with LAMP primers from Objective 1 to simplify scoring of positive reactions. Plasmid or chromosomal DNA containing primers designed in Objective 1 will amplify complex structures with cauliflower-like stem loops during LAMP. Addition of FITC and ROX fluorescently labeled probes will enhance identification of the specific sequence amplified in the reaction. Expected results are precipitate which emits green fluorescence or red fluorescence when exposed to an ordinary hand held UV light resulting in sequence specific visual detection. When lamp products not related to the primers from Objective 1 are present, there will be no fluorescence due to lack of probe hybridization. Adding DNA intercalating dyes such as Sybr® green, Pico® green, or Eva® green, will enhance visual detection of double stranded DNA amplification as green fluorescence when exposed to UV light (Fig. 2, below).

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.

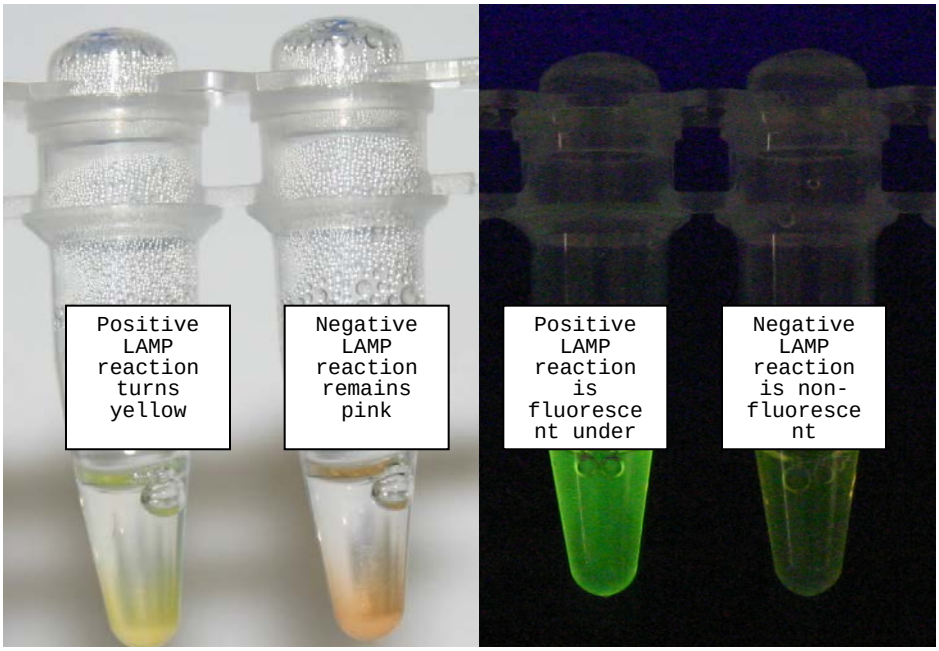


Figure 2. Positive and negative reactions for detection of *Erwinia amylovora* as indicated by addition of Pico® green at the end of a LAMP reaction.

Objective 3. 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 flower numbers at 0, 10, 100 or 1000 in 5 L of water in 19 L buckets. A 1 ml sample of the cell/flower suspension was used for serial dilution plating (three 100-fold dilutions) onto CCT (Ishamaru and Klos, 1984) to enumerate Ea153N and on Pseudomonas agar F (PAF, Difco laboratories) to enumerate total bacterial populations. One liter of the flower suspension was filtered through first 4 layers of cheesecloth and then passed through a 35 µm screen. Filtrates were concentrated by centrifugation and re-suspended

in 0.5 ml of water. Samples (5 µl) of the cell/flower suspension, filtrate, and concentrated suspensions were used for LAMP reactions (described above). DNA extraction of 0.5 ml samples (Instagene matrix, BioRad Inc.) prior to LAMP amplification was also performed. Representative populations of indigenous bacteria present on dilution plates are collected and stored for specificity reactions using LAMP reaction primers.

Objective 4. Use the LAMP reaction to detect *E. amylovora* in flower samples from inoculated orchard trees, and from surveyed commercial orchards in the Pacific Northwest. Eight flowers from each of 4 replicate trees of Bartlett pear or Golden Delicious apple were sampled for the detection of *E. amylovora* by dilution plating or LAMP reaction. Samples were taken on 4 and 9 days (pear only) and -5, 0 and 7 (apple only) days after pathogen inoculation (inoculum ~1 x 10^6 CFU per ml). Dilution plating was performed as described above with a detection limit of 100 CFU per ml. The LAMP reaction was performed on individual 1 ml blossom washes after a DNA extraction step; 100µl of the 1 ml blossom wash was boiled for 10 min in a solution of Triton-X 100 and EDTA (Shee et al., 1998) and compared to bacterial recovery on dilution plates.

Objective 5. Optimize sampling protocols for implementation by growers or farm service providers. We anticipate that the commercial orchard blocks selected for sampling will range in size from 2.5 to 7.5 ha; these blocks will be planted to important cultivars and will be susceptible to fire blight. Three sampling schemes will be evaluated in each orchard: 1) 'Thorough but efficient': A classic IPM sampling scheme where the scout makes five transects through the orchard walking on a 'W- pattern'. A bulk flower sample (200 to 1000 blossom cluster per sample) will be made on each sampling transect; 2) 'Targeted': Two bulk samples will be taken from areas of the orchard considered the highest risk for fire blight as determined by grower interview and orchard layout (e.g., where disease was most intense last year, and/or edge(s) of the orchard closest to an earlier-blooming susceptible cultivar); 3) 'Intensive': This scheme will include the first seven samples described just above plus an additional three bulked samples taken from areas not well sampled by either the 'thorough but efficient' or 'targeted' schemes. Because bees are an important vector for *E. amylovora*, these additional samples will be made either in the general vicinity of bee hives positioned in the orchard or along orchard perimeters where bees carrying the pathogen would first enter the block.

Results and Discussion:

Objective1. 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. An intercalating dye, Pico® green, which is a pink dye and integrates into double stranded DNA and turns yellow, was used to enhance the scoring of positive LAMP reactions. A positive LAMP reaction turns yellow while a negative reaction remains pink (Fig. 2). Binding of the intercalating dye to double stranded DNA causes the dye to become fluorescent and can be visualized using a hand-held ultraviolet light (Fig. 2). Although this technology allows easy visualization of a positive LAMP reaction, it is not specific to DNA of *E. amylovora*. To date, design and implementation of three specific fluorescent DNA probes to *E. amylovora* have failed to enhance visualization of the LAMP product.

Objective 3. Pathogen populations recovered from the spiked cell/flower suspensions ranged from 5 x 10^2 to 5 x 10^3 CFU per ml. Populations of indigenous bacteria in floral suspensions averaged 4.7 x 10^2, 4.3 x 10^3, and 1.3 x 10^5 CFU per ml for suspensions with 10, 100 or 1000 flowers per 5 L, respectively. Indigenous bacteria were not recovered from containers with no flowers. LAMP reactions for treatments containing no pathogen cells had 0% precipitate formation for apple and 8% precipitate formation for pear experiments. The false positive reactions for pear occurred at the high, 1000 flower concentration, and thus, potentially, the positive reactions were due to low level populations (below detection limit for plating) of *E. amylovora*.

For Bartlett pear, when *E. amylovora* was spiked into flower suspensions at 500 CFU per ml, an average of 63% of LAMP reactions were positive (Table 1). When 5000 pathogen cells were present, 96% resulted in positive LAMP reactions (Table 1). The number of pear flowers in the suspension had no effect on the incidence of positive LAMP reactions. Similarly for Gala apple, treatments of 500 and 5000 pathogen cells resulted in 61% and 89% positive LAMP reactions, respectively (Table 1).

Importantly, for flower suspensions with the pathogen spiked at 500 CFU per ml, both concentrating the cell/flower wash and DNA extraction from a 1 ml volume increased the incidence of positive LAMP reactions to > 90%. For the highest pathogen concentration (5000 CFU per ml), however, the method of sample preparation (boiled cell/flower wash, filtrate, concentrate, or DNA extraction) had no effect on incidence of a positive LAMP reaction for the.

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[b]		
Cultivar	**0**	**500**	**5000**
Bartlett pear	**8%[c]**	**63%**	**96%**
Gala apple	**0%**	**61%**	**89%**

[a] Floral tissue represents 0, 10, 100 or 1000 flowers per 5 L volume in a 19L bucket.

[b] Concentration of *E. amylovora* represents cell populations of no cells (0), 5 x 10^2 (500) to 5 x 10^3 (5000) CFU per ml.

[c] Incidence of positive LAMP reaction is the average of 2 (pear) or 3 (apple) bucket experiments where several methods of sampled preparation were employed: boiled flower wash, filtered then boiled, concentrate by centrifugation then boiled, and concentrate then DNA extraction.

Objective 4. On pear flowers, pathogen populations averaged 1.6 x 10^5 and 6.3 x 10^4 CFU per ml on 6 and 9 days after pathogen inoculation, respectively. Positive LAMP reactions were 97 and 100% for days 6 and 9, respectively. False negative reactions (i.e., no LAMP precipitate when *E. amylovora* was recovered on culture media) were 13% and 0%, respectively. False positive reactions (i.e., positive LAMP reaction when no pathogen was recovered) were 25% and 41% for day 6 and 9, respectively (Fig 3). The high incidence of false positive reactions may be due to low level (below detection limit) populations of *E. amylovora* on flowers. Pathogen populations on apple flowers averaged 1.0 x 10^2, 3.5 x 10^2, and 9.6 x 10^4 CFU per ml on -5, 0 and 7 days after pathogen inoculation, respectively (Fig. 3). Positive LAMP reactions were 97, 84 and 97%, false negatives were 3, 6 and 0%, and false positives were 94, 6 and 6% for days -5, 0, and 7, respectively (Fig. 3). *E. amylovora* was recovered from only 6% of flowers sampled 5 days prior to pathogen inoculation on apple but 97% of the samples had positive LAMP reactions on this day. The fact that the pathogen was detected suggests that a low level infestation of *E. amylovora* was present in the orchard, possibly introduced by foraging bees from the neighboring Bartlett pear block that had been inoculated prior to first flower sample in the Golden Delicious apple block.

Overall, when *E. amylovora* was present on flowers at moderate to high populations, the pathogen was consistently detected with the LAMP reaction. The LAMP reaction with flowers with low pathogen populations provided inconsistent results, perhaps because no concentration step was utilized to increase cell numbers for template preparation.

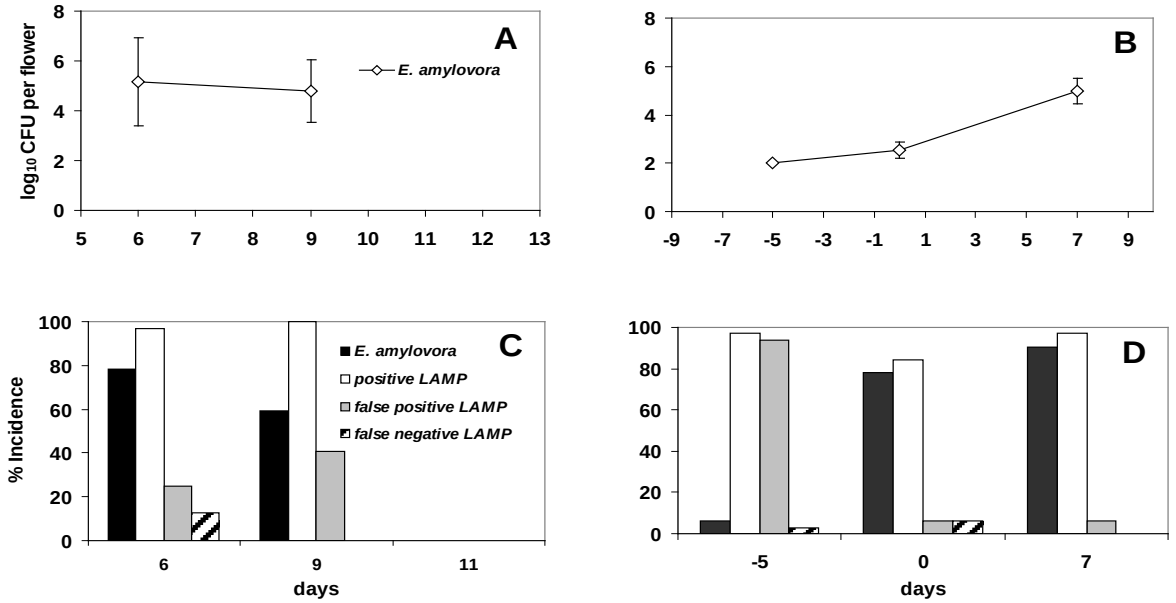


Figure 3. A and B: Population size (log_{10}) of *E. amylovora* on flowers sampled from orchards of Bartlett pear and Golden Delicious apple as a function of days from (or prior to) inoculation of the orchard with the fire blight pathogen. C and D: Incidence of *E. amylovora* on flowers as determined by dilution plating (black bar) and by LAMP reactions (white, gray, and slash bars) on individual floral washes. White bars depict percent positive LAMP reactions corresponding to a positive dilution plate reaction; gray bars depict a positive LAMP reaction corresponding to negative dilution plate; and slashed bars a negative LAMP reaction corresponding to a positive dilution plate. The high proportion of false positives on some sampling dates may be caused by the LAMP reaction having a lower detection limit (25 CFU) than the dilution plate assay (100 CFU).

Plans for 2008: Our focus will be on Objectives 3, 4 and 5. The objectives are concerned with evaluating LAMP technology in field samples. We will split our effort between experimental-type activities in artificially inoculationed samples and orchards, and survey-type activity in production orchards. The latter survey-type activities will become the primary research focus in the final year of the project (2009).

