Northwest Pear Research Review

Hood River Best Western, Hood River, OR

Thursday, February 10

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FINAL PROJECT REPORT
WTFRC Project Number: PR08-803

Project Title: Control of postharvest fruit rots in pears

PI: Chang-Lin Xiao
Organization: WSU Tree Fruit Research and Extension Center
Telephone: 509-663-8181 X229
Email: clxiao@wsu.edu
Address: 1100 N. Western Ave.
City: Wenatchee
State/Zip: WA/98801

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

Total 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
Crew labor	0	0	0
Shipping	0	0	0
Supplies	0	0	0
Travel	0	0	0
Miscellaneous	0	0	0
Total	3,184.21	3,184.21	3,184.21

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

Budget History:
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:

- When Pristine was applied to d'Anjou pear fruit one week before harvest, residual protection of the fruit by preharvest Pristine was still evident two months after harvest.

- On the fruit treated with Pristine in the field and packed one week after harvest, preharvest Pristine alone without any postharvest fungicides at packing reduced blue mold incidence by 56% in 2008, 87% in 2009, and 21% in 2010 in comparison with the nontreated control. Residual effects of Pristine were more pronounced in 2008 and 2009 than in 2010. The decline in residual effects of Pristine in 2010 likely resulted from the wash-off of residues because there was a rain event within three hours after Pristine application in the field, whereas no rain events occurred after Pristine spray in 2008 and 2009. The results suggest that rain events shortly after Pristine application could compromise its residual effects on decay control.

- For d'Anjou pear fruit that were sprayed with Pristine seven days before harvest and packed and inoculated with *Penicillium expansum* two months after harvest, Pristine alone, without any postharvest treatments, reduced blue mold incidence by 47-70% compared with the control eight weeks after packing, indicating that residue of Pristine on/in d'Anjou pear fruit can last for at least two months during storage.

- The biocontrol agent BioSave alone applied at packing significantly reduced blue mold on the fruit packed one week after harvest, but 30-61% of the fruit treated with BioSave alone still had blue mold. BioSave was less effective than postharvest fungicides Scholar and Penbotec for blue mold control.

- An integrated program consisting of Preharvest Pristine applied one week before harvest and postharvest BioSave applied at packing was superior to Pristine alone or BioSave alone and further reduced blue mold incidence to a much lower level (1-15%) on wounded- and inoculated fruit.

- The effectiveness of preharvest Pristine in combination with postharvest BioSave was reduced after the fruit had been stored at room temperature for one additional week after cold storage. Nonetheless, our results suggest that preharvest Pristine plus postharvest BioSave could be a promising program for blue mold control when fruit are packed shortly after harvest and have not been drenched prior to storage.

- 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 residual activity of these two postharvest fungicides on d'Anjou pear fruit was also less consistent than that on Red Delicious and Fuji apples we previously observed. This may be due to the differences in cuticles between apple and pear fruit, which may affect the uptake of fungicides or penetration of the fungicides.

- BioSave alone did not provide adequate control of blue mold on pear fruit that had been stored for four or six months after harvest. However, BioSave significantly reduced blue mold incidence on pear fruit that had been stored for two months or less. Fruit senescence four or six months after harvest may increase the susceptibility of fruit to blue mold and thus affect the efficacy of BioSave. Additional benefits from BioSave applied at packing were not consistent on Scholar- or Penbotec-drenched fruit for blue mold control and not present on TBZ-drenched fruit.

- Preharvest Pristine or Topsin applied seven days before harvest and a postharvest drench with Scholar, Penbotec or Mertect significantly reduced Phacidiopycnis rot compared to the nontreated control on the fruit infected in the field by *Phacidiopycnis piri* five weeks before harvest. The efficacy of a postharvest drench with Scholar or Penbotec was generally more consistent than those of other treatments.

- Conventional and real-time PCR assays were 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 PCR-based assays 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 BioSave or fungicides for blue mold control was evaluated on d'Anjou pears. Pristine was applied seven days before harvest. Fruit were harvested and stored in RA. Part of the fruit was removed from RA at one week and two months after harvest. Fruit were run through a research packingline and inoculated with *P. expansum*. Part of the inoculated fruit was treated with each of the three postharvest fungicides (TBZ, Penbotec and Scholar) or the biocontrol agent BioSave after inoculation. Nontreated fruit were used as controls. All fruit were stored in cold storage for eight weeks and then for seven 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 five weeks before harvest. For preharvest fungicide treatments, fungicides Pristine and Topsin M were applied within two 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 two weeks for five 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 four and six 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 BioSave after inoculation. Nontreated fruit will be used as controls. All fruit were then stored in cold storage for eight weeks and then for seven 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 a packinghouse.

Results and Discussion

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

Experiments were conducted for three years (2008-2010). For the fruit packed one week after harvest, similar results were obtained (Table 1 and Table 2). Residual protection of the fruit by preharvest Pristine was evident after harvest. In comparison with the nontreated control, preharvest Pristine alone without any postharvest fungicides at packing reduced blue mold from 97.5% to 42.5% in 2008, from 90% to 11.3% in 2009, and from 100% to 78.8% in 2010 (Table 1). Residual effects of Pristine were more pronounced in 2008 and 2009 than in 2010. The decline in residual effects of Pristine in 2010 likely resulted from the wash-off of residues because there was a rain event within three hours after Pristine application in the field, whereas no rain events occurred after Pristine spray in 2008 and 2009. The results suggest that rain events shortly after Pristine application could compromise its residual effects on decay control.

BioSave alone applied at packing significantly reduced blue mold, but 30-61% of the fruit treated with BioSave alone had blue mold (Table 1). However, preharvest Pristine plus postharvest BioSave was superior to Pristine alone or BioSave alone and further reduced blue mold incidence to a much lower level (1-15%). However, the effectiveness of preharvest Pristine in combination with postharvest BioSave was reduced after the fruit had been stored at room temperature for one additional week (Table 2). Our results indicate that preharvest Pristine plus postharvest BioSave could be a promising program for blue mold control when fruit are packed shortly after harvest and have not been drenched prior to storage.

For the experiment with fruit from the 2009 crop that were stored for two months before packing, the results were generally consistent with those observed on the fruit packed one week after harvest (Table 3). Preharvest Pristine without any postharvest treatments reduced blue mold incidence from 84% in the nontreated control to 25%, indicating residual effects of preharvest Pristine was still evident two months after harvest. Similarly, preharvest Pristine plus postharvest BioSave provided better control of blue mold than Pristine alone or BioSave alone for the fruit packed two months after harvest.

Table 1. Preharvest Pristine in combination with postharvest fungicide and biocontrol agent for control of blue mold in d'Anjou pears – decay incidence 8 weeks at 32°F after packing during 2008-2010

Preharvest Treatment	Fungicide applied 1 week after harvest	Incidence of blue mold (%) 8 weeks at 32F after packing		
		2008 season	2009 season	2010 season
Nontreated	No fungicide	97.5 a	90.00 b	100.0a
	Scholar	0.0 d	0.00 e	0.0f
	Penbotec	0.0 d	0.00 e	0.0f
	TBZ	100.0 a	96.25 a	95.0b
	BioSave	61.3 b	30.00 c	60.0d
Pristine	No fungicide	42.5 c	11.25 d	78.8c
	TBZ	67.5 b	27.50 c	76.3c
	Scholar	0.0 d	0.00 e	0.0f
	Penbotec	0.0 d	0.00 e	0.0f
	Biosave	2.5 d	1.25 e	15.0e

[z] Values within the same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio *t* test at K ratio = 100 ($P = 0.05$).

Table 2. Preharvest Pristine in combination with postharvest fungicide and biocontrol agent for control of blue mold in d'Anjou pears - decay incidence one additional week at room temperature after cold storage during 2008-2010

Preharvest Treatment	Fungicide applied 1 week after harvest	Incidence of blue mold (%) one additional week at room temperature after 8 weeks in cold storage		
		2008 season	2009 season	2010 season
Nontreated	No fungicide	98.8 a	95.00 b	100.0a
	Scholar	0.0 d	0.00 g	0.0f
	Penbotec	0.0 d	0.00 g	0.0f
	TBZ	100.0 a	98.75 a	97.5b
	BioSave	70.0 b	56.25 d	62.5d
Pristine	No fungicide	70.0 b	41.25e	85.0c
	TBZ	96.3 a	77.50 c	81.3c
	Scholar	0.0 d	0.00 g	0.0f
	Penbotec	0.0 d	0.00 g	0.0f
	Biosave	17.5 c	5.00 f	23.8e

[z] Values within the same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio *t* test at K ratio = 100 ($P = 0.05$).

Table 3. Preharvest Pristine in combination with postharvest fungicide and biocontrol agent for control of blue mold in d'Anjou pears in 2009-10 season (2 months after harvest of 2009 crop)

Preharvest Treatment	Fungicide applied 2 months after harvest	8 weeks at 32°F after packing		1 week at room temp after cold storage	
		% decay	Lesion (mm)	% decay	Lesion (mm)
Nontreated	No fungicide	83.8a	24.4a	97.5a	57.0a
	Scholar	0.0e	0.0e	0.0d	0.0d
	Penbotec	0.0e	0.0e	0.0d	0.0d
	TBZ	67.5b	23.0a	93.8a	49.3b
	BioSave	55.0b	15.9b	70.0b	46.0b
Pristine	No fungicide	25.0c	12.3c	68.8b	25.6c
	TBZ	27.5c	11.0c	78.8b	23.6c
	Scholar	0.0e	0.0e	0.0d	0.0d
	Penbotec	0.0e	0.0e	0.0d	0.0d
	Biosave	11.3d	5.4d	18.8c	22.1c

[z] Values within the same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio *t* test at K ratio = 100 ($P = 0.05$).

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

Experiments were conducted on 2008, 2009 and 2010 crops. Results from 2008 and 2009 crops were generally consistent. The 2008 results were reported last year. The results on 2009 crop are presented in Table 4. Four and six months after harvest, blue mold incidences were significantly lower on Scholar-drenched and Penbotec-drenched pear fruit that were inoculated at packing with *P. expansum* four or six months after harvest and did not received any other treatments at packing in comparison with the nontreated control (Table 4). However, the spectrum of residual effects of Scholar on blue mold control on d'Anjou pears was smaller on 2009 crop than on 2008 crop. The residual activity of these two postharvest fungicides on d'Anjou pear fruit was also less consistent than that on Red Delicious and Fuji apples we previously observed. This may be due to the differences in cuticles between apple and pear fruit, which may affect the uptake of fungicides or penetration of the fungicides. Nonetheless, 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.

BioSave alone did not provide adequate control of blue mold on pear fruit that had been stored for four or six months after harvest (Table 4). However, BioSave significantly reduced blue mold incidence on pear fruit that had been stored for two months or less (Tables 1-3). Fruit senescence four

or six months after harvest may increase the susceptibility of fruit to blue mold and thus affect the efficacy of BioSave. Additional benefits from BioSave applied at packing were not consistent on Scholar- or Penbotec-drenched fruit for blue mold control and not present on TBZ-drenched fruit (Table 4).

Research has been set up to repeat this experiment on the 2010 crops. The fruit are currently in CA. Part of the fruit will be removed from CA four or six months after harvest. The fruit will be run through a research packing line and inoculated with *Penicillium expansum*. The experiment will end in spring 2011. Results will be forthcoming.

Table 4. Residual effects of Scholar and Penbotec on blue mold on d'Anjou pears that were inoculated at packing with *Penicillium expansum* and treated with BioSave, 2009-10 season

		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 0ºC post packing	% infected fruit at one additional week at room temperature after storage	% infected fruit at 8 weeks at 0ºC post packing	% infected fruit at one additional week at room temperature after storage
		TBZ-R	TBZ-R	TBZ-R	TBZ-R
Nontreated	No fungicide	100.0a[z]	100.0a	100.0a	100.0a
	Scholar	1.3e	1.3c	2.5de	11.3d
	Penbotec	0.0e	0.0c	0.0e	0.0e
	Mertect	97.5b	98.8a	98.8a	98.8a
	Bio-Save	100.0a	100.0a	88.8b	100.0a
Scholar	No fungicide	61.3c	100.0a	36.3c	92.5b
	Bio-Save	38.8d	73.8b	25.0c	67.5c
Penbotec	No fungicide	2.5e	2.5c	5.0d	5.0d
	Bio-Save	1.3e	1.3c	5.0d	6.3d
TBZ	No fungicide	100.0a	100.0a	98.8a	100.0a
	Bio-Save	100.0a	100.0a	92.5b	100.0a

[z] Values within the same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio *t* test at K ratio = 100 ($P = 0.05$).

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

Experiments were conducted in a research orchard in 2008-09 and 2009-10 seasons 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 five weeks before harvest. All selected fungicide treatments significantly reduced Phacidiopycnis rot on pear compared to the nontreated control (Fig. 1). No Phacidiopycnis rot was observed on pear fruit treated with postharvest Scholar or Penbotec. Although Phacidiopycnis rot occurred on the fruit treated with Pristine, Topsin M or TBZ, the incidence of Phacidiopycnis rot was low and was not significant among all fungicide treatments.

The results from 2009-10 season were similar to those in 2008-09. Taken these together, it appeared that the efficacy of a postharvest drench with Scholar or Penbotec was generally more consistent than those of other treatments.

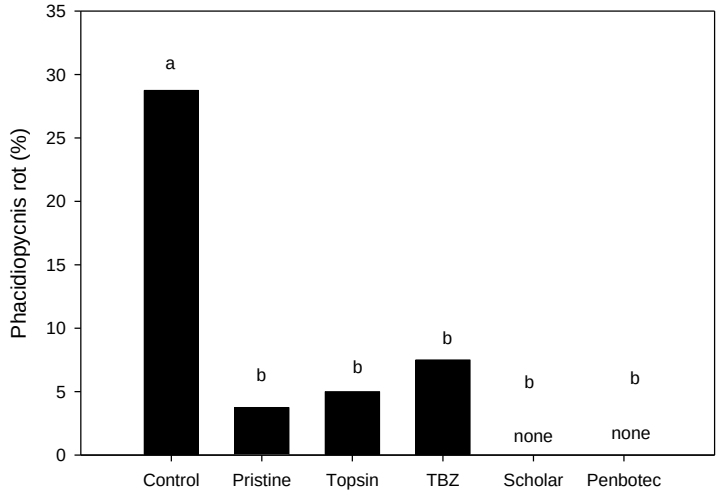


Fig. 1. Effectiveness of pre- and postharvest fungicides in controlling Phacidiopycnis rot on d'Anjour pear fruit that were inoculated with the Phacidiopycnis fungus 5 weeks before harvest in the orchard in 2009-10.

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

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

The first PCR assay was based on specific primer sets designed based on the sequences of ITS region. One primer set per target pathogen was selected and applied in both conventional and real-time PCR in this study. Specificity of the three primer sets against target pathogens and non-target pathogens or fungi was tested (data reported previously). At specified annealing temperatures, amplification for each pathogen was strong and detected only with its own isolates, while no amplifications were detected with other fungi and the fruit DNA. Validation with stem-end rot and calyx-end rot samples collected from a packinghouse indicated that ITS-based PCR assay and isolation-based assay yielded consistent results in the identification of causal agents of decayed fruit (Table 5).

Real-time PCR for diagnosis. 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 5).

Table 5. Identification of causal agents of naturally infected pear fruit using three different approaches

Samples[a]	Symptoms (# of samples)	Causal agent	Approaches		
			Isolation	PCR-based assays	
				ITS-based conventionalPCR	Real-time PCR
1	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
2	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
3	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	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.

Executive Summary

This report is a summary of a three-year project conducted from 2008 to 2010. The focus of this research project was on the integration of identified decay-control tactics, including field and postharvest components, for control of major postharvest diseases.

Pristine as a preharvest spray is being implemented by growers for postharvest decay control. In this project, we further found that when Pristine was applied to d'Anjou pear fruit one week before harvest, residual protection of the fruit by preharvest Pristine was still evident during storage two months after harvest. On the fruit treated with Pristine in the field and packed one week after harvest, preharvest Pristine alone without any postharvest fungicides at packing significantly reduced blue mold incidence in comparison with the nontreated control. For d'Anjou pear fruit that were sprayed with Pristine one week before harvest and packed and inoculated with *Penicillium expansum* two months after harvest, Pristine alone, without any postharvest treatments, reduced blue mold incidence by 47-70% compared with the control eight weeks after packing.

The biocontrol agent BioSave alone applied at packing significantly reduced blue mold on the fruit packed one week after harvest but did not provide satisfactory control as 30-61% of the fruit treated with BioSave alone still had blue mold. BioSave was less effective than postharvest fungicides Scholar and Penbotec for blue mold control.

However, an integrated program consisting of Preharvest Pristine applied one week before harvest and postharvest BioSave applied at packing was superior to Pristine alone or BioSave alone and further reduced blue mold incidence to a much lower level (1-15%) on wounded- and inoculated fruit. The effectiveness of preharvest Pristine in combination with postharvest BioSave was reduced after the fruit had been stored at room temperature for one additional week after cold storage. Nonetheless, our results suggest that preharvest Pristine plus postharvest BioSave could be a promising program for blue mold control when fruit are packed shortly after harvest and have not been drenched prior to storage.

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 residual activity of these two postharvest fungicides on d'Anjou pear fruit was less consistent than that on Red Delicious and Fuji apples we previously observed. This may be due to the differences in cuticles between apple and pear fruit, which may affect the uptake of fungicides or penetration of the fungicides.

