Northwest Pear Research Review

Red Lion Hotel, Yakima

Thursday, February 18

Time	Page	PI	Title	Funding period
8:00		T. Schmidt	Wecome & housekeeping	
8:05		Schmitten	Introduction	
			Final Project Reports	
8:15	1	Kupferman	Commercial Anjou ripening and conditioning methods: extension	08
8:30	12	Mattheis	Factors influencing development of d'Anjou pear scald and speckling	07-09
8:45	20	Sugar	Pear fruit quality improvement	07-09
	30	Spotts	Decay risk models and novel decay control methodology: Written report only	08-10
9:00	36	Johnson	Rapid detection of fire blight pathogen: No cost SCRI report	07-09
9:15			Break	
9:30	47	Horton	Volatile sex attractants in pear psylla	08-09
9:45	54	Horton	Quantifying biological control of pear psylla in a cover crop system	07-09
10:00	63	Dhingra	Pear genome project	
10:15	68	Dhingra	Gene discovery & controlled sport induction (CSI) for pear improvement	07-09
			Break	
Group #			Continuing Project Reports: 10:45 - 12:00	
1	75	Einhorn	Cold hardiness of quince	09-11
1	83	Einhorn	Horner rootstock grower trials	09-11
1	91	Smith	Pacific Northwest pear rootstock trial	09-11
1	97	Evans	Pear rootstock breeding	09-11
2	100	Xiao	Control of postharvest fruit rots in pears	08-10
2	106	Johnson	Evaluation of integrated fire blight control technologies	09-11
2		Schmidt	Technology Committee: See tech reports in appendix	
2	113	Shetty	Health benefits of Washington and Oregon pears	09-10
2	120	Schmidt	Pear crop load management and rootstock field testing	09-11

FINAL PROJECT REPORT
WTFRC Project Number: PR08-804 (WSU Project # 13C-4164-1211)

Project Title: Comparison of commercial Anjou ripening and conditioning methods

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Cooperators: Select packinghouses

Other funding sources

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

WTFRC Collaborative Expenses: None

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

Budget History:

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

Footnotes:

[1] Chris Sater, Associate in Research.

[2] Fruit, laboratory supplies.

[3] Travel to warehouses and to Portland for the consumer tests.

[4] Fee for two consumer tests done at the Food Innovation Center, Portland.

GOAL

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

OBJECTIVES

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

SIGNIFICANT FINDINGS

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

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

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

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

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

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

Consumer Experiment 3—Anjou Pears That Had Met Their Chilling Requirement (Late-Season)
Treatments were: 1 day in ethylene, 1 day in ethylene plus 1 day in warm air, 1 or 2 days in warm air or 5 days ripening (no conditioning). Following conditioning all fruit was held in cold storage (33 ºF) for 7 or 8 days to simulate transit to retail market. Three days prior to consumer evaluation all fruit was removed from cold storage and held at 72 ºF until testing. The ripening only treatment was removed from cold storage 5 days prior to consumer evaluation and held at 72 ºF until testing. Consumers scored the 2-day conditioned fruit (2-day air and 1-day ethylene + 1-day air) higher in pear flavor, sweetness, juiciness and texture as compared with the other treatments.

- The 2-day air and 1-day ethylene + 1-day air pears were ranked first ("best') by a 2:1 margin over the 1-day air and 5-day ripening pears (30% and 32% vs. 17% and 16%, respectively). The 1-day ethylene pears came in last.
- The 2-day air and 1-day ethylene + 1-day air pears scored significantly higher in the sweetness liking category, even though the soluble solids for all treatment was the same.

Commercial Conditioning Systems
Conversations with packers in Wenatchee, Yakima and Hood River determined that all packers who condition pears use a similar system: 12 to 24 hours warming with forced air to a pulp temperature of 65 ºF, followed by 24 hours of 100 ppm ethylene at 65 ºF. Because previous research by our lab has shown that ethylene will penetrate all box types (Euro, standard, poly-lined) equally, it was not necessary to ripen Anjou pears in commercial chambers.

MATERIALS AND METHODS

To address objectives 1 and 2, consumer testing on conditioned Anjou pears was done three times: once with Anjou pears that had not met their chilling requirement (October) and twice with pears that had met their chilling requirement (December and April).

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

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

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

Anjou pears from a single grower lot harvested between Sept 15th and Sept 21st were packed into Euro boxes with plastic trays by a commercial packer. The pears were obtained on Sept 29th so they had not received sufficient time in storage to have completed their chilling requirement. Fruit quality was evaluated at time of receipt, with the pears averaging 13.4 lbf with a color rating of 4.8 on a 1 to 10 scale (1 = dark green to 10 = yellow).

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

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

Consumer Experiment 2—Anjou Pears That Had Met Their Chilling Requirement (Mid-Season)

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

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

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

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

	Pallet shroud				Boxes			
	24 hrs	End of treatment			24 hrs	End of treatment		
Treatment (days)	C_2H_4 (ppm)	C_2H_4 (ppm)	O_2 (%)	CO_2 (%)	C_2H_4 (ppm)	C_2H_4 (ppm)	O_2 (%)	CO_2 (%)
1	252	252	19.5	1.4	179	179	18.4	2.5
2	255	490	18.2	2.5	241	411	16.9	3.9
4	262	988	15.4	4.8	270	816	14.5	5.8

Consumer Experiment 3—Anjou Pears That Had Met Their Chilling Requirement (Late-Season)

Anjou pears from a single grower lot harvested between Sept 27th and Sept 29th were packed into Euro boxes with plastic trays by a commercial packer. The pears were obtained on March 16th. Fruit quality was evaluated at time of receipt, with the pears averaging 12.6 lbf.

There were five conditioning treatments for the consumer trial in Portland: conditioning for 1 day with ethylene, 1 day ethylene plus 1 day in warm air, 1 or 2 days in warm air and 5 days ripening (no conditioning). Prior to conditioning all fruit was stored in the cold (33 ºF). Twenty-four hours prior to conditioning the fruit was placed into a warm room (72 ºF). Conditioning was done in shroud covered box pallets using Ethylene Release Canisters (ERCs) (Balchem Corporation, New Hampton, NY). The conditioning treatments reached at least 50 ppm ethylene within 6 hours in the shrouds. Following conditioning, all fruit was returned to cold storage (33 ºF) for 7 or 8 days to simulate transit to retail market. Three days prior to consumer evaluation all fruit was removed from cold storage and held at 72 ºF until testing. The ripening only treatment was removed from cold storage 5 days prior to consumer evaluation and held at 72 ºF until testing.

RESULTS AND DISCUSSION

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

The 6-day ethylene pears scored highest in every preference category and were ranked first ("best") by 74% of consumers (Table 2). Reasons for liking and disliking are listed in Table 8. Overall, 32 out of 480 pears were given an overall liking score of 9 (highest possible score) and 26 pears were given an overall liking score of 1 (lowest possible score). The average scores for all the preference categories, along with the average firmness and soluble solids values for these fruit are shown in Table 3.

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

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

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

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

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

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

Fruit with an overall liking score of 9 (liked extremely) consisted mostly of 6-day ethylene-treated fruit (81%) and was most often described as "sweet" (53%). Other words consumers used to describe the characteristics of this fruit included, "perfect pear," "sweet and juicy," and texture that "melts." The soluble solids levels of the highest scored fruit, was actually slightly lower than that of the lowest

scored fruit, so the characteristic described as "sweet" by consumers is not necessarily related to the measurable solids content of the fruit.

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

Consumer Experiment 2—Anjou Pears That <u>Had Met</u> Their Chilling Requirement (Mid-Season)
The 4-day ethylene pears scored highest in every preference category and were ranked first ("best") by 50% of consumers (Table 4). Reasons for liking and disliking are listed in Table 8.

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

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

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

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

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

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

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

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

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

Consumer Experiment 2—Anjou Pears That <u>Had Met</u> Their Chilling Requirement (Late-Season)
The 2-day air and 1-day ethylene + 1-day air pears were ranked first ("best') by a 2:1 margin over the 1-day air and 5-day ripening pears (30% and 32% vs. 17% and 16%, respectively). The 1-day ethylene pears came in last (Table 6). Reasons for liking and disliking are listed in Table 8.

Table 6. Consumer liking scores for six Anjou pear attributes and pear quality measurements for each treatment; consumer sensory trials at the OSU FIC, Portland Oregon, **March 31 – April 1, 2009**.

	Consumer Liking Scores*							Pear Quality	
Treatment	**Overall**	**Pear flavor**	**Sweetness**	**Juiciness**	**Firmness**	**Texture**	**Ranked First****	**Soluble solids (%)**	**Firmness (lbf)**
1-day air	6.2 a	6.0 abc	5.0 b	5.6 b	6.3 a	6.1 ab	17%	14.0	3.9
1-day ethylene	6.1 a	5.8 c	5.2 b	5.6 b	6.2 a	5.8 b	6%	13.9	4.0
2-day air	6.6 a	6.6 a	6.0 a	6.8 a	6.6 a	6.7 a	30%	14.1	3.1
1-day ethylene + 1-day air	6.6 a	6.4 ab	6.1 a	6.7 a	6.7 a	6.6 a	32%	14.0	3.1
5-day ripening	6.0 a	5.9 bc	5.3 b	5.8 a	6.2 a	5.8 b	16%	13.9	3.4

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

Overall, 26 out of 600 pears were given an overall liking score of 9 (highest possible score) and 22 pears were given an overall liking score of 1 or 2 (lowest scores). The average scores for all the preference categories, along with the average firmness and soluble solids values for these fruit, are shown in Table 7.

Fruit with an overall liking score of 9 (liked extremely) was split evenly between 1-day ethylene + 1-day air, 2-day air and 5-day ripening treatments. This fruit was described as "perfect" or "nearly perfect" by 65% of consumers.

Fruit with an overall liking score of 1 or 2 (disliked extremely and disliked very much) was split evenly between 1-day ethylene + 1-day air, and 1-day ethylene treatments. The most common reason given for disliking the fruit was a flavor component (82%). Words consumers used to describe the characteristics of this fruit included, "too tart," "bitter," and "not ripe."

Table 7. Average preference scores, firmness values, and soluble solids levels for the highest (9) and lowest (1 and 2) scored fruit over two days of sensory testing at the OSU FIC, Portland Oregon, **March 31 – April 1, 2009**.

Overall Liking	Pear Flavor	Sweetness	Juiciness	Firmness	Texture	Firmness (lbf)	Soluble solids (%)
9.0	8.7	8.3	8.5	8.5	8.5	3.1	14.2
1.6	2.0	1.8	3.2	3.9	3.3	4.1	13.6

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

Consumer Comments

In Experiment 1 (October), the most common reason for liking was juiciness (42%), followed by sweetness (35%). In Experiment 2 (December), these attributes were reversed, with sweetness the most common (42%) followed by juiciness (23%). The results for Experiment 3 (April) were similar to Experiment 2, with sweetness by far the most common reason for liking (56%). It is interesting that firmness is only the third most common reason for liking, below sweetness and juiciness. In all three experiments, lack of flavor was the most common reason for disliking (33%, 35% and 43%, respectively) (Table 8).

Table 8. Reasons for liking and disliking pears

	Reasons for Liking/Disliking Fruit		
	Experiment 1, Oct. 2008	**Experiment 2, Dec. 2008**	**Experiment 3, Apr. 2009**
Reasons for Liking			
Juiciness	**42%**	23%	11%
Sweetness	35%	**42%**	**56%**
Firmness	17%	21%	13%
Tartness/sourness	3%	5%	8%
Other	3%	8%	9%
Smell/aroma	1%	0%	3%
Reasons for Disliking			
Lack of flavor	**33%**	**35%**	**43%**
Too hard	29%	18%	8%
Gritty texture	16%	13%	11%
Too soft	7%	14%	3%
Lack of sweetness	5%	2%	8%
Too tart or sour	4%	7%	16%
Lack of juiciness	4%	6%	3%
Other	2%	3%	4%
Not tart or sour enough	0%	1%	2%
Skin color	0%	1%	3%

Ripening Expectation

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

Days to Ripen	Dec. 2008 Test	Apr. 2009 Test
1 to 2	36%	27%
3 to 4	54%	58%
5 to 6	10%	15%

Response to Ethylene as a Conditioning Agent

Ethylene used during conditioning speeds the ripening of Anjou pears as compared to warm room conditioning as shown in Tables 2 and 4. An additional contribution of ethylene conditioning is the promotion of uniformity of ripening. Thus a box, pallet or truckload of Anjou pears conditioned with ethylene can be expected to ripen more uniformly. This can be seen by comparing the standard deviations in both the tests performed with the fruit conditioned for the consumer trials, but also in more detailed laboratory studies that were run concurrently (Tables 9 and 10).

Table 9. Comparisons of firmness and standard deviations between the most acceptable ethylene and the most comparable air conditioned fruit used in the consumer trials.

	Experiment 1		Experiment 2		Experiment 3	
Firmness	**6-day ethylene**	**7-day air**	**4-day ethylene**	**5-day air**	**1-day ethylene +1-day air**	**5-day ripe only**
Minimum	1.4	6.4	1.4	1.3	1.8	1.9
Maximum	4.8	19.3	4.1	7.0	5.0	6.4
Average	**2.4**	**11.6**	**2.5**	**2.7**	**3.1**	**3.4**
Std Dev	***0.6***	***1.9***	***0.5***	***1.3***	***0.6***	***0.9***

Table 10. Standard deviations after various conditioning treatments followed by ripening to ideal eating firmness of approximately 2 lbf.

	Days			Firmness (lbf)			
Treatment	**Conditioned**	**Cooling**	**Ripening**	**Minimum**	**Maximum**	**Average**	**Std Dev**
Air	7	3	1	1.54	6.07	**2.58**	***1.30***
Air	7	3	3	1.01	1.72	**1.36**	***0.23***
Air	5	3	3	1.17	6.54	**2.55**	***1.73***
Air	5	3	5	1.38	4.10	**2.45**	***0.86***
Ethylene	4	0	0	1.78	4.88	**2.83**	***0.90***
Ethylene	4	3	1	1.70	2.99	**2.13**	***0.39***
Ethylene	2	3	3	2.44	3.09	**2.85**	***0.23***
Ethylene	2	3	5	1.36	2.68	**1.83**	***0.38***
Ethylene	1	3	7	1.67	2.84	**2.03**	***0.37***

EXECUTIVE SUMMARY

This research centered on comparing different conditioning treatments at three time periods: immediately after harvest when chilling has not been met (early October), after mid-term storage (December) and after long term storage (April), when chilling requirements had been met. Pears were conditioned and ripened for 3 to 4 days then served to consumers (120 consumers per date). This large number of consumers allowed us to obtain solid information on conditioning preference, the expectation for time to ripen and ideal firmness when ripe and to pair this data with our objective evaluations of pear quality.

Defining the Target.
Consumers (360) preferred Anjou pears that were between 2.2 and 3.9 lb firmness at time of consumption. They defined an excellent quality pear as being sweet and juicy. They desired a pear that will ripen to that firmness within 4 days of purchase. Consumers gave sweetness as the most important reason for liking a pear—above firmness or juiciness. Lack of flavor was the principle reason stated for disliking a pear.

Conditioning to Reach the Target
Early season (October) Anjous at this time had not obtained sufficient chilling to ripen quickly. Therefore, conditioning treatments were 2, 4, or 6 days with ethylene or 7 days without ethylene followed by 3 days of ripening. The fruit not conditioned with ethylene did not reach edible firmness and remained at 11 lbf even after a total of 10 days in warm air. Ethylene accelerated conditioning, but the pears conditioned for 6 days were the only ones that reached the target firmness (2.2 lbf). These fruit scored higher than that of any other treatments.

Mid-season (December) Anjous were easier to condition. Conditioning treatments were 1, 2 and 4 days with ethylene compared with 5 days in warm air without ethylene followed by ripening, Pears conditioned with ethylene for 4 days were 2.5 lbf after ripening and scored highest in all categories.

Long-term stored (March) Anjous were also easy to condition. Conditioning treatments were 1 day in ethylene, 1 day in ethylene followed by 1 day in warm air, or 1 and 2 days in warm without ethylene followed by ripening, compared with 5 days ripening only (no conditioning). There was no difference in overall liking or firmness liking for any treatment. Consumers scored the 2-day conditioned fruit (2 days in air and 1 day in ethylene followed by 1 day in air) higher in pear flavor, sweetness, juiciness and texture as compared with the other treatments. They also ranked fruit in these treatments higher. Thus, ethylene conditioning did not improve consumer liking or ranking of the 2-day conditioned fruit at this time of year.

Consumers preferred ethylene-treated fruit to those conditioned with warm air even at the same firmness during the first two trials (October and December). In the third trial (April) consumers preferred fruit that had been conditioned for a total of 2 days (with or without ethylene) over fruit that was conditioned for 1 day or ripened only.

FINAL PROJECT REPORT
WTFRC Project Number: PR-07-706

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

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Cooperators: none

Other funding sources: None

WTFRC Collaborative expenses: none

Budget History:

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

Footnotes: *0.5 salary for GS11 postdoctoral research associate

Objectives:

1. Characterize what if any relationship exists between fruit physiological status (including peel metabolic profiles) at harvest and lot to lot susceptibility to low O_2-induced peel speckling or superficial scald development during storage.

2. Identify changes in pear metabolic profiles that are coincident with development of speckling and superficial scald induced using postharvest environments or protocols known to enhance speckling or scald development.

3. Develop postharvest protocols to manage scald and speckling development using available or new postharvest technologies as appropriate.

The risk of peel disorder development, particularly superficial scald (scald), during storage of d'Anjou pear is a significant factor influencing postharvest management strategies for this cultivar. Issues (efficacy, expense, logistics, residues) with current disorder control strategies based on antioxidant chemical application suggest development of additional strategies less reliant on chemical use would be of benefit to the industry. Storage of d'Anjou fruit at less than 1% O_2 has prevented scald development under experimental conditions, however, the risk of development of another peel disorder, speckling, and the internal disorder pithy brown core, increase when fruit are stored at less than 1% O_2. Previous research indicates storage at less than 1% O_2 can effectively control scald while avoiding anaerobiosis and potential development of off-flavors. A comprehensive survey of peel metabolism based on a metabolic profiling approach has potential to identify perturbations in metabolic pathways that may be linked to speckling development. Development of speckling is lot specific, but factors that initiate and influence the occurrence of speckling and lot to lot susceptibility have not been characterized. Identification of factors that influence speckling development may provide a means to develop low O_2-based storage protocols that control speckling while also controlling scald. Effective speckling control strategies that allow the use of less than 1% O_2 during d'Anjou storage have the potential to alter postharvest management of this cultivar to promote retention of fruit quality while avoiding development of peel disorders.

Significant Findings:

- Ultra-low O_2 at 0.5% or lower prevented scald in all lots.
- Speckling developed on 1 in 8 lots during the first two years of the project.
- Core browning and core cavitation developed in all lots stored at or below 0.8 % O_2.
- Delaying CA up to 10 days after harvest did not prevent low O_2 core disorders.
- Fruit stored at the critical O2 concentration developed calyx browning.
- Differences in peel metabolic profiles for fruit stored in 0.4% or 1.5% O_2, or air were detected at one month and continued through 9 months.
- Some lots stored in UA through 4 months did not soften to eating ripe during 7 days at 68 ^{o}F.

Results and Discussion

Of the nine orchard lots used in the three years of this project, the O_2 concentration at which a change in chlorophyll fluorescence was detected was 0.2% for 7 lots and 0.3% for the other 2 lots. Fruit maturity at harvest based on firmness, soluble solids, titratable acidity and color was not associated with the different critical O_2 concentrations. 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 with Anjou pears.

Scald did not develop on fruit stored in O_2 at or below 0.5%. Scald development on fruit from all lots stored at 1.5% O_2 was lower compared to fruit stored in air but higher than fruit stored at lower O_2 concentrations. Delaying CA for up to 10 days did not affect efficacy of scald control regardless of the CA O_2 concentration, and an initial low O_2 stress at 0.1% between days 7 and 13 after harvest did not change scald efficacy or result in tissue injury.

Peel speckling was observed on one lot in the 2008-09 season (Table 1). Speckling developed during the 7 day warm room period after removal from storage after 9 months. Symptoms were present on fruit previously stored in air (89% incidence) and on fruit stored in 0.2% O_2 (11%) or 1.1% O_2 (15%). The lack of symptoms at removal from storage is not typical compared to previous experiments. Fruit in the same experiment stored at 0.5, 0.8, or 1.4% O_2 did not develop speckling in contrast to previous reports where speckling increased as CA O_2 concentration decreased. Fruit on which speckling developed were not subjected to metabolic analysis was not part of this experiment.

Core disorders (Figure 1) were present in fruit from all lots stored at or near the critical O_2 concentration at which a change in chlorophyll fluorescence was detected (Table 2). In no case was a change in fluorescence evident after the initial O_2 increase to the final O_2 setpoint. This indicates that metabolic stress occurring at the low O_2 setpoint over the course of the experiments is not detectable by monitoring chlorophyll fluorescence. Core disorders ranging from browning of the seed cavity walls to severe cavitation were evident in as little as 2 months after harvest for many lots. While seed cavity wall browning could by some be considered a minor defect as it impacts fruit tissues typically discarded, the symptoms were very noticeable when present.

Calyx-end browning (Figure X) occurred only on fruit stored at the critical low O_2 concentration. The affected area had a russet-like appearance beginning around the calyx opening and increasing in size over time. Symptoms were observed as early as 2 months after harvest. Decay spread through the affected tissues at later (6-8 months) storage durations.

Shrivel was an issue in some experiments for fruit stored at or below the critical O_2 concentration (Table 1). For example, all fruit stored at 0.05 or 0.2% O_2 for 6 or 8 months had some shrivel in a 2008/09 experiment. Fruit stored in air or in 0.5 to 1.4% O_2 did not show shrivel. It is possible fruit stored at the lowest O_2 concentrations experienced higher water loss due to lack of cuticle development under low O_2 conditions.

Fruit from 5 of the 9 orchards stored under UA conditions softened slower relative to fruit stored in air or CA after 2 months storage. This residual impact of low O_2 CA has been observed in our previous work with pears. A possible means to overcome the low O_2- induced softening delay may be to delay CA. An experiment where half of a lot of fruit was held 7 days at 33 ^oF prior to establishment of low O_2 CA softened normally after 2 months compared to fruit from the same lot for which CA was established within 36 hours of harvest.

Ethanol accumulation during low O_2 stress has been associated with physiological disorders in other studies. We did not find a clear relationship between ethanol content and injury development in fruit stored near the critical low O_2 concentration until after symptoms were observed (Figure 2). Fruit from 5 lots were stored 0.2% O_2 above the critical O_2 concentration as determined by chlorophyll fluorescence, or were stored at 1.5% O_2. Fruit from all lots stored close to the critical O_2 concentration developed core disorders, but in only 3 of 5 lots was ethanol determined to be higher at some point during storage in the low O_2 fruit. While several lots stored using HarvestWatch technology had higher ethanol after 12 weeks compared to fruit stored in standard CA, earlier

occurrence of core browning indicates ethanol accumulation in this case may be and effect rather than a cause of the disorder.

Analyses of many fruit compounds indicated fruit could be chemically differentiated by storage treatment after one month (Figure 3). The differences in these profiles were due to a number of individual compounds including but not limited to amino and organic acids, sugars, vitamins, antioxidants, sterols, and pigments. Patterns of some individual compounds included a large reduction in vitamin C during the first 4 weeks after harvest regardless of storage regime and a more moderate reduction in vitamin E throughout the storage period. None of the storage treatments effectively slowed loss of any of these compounds over the course of the storage period (Figure 4). Core browning occurred in fruit stored in UA at 2 months and after indicating levels of these 3 compounds with anti-oxidant activity do not appear to be related to development or resistance to core browning.

A number of peel chemicals including including ursolic acid and β-sitosterol with putative cholesterol-lowering and antioxidant capacity were found in the peel and were differentially impacted by both storage atmosphere and storage duration. A number of related compounds that may be sterols or other triterpenoids were also detected. Considerable clinical evidence in the medical literature links phytosterols with positive health effects related to their antioxidant properties. These compounds were also differentially impacted by storage environment with both increase and decreased content observed related to storage environment and storage duration. We have recently observed similar compounds in apples have patterns related to superficial scald development. More work is needed to confirm the identity of these possible sterols and to determine what if any relationship they have to peel disorders. At harvest indicators indicating the potential for scald or other disorders were not identified during this project. Additional studies with multiple lots are needed to further investigate the potential for at-harvest prediction of disorder susceptibility.

Table 1. Peel speckling on Anjou pears. CO_2 in all CA treatments =0.5%. Fruit stored 9 months at 33 ^{o}F and evaluated after 7 days at 68 ^{o}F.

% O_2	% speckling	% scald	% core browning	% shrivel
Air	89	56	0	0
0.05	0	0	0	100
0.2	11	0	22	100
0.5	0	0	24	0
0.8	0	29	24	0
1.1	15	46	6	0
1.4	0	43	29	0

Table 2. Core browning incidence(%) in Anjou pears stored in controlled or ultra-low O_2 atmospheres or air. Ultra-low O_2 concentration = O_2 concentration at which change in chlorophyll fluorescence detected + 0.2%. *:stored in 0.5% O_2. **: evaluated after 3, 6, or 9 months.

Storage	1.5% O_2, 0.5% CO_2			0.4% O_2, 0.5% CO_2			air	
Months	2	4	6	2	4	6	2	4
Orchard 1*	0%	0	0	39	6	28	0	0
2	0	0	0	11	89	67	0	0
3	0	0	0	33	39	39	0	0
4*	6	11	6	83	56	67	0	0
5	17	28	33	61	63	67	0	0
6	0	0	0	56	39	94	0	0
7	17	17	6	17	18	22	0	0
8**	0	12	29	0	6	24	0	0
9	17	0	19	50	50	50	0	0

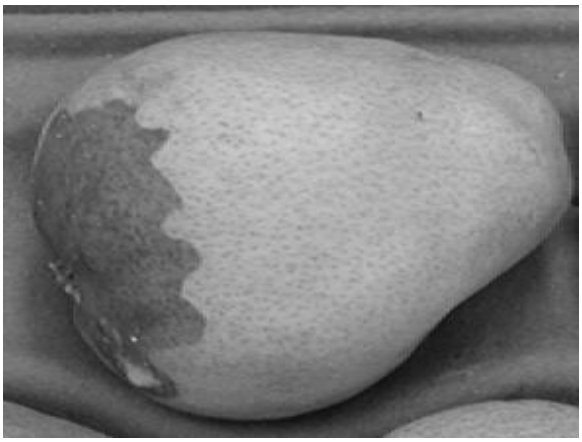
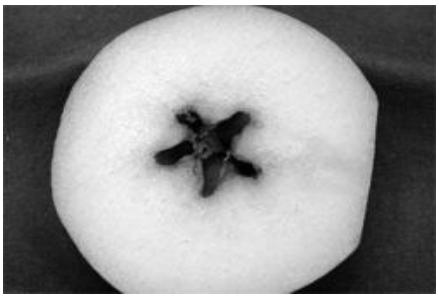
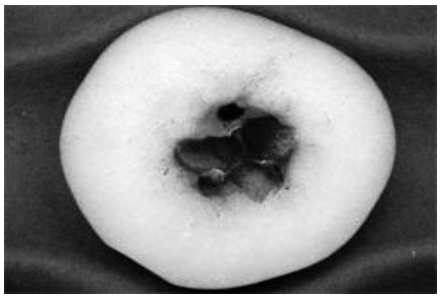


Figure 1. A. Calyx-end breakdown. B. Undamaged pear.



C. Core browning.



D. Core browning with cavitation.

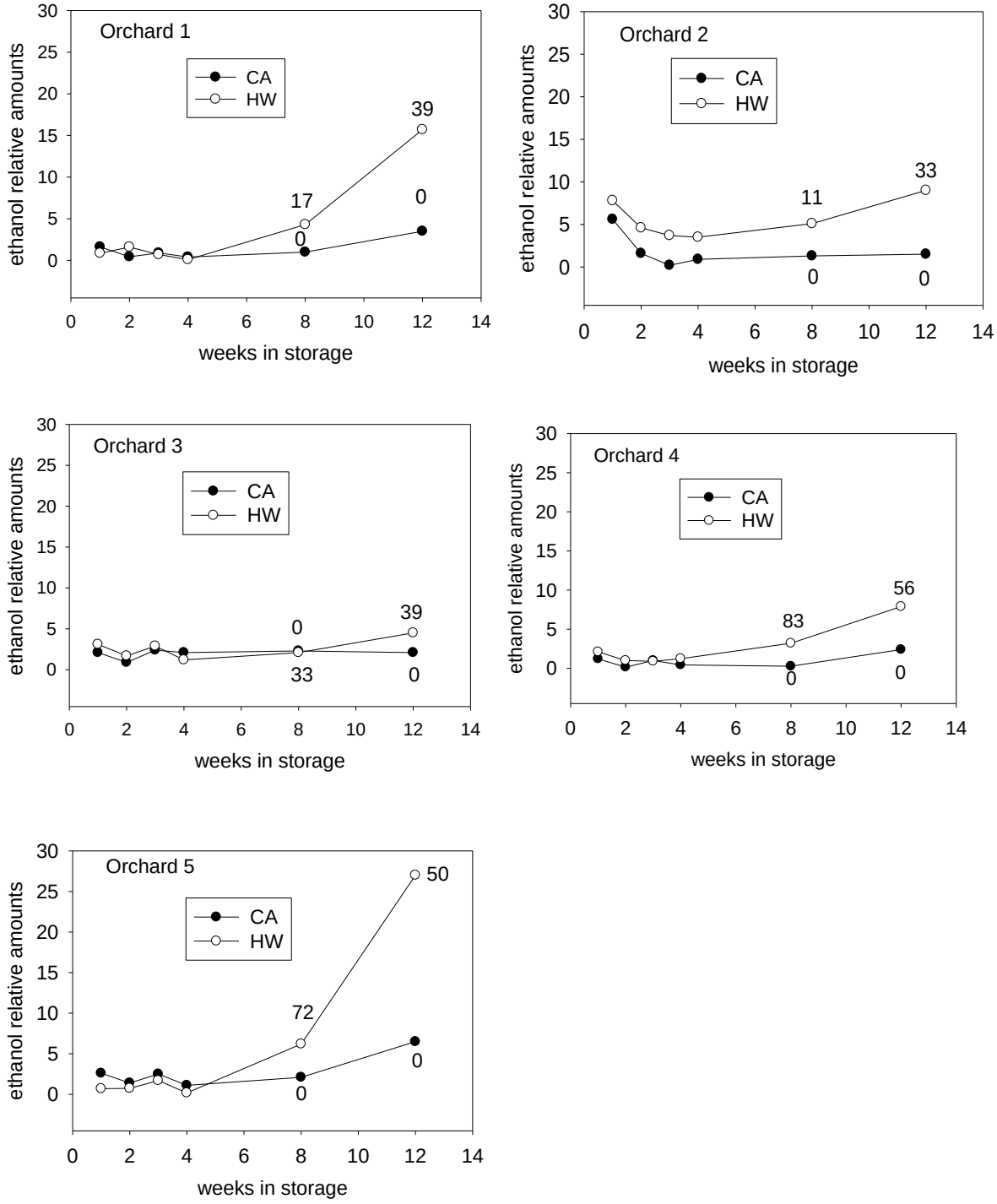


Figure 2. Ethanol content of Anjou pears after storage in 1.5% O_2 (CA) or 0.4% (orchards 2,3,5) or 0.5% O_2 (orchards 1,4) with 0.5% CO_2. HW: Harvest Watch: chlorophyll fluorescence monitoring equipment used to determine low O_2 setpoint.

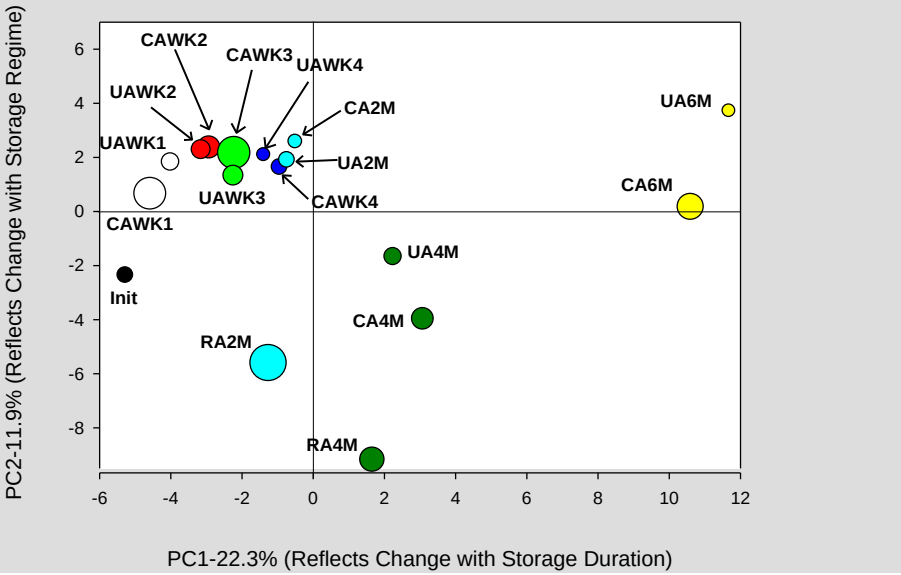
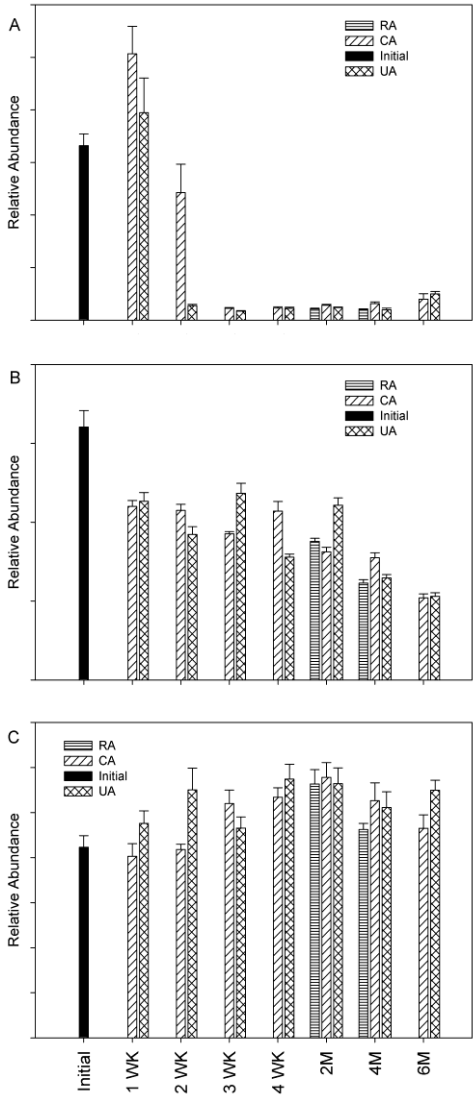


Figure 3. Separation of Anjou peel metabolic profiles based on storage duration and storage atmosphere. Fruit were stored up to 6 months in air (RA) or 0.5% CO_2 with 1.5 (CA) or 0.5 (UA) % O_2 at 33F.

Figure 4. Content of Anjou pear (A) vitamin C, (B) vitamin E, and (C) β-carotene during storage. Fruit were held at 33 ^{o}F in air (RA), 1.5% O_2 with 0.5% CO_2 (CA), or 0.5% O_2 with 0.5% CO_2 (UA). Samples were collected the day fruit were removed from storage.



Executive Summary

Monitoring chlorophyll fluorescence did not predict development of Anjou internal disorders. While some lots did not develop scald when stored based on identification of the critical O_2 concentration at which a change occurred in chlorophyll fluorescence, the risk of core disorders was high for fruit stored at less than 1% O_2. Other potential issues to be addressed prior to commercialization of this technique include a lack of early season (2-4 months after harvest) softening as well as calyx breakdown and shrivel should the critical O_2 concentration be underestimated. Implementation of low O_2 storage for scald control will require further research to identify risk factors at harvest or during storage that can provide a means to avoid low O_2 induced disorders while affording scald control. The lack of typical peel speckling in multiple lots and seasons is puzzling but was offset in these studies by the propensity for core disorder development in fruit stored under low O_2. The lack of peel speckling in these studies in our opinion does not allay the risk of this disorder that has been documented to occur under low O_2 storage. This is due to the lack of known system-wide changes in Anjou production practices that would have eliminated susceptibility to this disorder. Until more information regarding speckling and its causes are known, additional research focused on Anjou responses to low O_2 are likely to encounter the disorder.

Ethanol has long been associated with fruit disorders ranging from off-flavors to a variety of peel and internal browning or breakdown. The association has typically been observed due to an accumulation of ethanol in fruit with one or more quality problems. High concentrations of ethanol in fruit are typically the product of periods of O_2 stress when the O_2 concentration is too low to support normal metabolism. However, ethanol is usually present during normal ripening, a fact that prevents establishment of an unquestionable cause and effect relationship between ethanol accumulation and browning disorder development. The studies conducted for this project do not support a direct relationship between ethanol and subsequent development of injury as the largest accumulation occurred after injury began to be observed. While further studies of ethanol and related metabolism are needed in relation to development of commercial low O_2 storage for Anjou, the current results indicate at least that monitoring of ethanol alone is likely to not be sufficient to avoid disorder development.