Literature review:

Ishimaru, C. A., and Klos, E. J. 1984. New medium for detecting *Erwinia amylovora* and its use in epidemiological studies. Phytopathology 74:1342-1345.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., and Hase, T. 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Research 28:*e*63.

Shee, E. L., Soo, Y. K., Sei, J. K., Hyun, S. K., Jong, H. S., Sang, H. C., Sun, S. C., and Joon, H. R. 1998. Direct Identification of *Vibrio vulnificus* inClinical Specimens by Nested PCR. J. Clinical Microbiology 36:2887-2892.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-07-703

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

Cooperators: R.A. Spotts, C. L. Xiao, E.E. Sanchez

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

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 enhancing fruit size, reducing or enhancing fruit russet, and managing fruit ripening capacity and postharvest decay.

Significant Findings:

Postharvest decay control programs.

1. An experiment tested the ability of pre-harvest treatments to reduce decay in Bosc pears in the event that postharvest line-spray fungicide treatments are delayed due to prolonged storage in field bins prior to packing. While decay increased with longer delay until postharvest Mertect was applied, summer calcium sprays reduced the rate of decay increase; Pristine applied 1 week pre-harvest reduced the rate further; calcium and Pristine combined reduced the rate of decay increase to relatively low levels.

2. Results of trials of pre-harvest fungicide sprays for postharvest decay control from the research programs of Sugar, Spotts, and Xiao in the past 4 years were analyzed together. Over a large number of trials, the average effectiveness of Topsin M, Flint, and Pristine was comparable, in the range of 60-70 % decay control as compared to using no pre-harvest treatment.

3. In 2007 trials of pre-harvest Topsin M, Flint, and Pristine, all treatments were effective in reducing postharvest decay, but decay control was not significantly improved by combining sprays with NutraPhos 24, preceding the sprays with ziram (1 month before harvest), or both preceding pre-harvest sprays with ziram and combining them with NutraPhos 24.

Pear fruit quality enhancement.

1. **Developing ripening capacity in Comice and Bosc 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 and Bosc. 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. Furthermore, ripening capacity was induced in a shorter period of time at 41 °F than at 31 °F, and in an even shorter time at 50 °F.

2. **Fruit size**. Over 4 years of trial, urea sprays at 80% bloom increased fruit size in Bartlett pears. 7.5% urea was more effective than 5% urea. The 5% urea spray did not increase fruit size in Bosc, Red Anjou, or Comice. MaxCel treatments increased fruit size in Bartlett; urea did not enhance the MaxCel program.

3. **Russet management**. Russet severity in Comice best correlated with weather parameters in the period from 15-21 days after full bloom. Evapotranspiration (ET) was the most predictive measure. In Bosc, ET during the period 8-14 days before full bloom correlated most with the frequency of high-russet fruit.

Methods:

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

Results and Discussion:

Postharvest decay.

1. Previous research in this project has shown that the longer the delay after harvest before application

of postharvest decay control materials, the less effective they are in controlling decay at wounds occurring during harvest and transport. By 6-9 weeks after harvest, treatments may have little benefit. However, several marketing and logistical factors have resulted in large volumes of winter pears being stored for long periods of time in field bins without protective postharvest treatments. This situation emphasizes the importance of pre-harvest sprays for protection against postharvest diseases. In 2007, an experiment was designed to test the interaction of a pre-harvest spray program consisting of summer calcium sprays and Pristine fungicide applied one week before harvest with a series of postharvest Mertect application timings. The result of each delay in application of Mertect was an increase in the incidence of decay. However, the rate at which decay incidence increased over the delay was slower when the fruit had received calcium sprays in the orchard, slower yet when the fruit had received Pristine one week before harvest, and slowest when the fruit had received both calcium and Pristine treatments (Fig. 1). This demonstrates that the risk associated with a delay in application of the postharvest fungicide treatment can be partially mitigated by the appropriate use of pre-harvest treatments.

2. Results of pre-harvest fungicide trials for control of postharvest pear decay were accumulated from researchers at Oregon State University (Sugar and Spotts) and Washington State University (Xiao) for the years 2003-2006. Although trial methods varied, results could be grouped in a way that shows the overall experience with various pre-harvest fungicide programs (Fig. 2). Over a large number of trials, results varied widely, but the average efficacy of Topsin M, Flint, and Pristine was comparable, in the range of 60-70 % decay control. Ziram was less effective, but the value of ziram may depend on the types of decay associated with specific orchards. Adding NutraPhos 24 to Topsin M was not clearly superior, but the number of trials was limited. NutraPhos 24 alone did not control decay substantially.

3. In 2007, pre-harvest programs were combined with either no postharvest treatment, postharvest BioSave 110, or postharvest Scholar (Table 1). Both BioSave 110 and Scholar were highly effective postharvest treatments (Table 2). The effectiveness of the fungicides applied one week pre-harvest, as a group, was not consistently improved by being preceded by a ziram spray, by being combined with and preceded by NutraPhos 24, or by combined with a ziram + NutraPhos 24 program (Table 3).

Fruit Quality.

1. **Ripenability**. Important discoveries were made regarding the relationship of maturity at harvest to the length of “chill” time needed to induce ripening capacity in Comice and Bosc pears. In Comice, at the beginning of the maturity range (13 lbf), approximately 30 days are required at 31 °F. However, we found that for each day later that the fruit are harvested, the chilling requirement reduces by approximately 0.6 day (Table 4). In Bosc harvested at 16 lbf, approximately 15 days are need at 31 °F, but the requirement reduces by about 0.3 day for each day later that the fruit are harvested. Furthermore, across all harvest dates, in both varieties the requirement is substantially reduced if fruit are chilled at 41 °F, and reduced further if chilled at 50 °F. Adding 48 hours in ethylene into the program, Comice pears harvested at the beginning of the maturity range and given 2 days in ethylene + 3 days chilling at 41 °F + 5 days at room temperature ripened to very good quality.

2. **Fruit size**. Using combined data from 4 years of trials, urea applied at 80% bloom significantly improved fruit size of Bartlett pears when applied at concentrations of 5 or 7.5% (Table 5). The higher rate was generally more effective. Urea treatments tended to reduce fruit set and overall yield, while increasing the yield of fruit of size 90 or larger relative to the yield of unthinned control trees. Urea 5% sprays did not increase fruit size in Bosc, Comice, or Red Anjou pears in 2007. In 2006 and 2007 Bartlett trials, a treatment of 5% urea at petal-fall was also included, and appeared to enhance

fruit size similarly to the same rate applied at 80% bloom (Table 6). This suggests that the effect of urea may be more a nutritional boost to the developing fruitlets than an effect of thinning. Treatments with the plant growth regulator MaxCel at 125 ppm increased the average fruit weight of Bartlett pears but did not consistently increase the total yield of large fruit (Table 7). Urea sprays followed by MaxCel did not consistently increase fruit size over MaxCel alone.

3. **Russet management**. Data on russet severity in Comice and extent of russet coverage in Bosc from previous and current years of research were tested for correlation with weather parameters during various one-week periods before and after bloom (Table 8). Russet severity in Comice best correlated with weather parameters in the period from 15-21 days after full bloom. During this period, maximum temperature, rainfall, and evapotranspiration (ET) correlated well with russet severity. The most predictive was ET, which is calculated from moisture content of the air, temperature, wind, and solar radiation, all factors that may influence cuticle development, and in turn sensitivity to russet. This reinforces the importance of this post-petal-fall period for protection against russet. In contrast, in Bosc pears the period 8-14 days before full bloom had the only significant correlation between the frequency of high-russet fruit and ET. This suggests the possibility that pre-bloom conditions could be predictive of the need for treatments to supplement natural russet in Bosc. The effectiveness of copper applied at petal-fall was correlated with rainfall 22-28 days after full bloom, indicating that copper treatments are more effective when more rainfall occurs during this period. Dithane and Pristine in spring, but not Procure, decreased russet on Comice pears, but did not enhance the fruit cuticle mass (Table 9).

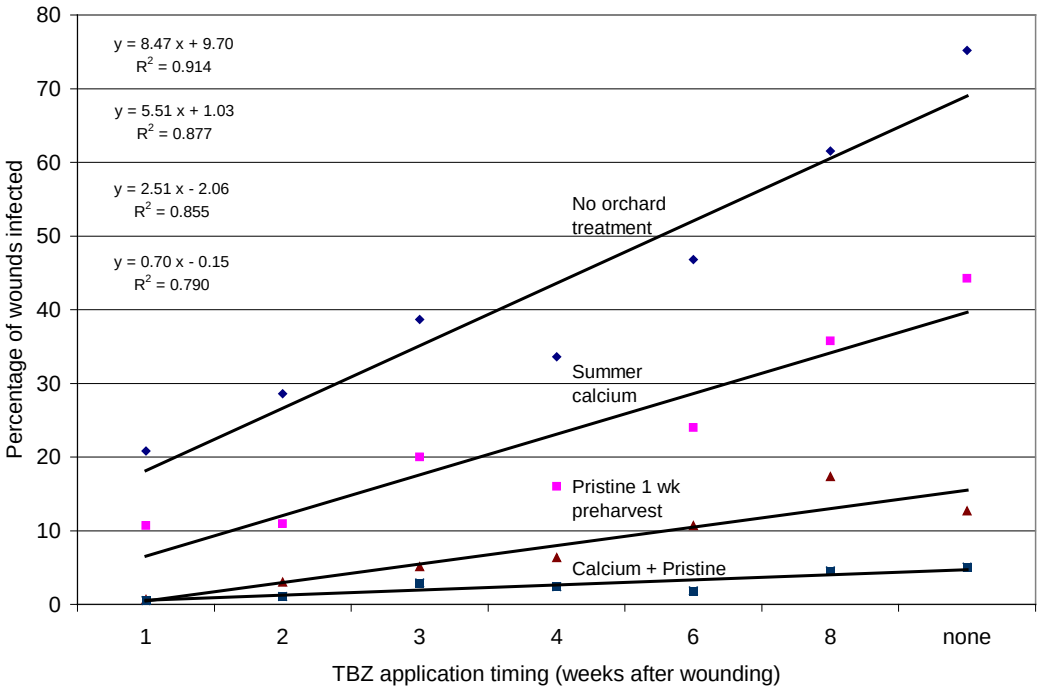


Fig. 1. Effect of summer treatments with calcium chloride and pre-harvest treatments with Pristine fungicide, and timing of postharvest TBZ (Mertect) application on the incidence of postharvest decay at wounds in Bosc pears.

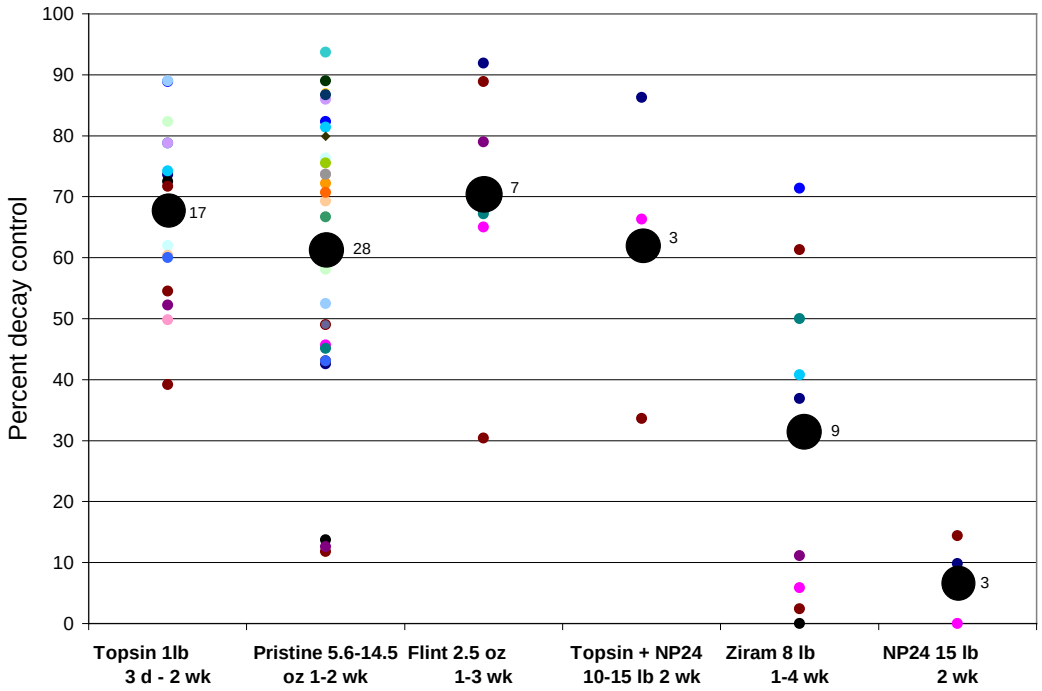


Fig. 2. Summary of effectiveness of pre-harvest orchard fungicide treatments on postharvest decay of winter pears. Combined results of 2003-2006 from Sugar, Spotts, and Xiao, including both Anjou and Bosc pears and a range of treatment parameters. Each small dot in columns represents the average decay control in a single trial. The large dot represents the grand average of all trials in the column, and the number adjacent to the large dot indicates the number of trials included in the average.

Table 1. Effects of orchard and postharvest treatments on the incidence of postharvest decay at wounds in Bosc pears.