BioSave alone did not provide adequate control of blue mold on pear fruit that had been stored for 4 or 6 months after harvest. However, BioSave significantly reduced blue mold incidence on pear fruit that had been stored for two months or less. Fruit senescence four or 6sixmonths after harvest may increase the susceptibility of fruit to blue mold and thus affect the efficacy of BioSave. Additional benefits from BioSave applied at packing were not consistent on Scholar- or Penbotec-drenched fruit for blue mold control and not present on TBZ-drenched fruit.

Preharvest Pristine or Topsin applied seven days before harvest and a postharvest drench with Scholar, Penbotec or Mertect significantly reduced Phacidiopycnis rot compared to the nontreated control on the fruit infected in the field by *Phacidiopycnis piri* five weeks before harvest. The efficacy of a postharvest drench with Scholar or Penbotec was generally more consistent than those of other treatments. It appeared that a postharvest fungicide drench is a better measure for control of Phacidiopycnis rot.

Conventional and real-time PCR assays were 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 PCR-based assays and the isolation-based assay yielded consistent results in the identification of causal agents of decayed fruit.

FINAL PROJECT REPORT

Project Title: Health benefits of Oregon & Washington pears

PI: Kalidas Shetty
Organization: University of Massachusetts at Amherst
Telephone: 413-545-1022
Email: kalidas@foodsci.umass.edu
Address: Chenoweth Laboratory
Address 2: 102 Holdsworth Way
City: Amherst
State/Zip: MA 01003

Co-PI: Duane W. Greene
Organization: University of Massachusetts at Amherst
Telephone: 413-545-5219
Email: dgreene@pssci.umass.edu
Address: Bowditch Hall
Address 2: Holdsworth Way
City: Amherst
State/Zip: MA 01003

Cooperators: NA

Other funding sources:
UMass supported Teaching Assistant also worked on this project.
Agency Name: University of Massachusetts at Amherst

Amount awarded: Year 1: $ 47,050 Year 2: $ 51,400

Total Project Funding: $ $98,450 for 2 years

Budget History:

Item	**(Year 1)**	**(Year 2)**
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

RECAP OF 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 of 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 (anti-diabetes potential) and inhibit ulcer bacteria (*Helicobacter pylori*) for better digestive and gut health.

The objectives were 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

SIGNIFICANT FINDINGS:

A) Fresh Variety Analysis: (Manuscript 1 being written for publication in J. Medicinal Food)

1) Important pear varieties Bartlett, Comice, Bosc, Starkrimson, Concorde, Red Anjou and Anjou have significant total soluble phenolic antioxidants distributed between pulp and peel and on an average 250 gram fresh pear have between 40 to 70 mg of total soluble phenolics. At the higher end for Concorde the content was about 75 mg from one 250 gram serving of fruit.

2) There is positive correlation between total soluble phenolic content and free radical scavenging-linked antioxidant activity. The peel extracts of Starkrimson and Concorde especially stand out in terms of antioxidant activity. Overall all varieties do have significant soluble phenolics-linked antioxidant activity.

3) High α-glucosidase and α-amylase inhibitory activity (reflection of *in vitro* anti-diabetes potential through the ability to inhibit key enzyme involved in carbohydrate digestion and uptake) was observed in all pear cultivars during both years. Pulp extraction showed higher inhibitory activity of these enzymes compared to peel extracts.

4) A positive correlation was observed between α-glucosidase inhibitory activity and total soluble phenolic content in peel and pulp extractions of majority of pear cultivars. Significant dose-dependent response (a key factor for pharmaceutical relevance of drugs like acarbose) in the enzyme inhibitory activity was also observed in all pear cultivars. Red pear, Red Anjou, Comice, Starkrimson and Concorde showed higher α-glucosidase inhibitory activity in both peel and pulp extracts. This is significant since the entire fruit has high anti-diabetic potential.

5) The major phenolics found in fresh and stored varieties were protecatechuic acid, quercetin dervitives (Rurin), gallic acid and small levels of resveratrol in peel and protocatechuic acid and gallic acid in pulp extracts.

B) Fermentation and Probiotic Benefits of Pears: (Manuscript 2 on fermented anti-diabetic potential being written for Journal of Food Biochemistry and Manuscript 3 on anti-ulcer and probiotic benefits being written for publication in International Journal of Food Microbiology)

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

2) 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 acarbose.

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

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

5) Epicatechin and quercetin derivatives were the major phenolic compounds detected in the fermented samples.

6) Juice extraction and concentration increases ACE (angiotenin converting enzyme) inhibitory activity, which is a target for reducing high blood pressure, which a macro vascular complication of type 2 diabetes.

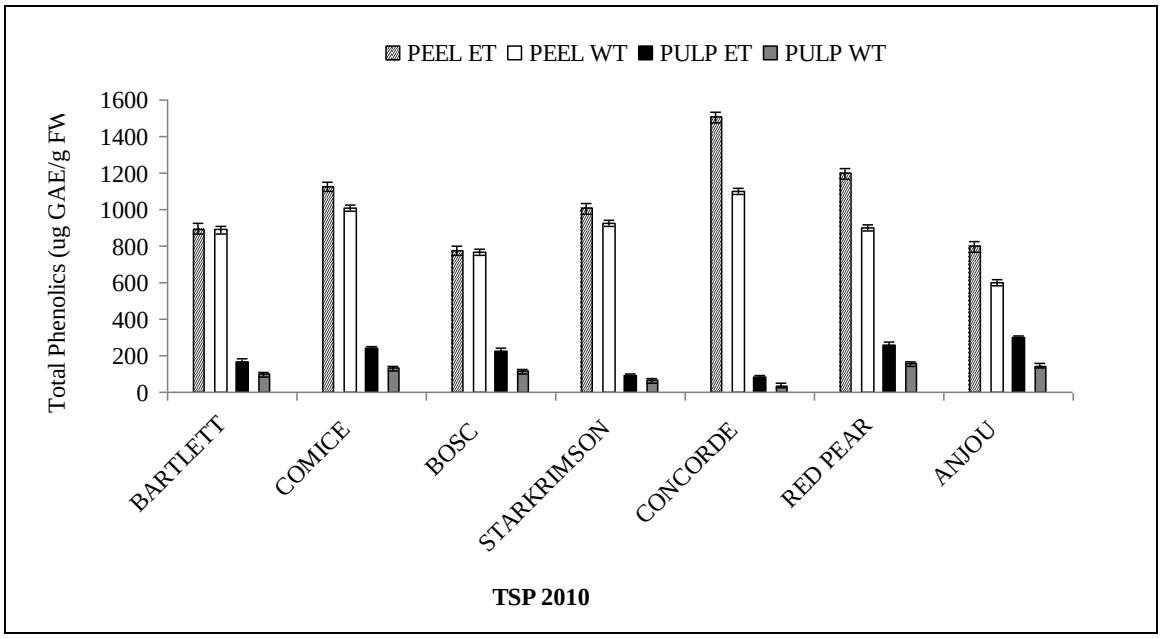
RESULTS & DISCUSSION:

Six pear cultivars (Bartlett, Comice, Bosc, Starkrimson, Red Anjou and Anjou) form Northwest obtained through US Pear were evaluated in 2009 whereas seven different pear cultivars (Bartlett, Comice, Bosc, Starkrimson, Concorde, Red pear and Anjou) were evaluated in 2010. Pears were first peeled then cut and weighed. Peel and pulp of each variety were extracted using water and 12% ethanol (20 g of peel in 50 mL of water and 5 g of peel in 15 mL of 12% ethanol and 100 g of pulp in 50 mL of water and 10 g of pulp in 20 mL of 12% ethanol). All the samples were homogenized using a Waring blender for 3 min. All samples were adjusted to pH 6.00 to reflect the enzyme contents of pancreatic alpha-amylase and intestinal alpha-glucosidase. The samples were centrifuged at 15,000 g for 1 min. Supernatant were collected and kept at -20˚C during the period of study.

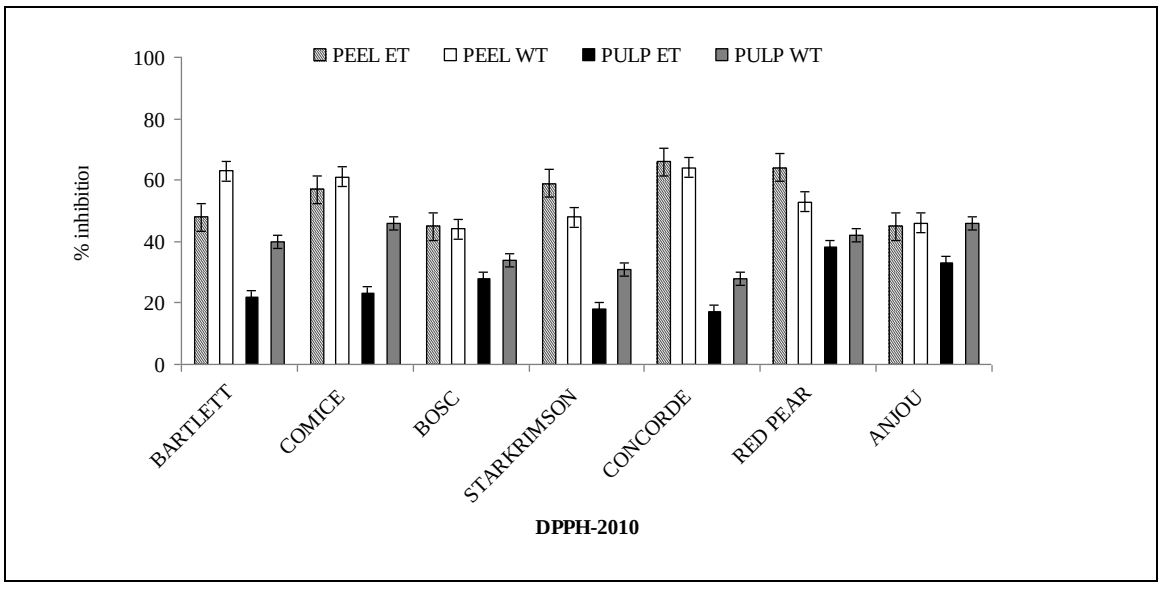
Total Soluble Phenolic and DPPH Free Radical Scavenging-Linked Antioxidant Activity:

Total soluble phenolic content of peel varied between 577- 1270 ug GAE/ g F.W. The higher total soluble phenolic content was observed in ethanol extraction of Starkrimson followed by Bosc, Bartlett, Red Anjou, Comice and Anjou. Whereas total soluble phenolic content of pulp varied between 64-204 ug GAE/ g F.W. and higher soluble phenolic was observed in ethanol extraction. High soluble phenolic content of pulp was observed in Red Anjou followed by Anjou, Bosc, Comice, Bartlett and Starkrimson. Similarly, in 2010 out of all seven cultivars highest total soluble phenolic was observed in ethanol extraction of Concorde peel (1505 ug GAE/ g F.W.) followed by Red pear, Comice, Starkrimson, Bartlett, Bosc and Anjou. The higher soluble phenolic in pulp was observed in Anjou followed by Red Anjou, Bosc, Comice, Bartlett, Concorde and Starkrimson. Results from both years showed similar general trend. Only Comice showed significantly higher soluble phenolic content in peel in 2010 compared to 2009.

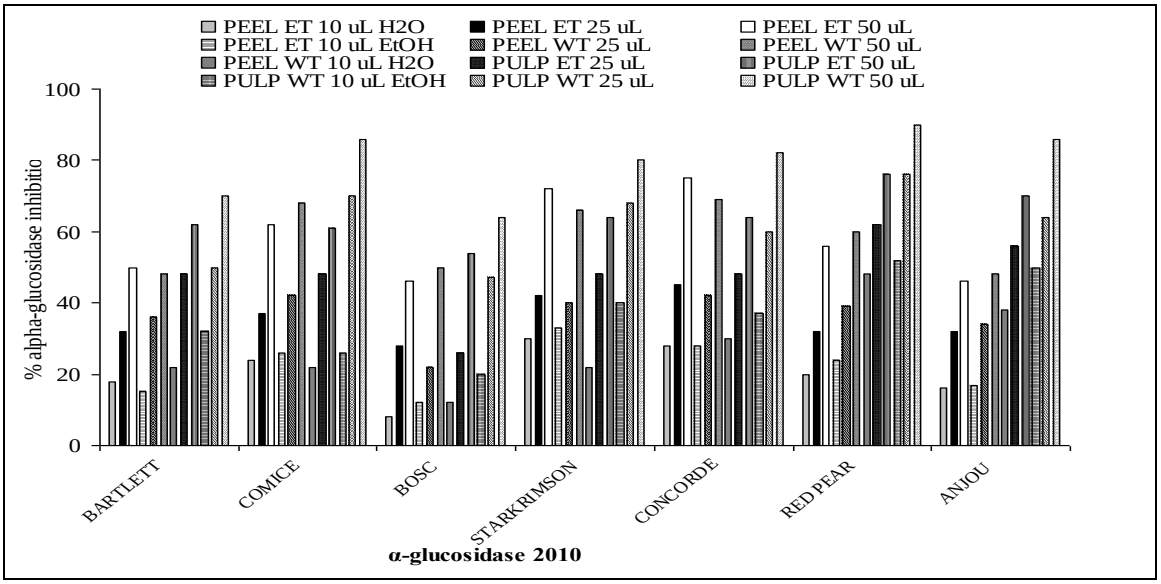
An average 250 gram fresh pear has between 40 to 70 mg of total soluble phenolics. At the higher end for Concorde the content was about 75 mg from one 250 gram serving of fruit.

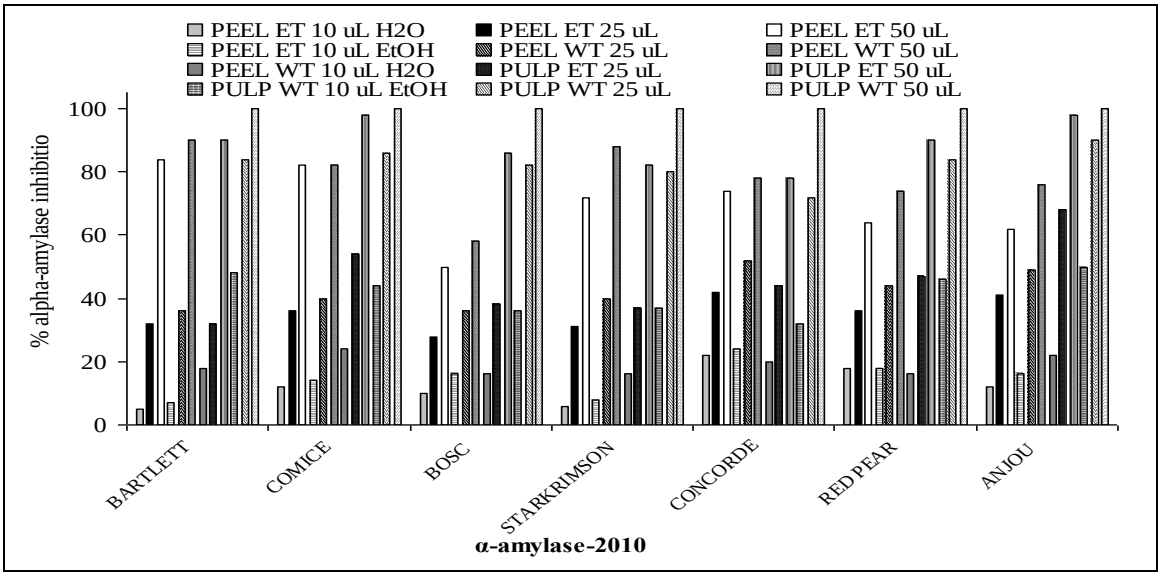


Like total soluble phenolics, total antioxidant activity (DPPH radical inhibition) of pear cultivars also showed similar trend in both years (2009-2010). Significant positive correlations were observed between total phenolic content and total antioxidant activity in peel and pulp extractions of all pear cultivars. High total antioxidant activity was observed in peel extraction of Starkrimson (74% inhibition of DPPH oxidation) and pulp extraction of Red Anjou (38%) similar to total soluble phenolic content in 2009. During 2010, high total antioxidant activity was observed in Concorde peel extracts (66%) and pulp extracts of Anjou (46%) and Comice (46%). High phenolic content and high total antioxidant activity of these pear cultivars particularly in peel clearly show promise for using whole pear fruit for health benefits and for enhancing overall fruit and vegetable intake in the United States from less than 1 serving per day to the needed 7-8 serving per day. **Pear due to it ease delivery as whole fruit combined with food such as yogurts can at least meet the needs of 2 servings per day per person of total fruit and vegetable in take..**



Alpha- glucosidase and Alpha- amylase activity: High α-glucosidase and α-amylase inhibitory activity are reflections of anti-diabetic potential through inhibitions of these key enzymes involved in carbohydrate breakdown and uptake. The inhibitory activity was observed in all pear cultivars during both years. Pulp extraction showed higher inhibitory activity of these enzymes compared to peel extracts. A positive correlation was observed between α-glucosidase inhibitory activity and total soluble phenolic content in peel and pulp extractions of majority of pear cultivars. Significant dose response (reflection of structure-function pharmaceutical potential) in the enzyme inhibitory activity was also observed in all pear cultivars. Red pear, Red Anjou, Comice, Starkrimson and Concorde showed higher α-glucosidase inhibitory activity in both peel and pulp extracts.