Metabolic analyses revealed a number of compounds not known to be present in pear fruit that based on clinical trials have positive effects on human health. These compounds are sterols and are referred to as phytosterols in the relevant medical literature. Our work showed the compounds we think are sterols exhibit various patterns during storage in relation to duration and atmosphere. Both increased and decreased trends were found for different compounds, and further work to characterize these compounds in relation to storage environments as well as the onset and progression of disorders may provide a means develop at harvest or during storage information that can predict and/or diagnose disorders. This information, particularly at harvest, would have utility in postharvest management to assist in decisions related to at-harvest treatment for scald. Similar compounds in apple fruit have recently been identified by Dave Rudell as acylated sterol glycosides and their metabolism appears to be very responsive to low temperature as well as controlled atmospheres. There is evidence to support a role for some of the apple compounds as a means to resist stress or as indicators stress has occurred. Based on those results, future work with pears to determine if similar properties exist among the pear compounds found to date may be a means to explore ways to mitigate the negative responses to low O_2 storage.

FINAL PROJECT REPORT

Project Title: Pear fruit quality improvement

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City: Medford
State/Province/Zip Oregon 97502

Total Project Funding: **Year 1:** 28,997 **Year 2:** 28,997 **Year 3:** 28,997

Budget History:

Item	2007	2008	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

Significant Findings:

1. **Developing early ripening capacity in winter pears**.

- 50°F was the most efficient temperature for conditioning (or "satisfaction of the chill requirement") for Comice and Anjou pears among a range of temperatures tested between 31 and 65°F. Both colder and warmer temperatures required longer times for chilling satisfaction.
- As fruit maturity advances through the harvest season, the minimum conditioning time decreases. Relatively short periods at 41°F may be used to condition later-harvested Anjou pears while maintaining adequate firmness for shipping.
- Combinations of ethylene conditioning and intermediate temperature conditioning can substantially reduce the time between harvest and development of ripening capacity in winter pears.

2. **Postharvest decay control programs**.

- Orchard-based decay management programs using summer calcium chloride sprays followed at 1 week pre-harvest with fungicides Pristine, Flint, or Topsin M can both reduce overall postharvest decay levels and reduce the impact of a significant delay in application of postharvest fungicides due to storage of field-run fruit.
- A single-bin drench system for applying postharvest fungicide to fruit in bins prior to truck loading in the orchard may contribute to an orchard-based decay management program.

3. **Fruit size enhancement**. Over six years of study, urea treatments applied as 5% solutions at full bloom to Bartlett pears increased average fruit weight and the tons per acre of fruit size 90 and larger. Applications at 7-7.5% concentration were more consistent than 5% solutions. Over three years of study, MaxCel applied at 125 ppm 6-benzyladenine enhanced fruit size in Bartlett pears when applied 10 days after petal-fall. Fruit size distribution for untreated, unthinned trees peaked at size 100, while fruit size distribution from trees treated with MaxCel 125 ppm at 10 days after petal-fall peaked at size 80.

4. **Russet management**. Long-term correlations between russet and weather factors point to the period 2-3 weeks after petal-fall as the critical period for russet susceptibility. Spray mixtures consisting of any two of the three products Pristine, mancozeb, or Surround during this period were more effective in reducing Comice russet than either material alone.

Results and Discussion:

1. **Developing early ripening capacity in winter pears**. New knowledge about the responsiveness of Anjou and Comice pears to intermediate conditioning temperatures should be useful in getting fruit to market sooner after harvest. The combination of ethylene conditioning and temperature conditioning can significantly reduce total conditioning time. Some treatment combinations that speed conditioning may result in fruit that are too soft for long-distance shipping after treatment, but many treatment options result in the fruit maintaining good shipping firmness.

a. **Conditioning with intermediate storage temperatures.** This project identified the most efficient temperature for fastest satisfaction of the chill requirement for Comice and Anjou pears as 50 °F among a range of temperatures tested between 31 and 65 °F. Both colder and warmer temperatures required longer times for chilling satisfaction.

Table 1. Effects of different conditioning temperatures and exposure durations on the ripening capacity of Anjou pears. Firmness values below 3.0 typically reflect a buttery-juicy texture.

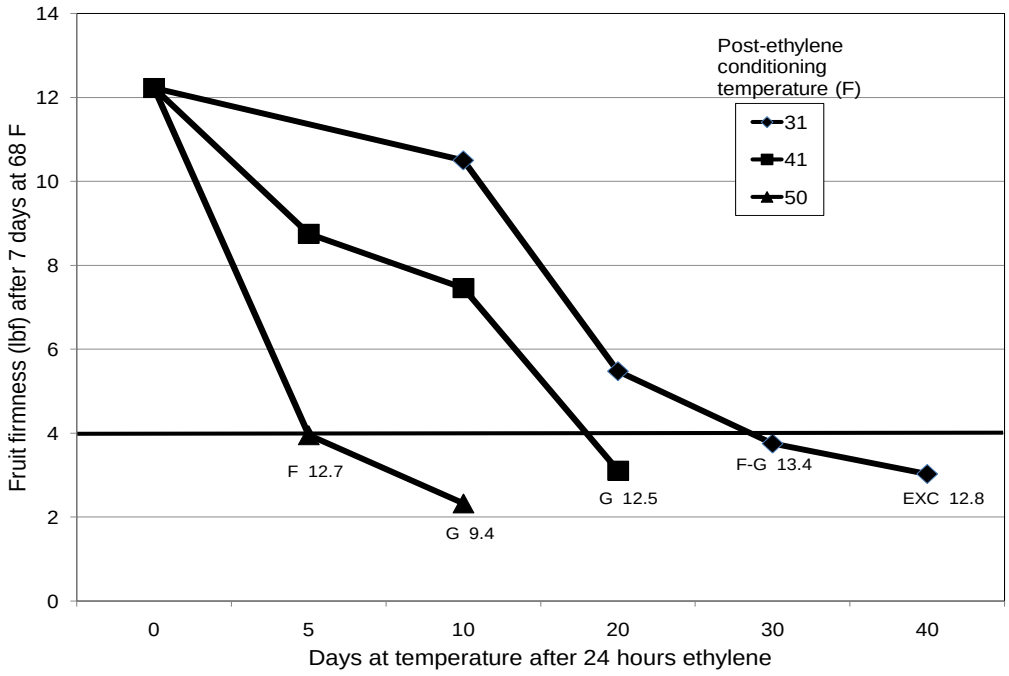
Conditioning Time (days)	Average firmness (lbf) of Anjou pears after conditioning at different temperatures followed by 7 days of ripening at 68 °F.							
	Conditioning temperature							
	31	**41**	**46**	**50**	**54**	**57**	**61**	**64**
10	13.6	12.8	9.6	4.8	7.9	8.2	7.5	7.7
20	12.1	7.2	5.8	1.7	4.7	5.4	5.3	5.5
30	7.8	5.2	3.6	1.7	2.8	4.1	3.7	4.8
40	5.3	2.7	2.0	1.6	1.8	2.7	2.5	2.9

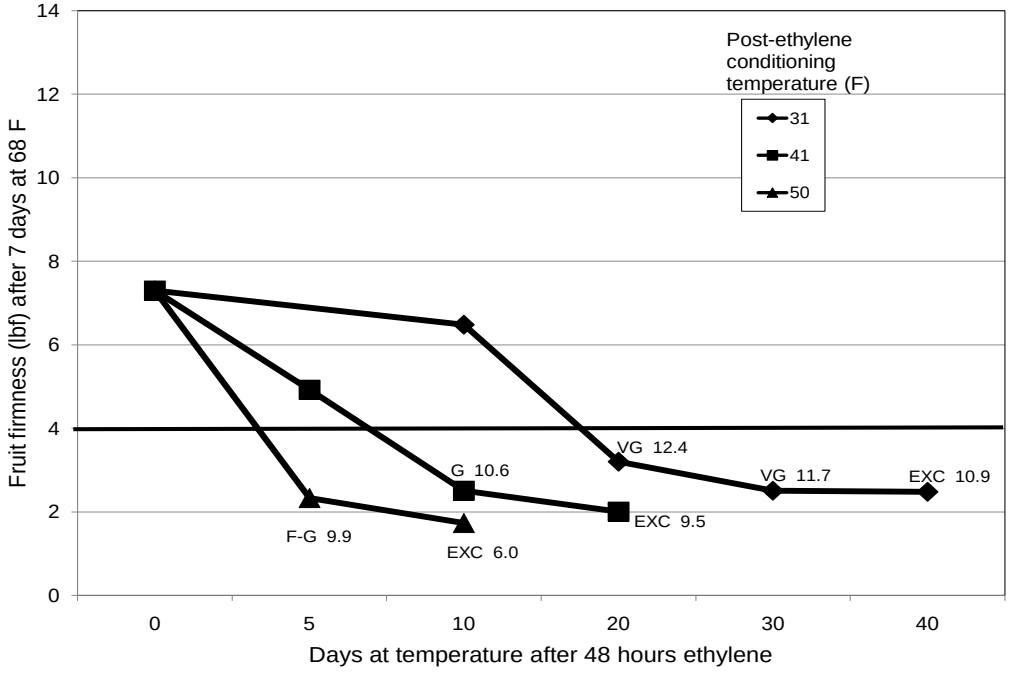
b. **Interaction of fruit maturity at harvest with temperature and duration of postharvest conditioning.** Later- harvested fruit required less conditioning time at all conditioning temperatures. Anjou pears harvested at 12 lbf or less needed only 10 days at 50°F to develop the capacity to ripen to acceptable quality, while retaining shipping firmness $\geq$ 8 lbf. Earlier harvested fruit required 20 days conditioning at 50°F, and consequently were too soft for shipping at the end of temperature conditioning. Shipping firmness was well-retained by conditioning at 41°F.

Table 2. Effects of harvest maturity and conditioning temperature on development of ripening capacity in Anjou pears.

Anjou 2009 Harvest			Minimum conditioning days needed to allow ripening to acceptable quality			Fruit firmness after conditioning (lbf) (shipping firmness)		
Day	Date	Firmness	**31°F**	**41°F**	**50°F**	**31°F**	**41°F**	**50°F**
0	Sept 14	14.1	> 60	40	20	-	13.0	4.8
7	Sept 21	13.2	> 60	40	20	-	12.3	3.9
14	Sept 28	12.0	> 60	30	20	-	11.1	4.5
21	Oct 5	11.9	60	20	10	11.8	11.2	9.1
28	Oct 12	10.8	30	20	10	10.2	9.7	8.0

c. **Interaction of ethylene conditioning and temperature conditioning to induce ripening capacity in Anjou pears**. Anjou pears harvested at 14.1 lbf average and exposed to ethylene 100 ppm at 68°F for 24 hours still needed an additional 30 days at 31°F in order to ripen to acceptable quality in 7 days at 68°F. However, fruit from the same 24 hour ethylene treatment needed only 20 additional days at 41°F, or 5-10 days at 50°F. If ethylene treatment was increased to 48 hours at 68°F, an additional 20 days were necessary at 31°F, 10 days at 41°F, and 5 days at 50°F.
In the figures below, treatments that resulted in successful ripening are accompanied by quality ratings (fair, good, very good, excellent) and the average fruit firmness at the end of the conditioning period (ethylene conditioning + further temperature conditioning), which approximates the shipping firmness.





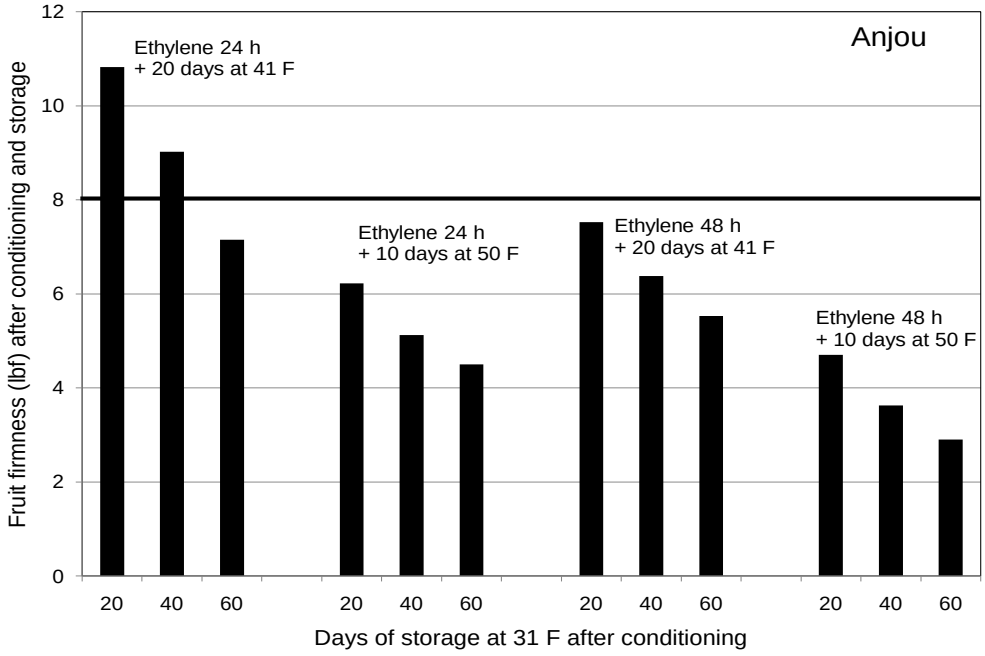
d. **Ethylene conditioning + temperature conditioning summary.** Experiments with Anjou, Bosc, and Comice pears in the course of this project have generated sufficient information to estimate the

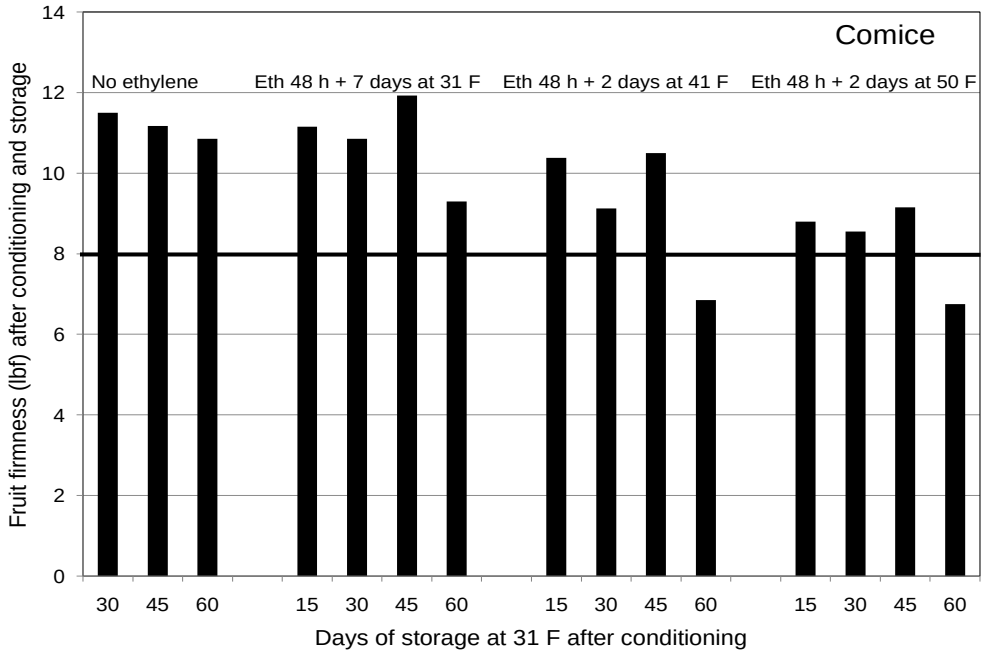
amount of time necessary to condition pears harvested near the top of the maturity range, using various combinations of ethylene and temperature conditioning.

Table 3. Summary of minimum conditioning times for winter pear varieties using both ethylene conditioning and temperature conditioning.

	Approximate number of conditioning days needed to induce ripening capacity (Fruit harvested at beginning of maturity range)								
	No ethylene			**24 h ethylene at 68°F**			**48 h ethylene at 68°F**		
	31°F	**41°F**	**50°F**	**31°F**	**41°F**	**50°F**	**31°F**	**41°F**	**50°F**
Bosc	15	10	5	0	0	0	0	0	0
Comice	30	20	12	15	10	5	10	5	4
Anjou	>60	40	20	40	20	10	20	10	5

d. **Post-conditioning storage life**. Anjou pears harvested at 14.1 lbf and treated with ethylene for 24 hours followed by 20 days at 41°F had a shipping firmness near 11 lbf after 20 days further storage at 31°F, and over 8 lbf after 40 days further storage at 31°F. However, shipping firmness values were below 8 lbf following 24 hour ethylene treatment plus 10 days at 50°F, or 48 hours ethylene treatment plus 10 days at 50°F or 20 days at 41°F.





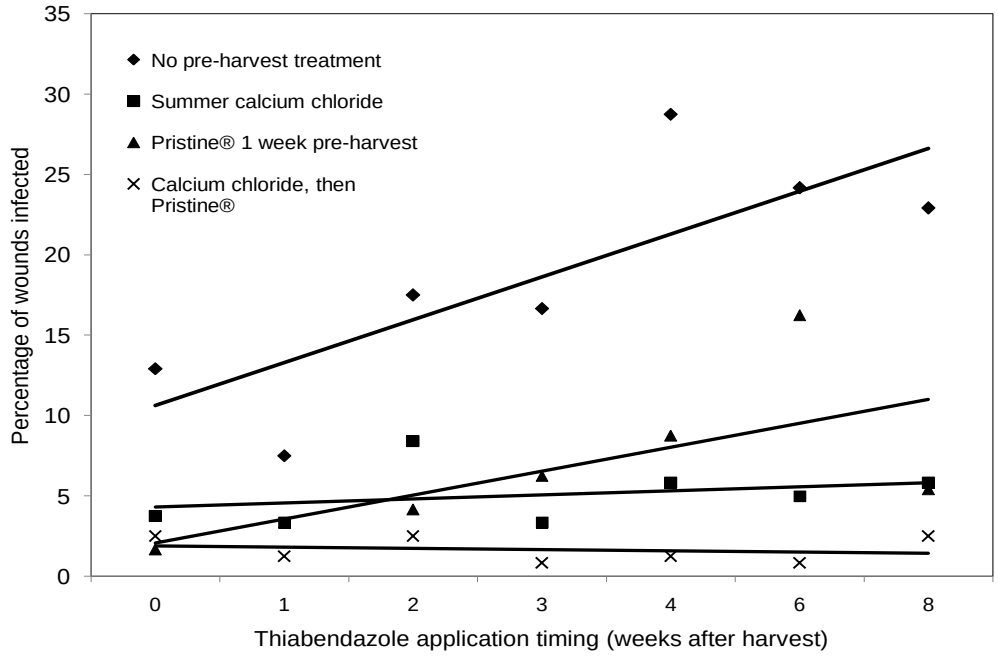
2. **Postharvest decay control programs**. Two conflicting facts drove the design of these decay studies: (1) postharvest fungicide and biocontrol treatments lose effectiveness in controlling decay if they are applied more than 3 weeks after harvest; and (2) there is a trend in the pear industry to delay postharvest line-spray fungicide treatments due to prolonged storage in field bins prior to packing. The research results demonstrated the high value of a programmatic approach in which summer calcium treatments are followed by application of appropriate fungicides approximately one week before harvest in reducing decay incidence when postharvest fungicide treatment is delayed. A single-bin drench system for applying postharvest fungicide to fruit in bins prior to truck loading in the orchard may also contribute to an orchard-based decay management program.

a. **Individual bin field drenching**. A powered hand-operated single-bin drencher was tested for field application of Scholar fungicide at 4 fl oz per 100 gallons but varying treatment volumes, without recirculation. Wound-inoculated fruit were buried near the top and bottom of each bin before treatment. Treatments reduced decay, and increasing treatment volume generally improved control. (In cooperation with Syngenta, Wilbur-Ellis, and Dr. Jim Adaskaveg, UC Riverside).

Table 4. Decay in wounded, inoculated Bosc pears buried in standard bins and treated Scholar fungicide using a hand-operated single-bin drenching system at varying gallonage.

	Percentage of wounds infected			
	Botrytis (gray mold)		*Penicillium* (blue mold)	
	Top	Bottom	Top	Bottom
Scholar dip	0.0 c	0.0 d	2.1 c	2.1 c
Water check	100.0 a	100.0 a	95.8 a	89.8 a
Scholar 4 oz in 2 gal water per bin	27.8 b	61.4 b	31.5 b	60.7 b
Scholar 4 oz in 4 gal water per bin	40.3 b	51.8 bc	37.5 b	59.3 b
Scholar 4 oz in 8 gal water per bin	13.0 c	35.4 c	21.5 c	19.9 c

b. **Orchard-based decay management programs**. The figure below shows the response of Bosc pears from different orchard decay management programs to postharvest line-spray fungicide treatment applied from 0 to 8 weeks after harvest. Fruit were wounded immediately after harvest, then stored at 31°F before and after line-spray treatment. Both summer calcium chloride sprays (3 sprays of 3 lb actual calcium per 100 gallons, 2 weeks apart during July and August) and fungicide applied one week before harvest were very effective in compensating for the delay in application of postharvest line-spray. Results show with Pristine were similar to those found with Flint or Topsin-M as the pre-harvest fungicide.



3. **Fruit Size Enhancement**. Urea treatments at full (80%) bloom resulted in increased average fruit weight and in increased yield of fruit size 90 and larger over the six years of evaluation in Bartlett pear (Table 1). The treatment was dosage-dependent; although 5% urea treatments were highly effective over the course of the study, 5% urea only enhanced Bartlett average fruit weight significantly in two of the six individual years, while 7-7.5% urea treatment enhance Bartlett average fruit weight every year. The overall reduction in fruit set and yield was compensated for by the increase in fruit size, resulting in a net gain of large-sized fruit. We suspect that a nitrogen boost to developing fruitlets may have worked in tandem with thinning to produce the size-enhancement effect. The plant growth regulator MaxCel, applied at 125 ppm 6-benzyladenine 10 days after petal-fall (8-10 mm average fruit diameter) was also highly effective in enhancing Bartlett pear fruit size and tons per acre of fruit size 90 and larger, despite reducing fruit size and total yield (Table 2).

Table 5. Effects of urea treatments applied at 80% bloom to Bartlett pear trees on production characteristics averaged over six years of study (2004-2009).

Treatment	Fruit set /100 clusters	Total yield (tons/acre)	Avg. fruit weight (g)	% of fruit size 90 & larger	Tons/acre size 90 & larger
Untreated	66.2 a	18.8 a	192 c	30.7 c	5.5 b
Urea 5%	56.8 b	17.5 ab	210 b	45.4 b	7.8 a
Urea 7-7.5%	45.0 c	16.1 b	223 a	56.1 a	8.9 a

Table 6. Effect of MaxCel 125 ppm treatment timing on fruit and production performance in Bartlett pear. Results averaged over 2008 and 2009 trials.

Treatment	Timing	Fruit diam. at treatment	Fruit set /100 clusters	Total yield (tons/acre)	Avg. fruit weight (g)	% of fruit size 90 & larger	Tons/acre size 90 & larger
Untreated			89.3 a	17.2 a	202 c	38.2 c	6.6 b
MaxCel 125 ppm	Petal-fall + 5 days	5-7 mm	82.7 ab	14.1 ab	223 b	56.3 b	7.7 ab
MaxCel 125 ppm	Petal-fall + 10 days	8-10 mm	68.5 b	13.1 b	246 a	72.0 a	9.5 a
MaxCel 125 ppm	Petal-fall + 15 days	12-14 mm	75.7 ab	13.3 b	239 a	67.8 a	8.7 ab

4. **Russet Management**. Experiments bagging Comice pear fruit weekly after petal-fall in 2008 and 2009 demonstrated that fruit are most susceptible to developing russet when wet during any of the first three weeks after petal-fall. Greatest susceptibility appears to occur during the first two weeks after petal-fall. Application of mancozeb, Pristine, or Surround during this period has been effective in reducing russet, and combinations (tank-mixes) of any two of the three products during this period appears to reduce russet to a greater extent than either product alone. Correlation studies comparing various weather factors to incidence of russet in Comice pears over the past 11 years showed that the strongest predictors of russet incidence were cool temperatures and low evapotranspiration during the second and third weeks after petal-fall.

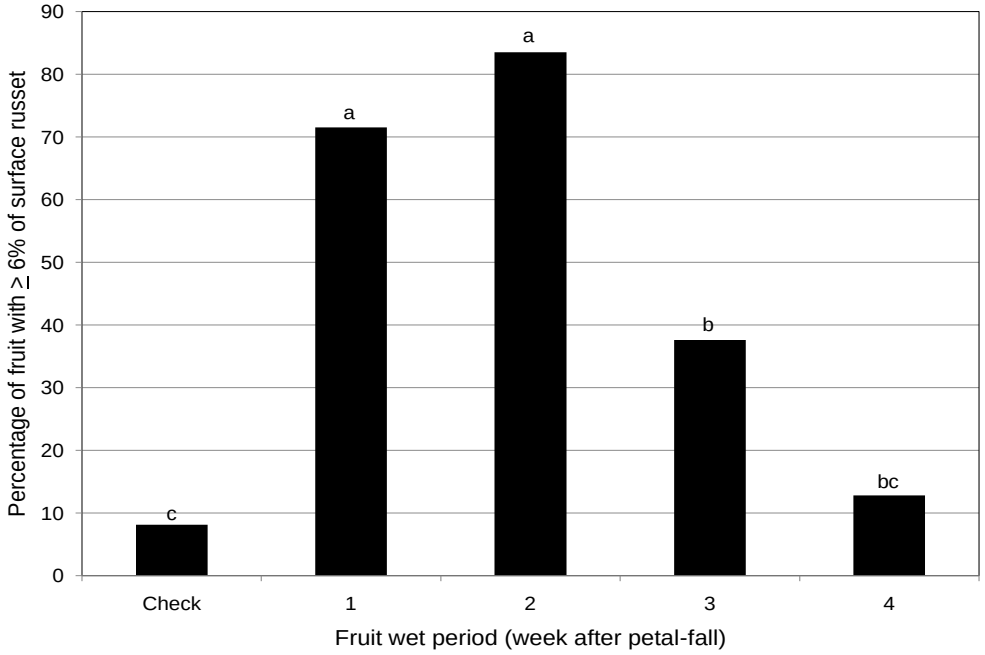


Table 7. Control of russet in Comice pears with treatment programs applied at petal-fall and 2 and 4 weeks after petal-fall (Mancozeb is active ingredient in products Dithane and Manzate).

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

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

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

Executive Summary:

The most efficient temperature for fastest satisfaction of the chill requirement for Comice and Anjou pears was 50 °F among a range of temperatures tested between 31 and 65 °F. Both colder and warmer temperatures required longer times for chilling satisfaction. Anjou pears harvested at 14.1 lbf average and exposed to ethylene 100 ppm at 68°F for 24 hours still needed an additional 30 days at 31°F in order to ripen to acceptable quality in 7 days at 68°F. However, fruit from the same 24 hour ethylene treatment needed only 20 additional days at 41°F, or 5-10 days at 50°F. If ethylene treatment was increased to 48 hours at 68°F, an additional 20 days were necessary at 31°F, 10 days at 41°F, and 5 days at 50°F. Conditioning requires less time as pear harvest maturity advances, as ethylene exposure time increases, and as post-ethylene temperature increases up to 50°F. These findings need to be followed up with studies of ethylene-temperature conditioning regimes at different fruit maturities, and predictions of the shipping firmness and storage potential of pears following various conditioning regimes.

Bartlett pear fruit size was enhanced by either urea at full bloom or MaxCel at 125 ppm applied 10 days after petal-fall. Urea was more effective and more consistent in improving fruit size when applied at a 7.5% concentration than at 5%. Fruit size enhancement was similar with 7.5% urea or with MaxCel 125 ppm. Fruit russet was most affected by weather conditions during the 2-3 weeks after petal-fall. Combinations of any two materials among mancozeb, Pristine, or Surround beginning at petal-fall were more effective in reducing russet than any product alone.

FINAL REPORT

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

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CoPI: Chang-Lin Xiao
CoPI: David Sugar
Organization: OSU Mid-Columbia Ag Research and Extension Center
Telephone/email: 541-386-2030 ext. 15/robert.spotts@oregonstate.edu

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

Total project funding: 43,822

Budget: **2008**: 43,822

Significant findings:

- Two years of validation of the gray mold risk prediction model showed that decay risk was accurately predicted for field run fruit, but decay levels were too low in fruit that had been run over the packing line and treated with disinfectants and fungicides for accurate risk prediction.
- The factors that are important for gray mold prediction did not affect blue mold similarly. Blue mold is more closely related to contamination of packinghouse surfaces and water.
- Resistance of pear fruit to decay changes yearly and can be quantified.
- The relationship between decay and spore load is important for establishing action thresholds for packinghouse water systems. Results emphasize the importance of good sanitation.
- Topsin, Pristine, and Ziram reduced gray mold, while Topsin reduced blue mold.

Results and discussion:

1. Validate gray mold decay risk prediction model

The first complete year of validation was in 2007-2008 and included pear fruit from 34 orchards in OR and WA (Table 1). The second year of validation was in 2008-2009 and used fruit from 37 orchards (Table 2).

Pear fruit from 9 and 6 orchards in 2007-8 and 2008-9, respectively, were stored field-run. Gray mold in this fruit ranged from 0.4 to 8.9% in 2007-8 and 4.5 to 21.3% in 2008-9 (Table 3). The model predictions matched well with the levels of gray mold in both years.

Fruit from commercial storages had 0.07 to 0.32% gray mold in 2007-8 and 0.03 to 2.68% in 2008-9 (Table 4). Percent gray mold was reduced from 90 to 99% when run over the packing line and placed in commercial storage when compared with field run fruit from the same orchards without any postharvest treatments. Postharvest treatments and cold storage conditions varied considerably among packinghouses, and the model predictions were not useful with the low levels of decay that are typical of commercial conditions.

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

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

Packer	Orchard	2007 Harvest Date	DNA	Preharvest fungicide	Rain	Orchard Rating	Time stored (mo)	Predicted Risk	Total %Bot
A	1	9/15	L	Yes	Yes	1	ND	L	ND
A	2	9/12	L	Yes	Yes	2	ND	M	ND
A	3	9/24	L	Yes	No	1	ND	L	ND
A	4	9/6	L	Yes	Yes	2	ND	M	ND
A	5	9/4	L	No	Yes	2	6*	H	2.85*
A	6	9/7	L	No	Yes	2	6*	H	3.29*
A	7	9/18	L	Yes	Yes	1	ND	L	ND
B	1	9/20	L	Ziram	Yes	2	7	M	0.33
B	2	9/24	L	Ziram	No	2	6	L	0.51
B	3	9/8	L	Ziram	Yes	2	8	M	0.29
B	4	9/8	L	Ziram	Yes	2	8	M	1.39
B	5	9/8	L	Ziram	Yes	2	7.75	M	0.22
B	6	9/19	L	Ziram	Yes	2	6*	M	0.88*
B	7	9/20	L	Ziram	Yes	2	7.75	M	0.37
C	1	9/8	L	Yes	Yes	2	4.5	M	0.22
C	2	9/10	L	Yes	Yes	3	5.25	H	0.39
C	3	9/8	L	Yes	Yes	2	4.5	M	0.08
C	4	9/15	L	Yes	Yes	1	7.5	L	0.04
C	5	ND	L	Yes	Yes	2	ND	M	ND
C	6	ND	L	Yes	Yes	3	4.5	H	0
C	7	9/11	L	Yes	Yes	1	4.5	L	0
C	8	9/13	L	Yes	Yes	3	4.5	H	0.28
D	1	9/17	L	Topsin	Yes	2	ND	M	ND
D	2	9/17	L	Topsin	Yes	2	6.25	M	0.01
D	3	9/21	L	Topsin	Yes	2	ND	M	ND
D	4	9/10	L	Topsin	Yes	2	6.5	M	0.24
D	5	9/14	L	Topsin	Yes	3	6*	H	3.48*
D	6	9/19	L	Yes	Yes	2	6.5	M	0.21
D	7	9/14	L	No	Yes	3	6*	E	9.9*
D	8	9/14	L	No	Yes	3	6*	E	7.8*
E	1	9/6	L	No	No	2	4	M	0.54
E	2	9/6	L	Ziram	No	2	4	L	0.18
E	3	9/6	L	No	No	2	4	M	0.18
F	1	8/30	L	Yes	Yes	2	6*	M	0.15*
F	2	9/17	L	No	No	2	6*	M	0.07*
F	3	9/17	L	Yes	No	2	6*	L	1.3*

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

Table 2. Gray mold risk model validation orchards 2008-9

Packer	Orchard	2008 Harvest Date	DNA	Preharvest fungicide	Rain	Orchard Rating	Time stored (mo)	Predicted Risk	Total %Bot
A	1	9/27	L	Topsin	Yes	2	8(4.5)	M	0.23
A	2	9/17	L	Yes	No	2	8	L	0.11
A	3	9/28	H	Topsin	Yes	2	8.7	H	1.02
A	4	9/22	L	Topsin	Yes	2.5	8	M+	0.51
A	5	9/27	L	Topsin	Yes	1	8.75	L	1.07
A	6	9/20	L	Topsin	Yes	2	8(4.5)	M	0.07
A	7	9/19	H	Topsin	Yes	2	5	H-	0.48
A	8	9/29	L	Topsin	Yes	1.5	4.5	L+	0.17
A	9	9/28	L	Topsin	Yes	2	8.75	M	0.16
B	1	9/5	L	Yes	No	2	6	L	1.75
B	2	9/15	L	No	No	2	6	M	0.25
B	3	9/12	L	No	No	2	6	M	0.67
B	4	9/16	L	No	No	2	6	M	2.4
B	5	9/15	L	No	No	2	6	M	2.68
B	6	9/12	L	Yes	No	2	6	L	0.12
C	1	9/29	L	Topsin	Yes	2	5.5	M	1.79
C	2	9/16	L	Topsin	No	1	7	L	1.19
C	3	10/7	L	Topsin	Yes	2	5	M	2.36
C	4	9/15	L	Topsin	No	2	6	L	0.03
C	5	17-Sep	L	Topsin	No	2	7	L	0.12
D	1	7-Oct	L	Yes?	Yes	2	6.5	M	0.56
D	2	10/9	L	Yes	Yes	2	5.5	M	0.1
D	3	10/8	L	ziram	Yes	2	5	M	0.24
D	4	10/8	L	Yes	Yes	2	5	M	0.1
D	5	9/23	L	Topsin	Yes	3	6.75	H	0.14
E	1	9/23	L	No+	No	2	5.25	M-	1.06
E	2	9/15	L	No+	No	2	5	M-	0.5
E	3	9/15	L	No+	No	2	5	M-	0.38
E	4	9/15	L	Topsin	No	2	5	L	1.2
E	5	9/23	L	Ziram	No	2	5.25	L	1.02
E	6	9/15	H	No	No	2	5	H	0.42
F	1	9/11	L	No	No	2	8	M	5.1*
F	2	9/17	L	No	No	2	8	M	4.6*
F	3	9/19	L	Organic-No	No	3	8	H	13.2*
F	4	9/23	L	Topsin	Yes	2	8	M	4*
F	5	9/19	H	Organic-No	No	3	8	E	21.*3
F	6	10/2	L	Yes	Yes	2	8	M	4.2*

*=fruit not in commercial storage but field run in MCAREC room.

Table 3. Anjou pears stored field-run at MCAREC and SOREC for gray mold risk model validation 2007-8 and 2008-9

	2007-8		2008-9	
Predicted risk level	Avg. gray mold (%)[z]	No. orchards	Avg. gray mold (%)[z]	No. orchards
Low	1.3a	1	-	-
Moderate	0.3a	3	4.5a	4
High	3.2b	3	13.2b	1
Extreme	8.9c	2	21.3c	1

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

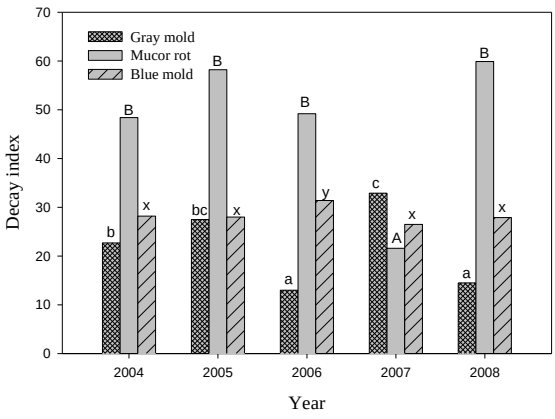
Table 4. Pears run over commercial packing lines and stored in commercial cold rooms for gray mold risk model validation 2007-8 and 2008-9

	2007-8		2008-9	
Predicted risk level	Avg. gray mold (%)[z]	No. orchards	Avg. gray mold (%)[z]	No. orchards
Low	0.46a	4	0.68a	10
Moderate	0.35a	12	0.83a	17
High	0.44a	4	0.51a	4

[z]Fruit stored 4 to 8.75 months; different letters indicate statistical differences at $P = 0.05$.