	Postharvest line-spray treatment		
	% of wounds infected (total decay)		
Orchard treatment and timing	Water	BioSave110	Scholar
Untreated	45.8 a	6.7 a	0.0 a
NutraPhos 24 1 month + 1 week pre-harvest (NP24)	39.2 ab	3.4 abc	0.4 a
Topsin 1 week pre-harvest + NP24	30.4 bc	2.5 bc	0.4 a
Ziram 1 month pre-harvest	27.1 cd	2.1 bc	0.0 a
Ziram 1 month, then Topsin 1 week pre-harvest	22.8 cde	1.7 bc	0.4 a
Ziram 1 month, then Topsin + NP24 1 week	19.8 cdef	3.3 abc	0.8 a
Topsin 1 week pre-harvest	18.3 defg	4.6 ab	0.0 a
Flint 1 week pre-harvest	15.0 efg	1.3 bc	0.4 a
Ziram 1 month, then Pristine1 week pre-harvest	11.7 efg	0.4 c	0.0 a
Pristine 1 week pre-harvest + NP24	11.3 efg	2.5 bc	0.4 a
Flint 1 week pre-harvest + NP24	10.9 efg	1.3 bc	0.8 a
Ziram 1 month, then Flint 1 week pre-harvest	10.4 fg	0.4 c	0.0 a
Pristine 1 week pre-harvest	9.2 fg	2.5 bc	0.0 a
Ziram 1 month, then Flint+NP24 1 week pre-harvest	7.1 g	0.8 c	0.8 a
Ziram 1 month, then Pristine+NP24 1 wk pre-harvest	6.8 g	0.4 c	0.0 a

Table 2. Effect of postharvest treatments on decay of Bosc pears, across all orchard treatments.

Postharvest treatment (across all orchard treatments)	% of wounds infected (total decay)
Water	19.1 a
BioSave 110	2.3 b
Scholar	0.3 b

Table 3. Comparison of decay control provided by fungicides (Topsin M, Pristine, and Flint) applied one week pre-harvest with the same treatments as part of programs including ziram (1 month pre-harvest), NutraPhos 24 (1 month and 1 week pre-harvest), or both ziram and NutraPhos 24.

	% of wounds infected	
Orchard treatment	Across all postharvest treatments	Postharvest water only (check)
Untreated	17.5 a	45.8 a
Fungicide 1 wk pre-harvest	5.7 b	14.2 b
Fungicide + NutraPhos 24 10 lb/acre	6.7 b	17.5 b
Fungicide + Ziram 8 lb/acre	5.3 b	15.0 b
Fungicide + Ziram and NutraPhos24	4.4 b	11.2 b

Table 4. Relationship between harvest maturity and conditioning ("chill") temperature for inducing ripening capacity in Comice pears.

	Days needed to induce ripening capacity		
	Conditioning temperature		
Days after entering maturity range	31°F (-0.5°C)	41°F (5°C)	50°F (10°)
0	30	19	12
7	26	16	10
14	22	13	8
21	17	11	6
28	13	9	4

Table 5. Summary of 4 years of trial evaluating the effect of urea sprays at 80% bloom on production characteristics of Bartlett pears.

Treatment	Yield (tons/acre)	Average fruit weight (g)	% of fruit ≥ size 90	Tons/acre ≥ size 90	Fruit set /100 clusters
Check	19.7 a	187.4 c	26.9 c	5.0 b	81.1 a
Urea 5%	17.4 ab	208.4 b	43.9 b	7.5 a	69.3 ab
Urea 7.5%	15.8 b	223.6 a	56.2 a	8.7 a	58.4 b

Table 6. Summary of 2 years of trial comparing urea treatments at 80% bloom with a treatment at petal-fall in Bartlett pears.

Treatment	Yield (tons/acre)	Average fruit weight (g)	% of fruit ≥ size 90	Tons/acre ≥ size 90	Fruit set /100 clusters
Check	17.3 ab	185.2 c	25.5 c	4.0 b	83.8 ab
Urea 5% at 80% bloom	19.0 ab	202.5 b	39.2 b	7.4 a	83.7 ab
Urea 7.5% at 80% bloom	16.5 b	220.0 a	52.5 a	8.3 a	67.1 b
Urea 5% at petal-fall	20.4 a	204.2 b	41.5 b	8.2 a	92.3 a

Table 7. Effect of the plant growth regulator MaxCel, with and without prior urea treatments, on production characteristics of Bartlett pears.

Treatment	Yield (t/acre)	Average fruit weight (g)	% fruit size ≥ 90	Tons/acre ≥ size 90	Fruit set /100 clusters
Check	15.4 a	206.2 c	42.1 c	6.3 b	68.4 a
MaxCel 125 ppm	14.8 a	242.0 ab	67.5 ab	9.9 ab	44.5 ab
MaxCel 150 ppm	11.6 a	221.6 bc	54.2 bc	6.5 b	49.1 ab
Urea 5%, then MaxCel 125 ppm	11.8 a	248.4 ab	69.7 ab	7.9 ab	30.0 b
Urea 5%, then MaxCel 150 ppm	12.5 a	248.1 ab	71.3 ab	9.2 ab	30.6 b
Urea 7.5%, then MaxCel 125 ppm	14.7 a	252.5 a	75.5 a	11.1 a	42.2 b
Urea 7.5%, then MaxCel 150 ppm	10.9 a	268.2 a	79.1 a	8.4 ab	31.3 b

Table 8. Correlations between russet coverage of Comice and Bosc pears and environmental measures during periods before and after bloom.

Variety	Years of russet data	Significant factors	Period of greatest effect	Correlation measure	Statistical significance
Comice	9	Temperature Rainfall EvapoTransp.	15-21 d after full bloom	-0.744 0.697 -0.842	0.022 0.037 0.004
Bosc	6	EvapoTransp.	8-14 d before full bloom	-0.933	0.007
Bosc + copper at petal-fall	6	Rainfall	22-28 days after full bloom	0.875	0.022

Table 9. Effect of fungicide treatments (2 sprays after petal-fall) on incidence of russet in Comice pears and on the final mass of fruit cuticles.

Treatment	Rate	% of fruit with ≥ 6 % russet	Weight of 10 cuticles (mg)
Untreated	-	54.1 ab	10.2 a
Procure	12 fl oz/acre	59.2 a	10.3 a
Procure + Dithane	12 fl oz + 3 lb/acre	33.2 bc	9.4 a
Dithane	3 lb/acre	31.2 c	9.9 a
Pristine	14.5 oz/acre	26.5 c	10.0 a
Captan	1 qt/100 gallons	38.6 bc	9.7 a

CONTINUING PROJECT REPORT **YEAR**: 2 OF 3
WTFRC Project Number: PR-07-705 (WSU Project #13L-4164-1210)

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

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Co-PI: Peter Sanderson
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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; Ron Gonsalves, Blue Bird Growers.

Total project funding request: **Year 1:** 51,511* **Year 2: 38,060** **Year 3:** 41,864
Notes: *Year 1 received grant for $38,633.

Other funding Sources

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

The financial information provided in addition to sponsor support simply communicates research program support costs vs. specific project cost-share commitment.

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:

Organization: WSU TFREC **Contract Administrator:** ML Bricker; Kevin Larson
Telephone: 509-335-7667; 663-8181 x221 **Email:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	Year 1: 2007	**Year 2: 2008****	Year 3: 2009
Salaries[1]	13,125	**10,920**	11,466
Benefits (47.3%)	6,208	**5,165**	5,423
Wages[2]	9,000	**7,600**	7,600
Benefits (11.5%)	1,035	**875**	875
Supplies[3]	21,643	**12,000**	15,000
Misc[4]	0	**1,000**	1,000
Travel	500	**500**	500
Total	51,511*	**38,060**	41,864

*Year 1 award was $38,633.

**Year 2 budget includes $2,000 for Objective 2 (double-drenching).
[1]Salary for Chris Sater.
[2]Time-slip help.
[3]Supplies: primarily fruit costs and lab supplies. Year 2 fruit will be cull fruit to reduce cost. Chemicals and residue analysis will be provided at no cost from Pace International and other suppliers.
[4]Room cleaning and fruit disposal fees.

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. There is evidence (limited) that indicates that one application of thermofogger-applied ethoxyquin immediately after harvest can control scald. Our experiments, as well as those of Dr. Chen, have shown that the most effective timing for the application of an antioxidant is immediately after harvest. However, it is often not commercially possible to fill a room in one day. Tests will be made to examine the length of time after harvest in which ethoxyquin can be applied by thermofogging to control scald.

    b. Determine the effect of multiple, lower concentration applications on residue levels. It is critical to have residue levels on all fruit below the maximum tolerance, but above the target rate. Previous experiments with thermofogged ethoxyquin have shown excessively high residues on certain fruit in the room. Two applications of antioxidant at lower concentrations will be tested to see if this provides effective scald control while avoiding excessive residue.

    c. Evaluate and improve ethoxyquin dispersion in storage room. A challenge to thermofogging is the distribution of chemical throughout the storage room because the chemical is a mist of particles and not a gas. Lack of uniform dispersion can lead to excessive residue levels on fruit in bins on the sides of the storage rooms. This may be mediated through the use of a manifold placed in the room. Some researchers believe that the fans should be running during application while others suggest that the fans should be off (Hurndall, Sive). Gentle air movement during application may assist the distribution of the material. Effects of fan operations and manifolds on chemical residue distribution within the room will be tested using multiple strategies.

2. **Determine whether the phytotoxicity of ethoxyquin can be reduced by rinsing the fruit after it has been drenched ("double-drenching").** The application of ethoxyquin as a liquid drench immediately after harvest is an effective way to control storage scald. However, the risk of staining fruit is great and varies from orchard to orchard, which is why drenching is not widely used on pears. In our previous work on scald there was a pink residue on the fruit immediately after drenching which was easily removed by rubbing or rinsing. It is possible that this excessive residue causes staining. Double-drenching could minimize this staining by allowing the storage operator to apply ethoxyquin as a first drench and then apply the fungicide as a second drench either on the same day, or within 6 weeks after harvest. The second drench might remove enough ethoxyquin residue so that phytotoxicity would not be problematic.

Specific Goals and Activities for 2008-2009

Refine thermofogging technique and procedures to improve dispersion and reduce preferential deposition of ethoxyquin. Research performed on the 2007 fruit utilized a combination of commercial quality fruit and culls. The commercial quality fruit was treated and stored until spring 2008 to allow evaluation of scald after long-term CA storage. The cull fruit was examined for chemical residue and phytotoxicity immediately after treatment and again after short-term CA storage (December). Good progress was made in understanding the dynamics of how the fogged material moves within a storage room. These findings will be validated and methods will continue to be refined to disperse the chemicals throughout the room. This will be the main thrust of our approach in 2008-09. Activities that will be conducted to improve dispersion, uniformity of application and residue control include:

1. Internal adjustments to the fogger to ensure repeatability;
2. Manifold placement and modification for uniform dispersion;

3. Applying the fog in short intervals over a period of time to allow the fog to disperse between applications;
4. Covering the top layer of bins with either permeable or solid materials to prevent over-deposition on the top of the stack;
5. Comparing plastic (vented) vs. wooden bins on chemical dispersion;
6. Residue analysis based on fruit temperature at time of fogging;
7. Determining the degradation of ethoxyquin residue over time in storage.

Additional split-application and timing studies (Objectives 1a and 1b from the recap) will be performed in 2009-2010, following refinements to the fogging methodology, as described above.

Correlate residue levels with incidence of storage scald. Determine a benchmark residue level for ethoxyquin that effectively controls scald. New procedures for measuring ethoxyquin residue have been developed by Pace International, making it possible to correlate residue levels and the incidence of storage scald. Formerly, it was prohibitively expensive to determine ethoxyquin concentration. When linked with residue degradation rates and incidence of phytotoxicity, an appropriate dosing level for thermofogged ethoxyquin can be determined.

Repeat double-drenching of ethoxyquin and fungicide to account for seasonal and orchard variability. Based on preliminary work done this year on one orchard, double-drenching has the potential to control scald while mitigating the risk of phytotoxicity due to ethoxyquin deposition. This trial will be repeated in 2008-2009, based on residue levels and scald incidence from the 2007 crop.

Schedule

September 2008	Treat the fruit from thermofog activity 7, store in CA, and pull samples at regular intervals for residue analysis throughout the fall.
	Drench the fruit at harvest and apply repeat drenches of fungicide at regular intervals over 6 weeks, store bins in CA.
Sept.-Dec. 2008	Perform thermofog activities 1 through 6 using cull fruit obtained from local warehouses, sample fruit for residue levels.
Spring 2009	Evaluate thermofogged and drenched fruit for scald, residue and phytotoxicity.
	Correlate scald incidence with ethoxyquin residue levels at harvest.

SIGNIFICANT FINDINGS FROM THE 2007 CROP:

Objective 1—Refine the methodology of thermofogging of ethoxyquin.

a. Determine the effect of time between harvest and ethoxyquin application. Commercial quality fruit was treated 2, 11 and 15 days after harvest, and will be examined for phytotoxicity and scald in spring 2008.
   - Residue sampling shows highest residue on top layer of fruit in the bin.
   - Fruit on the bottom and middle of the bins had uniform residue levels but were lower than the top layer of fruit.

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 fruit will be examined for phytotoxicity and scald in spring 2008.
   - 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).
   - The top layer of fruit had higher residue levels than fruit in the middle of the bins.

c. Evaluate and improve ethoxyquin dispersion in storage room. Strategies used to improve dispersal of ethoxyquin included utilization of a free-flowing air manifold to move ethoxyquin through the storage room and the use of porous and non-porous bin covers.
    - The free-flowing manifold improved dispersion of ethoxyquin throughout the room, within the bins, and the stacks; however, uniformity of residue was not improved. The concentration of residue was increased by using the manifold but the variability in concentration from bin to bin and within the bins increased as well. The top layer of fruit had the highest concentrations of ethoxyquin.
    - Both the porous cover (shade fabric) and non-porous cover (plastic elevated approximately 3 inches above bin level) significantly reduced deposition of ethoxyquin on the top bin of each stack. The uncovered bins (at the top of stacks) showed the highest residue levels, especially when the manifold was operating. However, in the uncovered bins, many residue levels exceeded the legal limit for ethoxyquin (3 ppm).