Significant α-amylase activity was observed in all pear cultivars and particularly in pulp extractions. Cultivars like Starkrimson, Red pear, Red Anjou and Concorde showed comparatively higher α-glucosidase inhibitory and low α-amylase inhibitory activity with higher total soluble phenolic content. These observations showed higher promise for better type 2 diabetes management

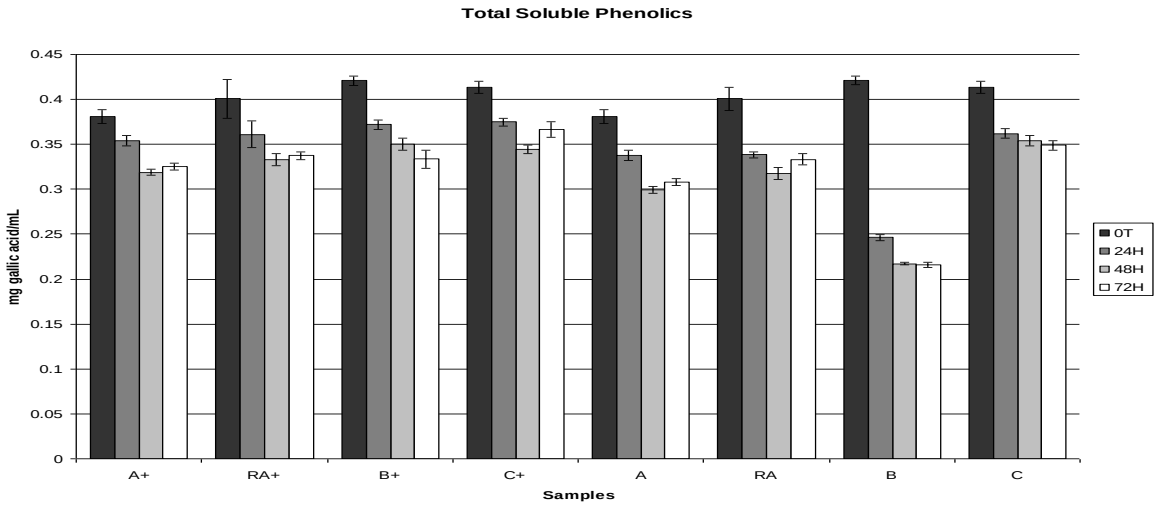
in these cultivars since these can potentially overcome the side effects undigested starch observed in currently used pharmaceutical drugs.

No to negligible ACE (Angiotensin-I converting enzyme) inhibitory activity was observed in all pear cultivars. The activity of found is important as we could use fruits to help long term management of blood pressure, which is a macro vascular complications of type 2 diabetes.

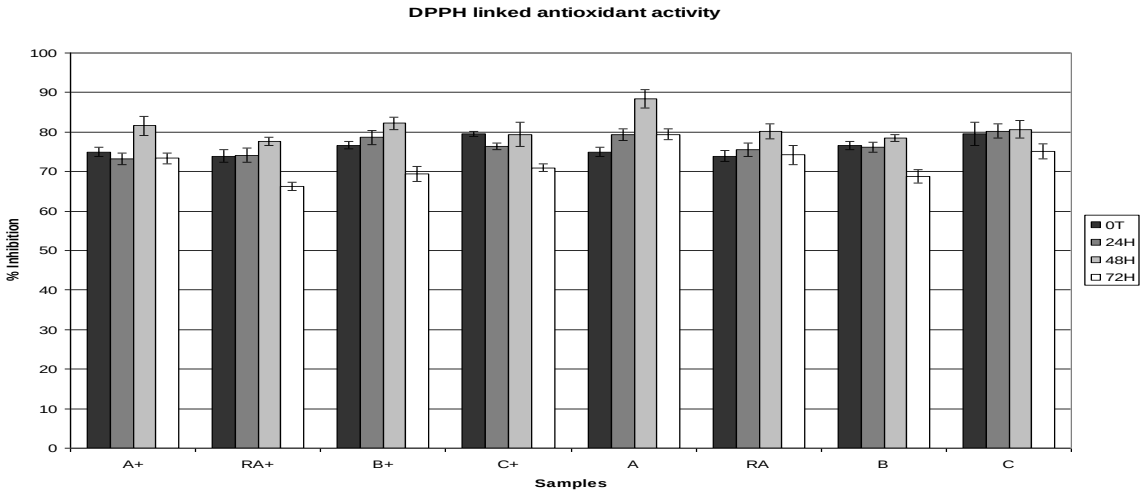
B) Anti-Diabetic, Probiotic and Ulcer Bacteria Inhibiting Benefits of Fermented Pears:

Type 2 diabetes and other chronic diseases are oxidation-linked diseases that are emerging challenges globally and fruits have been shown to have good potential in managing oxidation linked diseases. Four cultivars (Anjou, Red Anjou, Bartlett and Comice) of pear were homogenized to extract their juice, fermented using *Lactobacillus acidophilus* for 0, 24, 48 and 72 h juice and changes in bioactive functionality related to total phenolics, antioxidant potential, inhibition of key enzymes in carbohydrate metabolism and hypertension linked ACE were evaluated. The pH of the juices was adjusted to 6.0-7.0 before fermentation and assays at each time point were carried out at fermented acidic pH and by adjusting the pH to 6.0-7.0. Total phenolics decreased with fermentation and DPPH-linked antioxidant activity increased until 48 h and then it decreased for most samples at 72 H. α-Glucosidase inhibitory activity did not change significantly for pH adjusted samples whereas for pH not adjusted samples there was a significant increase in inhibition with fermentation for most samples. α-Amylase inhibition was not observed since the sample was diluted due to high sugar content. ACE inhibitory potential was high at 0 H and decreased with fermentation for all samples. Protein content decreased or remained constant for all samples. Epicatechin and quercetin derivatives were the major phenolic compounds detected in the fermented samples This suggests that fermentation of pear juice is a good strategy to enhance antioxidant potential and α-glucosidase inhibitory potential to reduce post-prandial rise in blood glucose linked to type 2 diabetes.

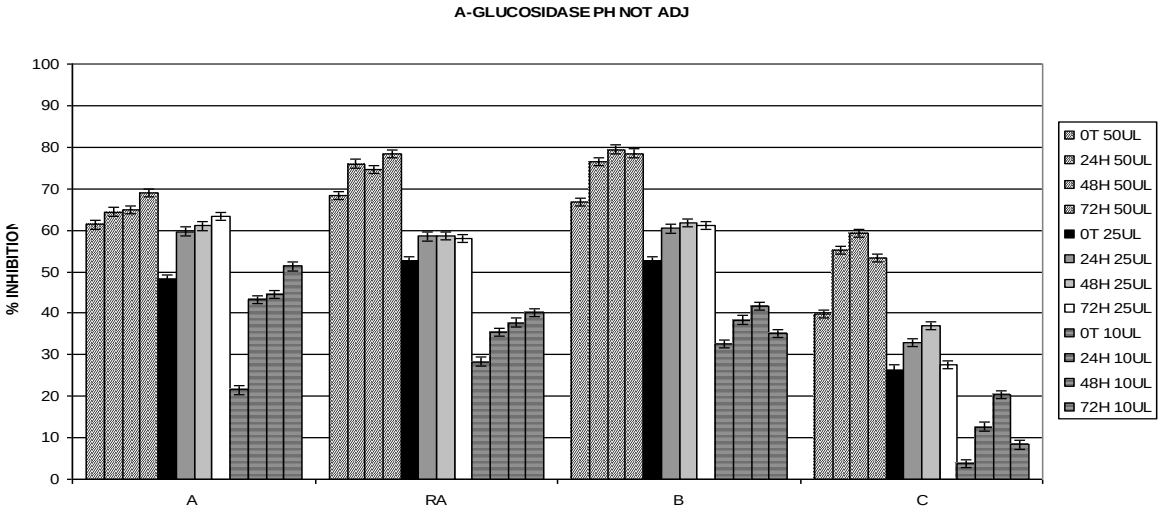
Soluble Phenolics: Soluble phenolics were readily utilized by probiotic *Lactobacillus acidophilus* without its growth being inhibited. Over a 72 H growth period the level of phenolics falls and type of phenolics also vary. With pH adjustment the phenolic profiles change in acidic environment (analogous 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.



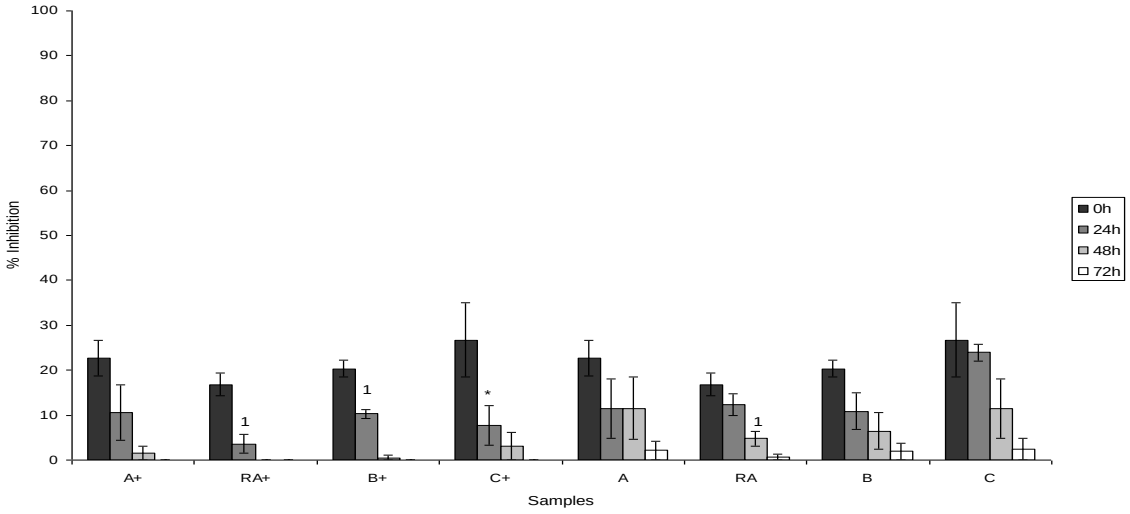
Free Radical Scavenging-Linked Antioxidant Response: Following phenolic mobilization in the initial 48 H the antioxidant activity increases before falling at 72 H. This indicates the potential of polymeric antioxidant phenolics being generated from pear by probiotic LA that is more bioactive. This indicates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



Alpha-Glucosidase Inhibition Response: *Lactobacillus acidophilus* 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 with pharmacological potential. 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 acarbose 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. Pear combinations with yogurts would be of great interest. This will be further investigated in community school lunch program in Massachusetts.**



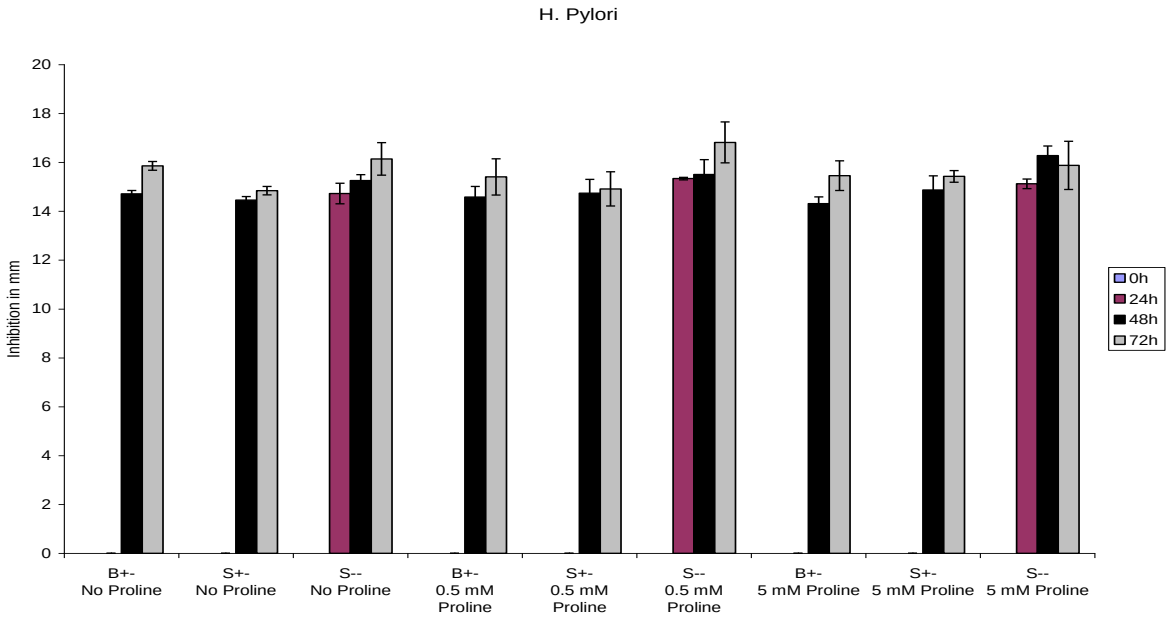
Extracted juice at 0 hours had ACE (angiotensin converting enzyme) inhibitory activity, which is target of reducing high blood pressure.



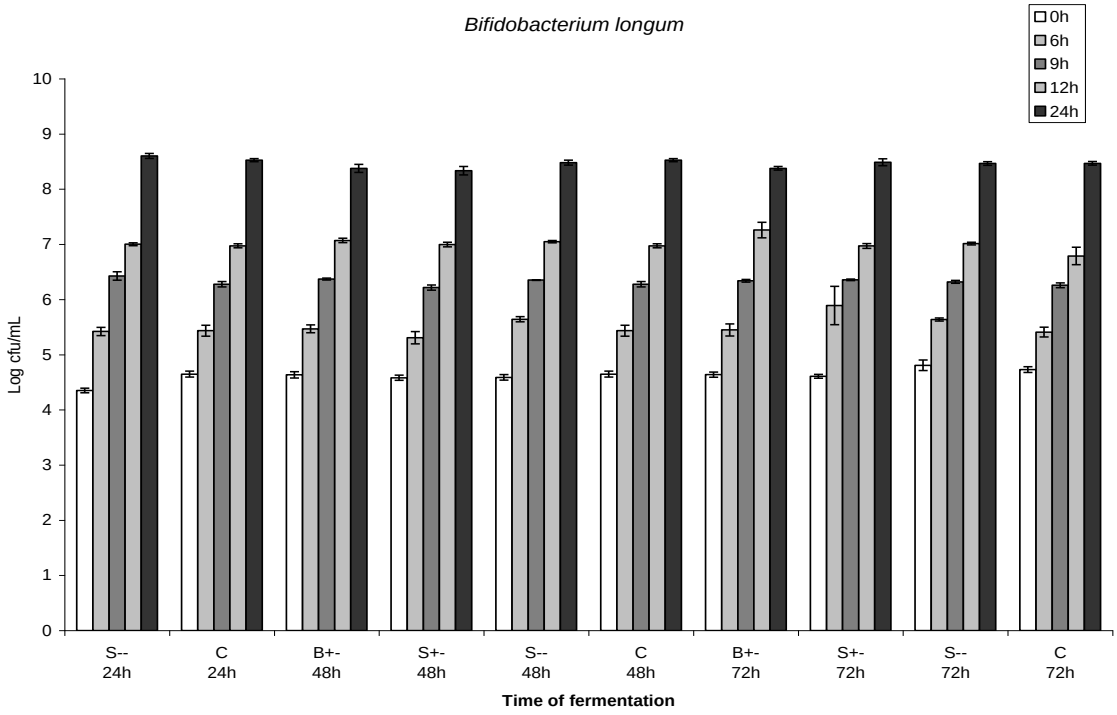
Ulcer Bacteria Inhibition: *Helicobacter pylori* has been identified as an etiological agent in the development of gastric ulcer, peptic ulcer, gastritis and many other stomach related diseases. Recent research has shown that fermented extracts of lactic acid bacteria has potential in inhibiting the stomach ulcer causing pathogen. Two cultivars of pear (Bartlett: B; Starkimson: S) were homogenized to extract their juice and then fermented using *Lactobacillus acidophilus* by adjusting the pH before and after fermentation. *H. pylori* inhibition was observed for all samples (B+-, S+-, S--) at 48 and 72 H where final pH was not adjusted except Bartlett where both initial and final pH were not adjusted (B--). In case of Starkimson where initial and final pH was not adjusted (S--) inhibition was observed at 24 H. Samples which showed *H. pylori* inhibition were further tested to evaluate their effects on lactic acid bacteria with probiotic potential. No inhibition and in some cases proliferation was observed in case *Bifidobacterium longum* which indicates fermented pear juice may be able to inhibit *H. pylori* without affecting or in some cases stimulating the probiotic lactic acid bacteria. **Fermented pear juice therefore would offer a low cost dietary support food system in the management of *H. pylori* without affecting the beneficial lactic acid bacteria in the colon. Therefore a health-based yogurt or probiotic-based pear whole fruits or juice products can be developed for multiple bioactive health functions.**

Overall *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 therefore 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 healthy food design and product application strategies can be developed from this insight and provides rationale for enhancing healthy fruit intake in the

American diet. In particular range of yogurt and probiotic beverage-based products using pear and pear products are feasible for school lunch and community health programs where obesity is high.