2. Develop blue mold decay risk prediction model

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

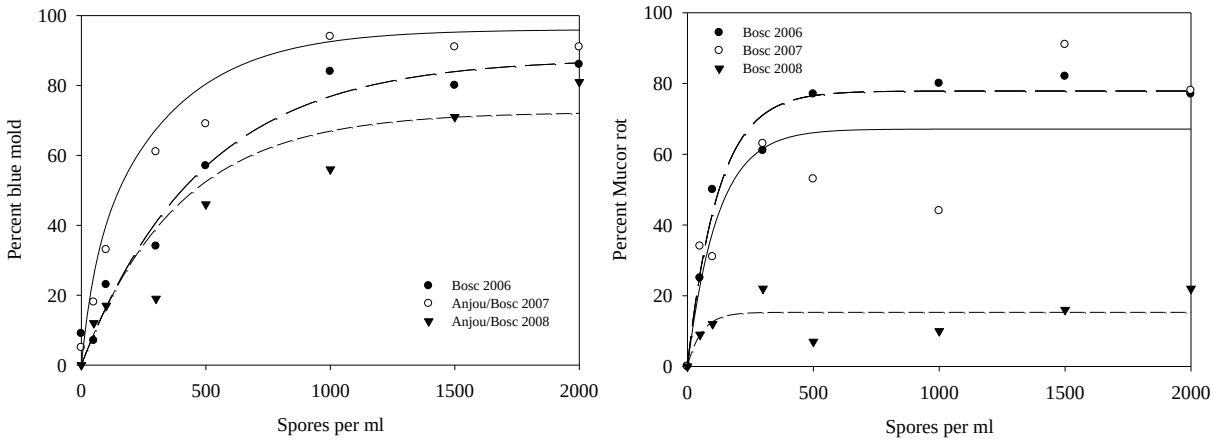


Resistance of pear fruit to decay changes yearly. We developed a test to measure this at the beginning of each packing season. Fruit resistance eventually needs to be incorporated into gray mold and blue mold risk prediction models.

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

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

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



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

In 2007-8, all tested preharvest fungicides reduced gray mold. In both years, Topsin was the most effective preharvest fungicide for control of blue mold (Table 5).

Table 5. Preharvest fungicides for control of postharvest decay of d'Anjou pear fruit

	2007-8		2008-9
Fungicide and rate/A	Gray mold (%)	Blue mold (%)	Blue mold (%)
Topsin 70WP 1/0 lb	2.2a	6.2a	15.5a
Pristine 38WG 14.5 oz	3.1a	21.3b	25.2ab
Ziram 76DF 8.0 lb	2.9a	19.4b	---
Yucca Ag Aide 2%	---	---	24.6ab
Silmatrix 2%	---	---	46.9c
Unsprayed	7.5b	26.0b	33.7bc

In 2007, all fungicides contained Nutraphos 24. In 2008, Pristine used at 18.5 oz with Silgard 4.0 oz. Fungicides applied 2 wks before harvest and evaluated after 3, 6, and 8 months at 30ºF. Numbers followed by the same letter within columns are not significantly different at $P = 0.05$ according to protected LDS.

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

Dr. Xiao will report the results for this objective.

Executive Summary:

Gray mold is one of the most serious decay problems of pear fruit in the Pacific Northwest and is estimated to cost the pear industry about $6 million per year. The main thrust of this project was to develop a model to predict, at harvest, the risk of gray mold for pear fruit in long-term cold storage. The model is driven by four factors that include: i) preharvest fungicide application, ii) preharvest rainfall, iii) an orchard management rating, and iv) amount of DNA of Botrytis on the fruit surface. A simplified version without the DNA factor also was developed. The model classifies gray mold risk as low, moderate, high, or extreme. It is important to note that **orchard rating** (orchard condition) was the most significant predictor of gray mold risk. Problem orchards often had old trees with dead limbs and poor weed control. Fruit on lower limbs often were intermingled with various weeds and grasses. **Preharvest fungicide** application was the second most important predictor of gray mold risk. This project has identified effective preharvest fungicides for gray mold. The model works best for field run fruit rather than for fruit run over the packing line that has been subjected to various postharvest treatments. Gray mold risk prediction at harvest is a valuable tool for packinghouse managers to determine which fruit is most suitable for long-term storage. The prediction also is useful to growers to help understand the factors that cause fruit to be at risk of decay and to make the necessary changes in horticultural and pest management practices to lower the risk of gray mold.

FINAL PROJECT REPORT

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
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City: Corvallis
State/Province/Zip OR 97331-2902

Cooperators: Virginia Stockwell, David Sugar, Steve Castagnoli, Bob Spotts, Clive Kaiser, Kent Evans (Utah), Rachel Elkins (California), others.

Other funding sources

Agency Name: UDSA Western Region Integrated Pest Management Competitive Grants Program
Amount awarded: $60,000
Notes: Awarded for implementation research in ’09 and ’10.

Total project funding: Year 1: $31,369 **Year 2:** $32,310 **Year 3:** No Cost

Budget 1:

Organization: OSU Agric Research Foundation		**Contract Administrator:** Dorothy Beaton	
Telephone: 541 737-3228		**Email:** dorothy.beaton@oregonstate.edu	
Item	**2007**	**2008**	**2009**
Salaries 6 mo. FRA	16,500	16,995	
Benefits 67%	11,055	11,387	
Wages			
Benefits			
Equipment			
Supplies	2814	2928	
Travel	250	250	
Miscellaneous plots	750	750	
Total	$31,369	$32,310	**no cost**

Objectives:

2007 to 2009:

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

Significant findings:

- **We developed two LAMP primer sets with high specificity to *E. amylovora*.** Two DNA primer sets are being used with field samples (from 45 that we designed and evaluated). One set is targeted to plasmid pEA29 and the other to the chromosomal gene *amsL*. LAMP reactions are highly specific for *E. amylovora*, and test negative with other bacteria recovered from flowers.

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

- **Mixed LAMP results were attained after adding a single flower infested with 10^5-10^7 CFU of *E. amylovora* to 100 floral clusters.** Single, pathogen-infested flowers when mixed in 1.5 L water yielded concentrations of 1×10^2 to 5×10^4 CFU per ml. LAMP reactions were positive when *E. amylovora* populations were $\geq 1 \times 10^3$ CFU per ml. Concentrating the wash with a filter improved detection.

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

- **LAMP detected the fire blight pathogen in flower samples from commercial orchards.** A total of 43 commercial orchards from Oregon, Washington, California and Utah were surveyed. LAMP reactions were negative in 11 orchards with no blight developing in 9, and a few strikes in 2. Positive LAMPs were obtained in 30 orchards; 20 of which developed fire blight. In several cases, communication of positive LAMP test to orchardists resulted in intensified control efforts.

- **With in-state support, orchardists in Utah and California are using LAMP-based scouting in 2010.** Utah will use the technology industry-wide to time initiation of spray programs. California is using LAMP to re-evaluate the value of delayed dormant copper treatments for blight suppression.

Results and Discussion:

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

Two DNA primer sets are being used with field samples (from 45 that we designed and evaluated). One set is targeted to plasmid pEA29 and the other to the chromosomal gene *amsL* (Table 1).

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.

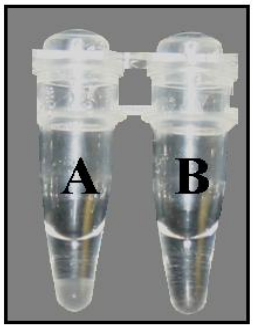

Figure 1: Comparison of positive and negative LAMP reactions. Tube (A): positive reaction seen as a cloudy white precipitate, and tube (B): negative reaction seen as clear liquid.

LAMP reactions run in a constant temperature heat block or water bath (65°C), and do not require expensive, precision instruments (a thermocycler followed by gel electrophoresis) to visualize the results.

Table 1. LAMP primers for detection of *Erwinia amylovora.* The full LAMP protocol is available from us upon request.

Primers to detect plasmid pEA29:

Name	Tm	5' to 3' primer sequence
Ea29 Fip	60°C	TCGTGGTTATGCGATAACGCGTCAGGAACTCCAGGGAGGTC
Ea29 Bip	60°C	TGTGTCACGATCCAGAGCACACGGTCATATGCAGGAGCAAGT
Ea29 F	59°C	ACGCAAGCCTTCTAAAGCT
Ea29 B	59°C	ATGGCCCGTGAAAAAGTCA
Ea29 Loop	60°C	GGGGGAGAGTCCATTTGGA

[a] Primers Fip and Bip were used at 2.4 µM, primers F and B at 0.2 µM, and Loop primer at 0.4 µM final concentrations .

Primers to detect amsL B:

Name	Tm	5' to 3' primer sequence
ALB Fip	60°C	CTGCCTGAGTACGCAGCTGATTGCACGTTTTACAGCTCGCT
ALB Bip	60°C	TCGTCGGTAAAGTGATGGGTGCCCAGCTTAAGGGGCTGAAG
ALB F	58°C	GCCCACATTCGAATTTGACC
ALB B	58°C	CGGTTAATCACCGGTGTCA

[a] Primers Fip and Bip were used at 2.4 µM, primers F and B at 0.2 µM final concentrations .

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

Laboratory strains of *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.

Freeze-dried cells of *E. amylovora* were suspended in water (a 3-L volume in food grade plastic pails) at concentrations of 2.5×10^2 and 1.8×10^3 CFU per ml (as determined by dilution

plating). Flowers of pear or apple were added to the pails as a treatment, which increased the population of indigenous (naturally occurring) bacteria in the suspensions to 10^2, 10^4, and 10^6 CFU per ml for pails with 10, 100 or 1000 flowers per 3 L, respectively. Indigenous bacteria were not recovered from wash suspensions without flowers.

For both Bartlett pear and Gala apple, 100% of LAMP reactions were positive if *E. amylovora* was spiked into flower suspensions (Table 1). The number of pear flowers in the suspension had no effect on the incidence of positive LAMP reactions. All LAMP reactions for wash suspensions containing no pathogen cells were negative.

Table 2. Percentage of positive LAMP detection as influenced *E. amylovora* concentration and a flower density in the wash.

Cultivar	*E. amylovora* concentration in suspension	Flower density in wash suspension [a]			
		0	10	100	1000
Bartlett pear	0	0%[b]	0%	0%	0%
	2.5×10^2 [b]	100%	100%	100%	100%
	1.8×10^3	100%	100%	100%	100%
Gala apple	0	0%	0%	0%	0%
	2.5×10^2	100%	100%	100%	100%
	1.8×10^3	100%	100%	100%	100%

[a] CFU per milliliter in 3 L volume of water.
[b] Average of 5 experiments.

Objective 3. Determine the sensitivity of LAMP reaction when one flower with a natural infection of *E. amylovora* is added to 100 flower clusters.

Single apple or pear flowers on which *E. amylovora* had been inoculated and allowed to incubate for 24-72 hours were suspended 0.3 (2009) or 1.5 L (2008) of water. Populations of *E. amylovora* in the suspensions ranged from 8.9×10^2 to 4.7×10^6 CFU per ml per ml. Over this range of concentrations, LAMP reactions were a mix of positive and false negatives if populations of the pathogen were below 1×10^3 CFU per ml. Concentrating 30 ml of the wash suspension by embedding onto a 0.2 micron membrane and resuspending into a 1 ml volume of water increased pathogen cell density by one log unit (as determined by dilution plating). Also, DNA extraction with the InstaGene™ Matrix and a mini-elute column increased improved pathogen detection with LAMP.

Following this concentration and extraction protocol, LAMP yielded a positive result with all pathogen-inoculated flowers regardless if an additional (non-inoculated) 100-flower clusters were added to the wash suspension (Table 3). Indigenous bacteria were recovered in all wash volumes to which 100 flower clusters had been added (ranging from 2×10^2 to 4×10^6 CFU per ml). Water- or water and flower cluster only samples were negative for detection of *E. amylovora* by LAMP or dilution plating (Table 3.)

Table 3. Percentage of positive LAMP detections from single *E. amylovora*-colonized *flower* as influenced by presence or absence of pear or apple flowers in the wash[a].

	Treatment added to wash			
Cultivar	**Nothing**	**100 flower clusters**	**Single flower colonized by *E. amylovora***	**Single flower colonized by *E. amylovora* and 100 flower clusters**
2008				
Bartlett pear	**0%[b]**	**0%**	**100% (4.0 ± 0.96)[c]**	**100% (4.2 ± 0.34)**
Gala apple	**0%**	**0%**	**100% (3.4 ± 0.62)**	**100% (3.8 ± 2.05)**
2009				
Bartlett pear	**0%**	**0%**	**100% (4.8 ± 0.71)**	**100% (5.3 ± 1.92)**
Gala apple	**0%**	**0%**	**100% (5.3 ± 1.84)**	**100% (5.2 ± 1.20)**

[a] 100 flower clusters per 0.3 (2009) or 1.5 L (2008) volume in a re-sealable plastic bag.
[b] Percentage of positive LAMP reaction is the average of 2 or 3 experiments each year.
[c] Average $\log_{10}$ population size (CFU per ml) and standard deviation of *E. amylovora* in the wash suspension after addition of a single pathogen-infested flower to 0.3 (2009) or 1.5 L (2008) water followed by concentrating 30 ml of the wash suspension onto a 0.2 micron membrane and resuspending into a 1 ml volume of water.

Objective 4. Use LAMP to detect *E. amylovora* in flower samples from inoculated and non-inoculated orchard trees.

In both 2008 and 2009, all 100-flower cluster samples from apple and pear orchards inoculated with *E. amylovora* 153N had positive LAMP reactions at full bloom and petal fall (Table 4). Pathogen populations in these samples, as estimated by dilution plating, ranged from 1.2×10^3 to 4.7×10^5 CFU per ml.

In nearby orchard blocks that were not inoculated with the pathogen, all 100-flowers cluster samples sampled processed in 2008 were negative for *E. amylovora* as determined by LAMP and by dilution plating. In 2009, floral clusters sampled at full bloom from non-pathogen-inoculated orchards also were negative, but the petal fall sample had positive LAMP reactions in 2 walks from a Bartlett pear block and 1 walk from a Braeburn apple block; these blocks were located < 100 meters from a inoculated pear block. *E. amylovora* was not detected by dilution plating method from any samples from the non-inoculated orchards (10^2 CFU per ml detection level). Populations of other bacteria in the flowers washes averaged approximately 1×10^6 CFU per ml (Table 4).

Table 4. LAMP results of 100 blossom cluster samples[a] from experimental pear and apple orchards inoculated with or without *E. amylovora*.

Cultivar	**Inoculated [b]**	**No. samples per orchard with positive LAMP [c]**	***E. amylovora* population [d] Log_{10}(CFU/ml)**	**Total bacteria population Log_{10}(CFU/ml)**
2008				
Bartlett pear	**No**	**0**	**Not detected**	**6.7 ± 0.10**
Fuji apple	**No**	**0**	**Not detected**	**5.7 ± 0.29**
Jonathon apple	**No**	**0**	**Not detected**	**5.7 ± 0.15**
Bartlett pear	**Yes**	**6**	**3.8 ± 0.70**	**6.3 ± 0.30**
Gala apple	**Yes**	**6**	**4.8 ± 0.53**	**5.5 ± 0.24**
Golden Delicious apple	**Yes**	**6**	**5.5 ± 0.16**	**6.1 ± 0.28**
2009				
Bartlett pear	**No**	**2 (at petal fall)**	**Not detected**	**5.8 ± 0.41**
World pear	**No**	**0**	**Not detected**	**6.2 ± 0.16**

Braeburn apple	**No**	**1 (at petal fall)**	**Not detected**	**6.2 ± 0.08**
Bartlett pear	**Yes**	**6**	**5.3 ± 0.07**	**5.0 ± 0.24**
Red Delicious apple	**Yes**	**6**	**7.7 ± 0.05**	**6.9 ± 0.05**

[a] 100 flower clusters per sample were suspended in 0.3 (2009) or 1.5 L (2008) volume of water in a re-sealable plastic bag.

[b] Indicates if experimental orchard was inoculated with *E. amylovora* (1 x 10^6 CFU per ml).

[c] In each orchard, 6 samples were taken; 3 at full bloom and 3 at petal fall.

[d] Average $\log_{10}$ population size (CFU per ml) and standard deviation of *E. amylovora* or the total bacteria recovered recover in the floral washes.

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

Selected commercial orchards were typically 3 to 5 hectares in size, and five 100 flower cluster samples were taken in each orchard on each sampling date. Each orchard was sampled three times: mid-bloom, full bloom, and petal fall. In 2008, the volume of water used to wash collected flower clusters was 1.5 L, whereas in 2009, this volume was reduced to 0.3 L. In addition, in 2009, 15 ml of the wash volume was concentrated on a 0.2 um filter, then the bacteria trapped on the filter were resuspended in 1 ml prior to DNA extraction. In 2008, extracted DNA was concentrated by high speed, low temperature evaporation.

2008:

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

Hood River Valley (Parkdale). Bloom at higher elevations in the Hood River Valley coincided with a period of extreme fire blight risk (Fig. 2). The first samples (30% bloom in pear) occurred at low risk, and *E. amylovora* was not detected. For the 3rd sample time (May 19), which occurred during the high risk period, *E. amylovora* was detected by LAMP in 4 of 5 orchards, all of which developed some fire blight (Table 3). Positive pathogen detection by LAMP in 3 of 4 orchards, however, required concentration of the extracted DNA by high speed, low temperature evaporation. This result was the reason the wash volume was reduced and further concentrated in 2009.

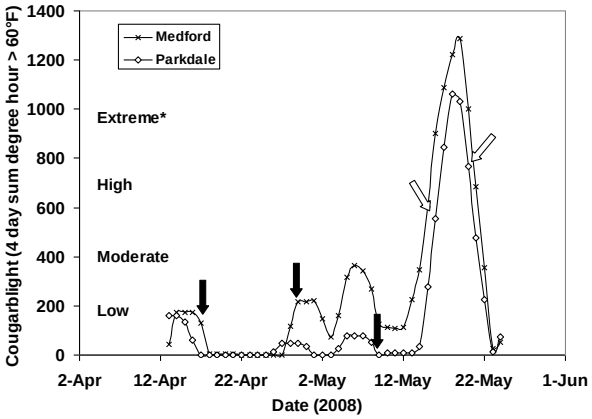

Fig. 2. Fire blight risk in spring 2008 based on temperatures measured at Medford and Parkdale, Oregon. Arrows indicate samples dates.

2009:

The survey was expanded to apple and pear production areas in four states: Oregon, California, Washington, and Utah (Table 5). With the exception of Utah, all samples were processed at Oregon State University.

In Oregon, a total of 10 pear and 6 apple orchards were sampled from Hood River, Medford, Milton-Freewater, and Parkdale. Positive LAMP reactions were obtained from 14 of 16 orchards, Summer fire blight evaluation revealed light disease development in 8 of the 16 orchards, 7 of which were positive for LAMP (Table 3).

In California, three pear orchards were sampled from Lake County at mid-bloom, full bloom, and petal fall. Two of three orchards had positive LAMP reactions with light disease developing in one of the two positive orchards (Table 3). *E. amylovora* was isolated on culture media in only one orchard with an average population of 2.6×10^4 CFU per sample.

In Washington, a total of 3 pear and 3 apple orchards were sampled; these orchards were located in the Yakima, Zillah, Wenatchee, and Okanogan districts. Positive reactions were obtained in three of six orchards; light disease developed in one orchard in which *E. amylovora* was detected, and in one orchard in which it was not detected (and disease data were not obtained for 2 of the six orchards).

In Utah, 7 apple orchards located south of Provo were sampled. At this location, the orchards were sampled from 4 to 12 days in a row with 6 orchards being sampled at least 10 days in a row (Table 3). Positive LAMP reactions resulted from all orchards with populations of *E. amylovora* that ranged from 2.4×10^3 to 3.2×10^7 CFU per sample. Stigma imprints were performed on 4 (one orchard) or 8 (6 orchards) of 10 sample days and resulted in detection of *E. amylovora* in 4 of the 7 orchards (Table 3). In all orchards, fire blight developed in degrees varying from light to heavy (Table 3).

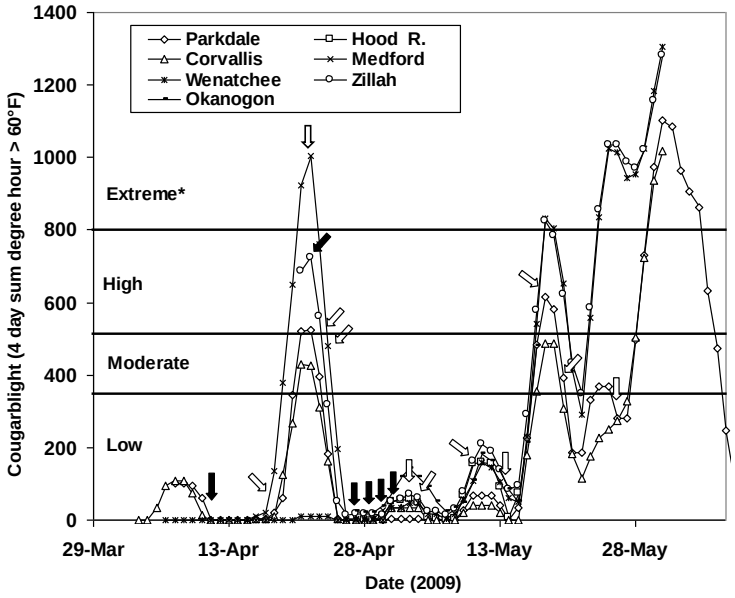

Figure2: Cougarblight model consisting of 4-day sum of degree hours greater than 15°C (60°F) plotted against dates in spring in 2009. Risk of disease outbreak is based on the assumption of "blight present in the region but not near the orchard last year" where 0 to 350 is low risk, 350 to 500 is moderate risk, 500 to 800 is high risk, and 800+ is extreme risk. Black arrows indicate negative detection and white arrows indicate positive detection of *E. amylovora* by loop mediated isothermal amplification.

Table 5. LAMP results of 100 blossom cluster samples[a] taken from commercial orchards in the Pacific Northwest of the United States of America.

			No. of samples with Positive LAMP			Media isolationb	
State		Cultivar	Mid-bloom	Full bloom	Petal fall	(Avg. Log10)	Blight[c]
2008							
OR	Medford	Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
		Bosc & Red d'Anjou pear	0 of 5	0 of 5	0 of 5	No	No
		Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
		Red Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
	Parkdale	Red d'Anjou pear	0 of 5	0 of 5	0 of 5	No	No
		Bartlett, d'Anjou, & Bosc pear	0 of 5	2 of 5 *	5 of 5 *	Yes (3.3)	Yes Moderate
		JonaGold apple	2 of 5 *	no data	no data	No	Yes Moderate
		Bartlett & Bosc pear	0 of 5	1 of 5 *	2 of 5 *	Yes (2.7)	Yes light
		Gala apple	5 of 5 *	no data	no data	No	Yes light
2009							
OR	Medford	Bartlett pear	1 of 5	0 of 5	0 of 5	Yes (2.3)	No
		Bosc & Red d'Anjou pear	0 of 5	0 of 5	1 of 5	Yes (6.0)	No
		Bartlett pear	4 of 5	1 of 5	2 of 5	Yes (6.0)	Yes light
		Red Bartlett pear	0 of 5	1 of 5	0 of 5	No	No
	Parkdale	Red d'Anjou pear	2 of 5	0 of 5	2 of 5	Yes (4.0)	No
		Bartlett, d'Anjou, & Bosc pear	2 of 5	0 of 5	1 of 5	Yes (3.7)	No
		JonaGold apple	0 of 5	2 of 5	2 of 5	Yes (4.0)	No
		Bartlett & Bosc pear	0 of 5	0 of 5	0 of 5	N	Yes light
		Gala apple	0 of 3	0 of 3	2 of 3	Yes (4.0)	Yes light
	Milton-Freewater	Gala apple	0 of 5	1 of 5	4 of 5	Yes (5.5)	Yes light
		Gala apple	1 of 5	0 of 5	1 of 5	No	No
		Pink Lady apple	0 of 5	0 of 5	4 of 5	Yes (5.2)	Yes light
		Pink Lady apple	0 of 5	3 of 5	5 of 5	Yes (5.1)	Yes light
	Hood River	Forelle pear	2 of 5	4 of 5	5 of 5	Yes (7.0)	Yes light
		Bartlett pear	1 of 5	2 of 5	0 of 5	No	Yes light
		Bartlett pear	0 of 5	0 of 5	no data	No data	No
CA	Lake County	Star Crimson pear	1 of 5	1 of 5	1 of 5	Yes (3.1)	Yes light
		Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
		Bartlett pear	2 of 5	1 of 5	0 of 5	Yes (2.5)	No
WA	Yakima	Gala apple	0 of 5	0 of 5	0 of 5	No	Yes light
	Zillah	Gala apple	0 of 5	0 of 5	1 of 5	No	no data
		Pink Lady apple	no data	no data	1 of 1	Yes (5.0)	no data

	Wenatchee	**d'Anjou pear**	**0 of 5**	**0 of 5**	**0 of 5**	**No**	**No**
		d'Anjou pear	**0 of 5**	**0 of 5**	**0 of 5**	**No**	**No**
	Okanogan	**Bosc pear**	**0 of 4**	**0 of 6**	**2 of 4**	**Yes (5.3)**	**Yes light**
UT				**LAMP[d]**	**Stigma imprint[e]**		
	Provo	**Gala apple**		**10 of 12**	**8 of 8**	**Yes (4.8 ± 1.00)**	**Yes heavy**
		Gala apple		**8 of 10**	**4 of 8**	**Yes (5.3 ± 0.71)**	**Yes heavy**
		Fuji apple		**2 of 10**	**0 of 8**	**Yes (2.9 ± 0.24)**	**Yes light**
		Gala apple		**5 of 10**	**5 of 8**	**Yes (3.6 ± 1.12)**	**Yes moderate**
		Fuji apple		**5 of 10**	**0 of 8**	**Yes (4.0 ± 2.02)**	**Yes moderate**
		Jonathon apple		**9 of 10**	**8 of 8**	**Yes (6.0 ± 0.24)**	**Yes heavy**
		Gala apple		**2 of 4**	**0 of 4**	**Yes (4.3 ± 1.87)**	**Yes light**

[a] 100 flower clusters per sample were washed in 0.3 (2009) or 1.5 L (2008) of water in a re-sealable plastic bag.
[b] Average $\log_{10}$ population size of *E. amylovora* (CFU per ml) recovered from floral washes.
[c] Whether or not fire blight developed in the orchard, and if yes, the disease rating applied to the orchard: light = 1 strike per tree, moderate =2 to 5 strikes per tree, and heavy $\geq$ 6 strikes per tree).
[d] Incidence of positive LAMP reaction is the average of up to 12 floral samples in Utah taken daily from orchards from mid-bloom to petal fall.
e Incidence of positive isolation of *E. amylovora* from imprinting stigmas of pear or apple flowers onto CCT media.

In summary, *E. amylovora* was detected in 30 of 41 commercial orchards, 20 of which developed fire blight. Detection of *E. amylovora* in commercial orchards typically coincided with full bloom to petal after heat units had begun to accumulate on a COUGARBLIGHT risk curve. Nonetheless, E. amylovora was detected in 9 orchards at the early (mid-bloom) sample. In several cases, information that *E. amylovora* was present in flowers in an orchard intensified the orchardist's fire blight management activities.

Discussion:

Given the sensitivity of LAMP and our preliminary results, we expected that our sampling scheme would readily detect *E. amylovora* at high levels of infestation, which proved true. In addition, through refinement of the method we used to wash bulk flower samples, detection of *E. amylovora* at lower levels of infestation also was improved.

The important question raised by the data concerns whether or not LAMP-based scouting for *E. amylovora* is worth the effort. In our view there are several answers to this question:

a) In cases where we detected either a high-infestation levels of the pathogen (mostly Utah in 2009) or the pathogen was detected early in the bloom period (Hood River and Parkdale, OR and Lake County, CA in 2009), orchardists responded to positive LAMP results by intensifying their control efforts. This intensification following the information provided by LAMP was in our view the most beneficial aspect of early scouting for *E. amylovora.* It is likely that through early knowledge of the pathogen's presence, at least some orchardists reduced fire blight damage.

b) In numerous orchards we detected the pathogen but late in bloom and in only one or two samples from an orchard (i.e., a relatively low level of infestation, which was also evidenced by relatively low levels of blight during the summer). The later bloom samples were taken at generally higher CougarBlight heat unit accumulations, and thus knowledge of fire blight risk was available using a simpler and cheaper method. In these cases, it is unlikely that LAMP based scouting provided value beyond that provided by CougarBlight. Nonetheless, one grower expressed a level of 'peace-of-mind' from negative results:

> "The information we received from the 2009 fire blight program was invaluable. Knowing that we had fire blight in the orchard but, more importantly, knowing where it was, saved us money. We didn't just spray all the pears like we usually do. Besides saving money, resistance might be further delayed. We would be interested in participating in the 2010 program also."

This statement shows potential for additional value from LAMP-based scouting; however, in our opinion, we think the LAMP scouting database is still too small to make the judgment "to not spray all the trees like we usually do."

c) Finally, both a) and b) are conditioned on the current state of molecular-based detection technology (in this case LAMP) and its relative ease of use. Currently, we feel that the LAMP protocol to detect the fire blight pathogen in flower samples needs to be done by an individual who is trained and experienced with the methods and aware of the potential problems (such as minimizing molecular contamination, and inclusion/interpretation of controls). However, it is likely that in the not-to-far-off future, advances in technology will make assays like LAMP easier to deploy at an on-site location by a less experienced user. An example that coincides with the submission of this report is:

> Tomlinson, J. A., Dickinson, M. J., and Boonham, N. 2010. Rapid detection of *Phytophthora ramorum* and *P. kernoviae* by two-minute DNA extraction followed by isothermal amplification and amplicon detection by generic lateral flow device. Phytopathology 100:143-149.

Results summary:

- We developed two LAMP primer sets for specific detection of *E. amylovora*.
- The detection limit with pure cultures is ~25 pathogen cells per ml. Practical detection limit with field samples is ~10,000 cells per 100 flower cluster sample.
- We consistently detect *E. amylovora* in spiked washes, and inoculated field trials.
- *E. amylovora* was detected in commercial orchards using a sample size of 100 flower clusters (sampled into a re-sealable plastic bag) taken at a frequency of one sample per hectare (typically 5 samples per orchard).
- Consistent detection of *E. amylovora* was achieved when 100-flower cluster samples were washed in 0.3 L water, and 15 ml of this wash was concentrated to 1 ml prior to DNA extraction.
- *E. amylovora* was detected in 30 of 41 commercial orchards, 20 of which developed fire blight. Detection of *E. amylovora* in commercial orchards coincided with full bloom after heat units had begun to accumulate on a COUGARBLIGHT risk curve. In several cases, information that *E. amylovora* was present in flowers in an orchard intensified the orchardist's fire blight management activities..

Executive Summary:

We have developed a LAMP-PCR method for detection of the fire blight pathogen, *Erwinia. amylovora*, from pure cultures, laboratory experiments in floral washes, and from bulked floral samples obtained from experimental and commercial orchards.

Early detection of the fire blight pathogen in commercial orchards involves sampling bulked, 100-flower cluster samples (~ 1 per hectare) and processing the sample wash with LAMP, which requires 1–2 hr to complete. The method reliably detects a single pathogen-colonized flower in a sample of 100 clusters (~600 flowers). In three experimental orchards inoculated with *E. amylovora*, positive LAMP reactions were attained from nine of nine 100-flower cluster samples.

A two year study evaluated LAMP-based scouting for the fire blight pathogen in 41 pear and apple orchards in of Oregon, Washington, California and Utah. *E. amylovora* was detected by LAMP in flower samples from 30 orchards, of which 20 developed fire blight. In another eleven orchards, all floral washes were negative for *E. amylovora* by LAMP and by dilution plate; of these, light disease was observed in two orchards during the summer.

Overall, detection in commercial orchards coincided with full bloom after heat units had begun to accumulate on a COUGARBLIGHT risk curve, indicating that the heat unit model works well to forecast fire blight risk, and may well be a sufficient measure of risk for many orchardists. On the other hand, several growers were able to use information provided by LAMP- based scouting to intensify or modify their control practices. For example, one grower cooperator wrote:

> "The information we received from the 2009 fire blight program was invaluable. Knowing that we had fire blight in the orchard but, more importantly, knowing where it was, saved us money. We didn't just spray all the pears, like we usually do. Besides saving money, resistance might be further delayed. We would be interested in participating in the 2010 program also."

Implementation of LAMP 'on-site' (e.g., an orchardist's kitchen) is not a feasible currently, but use by regional extension or a field station unit is a viable option. For example, in 2010, Utah through cooperative extension personnel will implement LAMP technology industry-wide to time initiation of spray programs. Growers and extension personnel in Lake Co., CA are using LAMP in 2010 to re-evaluate the value of delayed dormant copper treatments for blight suppression. The ease of implementing LAMP-based detection on-site is expected to improve in the coming years.

FINAL PROJECT REPORT: PR-08-800

Project Title: Volatile sex attractants in pear psylla

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Agency Name: USDA-CSREES-NRI
Amount awarded: $233,473 (FY 2006-2008)
Personnel: Horton, Guédot, Landolt, Millar

Agency Name: Binational Agricultural Research and Development Fund (BARD)
Amount awarded: $273,000 (FY 2008-2010)
Personnel: Horton, Guédot, Landolt, Soroker, Zada

Total Project Funding: $30,000

Budget History:

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

OBJECTIVE:
Test compounds extracted and identified from cuticular washes of female psylla for attractiveness to male pear psylla

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS:

- Demonstrated that surface extracts from females are as attractive to males in olfactometer trials as an equivalent number of live females
- Used GC-MS to identify chemicals present in cuticular washes of diapausing and post-diapause male and female winterforms
- Synthesized one chemical (13-methylheptacosane) that was found to occur at levels 3-fold higher in post-diapause female winterforms than male winterforms
- Demonstrated attractiveness of 13-methylheptacosane to male winterform psylla in olfactometer trials and in field trials
- Identified 3 chemicals (including 13-methylheptacosane) that were found to occur at levels 2.7- to 8.6-fold higher in female summerforms than male summerforms

RESULTS AND DISCUSSION:

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

GC-MS analysis of cuticular extracts from winterform psylla. A GC-MS analysis of cuticular extract from male and female diapausing and post-diapause winterform psylla was done **(Figure 2: upper panel – postdiapause psylla, lower panel – diapause psylla)**. We identified many of the peaks in both traces **(Table 1)**, and determined quantities of the chemicals in females and males (shown as female:male ratios in **Table 1**). One chemical (13-methylheptacosane) occurred at a level 3-fold higher in females than males for post-diapause winterforms, but was found to occur at similar levels between sexes in diapausing winterforms **(Table 1)**.

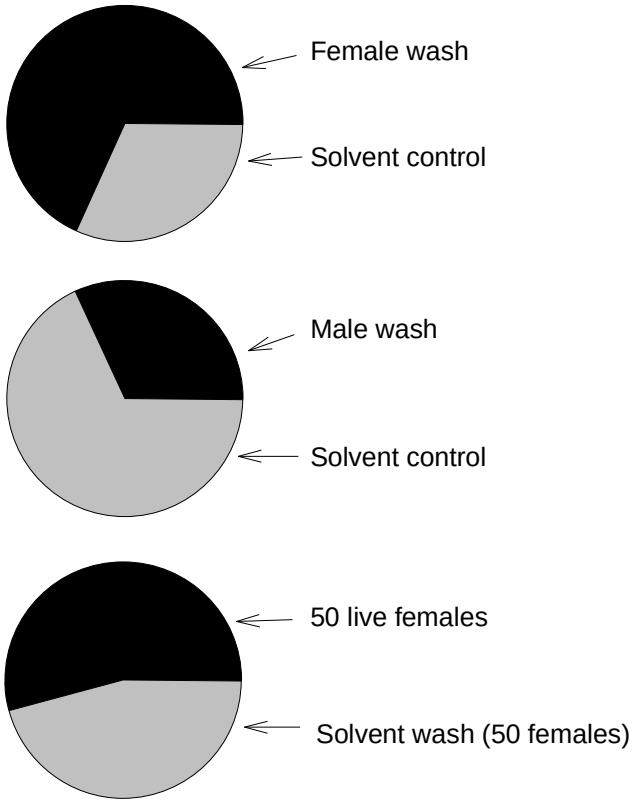
Olfactometer trials with post-diapause winterforms and 13-methylheptacosane. Olfactometer trials were conducted with post-diapause winterform psylla and 13-methylheptacosane **(Figure 3)**. The chemical was synthesized by J. Millar, and forwarded to Wapato cooperators. Our first assay assessed attractiveness of the chemical (vs solvent blank) to male and female psylla. Male psylla (but not female psylla) were significantly attracted to the chemical **(Figure 3, top panel)**. A second series of assays was then done to compare attractiveness of the synthesized 13-methylheptacosane versus the crude cuticular extract **(Figure 3, bottom panel)**. Again, the synthesized chemical was attractive to male psylla when assayed against a solvent control. The crude extract was also attractive. Lastly, male psylla did not discriminate between 13-methylheptacosane and the crude extract, which suggests that the individual chemical was as attractive to male psylla as the full cuticular wash.