Objective 2 - Split drenching of ethoxyquin and a fungicide.

- 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 (0, 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.

METHODS FOR 2008-2009:

Objective 1—Refine the methodology of thermofogging of ethoxyquin.

Obtaining appropriate levels of residue that are evenly distributed is a challenge when the thermofogger is used with ethoxyquin on pears. The following experiments will develop a system that gives a uniform residue of the appropriate concentration throughout the room. Immediately following each application fruit will be sampled and analyzed for residue. The lab results may modify the experimental setup for the next trial. Cull fruit will be used to significantly cut costs, with the exception of experiment 7, which uses commercial quality fruit.

1. Adjustment of the fogger – two trials to validate our findings in 2007 that appropriate residue levels are reached and repeatable.
2. Manifold modification – four trials using different manifold placements in both active and passive modes.
3. 'Pulsing' the fogging – three trials in which the fog is applied in short bursts (pulses) over a period of time.
4. Covering – two trials in which the topmost bins in the room will be covered with either porous (cloth) material, solid material or left uncovered.
5. Plastic vs. wooden bins – two trials to compare the residue on fruit in different types of bins, wooden vs. plastic (vented).
6. Fruit temperature – two trials to compare the residue level on different temperatures of fruit (32 vs. 45 ºF).
7. Residue degradation – commercial quality fruit will be used to determine the role of packing procedures as well as the effect of time in storage on residue concentration. Currently, there is little data on the rate of decline in ethoxyquin residue on fruit in storage. Target residue levels at time of the initial drench could be modified based on the results of degradation research.

Objective 2. Split drenching of ethoxyquin and a fungicide.

In 2008, double-drenching will be expanded to get more information on the effect of the interval between drenches on residue and scald control.

RESULTS AND DISCUSSION

Objective 1—Refine the methodology of thermofogging of ethoxyquin.

a. Determine the effect of time between harvest and ethoxyquin application. The first goal of 2007 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 on pears 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 this was accomplished. The results of the tests were settings of air temperature and velocity that optimize particle size. Dummy bins (bins wrapped in plastic to prevent through-flow) were used to better mimic commercial treatments while minimizing the amount of fruit needed to complete these trials.

Ethoxyquin was applied to commercial quality fruit 2, 11 and 15 days after harvest. Residues at the top of each bin were higher than those in the middle or bottom (Table 1). The target residue for this experiment was between 1 and 3 ppm; in initial trials it was difficult to reach this level. However, since residue level results from commercial applications are not well known, it is possible that ethoxyquin residues of <1.0 ppm are effective in controlling scald. The fruit will be evaluated for scald in spring 2008 and the results will be correlated with the residue data.

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 60 day application showed very high ethoxyquin residues. Whether this is 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. Experiments 6 and 7 planned for 2008-2009 will help answer these questions.

Table 1. Residue results at each application date.

Location in bin	Ethoxyquin residue (ppm) at each application		
	+2 days	+11 days	+15 days
Top	0.7	0.6	1.0
Middle	0.3	0.5	0.6
Bottom	0.3	0.5	0.6

Table 2. Residues for low concentrations of ethoxyquin applied 2 and 60 days after harvest.

Location in bin	Ethoxyquin residue (ppm)	
	2 days	60 days
Top	0.4	5.6
Middle	0.2	3.2

c. Evaluate and improve ethoxyquin dispersion in storage room. The fruit at the top of the room (bin position 1 in Figure 1) retained the highest chemical concentration, at times in excess of maximum legal residue. In this experiment, a manifold was constructed from porous flexible plastic pipe and spread across the back wall and across one side of the room. A fan was fitted to the pipe to develop a pressure differential. The stacking pattern for the room is shown in Figure 1. Full bins of fruit were stacked in the 2, 3 and 4 positions under the cloth covered bins to determine chemical dispersion throughout the room. Residue levels were higher when the manifold was utilized (Table 3).

Table 3. Effect of moving air with a free-flowing manifold on deposition of ethoxyquin applied by thermofogging within bins of fruit

	Ethoxyquin Residue (ppm)

Manifold ON	2.2 a
Manifold OFF	1.3 b
	(P = 0.021)

Means separated using Tukey's HSD

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 was utilized (Table 4).

Another goal 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 to 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 5).

In 2008 the plan is to continue to develop scientifically valid information on how to reduce the variability of residue within a bin and between bins. Cull fruit will be utilized to reduce cost. The manifold will be modified to test both active and passive modes; fog will be pulsed into the room at various intervals; and additional coverings that will permit cooling after fogging and prevent over-deposition will be tested.

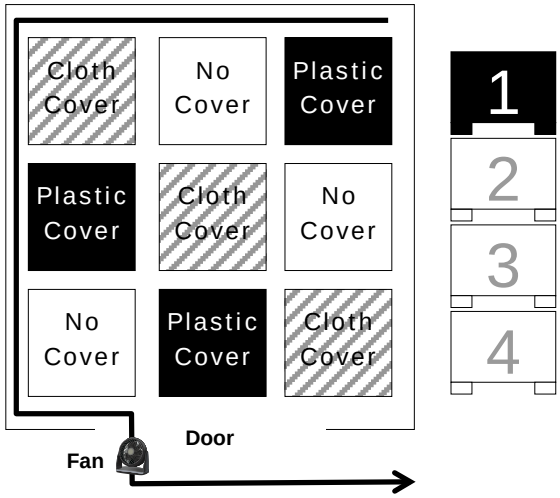

Figure 1. Stacking pattern for manifold thermofogging experiments in Stemilt RCA room. The manifold placement is indicated by the solid black line.

The hypothesis that traditional wooden bins are a barrier to reasonable air flow will be tested by comparing residue levels in plastic (vented) vs. wooden bins. It is hoped that the highly vented plastic bins will allow increased penetration of the chemicals into the bins, thereby increasing uniformity of application within each bin.

The effect of fruit temperature (32 vs. 45 ºF) on residue will be tested to anticipate the residues that might be expected at harvest or when fruit are coming from storage. Finally, commercial quality fruit will be used to determine the role of the packing process on ethoxyquin residue following fogging and storage. Samples will be tested out of CA storage to develop a residue curve over time.

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

Objective 2. Split drenching of ethoxyquin and a fungicide.

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

	Manifold	
Fruit location	**On**	**Off**
Top	4.6 a	2.1 b
Middle	2.5 b	1.7 b
		(P = 0.04

Means separated using Tukey's HSD

Table 5. 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

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 develops over time when 'liquid' ethoxyquin residue remains on the fruit (Figure 2). When this 'liquid' residue was removed by washing or brushing the burn did not develop. In 2007, we obtained sufficient ethoxyquin residue on pears whether the fruit were drenched once (with both ethoxyquin and fungicide), or first drenched with ethoxyquin alone and then drenched with a fungicide as long as 42 days later (Figure 3). This fruit will be evaluated for phytotoxicity and scald following long-term CA storage. In 2008, experiments will be done to determine how soon it is possible to apply the second drench after the first drench and still have optimum residues of both chemicals without the risk of phytotoxicity.

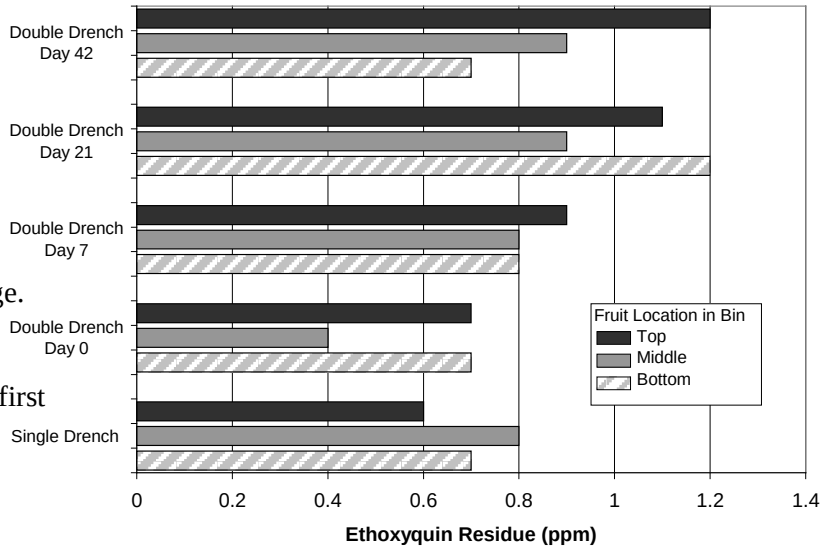

Figure 3. Ethoxyquin residue on fruit at top, middle and bottom of bins, after double-drenching (ethoxyquin followed by a fungicide) 0, 7, 21 and 42 days after harvest.

Figure 2. Phytotoxicity on Anjou due to ethoxyquin staining.

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

WTFRC Project Number: PR-07-706

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

PI:	Jim Mattheis	**Co-PI(2):**	Dave Rudell
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Total project funding request: **Year 1:**$25,875 **Year 2**:$27,200 **Year 3:** $27,990

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, other salary and benefits from Mattheis existing funds

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.

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

These objectives address and are consistent with 2007 research priority 1, Postharvest: storage technology, and scald. Information generated by the proposed studies may also be of use for identification of other fruit quality parameters including nutritive value (content of known antioxidants), firmness measurements as maturity/storability indicators (additional firmness/texture values generated by Digitest instrument), as well as alternative storage protocols.

SIGNIFICANT FINDINGS 2007 (through 4 months after harvest)

- Through 4 months storage, no speckling has occurred on fruit from any orchard. This is consistent with our previous work where speckling developed between 4 and 6 months after harvest.
- Superficial scald has developed on fruit from 2 of 5 orchards stored in air but not on fruit held in CA (1.5 or 0.5% O_2, 0.5% CO_2).
- Core browning (limited to seed cavity walls) has developed on fruit from all orchards stored at the low O_2 setpoint (0.4 or 0.5% O_2) and in fruit from 3 of 5 orchards stored in 1.5% O_2.
- Ethanol accumulation has been higher in fruit stored at the low O_2 setpoint compared to fruit stored in 1.5% O_2 or air. Ethanol accumulation increased between 2 and 4 months in storage. While well above the analytical detection limit, ethanol amounts detected to date are all relatively low.

METHODS

Speckling induction: Fruit obtained at commercial harvest in 2007 from 5 orchards in the Wenatchee area was placed in a 33 ^oF cold room. Fruit are being stored in air, CA (1.5% O_2/ 0.5% CO_2) established on day 1 after harvest, or a lower O_2 CA established with the same CO_2 concentration but an O_2 setpoint of the O_2 concentration at which a change in chlorophyll fluorescence occurred for each lot plus 0.2%. This value was determined on day 2 after harvest. Peel samples were collected the day of harvest and after 2 and 4 months; the last sampling will be at 6 months. Peel tissue is 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 have been 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 also after storage. Five orchards were sampled to increase the likelihood of speckling (we have previously observed lot to lot variability for this disorder). By using multiple orchards we hope to obtain peel samples from orchards with and without speckling for comparative analysis.

RESULTS & DISCUSSION

The five orchard lots obtained for this 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.

Results to date are consistent with our previous work where fruit stored in CA have at least delayed incidence of superficial scald and ripening compared to fruit stored in air. At 4 months, fruit from orchards 1-4 stored in 0.4 or 0.5% O_2 softened slower relative to fruit stored in air or 1.5% O_2 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 work.

The incidence of core browning in fruit from all lots stored at 0.4 or 0.5% O_2 has not been observed in our previous work at low O_2 concentrations (Figure 1). So far the disorder has been limited to the seed cavity walls. Ethanol content is also higher 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 core browning can be established.

Development of procedures for extraction and analysis of d'Anjou peel tissues indicated methods previously developed for use with apple peel were not directly transferable for pear. A method specific for pear was developed and peel sample collection, extraction and analysis is ongoing. The chromatographic systems available in our lab are working well, particularly the LC/MS where peak separation and resolution have been consistently good. We anticipate completion of the year one planned activities for this portion of the project (focused on respiratory metabolism) will occur as scheduled.

Relevance of this project to the industry continues to be in the potential use of atmospheres to control superficial scald, avoid speckling, and manage fruit quality. Our goal in the first year was to develop a model system to induce speckling to enable the metabolic basis of the disorder to be studied as well as to provide a basis for selecting other atmospheres/protocols to avoid speckling development. As this report is written speckling development has not occurred but that observation is consistent with previous results. Observations at 6 and 8 months will be used to gauge the success of our experimental plan for year one and as a guide to designing year 2 experiments.

Table 1. Summary at 4 months after harvest of d'Anjou fruit quality, disorders, and physiological measurements. All fruit stored at 33 ^oF and analyses were conducted after removal from storage plus 7 days at 68 ^oF. Generation of CA conditions was initiated 2 days after harvest.