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



EXECUTIVE SUMMARY:

Potential Health Benefits 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 40 to 70 mg soluble phenolics per 250 gram fruit. This also translates into high antioxidant capacity across all cultivars. There are multiple bioactive health application of the pear phenolics both in whole fruit form and also processed fruit form in form of canned fruits or juice concentrates since phenolic bioactives are spread well across peel and pulp.

An exciting discovery from this study is that overall all pear fresh varieties have phenolic bioactive factors which indicate anti-diabetic potential by inhibiting key enzyme such as alpha-glucosidase and alpha-amylase associated with hyperglycemia (high sugar in the blood). The phenolic bioactives which when fermented by lactic acid bacteria increase the bioactive function to potentially inhibit glucose uptake and therefore a pear-based probiotic drink could also have health benefits. The high antioxidant potential of phenolics both in fresh fruits and fermented juice have relevance for managing cellular oxidative stress related to management of diabetes complications from higher soluble sugars and control of glucose uptake (hyperglycemia).

A third exciting discovery is that the increased pear phenolic 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. This offers further evidence of multiple bioactive health potential from managing negative effects of high glucoses to inhibiting pathogenic bacteria while supporting beneficial probiotic bacteria.

All the results of this study 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. In the immediate next phase we are exploring the use of pear and pear products to increase the fruit intake in poor urban school and elderly communities where fruit intake is low. A yogurt-based pear design has the potential to not only increase healthy fruit intake but in the long run we want design experiments whether we can reduce the level of general infections and colds in children and elderly. Likewise in specific communities where obesity and associated type 2 diabetes is high we want to initially increase fruit intake using pears and apples and bring it to a range of 3-5 servings per day per person. 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 stimulating 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.

FINAL PROJECT REPORT **EARLY TERMINATOIN**
WTFRC Project Number: PR-099-903A

Project Title: PNW Pear Rootstock Trial

PI:	Timothy J. Smith	**Co-PI:**	Todd Einhorn
Organization:	Washington State University	**Organization:**	OSU-MCAREC
Telephone:	509-667-6540	**Telephone:**	541-386-2030 x13
Email:	smithtj@wsu.edu	**Email:**	todd.einhorn@oregonstate.edu
Address:	400 Washington Street	**Address:**	3005 Experiment Station Drive
City:	Wenatchee	**City:**	Hood River
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 (Tonasket Trial). Advisors: Fred Valentine, Tom Auvil, Greg Rains, Bob Gix.

Total Project Funding: **Year 1:** $9,876 **Year 2:** $8,611

Other funding sources

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

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

	2009	**2010**	
Salaries	$2,880	$3368	
Benefits	1,353	1582	
Supplies	300		
Travel	1,808		
Total	**$6,341**	**$4,950**	

Footnotes: Salaries and benefits are in support of 0.0769 FTE (20 days) of a full time technician.

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**	
Salaries[1]	$1,950	$2,028	
Benefits	$1,185	$1,233	
Wages			
Benefits			
Supplies	$300	$300	
Travel	$100	$100	
Total	**$3,535**	**$3,661**	

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

ORIGINAL OBJECTIVES:

The seven pear scions/rootstocks planted in 2002 and the six planted in 2005, now completing their 9th and 6th season, were evaluated on the following: 1. survival, 2. suckering, 3. vegetative growth potential (trunk size and tree diameter), 4 yield, and 5.fruit size.

SIGNIFICANT FINDINGS

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

- A number of potential rootstocks, including one that was being sold commercially in Washington and Oregon, were shown to be inferior due to disease or cold injury susceptibility, comparative yield, fruit size, the production of thorny root suckers, or a combination of these attributes. These findings stopped the sale of this rootstock, which helped potential buyers avoid great financial loss over time.

- The OHxF 87 performed well enough in the trial to become the industry standard semi-dwarfing rootstock until something better comes along. Nurseries responded by growing more trees on this root, rather than the easier to produce OHxF 97, a rootstock that induces much more vigorous, and larger, trees. This has resulted in availability of this root to pear growers who wish to take advantage of its benefits.

- Bartlett on Pyro 2-33 appears superior to Bartlett on OHxF 87, and especially to those on Pyrodwarf. Superiority is due to a more balanced fruit set, leading to less hand thinning, superior fruit size, and yields equal to or slightly higher than produced by OHxF 87. Several growers now have Bartlett on Pyro 2-33 planted.

- Planting of pears is relatively uncommon, and "traditional" pear growers usually see no need to change away from good quality orchards producing high yields of large fruit. However, if a grower has a reason to replace an orchard and wishes to grow pears, this rootstock trial has demonstrated that semi-intensive planting systems offer the best opportunity to achieve full production in less than eight years, rather than the traditional 14 to 16. Through the use of the best available rootstocks (OHxF 87 for most pears, or Pyro 2-33 for Bartlett), and good horticultural management, we have demonstrated and documented that a grower may be able to produce significant yields as early as the 5th or 6th season after planting.

- Planting D'Anjou and Bosc pears on OH x F 87 at 6 x 14 feet and training them on an upright trellis did not lead to production equal to that achieved by planting on the same rootstock at 8 x 15 feet and training to a free-standing central leader system. About 70% of the fruit could be harvested without a ladder in the mature free-standing system, which the harvesting crew considered a great advantage.

- Comparing the most productive (OH x F 87) to the least productive (Pyrodwarf) rootstock, full russet Bosc in a free standing system, by the 8th season the OH x F 87 orchard would have returned a gross total of $40,940 / acre, the Pyrodwarf rootstock orchard managed similarly would have returned $19,110 / acre. The Bosc on OH x F 87 will "break even" economically in the 9th or 10th year; about one-half of the necessary time for traditional pear planting spacing and rootstocks. The year that the Boscs on Pyrodwarf will break even is not yet possible to predict.

RESULTS & DISCUSSION

D'ANJOU

9th and Final Season (2010) Data on 2002 Planted Trees, PNW Pear Rootstock Trial, D'Anjou:

D'Anjou 2002, Cashmere 2010 Harvest	2010 Pounds Fruit/ Acre, 9th Year	Calc. Trees Per Acre	2010 1100 lb. Bins Fruit / A	2010 Avr. Box Size (fruit per 44 pounds)	2010 Lbs. Fruit per Tree	2010 Trunk Cross Section Area CM^2	2010 lbs. Fruit / CM^2 of Trunk
OHxF 87	80,104	323	72.8	70	248	148	**1.68**
OHxF 40	66,861	323	60.8	70	207	151	**1.37**
Pyro 2-33	52,003	323	47.3	74	161	135	**1.19**
Fox 16	42,401	389	38.5	72	109	128	**0.85**
708 - 36	38,122	389	34.7	76	98	125	**0.78**
Fox 11	33,065	389	30.1	73	85	129	**0.66**
Pyrodwarf	23,256	323	21.1	80	72	140	**0.51**

Table 1-1. 2010 Data from the 2002 planting of Green D'Anjou, (9th season), listed in descending order of total yield. Planting space was calculated at 9 x 15 feet for the 323 trees per A, and 8 x 14 feet for the 389 trees per acre.

8th Season Data on 2002 Planted Trees, PNW Pear Rootstock Trial, D'Anjou:

D'Anjou 2002, Cashmere 2009 Harvest	2009 Pounds Fruit / Acre, 8th Year	Calc. Trees Per Acre	2009 1100 lb. Bins Fruit / A	2009 Avr. Box Size (fruit per 44 pounds)	2009 Lbs. Fruit per Tree	2009 Trunk Cross Section Area CM^2	2009 lbs. Fruit / CM^2 of Trunk
OHxF 87	61,693	323	56.1	81	191	145	**1.32**
OHxF 40	49,419	323	44.9	89	153	141	**1.09**
Fox 16	37,733	389	34.3	86	81	97	**0.84**
Pyro 2-33	35,207	323	32.0	88	109	119	**0.92**
708 - 36	29,953	389	27.2	93	77	102	**0.75**
Fox 11	26,063	389	23.7	86	67	105	**0.64**
Pyrodwarf	20,026	323	18.2	95	62	120	**0.52**

Table 1-2. 2009 Data from the 2002 planting of Green D'Anjou, (8th season), listed in descending order of total yield. Planting space was calculated at 9 x 15feet for the 323 trees / A, and 8 x 14 feet for the 389 trees / acre.

BARTLETT

Bartlett 2002 Planted 2010 Harvest	Total Yield To Date in Pounds per Acre	2010 Yield In 1100 lb bins per Acre	Average Fruit Box Size 2010 (Fruit / 44 Pounds)	2010 Pounds Fruit Per Tree	Trunk Size in Sq. cm (Veg. Growth)	2010 Yield Efficiency Lb. Fruit / cm^2	Total Yield 04-2010 Efficiency Lb. Fruit / cm^2
Cashmere Pyro 2-33	194,875	61	69.3	172.5	117	1.47	**4.27**
Cashmere Pyrodwarf	141,805	46	84.1	129.5	116	1.12	**3.13**
Cashmere OHxF 87	179,787	61	70.9	173.3	109	1.59	**4.23**

Table 2-1. Summary data for Cashmere site, 2002 planted (9^{th} leaf) Green Bartlett pears, 2010 season and averages of all years. 7.5 x 15, 390 trees / Acre tree spacing.

Bartlett 2002 Planted	2009 Yield In lbs. per Acre	Total Yield To Date in Pounds per Acre	Average Fruit Box Size (Fruit / 44 Pounds)	Trunk Size in Sq. cm (Veg. Growth)	2009 Pounds Fruit Per Tree	2009 Yield Efficiency Lb. Fruit / sq. cm	Total Yield Efficiency Lb. Fruit / sq. cm
Tonasket Pyro 2-33 02 to 09	70,885 **8^{th} Leaf**	191,259	82	71.3	160	2.24	**6.04**
Tonasket Pyrodwarf 02 to 09	72,489 **8^{th} Leaf**	121,016	98	70.4	163	2.32	**3.87**

Table 2-2. 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 for 444 trees / Acre. Note: the higher the box size number, the smaller the fruit.

BOSC

The Bosc portion of this trial suffered a serious crop reduction, probably due to frost at full bloom, and was not evaluated in 2010.

8^{th} and Final Season (2009) Data on 2002 Planted Trees, PNW Pear Rootstock Trial, Bosc:

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	Average Fruit Box Size (Fruit / 44 Pounds)	2009 Pounds Fruit Per Tree	2009 Yield Efficiency Lb. Fruit / sq. cm	Total Yield Efficiency Lb. Fruit / sq. cm
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 3-1. Summary data for 2002 planted (8th leaf) Golden Russet Bosc pears, 2009 season and averages of all years.

2005 Planted Section of the Rootstock Trial:

In Tonasket, the 2005 Golden Russet Boscs are spaced 6 x 14 ft on an upright 4 wire trellis, and had variable production in their 3rd leaf, and significant production in their 4th and 5th growing season. The 6th growing season, 2010 was essentially a crop failure due to frost damage at time of full bloom, so no data is available. The D'Anjous in Cashmere, also trellised, had a very light yield so far. Data is provided below (Table 4-1) to illustrate the frustration of growing a pear cultivar that is inherently slow to come into bearing (D'Anjou) with a semi-dwarfing (at best) rootstock, on a site with good soil.

Trunk size (vegetative growth:,
Trunk size, Cashmere D'Anjou on trellis, cross-section area in square centimeters, in descending order of size: OHxF 87 (57.4), Horner 4a (57.2), BM-2000 (57.2), BU-2 (30.7), and BU-3 (27.6). The trunk sizes in inches of diameter were OHxF 87 (3.36), Horner 4a (3.35), BM-2000 (3.34), BU-2 (2.45), and BU-3 (2.31). The Bartlett pollenizers on Horner 4a have a trunk cross sectional area of 83 cm^2 (or a diameter of 2.9 inches). The Horner 4a and BM 2000 trees are growing more vigorously than those on OH x F 87, even though the trunk sizes are similar.

Survival of the tree:
The BU-2 and BU-3 in the 2005 trial appear to be affected by pear decline at the Cashmere D'Anjou and the Tonasket Bosc site. The Hood River site does not seem to have this pear decline problem, as even the 2002 planting of 708-36 did not become diseased, and that root that had significant pear decline problems in the northern Washington trial site. Tree survival at the Hood River trial has been virtually 100%. Temperatures of -10 to -15ºF, or lower occurred at the Tonasket trial site in December, 2008, and October 8 – 10 2009 low temperatures reached 10-12ºF. No cultivar/rootstock combination in the 2002 and 2005 rootstock trial has shown any symptoms of damage due to these two cold temperature events.

Root suckering:
No significant suckering was observed on any rootstock other than Pyrodwarf, and to a much lesser extent, 708-36. Pyrodwarf roots started developing suckers by their third season of growth. In the 2002 planting, these Pyrodwarf suckers became large, numerous and thorny by the 5th season of tree growth. Fewer, and much less thorny and vigorous suckers began to develop in the 2002 planted trees from the 708-36 roots by the 8th growing year.

Yields, fruit size and Efficiency:
The Bosc scion/rootstock combinations began to produce commercially significant yields in the 4th, and especially in 2009, their 5th season. Unfortunately, in 2010, frost greatly reduced yields. The D'Anjou rootstock trial has never set a crop worth picking, except for those trees on OHxF 87, which, in the 6th leaf may have been worth picking, but barely (Table 3.1). The BU-2 and BU-3 rootstocks are the most dwarfing of any pear rootstock tested in either the 2002 or 2005 sections of the trial. Unfortunately, both of these roots have disease/survival problems, and almost half of the trees in the D'Anjou trial had symptoms of pear decline disease, and then died.

D'Anjou-2005 Planting Cashmere (on a trellis)	**2010 Pounds Fruit/ Acre, 6th Year**	**2010 Bins Fruit / Acre**	**2010 Average Fruit Box Size**	**2010 Trunk Cross Sectional Area in CM^2**	**2010 Lbs. Fruit / Tree**	**Total lbs. Fruit per CM^2 of Trunk (Efficiency)**
OHxF 87	13,129	11.94	69.6	57.4	21.7	0.378
BM 2000	2.481	2.26	74.3	57.2	4.1	0.072
Horner 4a	5,264	4.79	73.4	57.2	8.7	0.152
BU-2	1,876	1.71	78.3	30.7	3.1	0.101
BU-3	4,477	4.07	74.3	26.7	7.4	0.277

Table 4-1**. 2010 harvest of the 2005 planting, D'Anjou** pear, Cashmere, (6th season), 6 x 12 ft. on 4-wire upright trellis (605 trees/A).

Bosc-2005 Planting Tonasket (on a trellis)	2007-08 Pounds Fruit/ Acre, 3rd and 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 Sectional 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
Bartlett Horner 4a	10,231	17,160	27,391	24.9	81	28.7	33.3	1.16
2002 Free-standing Bosc in 4th & 5th Leaf OHxF 87	*8,814*	*31,763*	*43,338*	*36.9*	*78*	*46.4*	*81.4*	*2.39*

Table 4-2. 2009 yields, 2005 planting of Golden Russet Bosc pear, Tonasket, (5th season), 6 x 12 ft. on 4-wire upright trellis. Bartlett is pollenizer, every 5th tree. 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.

No new 2005 trial rootstock was sufficiently productive by the end of the 6th leaf (fall 2010), so this trial was terminated.

EXECUTIVE SUMMARY

02 Bosc	Survival	Tree Size (dwarfing)	Suckering (no)	Yield	Fruit Size	Efficiency	Average
Multiplier	***5***	***3***	***5***	***4***	***3***	***3***	
OHxF 87	25	0	25	20	15	15	**16.7**
OHxF 40	25	0	25	12	3	9	**12.3**
Pyro 2-33	25	0	25	4	15	3	**11.8**
Fox 16	0	9	25	0	9	0	**7.2**
Fox 11	0	9	25	0	9	0	**7.2**
Pyrodwarf	25	0	0	0	0	0	**4.2**
708-36	0	15	0	0	0	0	**2.5**

Bosc 2002 Trial through 8th crop. Assigned score value re: place in factor ranking, 1st = 5, 2nd = 3, 3rd = 1, 4-7th = 0, then multiplied that score by multiplier factor valuing relative importance. 5 = Must have, 4 = Very important, 3 = Important.

2002 D'Anjou	Survival	Tree Size (dwarfing)	Suckering (no)	Yield	Fruit Size	Efficiency	Average
Multiplier	***5***	***3***	***5***	***4***	***3***	***3***	
OHxF 87	25	0	25	20	15	15	**16.7**
OHxF 40	25	0	25	12	9	9	**13.3**
Fox 16	25	9	25	0	3	0	**10.3**
Fox 11	25	9	25	0	0	0	**9.8**
Pyro 2-33	25	0	25	4	0	3	**9.5**
Pyrodwarf	25	0	0	0	0	0	**4.2**
708-36	0	9	0	0	0	0	**1.5**

D'Anjou 2002 Trial through 9th crop. Assigned score value re: place in factor ranking, 1st = 5, 2nd = 3, 3rd = 1, 4-7th = 0, then multiplied that score by multiplier factor valuing relative importance. . 5 = Must have , 4 = Very important, 3 = Important.