Field trial with winterform psylla and 13-methylheptacosane. Field trials were done in late March and early April at the Moxee experimental farm. Sticky traps, composed of nylon mesh coated with tanglefoot **(Figure 4)**, were used to assess attractiveness of 13-methylheptacosane to male and female winterform psylla. Gray septa were used to dispense 3 concentrations of the chemical (10, 100, or 1000 µg of the product); solvent-loaded septa were used as controls. Each treatment was replicated 11 times. Numbers of males and females per trap were determined after 5 days in the field. There was marginally significant evidence that males preferentially accumulated on the 13-methylheptacosane traps during the late March test **(Figure 5, top panel)**. More conclusively, our

early April trial provided very strong and highly significant evidence that males were attracted to the chemical **(Figure 5, bottom panel)**. Females exhibited no obvious patterns in trap catch, other than some weak evidence for avoidance of higher concentrations of the chemical in the first trial.

GC-MS analysis of cuticular extracts from summerform psylla. A GC-MS analysis of cuticular extracts from male and female summerform psylla identified 3 chemicals that occurred at higher levels in the female extract than the male extract (2.7- to 8.6-fold higher in females). The chemical with the largest female:male ratio was again 13-methylheptacosane (data not shown). Assays with these 3 chemicals are planned for 2010.

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



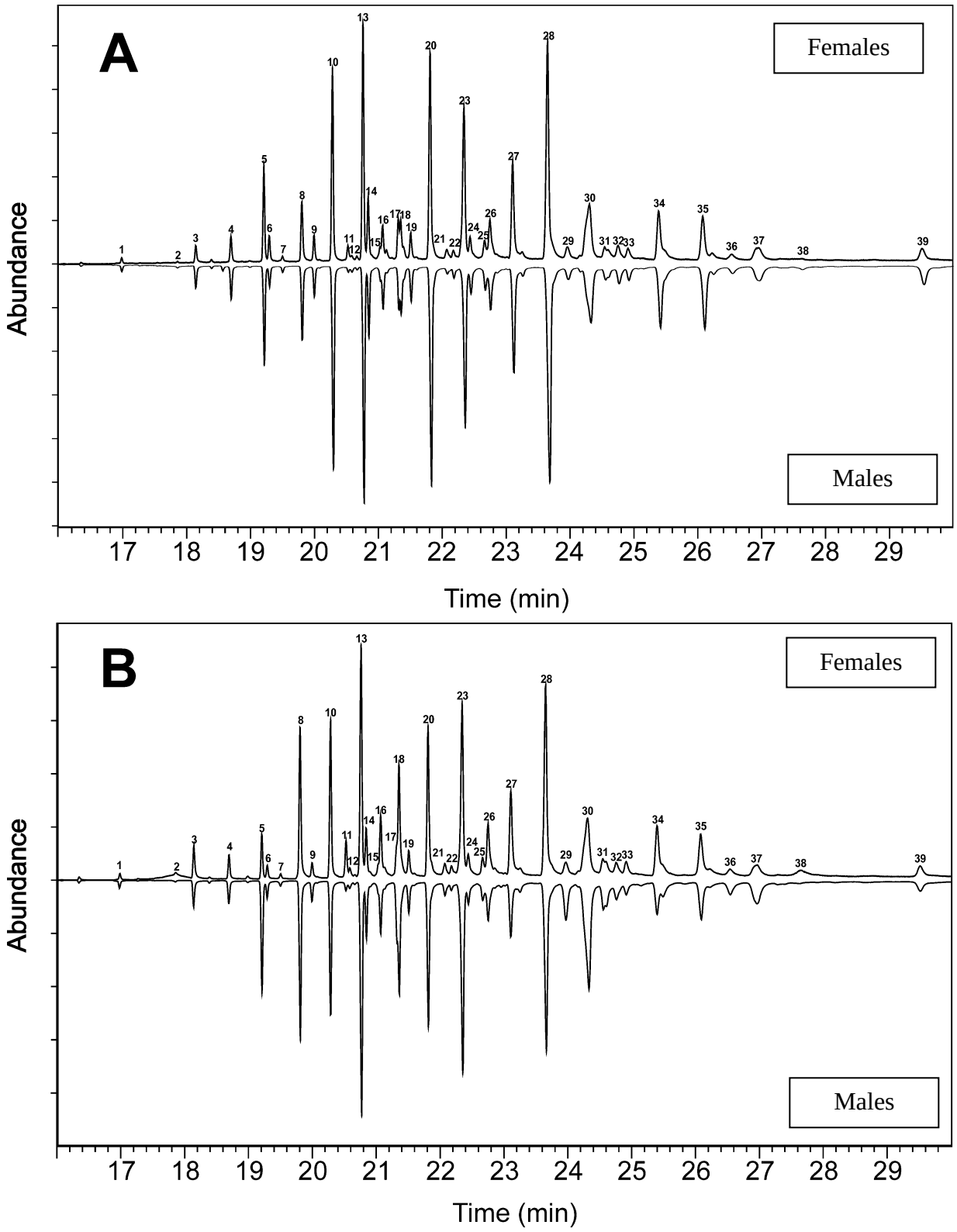


Figure 2. GC-MS traces of solvent extracts from diapausing female and male psylla (Panel A), and post-diapause female and male psylla (Panel B); in both panels, upper trace is female wash and lower trace is male wash. Peak #11 corresponds to 13-methylheptacosane.

Table 1. Identification of hydrocarbons and related compounds in cuticular extracts of winterform *Cacopsylla pyricola.* Bold font designates the 13-methylheptacosane.

Peak #	Retention time (min)	Identification	Ratio female/male	
			Diapause	Post-diap
1	16.99	tricosane	1.1	1.0
2	17.86	tetracosane	tr	tr
3	18.15	docosanal	1.1	1.0
4	18.70	pentacosane	1.1	1.0
5	19.21	2-methylpentacosane	1.2	0.6
6	19.36	3-methylpentacosane	1.3	1.1
7	19.51	hexacosane	1.0	tr
8	19.81	tetracosanal	1.2	0.8
9	20.00	2-methylhexacosane	1.0	1.0
10	20.29	heptacosane	1.1	1.2
11	**20.53**	**13-methylheptacosane**	**1.5**	**3.2**
12	20.59	pentacosanal	1.0	1.1
13	20.77	2-methylheptacosane	1.1	1.2
14	20.85	3-methylheptacosane	1.0	1.1
15	21.04	octacosane	1.0	1.2
16	21.07	unidentified	1.2	1.1
17	21.31	unidentified	1.1	0.9
18	21.36	hexacosanal	1.1	1.0
19	21.51	2-methyloctacosane	0.9	1.0
20	21.81	nonacosane	1.1	1.1
21	22.08	11-, 13-, and 15-methyl-nonacosane	1.1	1.2
22	22.18	heptacosanal	1.0	1.1
23	22.35	2-methylnonacosane	1.1	1.0
24	22.44	3-methylnonacosane	1.0	1.1
25	22.67	triacontane	1.0	1.1
26	22.75	unidentified	1.2	1.2
27	23.11	octacosanal	1.2	1.1
28	23.66	hentriacontane	1.1	1.1
29	23.97	11-, 13-, and15-methyl-hentriacontane	1.0	0.8
30	24.31	11,15- and 13,17-dimethyl-hentriacontane	1.2	0.8
31	24.55	unidentified	1.0	1.0
32	24.61	unidentified	1.0	1.0
33	24.76	dotriacontane	1.0	1.1
34	25.40	triacontanal	1.1	1.1
35	26.08	tritriacontane	1.1	1.1
36	26.54	11-methyltritriacontane	1.1	0.9
37	26.97	unidentified	1.3	0.9
38	27.66	tetratriacontane	1.0	1.0
39	29.52	pentatriacontane	1.0	1.0

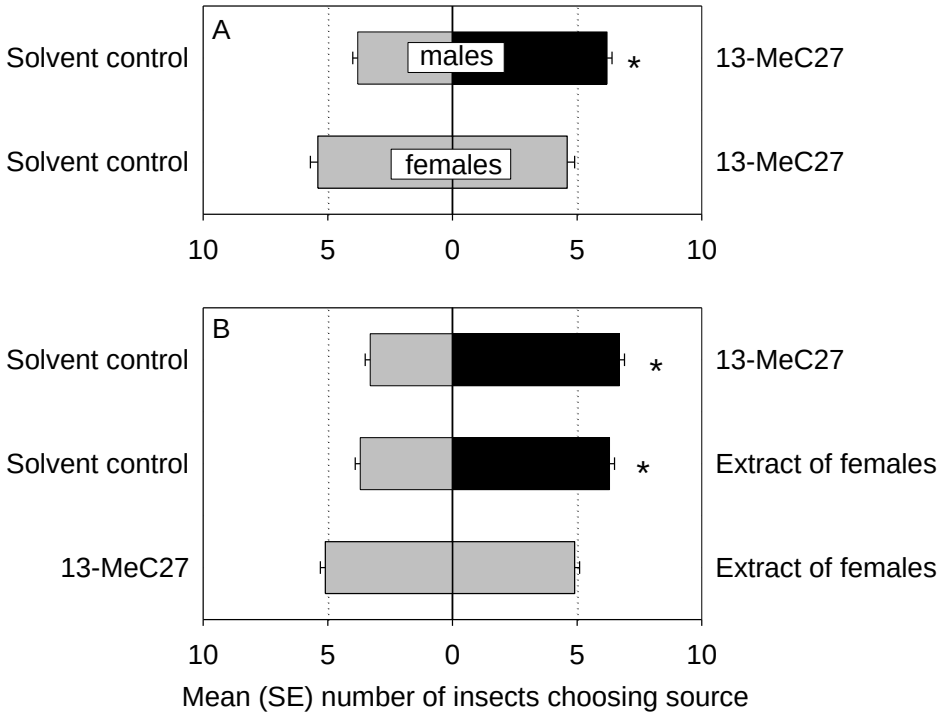


Figure 3. Olfactometer trials with 13-methylheptacosane

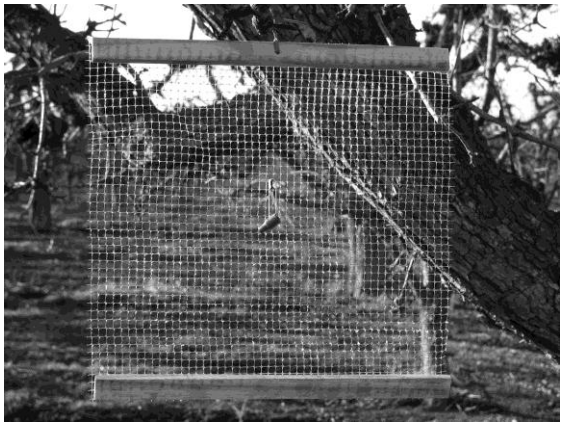


Figure 4. Trap design for field test

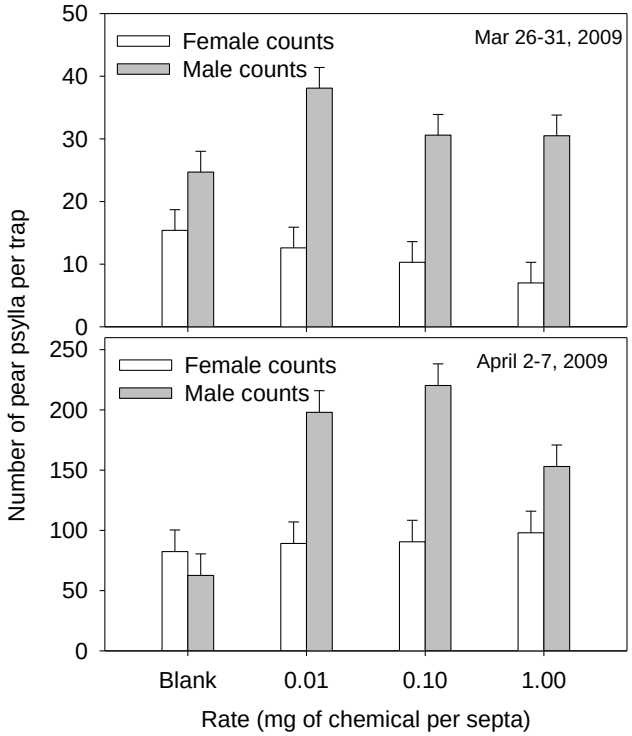


Figure 5. Results of field test

EXECUTIVE SUMMARY

A chemical (13-methylheptacosane) found to occur at higher levels in cuticular washes of post-diapause winterform female psylla than in washes from males was tested for attractiveness to male winterforms. Specific findings:

- Cuticular extracts from post-diapause winterform female psylla attracted males in olfactometer trials at a rate equivalent to attractiveness of an equivalent number of live females
- GC-MS analysis of extracts identified one chemical that occurs at a level several-fold higher in females than males (13-methylheptacosane)
- This chemical was synthesized by J. Millar, and then shown in laboratory and field trials to attract male winterform psylla
- The same chemical and two other compounds were identified in cuticular washes of summerforms at levels several-fold higher in females than males

Plans for 2010

- Assess in field and laboratory assays whether attractiveness of the 13-methylheptacosane compound to male psylla depends upon time of year
- Continue assays with summerforms and three identified female-specific compounds

PUBLICATIONS

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

Guédot, C., J.G. Millar, D.R. Horton, and P.J. Landolt. 2010. Identification of a sex attractant pheromone for male winterform pear psylla, *Cacopsylla pyricola*. *Journal of Chemical Ecology* (in press).

FINAL PROJECT REPORT
WTFRC Project number: PR-07-702

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

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Other funding sources

Agency Name: Western SARE
Amount awarded: $121,092 (2008-2009)
Notes: Expands the sampling part of the study to 3 commercial organic pear orchards

Agency Name: WSU Center for Sustaining Agriculture and Natural Resources (CSANR): Organic Cropping Research for the Northwest
Amount awarded: $63,807 (2009-2010)
Notes: Expands the sampling part of the study to 3 commercial organic pear orchards

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

Budget History: USDA-ARS

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

Budget History: WSU

Item	2007	2008	2009
Salaries	3,148	3,273	
Benefits	1,133	1,178	
Supplies	719	549	
Total	5,000	5,000	0

OBJECTIVES:

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

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS:

- Densities of generalist predators were substantially higher in understory of alfalfa plots than grass plots.
- Despite the high densities of predators in the alfalfa cover crop, we found no statistical increase during 3 yrs of sampling in densities of predators in the canopy of trees having the alfalfa understory, and no effects on psylla densities.
- Over 4000 specimens were collected from orchard floor and tree canopy for assessment of marker presence (markers applied to orchard floor vegetation). The specimens were assayed for presence of markers using ELISA. We saw evidence for movement from orchard floor into tree by several predator taxa, but no striking differences between alfalfa and control plots.
- Specimens are currently being analyzed with ELISA for gut contents (to assess presence of pear psylla remains).
- We observed increased nitrogen levels in trees having the alfalfa understory.
- Funding was obtained from Western SARE ($121,092) and CSANR-WSU ($34,178) to expand project into 3 commercial organic orchards; plots were set out and planted in spring 2008. Sampling began in 2009. We saw no effect of alfalfa on predator counts in trees. Psylla and predator counts were very low in all 3 orchards. Grower cooperators were e-mailed with sampling results at regular intervals.

RESULTS AND DISCUSSION:

Methods used in studies

Plot design. The studies were done at the Moxee farm (5-8 year old Bartlett trees). We had 4 blocks, each composed of an alfalfa cover crop plot (planted spring 2006) and a control grass plot (Figure 1). Plots were also established at 3 commercial organic orchards (Figure 2). Plots were each 0.3 to 0.5 acres in size. The alfalfa was planted in April 2008 as ½ meter wide strips down the centers of aisles. Movement and feeding studies were limited to the Moxee farm.

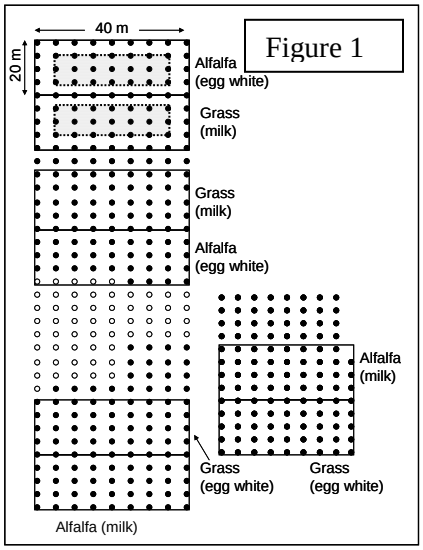


Figure 1

Psylla and predator densities. We monitored densities of prey and predators in trees and orchard understory. Pear psylla numbers were monitored with beat trays and leaf samples (eggs and nymphs). Predator numbers were assessed using beat trays (trees), sweep nets (understory), and sticky traps (Moxee only); the sticky traps were placed at two heights: 1 foot and mid-tree canopy.

Protein marker methods. At the Moxee site, cover crop and grass control plots were 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 received 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 were 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 were 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 were removed from the adhesive in the field using wooden toothpicks, and transferred singly into 1.5 ml microtubes. Similar methods were used to obtain arthropods from the ground covers, except that the vegetation was shaken over the top of the cardboard sheet. Marker presence was determined using ELISA (Jones); the same specimens are then to be assayed for presence of psylla proteins (Unruh).

Leaf nitrogen. Pear leaves were collected from control and cover crop plots for N-analysis.

Results and Discussion

Moxee site

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 several-fold higher in the alfalfa plots than the grass plots (Fig. 3). There was a significant presence all season of predators in the alfalfa cover crop, except immediately following mowing (Fig. 3). Tray counts of predators were, if anything, larger in the grass plots than the alfalfa plots (Fig. 4). Sticky trap catches of generalist predators are shown for canopy-height traps (Fig. 5, upper panel) and ground-level traps (Fig. 5, lower panel). Numbers of predators on traps were similar in the alfalfa and grass plots (Fig. 5). Trap catch was dominated by the true bugs and lacewings. Densities of adult psylla were statistically similar in grass and alfalfa plots (Fig. 6). Counts of psylla eggs and nymphs were also similar in grass and alfalfa plots (Fig. 7).

Marker results. Marker results are shown for the 2009, tree-collected specimens (Table 1); data from previous years and from the orchard floor specimens are still being analyzed. Results support the hypothesis that certain predatory taxa moved between orchard floor vegetation and the tree canopy. Lacewings may have been especially mobile. No striking differences were noted between alfalfa and grass plots.

Gut contents results. Specimens are still being assayed.

Leaf nitrogen. Pear leaves were collected from each plot on one date in both 2008 and 2009, and assayed for nitrogen content. Results suggest strongly that an alfalfa understory led to increased levels of nitrogen in the pear tree canopy (Fig. 8).

Commercial orchards

Counts of natural enemies and psylla were very low in all 3 orchards in 2009 (data not shown). Grower cooperators were updated with e-mail at regular intervals summarizing results of the sampling.

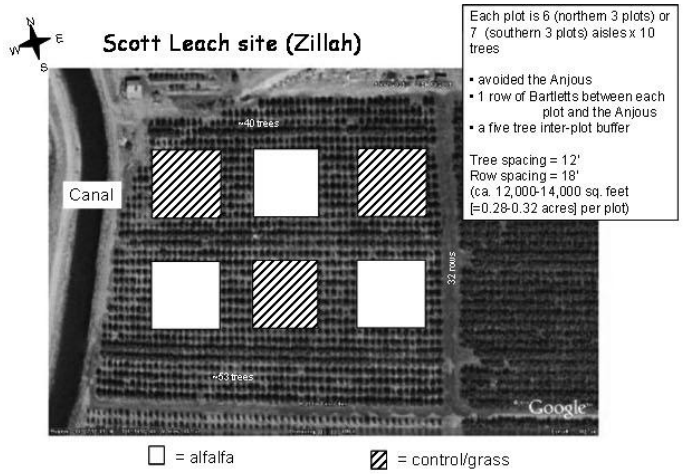
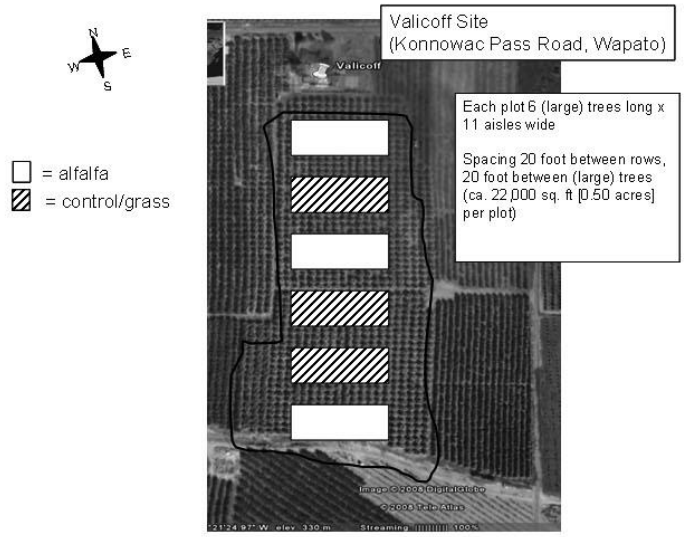
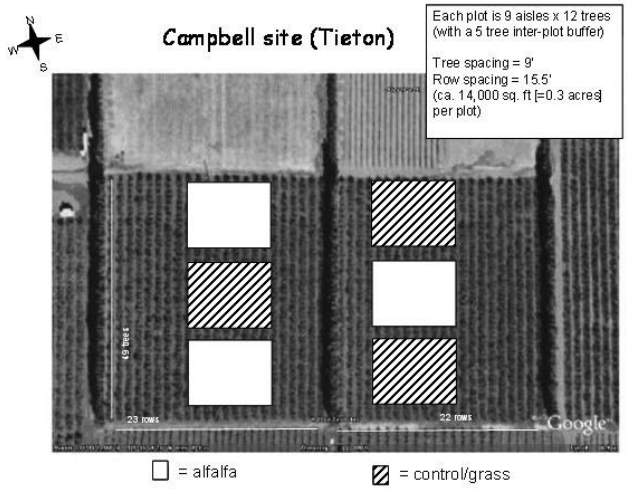


Figure 2. Plot design at each of 3 commercial organic orchards.





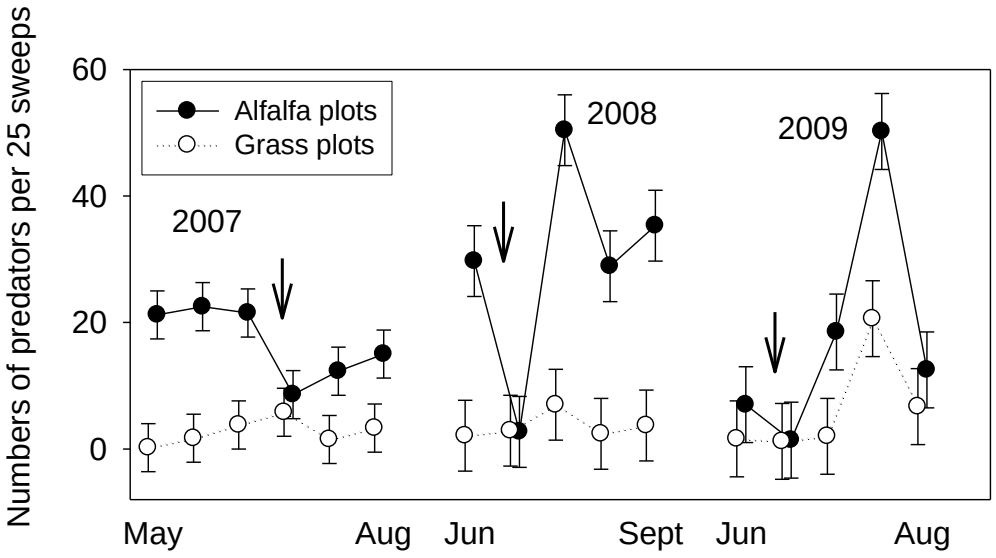


Figure 3. Predators per 25 sweeps

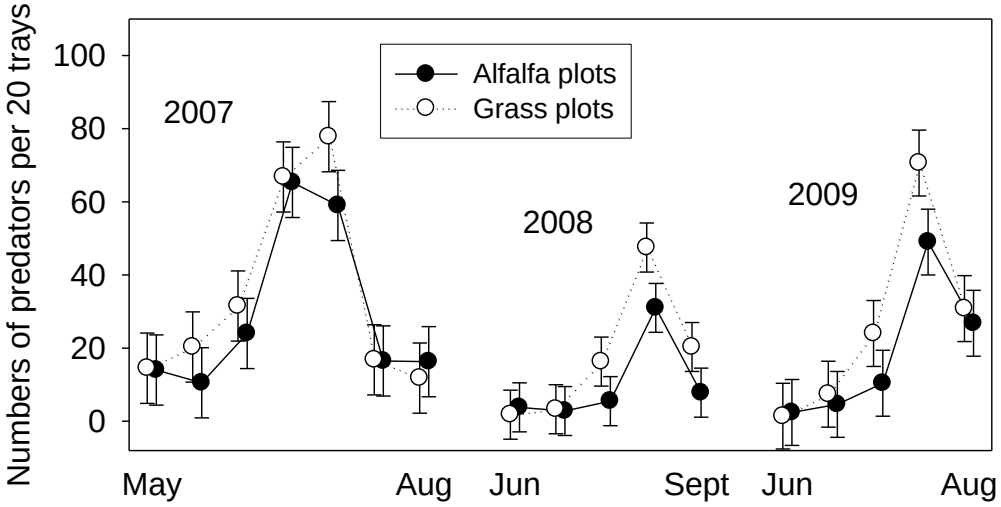


Figure 4. Predators per 20 trays

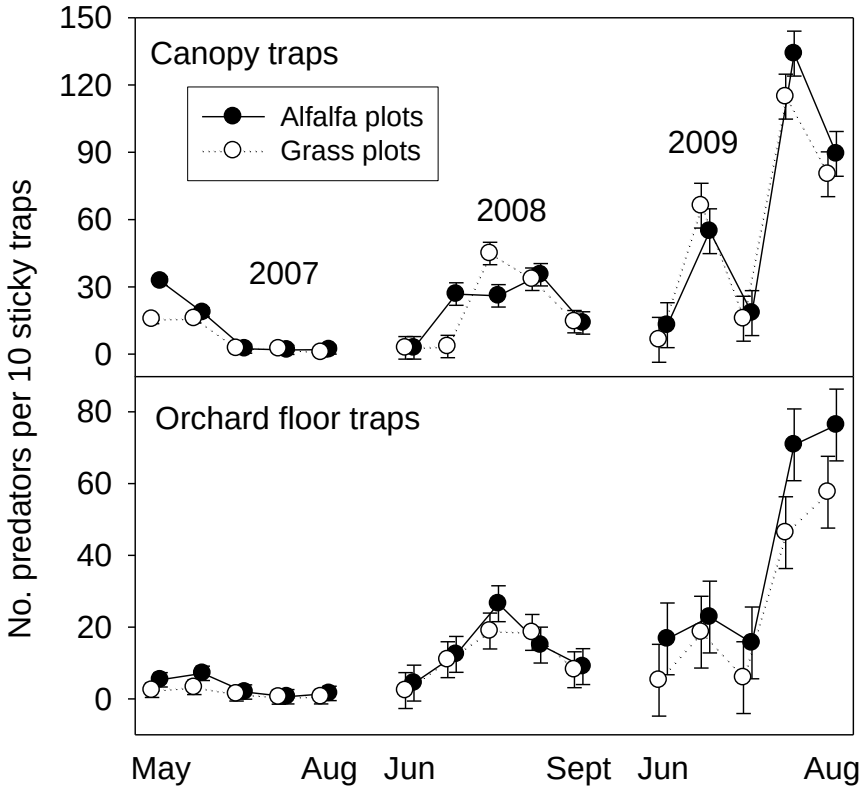


Figure 5. Predators per 10 sticky traps

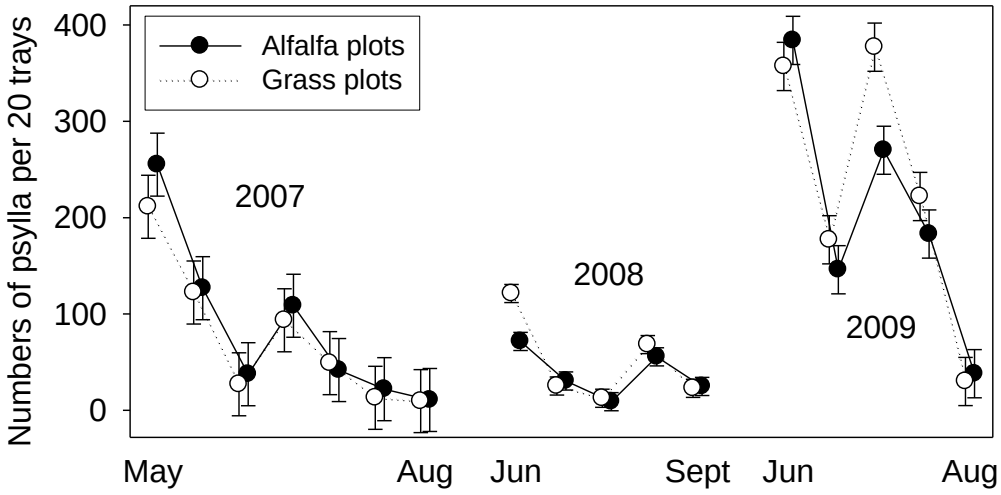


Figure 6. Adult psylla per 20 trays

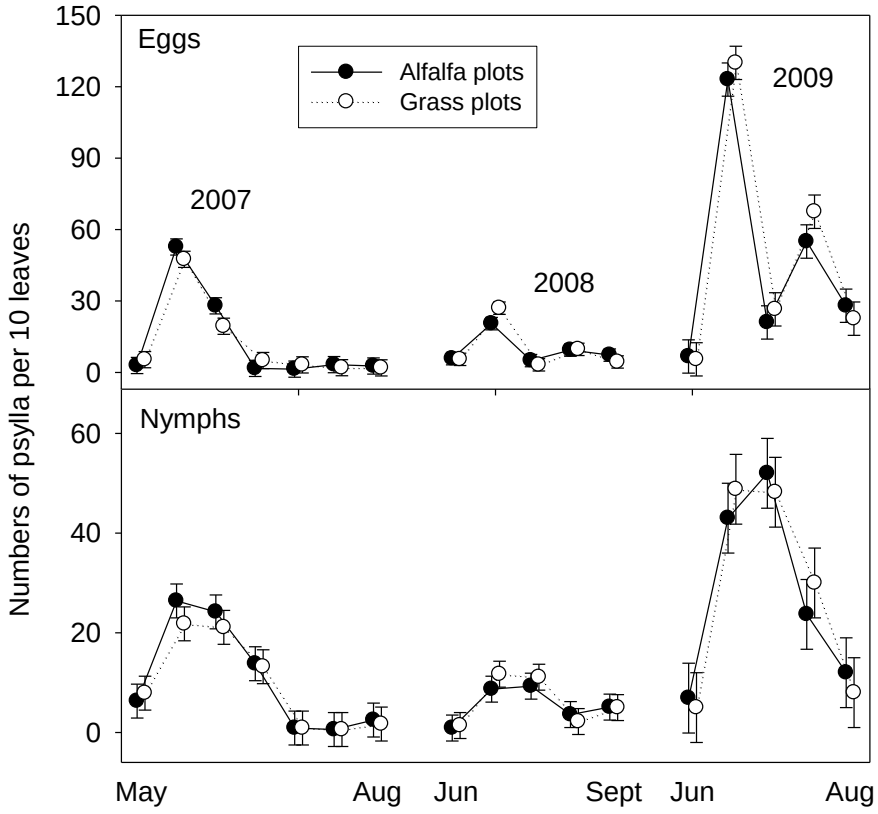


Figure 7. Immature psylla per 10 leaves

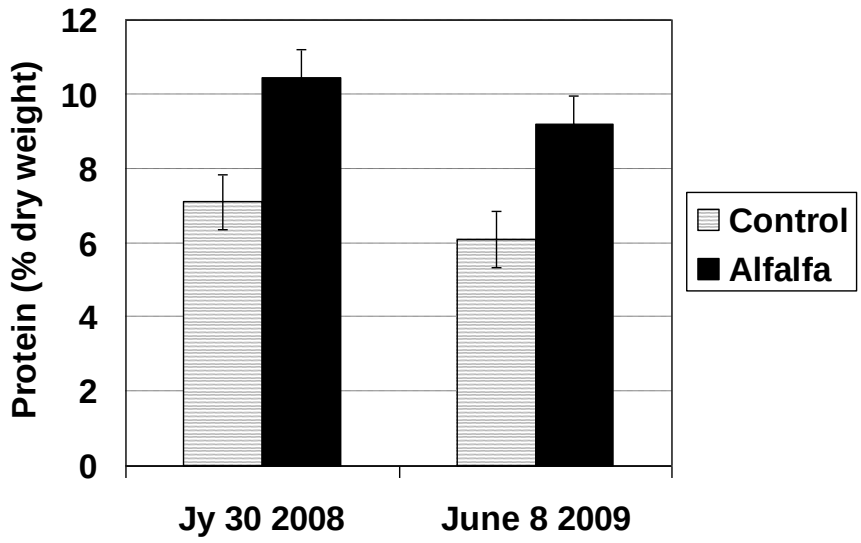


Figure 8. Nitrogen levels in pear leaves

Table 1. Number of marked / total (% marked) predators collected from tree canopy in alfalfa and control plots; summer 2009 data.

	June 2009			August 2009	
	Alfalfa	Control		Alfalfa	Control
TRUE BUGS					
Anthocoris	1/28 (3.6)	0/8 (0)		4/71 (5.6)	8/101 (7.9)
Deraeocoris	5/127 (3.9)	0/93 (0)		12/85 (14.1)	4/81 (4.9)
Orius	0/4 (0)	0/5 (0)		0/2 (0)	0/3 (0)
Nabis	--	--		1/1 (100)	--
TOTAL	**6/159 (3.8)**	**0/106 (0)**		**17/159 (10.7)**	**12/185 (6.5)**
LACEWINGS					
Hemerobius	0/1 (0)	0/1 (0)		0/1 (0)	--
Eremochrysa	4/31 (12.9)	5/23 (21.7)		18/48 (37.5)	5/16 (31.3)
C. plorabunda	6/17 (35.3)	1/17 (5.9)		5/35 (14.3)	5/14 (35.7)
C. nigricornis	1/1 (100)	0/3 (0)		5/23 (21.7)	0/3 (0)
C. coloradensis	0/1 (0)	1/10 (10.0)		--	--
TOTAL	**11/51 (21.6)**	**7/54 (13.0)**		**28/107 (26.2)**	**10/33 (30.3)**
LADYBIRD BEETLES					
Hippodamia	0/1 (0)	0/1 (0)		6/27 (22.2)	8/42 (19.0)
Stethorus	0/2 (0)	0/1 (0)		1/5 (20.0)	0/12 (0)
C. transversoguttata	--	0/1 (0)		--	--
Harmonia	--	--		2/6 (33.3)	3/17 (17.6)
Chilocorus	--	--		0/1 (0)	1/6 (16.7)
Hyperaspis	--	--		14/65 (21.5)	0/5 (0)
C. septempunctata	--	--		2/8 (25.0)	1/6 (16.7)
unknown	--	--		0/1 (0)	0/4 (0)
TOTAL	**0/3 (0)**	**0/3 (0)**		**25/113 (22.1)**	**13/102 (12.7)**
SPIDERS	**11/99 (11.1)**	**1/76 (1.3)**		**5/84 (5.9)**	**5/68 (7.4)**

EXECUTIVE SUMMARY

Effects of an alfalfa cover crop on biological control of pear psylla and tree nutrition was assessed. The bullet points below summarize findings.

Experimental Orchard (Moxee)

- substantial increase in predator densities on orchard floor associated with alfalfa cover crop
- no correlative effect on predator numbers in trees
- no effects of cover crop on psylla densities
- ca. 2% increase in pear leaf nitrogen in alfalfa plots
- evidence for movement between orchard floor and tree by some predator taxa (especially lacewings and ladybeetles), but no striking differences between cover crop and grass plots (data still being analyzed)
- gut contents of predators that moved from orchard floor to tree to be assessed using ELISA (specimens still being assayed)

Commercial Orchards

- Study expanded to 3 commercial organic orchard (SARE and CSANR funding)
- Minimal build-up of natural enemies in alfalfa, apparently due to frequent mowing
- Very low pest and predator densities in trees

Plans for 2010 (CSANR)

- Determine whether mowing of alfalfa prompts movement by natural enemies into tree

PUBLICATIONS

Horton, D.R., V.P. Jones, and T.R. Unruh. 2009. Use of a new immunomarking method to assess movement by generalist predators between a cover crop and tree canopy in a pear orchard. *American Entomologist* 55(#1): 49-56.