Orchard 1

Storage	1.5% O_2, 0.5% CO_2		0.5% O_2, 0.5% CO_2		air	
Months	2	4	2	4	2	4
Titr.Acidity %	0.201	0.160	0.244	0.185	0.184	0.165
Peel Color[1]	1	1	1	1.3	1.7	1.7
core browning %[2]	0	0	39	6	0	0
scald %[2]	0	0	0	0	0	0
lbs	4.8	3.0	5.4	5.3	2.5	2.2
CO_2 umol	410	540	460	550	530	630
ethylene umol	0.10	0.30	0.13	0.37	0.30	1.0
ethanol umol	9.9	35	43	157	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	2	4	2	4
Titr.Acidity %	0.241	0.224	0.272	0.281	0.237	0.236
Peel Color[1]	1.1	1	1	1	1.6	1.4
core browning %[2]	0	0	11	89	0	0
scald %[2]	0	0	0	0	0	6
lbs	8.1	2.5	7.0	7.5	2.8	2.9
CO_2 umol	290	390	340	300	420	580
ethylene umol	nd[3]	0.05	0.01	0.01	0.01	80
ethanol umol	13	15	51	86	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	2	4	2	4
Titr.Acidity %	0.244	---	0.242	---	0.213	---
Peel Color[1]	1.1	1	1	1.33	1.4	1.4
core browning %[2]	0	0	33	39	0	0
scald %[2]	0	0	0	0	0	6
lbs	6.7	3.2	6.1	7.2	2.3	2.7
CO_2 umol	340	360	340	320	560	450
ethylene umol	nd[3]	0.12	0.03	0.03	0.04	0.35
ethanol umol	22.7	21.4	21.2	45.3	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	2	4	2	4
Titr.Acidity %	0.264	0.183	0.226	0.277	0.231	0.154
Peel Color[1]	1	1.2	1	1.1	1.8	2.2
core browning %[2]	6	11	83	56	0	0
scald %[2]	0	0	0	0	0	0
lbs	5.5	2.9	5.6	4.8	2.4	1.78
CO_2 umol	375	500	470	430	530	690
ethylene umol	0.04	0.53	0.19	0.1	0.26	1.3
ethanol umol	2.7	23.9	32.1	79	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	2	4	2	4
Titr.Acidity %	0.207	*	0.197	*	0.180	*
Peel Color[1]	1.1		1		2.1	
core browning %[2]	17		61		0	
scald %[2]	0		0		0	
lbs	4.6	2.0	5.4	2.9	2.3	2.2
CO_2 umol	330		330		460	
ethylene umol	nd[3]		nd		nd	
ethanol umol	21.3		62		15.5	

[1] Peel Color: 1=green; 5=yellow

[2] Core browning and scald values are % of fruit with symptoms.

[3] nd: not detected

---: results not available due to titrator malfunction

*: results not available when report prepared

A **B**

Figure 1. d'Anjou pear core browning. A: fruit without symptoms. B: core browning, symptoms limited to seed cavity area.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-06-606

Project Title: Field evaluation of new pear rootstocks

PI Clark Seavert
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Co-PI (2): Tom Auvil
Organization: WTFRC
Telephone/email: 509-665-8271, auvil@treefruitresearch.com
Address: 1719 Springwater Drive
City: Wenatchee
State/Province/Zip WA 98801

Cooperators: Tim Smith
Total project funding request: $34,659

Budget 1:
Organization Name: Agricultural Research Foundation **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-4068 **Email address:** dorothy.beaton@oregonstate.edu

Item	**Year 1:** 2006	**Year 2**: 2007	**Year 3**: **2008**
Salaries[1]	15,239	17,508	19,850
Benefits (61%)	9,296	10,680	12,109
Wages	1,200	2,000	
Benefits (8.2%)	98	164	
Equipment			
Supplies[2]	4,600	2,000	2,000
Travel	300	1,000[3]	700
Miscellaneous	200	200	
Total	30,933	33,552	**34,659**

Footnotes:
[1] 0.50 FTE of a technician
[2] Nursery tree orders and liners commercially propagated.
[3] One quarter of the cost to travel to International Pear Symposium In May 2007.

Objective 1 Initial screening and evaluation of the Horner rootstock series and evaluate untested rootstocks at OSU-MCAREC

- Three separate Horner trials will be planted in 2004, 2005, and 2006. These three trials represent 428 different Horner clones.
- Old Home by Farmingdale 87 will be used as a control rootstock.

MCAREC High Density COS Block

- Selections of P2535, Bet # 2291, 517-9, 708-13, 96FI11, 96FI12, 96FI14, 96FI15, Horner 4, OH 11, OHxF 87, Pyronia, and Q29859 will be planted.
- Trees planted in a 12 ft x 4 ft vertical fruiting wall. (907 trees/acre)
- Old Home by Farmingdale 87 will be used as a control rootstock
- Trees will be managed to encourage early fruiting and trained to facilitate mechanical assist harvesting.

Objective 2 A comprehensive evaluation of the Horner rootstock series and untested rootstocks to be implemented in COS 2015 Trials.

- These trials will be conducted on a small scale located at the OSU-MCAREC, and grower sites in Hood River, Yakima and Wenatchee.
  - Finished trees will be available for grower sites in 2009
  - Planting system and scion will be grower's choice
  - Liners will be available for two plantings of plant-in-place, late spring 2007.
- Ten tree replicates per rootstock
- 5 replications per rootstock
- Trees planted in a 12 ft x 4 ft vertical fruiting wall. (907 trees/acre)
- A cultivar that is a good indicator of the characteristics of interest for each growing region, (e.g. Anjou for Hood River, Bartlett for Yakima, Bosc for Wenatchee) will be used.
- Old Home by Farmingdale 87 will be used as a control rootstock
- Trees will be managed to encourage early fruiting and trained to facilitate mechanical assist harvesting.

Objective 3 Identification of new rootstocks for future evaluation

- An international search will be initiated to identify potential rootstocks for evaluation in future trials.
- Selections will be made in collaboration with the Northwest Pear Rootstock Advisory Committee (see below).
- Contacts will be made with the international breeding programs in East Malling UK, Pillnitz Germany, and Angers France to select at least three new clones and begin the process of transferring material to our initial field trials.
- Liners will be propagated after the material is released from quarantine.

Objective 4 <u>Advisory Committee</u>

An advisory committee will be formed with representation from the main pear districts in Washington and Oregon. This committee will meet at multiple times during the year to discuss progress and provide input on future direction of the program. COS 2015 tours in each growing region will

provide opportunities to observe pear rootstock trials in other locations.

Progress to Date

Objective 1 Horner and untested rootstock screening

Horner 2004

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 harvest could clarify the choices. The 22 selections made from the 2006 data weren't validated by the 2007 harvest data, with none of the 2006 selections producing enough fruit to be of import. Although harvest in 2007 did not set any trends in rootstock performance, there were 19 pairs of clones that produced 5 fruit/tree or more and averaged box size 90 and larger. Cumulative yield has been extrapolated to represent yield per acre with spacing of 10x16, (272 trees/acre). (Table1) The fruit set in the spring of 2007 was overshadowed by severe spring temperatures that damaged blossoms. This may have been a factor in the variability of fruit set. The only pair that increased in yield and fruit size from 2006 to 2007 was Horner #119. (Table 1)

- Cumulative data for 2006-2007 shows that there were 180 trees without fruit for both years.
    - Forty eight pairs of clones were lacking in fruit from this number.
    - Eighteen single trees had no blooms or fruit for both years (Table 2)
    - Sixty-six single trees had bloom but did not set fruit.

It has been agreed to by the Advisory committee to postpone the selection process for on farm trials until the spring of 2008.

Horner 2005

The bloom and fruit set in the third leaf of the Horner 2005 trial was sparse, but branch angle, bloom, harvest and trunk measurements were completed this year.
There were no sets of clones that produced fruit on all trees.

Horner 2006

Trees have been maintained according to advisory committee recommendations for the Horner trials and data has been collected. There were few blooms and no fruit set this year.

MCAREC High Density COS Block

To achieve maximum performance, continuous growth of 5 inches per week on the central leader was necessary through the growing season. The finished trees have reached and in some cases exceeded the top wire of the trellis system. (Top wire 13 feet).Cultural techniques to encourage branching at wires and to slow the vertical growth will need to be employed in 2008. Trunk measurements have been taken and the first step to creating a Cordon system in two of the rows has been initiated. Soil and leaf samples have been collected and we are awaiting the results.

Objective 2 COS 2015 On-Farm Trials

Two clones, Horner 4 and Horner 10, and the standard OHxF 87 have been selected and have been budded in the summer of 2007 by Van Well's nursery. They will be available for planting on farm in 2009. DNA analysis has been completed for Horner 10 and it has been verified that the material used for propagation is identical to the donor plants from MCAREC.

Objective 3 Identification of new rootstocks for future evaluation

- The three Khazakstan rootstocks (Q29857, Q29858, and Q29859) are the latest clones to be propagated and 10 plants of each clone were planted in the COS block. The anticipated

quantities of the three clones were reduced by rootstock failure, and have not been planted in a replicated trial as previously planned.

Quince Rootstock search

- The commercial Quince rootstocks currently available offers size control but unfortunately have some drawbacks, such as incompatibility with cultivars and lack of cold hardiness. There are other Quince selections that haven't been tested, and they are reported to be cold hardy. These are the Pigwa selections from Skierniewice Poland- 'S1', 'S2', and 'S3'. Preliminary plans for a trial with OHxF87, the Polish selections, and possibly 'Sydo' are being discussed.

Objective 4 Rootstock Advisory Committee meetings

The Advisory Committee met in April 2007 and toured the rootstock trials during bloom. No choices were made for propagation for on farm trials at this time. Committee members attended the Extension Field Day on Rootstocks and Training Systems at MCAREC in July. After reviewing harvest data for the Horner 2004 block for 2006 and 2007, it was apparent that the data was not showing any significant trends in productivity, so selections for propagation were postponed until the spring of 2008.

Table 1
Clones with both trees that had five fruit or more and fruit size greater than or equal to box size 90 are included in this table. Per acre yield had been extrapolated using 272 trees/acre, or 10x16' spacing.

Per acre extrapolated yield for Horner Harvest 2006-2007							
Horner	Trees/acre	lbs./fruit	44lb.Box/acre	2006-07	Average	TCSA	YE
Rootstock ID	10x16 spacing	per acre	80% packed	Yield (lbs)	Box Sz	cm2	lbs fruit/cm2
81	272	3400	62	12.5	92	33.8	0.370
81	272	1278	23	4.7	75	34.8	0.135
118	272	1278	23	4.7	84	29.9	0.157
118	272	1360	25	5.0	70	35.4	0.141
119	272	1746	32	6.4	80	38.5	0.167
119	272	1352	25	5.0	77	33.4	0.149
135	272	1795	33	6.6	73	32.8	0.201
135	272	843	15	3.1	71	43.2	0.072
145	272	1115	20	4.1	86	33.1	0.124
145	272	2475	45	9.1	73	41.0	0.222
148	272	2938	53	10.8	86	20.4	0.530
148	272	1601	29	5.9	81	32.8	0.180
160	272	762	14	2.8	79	27.5	0.102
160	272	1088	20	4.0	88	23.0	0.174
186	272	1686	31	6.2	85	32.2	0.193
186	272	789	14	2.9	91	27.8	0.104
188	272	1074	20	3.9	78	32.5	0.122
188	272	5440	99	20.0	75	32.2	0.622
220	272	2176	40	8.0	88	35.1	0.228
220	272	2557	46	9.4	66	43.2	0.218
236	272	808	15	3.0	89	38.5	0.077
236	272	979	18	3.6	86	35.8	0.101
238	272	1442	26	5.3	75	25.2	0.210
238	272	816	15	3.0	88	36.1	0.083
252	272	816	15	3.0	73	36.8	0.082
252	272	2530	46	9.3	85	25.5	0.365
254	272	1414	26	5.2	76	39.2	0.133
254	272	4053	74	14.9	71	51.7	0.288
270	272	1686	31	6.2	71	32.5	0.191
270	272	816	15	3.0	73	28.7	0.104
317	272	2013	37	7.4	77	41.0	0.180
317	272	925	17	3.4	78	27.8	0.122
328	272	1115	20	4.1	75	19.6	0.209
328	272	1306	24	4.8	92	19.6	0.245
330	272	2176	40	8.0	83	38.9	0.206
330	272	1523	28	5.6	87	24.4	0.230
232B	272	1822	33	6.7	85	20.4	0.329
232B	272	2150	39	7.9	90	32.2	0.246

Table 2
These Horner clones from the 2004 planting have not had any bloom or fruit in 2006 or 2007.

Trees without bloom or fruit in 2006-2007			
ROW	TREE	H-ID#	
8	32	13	dead
16	34	13	
19	30	27	
11	21	45	
14	2	110	
12	18	121	
3	22	127	
17	29	153	
9	5	168	
9	14	193	
10	18	196	dead
5	12	217	
11	10	246	
10	11	257	
18	2	258	
17	1	259	
15	12	283	
19	22	295	
2	26	320	

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-06-607A

Project Title: PNW Pear rootstock trial

PI: Timothy J. Smith
Organization: Washington State University
Telephone/email: 509 667 6540
Address: 400 Washington Street
City: Wenatchee
State/Province/Zip WA 98801

Cooperators: Clark Seavert & Janet Turner, OSU (Hood River Trial)
Ed and Darrin Kenoyer (Cashmere Trial)
Geoff and Tyler Thornton (Tonasket Trial)

Total project funding request:	***Year 1:2006***	***Year 2:2007***	**Year 3:2008**
	$13,406	$13,306	**$12,403**

Other funding Sources: None
WTFRC Collaborative expenses: None

Budget Summary of Total Project:

Projects by Site	Year 1: 2006	Year 2: 2007	**Year 3: 2008**
Cashmere	7,618	7,291	**6,157**
and Tonasket			
Hood River	5,788	6,015	**6,246**
Total:	13,406	13,306	**12,403**

Budget 1:
Organization Name: WSU **Contract Administrator:** Jennifer Jansen
Telephone: 509-335-2867 **Email address:** jjansen@wsu.edu

Item	**2008**		
Salaries	2,873		
Benefits	1,293		
Wages	0		
Benefits	0		
Equipment	0		
Supplies	400		
Travel	1,591		
Miscellaneous			
Total	**6,157**		

Footnotes: 2008 - 0.0833 (one month) FTE Extension Coordinator (Tonasket, Cashmere)
Travel 3280 miles at 0.485/mi.