	Survival	Tree Size	Suckering	Yield	Fruit Size	Efficiency	Average
OHxF 87	100	53	100	100	97	100	
OHxF 40	100	63	100	83	92	99	
Pyro 2-33	100	67	100	76	93	97	
Fox 16	70	86	100	53	100	70	
Fox 11	70	84	100	64	93	88	
708-36	80	100	80	64	85	98	
Pyrodwarf	100	57	0	51	91	57	

Bosc 2002 Trial through 8th crop. Percentage re best performing root within the category. Higher average is better. Tree size = smallest tree / tree being scored. Fruit size = largest size / size from rootstock being rated. Higher score is better.

	Survival	Tree Size	Suckering	Yield	Fruit Size	Efficiency	Average
Importance Wt. Factor	5	3	5	5	4	3	**Total score / 6**
OHxF 87	500	159	500	500	388	300	**391**
OHxF 40	500	189	500	415	368	297	**378**
Pyro 2-33	500	201	500	380	372	291	**374**
Fox 16	350	258	500	265	400	210	**331**
Fox 11	350	252	500	320	372	264	**343**
708-36	400	300	300	320	340	294	**326**
Pyrodwarf	500	171	0	255	364	171	**244**

Above table with score weighted re importance of factor. 5 = Must Have ,4 = Very important, 3 = Important 1. Not wanted 0 =. A no-no. Bosc 2002 Trial through 8th crop. Percentage re best performing root within the category. Higher average is better. Tree size = smallest tree / tree being scored. Fruit size = largest size / size from rootstock being rated. Higher score is better

	Survival	Tree Size	Suckering	Yield	Fr. Size	Efficiency	Average
Importance	***1***	***3***	***5***	***1***	***2***	***2***	
OHxF 87	1		0	2	6	4	
OHxF 40	1		0	4	15	8	
Pyro 2-33	1		0	6	12	16	
Fox 11	5		0	12	9	20	
Fox 16	6		0	8	3	24	
708-36	7		30	10	21	12	
Pyrodwarf	1		35	14	18	28	

Lower score is better. 5 = Must Have ,4 = Very important, 3 = Important 1. Not wanted 0 =. A no-no. Score is weight factor x percent relative to the best performance root in that category

	Survival	Tree Size	Suckering	Yield	Fruit Size	Efficiency	Average
OHxF 87	1	7	1	1	2	1	**2.17**
OHxF 40	1	5	1	2	5	2	**2.67**
Pyro 2-33	1	4	1	3	4	4	**2.83**
Fox 16	5	2	1	4	1	6	**3.17**
Fox 11	6	3	1	6	3	5	**4.00**
708-36	7	1	6	5	7	3	**4.83**
Pyrodwarf	1	6	7	7	6	7	**5.67**

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

Project Title: Cold hardiness of quince

PI:	Todd Einhorn	**Co-PI(2)**:	Joseph Postman
Organization:	OSU-MCAREC	**Organization**:	USDA/ARS
Telephone:	(541) 386-2030 x13	**Telephone**:	(541) 738-4220
Email:	Todd.einhorn@oregonstate.edu	**Email**:	joseph.postman@ars.usda.gov
Address:	3005 Experiment Station Drive	**Address**:	33447 Peoria Road
City:	Hood River	**City**:	Corvallis
State/Zip:	Oregon 97031	**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: Requested amount of $12,192.00, Awarded amount, $9,750.00.
Notes: USDA Plant Germplasm Evaluation Program approved funding March 19, 2010 to develop a precise system for determining the lowest survival temperatures of quince and pear accessions tested herein. We have successfully developed and implemented differential thermal analysis (DTA) techniques for measuring plant hardiness, and will be correlating those data with results from this project.

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

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

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

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

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

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

Objectives

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

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

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

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

Significant Findings Sep 2009-Jan 2011

- Following cold acclimation ~50% (25) of the quince accessions tested were capable of withstanding -30 °C [-22° F] without accompanying freeze damage to tissues, and roughly 20 % showed likely survivability following exposure to -40 °C [-40° F].
- Many quince accessions were capable of attaining maximum hardiness levels greater than or equal to our current commercial Pyrus rootstock candidates.
- For the 2009-2010 sampling period [Sept 2009-April 2010], plants reached their peak hardiness levels in December 2009. Beginning in January, 2010 de-acclimation was evident as tissue injury was observed at higher temperatures.
- The cambial zone was observed to be more sensitive to cold injury throughout the measurement period, followed by xylem. Phloem developed the greatest hardiness in mid-winter.
- Sep 2010-January 2011 temperatures were warmer on average than those observed from Sep 2009-April 2010, and did not provide 'good' acclimation conditions. Importantly, mean temperatures for the period only fell below freezing (~28 °F) for two days (Nov 23-24, 2010), and minimum temperatures did reach values below 23 °F during that period. Despite poor acclimation conditions, plants acclimated similarly to 2009, and reached equivalent hardiness values in December. Importantly, we have excellent agreement for hardiness values of individual accessions between years, indicating repeatability.
- 30 quince genotypes that had been successfully propagated from soft-wood cuttings will be used to test compatibility using 'Bartlett' and 'd'Anjou' as scion.
- We have developed a DTA system to precisely identify low temperature freeze points of quince and pear tissue.

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) and shipped next-day to MCAREC. Tissue was sampled at ~three-four week intervals, beginning in late September. Sampling continued until bud-break (March-April), and is being repeated in year 2. 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, -4, -22, -40° F]), with the exception of the first sample period [Sep 2009], when samples were subjected to 0, -10, -25, and -40° C to account for a shortage of shoot material. Each of the four replicates was run on a separate date.
- 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, [(Fresh Weight - Dry Weight)/Fresh Weight] *100

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

*Objective 4:*We have used funding awarded from the USDA to design a precise system (termed differential thermal analysis [DTA]) for detection of plant kill points (acknowledgements to Dr. Markus Keller's lab group at WSU for providing invaluable insights into DTA system design, and Dr. David Gibeaut (OSU-MCAREC) for building the system and developing the protocol). Due to the robustness and previously determined precision of this technique we have substituted it for the electrolyte leakage studies that we originally proposed to develop. Briefly, tissue is harvested and shipped from Corvallis as described above, wrapped first in a piece of damp paper towel then aluminum foil and placed on thermal plates equipped with > 100 thermocouples. Tissue is subjected to identical freeze rates and temperature treatments as outlined above. Thermalplates are wired into a data logger and data are recorded at 30 second intervals. The change in temperature associated with freezing water (heat release), is detected as a voltage change and plotted against temperature. A thorough description of the system will be provided in the final report where more space is provided.

Results and Discussion

Objectives 1 and 3: Early fall 2010 temperatures recorded near the NCGR field site did not decline as quickly, nor did they attain the lows observed in 2009 (Fig 1). No frost events were recorded prior to November 21, 2010, as compared to the light frosts observed on October 6 and 12, 2009. Mean and minimum [24 °] temperatures were lowest on Nov 23-25, 2010, and were immediately followed by

unseasonably warm temperatures. A seasonal, gradual progression of declining minimum and mean temperatures followed by hard freeze events as observed in 2009 are conducive to cold acclimation, a process by which plants acquire hardiness through exposure to increasingly lower temperatures, albeit, in the Willamette Valley (NCGR) this process occurs later than in most pear growing regions of the PNW. 2010 early to late fall temperatures would be expected to equate to delayed onset and poor development of cold hardiness.

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 < 25 % browning] (Fig 2). Our estimates of minimum hardiness levels are made at ~25 % observable browning, a point that is highly debatable since anecdotal evidence from cutting wood following winter freeze events suggests that pear re-growth the following spring is not impaired at such light browning levels. Subsequently, our estimates of maximum hardiness are extremely conservative. Our first sampling date in September, 2009 indicates that a portion of the plants were capable of handling temperatures as low as -10 °C (14° F), prior to detection of injury (Fig 2, top left). Very little segregation occurs among accessions, since plants have not yet developed sufficient hardiness early in the season. Increasing hardiness can be seen with each subsequent sampling date for all accessions, reaching maximum levels in December (Fig 2). Over half of the accessions that were collected on December 7, 2009 (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 2, December) that were equal to, or hardier 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. Despite mild acclimation temperatures at the repository, cold hardiness tests this year (Sept-December, 2010) are in good agreement with 2009 results (data not shown due to space).

Following December sampling, maximum hardiness values decline (Fig 2), until hardiness is completely lost in April (not shown). Interestingly, two quince accessions had significant delays in bud break, and remained hardy to -10 °C on April 15, 2010. During the de-acclimation period it will be important to identify accessions which 'come out' of dormancy slowly.

As the season progressed, cambial tissue (meristematic tissue responsible for cellular division, lateral trunk growth and ultimately new xylem and phloem tissue) appeared to be consistently more sensitive to sub-freezing temperatures than either of the vascular tissues [i.e., phloem or xylem] (data not shown). At the maximum hardiness level [December] phloem tissue was hardier for 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.

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

Overall results are very encouraging. A large group of quince taxa exist with the apparent capacity to acclimate and attain sufficient levels of cold-hardiness for many regions of the PNW, and these data

are further supported from our 2011 November and December samples. Additionally, previous reports have suggested that full expression of hardiness is associated with exposure to temperatures below 14° F for several weeks (hashed line in Fig 1). Temperatures at the test orchards did not attain these values for any extended period of time, indicating that greater cold tolerance is entirely possible when planted in colder climates.

Objective 2: Roughly 300 rooted cuttings have been established from 31 quince genotypes (~half of the sample population) having an average of 8 rooted cuttings per clone. We will use a portion of these potted plants to test compatibility with 'd'Anjou' and 'Bartlett' this winter via chip budding. Following another season of growth these plants will be used in whole plant freeze studies.

Objective 4: Preliminary data from late December, 2010 samples is provided in Figure 4 for four genotypes: 1) 'OH x F 87', 2) *Pyrus pashia* (cold sensitive pear), 3) *Pyrus ussuriensis* (cold hardy pear) and 4) Quince 'Akhtubinskaya'. The first peak (occurring at ~ -5 °C) signifies the freezing of extracellular water in the tissue, and occurs similarly across genotypes. It is termed the high temperature exotherm and is a non-lethal, non-injurious event. The next series of peaks represent cellular freezing (low temperature exotherms), and these are highly associated with tissue death. The quince clone 'Akhtubinskaya' did not freeze until -38 °C, and can be seen to possess equivalent hardiness as *Pyrus ussuriensis* (Fig 4). The cold sensitivity of *Pyrus pashia* relative to other accessions can also be observed (Fig 4). In fact, *Pyrus pashia's* freeze point determined by DTA (-22 °C [-8 °F]) was in agreement with our oxidative browning analysis [rated a 3 at -10 °C, and a 5.5 at -20 °C], and this agreement appears to be the fairly uniform across the sample population. Interestingly, we have often observed multiple low temperature peaks, as can be seen for 'OH x F 87' in Figure 4, and hypothesize that these reflect freezing of individual tissue zones (i.e., xylem, cambium, phloem). We are presently aligning these data with our oxidative browning results.

Figures

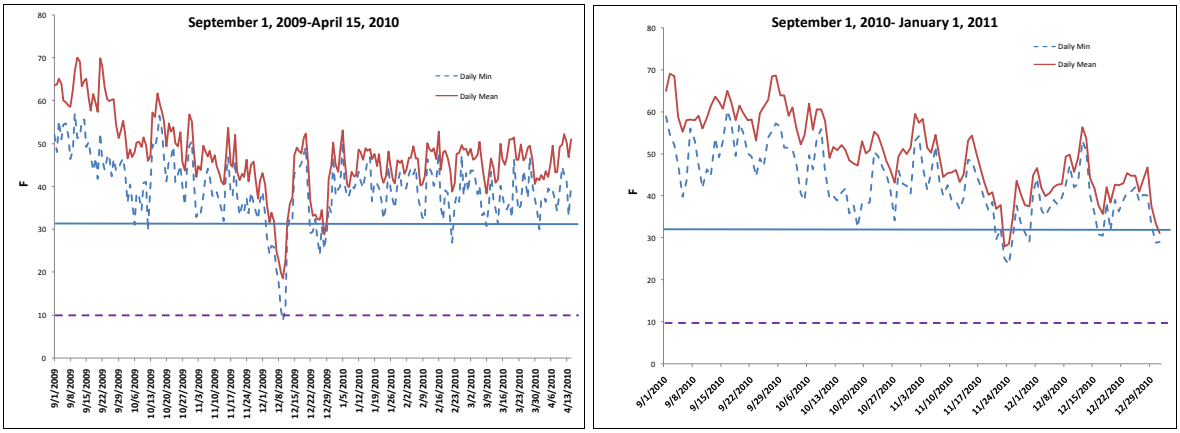


Figure 1. Daily mean and minimum temperatures (° F) from September 1, 2009 through April 15, 2010 (left), and September 1, 2010 through January 1, 2011 (right) , recorded at the Hyslop farm located ~ 6 miles N.E. of the NCGR quince site. Horizontal line signifies freezing (32° F). Hashed line represents previously published threshold temperature necessary for woody plants to attain maximum hardiness levels, if exposure persists for a multiple week period.

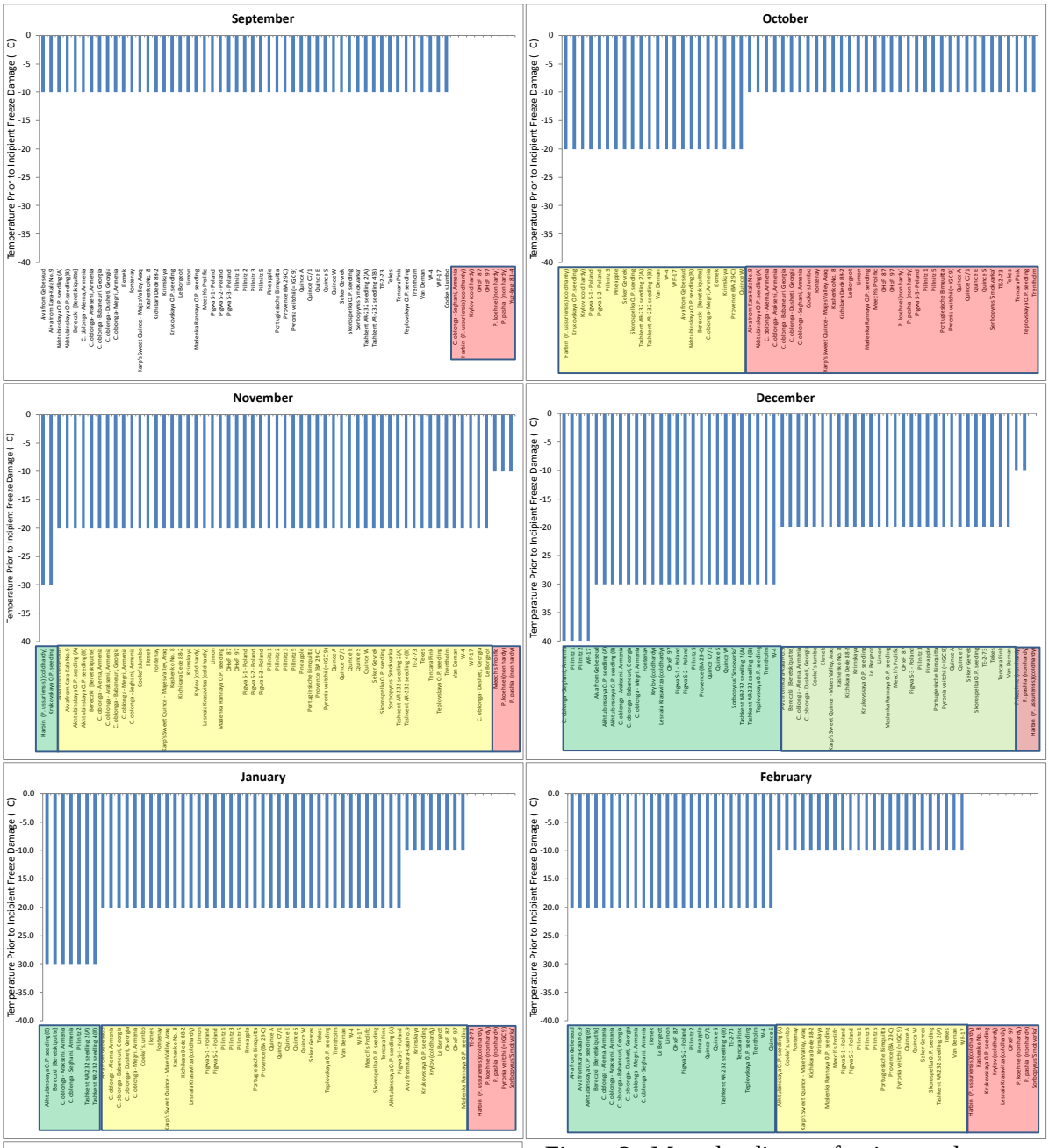


Figure 2. Mean hardiness of quince and pear accessions Sept, 2009-April 2010. Data are lowest temperature at which no injury was detected (see text). Where blue data bars are not apparent, tissue tolerance was 0° C [32 °F]. When applicable, accessions along the x-axis were grouped into three broad categories for a given sample period: 1) left [non-hardy], 2) center [mid to hardy], and 3) right [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.