FINAL PROJECT REPORT

Project Title: Pear genome project

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City: Pullman
State/Zip: WA 99164

Cooperators: Ananth Kalyanaraman, WSU**;** Todd Einhorn, OSU; Marie-Helene Simard (Pear Breeder at INRA), Yves Lespinasse, Charles-Eric Durel, Elisabeth Chevreau, INRA at Angers, France; Richard Bell, USDA; Kate Evans, WSU; Robert Bogden, Amplicon Express, Riccardo Velasco, IASMA, Italy; Jasper Rees, Univ of Western Cape, SA.

Other funding sources

Agency Name: Andres Bello University – Herman Silva and Lee Meisel
Amount awarded: $10,000
Notes: Funds to generated additional genome sequence

Agency Name: IASMA, Italy
Amount awarded: $20,000
Notes: Funds being used in Italy to generate additional sequence from BAC DNA library constructed as part of this project.

Agency Name: Roche Inc.
Amount awarded: $30,000
Notes: Funds being used at 454 to generate scaffold DNA libraries and sequencing to enable efficient assembly of the genome.

Agency Name: USDA - NRI
Amount awarded: $ 224,000
Notes: Supplemental funding provided by USDA for scaffold sequencing in apple. The method developed with the apple funds will be utilized for rapid and efficient assembly of the pear genome.

Total Project Funding: $ 57,000

Budget History:

Item	Year 1:	Year 2:	Year 3:
Equipment			
Supplies	44,000		
Travel			
Miscellaneous	13,000		
Total	57,000		

ORIGINAL OBJECTIVE

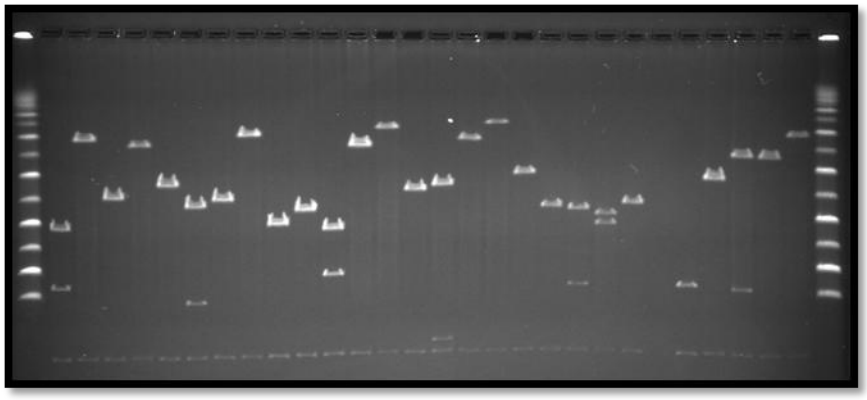
1. Generate sequence information from the double haploid (DH) Comice pear to establish a high quality draft sequence of the pear genome.

SIGNIFICANT FINDINGS

Definition: BAC Library – It is a method of capturing the large mass of the genome or DNA from any cell and breaking it into small pieces. This needs to be done so that the genome can be studied in manageable pieces.

The basic purpose of this project was to generate a draft assembly of the DH Comice pear genome sequence. Support from this project has allowed us to construct a high quality BAC DNA library that has large pieces of genomic DNA captured in a way that we can multiply them individually. The average size of the genomic DNA in these clones is 145 Kb, which is far above the average size other libraries have (Fig. 1). In comparison, apple BAC library has 130 kb average size of DNA fragment in it.

Figure 1:



Pear BAC Library. The figure shows DNA as bright bands. Using a measure of size on the sides, we can determine the average size of genome captured in a library.

Our original plan was to generate sequence information from random DNA pieces derived from the pear genome. Thereafter, we would use the redundancy or sequence similarity at the ends to develop the entire genome.

While the work was in progress, we obtained additional funding from USDA-NRI program to develop a new method that allows us to generate sequence in a way that not only represents the entire genome; it also builds a scaffold simultaneously. This requires a computational program that we are refining for apple and will be directly applicable to pear once completed in the next coming months.

The objective of generating the sequence information is currently in progress. We are also in the process of refining the computational methods to integrate random and scaffold sequencing data for building a complete assembly of the pear genome.

We have also provided the DH Pear genome DNA library to IASMA for generating sequences from

the ends of DNA fragments. All this data will aid in our final goal of assembling the DH pear genome. They have committed their own funds to generate this information as part of our ongoing collaboration.

RESULTS & DISCUSSION

Sequence information can be rapidly utilized for developing molecular markers for the pear improvement program. It can also provide complete sequence information for genes where we only have partial information. Over the last year we have utilized the preliminary assemblies for mining such information for various colleagues at WSU. Most importantly this information has been the basis of identifying the complete coordinates and sequence for a putative gene believed to regulate the 2nd stage ethylene biosynthetic burst associated with the onset of ripening in winter pear that we identified within another project funded by WTFRC. Knowledge of genes underlying important traits can also serve as targets for improving existing varieties using controlled sports induction (CSI) using non-transgenic approaches. We have a continued emphasis on refining the CSI approach in our program to improve existing varieties thereby circumventing marketing and retail shelf space issues.

The significance of this information will far outlive the duration of this project. Each economically important trait or desirable quality in the fruit tree is controlled at some level by genes. An accessible genomic blueprint of pear enables us to pinpoint what gene or group of genes are responsible for agriculturally important traits. This information will guide pear improvement in both the short and long term future. Another testimony to this fact is that scientists have now discovered the gene underlying skin and lung cancer in humans utilizing human genome information. As in the case of humans, the potential economic benefits to the industry are apparent. With the pear genome sequence in hand, we can develop unique varieties for the PNW combining all priority traits that can create lucrative economic opportunities ranging from production to post-harvest stages.

BROADER IMPACTS

Presentations: The pear genome information has been highlighted at several forums over the last year including WSHA meetings. In 2009, the PI was invited to speak at the Hort Show about Enabling Economic Resilience through Genomics Research. Besides that, the work has been shown as poster presentations at annual international meetings like American Society of Plant Biology and Plant and Animal Genome Meeting.

Publications: The data generated from WTFRC and WSU-supported DH pear genome will be integrated with the sequence information generated at IASMA and a manuscript will be submitted by June 2010.
Research: The apple and pear genome sequencing projects have enabled us to now begin sequencing work in order to obtain the cherry genome. We are also a part of the strawberry and peach genome project consortia.

Training opportunities: This project has been steered by graduate student Christopher Hendrickson. Our program has graduated a computer science student (Vandhana Krishnan) who utilized both apple and pear genome data for her MS thesis.

Community building: A survey was conducted to gauge the ongoing interest of several pear researchers worldwide and assess their interest in the use of pear genome information. A template of the survey is provided below. Out of 12 groups worldwide, 10 responded and have committed to participating not only in increasing the genomic information for pear but collaborating on providing genotypes, that will include dwarfing trait, other types of genomic information that will help in

advancing the science of pear improvement further.

"Pear Genome Project Collaborators
The PNW Pear Research Bureau has provided support to initiate genome sequencing of the Double Haploid Pear Genome. You are receiving this message as you had indicated to participate as a collaborator on this project.
There are two main objectives of the approved project
1. Prepare a 6X BAC library – *The BAC library has been constructed.*
2. Obtain 4X genome coverage using de novo sequencing on the 454 platform

While these objectives will establish the much needed nucleus for the project, there is a need for additional support to complete the genome. In particular, following resources are needed
1. Genome Scaffolding using 3kb and 20 kb paired-end reads.
2. Solexa-based paired-end read sequencing for fine-scaffolding.
3. BAC-end sequencing.
4. Transcriptome sequencing for functional annotation.

We will be happy to send the 454 Titanium library to any of the colleagues who are capable of performing the sequencing in house. Alternatively, a bulk run on 454 Titanium post-library preparation costs $7193 and we have a mechanism in place to charge the collaborators if they decide to sponsor some runs. I would like to present a report to the PNW Pear Research Bureau and to facilitate the preparation of this report your feedback is important. I have prepared a feedback form that you can fill out indicating how the pear genome sequence will help your research. This will enable us to apply for funding to federal agencies in the coming months. Please feel free to add as much information as you would like to add. Thanks!
1. Name and area of research in pear biology
2. How do you plan to utilize the pear genome sequence in your ongoing research?
3. Please list synergistic projects and funding amount of these projects that will benefit from the Pear genome sequence.
4. Would you be able to contribute to the pear genome project? If yes, in what capacity?
5. Would you be interested in performing or sponsoring any of the additional tasks listed above?"

EXECUTIVE SUMMARY

Significant progress: The objective of generating pear genome sequence coverage is currently in progress. We have devised a new method of generating far more useful and complete information using a novel scaffold-sequence approach. At present we continue to refine the computational methods for creating a complete and efficient pear genome assembly. It is a reiterative process owing to the computational constraints that involves testing different parameters to arrive at the best and most accurate genome assembly possible. Collaborations with IASMA, Italy; Roche Inc. and Andres Bello University have provided extra funds to develop a much finer assembly of the pear genome.

Outcomes and summary of finding: Preliminary DH pear genome sequence data are available that are being used by our program to identify coordinates and sequence information of important genes linked to desirable traits; one important one being the cold-induced ripening gene. In summary this is just the start of the most efficient way of connecting traits to genes, an emphasis of our fruit crop genomics program.

Future directions: We have two proposals under review at NSF and USDA, and others at various stages of writing to build upon this foundational information. Our programmatic approach is to connect traits with genes using function information. Future projects are aimed at applying this approach in new and novel ways to the improvement of pears.

FINAL PROJECT REPORT

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

Budget History

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

Pear is an important PNW crop as well as an important member of the Rosaceae family. However, at the beginning of this project, pear genomic resources were scarce. This project was aimed at setting up a platform to identify a gene or set of genes underlying important physiological problems. This knowledge has application in today's pear orchards as well as it can serve as a foundation for future pear improvement via breeding. Recognizing the fact that there is only one pear breeding program in the US and that improvement via breeding can take several decades, the second aspect of this proposal was aimed at establishing a platform that will help in rapid improvement via sport induction that is not random but targeted. For the success of the second activity, we first need to identify trait-gene relationship. Therefore the two major objectives of the proposal were highly complementary.

At the onset of this project two very important traits that affect pear consumption and its production were short-listed. First one was improvement of *post-harvest quality* and storage abilities of pear varieties grown in the Pacific Northwest and second longer term goal was to fulfill the urgent need to develop a *dwarfing pear rootstock.*

ORIGINAL OBJECTIVES: Proposed objectives of the project were:

1. Prioritization of economically important pear traits.
2. Gene discovery for establishing trait-gene relationships using an economical yet high-throughput methodology called 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.

SIGNIFICANT FINDINGS

Objective 1: Prioritization of economically important pear traits.

After constant feedback from the industry over the past three year, it was found that the priority traits for pear improvement have not changed much since the late George Ing published an article in Acta Hort in 1993. It was intriguing why similar issues remain. Partly it is due to the fact that pear varieties remain the same, they have a long generation time and another that scientists have always attempted at using solutions generated for apple in pear. As is apparent, pears are a different organism and some information can be borrowed from apples however, pear-specific research will be urgently needed to resolve pear-specific issues.

Pears are not apples and apples are not pear.
Although their family they may share…..

After several industry visits and discussions with growers and packers, one important issue is lack of consistency of the product that reaches the shelf. Not much effort has been made to understand the underlying genomic or genetic reason for most physiological disorders. The solutions for several disorders have brought the industry into a profitable entity however for further progress, effort will have to be focused on understanding how the existing pear trees respond to chemical treatments, how to handle pears like pears and not modify apple processing lines and suffer nearly 30% loss due to scuffing or post-harvest damage.

The pear enterprise can be divided into three parts: production, processing and post-harvest stages. Each of these stages requires minor adjustments to reduce the losses that the industry has to face. These issues have become the cornerstone of a larger, team-based and interdisciplinary proposal

submitted to the NW Pear Bureau. We would like to take these issues to the USDA-SCRI or NIFA proposal this year too.

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

A comparative gene profiling between Bartlett and D'Anjou fruit peel and core has rapidly yielded information about genes that control several quality aspects of the fruit. The first and the one of the important ones we have identified and continue to work on is a gene that is the proposed cold-induced ripening gene. This gene has not been described in the past and is expected us to enable develop effective ripening strategies for pear. Also, it would serve as a target for controlled sport induction in Bartlett pear to change its shelf life.

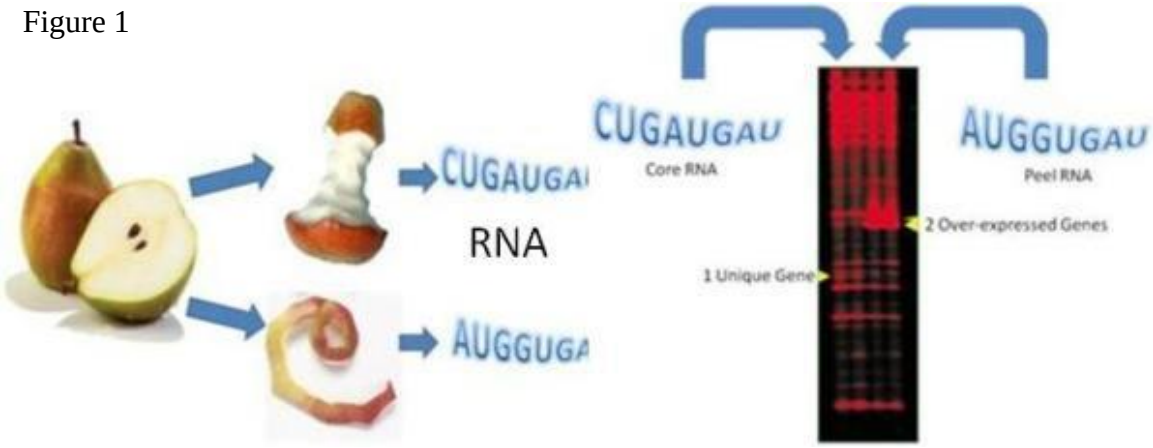
3. Controlled Sport Induction using tissue culture derived propagules combined with high-throughput screening of allelic diversity for genes responsible for desirable trait.

We have established the method of generating new plants from leaf material from Bartlett and D'Anjou pear. The methods for generating targeted mutations are being refined for pear. In addition, we have perfected the techniques for rootstock micropropagation that can enable rapid multiplication of any sport scion that is generated through our CSI approach.

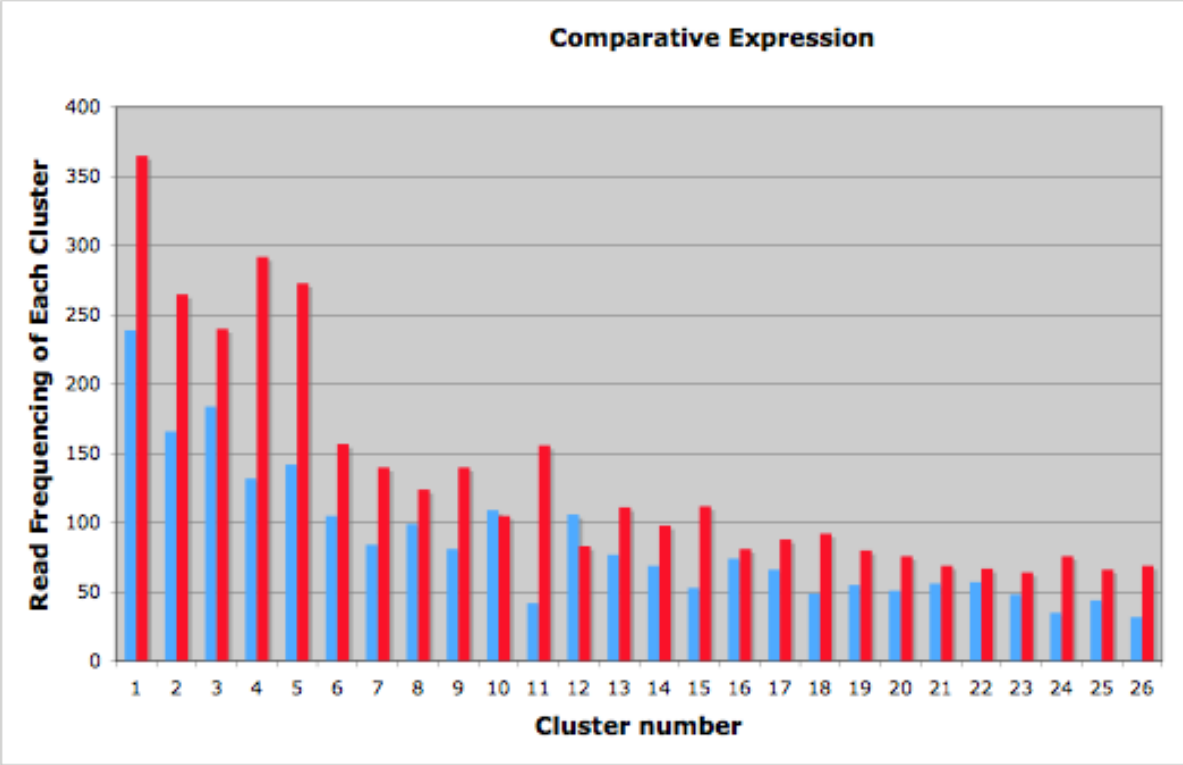
RESULTS AND DISCUSSION

The methods employed in gene discovery in pear are depicted in Figure 1. Peel and core samples were taken from fruit sterilized with ethanol. These samples were transferred in liquid nitrogen for return to the laboratory. By grinding samples in the Spex SamplePrep 6870 freezer mill we were able to obtain high quality RNA ready for analysis.

Figure 1



RNA was isolated from the ground tissue using a Qiagen RNA extraction kit. We performed differential display experiment as previously planned. Besides the gel based differential display, we have performed comparative RNA profiling using the 454 next-generation sequencer. It provides sequence based information on genes and represents the entire transcriptome at the same time. In short we can capture the response of the entire transcriptome in one shot. The graph on next page shows such a snapshot where a cluster represents a single gene and read frequency indicates its abundance. The bars in blue and red represent two different genotypes. The data was obtained from core and peel tissues of Bartlett and D'Anjou harvested at a comparative developmental stage.



By applying custom-developed computational script we analyzed this data for identification of novel differentially expressed genes in the tissue. This revealed the differential expression of numerous unknown genes, as expected in such a relatively uncharacterized organism. However, we discovered a differentially expressed gene (termed *Pyrus communis* membrane-integral protein, or PcMIP). Further computational analysis of this gene shows that it likely serves as a signal receptor, transmitting the cold signal in winter pear. This gene was found 8 times in D'Anjou peel tissue only, not in Bartlett. A review of literature related to this gene suggests a role in ethylene signaling and regulation. Based on this computational and literature analysis, we hypothesize this newly discovered gene in pear to regulate the 2nd-stage ethylene biosynthetic burst, and subsequent ripening in pear. Recent work by Sugar (OSU), Mitcham (UC-Davis), and Kupferman (WSU) support this model by revealing that exogenous ethylene application can circumvent this proposed regulatory mechanism. This gene will serve as a target for our CSI approach and as a powerful molecular marker in pear research and breeding efforts, allowing for rapid crop improvement.

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

DNA-RNA hybrid molecules as shown in figure on previous page. DNA bases in dark have been modified to induce mutations.

For development of callus, we have tested several parameters as outlined in Figure below.

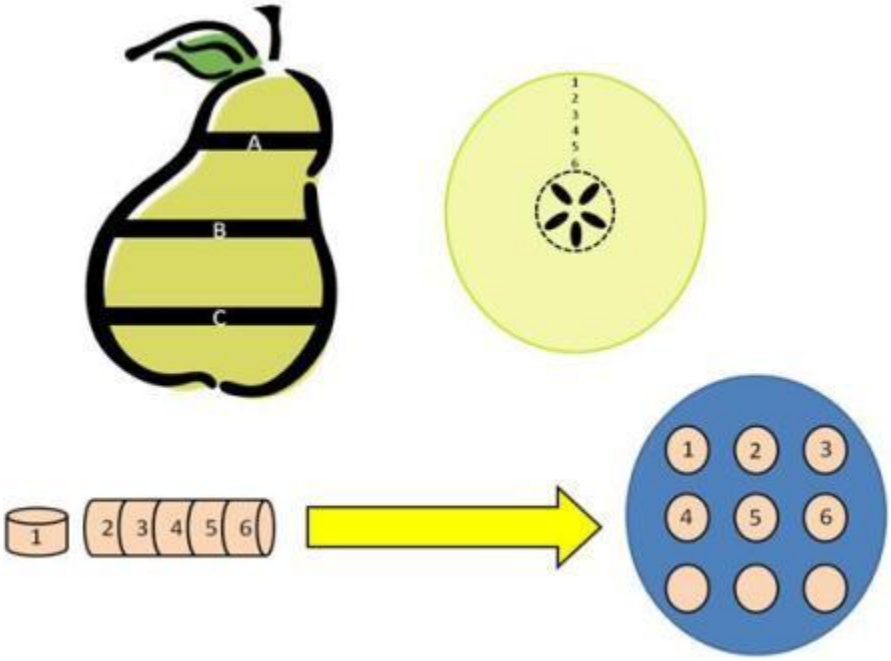


Figure on pear callus production depicts the procedures employed to assess the productivity of callus formation by various parts of the pear fruit. Cores were taken from each of three selected parts of the pear; the top (A), middle (B), and bottom (C). The samples cores were cut into multiple sections 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.

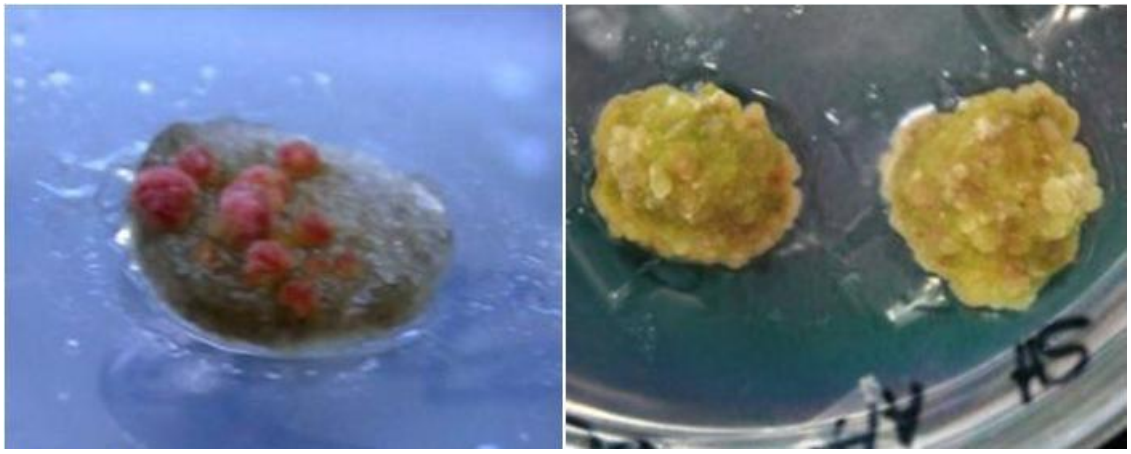
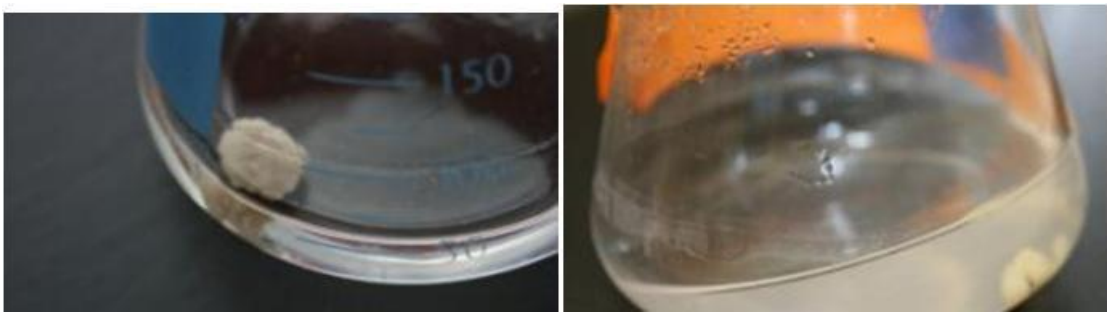


Figure above displays early callus growth (left) on pear tissue discs after two weeks of growth and a later stage of callus growth (right) after two months of growth. **Right panel** displays cellular growth after 40 days of inoculation. After sufficient callus was produced, callus tissue was transferred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



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. It was also features at the WSHA invited presentation in 2009.
2. The work was presented in an invited talk at the AEMP 2007 meeting in Portugal in September 2007 and AEMP 2008 in India.
3. The preliminary concepts were presented at the WSHA meeting in 2007, 2008 and 2009 by Scott Schaeffer and Chris Hendrickson graduate students in the Dhingra lab.
4. This work was presented at the Annual Rosaceae Genomics Conference in Chile in March 2008 and American Society of Plant Biologist annual meeting in July 2008 and 2009.

EXECUTIVE SUMMARY

Significant Progress and outcomes and summary of findings

Some of the major steps we have accomplished to sustain the pear improvement efforts are to have established a strong feedback mechanism from the industry, established a strong community network of pear researchers worldwide and established genomic resources previously missing in the community. We have identified traits where we can implement the knowledge of genomics today for improving the existing pear orchards. Discovery of the putative cold-induced ripening gene in D'Anjou is a major accomplishment without years of phenotyping on diverse genetic material. We have also established methods for generating new plants from leaf tissue of scion varieties and micropropagation rootstock material. This accomplishment will aid in developing novel pear varieties using controlled sport induction.

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.

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.

Future directions

We plan to further characterize other genes that we have already identified as differentially expressed between two fruit types. Some of these genes are directly related to fruit quality traits. The results and methods established in this proposal are serving as a basis for a larger proposal submitted to NW Pear Bureau. The funding will be utilized for a larger USDA-SCRI proposal. Since gene discovery and its characterization is a basic research component, we are submitting a proposal to NSF to understand the ripening mechanism in further detail.

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

Project Title: Cold hardiness of quince

PI: Todd Einhorn
Organization: OSU-MCAREC
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Email: Todd.einhorn@oregonstate.edu
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City: Hood River
State/Zip: Oregon 97031

Co-PI(2): Joseph Postman
Organization: USDA/ARS
Telephone: (541) 738-4220
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City: Corvallis
State/Zip: Oregon 97333

Cooperators: Amit Dhingra, Kate Evans

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

Other funding sources

Agency Name: National Plant Germplasm System
Amt. requested: $12,192
Notes: A proposal was submitted to NPGS November 10, 2009 to complement the present study by more precisely defining the lowest survival temperatures for the quince and pear accessions tested herein, through the development and implementation of differential thermal analysis (DTA) techniques. Our proposal was ranked 1st. Final funding decisions will be announced ~ April, 2010. If funded, work would then be performed beginning September, 2010.

WTFRC collaborative expenses: None

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

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

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

Budget 2 Joseph Postman
Organization Name: USDA/ARS **Contract Administrator**: Dorothy Beaton
Telephone: 541 737-3228 **Email address**: dorothy.beaton@oregonstate.edu

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

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

Objectives

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

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

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

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

Significant Findings

- Genetic diversity, relative to cold-hardiness, exists in the core-collection of quince
- Following cold acclimation ~50% (25) of the quince accessions tested were capable of withstanding -22° F without accompanying freeze damage to tissues
- Following cold acclimation 13 quince accessions were categorized as having low levels of tissue browning (likely survivability) following exposure to -40° F
- None of the pear accessions tested, including four previously reported cold-hardy accessions, appeared capable of withstanding -40° F
- Our model for freezing and visual assessment of injury proved reliable, as our data strongly aligned with previously characterized hardiness levels of both cold tolerant and cold sensitive Pyrus selections (i.e., used in our study as controls)
- Minimum and mean temperatures observed near the collection orchard gradually declined throughout early fall, providing good conditions for onset of cold acclimation and development of hardiness
- Sensitivity to sub-zero temperatures was similar among xylem, phloem and cambial tissue, although cambial tissue appeared to be the least hardiest tissue zone, and this response occurred independent of the accession.
- Relative water content (RWC) of sample tissue did not change significantly as the season progressed, and averaged around ~ 50 %
- 31 quince genotypes were successfully propagated (root initiation, and healthy stem tissue) from soft-wood cuttings. These plants will be grown out for the 2010 growing season prior to undergoing whole plant freeze studies in 2010/2011

Methods

Objectives 1 and 3:
Mature, current season shoots from eight Pyrus (Pear) clones and 50 quince clones, were collected from trees located in the NCGR orchards (Corvallis, OR). Tissue was sampled at ~three-four week intervals, beginning in late September. Sampling will continue until bud-break (March-April). The protocol is briefly outlined below.

- One-year-old shoots were harvested from trees and shipped next-day to MCAREC. Upon receipt, samples were placed in 42° F storage, and sectioned into one-inch pieces. Samples were weighed, and their fresh weights recorded. Four replicate stem pieces per accession per treatment (i.e., temperature) were made. These replicates also accounted for the likely biological differences occurring within a shoot (i.e., rep 1 was always taken from the thicker,

earlier growth at the basal portion of the one-year-old shoot, rep 2 with increasing distance toward the tip, rep 3 further, and rep 4 comprised the apical region, not including the terminal two inches of the shoot).

- Stem pieces were loaded into a programmable Tenney T2C Freeze Chamber, and subjected to freezing at a rate of 4° C per hour. Samples were removed following a one hour 'soak' at each of five treatment temperatures (0, -10, -20, -30, and -40° C [32, 14, -13, -22, -40° F]), with the exception of the first sample period, when samples were subjected to 0, -10, -25, and -40° C to account for a shortage of shoot material. Each of the four replicates was run on a separate date.
- Once removed from the freeze chamber, stem samples were placed in sealed plastic bags with moistened paper towel, and allowed to incubate at room temperature for one week prior to microscopic evaluation of injury.
- Transverse sections of stems were made midway into the one-inch sample, placed under a stereomicroscope, and individual tissue zones (phloem, cambium, and xylem) were rated according to the degree of oxidative browning observed using a six point scale, where 1, no damage [white]; 2, no damage [off-white]; 3, ~ 25 % area lightly browned; 4, ~ 50 % area browned; 5, >75 % area browned; 6, 100 % completely oxidized [black]. Visual assessment of freeze injury was performed by one technician, and all samples were prepared and rated in a double blind manner. The lowest exposure temperature which resulted in the absence of any observable levels of injury (i.e., a rating < 3) was termed the temperature prior to incipient damage.
- Following analyses, sample pieces were dried in an oven at 70° C and weighed until a constant weight was attained (i.e., dry weight). Relative water content was derived from fresh and dry weights as, [(Final Weight-Initial Weight)/Initial Weight] *100

Objective 2:
In late May and early June, 2009, softwood cuttings were taken from 53 quince clones and one clone each of *Pyronia veitchii* (Pyrus x Cydonia) and *Sorbopyrus auricularis* (Sorbus x Pyrus), with the goal of generating 10 self-rooted trees of each. These trees will be grown on in pots and used for whole-tree de-acclimation and cold hardiness trials in growth chambers during winter 2010-11. Sixteen cuttings were initially made for each genotype. Each cutting contained at least 3 nodes (~ 6 cm), and the base was dipped in a powdered rooting product containing 0.8% IBA before sticking in Oasis® Rootcubes and rooted under mist with bottom heat to keep media temperature at about 24° C. For genotypes that failed to thrive or produce any roots after 4-6 weeks, a second set of cuttings was made in July, 2009.

Objective 4:
Following the freeze treatments outlined above, a subset of tissue will be placed in individual wells of a 100-well electrolyte leakage chamber (Neogen, Lansing, MI). Each well contains water and the conductivity of the well solution is measured at 10 minute intervals. Maximum electrolyte leakage and leakage rate will be derived from standard curves following 24 hours of soaking and normalized to the dry-weight of the sample tissue. Tissue damage assessed by leakage will be correlated with ratings generated from visual assessments.

Results and Discussion

Objectives 1 and 3:
Temperatures recorded near the NCGR field site declined steadily from August 1 through October 12 (Fig 1). Light frosts were recorded on October 6 and 12, and were followed by a warm spell in mid October. Temperatures continued to decline from mid October reaching a season low of 8° F on

December 6, 2009 (Fig 1). The seasonal, gradual progression of declining minimum and mean temperatures are conducive to cold acclimation, a process by which plants acquire hardiness through exposure to increasingly lower temperatures, albeit, in the present study this process occurs later than in most pear growing regions of the PNW.

Our first sampling date in September indicates that plants were capable of handling temperatures as low as 14° F, prior to detection of injury (Fig 2A). Very little segregation occurs among accessions, since plants have not yet developed sufficient hardiness early in the season. However, one quince accession, two cold-sensitive pear clones, and, interestingly, OHxF 87 and 97 (Fig 2A) appeared to be more sensitive to sub-freezing temperatures, in which case slight browning was apparent at 14° F. Increasing hardiness can be seen with each subsequent sampling date for all accessions, reaching maximum levels in December (Fig 2B-D). In cooler regions at latitudes similar to those of PNW pear districts, maximum hardiness levels of Rosaceae tree crops are typically observed to occur in mid December. Over half of the accessions that were collected on December 7 (immediately following the 8° F recorded the previous night) were capable of tolerating -22° F (-30° C) without any signs of injury. This group consists of 25 quince selections (Fig 2D) that were equal to, or more hardy than, the cold-hardy pear genotypes tested as controls. In fact, Pillnitz 2 was capable of handling -40° F without detectable levels of tissue damage.

For each sample date, we have determined the warmest temperature at which injury was observed (temperature of incipient damage), and report minimum hardiness level as that temperature which immediately preceded the temperature of incipient damage [i.e., lowest exposure temperature resulting in freedom of visual levels of browning] (Fig 2). Several points must be made when interpreting these data: 1) Our estimates of minimum hardiness levels are extremely conservative. Lower levels of oxidative browning following freeze events have been observed to occur in temperate-zone tree fruit crops, without the concomitant expression of poor, or retarded, growth and development the following spring. However, until we are capable of aligning our freeze chamber results with those from whole plant freeze tests [scheduled for next year] we will remain cautious in our estimation of hardiness levels, 2) Because the increment between measurements is 10° (a fairly wide range), and in several cases the first temperature at which injury is detected results in quite significant browning (i.e., much higher levels of injury [score of 4-6]), the data reveals little about the qualitative nature of the temperature of incipient damage. Representative data collected for four genotypes is provided in Figure 3 to illustrate this point. Furthermore, once tissue damage is observed, we are unable to define whether the actual injurious event, or kill point, occurs following a 1° or a 9° lowering of the temperature from the previous test temperature. Indeed, this was the rationale used for our recent proposal submission to the NPGS, in which we propose to utilize more sensitive techniques such as differential thermal analysis (DTA) to detect precise kill points. Having said all of this, these results are very encouraging, because they identify a large group of quince taxa with the apparent capacity to acclimate and attain sufficient levels of cold-hardiness for many regions of the PNW. Additionally, previous reports have suggested that full expression of hardiness is associated with exposure to temperatures below 14° F for several weeks. Temperatures at the test orchards did not attain these values for any extended period of time, indicating that greater cold tolerance is entirely possible when planted in colder climates.

All tissue zones assessed (cambium, xylem, phloem) developed hardiness quite similarly, though cambial tissue (meristematic tissue responsible for cellular division, lateral trunk growth and ultimately new xylem and phloem tissue) appeared to be consistently more sensitive to sub-freezing temperatures than either of the vascular tissues [i.e., phloem or xylem] (data not shown). Within genotype, the sensitivity of the different vascular tissues to freezing was quite similar. At the maximum hardiness level [so far, the December samples] phloem tissue was hardier for 25 accessions, xylem hardier for 19, and 15 accessions scored equivalent values (data not shown).

Differences between oxidative browning ratings for xylem and phloem rarely exceeded 1. Whether genotypes and individual tissue zones have reached their maximum hardiness level will be partly explained following analysis of the January samples [run through the freeze chamber and presently incubating, prior to microscopic assessment January 25]. We will continue hardiness assays until bud-break (early spring), as plants accelerate through the de-acclimation process, progressively loosing hardiness. Data on freeze injury during the highly sensitive de-acclimation period will be valuable.