Budget 2:
Organization Name: OSU **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-4068 **Email address:** dorothy.beaton@oregonstate.edu

Item	**2008**		
Salaries	2,852		
Benefits	1,740		
Wages	692		
Benefits	62		
Equipment	0		
Supplies	500		
Travel	400		
Miscellaneous	0		
Total	**6,246**		

Footnotes: [1a] 0.1 FTE Technician. [2] Time slip wages. [3] Includes miscellaneous supplies; MCAREC supplies includes packing line charges. [4] Travel to Washington and local field plots.

Objectives:
This effort was initiated in the 1990's with the premise that pear growers had limited access to semi-dwarfing pear rootstocks, and all of the horticultural benefits that come with a shift away from large, vigorous trees. Various pear rootstocks were obtained, though not all available semi-dwarf rootstocks were included. The most obvious missing rootstock is OHxF 69, which has performed well in some trials. The plots were spread across Washington and northern Oregon pear growing regions to enhance evaluation, as Hood River has unique growing conditions, and there was value in the rootstocks being exposed to colder winters, hotter summers, and various central Washington soils.

2008: We will continue evaluation of 2002 and 2005-planted pear rootstocks, with continued emphasis on tree survival, root suckering, vegetative growth potential of the scion, fruit yield, and fruit size. Additional evaluation will be possible re: biennial bearing. We will be contrasting yields of 4th season D'Anjou on the 2005 rootstocks on trellis in Cashmere vs. free standing in Hood River. The 2005 Bosc trial may also provide a good 4th leaf performance contrast of Bosc/rootstock on free standing vs. upright trellis.

Significant Findings/Changes:
Some of the 2002 planted rootstock/scion combinations had sixth season yields of high quality fruit in excess of those expected in average mature Washington pear orchards. The Golden Russet Bosc on OHxF 87 has been the top combination, on the Bartlett; Pyro 2-33 has induced the highest yields and largest fruit without over-setting.

While there is great variation in tree growth related to rootstock, there is little correlation of smaller tree size to precocity and yield.

Some of the rootstocks are exhibiting important negative attributes, such as probable susceptibility to pear decline disease, low young tree tolerance to winter cold, excessive root suckering, relatively low precocity and yield, and tendency toward production of smaller than plot average fruit size. Though it produces a healthy tree, Pyrodwarf continues to have the worst mix of many of these bad habits.

The Yakima Bartlett rootstock trial has had severe fire bight in three of its' six seasons, 2004, 2005 and 2007, rendering it useless for yield or fruit quality data. Fire blight infection had little correlation to rootstock, although the more vigorous rootstocks induced larger tree size. Larger trees were more likely than smaller trees to withstand blight damage without subsequent tree death. None of the rootstocks were more sensitive than the Bartlett scion, so tree death occurred when the trunk died. Further evaluation of this plot would result in misleading horticultural data, so the plot has been discontinued. Valid Bartlett/rootstock data is being evaluated on OHxF 87, Pyro 2-33, Pyrodwarf, and Horner 4 at the other plot sites.

Results and Discussion
Yields and Fruit Size:
The D'Anjou trials in Hood River and Cashmere have been slow to come into production for various reasons, some unrelated to the rootstock. The trees appear to be in condition to produce a good test yield in 2008, the 7th year of growth.

The Bartletts that remain in the trial are producing relatively well, and some yield and fruit size differences are evident. The best production/fruit size/balanced vigor combination in the Bartletts is achieved with Pyro 2-33. Pyrodwarf results in smaller fruit and lower yields, and OHxF 87 generally oversets, though careful hand-thinning and careful pruning leads to adequate tree growth and fruit

size. Thinning fruit on Bartlett/Pyro 2-33 is much less time consuming, and the trees have had no tendency to stunt when fruited heavily in the 4th – 6th leaf.

The best production, leading to the most comprehensive data, is in the Tonasket Bosc Trial. Some of these trees started producing fruit in the 3rd leaf, and yields continue to increase significantly each season. As the upper 20% of the trees bearing surface has not come into production yet, higher yields appear possible. Extensive scaffold support was used in 2007 to reduce limb breaking. Production reached or exceeded industry averages in 2007, though there were very significant differences amongst the rootstocks. As Bosc tend to bear large crops in alternate years, it will be important to note the consistency of production now that most of the individual trees are approaching full production. The trees on OHxF 87 had a relatively large crop in 2006, but were even more heavily cropped in 2007. It is also common that the size of fruit is large on a first significant cropping year, then declines the next season. This did not occur on the OHxF 87 trees this year after the near-full crop of 2006. It should be noted that most of the other rootstocks in this trial are producing their first significant crop, so evaluation of size and return bloom in 2008 may lead to important differences.

The 2005 plantings appear to be set for their fist significant crop in 2008. The Boscs and one of the two D'Anjou plots have been trained to a trellis. Some Bartlett on OHxF 87 and Horner 4 are also trellised as pollenizers.

Tree Survival / Health:

During the first three seasons after planting, a significant percentage of some 2002 planted rootstocks/cultivars died from either pear decline or winter damage. Since that time, there has been no rootstock related tree loss. Of the rootstocks planted in 2005, some trees on the BU-2 and BU-3 appear to be struggling to survive. While they appear healthy in Tonasket and Hood River, there have been very early leaf drop and possible pear decline symptoms in the Cashmere D'Anjou trial.

Suckers: The only trees that have significant root suckers in the 2002 trials are on Pyrodwarf. No 2005 planted rootstock is suckering yet.

Plan for the next season:

2008: We will take fruit measurements on the 2002 planted rootstocks with economic potential, and will take 4th season measurements on 2005 planted rootstocks. We will need to plan for the 5th, 6th and 7th seasons of 2005 planting rootstock evaluation, if deemed necessary. As the lesser rootstocks in the 2002 plantings may be fully evaluated, the actual time necessary to maintain and evaluate the 2002 trial blocks will be reduced as the obviously less interesting roots are eliminated from consideration after the 2007 and 2008 evaluations. It is likely that the PI will continue to maintain the trees planted in 2002, and evaluate the apparent better choices for consistency of production and fruit size. The level of effort will therefore decline somewhat in the 2002 plots, and greatly increase in the 2005 planted trial, as their production is expected to increase rapidly.

2007 Data:

Tonasket GR Bosc-2002 Rootstock Trial	2007 Pounds Fruit/ Acre, 6th Year	Calc. Trees / Acre	2007 44 lb. Box/ Acre, if 90% Packed	2007 Average Box Size 44 / Fr. Wt.	2007 % 90's and Larger	Total Bins Fruit /A 2004 to 2007	2007 Lbs. Fruit per Tree	Trunk Cross Sect. Area CM2	2007 lbs. Fruit / CM2 of Trunk x-sec (Efficiency)
OHxF 87	56,723	390	1159	75	89	97	145.4	72.8	2.00
OHxF 40	44,148	390	902	80	84	72	113.2	65.1	1.74
Pyro 2-33	43,290	390	886	75	90	65	111.2	70.7	1.57
708 – 36	28,050	477	560	88	70	49	73.4	45.4	1.62
Pyrodwarf	29,510	390	604	75	84	39	75.7	69.5	1.09
Fox 11	26,418	444	540	75	90	36	85.0	52.6	1.62
Fox 16	21,895	477	448	70	97	29	65.6	50.3	1.30

Table 1-1. 2002 planting of Golden Russet Bosc, (6th season).

Tonasket Bosc-2005 Trial (on a trellis)	2007 Pounds Fruit/ Acre, Third Year	2007 44 lb. Box/ Acre, 90% Packed	2007 Average Box Size 44 / Avr. Fr. Wt.	2007 Total Bins Fruit / Acre	Trunk Diam-eter Inches	2007 Trunk Cross Sectional Area in CM2	2007 Lbs. Fruit / Tree	2007 lbs. Fruit per CM2 of Trunk X-Section (Efficiency)
OHxF 87	7,331	150	68	6.7	2.10	23.8	11.8	0.496
Pyro 2-33	2,275	47	69	2.1	1.67	15.3	3.7	0.242
Pyrodwarf	4,546	93	72	4.3	2.01	20.9	7.3	0.349
BM 2000	3,441	70	67	3.1	1.87	18.6	5.5	0.296
Horner 4a	3,197	65	62	2.9	2.00	20.3	5.1	0.251
BU-3	1,467	na	too few fruit	1.3	1.13	6.7	2.4	0.358
Bartlett Horner 4a	3,582	73	73	3.3	2.09	23.0	6.9	0.300

Table 1-4. 2005 planting of Golden Russet Bosc pear, Tonasket, (3rd season), on upright trellis.

Hood River MCAREC 2002 D'Anjou Rootstock Trial	2007 Pounds Fruit/ Acre, Sixth Year	Calc. Trees / Acre	2007 44 lb. Box/ Acre, 80% Packed	2007 Average Box Size 44 / Avr. Fr. Wt.	2007 % 80's and Larger	Total 1100 lb. Bins /Acre 2006 & 2007	2007 Lbs. Fruit / Tree	Trunk Cross Sect. Area CM2	2007 lbs. Fruit/ CM2 Trunk - Efficiency
OHxF 87	28,424	390	517	85	73	32.5	72.9	83.1	0.877
OHxF 40	18,201	390	331	85	69	21.4	46.7	80.4	0.581
Pyro 2-33	23,012	390	418	91	48	26.1	59.0	79.2	0.745
708 – 36	22,074	445	401	89	54	31.2	49.6	71.9	0.690
Pyrodwarf	19,052	390	346	87	46	22.8	48.9	85.7	0.571
Fox 11	21,340	480	388	82	69	23.0	44.5	73.1	0.609
Winter Nellis	19,960	390	363	85	65	24.2	51.2	84.9	0.603

Table 2-1. 2002 planting of D'Anjou pear, Hood River MCAREC, (6th season).

Cashmere 2002 D'Anjou	2007 Pounds Fruit/ Acre, Sixth Year	Calc. Trees / Acre	2007 44 lb. Box/ Acre, 80% Packed	2007 Average Box Size 44 lb. / Avr. Fr. Wt.	2007 % 90's and Larger	Total Bins Fruit /A 04 to 07 (No 06)	2007 Lbs. Fruit / Tree	Trunk Cross Sect. Area CM2	2007 lbs. Fruit/ CM2 Trunk - Efficiency
OHxF 87	13,845	390	252	83	82	17.9	35.5	77.3	0.459
OHxF 40	12,751	390	232	85	84	15.6	32.7	87.0	0.376
Pyro 2-33	12,567	390	228	80	93	14.0	32.2	56.6	0.567
708 – 36	7,992	445	145	92	75	9.8	18.0	70.2	0.256
Pyrodwarf	2,460	390	45	116	25	3.6	6.3	70.2	0.090
Fox 11	11,120	480	202	83	86	12.3	23.2	64.0	0.363
Fox 16	7,400	445	135	86	84	6.7	16.7	68.0	0.246

Table 3-1. 2002 trial D'Anjou pear, Cashmere, (6th season), yield, extrapolated yield, fruit size,

D'Anjou - 2005. Cashmere trellis	2007 Pounds Fruit/ Acre	2007 44 lb. Box/ Acre	2007 Average Box Size	2007 Total Bins / Acre	2007 Trunk X-Sec. Area in CM^2	2007 Lbs./ Tree	2007 lbs. per CM^2 of Trunk
OHxF 87	147	2.7	70	0.13	17.6	0.28	0.016
BM 2000	154	2.8	65	0.14	12.6	0.30	0.024
Horner 4a	231	4.2	73	0.21	15.2	0.44	0.029
BU-2	0	0	na	0	9.3	0	0
BU-3	0	0	na	0	7.0	0	0
Bartlett / H4a	946	17.2	85	0.86	10.3	1.82	0.177

Table 3-2. 2005 planting of D'Anjou pear, Cashmere, (3rd season), on upright trellis, 520 trees /A

Bartlett 2002 Planting Tonasket	2007 Pounds Fruit/ A	Calc. Trees / Acre	2007 44 lb. Box/ Acre,	2007 Average Box Size	2007 % 100's & +	Total Bins /A 04 - 07	2007 Lbs. / Tree	Trunk X-Sec Area CM2	2007 lbs. per CM2 of Trunk
Pyro 2-33	38,663	390	746	74	98	82	99.1	50.6	1.96
Pyrodwarf	24,668	390	476	90	88	53	63.3	45.5	1.39

Table 4-1. 2002 planting of Bartlett pear, Tonasket, (6th season).

Bartlett 2002 Planting Cashmere	2007 Pounds Fruit/ Acre, 6th Year	Calc. Trees / Acre	2007 44 lb. Box/ Acre, 80% Packed	2007 Avr. Box Size	2007 % 100's and Larger	Total Bins Fruit /A 04 to 07 (NO 2006)	2007 Lbs. Fruit / Tree	Trunk Cross Sect. Area CM2	2007 lbs. Fruit / CM2 Trunk - Efficiency
Pyro 2-33	31,005	390	564	96	66	37	79.5	60.0	1.33
Pyrodwarf	21,298	390	387	116	39	26	54.6	54.1	1.01
OHxF 87	24,351	390	443	104	62	29	62.4	57.6	1.08

Table 4-2. 2002 planting of Bartlett pear, Cashmere, (6th season).

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-07-708

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:** 54,300 **Year 2:** 59,492 **Year 3:** 63,252

Other Funding Sources: NONE

Budget 1:
Organization Name: Washington State University **Contract Administrator:** ML 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:
[4]Travel:

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 are being 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.
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.

   Proposed activities for 2008:

- Expand collection of pear propagules to include Bosc, Comice, Red D'Anjou, Red Bartlett, and Seckel during the 2008 growing season and establish suspension cultures of aforementioned varieties by December 2008. Maintain and increase stocks of all pear suspension cultures for downstream experiments.

- Perform random mutation experiments and CSI associated with desirable pear traits by the end of 2008.