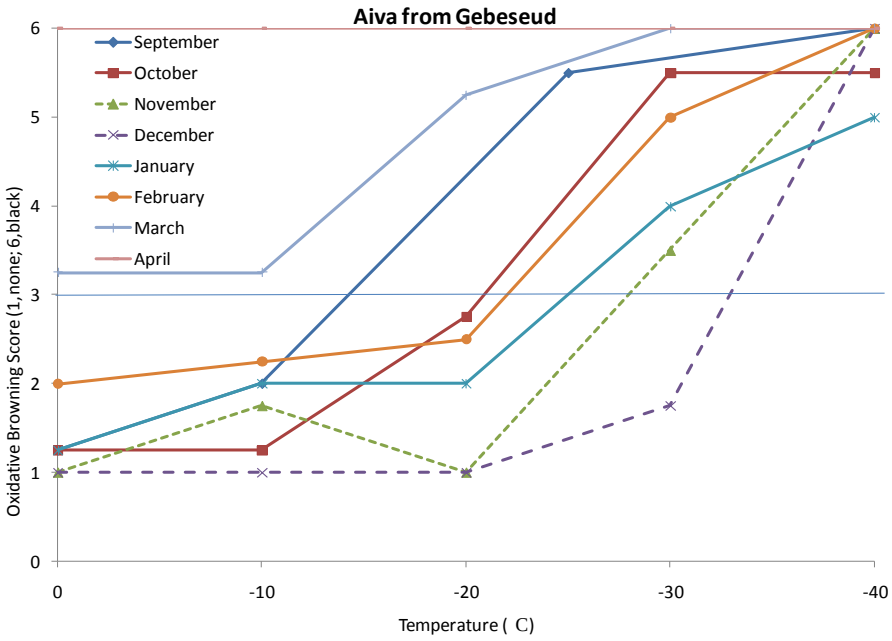


Figure 3. Oxidative browning rating of vascular tissue (where, 1, no damage [white]; 2, no damage [off-white]; 3, ~ 25 % area lightly browned; 4, ~ 50 % area browned; 5, >75 % area browned; 6, 100 % completely oxidized [black]) of the quince accession 'Aiva from Gebeseud' from Sep 2009-April 2010. The horizontal line signifies the threshold for injury (i.e., ~ 25 % area lightly browned).

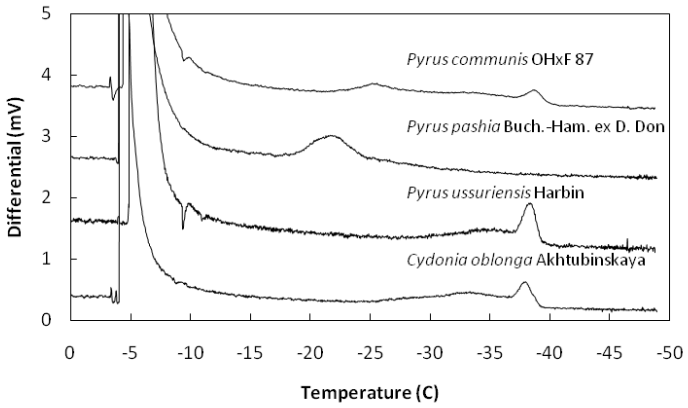


Figure 4. Differential thermal analysis graphs for 'OH x F 87', *Pyrus pashia* (cold sensitive pear), *Pyrus ussuriensis* (cold hardy pear) and Quince 'Akhtubinskaya'. Data lines are offset for clarity of presentation. The y-axis is voltage (temperature) differential and is plotted against temperature. Peaks represent heat released during freezing of water (see text for explanation).

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

Project Title: Horner rootstock grower evaluation trials

PI: Todd Einhorn
Organization: OSU-MCAREC
Telephone: (541) 386-2030 x13
Email: Todd.einhorn@oregonstate.edu
Address: 3005 Experiment Station Drive
City: Hood River
State/Zip: OR 97031

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

CO-PI: Steve Castagnoli
Organization: OSU
Telephone: 541-386-3343
Email: Steve.castagnoli@oregonstate.edu
Address: 2990 Experiment Station Drive
City: Hood River
State/Zip: OR 97031

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: $18,552**

Other funding sources: None

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

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

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

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

Item	2009	2010	2011
Salaries[1]			6,000
Benefits[1]			1,900
Wages[1]			2,300
Benefits			700
Equipment	See Schmidt Report	See Schmidt Report	
Supplies			
Travel[1]			1000
Miscellaneous			100
Total			**$12,000**

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

Objectives:

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

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

Significant Findings 2010:

- Of the five trial sites planted, four are performing well. A fifth site was inadvertently subjected to herbicide damage.
- The mortality rate for all sites was 6 %, but varied markedly among sites (e.g., range of 1 % to 12 %).
- Averaging across scion cultivars and sites, Horner 4 sustained the greatest rootstock mortality rate [10 %], Horner 10 was intermediate [7 %], and OHxF 87 had the fewest losses [3 %] (Table 1). Causes of individual tree losses varied according to site, and were inconsistent.
- 'Bosc' tree size in fall of 2010 was roughly equivalent for all rootstocks.
- Relative tree size for 'Bartlett' was only slightly, and non-significantly, affected by rootstock [relative tree size was Horner 10 < OHxF 87 < Horner 4].
- For 'd'Anjou', OHxF 87 and Horner 10 produced trees similar in size, and ~ 75-80 % the size of trees on Horner 4.
- Root suckering was not observed.

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 (Parkdale). Grower cooperators, researchers and technicians continued to collaborate 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, fall of 2010 and fall of 2011 [fall measurements were taken following leaf drop]. Relative annual 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:

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

1. 'd'Anjou'. Horner 4 produced a larger tree than either Horner 10 or OHxF 87, irrespective of site (Fig 1, top), though the magnitude of this difference was influenced by site. Bridgeport was characterized as a low vigor site due to the presence of gravel bars within the soil profile, indicating that the higher vigor Horner 4 may indeed have early canopy establishment benefits on poor sites. In fact, Bridgeport Horner 4 trees were smaller than those on Horner 10 or OHxF 87 at both Oregon sites (Fig 1, top) however, herbicide injury confounded results at the Bridgeport site. The inherent vigor of Horner 4 can be seen at Bridgeport, even in the presence of herbicide damage (Fig 1, bottom). The growth rate of trunks at Hood River and Parkdale was adequate (60-80 % increase) and similar for the 2010 season, irrespective of rootstock (Fig 1, bottom). Trees entering the third leaf (2011) will offer a potential first measure of precocity.

   Mortality rates were quite high at the Hood River site (11 %). Eight individual tree losses were observed on Horner 10, and 2 on OHxF 87. Trees on both of these roots were

significantly smaller than those on Horner 4 at the end of year one, and it appeared that these 'weaker' trees succumbed to a combination of environmental factors and disease pressure. In addition, several trees on Horner 10 and OHxF 87 were observed to senesce early (i.e., reddening leaves in early fall). These observations were not observed at the two other 'd'Anjou' sites, indicating that site specific issues were likely responsible for the higher mortality rates of Horner 10. On the other hand, Horner 4 at Bridgeport experienced ~ 30 % mortality.

2. 'GR-Bosc'. Interestingly, unlike the results for 'd'Anjou', differences in tree size were not observed for the different rootstocks following year two in the field, and this was true for both sites (Fig 2, top). Similarly to 'd'Anjou', the Bridgeport trees suffered from the combination of a low vigor site, and herbicide damage, consequently trees were much smaller at Bridgeport. As was reported in year one, the relative growth rates at Wapato were nearly double those at Bridgeport (Fig 2, bottom). For a given site, Horner 4 appeared to have slightly lower growth rates than trees on the other rootstocks, though these differences were not significant (Fig 2, bottom).
3. 'Bartlett'. Trees at Wapato are slightly larger than those at Methow following year two (Fig 3, top). Growth rates were slightly higher at Methow (opposite of year one results), and subsequently Methow trees have somewhat 'caught up' to those at Wapato. Rootstocks do appear to be segregating showing slight differences in relative tree size [i.e., Horner 10 < OHxF 87 < Horner 4].

Decisions regarding tree training will continue to be a collaborative process as we proceed into year three. Measurements for 2011 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 flowering and early production in 2011, and will add the following measurements to those outlined above:

- 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

Tables:

Table 1. Mortality rates of rootstock clones. Data are sums of all sites and scions.

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

Figures:

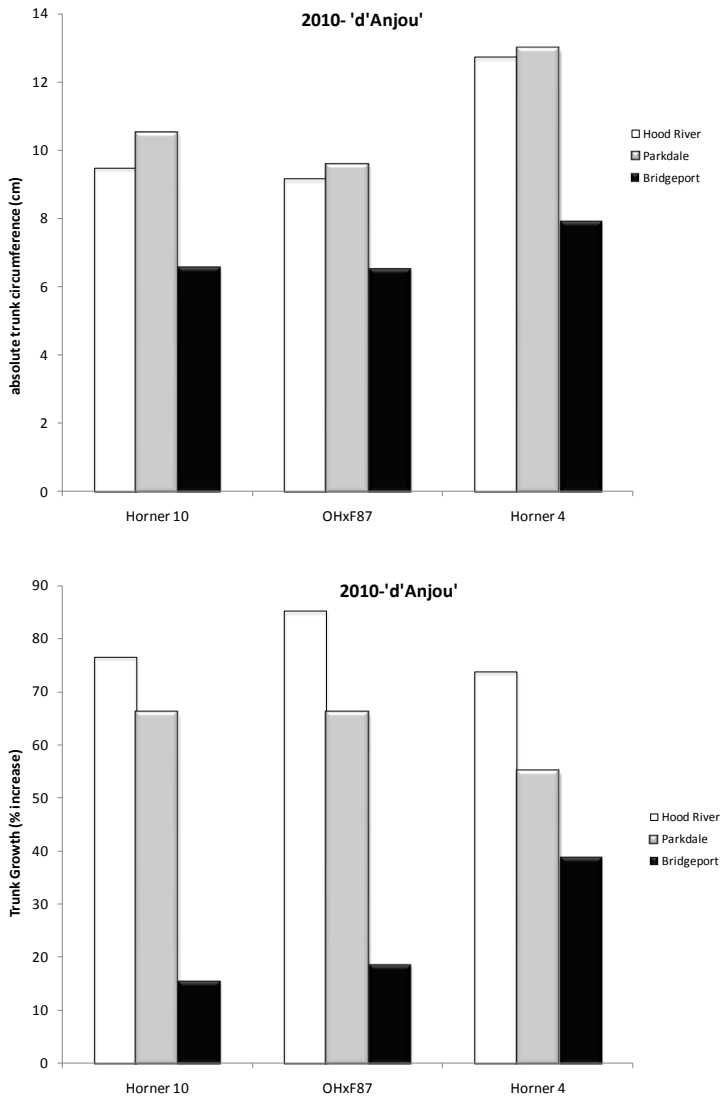
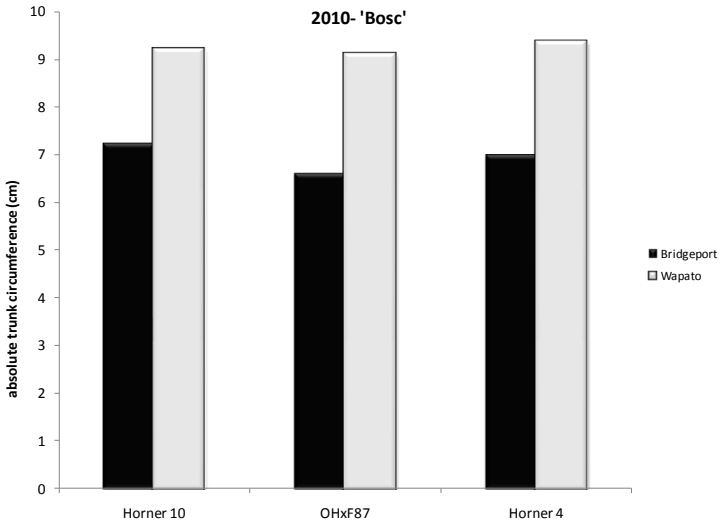


Figure 1. Trunk circumference (cm) following the 2010 growing season (top), and trunk growth as % increase from spring 2010 through fall 2010 (bottom), of 'd'Anjou' pear trees grafted on three different rootstocks, and at three different locations.



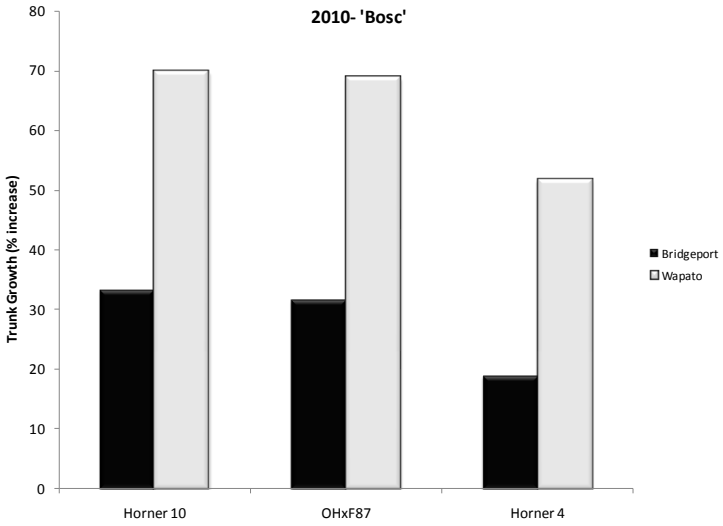


Figure 2. Trunk circumference (cm) following the 2010 growing season (top), and trunk growth as % increase from spring 2010 through fall 2010 (bottom), of 'GR Bosc' pear trees grafted on three different rootstocks, and at two different locations.

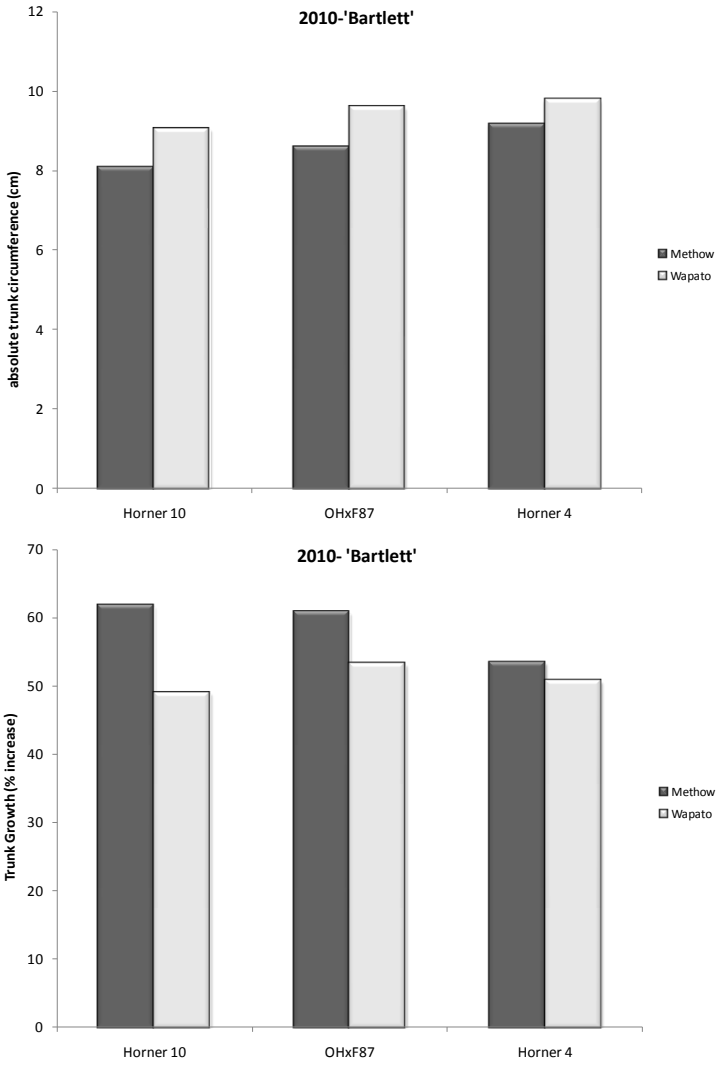


Figure 3. Trunk circumference (cm) following the 2010 growing season (top), and trunk growth as % increase from spring 2010 through fall 2010 (bottom), of 'Bartlett' pear trees grafted on three different rootstocks, and at two different locations.

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

Project Title: Pear rootstock breeding

PI: Kate Evans
Organization: WSU Tree Fruit Research and Extension Center
Telephone: 509-663-8181 x245
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee/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 Request: Year 1: $4,500 **Year 2:** $12,300 **Year 3: $3,500**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Mary Lou Bricker and Kevin Larson
Telephone: 509-335-7667, 509-663-8181 x221 **Email:** mdesros@wsu.edu, kevin_larson@wsu.edu

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

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. Rootstock germplasm was selected at the pear collection in Corvallis and budded for the parental collection at Sunrise orchard.
2. Alternative sources of useful rootstock germplasm have been located, ready for budwood supply in summer 2011 for propagation of further parental trees.
3. Pear rootstocks and selection techniques have been highlighted as an area of possible study in a new SCRI rootstock proposal.

Methods:
1. A pear rootstock advisory committee made up of industry and research experts will provide input on the objectives, activities and future planning for a pear rootstock research project.
2. Use internet searches, literature and informed contacts to review wide-ranging pear germplasm to identify possible accessions for a new rootstock parental collection.
3. Access germplasm for propagation from collections and other breeding programs, arrange for importation and propagation at commercial nursery.
4. Meet with 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 & Discussions:
After reviewing the literature and cross-referencing with the USDA pear repository germplasm list, Dr. Evans visited with Dr. Joseph Postman in Corvallis, Oregon and selected a range of interesting rootstock parental material from the repository in July 2010.