Objective 2:
As of November 2009 about 300 rooted cuttings have been established with 31 quince genotypes (48%) having 8 or more rooted cuttings per clone. The rootstock clones tended to root especially well, with Quince A, Quince S, Quince W, Quince WF-17, and several of the Pillnitz and the Polish Pigwa S clones rooting at nearly 100 %. No self-rooted Pyronia or Sorbopyrus trees were established, and these will be propagated by grafting along with the standard pear cultivars during late winter 2010. Additional softwood cuttings will be made in spring 2010 for quince genotypes with fewer than 10 rooted cuttings. A wide range in plant height existed in the population of rooted cuttings (Photo 1), with differences being more or less associated with genotype. Those accessions which rooted more easily produced marked growth last summer/fall, while genotypes which were slow to root did not elongate, and set terminal buds. New rooting techniques which alter rooting hormone concentration and application, as well as changes in the length of the soft-wood cutting will be explored in 2010.

Objective 4:
The electrolyte leakage chamber is presently being assembled and tested. Method development is scheduled to begin using January, 2010 sampled tissue.

Figures

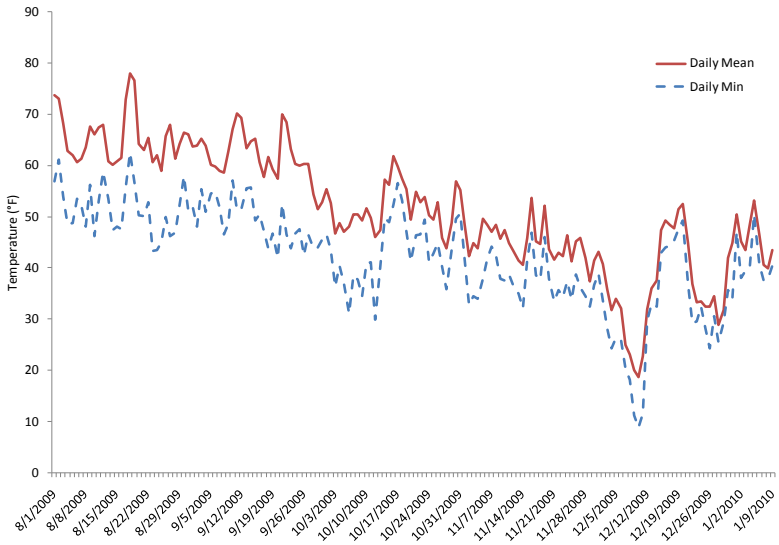

Figure 1. Daily mean and minimum temperatures (° F) from August 1, 2009 through January 9, 2010, recorded at the Hyslop farm located ~ 6 miles N.E. of the NCGR quince site.

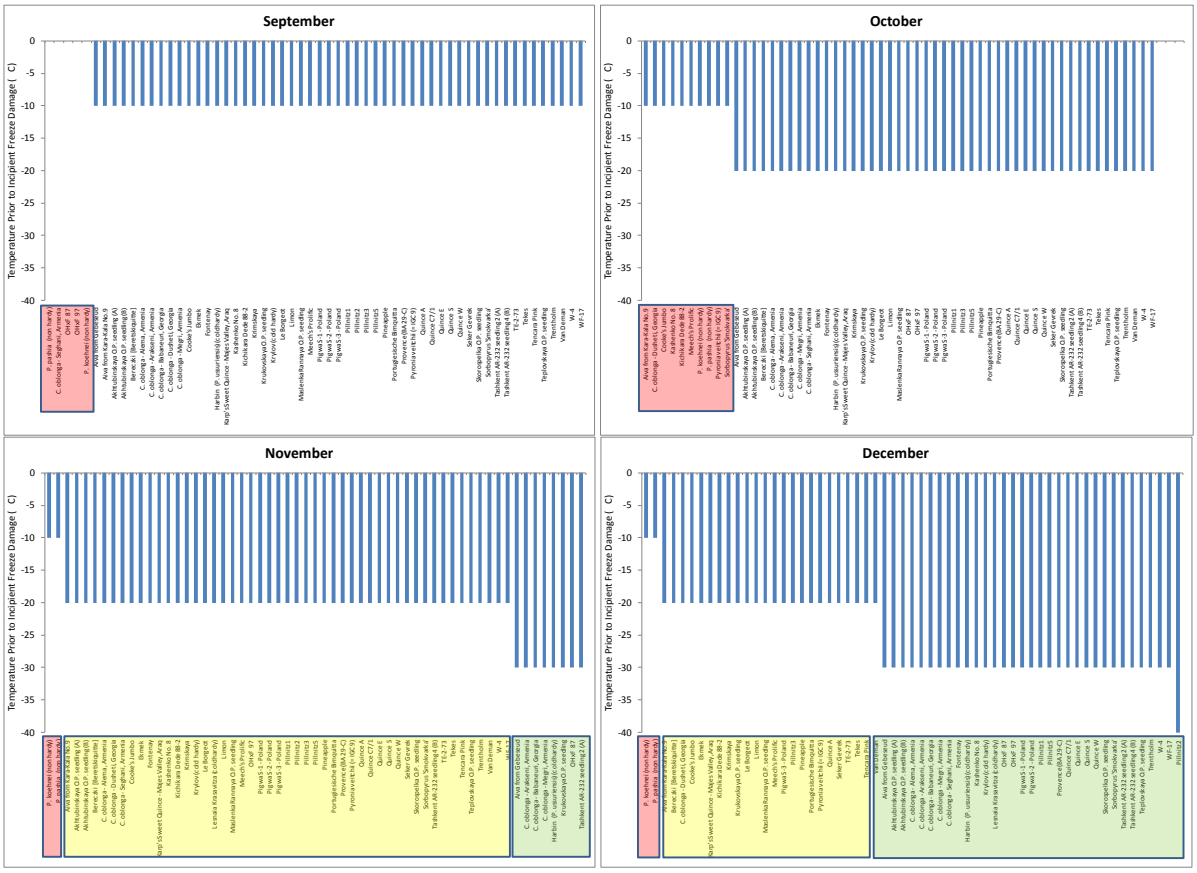

Figure 2. Mean hardiness of quince and pear accessions Sept-Dec, 2009. Data are lowest temperature at which no injury was detected for the most sensitive tissue assessed (see text). When applicable, accessions along the x-axis were grouped into three broad categories for a given sample period: 1) left grouping [non-hardy], 2) center grouping [mid to very hardy], and 3) right group [very hardy]. Data are means of 4 reps. Text on x-axis is not intended to be legible, but rather to show the range of diversity relative to hardiness level.

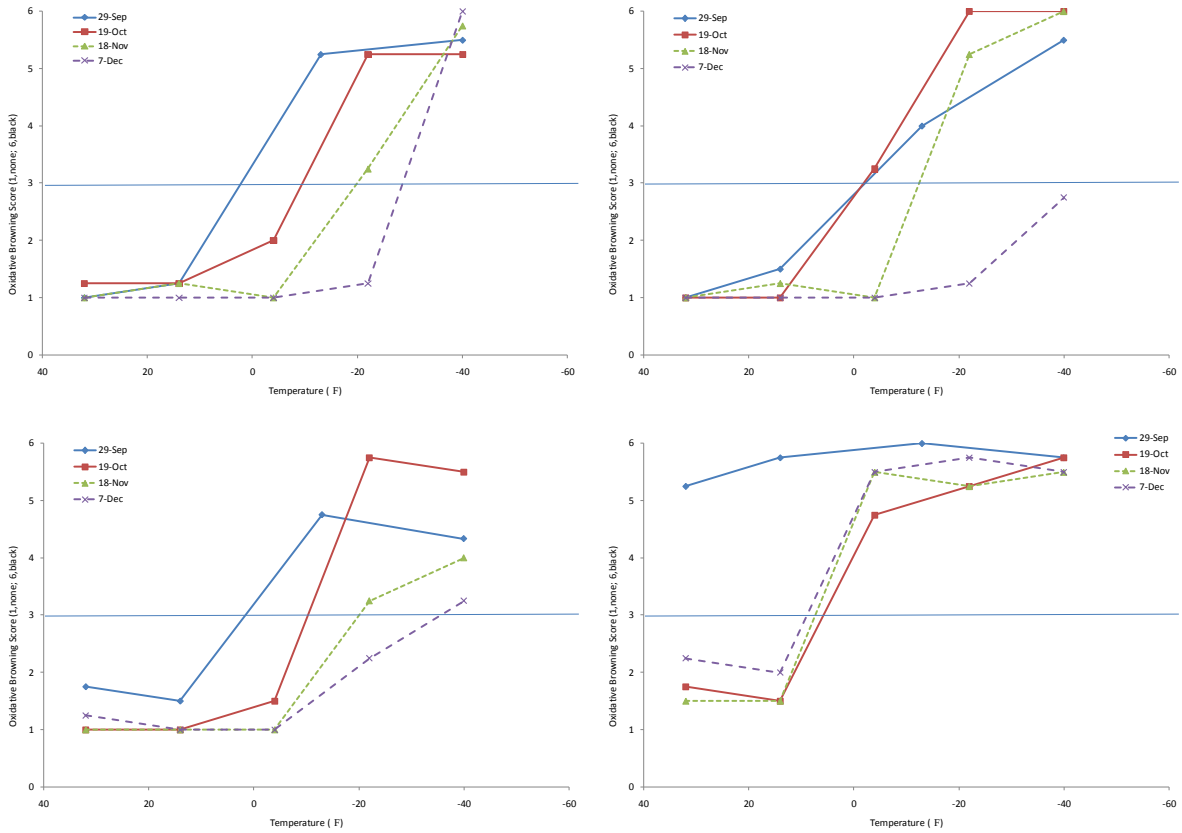

Figure 3. Oxidative browning rating of vascular tissue (where, 1, no damage [white]; 2, no damage [off-white]; 3, ~ 25 % area lightly browned; 4, ~ 50 % area browned; 5, >75 % area browned; 6, 100 % completely oxidized [black]) of three quince accessions and one pear accession subjected to five temperatures (32, 14, -4, -22 and -40 ° F) at four sampling dates (Sep., Oct., Nov., Dec.). Top left, 'Aiva from Gebeseud'; Top right, 'Pillnitz 2'; Bottom left, 'W-4; and Bottom right, cold-tender *Pyrus pashia*. The horizontal line on each graph signifies the threshold for injury.

Photo 1. Rooted cuttings of different quince genotypes. Cuttings were taken as soft-wood and rooted spring of 2009. Once growth resumes, plants will be grown for the remainder of the 2010 growing season, then brought to MCAREC for whole-plant freeze experiments.

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

Project Title: Horner rootstock grower evaluation trials

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

[1]Budget: **Year 1**: $6,170 **Year 2: $6,358** **Year 3:** $6,552

[1] $9,200, 10,600 and 12,000, in years 1, 2 and 3, respectively, has been requested, in advanced, as collaborative expenses in the WTFRC internal program.

Other funding sources

WTFRC Collaborative expenses:

Item	2009	2010	2011
Stemilt RCA room rental			
Crew labor			
Shipping			
Supplies			
Travel			
From WTFRC budget[1]	9200	10,600	12,000
Total	$9200	**$10,600**	$12,000

Footnotes: [1]Detailed budget for WTFRC is in the WTFRC proposal from Tory Schmidt/Tom Auvil

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

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

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

Objectives:

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

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

Significant Findings:

- Five trial sites were successfully planted with all rootstocks. A minimum of two sites were used for each cultivar. Sites varied in the training system and planting density.
- ~ 100 % survival rate was observed (the exception was a single OH x F 87 tree at one site)
- Horner 4 trees were on average 10-30 % larger than trees on either OH x F 87, or Horner 10 at time of planting (i.e., effects carried over from the nursery)
- Significant differences in relative trunk growth rate, and final size were observed among sites for a given cultivar
- Horner 10 and OH x F 87 produced trees of similar size, irrespective of site location and cultivar ('Bosc' being slightly smaller, albeit non-significantly)
- For all cultivars ('Bartlett', 'Bosc', and 'd'Anjou'), Horner 4 produced the largest trunk size, and this was fairly consistent across sites, with the interesting exception of one site, where final trunk size was similar among rootstocks.
- The combination of 'd'Anjou'/Horner resulted in the largest trees

Methods:

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

Hood River
- Spacing: 17' x 6'
- Scion: 'd'Anjou'
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Modified central leader/three wire support
- Replicates: Six, five-tree reps

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

Bridgeport Anjou
- Spacing: 16' x 6' (OHxF87 and Horner 10), 16' x 8' (Horner 4)
- Scion: 'd'Anjou'

- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Perpendicular "V"/wire support
- Replicates: Five, five-tree reps

Bridgeport Bosc
- Spacing: 16' x 5' (OHxF 87 and Horner 10), 16' x 7' (Horner 4)
- Scion: 'Bosc'
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Perpendicular "V"/wire support
- Replicates: Five, five-tree reps

Wapato
- Spacing: 10' x 4'
- Scion: 'Bartlett' and 'Bosc'
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Tall spindle fruiting wall/wire support
- Replicates: Five, five-tree reps

Methow
- Spacing: 12' x 4'
- Scion: 'Bartlett'
- Rootstocks: OH x F 87, Horner 4, Horner 10
- System: Tall spindle/wire support
- Replicates: Five, five-tree reps

Trunk circumference measurements were taken 20 cm above the graft union, immediately following planting, and again in the fall, following leaf drop. Relative growth rate of the trunks was derived from initial and final circumference measurements as [(Trunk Circum.$_{final}$ – Trunk Circum. $_{initial}$)/ Trunk Circum.$_{initial}$)*100]. Tree survival (or mortality) was determined following leaf drop in the fall. Evaluation of root suckering was performed by counting the total number of suckers per tree.

Results and Discussion:

Results will be presented based on cultivar (i.e., sites will be grouped according to cultivar).

1. Anjou. Relative growth rates were two to threefold higher at the Parkdale site, irrespective of rootstock (Fig 1). This resulted in larger trees (as defined by trunk size) at the Parkdale site relative to those at either the Hood River, or Bridgeport sites (Fig 1). Bridgeport was characterized as a low vigor site due to the presence of gravel bars within the soil profile, however results did not differ much from those observed at Hood River. The combination of 'd'Anjou' on Horner 4 produced the largest tree at both Hood River and Parkdale, but not at Brideport (Fig 1). Horner 10 and OH x F 87 produced trees of similar size (Fig 1).
2. Bosc. Relative growth rates at Wapato were nearly double those at Bridgeport (Fig 2). For a given site, growth rates were similar among rootstocks (Fig 2). Absolute trunk size was not significantly different between sites for a given rootstock, and trees on Horner 4 were slightly larger (Fig 2). Horner 10 and OH x F 87 produced trees of similar size (Fig 2).
3. Bartlett. Trees at Wapato had double to triple the growth rate observed at Methow, and this resulted in larger final tree size at the Wapato site (Fig 3). Within a site, however, little differences existed among rootstocks (Fig 3).

It is still too early to expect to see growth effects as a result of differences in in-row spacings among sites. Root volume and length would not be expected to result in competition among neighboring plants in the establishment year. Decisions regarding tree training will continue to be a collaborative process as we proceed into year two. Measurements for 2010 will include:

- Tree survival
- Root suckering
- Tree size (trunk cross-sectional area)
- Bloom observations (qualitative assessment of precocity)

Depending on the site and cultivar we would expect to come into production in year three, and will add the following measurements to those outlined above for 2011:

- Fruiting potential (based on the number of blossom clusters) [first two crops]
- Fruit set [first two crops]
- Annual and cumulative yield
- Fruit size and frequency distribution
- Fruiting efficiency

Figures:

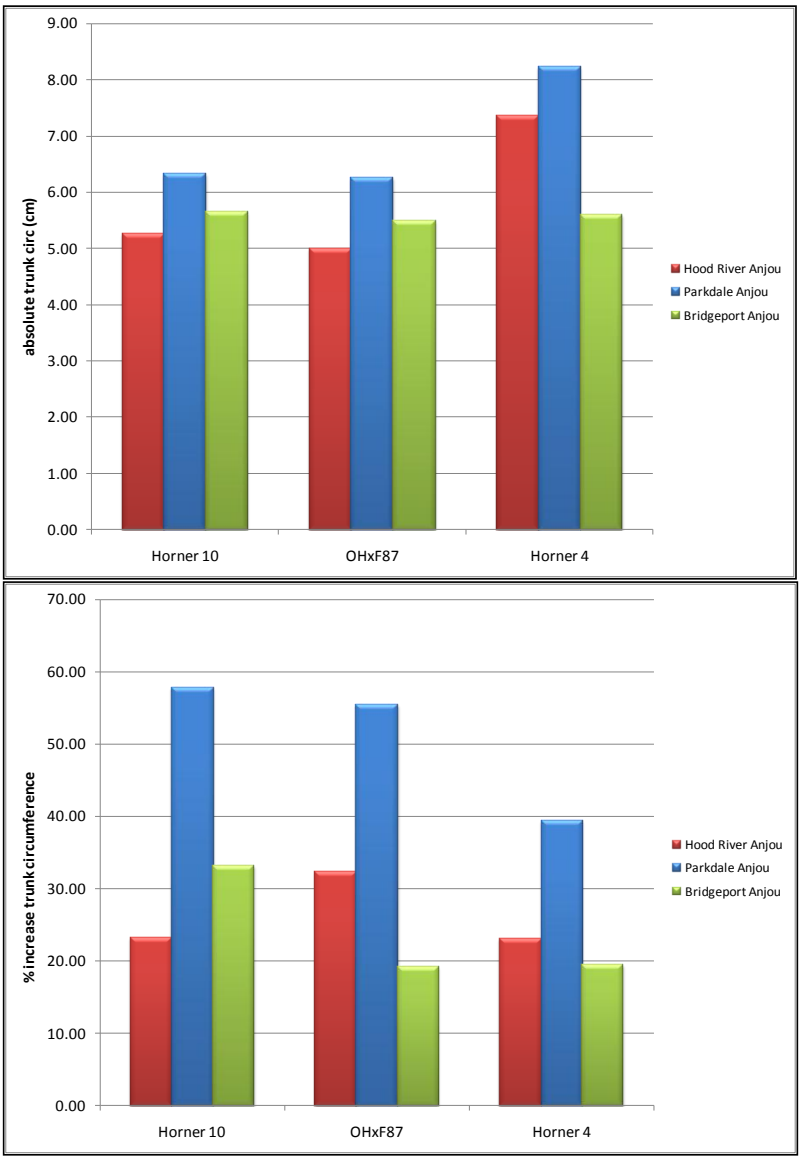

Figure 1. Trunk circumference (cm) following the 2009 growing season (top), and relative growth rate (%) of trunks (bottom), of 'd'Anjou' pear trees grafted on three different rootstocks, and at three different locations.

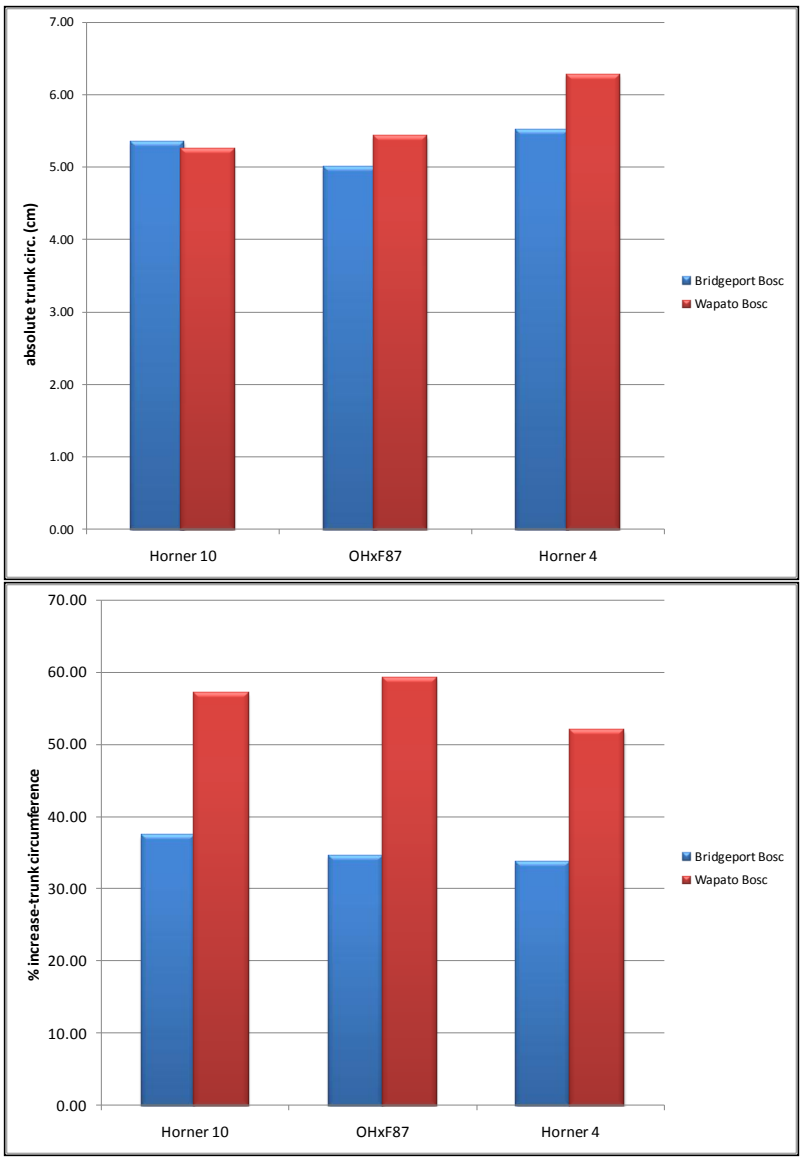

Figure 2. Trunk circumference (cm) following the 2009 growing season (top), and relative growth rate (%) of trunks (bottom), of 'GR Bosc' pear trees grafted on three different rootstocks, and at two different locations.

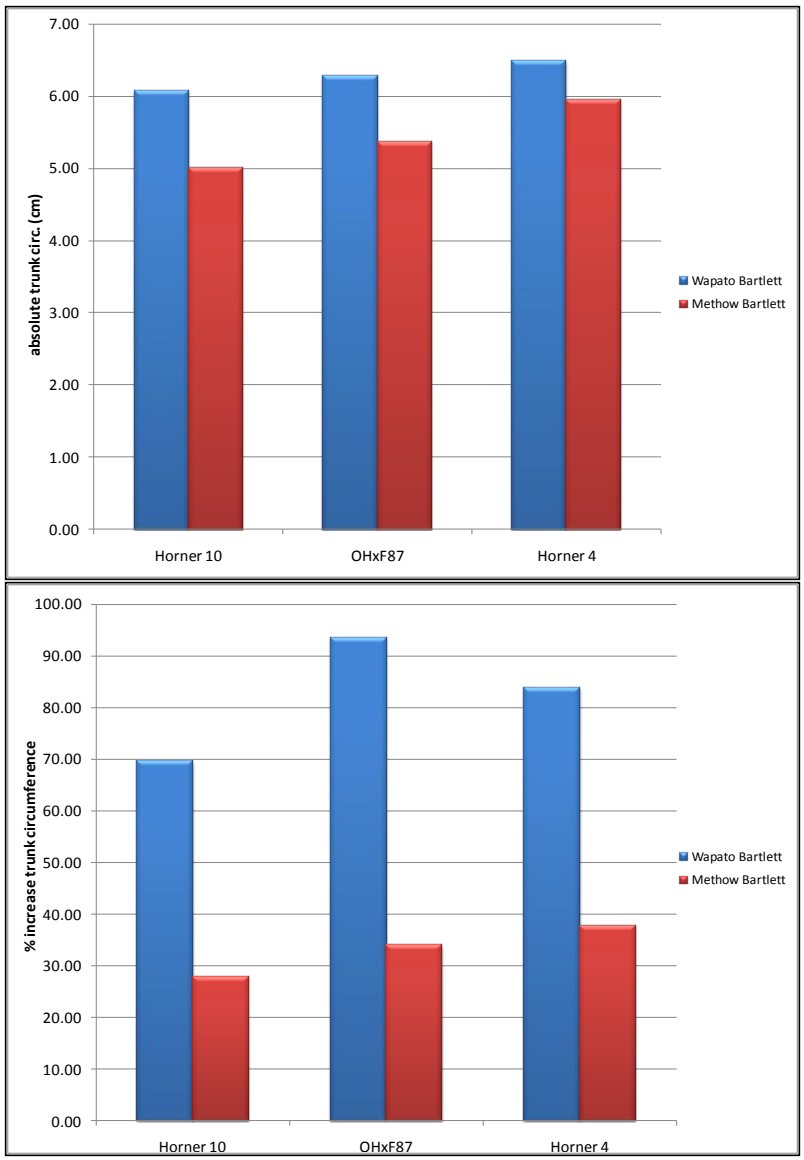

Figure 3. Trunk circumference (cm) following the 2009 growing season (top), and relative growth rate (%) of trunks (bottom), of 'Bartlett' pear trees grafted on three different rootstocks, and at two different locations.

CONTINUING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project Number: PR-06-607A

Project Title: PNW pear rootstock trial

PI:	Timothy J. Smith	**Co-PI:**	Todd Einhorn
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State/Zip:	WA 98801	**State/Zip:**	OR 97031

Cooperators: OSU: Steve Castagnoli and Janet Turner. WSU: Esteban Gutierrez. Growers: Ed and Darrin Kenoyer (Cashmere Trial), Geoff Thornton and site manager Dennis Lorz (Tonasket Trial). Advisors: Fred Valentine, Tom Auvil, Greg Rains, Bob Gix.

Total Project Request: **Year 1:** $9,876 **Year 2:** **$8,611** **Year 3:** $8,741

Other funding sources

$4,000 from the Northwest Nursery Improvement Institute was granted in support of the pear on trellis management demonstration at the Tonasket rootstock trial site.

WTFRC collaborative expenses: None

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

	2009	**2010**	**2011**
Salaries	2,880	2,160	2,160
Benefits	1,353	1,015	1,015
Supplies	300	300	300
Travel	1,808	1,475	1,475
Total	$6,341	**$4,950**	$4,950

Footnotes: Salaries and benefits are in support of 0.058 FTE (3 weeks) of a full time technician. Travel is to plots: Tonasket – 244 miles round trip, 10 trips = 2,440 miles. Cashmere – 20 miles x 12 trips = 240 miles @ $0.55/mile.

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

	Year 1 2009	**Year 2 2010**	**Year 3 2011**
Salaries[1]	1,950	2,028	2,109
Benefits	1,185	1,233	1,282
Wages			
Benefits			
Supplies	300	300	300
Travel	100	100	100
Total	$3,535	**$3,661**	$3,791

Footnotes: [1] 0.5 x FTE (2.5 weeks) of a full time technician (Hood River site.)

Objectives:
The pear scions/rootstocks will be evaluated on the following: 1. survival, 2. suckering, 3. vegetative growth potential (trunk size and tree diameter), 4. yield, and 5. fruit size. The current objectives are to carry this evaluation only on the rootstocks included in the 2005 phase of this trial.

Significant Findings:

- Although the trunk size measurements indicate the the semi-dwarfing OHxF 87 is one of the more vegetative in this trial, this root continues to set the standard in productivity, while maintaining comparatively good fruit size on both the eight and five year old trees.
- BU-2 and BU-3 are the most dwarfing of the rootstocks tested in the 2002 and 2005 trials.
- BU-2 and BU-3 have difficulty surviving high levels of exposure to the mycoplasma that induces pear decline disease. As with 708-36, those trees that survive the first four seasons appear to gain tolerance to this organism. Tree losses after planting would be too extreme for Washington growing areas, but perhaps not a problem near Hood River.
- The data continues to demonstrate that fruiting precocity is not tightly associated with the degree of dwarfing induced by pear rootstocks.
- The rootstocks that induce the most vegetative vigor in this trial (BM 2000 and Horner 4a) do not have trunk cross section areas much different than those of the more productive and fruit/vegetative balanced trees on OHxF 87.
- No rootstock in this trial has been the equivalent of any apple rootstock in current favor. The OHxF 87 is best compared to an EMLA 106, but without collar rot issues.
- Trellising has not yet given an advantage to the more dwarfing rootstocks over those rootstock that perform best in free-standing tree trials.
- And finally, good yields occurred on the d'Anjous at the 2002 planted Cashmere site, providing data that essentially duplicates the long-term order of yield ranking in the Tonasket Bosc rootstock trial. (Tables 2002-1, and 2002-4).

Methods:
In 2005, D'Anjou pears on various rootstocks were planted 10 feet apart in the row at the Mid-Columbia Agricultural Research and Extension Center in Hood River, and trained as a freestanding central leader. The D'Anjou pears in Cashmere and the Golden Russet Bosc in Tonasket were spaced 6 feet apart in the row and were trained on an upright trellis, 10 feet high.

Results and Discussion:
The 2005 planted PNW pear rootstock trial represents rootstocks that were not in sufficient quantity or quality, or obtained too late to be included in the 2002 planting. Some of the rootstocks in the 2002 trial were planted in the 2005 trial, including 0HxF 87, Pyro 2-33, Fox 11 and Pyrodwarf. Rootstocks that were not previously included were BM 2000, BU-2, BU-3, Horner 4, and 28-119.

In Cashmere and Tonasket, ten to thirty percent of the trees on 708-36, BU-2 and BU-3 have died by the third or fourth season after planting, very likely due to pear decline disease. No trees in Hood River have had this problem. The winter of 2008-09 brought temperatures of -10F (or maybe lower) to the Tonasket site, with no apparent damage to the exposed rootstocks. All remaining trees in the 2005 rootstock trial appear quite healthy.

Bosc- 2005 Planting Tonasket (on a trellis)	2007-08 Pounds Fruit/ Acre, 3rd + 4th Year	2009 Pounds Fruit/ Acre, 5th Year	Total Fruit Weight / Acre by 5th Season	07+ 08 + 2009 Total Bins Fruit / Acre	2009 Average Fruit Box Size	2009 Trunk Cross Section Area in CM^2	2009 Lbs. Fruit / Tree	Total lbs. Fruit per CM^2 of Trunk (Efficiency)
OHxF 87	19,342	24,844	44,186	40.2	84	43.8	47.8	1.95
Pyrodwarf	12,307	24,209	36,516	33.2	78	41.8	46.6	1.69
BM 2000	11,519	17,531	29,050	26.4	81	42.8	33.7	1.31
Pyro 2-33	9,689	16,640	26,329	23.9	77	30.6	32.0	1.66
Horner 4a	7,463	13,195	20,658	18.8	85	40.1	25.4	0.99
BU-3	3,761	5,920	9,681	8.8	65	16.5	11.5	1.13

Table 2005-1. 2005 planting of Golden Russet Bosc pear, Tonasket, (5th season), 6 x 14 ft. on 4-wire 10 foot upright trellis (518 trees/A) Note the comparison of the 4th and 5th leaf results in the 2002 free standing trial at a similar stage of development, lower row of table.

2005 D'Anjou Planting Hood River MCAREC	2009 Pounds Fruit/ Acre,	Total Bins Fruit / Acre	2009 Average Fruit Box Size	2009 Trunk Cross Sectional Area CM^2	2009 Lbs. Fruit per Tree	Total lbs. Fruit per CM^2 of Trunk (Efficiency)
708-36	7,659	6.96	102	34.3	21.1	0.616
OHxF 87	4,066	3.70	100	50.9	11.2	0.220
Fox 11	3,848	3.50	97	34.4	10.6	0.308
Pyro 2-33	3,665	3.33	99	30.6	10.1	0.330
Pyrodwarf	3,594	3.27	107	45.0	9.9	0.220
BU-2	2,251	2.05	95	47.0	6.2	0.132
Horner-4	1,597	1.45	96	66.7	4.4	0.066
BU-3	944	0.86	86	39.4	2.6	0.066
28-119	944	0.86	110	13.1	2.6	0.198
BM 2000	653	0.59	103	40.9	1.8	0.044

Table 2. Results of the Hood River MCAREC 2005 planted d'Anjou scion rootstock trial. Yield, fruit weight and yield efficiency in 2009. Yield per acre extrapolation assumes trees planted 7.5 x 16 feet, or 363 trees / acre.

The D'Anjous in Cashmere, also trellised, but have had an insignificant yield to date. Data not presented.

8th and Final Season Data on 2002 Planted Trees, PNW Pear Rootstock Trial:

Four pear rootstock trials were established in 2002, with D'Anjou as the scion cultivar in Cashmere, Washington and Hood River Oregon, Bartlett in the Yakima Valley and Bosc near Tonasket Washington. Three of the original four PNW pear rootstock trials were maintained and evaluated from 2002 through 2009; the Bartlett trial was dropped due to fire blight damage. The rootstocks included Old Home X Farmingdale 87 as the standard to be compared to Fox 11, Fox 16, Pyrodwarf, Pyro 2-33, OHxF 40, 708-36, and, in Hood River, Winter Nellis. The final results of the 2002 planting obtained in the eighth year are presented in tables below.

Bosc- 2002 Planted, Tonasket	**Trunk Size in Sq. cm (Veg. Growth)**	**2009 Yield In lbs. per Acre 8th Leaf**	**Total To Date Pounds per Acre**	**All Years Average Fruit Box Size**	**2009 Pounds Fruit Per Tree**	**2009 Yield Efficiency Lb. Fruit / CM^2**	**Total Yield Efficiency Lb. Fruit / CM^2**
OHxF 87	117	60,797	**204,696**	73	156	1.33	**4.49**
OHxF 40	98	45,513	**169,724**	77	117	1.19	**4.44**
Pyro 2-33	92	32,500	**156,243**	76	83	0.90	**4.35**
708 - 36	62	35,383	**131,057**	83	74	1.19	**4.40**
Fox 11	74	35,710	**130,434**	76	80	1.08	**3.97**
Fox 16	72	33.049	**108,602**	71	69	0.84	**3.16**
Pyrodwarf	108	28,557	**105,111**	78	73	0.68	**2.50**

Table 2002-1. Summary data for 2002 planted (8th leaf) Golden Russet Bosc pears, 2009 season and averages of all years.

Bartlett 2002 Planted	**Trunk Size in CM^2**	**2009 Yield In lbs. per Acre**	**Total Yield To Date in Pounds per Acre**	**Average Fruit Box Size (Fruit / 44 Pounds)**	**2009 Pounds Fruit Per Tree**	**Yield Efficiency Lb. Fruit / CM^2**
Cashmere Pyro 2-33	80.1	54,377	127,600	92	139	1.74
Cashmere Pyrodwarf	79.2	45,386	91,300	108	116	1.47
Cashmere OHxF 87	83.9	52,748	112,200	94	135	1.61

Table 2002-2. Summary data for 2002 planted (8th leaf) Green Bartlett pears, 2009 season and averages of all years. Note data is from two sites, Cashmere 7.5 x 15, 390 trees / Acre tree spacing and Tonasket 7 x 14 ft – 444 trees / A. Note: the higher the box size number, the smaller the fruit.

Bartlett 2002 Planted	Trunk Size in CM^2	2009 Yield In lbs. per Acre	Total Yield To Date in Pounds per Acre	Average Fruit Box Size (Fruit / 44 Pounds)	2009 Pounds Fruit Per Tree	Yield Efficiency Lb. Fruit / CM^2
Tonasket Pyro 2-33	71.3	70,885	191,259	82	160	2.24
Tonasket Pyrodwarf	70.4	72,489	121,016	98	163	2.32

Table 2002-3. Summary data for 2002 planted (8^{th} leaf) Green Bartlett pears, 2009 season and averages of all years. Note data is from two sites, Cashmere 7.5 x 15, 390 trees / Acre tree spacing and Tonasket 7 x 14 ft – 444 trees / A. Note: the higher the box size number, the smaller the fruit.

D'Anjou 2002 Planted, Cashmere	2009 Pounds Fruit/ Acre	Calc. Trees Per Acre	2009 1100 lb. Bins Fruit / A	2009 Average Box Size	2009 Lbs. Fruit per Tree	2009 Trunk X-Section Area CM^2	2009 lbs. Fruit / CM^2 of Trunk
OHxF 87	74,379	390	68	81	191	145	1.32
OHxF 40	59,774	390	54	89	153	141	1.09
Pyro 2-33	42,683	390	39	88	109	119	0.92
Fox 16	35,915	444	33	86	81	97	0.84
708 - 36	34,216	444	31	93	77	102	0.75
Fox 11	29,896	444	27	86	67	105	0.64
Pyrodwarf	24,072	390	22	95	62	120	0.52

Table 2002-4. 2009 Data from the 2002 planting of Green D'Anjou, (8^{th} season), listed in descending order of total yield. Planting space was calculated at 8 x 14 for the 390 trees / A, and 7 x 14 for the 444 trees / acre.

The 2002 D'Anjou rootstock trial in Cashmere had a light crop in 2005, its' 4^{th} season, but, despite a heavy bloom, for various frost and human-caused reasons, it did not set a crop again until 2009. In the absence of cropping, the trees grew relatively larger than the Boscs and Bartletts on the same rootstocks. The OHxF 87 rootstock, a semi-dwarf included as a "standard" in the trial, has been most productive and efficient, except with Bartlett, where Pyro 2-33 appears to have some advantages. The more dwarfing rootstocks (Fox 11 & 16, 708-36) induced neither precocity nor efficiency.