- Sample collection began post-bloom in 2007 in the Tukey Orchard. Samples were collected every 3-4 week until harvest. We are currently collecting samples from fruit in storage. This is being done to charter the course of development and identifying genes that play important role during the physiological progression of the fruit on the tree and during post-harvest storage. In 2008, another set of samples will be collected both at Tukey Orchard and in Wenatchee. Core and peel samples have been harvested from Bartlett and D'Anjou so far and the same genotypes will be used during the 2008 growing season.

- We plan to expand on the Differential Display experiments using the wider range of pear material mentioned earlier. This will aid in locating genes that are expressed at the different stages of fruit growth and potentially under different environmental conditions. The reason behind this exercise is to identify genetic elements that regulate post-harvest quality of the selected varieties.

SIGNIFICANT FINDINGS

- Callus (non-differentiated plant cells) from Bartlett and D'Anjou was readily grown from both varieties on an optimized tissue culture Schenk & Hildebrandt (SH) media.

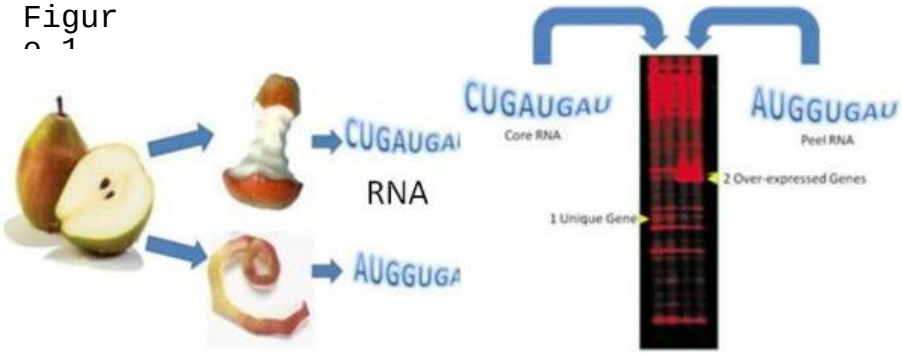
- The callus was able to grow from nearly all parts of the pear fruit that were tested and suspension cultures were successfully established for Bartlett and D'Anjou. Further details are provided in Results and Discussion section.

- Differential Display utilizes RNA (Ribonucleic acid) molecules that are produced from the genetic material, DNA in the genes. RNA is very susceptible to degradation with standard extraction techniques rendering these techniques unsuitable for our proposed use. We have shown that fruit samples ground in an SPEX SamplePrep Freezer Mill yielded high quality RNA after extraction. This specialized equipment (worth $ 12,000) has been procured from leveraged funds provided by the Agriculture Research Center and the Department. Additionally, another piece of equipment called a Bioanalyzer (worth $10,500 from WSU funds) has also been procured for the lab. This equipment assesses RNA quality which, as discussed earlier, is an essential component in performing accurate Differential Display.

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. This year we plan to collect samples every month in both Wenatchee, WA and Pullman, WA. These samples will be transferred in liquid nitrogen for return to the laboratory. By grinding samples in the Spex SamplePrep 6870 freezer mill we will produce high quality RNA ready for analysis.

Figur
e 1



RNA will be 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 can then be performed using the isolated and quality tested RNA**.**

Figur
e 2

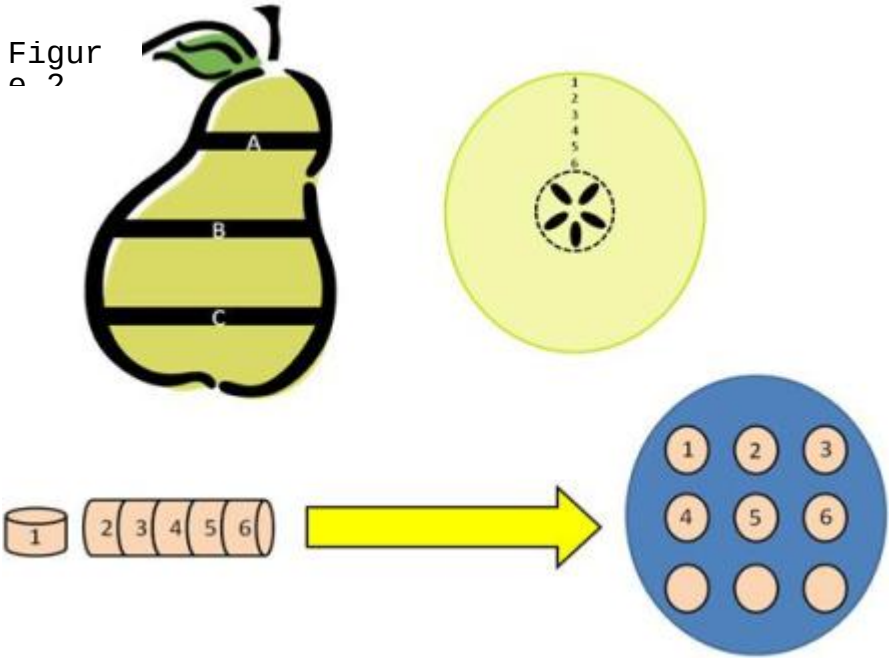


Figure 2 depicts the procedures employed to assess the productivity of callus formation by various parts of the pear fruit. Cores were taken from each of three selected parts of the pear; the top (A), middle (B), and bottom (C). The samples cores were cut into multiple sections and discs were cut from each section. Callus was able to grow from sections A, B, and C with no section showing any significant increased callus growth. While callus was derived from nearly all tissue, tissue nearest to the core (6 and 5) generally displayed the highest ability to grow callus. Optimal growth of callus was determined to occur by changing the SH media every three weeks.

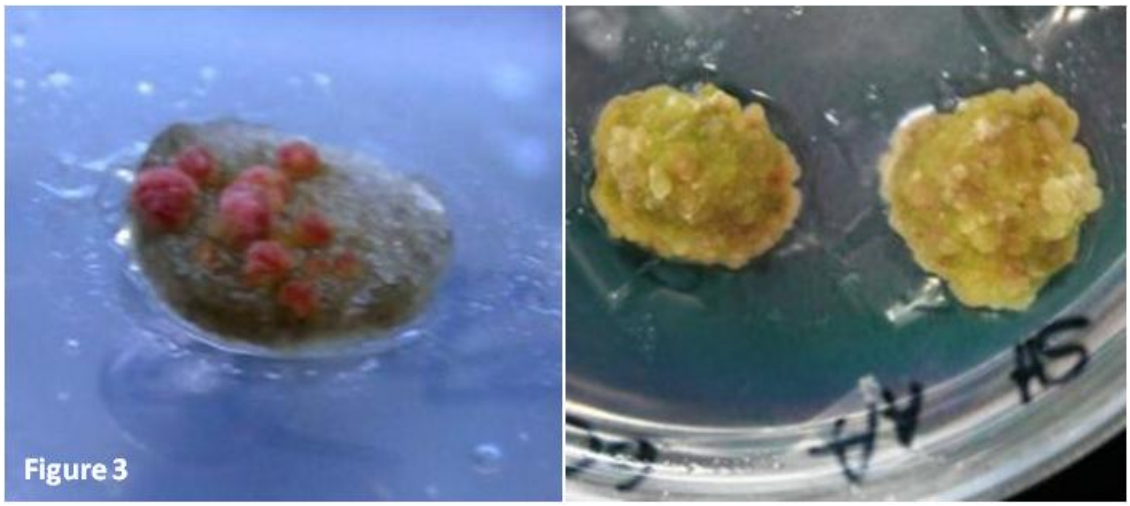
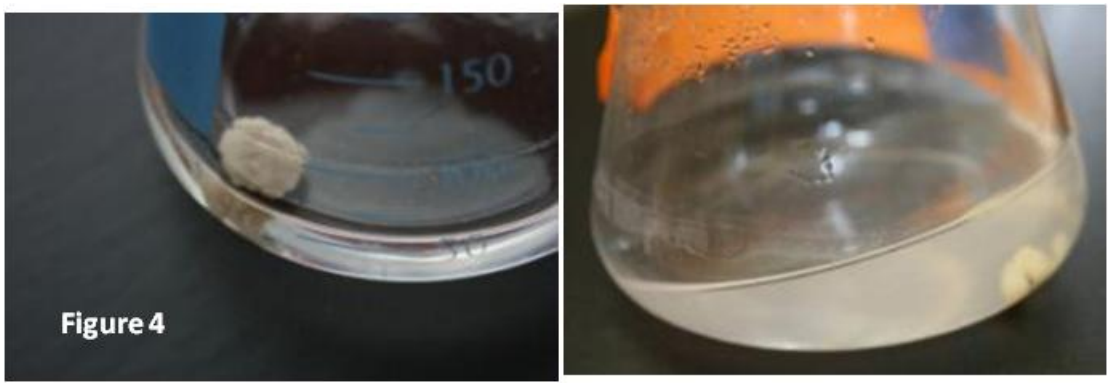


Figure 3 displays early callus growth (left) on pear tissue discs after two weeks of growth and a later stage of callus growth (right) after two months of growth. **Right panel** displays cellular growth after 40 days of inoculation. After sufficient callus was produced, callus tissue was transferred into liquid media (**Figure 4**). Cells were shaken to produce individual callus cells.



Single callus cells are required to perform CSI. Suspension cultures (**Figure 4**) have been established for Bartlett and D'Anjou. CSI can be utilized to produce pears with more desirable traits. These traits can potentially include longer storage ability, disease resistance, and dwarfing architecture. These non-transgenic improvements can be tailored to Pacific Northwest pears will increase consumer attraction and ultimately contribute to the local economy. With Bartlett and D'Anjou suspension cultures now established we can begin Controlled Sport Induction in 2008. We are currently in the process of increasing our stocks of the aforesaid suspension cultures. The method of CSI is a modified version of TILLING, a process that has been used in plants such as wheat, rice, Arabidopsis, and sugar beet.

In addition to Controlled Sport Induction, we plan to induce sports randomly via radiation. Some of our suspension cultures will be given to the WSU Nuclear Radiation Center. The samples will be bombarded with gamma irradiation. This irradiation causes random mutations in the plant DNA. Each individual cell will have a different single or combination of mutated genes. Mutated cells will be grown into plants and observations will be made upon the induction of desirable changes of the fruit and plant itself. Plants containing desirable mutations will be selected for by means of LICOR equipment acquired via a Genomics Matching Funds Grant from LiCOR Inc.

RESULTS AND DISCUSSION

Gene discovery is essential to identify the factors responsible for Pacific Northwest pear traits and can be exploited to improve the local economy's influence in domestic and international markets. Due to the narrow germplasm present in pears, a non-traditional program such as the Controlled Sport Induction method can be used exploit this knowledge to introduce new traits to existing varieties. New pear varieties could be developed to address immediate problems in the pear industry such as storage time and dwarfing as well as less immediate traits such as texture and color.

Controlled Sport Induction is becoming a realistic goal for improvement of pear traits. Samples of Bartlett and D'Anjou pear have been collected for the gene identification project. We have currently been successful in establishing a proficient RNA extraction technique in fruits. Within the year we plan to identify genes responsible for any number of fruit traits through the use of Differential Display. Development of callus and suspension cultures has been optimized for both Bartlett and D'Anjou. With the recent arrival of equipment we will begin CSI to target previously characterized genes 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.

Another exciting and vital development has been the initiation of apple genome sequencing by the PI at WSU. This is a public initiative that has attracted international partners. We also have access to genetically unique pear material from France that is highly amenable to genome sequencing. Due to the close relationship of pears and apples information generated in the latter species will accelerate biological research in pears as well.

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.
3. The preliminary concepts were presented at the WSHA meeting in 2007 by Scott Schaeffer, current lab manager and future graduate student in the Dhingra lab.
4. This work will be 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

Project Title: Crop load management and reflective fabrics in pears

PI: Internal staff
Organization: WTFRC
Telephone/email: Ines Hanrahan 509 669 0267 ines.hanrahan@co.yakima.wa.us
Tory Schmidt 509 669 3903 tory@treefruitresearch.com
Felipe Castillo 509 668 0043 castillo@treefruitresearch.com

Cooperators: Jonathan Toye (Extenday)

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

Item	2007	2008
Salaries	29,429	21,647
Benefits	9,417	6356
Wages	10,346	19390
Benefits (16%)	2,224	6331
Equipment	4,172	1500
Supplies		1508
Travel	5,000	548
Vehicle maint/ins.		5270
Miscellaneous		715
Less industry pmt.		3750
Total	60,588	**59515**

Footnotes: All chemicals and harvest supplies were provided by industry vendors.

WTFRC INTERNAL RESEARCH PROGRAM UPDATE

Summary

In 19 pear thinning trials conducted since 2003, ATS has been the most consistent bloom thinner, while urea has been ineffective under Washington conditions. BA products have consistently increased fruit size. Trials with Daybright reflective groundcover initiated in 2006 indicate yield gains in Bartletts by increasing fruit set and/or size.

1. Chemical thinner effects on pear crop load

Within a set of five trials we evaluated lime sulfur, ammonium thiosulfate (ATS), and urea as bloom thinners, as well as BA (Exilis Plus, MaxCel) and NAA as postbloom thinners. ATS (4%), LS (6%), and urea (5%) were applied at 80% bloom, BA (1%) and NAA (3.6oz/100gal) at 10 mm fruitlet size.

Table 1: Crop load effects of pear chemical thinning programs. WTFRC 2007.