Budwood was supplied in August to Willow Drive Nursery where the trees were propagated onto OHF 87 rootstock.

The parents selected include *Pyrus communis* 'Old Home', 'Farmingdale', OHF87 and 333 as well as other dwarf and compact *P. communis* scion varieties. Three of the Oregon series of 'P' fire blight resistant dwarf and semi-dwarf rootstocks were also accessed. A diverse collection of other *pyrus* species were also selected to include characteristics such as resistance to fire blight, tolerance to pear decline, resistance to *phytopthora*, resistance to woolly pear aphid, cold hardiness, ease of propagation and a range of different vigours. The parental collection will be planted in the WSU Sunrise orchard.

Further germplasm was assessed during a visit to WSU Puyallup in June. A large collection of interspecific *pyrus* hybrids, originally produced by Westwood, was established at the research and extension center in Puyallup for assessment and possible selection of urban ornamental trees by Dr. Rita Hummel. Dr. Hummel collected a considerable amount of potentially useful data on these trees which will enable the selection of a subset of further rootstock parental germplasm to be propagated in 2011.

Discussions are on-going regarding possible protocols for rootstock selection in seedlings. A visit to Stellenbosch, South Africa, in November 2010 provided the opportunity to discuss selection strategies with apple rootstock breeder Ken Tobutt. Pear rootstock breeding selection techniques have also been raised as an area needing more research in the SCRI rootstock proposal currently being prepared by Dr. Gennaro Fazio. Other more generic areas of possible research include developing a greater understanding of the propagation of rootstocks, something that is particularly pertinent to pear stocks. A second SCRI proposal (Dr. Amit Dhingra) will include the development of some key selection tools for pear rootstocks, for example DNA markers for dwarfing.

CONTINUING PROJECT REPORT **YEAR**: 2 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: $19,900****

Other funding sources: None

WTFRC collaborative expenses: None

BUDGET
Organization Name: OSU Agric. Research Foundation **Contract Administrator:** Cynthia Cox
Telephone: (541) 737-4066 **Email address**: Cynthia.Cox@oregonstate.edu

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

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 shift of Obj. 4 to WTFRC Apple Crop Protection.
**Budget reduced from original proposal owing to near completion of objectives 1-3.

OBJECTIVES:

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

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

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

4) Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets (this objective was funded from apple sources, but results are applicable to pear).

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

SIGNIFICANT FINDINGS

- We continued to achieve outstanding control of fire blight with Kasumin (kasugamycin) and with mixtures of Kasumin and Mycoshield (e.g., 80 ppm of each material).

- We continued to 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.

- For a second season, the not-yet-registered yeast material, Blossom Protect, provided excellent control of fire blight.

- 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.

- In the field, a combination of a foliar spray and soil drench of a SAR material (acibenzolar-*S* methyl) significantly reduced expansion of fire blight cankers in young Bosc pear.

METHODS:

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

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.

Field study. Kasumin 10L (kasugamycin 10% a. i., Arysta LifeScience North America, Cary, NC) was evaluated for control of fire blight in a 'Gala' apple orchard (11-yr-old) located at the OSU Botany and Plant Pathology Field Laboratory near Corvallis, OR. The experiment was arranged in a randomized, complete block design with 4 replications and 14 treatments applied to single tree plots (Table 1). In addition, a commercial formulation of the biological agent, *Pantoea agglomerans* C9-1S (BlightBan C9-1, NuFarm Americas, Burr Ridge, IL) was included as a component of some treatment combinations. Similarly, a kasugamycin-resistant selection of *P. agglomerans* C9-1S (designated C9-1^{Kr}) also was evaluated as a component of some treatment combinations. C9-1^{Kr} was

obtained in the laboratory by stepwise selection on increasingly higher rates of kasugamycin over a period of several weeks. Treatments were applied t during early morning at 30% bloom (water and *P. agglomerans* treatments), 70% bloom (all treatments), and full bloom (antibiotics). Treatment suspensions were sprayed to near runoff with a backpack sprayer equipped with a hand wand (~3 liters per tree). At full bloom a motorized, 25-gallon tank sprayer equipped with a hand wand was used to fog a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin-sensitive strain prepared at 5 x 10^5 CFU per ml) onto each tree (2 liters per tree). Establishment and population sizes of C9-1S and C9-1^{Kr} on flowers oversprayed with antibiotics were measured periodically during the bloom period by dilution plating of washes of the flowers onto culture media. Symptoms of fire blight were first observed in mid-May. Incidence of fire blight was determined by counting (and cutting) the number of blighted flower clusters (i.e. strikes) on each tree during detailed inspections made in late May and early June. Total number of blighted flower clusters per tree (log-transformed) and incidence of blighted floral clusters (diseased clusters divided by total clusters, arcsine-square root transformed) were subjected to analysis of variance.

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

Materials for organic suppression of fire blight were evaluated in a 'Golden Delicious' apple orchard (30-yr-old) located at the OSU Botany and Plant Pathology Field Laboratory near Corvallis, OR. The experiment was arranged in a randomized, complete block design with 4 replications and 12 treatments applied to single tree plots. Materials evaluated were chosen on the expectation they would meet potentially revised 2013 National Organic Program guidelines, and were tested in various combinations and treatment timings (Table 2). Treatment suspensions were sprayed to near runoff with a backpack sprayer equipped with a hand wand (3 liters per tree). Inoculation with the pathogen, measurement of fire blight, and statistical analysis were performed as described under objective 1.

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

Greenhouse study. Following the results obtained in 2009, we set up to repeat and expand the replicated greenhouse drench experiments with one-year-old potted Bosc Pear trees. However, 300 Bosc pear trees we received from a nursery were of such poor quality that they were unusable for experimentation. Moreover, in early June, the nursery replaced the first set trees, but tree quality was unimproved in second shipment. Apparently all trees we received had been freeze-damage shortly after being dug in fall 2009.

F**ield study.** One hundred Bosc pear trees (planted 2009) were inoculated on May 14 by cutting the terminal shoot with a razor blade; cells of the fire blight pathogen (10^9 colony forming units per ml) were painted onto the cut surfaces. A plastic bag was wrapped over the cut end and left in place for one week. As in the pear orchard trials, a very low rate of fire blight developed in the trees, perhaps due to cold nights after inoculation. The trees were re-inoculated on June 9 with a high rate of inoculation success. An experiment with five SAR-inducing treatments (including control) was arranged onto the infected trees. Single-tree plots of each treatment were replicated 14 to 28 times in an RCB design. In one treatment, the SAR-inducing material acibenzolar-S-methyl (ASM; Actigard 50WG, Syngenta Crop Protection) was drenched onto soil (75 mg a.i. per plant); in another treatment, ASM was applied as applied as a truck paint (15 g a.i./L in 2% PentraBark), and in the 3rd and 4th treatments, a foliar spray (0.25 g a.i./L) was applied to trees that had also been drenched or painted. ASM treatments were applied June 23, which was two weeks after the pathogen inoculation. Length of fire blight cankers on inoculated trees was measured July 27, which coincided with a slowing of new growth in the trees.

RESULTS

1) Integration of a new material, Kasumin, into blossom blight control programs for conventional orchards. The 2010 orchard experiment in Bartlett pear failed because of extreme cold weather during bloom (average high and low from march 28 to April 16 were 54ºF and 38Fº, respectively). A successful experiment was completed in apple, where the trees in the study averaged 965 flower clusters. Fire blight risk, as determined by COUGARBLIGHT, was low during the bloom with light precipitation occurring on 3 days between 80% bloom and petal fall. Nonetheless, disease intensity was high with water treated trees averaging 236 blighted clusters per tree (26%). Each treatment significantly reduced ($P < 0.05$) incidence of infection and total number of infected flower clusters per tree. Kasumin and the mixtures of Kasumin and Fireline provided excellent disease control. The integrated program of *P. agglomerans* C9-1S or C9-1^{Kr} in early bloom followed by Kasumin, or Kasumin and Fireline at full bloom also provided excellent control. Within integrated *P. agglomerans*/Kasumin programs, however, treatment with the kasugamycin-resistant strain C9-1^{Kr} did not enhance control relative to treatment with the kasugamycin-sensitive parental strain, C9-1S. Integrated programs and mixtures were evaluated as resistance management strategies, with the goal of providing effective disease control combined with a reduced likelihood of kasugamycin-resistance developing in the pathogen.

Table 1.

Treatment	Rate per 100 gallons water	Date treatment applied* 17 April 30% bloom	19 April 70% bloom	23 April Full bloom	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Water control	------	**X**[§]	**X**	**X**	**236**	**a**[#]	**26.0**	**a**[#]
Fireline 200 ppm	16 oz.	---	---	X	27	b	2.6	b
BlightBan C9-1	*10^8 CFU/ml*	X	X	---				
then Fireline 200 ppm	16 oz.	---	---	X	24	b	2.3	b
Kasumin 10L 100 ppm	12.8 fl. oz.	---	---	X	21	bc	2.7	b
Pantoea agglomerans C9-1^{Kr}	*10^8 CFU/ml*	X	X	---				
then Fireline 200 ppm	16 oz.	---	---	X	21	bc	2.3	b
Pantoea agglomerans C9-1^{Kr}	10^8 CFU/ml	X	X	---				
then Kasumin 10L 80 ppm	10.4 fl oz.	---	---	X				
with Fireline 80 ppm	6.4 oz.	---	---	X	19	bcd	2.1	bc
BlightBan C9-1	*10^8 CFU/ml*	X	X	---				
then Kasumin 10L 100 ppm	64 fl. oz.	---	---	X	18	bcd	2.0	bc
Kasumin 10L 80 ppm	10.8 fl. oz.	---	X	X				
with Fireline 80 ppm	6.4 oz.	---	X	X	17	bcd	1.8	bc
Kasumin 10L 100 ppm	12.8 fl. oz.	---	X	X				
with Fireline 100 ppm	8 oz.	---	X	X	16	bcd	1.7	bc
Pantoea agglomerans C9-1^{Kr}	*10^8 CFU/ml*	X	X	---				
then Kasumin 10L 100 ppm	12.8 fl. oz.	---	---	X	16	bcd	1.4	bc
Kasumin 2L 100 ppm	64 fl. oz.	---	X	X	14	cd	1.6	bc
FireWall 100 ppm	8 oz.	---	X	X	13	cd	1.4	bc
Kasumin 10L 80 ppm	10.4 fl. oz.	---	---	X				
plus Fireline 80 ppm	6.4 oz.	---	---	X	11	d	1.3	c
Kasumin 10L 100 ppm	12.8 fl. oz.	---	X	X	11	d	1.1	c

* Trees inoculated on 21 April 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 $P = 0.05$.

Integrated biological and chemical control. Evaluation of integrated control with *Pantoea agglomerans* as the biological component and Kasumin as the chemical component continued to show little interference of the Kasumin overspray on the establishment of antagonist populations on flowers. This result was observed with both the Kasumin-sensitive and -resistance strains of *P. agglomerans* (sensitive = strain C9-1S; resistant = strain C9-1^{Kr}). For example, in the 2010 'Gala' apple experiment, during full bloom, incidence of recovery for both C9-1S and C9-1^{Kr} averaged 80-95% of sampled flowers, and the population size of these antagonists on flowers from which it could be recovered ranged from 10^4 to 10^5 CFU per flower (Fig. 1). (Note: this is in contrast to pathogen populations, which are strongly suppressed by the Kasumi overspray [data not shown]). The results indicate that non-target effects of Kasumin on sensitive *Pantoea agglomerans* are relatively small, and that biological products (Bloomtime Biological, BlightBan C9-1) can be used in integrated fire blight control programs that include Kasumin.

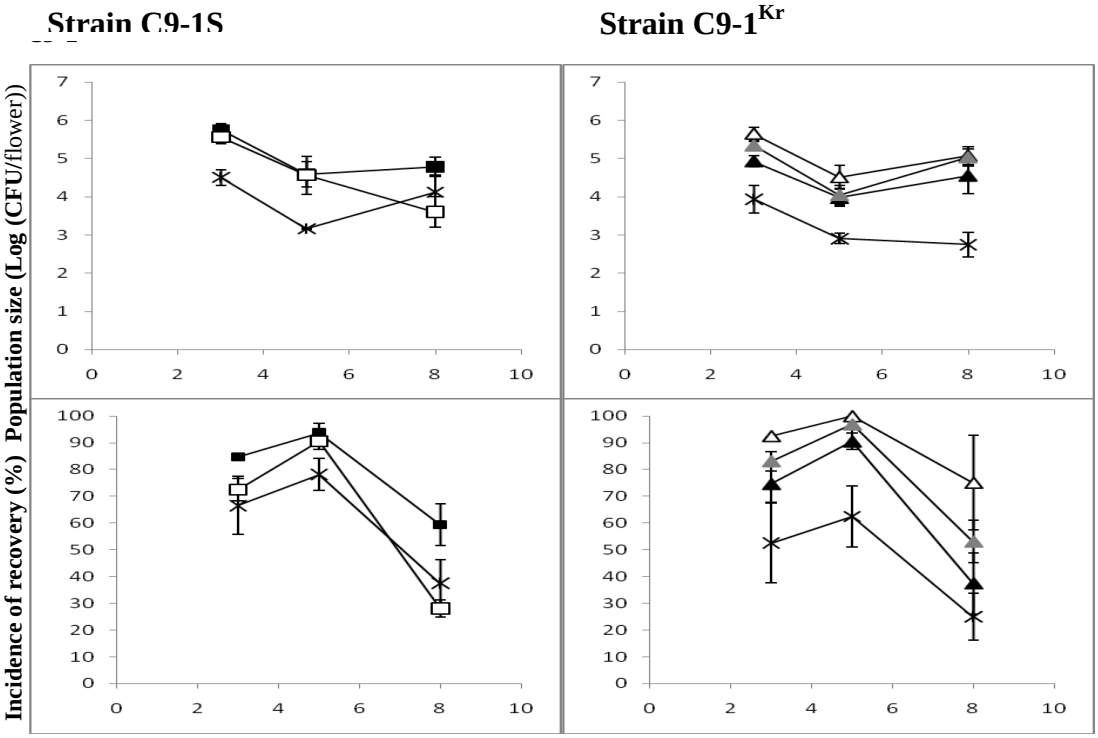


Fig. 1. Recovery of spray applied *Pantoea agglomerans* strains C9-1S and C9-1^{Kr} from flowers of Gala apple in 2010: populations of C9-1S oversprayed with oxytetracycline 200ppm (■), C9-1S oversprayed with Kasumin 100ppm (□), C9-1^{Kr} oversprayed with oxytetracycline 200ppm (▲), C9-1^{Kr} with oversprayed with Kasumin 100ppm (Δ) and C9-1^{Kr} oversprayed with oxytetracycline 80ppm and Kasumin 80ppm (▲). ∗ indicates data for combined *Pantoea* strains on flowers from the untreated control.

4) Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets. The 2010 orchard experiment in Bartlett pear failed because of extreme cold weather during bloom. A successful experiment was completed in apple, where the trees in the study averaged 780 flower clusters. Fire blight risk, as determined by COUGARBLIGHT, measured 'low' during the bloom period with light precipitation occurring on 3 days between 80% bloom and petal fall. Nonetheless, disease intensity was high with water-treated trees averaging 196 blighted clusters per tree (24%). Most treatment programs were designed to integrate the floral stigma-colonizing *Pantoea agglomerans* in Bloomtime Biological (which slows epiphytic build-up of pathogen populations) with materials hypothesized to provide better protection in the nectary where infection

occurs (Blossom Protect, Serenade Max and FireLine). All treatment programs provided significant suppression ($P < 0.05$) of floral infection with the exceptions of Actinovate, Rex Lime Sulfur mixed with Crocker's Fish Oil (only), and the single application Bloomtime Biological followed by one application of Serenade Max. Two applications of FireLine (streptomycin sulfate) provided 95% control relative to the water-treated check. Programs finishing with Blossom Protect at the full bloom and petal fall timings also provided > 90% control.

Table 2.

Treatment	Rate per 100 gallons water	Bloom stage treatment applied* 10% bloom	30% bloom	70% bloom	Full bloom	Petal fall	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Actinovate	3 oz.	X[§]	X	X	X	---	322	a[#]	38.6	a
Water control	------	---	**X**	**X**	**X**	---	**196**	**a**	**24.2**	**b**
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---	125	ab	17.5	b
[Bloomtime Biological	5.3 oz.	---	---	X	---	---				
then Serenade Max [##]	64 oz.	---	---	---	X	---	125	abc	15.5	bc
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---				
then Bloomtime Biological	5.3 oz.	---	---	---	X	---				
then Serenade Max	64 oz.	---	---	---	---	X	66	bcd	8.6	cd
[Bloomtime Biological	5.3 oz.	---	X	X	---	---				
then Serenade Max	64 oz.	---	---	---	X	X	54	cd	7.7	cd
[Bloomtime Biological	5.3 oz.	---	---	X	---	---				
then FireLine 200 ppm	16 oz.	---	---	---	X	---	35	de	4.4	de
[Bloomtime Biological	5.3 oz.	---	X	X	---	---				
plus/then Blossom Protect	1.34 lbs.	---	---	X	X	---				
with buffer A	9.35 lbs.	---	---	X	X	---				
then Serenade Max	64 oz.	---	---	---	X	X	31	def	4.2	de
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---				
then Bloomtime Biological	5.3 oz.	---	---	---	X	---				
plus/then Blossom Protect	1.34 lbs.	---	---	---	X	X				
with buffer A	9.35 lbs.	---	---	---	X	X				
then Serenade Max	64 oz.	---	---	---	---	X	17	ef	1.8	e
[Bloomtime Biological	5.3 oz.	---	X	X	---	---				
then Blossom Protect	1.34 lbs.	---	---	---	X	X				
with buffer A	9.35 lbs.	---	---	---	X	X	15	efg	2.1	e
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---				
then Blossom Protect	1.34 lbs.	---	---	---	X	X				
with buffer A	9.35 lbs	---	---	---	X	X	12	fg	1.5	e
[FireWall 100 ppm	8 oz.	---	---	X	X	---	9	g	1.3	e

Footnotes as in Table 1.
[= Bracketed treatments show results most applicable to pear (i.e., without bloom thinning).