D'Anjou 2002 Planted, Hood River	2009 Pounds Fruit/ Acre	Total Pounds Fruit/ Acre	Total Average Fruit Box Size	2009 Lbs. Fruit per Tree	Total Lbs. Fruit per Tree	2009 Trunk X-Section Area CM^2	Total lbs. Fruit / CM^2 of Trunk
OHxF 87	42,510	87,360	83	109	224	122	1.83
708 - 36	22,200	68,376	89	50	154	105	1.47
Pyro 2-33	33,540	67,860	88	86	174	112	1.56
Winter Nellis	35,490	65,520	89	91	168	119	1.41
OHxF 40	26,130	58,890	89	67	151	116	1.30
Fox 11	26,196	57,276	87	59	129	99	1.30
Pyrodwarf	18,720	49,140	92	48	126	127	0.99

Table 2002-5. 2009 and all years total data from the 2002 planting at Hood River MCAREC. Ranked in order of total yield. 390 trees/acre used for yield extrapolation, except 444 trees/acre for 708-36 and Fox 11.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR09-905 **(WSU Project # 13C-3655-3259)**

Project Title: Pear rootstock breeding

PI: Kate Evans
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State/Zip WA-98801

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

Total project funding request: **Year 1:** $4,500 **Year 2: $12,300** **Year 3:** $11,300

Other funding sources: None

WTFRC collaborative expenses: None

Budget
Organization: WSU-TFREC **Contract Administrator:** Mary Lou Bricker and Kevin Larson
Telephone: 509.335.7667, 509.663.8181x221 **Email:** mdesros@wsu.edu, kevin_larson@wsu.edu

Item	2009	2010	2011
Travel	1,000	2,500	500
Propagation[2]	3,500	8,800	8,800
Plot Fees[3]	0	1,000	2,000
Total	$4,500	**$12,300**	$11,300

Footnotes:
[1] Travel is budgeted in year 1 for Evans to visit the USDA pear germplasm collection at Corvallis, OR for plant material identification and for in-state travel. In years 2 and 3 travel is budgeted for in-state travel with the addition of a visit from Gennaro Fazio in year 2.
[2] Propagation is budgeted in year 1 assuming 70 selections (x 5 replicate trees) are identified for entry into the pear germplasm collection at WSU. In years 2 and 3, $7,800 is budgeted to enter three international accessions into the NRSP5 virus therapy program for quarantine entry into the U.S., and $1,000 is budgeted for re-propagation.
[3] Plot fees are calculated at $1,000/acre for planting at the WSU Sunrise Research Orchard.

OBJECTIVES

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

SIGNIFICANT FINDINGS

1. A pear rootstock advisory committee has been established and has provided useful input regarding key traits necessary for rootstocks for the Pacific Northwest pear industry.
2. A list of key traits for a potential Pacific Northwest pear rootstock breeding program has been compiled following input from the advisory committee and literature and internet searches.
3. Some sources of useful rootstock germplasm have been located and contact established, ready for budwood supply in summer 2010 for propagation of parental trees for the planned Sunrise orchard.

METHODS

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

RESULTS & DISCUSSION

A pear rootstock advisory committee has been established, membership includes Dr. Richard Bell, Dr. Todd Einhorn, Bob Gix, Ed Ing, John Ireland, Jim Koempel, Neal Manly, Chuck Peters, Ray Schmitten, Dr. Tim Smith, Dr. David Sugar and Janet Turner. To date, the majority of discussions have been by e-mail and has focused on the development of a list of key traits necessary in new rootstocks for the Pacific Northwest (see Table 1).

Table 1: List of key traits necessary in rootstocks for pear in the Pacific Northwest.

Primary traits

- size control similar to quince rootstocks
- induction of precocious bearing and consistent good yield
- good fruit size and skin finish
- resistance to fire blight and pear decline
- winter hardiness

Secondary traits

- adaptable to different scions
- ease of propagation by stool beds, hardwood or semi-softwood cutting
- resistance to *Phytophthora*
- resistance to woolly pear aphid

Visits to the Pacific Northwest rootstock trial in Cashmere (March) & the Hood River rootstock trials (April) were also useful opportunities for further discussion regarding rootstock objectives and methods of trialing new material.

Internet searches and literature reviews are underway to identify possible accessions for a new rootstock parental collection to be established at the Sunrise Orchard, Wenatchee. Some accessions have already been identified and budwood will be sourced in summer 2010 for propagation. Due to J. Olmstead's departure from WSU, progress in sourcing germplasm has been a little slower than anticipated; however, I am confident that we will be able to establish the required parental collection within the time-span of the project. The proposed visit to the germplasm collection at Corvallis was postponed from summer 2009 to summer 2010.

Discussions are on-going with Gennaro Fazio, apple rootstock breeder, Geneva, NY, and Ken Tobutt, apple rootstock breeder, Agricultural Research Council of South Africa, regarding methods of pre-selection in rootstock seedling progenies.

CONTINUING PROJECT REPORT **YEAR:** Year 2 of 3
WTFRC Project Number: PR08-803 **(WSU Project #: 13K-3661-6365)**

Project Title: Control of postharvest fruit rots in pears

PI: Chang-Lin Xiao
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State/Zip: WA/98801

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

Total project funding request: **Year 1:** $29,719 **Year 2:** $32,165 **Year 3: $33,187**

Other funding sources: None

WTFRC collaborative expenses:

Item	2008	2009	2010
Stemilt RCA room rental	3,184.21	3,184.21	**3,184.21**
Miscellaneous	0	0	0
Total	$3,184.21	$3,184.21	**$3,184.21**

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

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

Item	2008	2009	**2010**
Salaries[1]	19,778	17,844	18,193
Benefits	8,900	6,781	6,550
Wages (time slip)	3,000	3,000	3,000
Benefits	471	540	444
Equipment	0	0	0
Supplies[2]	4,000	3,000	4,000
Travel[3]	1,000	1,000	1,000
Miscellaneous	0	0	0
Total	$37,149 (approved 29,719)	$32,165	**$33,187**

Footnotes:
[1] Salary in 2010 is for Robin Boal (scientific assistant, 0.33 FTE) at 36% benefit rate.
[2] Supplies include cost of fruit purchased from commercial orchards or packers and lab supplies.
[3] We will be using a leased vehicle.

Objectives:

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

Significant Findings:

- For d'Anjou pear fruit that were sprayed with Pristine 7 days before harvest and packed and inoculated with *Penicillium expansum* 2 months after harvest, Pristine alone, without any postharvest treatments, reduced blue mold incidence by 47.2% compared with the control 8 weeks after packing, indicating that residue of Pristine on/in d'Anjou pear fruit can last for at least 2 months during storage.

- When Bio-Save was applied to the fruit at packing, preharvest Pristine plus postharvest Bio-Save was more effective than Pristine alone and reduced blue mold incidence by 82% and 66% compared with the nontreated control and Pristine alone, respectively. However, the effectiveness of preharvest Pristine in combination with postharvest Bio-Save was reduced after the fruit had been stored at room temperature for one additional week after cold storage. Our results indicate that preharvest Pristine plus postharvest Bio-Save could be a promising program for blue mold control.

- Preharvest Pristine and Topsin applied 7 days before harvest provided a similar level of control and reduced Phacidiopycnis rot by 55-64% compared to the nontreated control. The three postharvest fungicides (Penbotec, Scholar and Mertect) applied as a postharvest drench treatment were highly effective and reduced Phacidiopycnis rot by 91-100% compared to the nontreated control.

- Residues of Scholar and Penbotec on/in fungicide-drenched fruit persisted during storage, and residual activity of Scholar and Penbotec against *P. expansum* can last for at least 4-6 months during storage. However, the spectrum of residual effects of Scholar and Penbotec on blue mold control on d'Anjou pears was smaller than that on Red Delicious and Fuji apples we previously observed.

- A real-time PCR assay was developed for diagnosis and detection of Phacidiopycnis rot, gray mold and Sphaeropsis rot on d'Anjou pear fruit. Validation with stem-end rot and calyx-end rot samples collected from a packinghouse indicated that the real-time PCR assay and the isolation-based assay yielded consistent results in the identification of causal agents of decayed fruit.

Methods:

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

An experiment was conducted in a research orchard of d'Anjou pear near Wenatchee. To ensure a necessary disease level, fruit were inoculated with spore suspensions of the Phacidiopycnis fungus at 5 weeks before harvest. For preharvest fungicide treatments, fungicides Pristine and Topsin M were applied within 2 weeks before harvest, and a nontreated control was included. For postharvest fungicide treatments, fruit were not sprayed with preharvest fungicides. Treatments were arranged as a randomized complete block design with four replicates, with 1-2 trees per replicate. Fruit were harvested in mid-September. Fruit for postharvest fungicide treatments were treated with one of the three postharvest fungicides. All fruit were packed on fruit trays in cardboard boxes and stored in air at 32ºF. All fruit were visually examined for decay development (calyx-end rot and stem-end rot, etc.) every 2 weeks for 5 months, starting in December.

Commercially harvested d'Anjou pear fruit, without use of preharvest fungicides, were either not drenched or drenched with one of the postharvest fungicides. Fruit were stored in CA. Part of the fruit was removed from CA 4 and 6 months after harvest. Fruit were subjected to packing process and then inoculated with *P. expansum*. For each fungicide-drench treatment, part of the inoculated fruit was treated with Bio-Save after inoculation. Nontreated fruit will be used as controls. All fruit were then stored in cold storage for 8 weeks and then for 7 days at 68ºF at which time decay development was evaluated.

Molecular-based assays for diagnosis of Phacidiopycnis rot, gray mold and Sphaeropsis rot were developed. PCR based assays were validated using naturally infected fruit collected from packinghouses.

Results and Discussion

Preharvest Pristine in combination with postharvest biocontrol Bio-Save or fungicides for blue mold control

In 2008-09, for the fruit that were sprayed with Pristine before harvest, Pristine alone, without any postharvest treatments, reduced blue mold incidence by 47.2% 2 months after harvest compared with the control 8 weeks after packing, indicating the existence of residual activity of Pristine in pear fruit (Table 1). When Bio-Save was applied to the fruit at packing, preharvest Pristine plus postharvest Bio-Save was more effective than Pristine alone and reduced blue mold incidence by 81.9% and 65.8% compared with the nontreated control and Pristine alone, respectively. However, the effectiveness of preharvest Pristine in combination with postharvest Bio-Save was reduced after the fruit had been stored at room temperature for one additional week.

On the 2009 crops packed 1 week after harvest, similar results were obtained as those observed on the 2008 crops. Furthermore, the effects of preharvest Pristine alone and preharvest Pristine plus postharvest Bio-Save on blue mold control were greater than that observed on the fruit of 2008 crop packed 2 months after harvest (Table 2). Our results indicate that preharvest Pristine plus postharvest BioSave could be a promising program for blue mold control.

For the experiment with fruit from the 2009 crop that were stored for 2 months before packing, the fruit have been run and inoculated with the pathogen and are currently in storage for decay development. Results will be forthcoming.

Table 1. Preharvest Pristine in combination with postharvest fungicide and biocontrol agent for control of blue mold in d'Anjou pears (**2 months after harvest of 2008 crop**)

Preharvest Treatment	Fungicide applied 2 months after harvest	8 weeks at 32F after packing		1 week at room temp after cold storage	
		% decay	Lesion (mm)	% decay	Lesion (mm)
Nontreated	No fungicide	90.0 a	22.18 a	98.75 a	58.58 a
	Scholar	0.00 d	0.00 d	0.00 e	0.00 e
	Penbotec	0.00 d	0.00 d	0.00 e	0.00 e
	TBZ	91.25 a	21.63 a	98.75 a	57.05 a
	BioSave	60.00 b	15.33 b	78.75 c	47.88 b
Pristine	No fungicide	47.50 b	14.18 b	88.75 b	39.33 c
	TBZ	53.75 b	14.53 b	87.50 bc	38.65 c
	Scholar	0.00 d	0.00 d	0.00 e	0.00 e
	Penbotec	0.00 d	0.00 d	0.00 e	0.00 e
	Biosave	16.25 c	6.83 c	30.00 d	27.35 d

Table 2. Preharvest Pristine in combination with postharvest fungicide and biocontrol agent for control of blue mold in d'Anjou pears (**1 week after harvest of 2009 crop**)

Preharvest Treatment	Fungicide applied 1 week after harvest	8 weeks at 32F after packing		1 week at room temp after cold storage	
		% decay	Lesion (mm)	% decay	Lesion (mm)
Nontreated	No fungicide	90.00 b	20.73 a	95.00 b	53.35 a
	Scholar	0.00 e	0.00 d	0.00 g	0.00 d
	Penbotec	0.00 e	0.00 d	0.00 g	0.00 d
	TBZ	96.25 a	22.20 a	98.75 a	57.53 a
	BioSave	30.00 c	10.23 c	56.25 d	33.03 b
Pristine	No fungicide	11.25 d	8.53 c	41.25e	17.95 c
	TBZ	27.50 c	14.83 b	77.50 c	24.58 c
	Scholar	0.00 e	0.00 d	0.00 g	0.00 d
	Penbotec	0.00 e	0.00 d	0.00 g	0.00 d
	Biosave	1.25 e	2.5 d	5.00 f	23.13 c

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

This experiment was to determine whether preharvest fungicides applied at harvest and postharvest fungicide drench treatments were effective to control Phacidiopycnis rot on fruit that were infected by the Phacidiopycnis fungus 5 weeks before harvest. All selected fungicide treatments significantly reduced Phacidiopycnis rot on pear compared to the nontreated control (Fig. 1). Preharvest Pristine and Topsin applied 7 days before harvest provided a similar level of control and reduced Phacidiopycnis rot by 55-64% compared to the nontreated control. The three postharvest fungicides were highly effective and reduced Phacidiopycnis rot by 91-100% compared to the nontreated control.

The results indicated that a postharvest drench treatment with one of the three registered postharvest fungicides was more effective in controlling Phacidiopycnis rot than a preharvest spray with either Pristine or Topsin M.

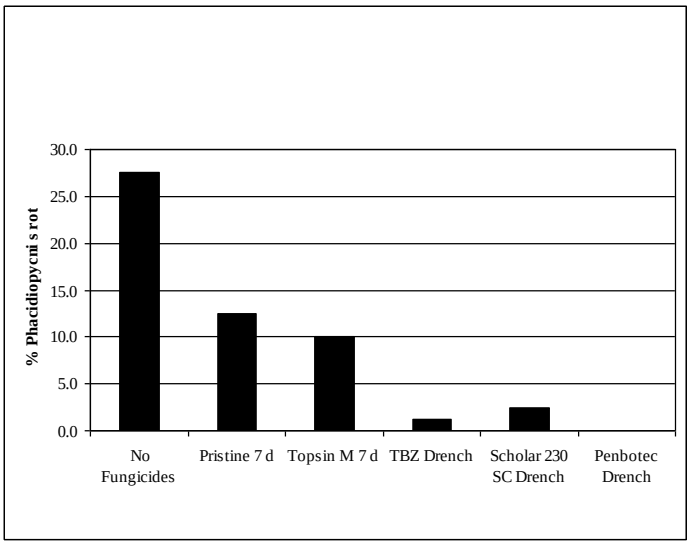


Fig. 1. Effectiveness of pre- and postharvest fungicides in controlling Phacidiopycnis rot on d'Anjour pear fruit that were inoculated with the Phacidiopycnis fungus 5 weeks before harvest in the orchard.

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

Four and 6 months after harvest, blue mold incidences were significantly lower on Scholar-drenched and Penbotec-drenched pear fruit that were inoculated with *P. expansum* 4 or 6 months after harvest and did not received any other treatments at packing in comparison with the nontreated control (Table 3). The results suggest that residues of Scholar and Penbotec on/in fungicide-drenched fruit persisted during storage and that residual activity of Scholar and Penbotec against *P. expansum* can last for at least 4-6 months during storage. However, the spectrum of residual effects of Scholar and Penbotec on blue mold control on d'Anjou pears was smaller than that on Red Delicious and Fuji apples we previously observed.

Bio-Save alone did not provide adequate control of blue mold on pear fruit that had been stored for 4 or 6 months after harvest. Fruit senescence 4 or 6 months after harvest may increase the susceptibility of fruit to blue mold and thus affect the efficacy of Bio-Save. Additional benefits from Bio-Save applied at packing on Scholar- or Penbotec-drenched fruit for blue mold control was not consistent.

Research has been set up to repeat this experiment on the 2009 crops. The fruit are currently in CA. Part of the fruit will be removed from CA 4 or 6 months after harvest. The fruit will be run through a research packing line and inoculated with *Penicillium expansum*. The experiment will end in spring 2010. Results will be forthcoming.

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

Phacidiopycnis rot, gray mold, and Sphaeropsis rot all can cause stem-end rot and calyx-end rot on pears. The symptoms of these three diseases are very similar, particularly in the early stage of symptom development. In addition to a conventional PCR-based assay developed for diagnosis and detection of these three diseases, we also developed a real-time PCR assay. Validation with stem-end rot and calyx-end rot samples collected from a packinghouse indicated that the real-time PCR assay and the isolation-based assay yielded consistent results in the identification of causal agents of decayed fruit (Table 4).

Table 3. Residual effects of Scholar and Penbotec on blue mold on d'Anjou pears that were inoculated at packing with *Penicillium expansum* and treated with Bio-Save

		4 months post drench treatments		6 months post drench treatments	
Drench treatment applied prior to storage	Fungicides applied at packing 4 or 6 months post drenching	% infected fruit at 8 weeks at 32ºF post packing	% infected fruit at one additional week at room temperature after storage	% infected fruit at 8 weeks at 32ºF post packing	% infected fruit at one additional week at room temperature after storage
Nontreated	No fungicide	100.0 a	100.0 a	100.0 a	100.0 a
	Scholar	0.0 e	0.0 g	0.0 d	0.0 e
	Penbotec	0.0 e	0.0 g	0.0 d	0.0 e
	Mertect	100.0 a	100.0 a	100.0 a	100.0 a
	Bio-Save	100.0 a	100.0 a	100.0 a	100.0 a
Scholar	No fungicide	7.5 d	72.5 c	28.8 b	90.0 b
	Bio-Save	15.0 c	38.8 d	20.0 b	43.8 c
Penbotec	No fungicide	17.5 c	22.5 e	3.8 c	11.3 d
	Bio-Save	1.3 de	2.5 f	6.3 c	12.5 d
Mertect	No fungicide	100.0 a	100.0 a	100.0 a	100.0 a
	Bio-Save	93.8 b	96.3 b	98.8 a	100.0 a

Table 4. Identification of causal agents of naturally infected pear fruit using three different approaches

Sample collection date [a]	Symptoms (# of samples)	Causal agent	Approaches		
			Isolation	PCR-based assays	
				ITS-based PCR	Real-time PCR
01/16/ 2008	Stem-end rot (20)	*Potebniamyces pyri*	9[b]	9	9
		B. cinerea	10	10	9
		S. pyriputrescens	1	1	1
	Calyx-end rot (20)	*Potebniamyces pyri*	16	16	16
		B. cinerea	4	4	4
		S. pyriputrescens	0	0	0
01/22/ 2008	Stem-end rot (20)	*Potebniamyces pyri*	8	9	9
		B. cinerea	11	11	11
		S. pyriputrescens	0	0	0
	Calyx-end rot (20)	*Potebniamyces pyri*	14	14	14
		B. cinerea	2	2	2
		S. pyriputrescens	4	4	4
04/14/ 2008	Stem-end rot (20)	*Potebniamyces pyri*	9	9	9
		B. cinerea	11	11	11
		S. pyriputrescens	0	0	0
	Calyx-end rot (14)	*Potebniamyces pyri*	8	9	9
		B. cinerea	3	3	3
		S. pyriputrescens	9[c]	9	9

[a] Samples were collected from a commercial packinghouse. At least 20 stem-end rot and calyx-end rot samples were included in each collection if available.

[b] Number of samples in which the pathogen was inferred as the causal agent.

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

Project title: Evaluation of integrated fire blight control technologies

PI: Ken Johnson
Organization: Oregon State University
Telephone/email: 541-737-5249 johnsonk@science.oregonstate.edu
Address: Dept. Botany and Plant Pathology
Address 2: 2082 Cordley Hall
City: Corvallis
State/Zip OR 97331-2902

Cooperators: Virginia Stockwell, BPP, Oregon State University, Corvallis

Total project request: **Year 1: $**39,100 **Year 2:** $31,484* **Year 3:** $32,428*

Other funding sources: None

WTFRC collaborative expenses: None

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

Item	**2009**	**2010**	**2011**
Salaries FRA 6mo	20,000	15,450	15,914
Benefits OPE 63%	12,600	9,734	10,026
Wages			
Benefits			
Equipment			
Supplies	4,000	3,800	3,914
Travel local	1,000	1,000	1,030
Miscellaneous plot fee	1,500	1,500	1,545
Total	$39,100	**$31,484***	$32,428*

Footnotes: Annually**:** FRA 4.5 mo plus fringe, $3.8K M&S, $1K local travel, $1.5K plot fee, 3% inflation.
*Budget reduced from original proposal owing to proposed shift of Obj. 4 to WTFRC Apple Crop Protection.

OBJECTIVES:

1) Evaluate mixtures of Kasumin and Mycoshield for fire blight suppression.

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

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

4) Evaluate a high frequency biocontrol program designed for fruit export to the European organic markets.

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

SIGNIFICANT FINDINGS

- We achieved outstanding control of fire blight of pear and apple with kasugamycin (Kasumin 2L) and with mixtures of Kasumin and Mycoshield (e.g., 80 ppm of each material).

- We were able to select mutants of the fire blight pathogen and of the beneficial bacterium, *Pantoea agglomerans* resistant to kasugamycin. Rates of mutation where in the range of 10^{-10} to 10^{-12}, which is somewhat less common than mutation to streptomycin resistance.

- We obtained very good suppression of fire blight with the beneficial bacterium *P. agglomerans* (BlightBan C9-1) followed by one treatment with Kasumin (i.e., integrated biological and chemical control). The high level of suppression was achieved regardless of whether the strain of *P. agglomerans* was sensitive or resistant to Kasumin.

- With the EU allowable organic materials, BlightBan C9-1 (*P. agglomerans*) and Serenade Max, doubling the frequency of treatment over a standard two treatment program significantly enhanced fire blight suppression. The experimental yeast material, Blossom Protect, provided significant disease control for a second season.

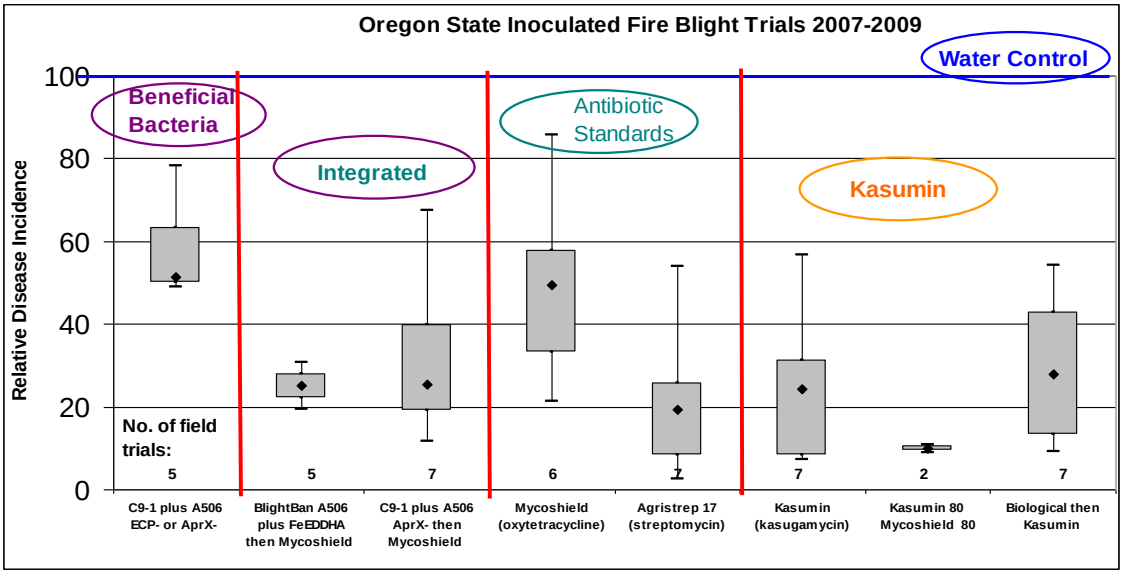
- A pot drench of a SAR material (acibenzolar-*S* methyl) dramatically slowed expansion of fire blight in young Bosc pear. In non-treated trees, the canker expanded nearly to the graft union (killing most trees), but scions on SAR-treated trees remained alive and continued to produce new shoot growth. The data suggest that application(s) of SAR materials in commercial pear orchards may slow advancement of fire blight cankers, and thereby prevent tree losses.

JUSTIFICATION: Management of fire blight is a research priority for WTFRC and FPC in 2009-2010. This proposal is addressing three areas of fire blight management: **a)** integration of a new product, Kasumin, into blossom blight control programs for conventional orchards; **b)** evaluation of improved control programs for blossom blight in orchards targeted to organic markets in the European Union, and **c)** rescue/protection of young pear trees from serious fire blight damage by soil and spray applications of inducers of systemic acquired resistance.

a) Below is a summary of recent results we have obtained with the antibiotic kasugamycin (Kasumin 2L). Over 7 trials, this material has resulted in a median 76% fire blight control, which compares

favorably to our long term median control of 51% and 81% with oxytetracycline (Mycoshield) and streptomycin (Agristrep 17), respectively, when targeted to antibiotic-sensitive pathogen strains (Fig. 1). We have been investigating properties of kasugamycin including effective dose, residual activity, and rates of mutation to resistant for both the fire blight pathogen and biocontrol strains; in this regard, kasugamycin has similarities to the related compound, streptomycin. Therefore, we are interested in developing a highly effective fire blight control strategy that also incorporates a judicious resistance management strategy should Kasumin be registered for use.

Fig. 1



b) In 2008, we evaluated an experimental yeast biocontrol product that required a high frequency of application (four treatments instead of the standard two). Surprising to us, the product was nearly as effective as Agristrep 17 in an orchard trial with very high disease pressure. Consequently, we have begun to look at combinations of materials allowable under EU organic standards, and whether or not increasing the frequency of treatment will improve blight control. The goal is to develop a recommendable fire blight suppression program for fruit exported to European organic markets where antibiotic use is not allowed.

c) A recent report used soil drenches of and inducer of systemic acquired resistance (SAR), acibenzolar-s-methyl (ASM), to obtain a remarkably high degree of protection of young citrus trees to bacterial canker (Fig. 2). If a similar response occurs in pears, a drench with a SAR-inducing chemical could potentially slow active fire blight in commercial orchards, thereby making pruning efforts more successful while reducing losses of major scaffolds, leaders and whole trees.

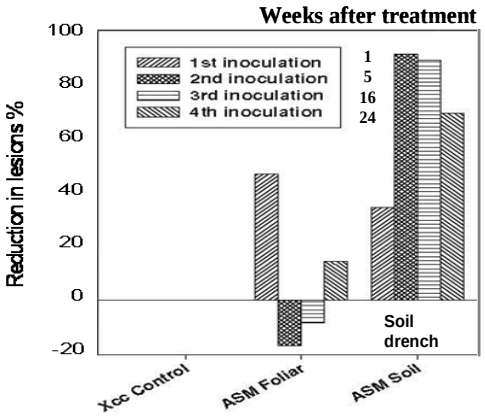


Fig. 2. Reduction of citrus canker lesions as a result of a single soil drench of acibenzolar-s-methyl (ASM) compared to a single foliar application of ASM and to a non-treated, inoculated (Xcc) control. Plants were inoculated with the pathogen, *Xanthomonas citri* spp. *citri*, at 1, 5, 16 and 24 weeks after chemical treatment. **Data from: Francis et al. 2009. Eur. J. Plant Pathology 124:283–292.**

METHODS:

Obj. 1) Evaluate mixtures of Kasumin and Mycoshield for fire blight suppression.
Obj. 2) Evaluate potential for the fire blight pathogen to become resistant to Kasumin.
Obj. 3) Evaluate an integrated biological/chemical control of fire blight with a spontaneous mutant of BlightBan C9-1 that is resistant to Kasumin.

Approach:
Laboratory studies. We have continued to select kasugamycin resistant mutants of *E. amylovora* and of the beneficial bacteria, *P. agglomerans* C9-1 (BlightBan C9-1) and 325 (Bloomtime Biological). The goal with the pathogen is to understand how quickly resistant strains could develop in the field. The goal with the beneficial bacteria is to determine if kasugamycin-resistant mutants enhance the compatibility and effectiveness of biological control when integrated in the orchard with oversprays of Kasumin.

Field studies: Antibiotic and integrated biological and antibiotic treatments for fire blight control are being evaluated in replicated orchard trials. The current emphasis is on evaluating mixtures of kasugamycin (Kasumin 2L) and oxytetracycline (Mycoshield or Fireline) with the rationale that a combination of these materials will increase the likelihood of kasugamycin remaining effective for a long period of time. Because we now have a better understanding of the minimum inhibitory concentrations for each material, we believe that in an effective mixture, kasugamycin should be used at or near the maximum label rate (80 to 100 ppm) but that we can reduce the amount of oxytetracycline to about half of its maximum label rate (to a range of 80 to 100 ppm).

Obj. 4) Evaluate a high frequency biocontrol program designed for fruit export to the European organic markets.

Approach:
Each season, combinations of biological products for organic fire blight control will be evaluated in orchard trials. The goal is to find combinations that best utilize and combine properties of individual products. For example, we know that beneficial bacteria (BlightBan) should be used early in bloom (because they need to colonize flowers), and that Serenade Max should be used late in bloom (because of the immediate antimicrobial properties of this product in suspension). Also, based on a result we obtained in 2008, we are interested frequency of application, and whether or not fire blight suppression can be enhanced by increasing the number of treatments.

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

Approach:
The current and future efforts will focus on the SAR material acibenzolar-S-methyl (ASM; Actigard 50WG, Syngenta Crop Protection) as imidacloprid (Admire Pro Systemic Protectant Bayer CropScience) did not show a strong SAR response in our 2009 experiment. We intend to repeat the replicated greenhouse drench experiments with one-year-old potted Bosc Pear trees. The goals in 2010 will be to obtain a better understanding of the ASM dose-response relationship, and whether or not disease suppression also can be observed with trunk paints of ASM.

In 2009, 200 Bosc pears were planted in the field (and additional trees will be procured in 2010 for field studies in 2011). Experimental design and treatments will be similar to the greenhouse study.

Additionally, thirty 8-year-old trees of 'Bartlett' and 'Commice' are available for SAR experiments. These trees will be inoculated and treated with ASM in summer 2010.

RESULTS AND DISCUSSION

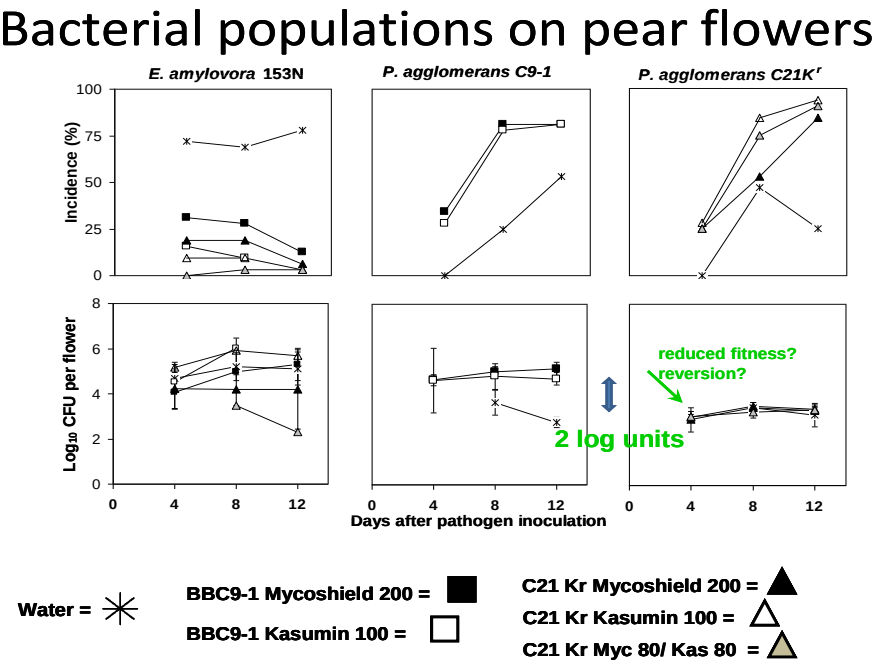
Obj. 1) Evaluate mixtures of Kasumin and Mycoshield for fire blight suppression.

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

Obj. 3) Evaluate an integrated biological/chemical control of fire blight with a spontaneous mutant of BlightBan C9-1 that is resistant to Kasumin.

Laboratory studies. The minimum inhibitory concentration (MIC) of kasugamycin to sensitive *E. amylovora* strain 153 is approximately 40 µg per ml (40 ppm; 80 to 100 ppm is the proposed dose). In addition, at twice the MIC (80 ppm), we have made estimates for mutation to resistant ranging from 10^{-10} to 10^{-11} (one resistant cell in 10 to 100 billion). Kasugamycin resistant mutants of *E. amylovora* grow well in the lab, but field fitness is unknown. We have conducted similar studies with *P. agglomerans* C9-1 (BlightBan C9-1) and found that while kasugamycin resistant strains grow also grow well in the lab, they do not appear to grow as well as sensitive strains on flowers in the orchard (Fig. 3). To date, we have obtained four mutants of C9-1 with stable resistance to kasugamycin, one of which we have worked with in the field (designated C9-1^{Kr} in Fig. 3 and Table 1). We also are working to obtain a kasugamycin resistant strain of *P. agglomerans* E325 (Bloomtime Biological), and if successful, we will include it in 2010 trials.

Fig. 3



Field studies. In 2009, we obtained excellent suppression of blossom blight with mixtures of Kasumin and Mycoshield, as well with Kasumin by itself (Table 1). We are also evaluating integrated biological and chemical control, where treatment with a beneficial bacterium (BlightBan C9-1) precedes Kasumin. This integrated approach yielded very good suppression of fire blight; a high level of suppression was achieved regardless of whether the strain of *P. agglomerans* was sensitive or resistant to Kasumin (Table 1).

EVALUATION OF KASUMIN FOR SUPPRESSION OF FIRE BLIGHT OF PEAR, 2009

Table 1. **BARTLETT PEAR, Corvallis, Oregon**

K.B. Johnson, T. N. Temple, and A.R. Hubbard, Oregon State University

		Date treatment applied*						
		16 April	18 April	21 April				
Treatment	Rate per 100 gallons water	30% bloom	70% bloom	Full bloom	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Water control	**------**	**X**[§]	**X**	X	**485**	**a**[#]	**44.0**	**a**[#]
Mycoshield 200 ppm	16 oz.	---	X	X	90	b	9.3	b
C9-1^{Kr} then	*10^8 CFU/ml*	X	X	---				
Mycoshield 200 ppm	16 oz.	---	---	X	66	bc	7.0	bc
C9-1then	*10^8 CFU/ml*	X	X	---				
Mycoshield 200 ppm	16 oz.	---	---	X	50	bc	5.1	bc
Kasumin 80 ppm &	52 fl. oz.	---	X	X				
Mycoshield 80 ppm	6.4 oz.	---	X	X	45	bc	4.8	bc
C9-1 then	*10^8 CFU/ml*	X	X	---				
Kasumin 100 ppm	64 fl. oz.	---	---	X	42	bc	4.0	bcd
C9-1^{Kr}	*10^8 CFU/ml*	X	X	---				
then Kasumin 100 ppm	64 fl. oz.	---	---	X	38	bc	3.5	bcd
Kasumin 100 ppm	64 fl. oz.	---	X	X	33	bcd	3.5	bcd
Kasumin 80 ppm &	52 fl. oz.	---	X	X				
Mycoshield 100 ppm	8 oz.	---	X	X	31	cd	3.3	bcde
C9-1^{Kr} then	*10^8 CFU/ml*	X	X	---				
Kasumin 80 ppm &	52 fl. oz.	---	---	X				
Mycoshield 80 ppm	6.4 oz.	---	---	X	23	de	3.0	cde
Kasumin 100 ppm &	64 fl. oz.							
Mycoshield 100 ppm	8 oz.	---	X	X	23	de	2.5	de
Agri-mycin 100 ppm	8 oz.	---	X	X	11	e	1.1	e

* Trees inoculated on 19April with 5 x 10^5 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin-sensitive pathogen strain).

** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.

*** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown.

[§] X indicates material was sprayed on that specific date; --- indicates material was not applied on that specific date.

[#] Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$.

(**Above treatments also were evaluated in an apple with similar results; data are available upon request).**

Obj. 4) Evaluate a high frequency biocontrol program designed for fruit export to the European organic markets.

In both pear (Table 2) and apple (data not shown), increasing the frequency of treatment of registered biological products improved blight control. The experimental yeast material, Blossom Protect, provided significant disease control for a second season; however, with this material, a similar result was obtained with both two and four applications. In apple, we also obtained significant suppression of fire blight with an organic bloom thinning protocol (lime sulfur plus fish oil).