TREATMENT	FRUITLETS/100 BLSM CLUSTERS	BLANKS (%)	SINGLES (%)	DOUBLES (%)	FRUIT (g)	BOX SIZE
Bartlett / Seedling - Sawyer						
ATS	72 b	52 a	31 ns	12 b	211 a	95
Exilis Plus	94 a	41 b	33	20 a	207 a	97
Control	96 a	42 b	29	21 a	196 b	102
Bartlett / Seedling - Buena						
ATS	70 a	48 b	38 a	12 a	175 b	114
MaxCel	44 b	65 a	27 b	7 b	198 a	101
Control	72 a	47 b	36 a	14 a	171 b	117
Bartlett / OH x F 97 - Monitor						
ATS	47 ns	62 ns	30 ns	8 ns	196 b	102
Exilis Plus	50	60	32	7	221 a	90
Lime sulfur	45	65	27	6	197 b	101
Urea	50	61	30	7	209 ab	96
Control	54	60	29	6	195 b	102
Bartlett / Seedling - Naches Heights						
ATS	116 ns	34 ns	28 ns	27 ns	no data	no data
Lime sulfur	103	39	31	20		
MaxCel	98	37	35	22		
NAA	90	40	36	19		
Urea	113	30	36	24		
Control	96	40	32	21		
Golden Russet Bosc / OH x F 97 - Tonasket						
ATS	58 a	54 b	35 a	10 a	248 ab	81
MaxCel	39 b	67 a	28 b	5 b	256 a	78
Urea	50 a	60 b	31 ab	9 a	241 bc	83
Control	58 a	56 b	31 ab	11 a	228 c	88

The Sawyer, Buena and Tonasket trials were sprayed by grower-cooperators, while those at Naches Heights and Monitor were applied with the WTFRC Proptec tower sprayer. Fruit set (fruitlets/100 clusters) was significantly reduced once (ATS, Sawyer), translating into higher individual fruit weight at harvest (Table 1). BA thinned fruitlets effectively in two of five trials, but always improved fruit size. Harvest fruit quality (finish, firmness, soluble solids, titratable acidity) was not affected by any treatment.

2. Effects of Daybright reflective fabric on pear fruit maturity and yield

Daybright, a reflective ground cover manufactured by Extenday, was applied for a second year in an established Bartlett block on a V-trellis (Sunnyside) and a young Bartlett block (Cashmere) from early bloom to harvest. 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. Significant results include (Table 2, 3):

- Overall yield was increased by 22% in Sunnyside, due to increased fruit set with no sacrifice in fruit size.
- Daybright increased individual fruit size by 13% in Cashmere.
- More fruit was observed in Daybright treatment tree centers at Cashmere.
- Sugars and acids were largely unaffected by Daybright.
- Treated fruit were more mature in Sunnyside, as indicated by decreased firmness.

Table 2: Effects of Daybright on pear fruit yield. WTFRC 2007.

		Barlett / OHxF Sunnyside		**Bartlett /OHxF 87 Cashmere**	
TREATMENT		Daybright	Control	Daybright	Control
Total yield (kg/tree)		45 a	37 b	46 ns	42
Total fruit ct (fruit/tree)		236 a	193 b	191 ns	179
Mean fruit wt (g)	1. pick	210 ns	209	272 a	240 b
	2. pick	183 ns	180	-	-
Percent of total fruit	1. pick	61 ns	61	-	-
	2. pick	39 ns	39	-	-
Yield efficiency					
Fruit/TCSA		1.9 a	1.5 b	2.6 ns	2.4
Kg/TCSA		0.4 a	0.3 b	0.6 ns	0.6

Table 3: Daybright effects on pear fruit maturity parameters. WTFRC 2007.

TREATMENT	SSC (% Brix)	TA (% Malic Acid)	Firmness (lbs)
Barlett / OHxF Sunnyside (first pick)			
Daybright	11.0 ns	0.289 b	16.5 b
Control	10.8	0.316 a	16.9 a
Barlett / OHxF Sunnyside (second pick)			
Daybright	10.8 ns	0.268 ns	15.0 b
Control	10.8	0.262	15.8 a
Bartlett /OHxF 87 Cashmere			
Daybright	12.4 ns	0.443 ns	17.5 ns
Control	12.5	0.430	17.6

Itemization of WTFRC projected expenditures

Item	2006-2007	2007-2008
Stemilt RCA room rental	24000	31842
Administration	58109	65085
Technology projects	55000	57514
Internal Research	48000	59515
Total	**185,109**	**213,956**

RCA Room rental
Xiao – Control of postharvest decay in pear
Xiao/Mattheis - Factors influencing development of d'Anjou pear scald and speckling (share one room)
Kupferman - Managing storage scald in Anjou pears **Total of 3 rooms**
- New technologies to control storage scald

Room rental cost: 5 @ $6368.42/year = $31,842.10

Technology funded projects

PI	Organization	Project Title	Technology
Yang	WSU	Chemical genomics	30000
Jones	WSU	Expanding and Stabilizing WSU-decision aid system	80309
Koselka	Vision Robotics	Automated picking hand development	27500
McConnell	Vision Robotics	Robotic tree fruit action system	360500
Whiting/ Dhingra	WSU Prosser	Factors affecting meristem fate in Rosaceae	22460
Schupp	Penn State	Mechanized blossom and green fruit thinning	17172
Kilmer	D&B Kilmer	Mechanized blossom and green fruit thinning - Darwin	14828
Hoheisel	WSU Extension	A database to aggregate research results and assess technologies	9078

561,847

Pear portion @ 9.4% $52,814

Projected funding -spring 2008: $50,000
Pear portion @ 9.4%: $ 4,700

Total estimated technology funding – Pear: $57,514

FINAL PROJECT REPORT
WTFRC Project Number: PH-05-500 (WSU Project #13C-3655-6299)

Project Title: Branch induction in pear trees with bioregulators

PI: Don C. Elfving
Organization: WSU Tree Fruit Research and Extension Center
Telephone/email: 509-663-8181 x252/delfving@wsu.edu
Address: 1100 N. Western Avenue
City: Wenatchee
State/Province/Zip: WA 98801

Cooperators: Dwayne Visser, WSU-TFREC

Other funding Sources

Agency Name: N/A

Total Project Funding: ***2005:*** 6,950 ***2006:*** 7,407 ***2007:*** 7,864

WTFRC Collaborative expenses: NONE

Budget History:

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	4,500	4,750	5,000
Benefits	1,530	1,615	1,700
Wages	200	220	240
Benefits	20	22	24
Equipment	0	0	0
Supplies	200	200	200
Travel	500	600	700
Miscellaneous	0	0	0
Total	6,950	7,407	7,864

Objectives of the project:

1. Determine the effectiveness of cyclanilide® as a soil-based, branch-induction treatment on young, vigorous pear trees in the year of planting in the orchard.
2. Determine whether proprietary cytokinin/gibberellin mixtures such as Promalin® or Maxcel® can be used prior to or at budbreak on vigorous, one-year-old wood to stimulate lateral branching in spring.
3. Compare pruning requirements for branched trees vs. those managed normally.
4. Establish one or more trials to assess the benefit of a multi-year branching treatment strategy on canopy development, pruning requirements and the onset of flowering and productivity.
5. Examine cytokinin and gibberellin treatments alone or in combination for stimulation of growth activity in latent buds on pear branches.

Significant findings 2005:

1. Application of cyclanilide to newly-planted 'Bosc' pear trees by soil drench resulted in minor growth effects on the central leader but no change in shoot or bud development in 2005.
2. Notching or scoring of bark on one-year-old, vigorous, upright 'Bartlett' pear shoots plus painting those cuts with 5,000 ppm Perlan (cytokinin/GA mixture) doubled branch development compared to untreated trees or trees receiving notching or scoring cuts only. Notching or scoring alone had no effects.
3. Increased fruit production in 2005 in 6th-leaf 'Bosc' trees was directly related to increased branching induced by spray applications of cyclanilide in June, 2003 (Fig. 1).
4. Soil drenches of cyclanilide as low as 50 – 150 milligrams of active ingredient per tree produced carryover effects on branching in the year following treatment applications. Pear trees are extremely sensitive to cyclanilide.
5. In a test of soil drenches of cyclanilide on newly-planted trees of five pear cultivars on several rootstocks in Oregon, cyclanilide again showed modest effects on shoot development, likely as a result of the relatively low vigor of these trees as they established their root systems in their first year in the orchard.

Significant findings 2006:

1. Application of cyclanilide to newly-planted pear trees by soil drench is ineffective for increasing lateral branching.
2. In a test of soil drenches of cyclanilide on newly-planted trees of five pear cultivars on several rootstocks at the Mid-Columbia Agricultural Research and Extension Center (MCAREC) in Hood River, Oregon, cyclanilide treatments at 5-20 mg/tree in 2005 produced no carryover effects in 2006.
3. Increased fruit production in 2005 in 6th-leaf 'Bosc' trees was directly related to increased branching induced by spray applications of cyclanilide in June, 2003. No effect of 2003 cyclanilide treatments on yield was observed in 2006.
4. Soil drenches of cyclanilide as low as 50 – 150 milligrams of active ingredient per tree produced carryover effects on branching in the year following treatment applications. Pear trees treated with 5-20 mg/tree of cyclanilide do not show carryover effects.

Significant findings 2007:

1. By the third year in the orchard, 'Golden Russet Bosc'/OHxF97 trees did not respond as strongly to soil applications of cyclanilide at 5, 10 or 20 mg/tree. Even trees treated in 2005, 2006 and 2007 with 20 mg cyclanilide per tree did not show strong secondary branching. Evidently more work needs to be done to determine how larger tree mass affects the sensitivity of pear trees to soil-applied cyclanilide.

2. Five pear cultivar/rootstock combinations treated with cyclanilide in fall 2006 with the same 5, 10 or 20 mg/tree did not produce the desired control over vigor and increase in branch development, emphasizing the idea that cyclanilide dose probably needs to be determined based on trunk cross-sectional area.
3. 'Bosc' trees treated in 2004 with higher doses of soil-applied cyclanilide (50 to 150 mg/tree) did not show any significant effect on fruit production in 2007 as a result of the very strong branching response in both 2004 and 2005 to cyclanilide applications.
4. Basal limb sections of both 'Bosc' and 'Kalle' (Red Clapp's) showed enhanced budbreak with applications of either thidiazuron (TDZ) at 2,500 ppm alone or TDZ + GA_{4+7} (ProVide) at 5,000 ppm. ProVide applied alone had no effect on latent bud development.
5. In an unusual observation, the combination TDZ/ProVide applications soaked under the flagging tape tied onto the limbs and stimulated cell division in the area under the flagging tape. This response is being explored for possible useful applications.

Results and discussion

A. Effectiveness of cyclanilide as a soil-based branching treatment (Objectives 1, 4).

Cyclanilide was shown to be ineffective when applied the year of planting. This result almost certainly is related to the virtual absence of a functional root system at the time of planting. By the time a root system has developed, the chemical product has dispersed or degraded. Applications starting in year 2 are very effective if the correct amount is applied. Overdosing is possible. Insufficient trials have been run to determine precisely how much cyclanilide is needed to produce good branching without overdosing. Also, as trees get larger, more cyclanilide may be needed. Spray application of cyclanilide to pear trees can produce a similar branching response without having to be concerned with absolute amounts of active ingredient,

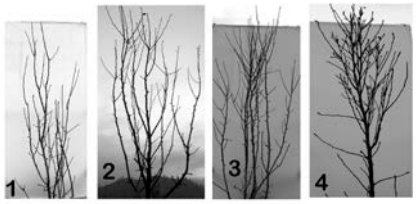


Fig. 1. 'Bosc' trees sprayed in June, 2003 with 0 (1), 5 (2), 10 (3) or 20 (4) ppm cyclanilide to induce branching. Photos taken fall, 2003.

B. Impact of cyclanilide on productivity (Objective 4).

Cyclanilide sprays on 4th-leaf 'Bosc' trees in 2003 led to yield increases in 2005 directly related to the amount of cyclanilide, which, in turn, was directly related to the amount of secondary branching developed (Fig. 1). Unfortunately, the improvement in yield seen in 2005 was not observed in either 2006 or 2007. Similarly, in another trial in which 'Bosc' trees received strong doses of cyclanilide, yield was not improved despite substantial effects for 2 years on shoot development. Since tree training was not practiced on trees in either trial, might implementation of better canopy-management strategies led to a different outcome?

C. Cytokinin/gibberellin effects on lateral branching (Objective 2).

Notching or scoring of one-year-old wood at budbreak did not improve branching. Painting either notches or scores with Perlan doubled the amount of branching over that occurring in control trees or those receiving only notching or scoring.

D. Compare pruning requirements for branched trees vs. those managed normally (Objective 3).

'Bosc' trees subjected to branching by soil-applied cyclanilide showed an increase in number of spring pruning cuts required per tree in direct proportion to the amount of applied product.

E. Stimulation of bud activity on "blind wood" in pear (Objective 5).

Thidiazuron (TDZ), a powerful cytokinin, was tested for efficacy in stimulating growth from latent buds on older limb sections (three to five year-old wood) of 'Kalle' (Red Clapp's Favorite) pear trees. TDZ at up to 1000 ppm did not produce significant changes in bud development on treated limb sections.

Acknowledgments:
The assistance and support of the following persons and organizations are gratefully acknowledged: Jeff Cawood, Mike Cawood, Dean Christie, Dick Cox, Rick Cox, Daryl Harnden, Jeff Henry, Jeff Holmer, Dr. Chris Ishida, Chris Olsen, Dwayne Visser, Don Weippert, Bayer CropSciences, Bayer Environmental Science, Cawood Orchards, Cox Orchards, Harnden Orchards, Holmer Orchards, Valent BioSciences, Washington Tree Fruit Research Commission, Weippert Orchards, WSU Agricultural Research Center.

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Elfving, D.C. and D.B. Visser. 2007. Bioregulators for managing growth, cropping and fruit quality in sweet cherry. Poster, Wash. State Hort. Assn. Annual Meeting, Wenatchee, WA.