To evaluate multi-material programs in 2010 and 2011, we are especially interested in evaluating the potential roles for Bloomtime Biological (a strain of *Pantoea agglomerans* manufactured by Northwest Ag Products, Yakima, WA) and Blossom Protect (a yeast produced in Germany to be sold through Westbridge Ag Products, Vista, CA). Moreover, because bloom thinning with lime sulfur appears to partially suppress fire blight, we will evaluate biological materials in conjunction with bloom thinning treatments. [Because of the increased effort on bloom thinning, we have made the proposal that this objective be transferred to apple sources of funds].

Table 2.

ORGANIC FIRE BLIGHT SUPPRESSION IN PEAR, 2009:
BARTLETT PEAR, Corvallis, Oregon
K.B. Johnson, T. N. Temple, and A.R. Hubbard, Oregon State University

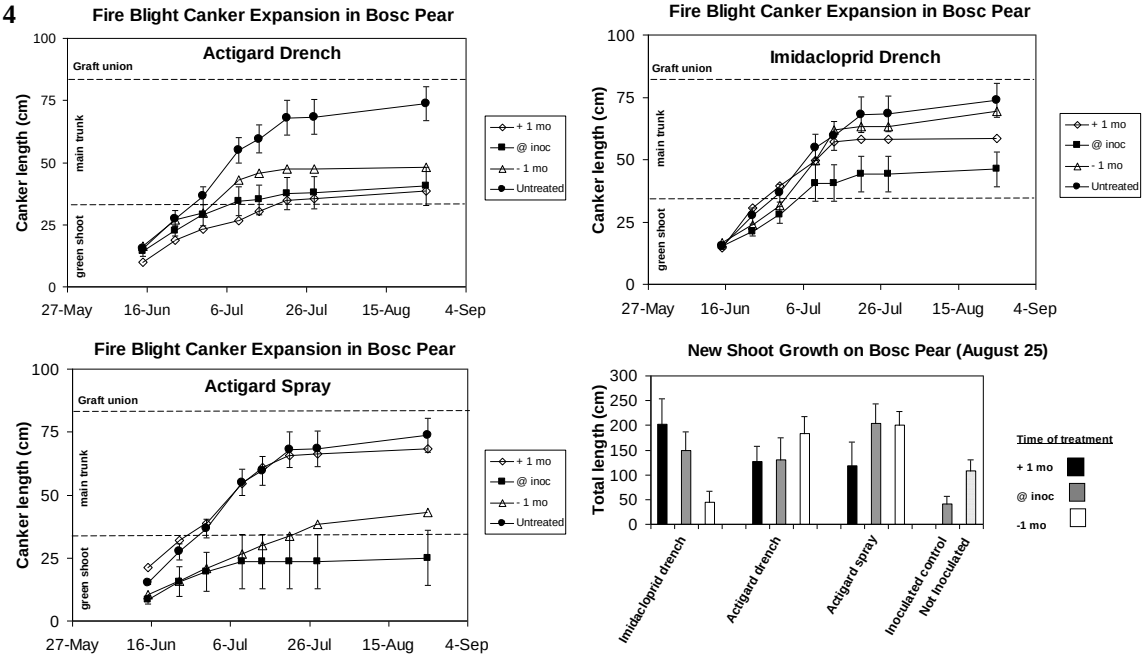
Treatment	Rate per 100 gallons water	Date treatment applied* 13 April 10% bloom	16 April 30% bloom	18 April 70% bloom	21 April Full bloom	25 April petal fall	Number of blighted clusters per tree****		Percent blighted floral clusters ***	
Water control	------	---	**X**[§]	**X**	**X**	---	**485**	**a**[#]	**44.7**	**a**[#]
BlightBan C9-1 plus	*5x10^7 CFU/ml*	---	---	X	---	---				
BlightBan A506 then	*5x10^7 CFU/ml*	---	---	X	---	---				
Serenade Max plus	64 oz.	---	---	---	X	---				
Nu-Film-P	6 oz.	---	---	---	X	---	178	b	19.1	b
Westbridge Yeast BCYP-B plus buffer A	1.34 lbs. 9.35 lbs.	---	X	X	---	---	120	cd	15.8	b
Westbridge Yeast BCYP-B plus buffer A	1.34 lbs 9.35 lbs	X	X	X	X	---	129	bc	15.1	bc
BlightBan C9-1 plus	*5x10^7 CFU/ml*	---	X	X	---	---				
BlightBan A506 then	*5x10^7 CFU/ml*	---	X	X	---	---				
Serenade Max plus	64 oz.	---	---	---	X	X				
Nu-Film-P	6 oz.	---	---	---	X	X	101	de	10.6	c
Mycoshield 200 ppm	16 oz.	---	---	X	X	---	90	de	9.3	c
BlightBan C9-1 then	*1x10^8 CFU/ml*	---	X	X	---	---				
Mycoshield 200 ppm	16 oz.	---	---	---	X	---	50	e	5.1	c
Agri-mycin 100 ppm	8 oz.	---	---	X	X	---	11	f	1.1	d

(Footnotes as in Table 1. Above treatments also were evaluated in an apple with similar results; data . are available upon request.)

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

A drench of a SAR material (acibenzolar-*S* methyl)(Fig. 4; top left) dramatically slowed expansion of fire blight in potted Bosc pear. In non-treated trees, the canker expanded nearly to the graft union. Scions on SAR-treated trees remained alive and continued to produce new shoot growth (Fig. 4 lower left). The data suggest that drenches (or well-timed sprays) of SAR materials in commercial pear orchards may slow advancement of fire blight cankers, and thereby prevent tree losses.

Fig. 4



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

Project Title: Health benefits of Oregon & Washington pears

PI: Kalidas Shetty
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Total Project Request: Year 1: $ 47,050 Year 2: $ 51,400

Other funding sources: None

WTFRC collaborative expenses: None

Organization Name: University of Massachusetts at Amherst
Contract Administrator: Jim Ayres (jayres@research.umass.edu)
Department contact: Beverly Kokoski (bkokoski@foodsci.umass.edu)
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Budget

Item	**2009**	**2010**	
Salaries: (50% Research Associate)	23,000	24,000	
Benefits (35% of Salary)	8,050	8,400	
Wages	NA	NA	
Benefits	NA	NA	
Equipment (HPLC Service Maintenance and Columns)	3,000	4,000	
Supplies (Reagents, Biochemicals & Enzyme)	7,000	8,000	
Travel	NA	NA	
Miscellaneous :(Orchard needs-preparation & extraction of samples)	6,000	7,000	
Total	$47,050	**$51,400**	

OBJECTIVES:

1) Determination of effect of whole pear phenolic and oligosaccharide bioactives from fresh and long-term CA stored pear fruit on stimulating growth of select lactic acid bacteria relevant for better digestive and gut health.

2) Determination pear and lactic acid bacteria fermented combinations to enhance bioactives that have antioxidant activity and which can slow enzyme activity relevant for carbohydrate metabolism-linked oxidation and inhibit ulcer bacteria (*Helicobacter pylori*) for better digestive and gut health.

The objectives will be carried out over 2 growing seasons with fresh pear varieties and one growing season with stored and commercial pear varieties following storage from markets in the East coast

Part of Year 1 Objectives have been accomplished and are outlined under Significant Findings below. Additional research on year 1 Objectives are on-going and results will be ready in early April, 2010.

Objectives for Next Year:

Do pear phenolics and related bioactives from long term stored pears to stimulate lactic acid bacteria important for digestive health?

Do pear phenolics and related bioactives following probiotic lactic acid bacterial fermentation have enhanced antioxidant activity and inhibitory activities against carbohydrate metabolizing enzymes in the stomach and ulcer bacteria *Helicobacter pylori.*

Do pear phenolics and related bioactives following probiotic lactic acid bacterial fermentation provide an environment for stimulating other beneficial gut health bacteria such as good health-linked *Bifidobacterium* spp.

SIGNIFICANT FINDINGS:

Objectives for year 1 are on schedule and following are the significant findings:

1) Important pear varieties Anjou, Red Anjou, Bosc and Comice have significant total soluble phenolic antioxidants uniformly distributed between pulp and peel and on an average 200 gram fresh pear has between 70 to 80 mg of total soluble phenolics. This is a high content from one serving of fruit

2) Probiotic bacteria, *Lactobacillus acidophilus* (LA) can grow effectively on all pear varieties and mobilize the soluble phenolics to higher antioxidant function over 72 hours.

3) The growth of probiotic LA on all pear varieties also enhances bioactives that inhibit carbohydrate metabolizing enzyme alpha-gluosidase which has relevance for management of early stages of type 2 diabetes similar to the drug acarabose.

4) The growth of probiotic LA on all pear varieties enhances bioactives that inhibit ulcer bacteria, *Helicobacter pylori.*

5) The growth of probiotic LA on all pear varieties enhances bioactives that enhance the growth of other beneficial bacteria such as *Bifidobacterium longum*.

METHODS:

Pear Varieties:

Pear varieties evaluated were supplied by USPear: Bosc (B), D'Anjou (A). Red D'Anjou (RA) and Comice (C). Other varieties recommended by the growers are also being investigated.

Bacterial cultures:

The most effective lactic acid bacteria strain, *Lactobacillus acidophilus* (LA) that is probiotic and has fermentation benefits was selected. This strain was supplied by Institute Rosel Inc. Montreal, Canada (Lot#: XA 0145, Seq#: 00014160) for detailed studies. Other lactic acid bacterial strains used in fruit and vegetable fermentations were also considered for initial studies. These were *L. plantarum* (LP1) (ATCC 9019), *L. plantarum* (LP2) (NRRL, B4496), *L. casei* (LC1) (ATCC 343, PLP-XYL) and *L. casei* (LC2) (PLP-3537). Isolates of *H. pylori* (strains ATCC 43579, which originated from human gastric samples) was obtained from the American Type Culture Collection (Rockville, MD) was used.

Fermentation of lactic acid bacterial strains with Probiotic potential with pear extracts:

Initially 100 µl of frozen stock from both strains was inoculated into 10 ml MRS broth for 16 h at 37^{o}C. Then 100 µl of the overnight grown sample was re-inoculated into 10 ml MRS broth for 16 h at 37^{o}C. An inoculum size of 10% of the overnight culture was added aseptically into 45 ml MRS medium with pear peel and pulp extracts providing 0.1% to 1% total phenolics. The 50 ml volume fermentation took place in 100 ml sterile Erlenmeyer flasks. Then the flasks were placed at 37^{o}C walk in incubator for 24 h. After the 24-72 h fermentation 100 µl from the appropriate dilution was plated on MRS Agar medium and incubated in an anaerobic jar for 72 h to determine the colony forming units-CFU/ml of each sample.

Sample preparation:

Sample size of 10 ml following fermentation was centrifuged two times at 10,000 x g for 10 min, and the supernatant was collected for further enzyme, ulcer bacterial inhibition and antioxidant analysis. All experiments were performed within 4 days of sample extraction and were kept at 4^{o}C.

Preparation of starter culture of H. pylori:

Standard plating medium was prepared by using 10 g of special peptone (Oxoid Ltd., Basingstoke, England) per liter, 15 g of granulated agar (Difco Laboratories, Detroit, Mich) per liter, 5 g of sodium chloride (EM Science, Gibbstown, NJ) per liter, 5 g of yeast extract (Difco) per liter, 5 g of beef extract (Becton Dickinson and Co., St.Louis, Mo) per liter of water. Antimicrobial activity against *H. pylori* was tested by the standard agar diffusion method.

Broth media was prepared by 10 g of special peptone (Oxoid Ltd., Basingstoke, England) per liter, 5 g of sodium chloride (EM Science, Gibbstown, NJ) per liter, 5 g of yeast extract (Difco) per liter, 5 g of beef extract (Becton Dickinson and Co., St. Louis, Mo) per liter of water. One hundred micro liters of stock *H. pylori* was added into test tubes containing 10 ml of broth media. They were incubated at 37 ^{o}C for 48 h before being used for inoculating by the spread plate technique. The active culture was then spread on *H. pylori* Agar plates to make bacterial lawn.

Agar diffusion assays was performed in order to determine the inhibitory effect of the pear and fermented pear extracts on *H. pylori*. Controls were disks with distilled water only. Each experiment was repeated three times.

Sample Extraction:

Soluble phenolics were extracted from the peel and pulp of each variety separately and also lactic acid fermented extracts of the same. Phenolics were extracted using distilled hot water and 12% ethanol. The water extractions were done with 20 g of peel in 50 ml of water and 100 g of pulp in 50 ml of water. The ethanol extractions were done with 5 g of peel in 15 ml of 12% ethanol and 10 g of pulp in 20ml of 12% ethanol. Pears were first peeled then cut and weighed. Peel and pulp were then mixed with either distilled water or 12% ethanol. This was then homogenized for 2 min using a blender. Resulting mix was collected and centrifuged for 5 min. Supernatant was collected and stored at -20°C. This will also be done for every stage of storage under both air room temperature storage and 4 ^{o}C storage for freshly picked pears and as when it arrives for CA storage.

Total Phenolics Assay:

The total phenolic content was determined by an assay modified in our laboratory. Briefly, one milliliter of extract was transferred into a test tube and mixed with 1 ml of 95% ethanol and 5 ml of distilled water. To each sample 0.5 ml of 50% (v/v) Folin-Ciocalteu reagent was added and mixed. After 5 min, 1 ml of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 725 nm. The absorbance values were converted to total phenolics and were expressed in micrograms equivalents of gallic acid per grams fresh weight (FW) of the sample. Standard curves were established using various concentrations of gallic acid in 95% ethanol.

Antioxidant Activity by 1, 1-Diphenyl-2-Picrylhydrazyl Radical (DPPH) Inhibition Assay:

To 3 ml of 60 μM DPPH in ethanol, 250 μl of each extract was added, the decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The readings were compared with the controls, which will contain 250 μl of 95% ethanol instead of the extract. The % inhibition was calculated by:

$$\%\ \text{inhibition} = \left(\left[\frac{A_{517}^{Control} - A_{517}^{Extract}}{\left[A_{517}^{Control} \right]} \right] \right) x100$$

α-Glucosidase Inhibition Assay:

α-Glucosidase (EC 3.2.1.20) was purchased from Sigma Chemical Co. A volume of 50 μl of sample solution and 100 μl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/ml) was incubated in 96 well plates at 25^{o}C for 10 min. After pre-incubation, 50 μl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25^{o}C for 5 min. Before and after incubation, absorbance readings were recorded at 405 nm by micro-array reader (Thermomax, Molecular device Co., Sunnyvale, CA) and compared to a control which had 50 μl of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as inhibition % and calculated as follows:

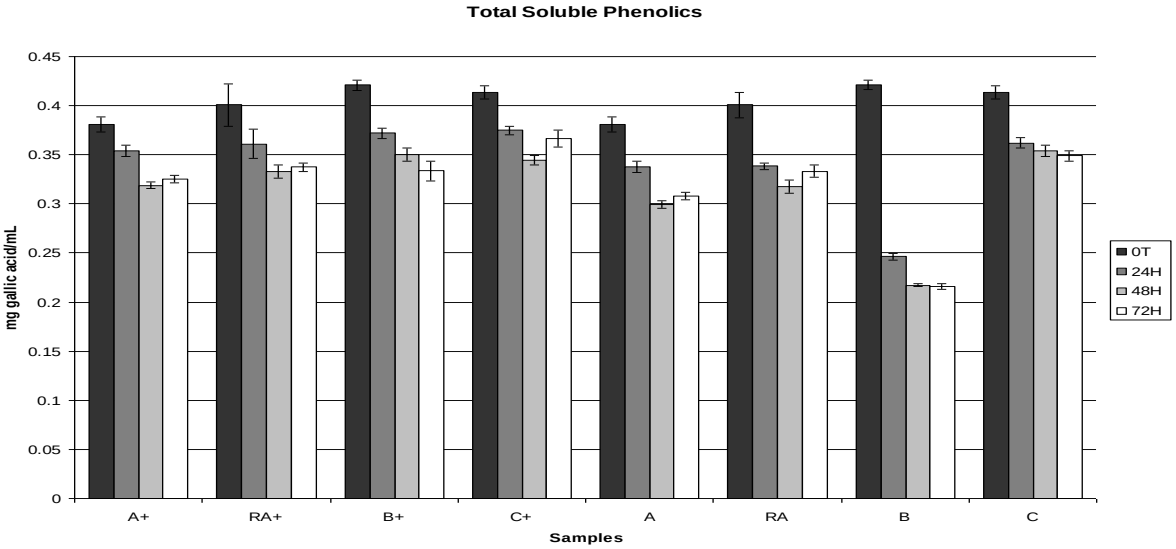
$$\%\ \text{inhibition} = \left(\left[\frac{\Delta A_{405}^{Control} - \Delta A_{405}^{Extract}}{\left[\Delta A_{405}^{Control} \right]} \right] \right) x100$$

Statistical Analysis:

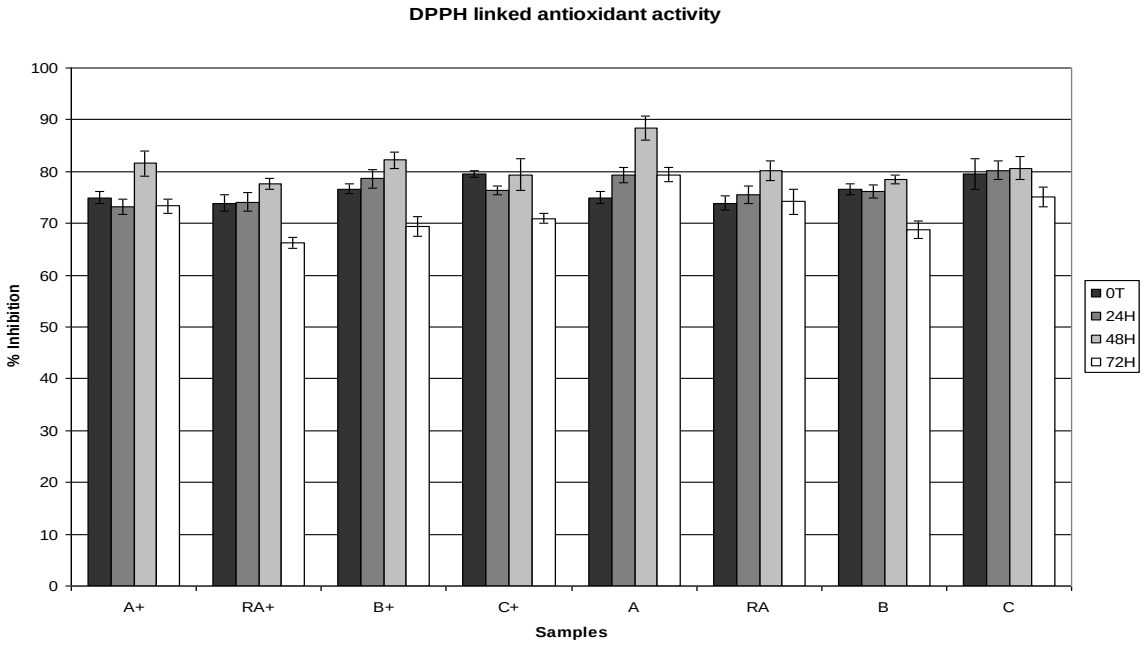
Analysis at every time point will be carried out in triplicates. Means, standard errors and standard deviations were calculated using Microsoft Excel.

RESULTS & DISCUSSION:

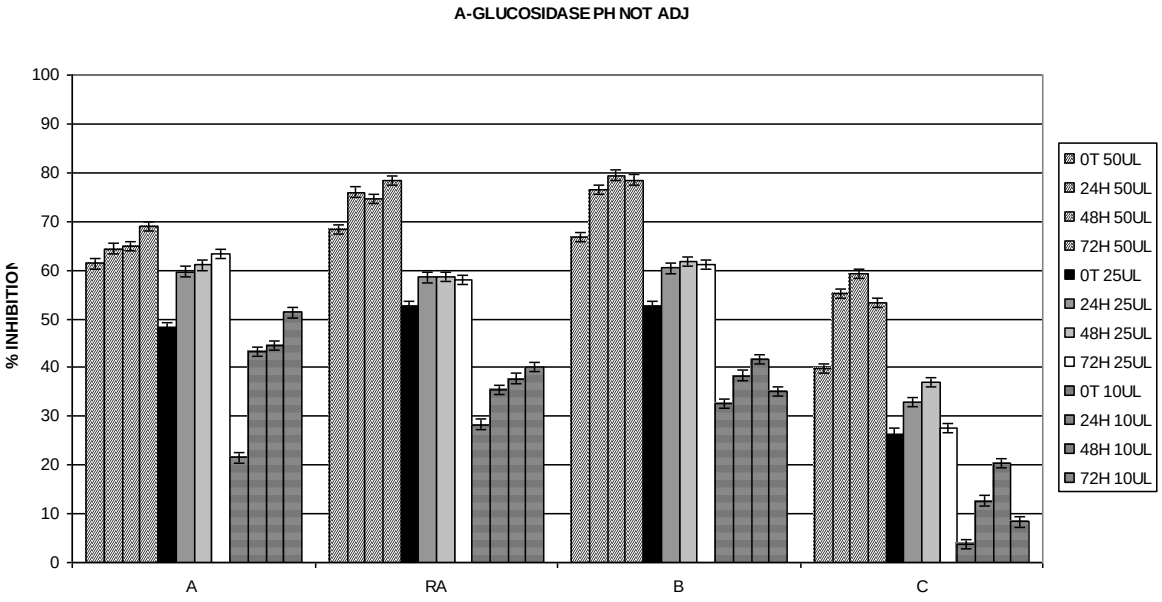
Soluble Phenolics: Soluble phenolics were readily utilized by probiotic *Lactobacillus acidophilus* without its growth being inhibited. Over a 72 H growth period the level of phenolics falls and type of phenolics also vary. With pH adjustment the phenolic profiles change in acidic environment (analogous to the stomach) to environment closer to pH 6.0 as in parts of the gut. Phenolic mobilization in Bosc is novel with rapid utilization and mobilization changes.



Free Radical Scavenging Antioxidant Response: Following phenolic mobilization in the initial 48 H the antioxidant activity increases before falling at 72 H. This indicates the potential of polymeric antioxidant phenolics being generated from pear by probiotic LA that is more bioactive. This indicates that pear following probiotic mobilization has excellent potential for supporting host antioxidant protective response.



Alpha-Glucosidase Inhibition Response: *Lactobacillus acidiphilus* fermentation of all pear varieties in this study enhanced alpha-glucosidase inhibitory activity over 72 H of growth. Further the activity was enhanced in a dose-dependent manner indicating a structure-function relationship. This indicates the probiotic bacteria either externally or when in the gut/intestine can enhance biological activity related to control of critical enzyme such as alpha-glucosidase activity involved with uptake of glucose from soluble sources in the diet. This critical enzyme is also the target of anti-diabetic drug acarabose prescribed for glucose control or hyperglycemia in early stages of type 2 diabetes. This study indicates that pear phenolic bioactives and beneficial probiotic bacteria can work synergistically to enhance bioactive function. Such synergistic combinations are needed for better efficacy and overall health benefits from the rapidly growing probiotics market (e.g. Goodbelly) globally. Such synergies are also essential for recovery of stomach, gut and intestinal health after repeated antibiotic therapy that reduces beneficial bacteria. Consumption of pears can not only help re-populating good beneficial bacteria but the enhanced antioxidant function has the potential to stimulate host cellular response to injury. Currently several fruit synergy products with probiotics are entering the market and pear is much superior to many of these choices.



Ulcer Bacteria Inhibition: *Lactobacillus acidophilus* fermentation of pear varieties resulting in phenolic enrichment also translates into inhibition of ulcer bacteria. These pathogenic bacteria need some level of oxygen and it is the metabolic pathway to using oxygen is blocked by pear phenolics, while leaving lactic acid bacteria unharmed as they do not need oxygen for energy production. These differences in oxygen requirements between pathogenic bacteria and beneficial lactic acid probiotic bacteria can be exploited by pear phenolics to help manage pathogens. Further with the antioxidant function of pears it enhances protective functions of host higher cellular form (eukaryotic cells like human and animal cells) to fight the bacterial pathogens better, while at the same time living harmoniously with probiotic beneficial bacteria. This is an exciting direction for phenolics from the family Rosaceae and especially pear. Many product and application strategies can be developed from this insight and rationale.

Beneficial Bifidobacterium stimulation: When 48 H or 72 H *Lactobacillus acidophilus* fermented pear extracts are used to grow highly anaerobic and well established probiotic bacteria, *Bifidobacterium longum* its proliferation was stimulated. Therefore pear improves product stability.

More detailed research of the Ulcer bacteria inhibition and Bifidobacterium stimulation is on-going based on pear varieties sent from Oregon and locally harvested pear varieties in Massachusetts. These results will be available in April-May 2010 in a manuscript format.

Discuss significance to the industry and potential economic benefits.

Health Benefits potential of pear has been defined at many levels using sound biochemical rationale and this has **significant impact on diverse use and applications of Pacific Northwest grown pear as a part of a healthy diet in the US and in the global market place.** Results clearly indicated the pears have significant soluble phenolic content in range of close to 350 ug to 400 ug per gram fresh weight including peel and this translates to about 70 mg to 80 mg soluble phenolics per 200 gram fruit. This also translates into high antioxidant capacity across all cultivars.

An exciting discovery from this study that particularly Bosc (B), D'Anjou (A) and Red D'Anjou (RA) have bioactive factors, which when fermented by lactic acid bacteria increase the bioactive function to potentially inhibit glucose uptake. This has potential for oxidative stress and management of diabetes complications from higher soluble sugars and control of glucose uptake (hyperglycemia).

The increased pear bioactive factors from lactic acid fermentation inhibit ulcer bacteria *Helicobacter pylori* while leaving good beneficial bacterial such *Bifidobacterium longum* unaffected or slightly stimulated in their growth.

All the above point to exciting ***health benefits of pear with phenolic-linked antioxidant protective functions*** that can influence positively the management of oxidative stress and management of infections. Therefore use of pear products can have impact on design of better diet to manage gut health and associated infections in combination with probiotics in food delivery systems such as fresh fruits, fruit smoothies or yogurts in dry or semi-solid form. We are further exploring the use of pear in military use for combating stress-related and excess antibiotic use-linked bacterial infections (*Clostridium difficile*), where recovery of good bacteria and inhibition of infections are important. These pear-based food designs along with apple and cherry also have implications for endurance management in sports activities and exercise due to high levels of relevant bioactive phenolics.

From these studies the health benefits of pears are better defined and this advances the wider use of pears as a part of healthy diet with enhanced fruits and vegetables. The bioactive potential indicates that pears particularly have relevance for not only managing oxidative stress but also beneficial bacteria, while at the same time being a hurdle to some form of bacterial infections. **Pear along with other important species in the family Rosaceae are essential to increase the per capta fruit intake for a healthy diet from the current US levels of 1 serving per day per person to close to 7-9 servings per day per person. This study provides clear new biochemical rationale for inclusion of *pear in a everyday healthy diet* for the American and global consumer.**

Additionally the phenolic-linked antioxidant regulation in pears has implications for innovative strategies for *post-harvest preservation based on natural phenolic regulation* using natural elicitors as pre-harvest sprays or combining with post-harvest treatments. We are exploring the use of oligosaccharides to enhance phenolics for both health benefits and better preservation.

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

Project Title: Pear crop load management and rootstock field testing

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Cooperators: Felipe Castillo, Ines Hanrahan, Jim McFerson, Dave Sugar, Todd Einhorn

Total Project Request: **Year 1:** 24,000 **Year 2:** 26,000 **Year 3:** 28,000

Other funding sources

All chemicals donated by companies

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

Item	2009	2010	2011
Salaries	10,500	12,000	13,500
Benefits	3300	3800	4300
Wages	5500	5500	5500
Benefits	1500	1500	1500
Equipment			
Supplies			
Travel	3000	3000	3000
Miscellaneous	200	200	200
CLM Subtotal	14,800	15,400	16,000
Rootstock subtotal *	9,200	10,600	12,000
Grand Total	$24,000	**$26,000**	$28,000

Footnotes: Rootstock expenses related to Einhorn Horner evaluation project; costs included here to reflect all expenses for WTFRC internal program pear projects

Objectives:

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

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

Significant findings:

- ATS applied during bloom and BA applied at 10mm fruitlet size often provide effective thinning and fruit size improvement of Bartlett and Bosc pears
- Inconsistent results are typical of chemical thinning studies across crops and suggest multiple applications (bloom and postbloom) offer greatest chance of successful crop load management
- NC99 shows potential as an organic blossom thinner of pear and warrants further testing
- Inclusion of spray oil with BA application showed no clear benefits in initial testing
- Application of AVG did not improve fruit set in D'Anjou trial; testing of different timings and concentrations are needed to validate anecdotal claims of higher yields
- Highlights of Horner rootstock evaluations are detailed in Einhorn's project report

Methods:

In 2009, we established three chemical thinning trials in commercial Bartlett orchards to be applied by grower-cooperators using their own spray equipment. Trials were designed as randomized complete blocks with plots comprised of 2-3 whole rows to simplify spraying. Initial bloom counts were recorded on tagged sample branches and each plot. All trials were successfully treated at appropriate timings at 100 gal water/acre. Fruit set counts were made on sample branches after June drop and before green fruit hand thinning, except one trial where crews inadvertently hand-thinned rows within the trial; results from that trial were compromised and are not reported here. Representative fruit from each plot were sampled within a few days of commercial harvest and evaluated in the WTFRC lab for size, firmness, sugar levels, acidity, and fruit finish.

We also established two other chemical thinning trials in commercial Bartlett blocks sprayed by WTFRC staff. Finally, a trial to evaluate fruit size and set effects of AVG and BA products was conducted on D'Anjou. These trials were applied at 100 gal water/acre using the WTFRC AccuTech sprayer on plots consisting of 5-8 trees. Data collection and harvest evaluations were identical to protocols described above.

Table 1. Typical Bartlett chemical thinning programs in 2009 WTFRC trials.

Material	Concentration	Timing(s)
ATS	5%	20% & 80% bloom
NC99	10%	20% & 80% bloom
BA (MaxCel, Exilis Plus, Genesis 6-BA)	128 oz/A	10 mm
BA + Superior oil	128 oz/A + 1%	10 mm

Results and discussion:

Results from 2009 chemical thinning trials confirm that both ATS and BA products can be effective crop load management tools for pear during and after bloom, respectively (Tables 2, 3). As in the past, our trials produced results in which treatments: 1. reduced fruit set with no effect on fruit size (White Salmon) 2. reduced fruit set and increased fruit size (Rock Island) and 3. did not affect fruit set, but increased fruit size (Cashmere). For the first time, we observed *decreases* in fruit size associated with thinning treatments (Buena), suggesting that trees in this trial may have been stressed by spray applications.

Table 2. Crop load effects of grower-applied bloom (ATS) and postbloom (BA) pear chemical thinning programs. WTFRC 2009.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
			%	%	g		%
Bartlett/OHxF.87	ATS	98 b	41 a	30 ns	168 ns	119	52 ns
- White Salmon	ATS; BA	89 b	46 a	27	170	118	61
	BA	122 a	33 b	28	165	121	56
	Control	132 a	30 b	26	163	123	52
Bartlett/Seedling	ATS	110 ns	42 ns	24 ns	173 b	115	20 ns
- Buena	ATS; BA	108	39	29	172 b	116	17
	BA	117	36	27	177 b	113	7
	Control	130	33	25	191 a	105	12

No treatments in the Cashmere trial (Table 3) produced significant results, but initial evaluation of NC99, an organic brine solution which has successfully thinned apple and peach in WTFRC trials, revealed numeric trends which suggest potential for decreased fruit set and increased fruit size from this product; we intend to follow up with more treatments of NC99 in 2010 trials.

Encouraged by the grower-cooperator from the Rock Island trial (Table 3) to "really push the envelope," we evaluated a thinning program featuring 2 applications of ATS during bloom followed by a full labeled rate of BA tank mixed with 1% spray oil at 10mm. Inclusion of the oil was based on anecdotal experience of another pear grower who felt use of oil had amplified the effect of BA on fruit size and set in a block he manages. Despite initial worries that we had been too aggressive with this program, the grower was ultimately very pleased with the outcome, which reduced hand-thinning and helped preserve fruit size in a season with heavy set in the trial block. ATS appeared to be the main thinning agent in all treatments in this trial, while BA apparently conferred the greatest increases in fruit size; the clearest effect of addition of the spray oil to the BA application was an apparent reduction in fruit size, perhaps due to tree stress.

Table 3. Crop load effects of WTFRC-applied bloom (ATS, NC99) and postbloom (BA, oil) pear chemical thinning programs. WTFRC 2009.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
			%	%	g		%
Bartlett/OHxF.87	ATS	43 ns	72 ns	17 ns	264 ab	76	27 ab
- Cashmere	ATS; BA	32	75	19	268 a	75	22 b
	BA	32	73	23	245 c	82	34 a
	NC99	36	74	18	263 ab	76	26 ab
	Control	43	71	18	253 bc	79	28 ab
Bartlett/OHxF.97	ATS	123 b	38 a	22 a	212 ab	94	22 ns
- Rock Island	ATS; BA	142 b	34 a	20 ab	222 a	90	16
	ATS; BA + oil	123 b	40 a	22 a	206 b	97	12
	Control	203 a	20 b	15 b	207 b	97	21

The inconsistency of trial results in our 2009 pear chemical thinning trials is comparable to prior seasons and our experience in chemical thinning research in other crops, including apple. With so much variability in trial outcomes, it can be tenuous to extrapolate results from individual trials to make broad assumptions about given programs. As such, we advocate evaluation of trial results across seasons, cultivars, and geographic regions to more accurately assess the efficacy of crop load management programs. Table 4 summarizes all WTFRC pear chemical thinning trials conducted since 2003; entries indicate how often various thinning agents have successfully achieved each of our three basic chemical thinning goals:

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

In this broader view, it is clear that ATS and BA products are the most consistent materials for reducing fruit set, while BA products most often confer larger fruit size; none of the programs tested reliably improve return bloom.

Table 4. Incidence and percentage of results significantly superior to untreated control. Pear chemical thinning trials WTFRC 2003-2009.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	9 / 29 (31%)	5 / 28 (18%)	2 / 21 (10%)
Urea	1 / 17 (6%)	3 / 17 (18%)	0 / 15 (0%)
Crocker's Fish Oil + lime sulfur	0 / 13 (0%)	1 / 13 (8%)	1 / 12 (8%)
Lime sulfur	1 / 13 (8%)	3 / 13 (23%)	0 / 13 (0%)
BA	3 / 15 (20%)	7 / 13 (54%)	2 / 22 (9%)
NAA	0 / 6	0 / 6	0 / 1

[1]Does not include data from 2009 trials.

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

Improved pear fruit size from use of BA products has the potential to significantly improve returns, but many D'Anjou growers are reluctant to use BA due to concerns about reducing fruit set and harvest yield. We conducted a trial evaluating reduced rates of BA applied at a later timing in hopes of conferring improved fruit size without affecting fruit set. Additionally, we included treatments

with AVG (ReTain) in hopes that it might improve fruit set or at least minimize any potential thinning effect of BA application. AVG is known to interfere with ethylene response in tree fruits and has been reported to decrease fruitlet abscission in pome fruits.

In this trial, both AVG and the 64 oz rate of BA provided classic thinning responses: decreased fruit set and increased fruit size (Table 5). The pairing of the chemistries muted both effects, producing milder thinning and fruit size increases. The lower rate of BA alone showed little treatment effect, suggesting that in this instance, 48 oz /acre was not adequate to elicit a response.

Table 5. Crop load effects of PGR programs. D'Anjou/OHxF.97, Monitor, WA. WTFRC 2009

Treatment	Application timing(s)	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russeted fruit
			%	%	g		%
48 oz BA	15 mm	92 a	48 bc	25 ab	248 c	81	30 b
64 oz BA	15 mm	61 b	62 a	20 b	288 a	69	42 a
AVG	70% bloom	62 b	59 ab	25 ab	266 b	75	41 a
AVG; 64 oz BA	70% bloom; 15 mm	86 a	44 c	32 a	251 bc	80	30 b
Control	---	98 a	43 c	28 a	245 c	82	45 a

A local representative for an agriculture chemical manufacturer reports that other pear growers believe AVG increased fruit set in their blocks in 2009; unfortunately, these "tests" were not replicated, did not include controls, and relied solely on the grower's subjective impressions, rather than objective fruit counts. These growers believe they gained approximately one bin per acre in yield, but that fruit size may have been compromised to do so. In 2010, we plan to develop a broader range of AVG concentrations and application timings in consultation with the product representative to conduct a more robust evaluation of its potential to increase fruit set. Depending on product availability and crop-destruct/registration constraints, we may also evaluate other plant growth regulators including CPA and/or CPPU, which have been reported to increase fruit set in cherry.

Budget figures associated with WTFRC's collaboration with the Horner rootstock evaluation project are included in this report for informational purposes only; please refer to Todd Einhorn's report for details on that project.