Obj. 5) Evaluate use of soil drenches of systemic acquired resistance inducers as a fire blight management tool in diseased pear trees. The drench treatment combined with a foliar spray of ASM (acibenzolar-*S* methyl) significantly slowed expansion of fire blight canker in 2-yr-old Bosc pear. The final sizes of cankers in the drench/spray treatment were 33% smaller than in the untreated control (Table 3). Cankers on all trees stopped expanding in mid/late-July, which coincided with an overall slowing of new shoot growth.

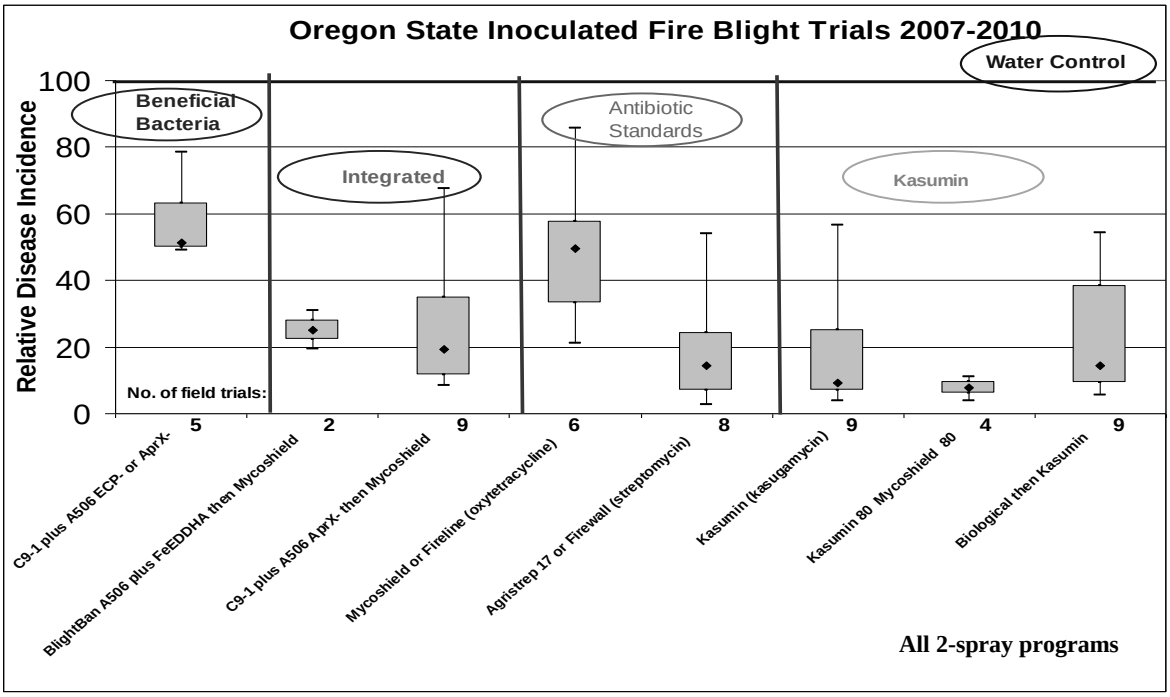
Table 3. Effect of ASM treatment on length of fire blight cakers on one-yr-old Bosc pear near Corvallis, OR in 2010.

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

[#] Standard error of the mean

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

Discussion: The chart below summarizes our results with Kasumin over the last 4 years. In addition to our results, others (Adaskaveg UC Riverside; Sundin Michigan State) also have ~4 years of efficacy trials with this material, and thus we believe it stands a reasonable chance for a section 3 registration with EPA; this application has been submitted is on track to be issued in spring 2012. **Note: the timing of the comment period for the proposed Kasumin label during is expected to be ~August 2011.** Some proposed label restrictions are more restrictive than prior labels for antibiotic materials (e.g., no alternate row spraying). Our research (and of others: e.g., Sundin, Michigan State) indicates some potential for *E. amylovora* to become resistant to Kasumin; this risk is likely intermediate to that observed with streptomycin (high) and oxytetracycline (low).



Plans for 2011. We are mostly retiring the objectives related to Kasumin as it is on track for a 2012 EPA registration; although we will include a few Kasumin (alone, mixture, integrated) as control standards in 2011orchards trials. The focus in the coming season will be on induction systemic acquired resistance with ASM to stop fire blight canker expansion in diseased trees. We will repeat greenhouse studies with 1-yr-old trees, and conduct field trials with a 3rd leaf planting of Bosc pear, and a an 11-yr-old orchard of Bartlett pear.

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

Project Title: Pear crop load management and rootstock field testing

PI:	Tory Schmidt	**Co-PI (2):**	Tom Auvil
Organization:	WTFRC	**Organization:**	WTFRC
Telephone:	(509) 665-8271	**Telephone:**	(509) 665-8271
Email:	tory@treefruitresearch.com	**Email:**	auvil@treefruitresearch.com
Address:	1719 Springwater Ave.	**Address:**	1719 Springwater Ave.
City:	Wenatchee	**City:**	Wenatchee
State/Zip:	WA 98801	**State/Zip:**	WA 98801

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

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

Other funding sources

All chemicals donated by companies
$3000 from Valent to support fruit set trials

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

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

Footnotes: 2011 expenses related to Einhorn Horner evaluation project have been removed from this budget
*Note: original budget total for 2010 was $26,000; current figure has been revised to reflect contributions to project from Valent

Objectives:

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

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

Significant findings:

- ATS applied during bloom and BA applied at 10 mm fruitlet size can provide effective thinning of Bartlett and Anjou pears; combined programs provide the best results
- Reduced rates of thinning materials were not sufficient to prevent Anjou pears from over-thinning
- Inclusion of neither spray oil nor carbaryl with BA application showed no clear benefits in initial testing
- Application of AVG, GA_3, GA_{4+7}, and BA + GA_{4+7} did not improve fruit set of Anjou or Red Anjou in 3 trials
- BA frequently improved harvest fruit size across chemical thinning and fruit set trials
- Budget and details for Horner rootstock evaluation in Einhorn's report

Methods:

Chemical thinning: We established chemical thinning trials in two mature solid Bartlett blocks and one mature Anjou block in 2010 that were applied by WTFRC staff with an AccuTech spayer. Trials were designed as randomized complete blocks with plot size ranging from 6-7 trees per plot. Initial bloom counts were recorded on tagged sample branches in each plot. All trials were successfully treated at appropriate timings at 100 gal water/acre (Table 1). Fruit set counts were made on sample branches after June drop and before green fruit hand thinning. Representative fruit from each plot were sampled within a few days of commercial harvest and evaluated in the WTFRC lab for size, firmness, sugar levels, acidity, and fruit finish.

Fruit set: Trials were established in two Anjou and one Red Anjou blocks with histories of poor fruit set. Growth regulators were applied by WTFRC staff at 100 gal water/acre with our AccuTech sprayer at timings and concentrations recommended by research staff of the chemical manufacturer (Table 5). Trials were designed as randomized complete blocks with 6-7 trees per plot. Initial bloom counts were recorded on tagged sample branches in each plot. Fruit set counts were made on sample branches after June drop. Representative fruit from each plot were sampled within a few days of commercial harvest and evaluated in the WTFRC lab for size, firmness, sugar levels, acidity, and fruit finish.

Table 1. Pear chemical thinning programs in 2010 WTFRC trials.

Material	Concentration	Timing(s)
ATS	Anjou: 4%; Bartlett: 5%	20% & 80% bloom
BA (MaxCel, Exilis Plus, Genesis 6-BA)	Anjou: 96 oz/A; Bartlett:128 oz/A	10 mm
BA + carbaryl	128 oz + 64 oz/A	10 mm
BA + Superior oil	128 oz/A + 1%	10 mm

Results and discussion:

Chemical thinning: We continue to see good performance with ATS as a bloom thinner and BA products (MaxCel, Exilis Plus, Genesis 6-BA) applied around 10 mm fruitlet size. Both materials can reduce fruit set and increase fruit size as stand-alone programs, but results are clearly improved when they are used in tandem (Tables 2, 3). Chemical thinning programs can often be confounded by poor weather or imprecise application timings and we generally find it advantageous to make multiple applications using different materials for the best chance for success.

The Monitor trial was established in a 30+ year old Bartlett block which the grower-cooperator and his field consultant believed would be less responsive to thinners than younger blocks planted at higher density. Fruit set was in fact reduced by the combination of ATS and BA (Table 2), but corresponding increases in fruit size were too subtle to be statistically significant.

As in 2009, our Rock Island cooperator wanted to evaluate very aggressive thinning treatments. As such, we tried mixing either superior oil or carbaryl (Sevin) with BA for postbloom thinning; all treatments significantly reduced fruit set and demonstrated positive effects on fruit size (Table 2). The addition of carbaryl to the postbloom spray tank neither improved nor harmed the performance of BA by itself. As we observed in 2009, the addition of oil to BA slightly improved thinning, but did not help fruit size as much as BA alone; even though these effects were not statistically significant in 2010, the trend once again suggests that even though use of oils as chemical thinners may help reduce fruit set, they may also stress trees sufficiently to hurt fruit size. No treatment in any trial significantly affected fruit finish (data not shown).

Table 2. Crop load effects of bloom (ATS) and postbloom (BA, oil, carbaryl) chemical thinning programs on Bartlett pears. WTFRC 2010.

Trial	Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
			%	%	g	
Bartlett/Seedling	ATS	81 ab	44 ab	36 ns	225 ns	89
- Monitor	ATS; BA	69 b	49 a	35	225	89
	BA	78 ab	47 a	32	215	93
	Control	90 a	38 b	38	216	92
Bartlett/OHxF.97	ATS; BA	37 b	72 a	20 ns	221 ab	90
- Rock Island	ATS; BA + carbaryl	39 b	70 a	23	222 a	90
	ATS; BA + oil	28 b	76 a	20	217 ab	92
	Control	68 a	57 b	25	207 b	97

Even with less aggressive chemical rates than in Bartlett (Table 1), both BA and the tandem of ATS and BA over-thinned our Anjou trial plots in 2010 (Table 3). These results reflect the conundrum of

crop load management in Anjou (and to a lesser degree, Bosc): while improved fruit size is desirable and achievable, chemical thinning programs typically reduce total yield too much to be considered profitable for growers. For this reason, we have increased efforts to explore programs that allow use of BA to increase fruit size while still preserving or improving yields of weak-setting pear varieties.

Table 3. Crop load effects of bloom (ATS) and postbloom (BA) chemical thinning programs on Anjou pears. WTFRC 2010.

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

Significant reductions in fruit set in all three of our 2010 trials might give the impression that these programs are more consistent than they actually are. As with most horticultural research, 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 2003-2010.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	9 / 31 (29%)	5 / 30 (17%)	3 / 25 (12%)
Urea	1 / 17 (6%)	3 / 17 (18%)	0 / 15 (0%)
Crocker's Fish Oil + lime sulfur	0 / 13 (0%)	1 / 13 (8%)	1 / 12 (8%)
Lime sulfur	1 / 13 (8%)	3 / 13 (23%)	0 / 13 (0%)
BA	4 / 19 (21%)	9 / 17 (53%)	3 / 27 (11%)
NAA	0 / 6	0 / 6	0 / 1

[1]Does not include data from 2010 trials.

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

Fruit set: We initiated work in 2009 to explore improvement of fruit set of Anjou pears with the use of AVG (ReTain) which is known to disrupt ethylene signaling in fruit. Our treatment was not successful in that initial trial, but we were hopeful that broader testing in 2010 would produce better results. After consulting with several scientists, we learned that European pear industries are known to apply a host of bioregulators, primarily gibberellins, following spring frosts to minimize fruitlet or flower abortion. As such, we developed protocols to assay several commercial plant growth regulators containing gibberellic acid (GA), as well as trying AVG again. In two of the three fruit set

trials, we overlaid applications of BA in factorial fashion in an effort to achieve our ultimate goal: improve fruit size without significantly reducing fruit set.

While BA consistently improved harvest fruit weights, it also consistently reduced fruit set (Table 5). None of our other materials were able to offset the thinning effect in either trial, in fact reducing fruit set by themselves in some cases; even though those effects were never statistically significant, they often reflected 20-30% reductions in fruit set (Cashmere Red Anjou and Monitor Anjou) which would represent meaningful losses in yield to the growers.

Despite these setbacks, we plan to explore these or similar programs for one more season. It is possible that our results were confounded by the vagaries of 2010's cold, wet, protracted bloom period and we feel that the potential benefits of developing programs like this are worth the additional effort to proceed with this line of research for another season.

Table 5. Crop load effects of PGR programs with and without BA applications (96 oz/acre at 10mm fruitlet size). WTFRC 2010.

PGR material/acre	Application timing(s)	BA application	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size
				%	%	g	
Anjou/unknown - Dryden							
10 g GA_3	20 & 80% bloom		62 ab	60 bcd	24 ab	214 bcd	93
10 g GA_3	20 & 80% bloom	Y	30 c	76 ab	18 ab	224 abc	89
6 oz BA + GA_{4+7}	20 & 80% bloom		57 abc	61 bcd	25 a	205 def	97
6 oz BA + GA_{4+7}	20 & 80% bloom	Y	34 bc	73 ab	21 ab	223 abc	90
32 g GA_{4+7}	20 & 80% bloom		71 a	52 d	29 a	195 f	102
32 g GA_{4+7}	20 & 80% bloom	Y	27 c	81 a	12 b	232 a	86
333 g AVG	80% bloom		65 a	57 cd	27 a	197 ef	101
333 g AVG	80% bloom	Y	32 bc	74 abc	21 ab	213 bcd	94
BA control	NA	Y	43 abc	69 abcd	21 ab	226 ab	88
Untreated control	NA		55 abc	62 bcd	25 a	211 cde	95
Red Anjou/OHxF.97 - Cashmere							
10 g GA_3	20 & 80% bloom		15 ab	87 ab	12 ab	233 ab	86
10 g GA_3	20 & 80% bloom	Y	12 b	89 a	11 b	242 a	83
6 oz BA + GA_{4+7}	20 & 80% bloom		19 ab	83 ab	15 ab	222 b	90
6 oz BA + GA_{4+7}	20 & 80% bloom	Y	15 ab	86 ab	13 ab	218 b	92
333 g AVG	80% bloom		19 ab	83 ab	16 ab	223 ab	90
333 g AVG	80% bloom	Y	14 ab	87 ab	12 ab	226 ab	88
BA control	NA	Y	17 ab	85 ab	13 ab	226 ab	88
Untreated control	NA		24 a	79 b	19 a	229 ab	87
Anjou/OHxF.97 - Monitor							
10 g GA_3	20 & 80% bloom		65 ns	61 ns	20 ns	260 ab	77
6 oz BA + GA_{4+7}	20 & 80% bloom		57	61	25	255 b	78
32 g GA_{4+7}	20 & 80% bloom		58	61	25	255 ab	78
333 g AVG	80% bloom		57	66	19	268 a	75
Control			75	56	22	256 ab	78

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

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

PI: Christelle Guédot
Organization: USDA-ARS
Telephone: 509-454-4446
Email: christelle.guedot@ars.usda.gov
Address: 5230 Konnowac Pass Road
City/State/Zip: Wapato/WA/98951

Co-PI (2): David Horton
Organization: USDA-ARS
Telephone: 509-454-5639
Email: david.horton@ars.usda.gov
Address: 5230 Konnowac Pass Road
City/State/Zip: Wapato/WA/98951

Co-PI(3): Peter Landolt
Organization: USDA-ARS
Telephone: 509-454-6570
Email: peter.landolt@ars.usda.gov
Address: 5230 Konnowac Pass Road
City/State/Zip: Wapato/WA/98951

Cooperators: Jocelyn Millar, University of California, Riverside

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

Other funding sources

Agency Name: Binational Agricultural Research and Development (BARD)
Amt. awarded: $273,000 (FY 2008-2010); $103,000 came to Wapato lab.
Notes: BARD provided partial funding support for C. Guédot; award terminated September 2010

WTFRC Collaborative expenses: None

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

Item	2010	2011
Salaries[1]	15,500	15,500
Benefits	4,500	4,500
Wages		
Benefits		
Equipment		
Supplies		
Travel		
Miscellaneous		
Total	$20,000	**$20,000**

Footnotes: [1] Partial support for GS-6 technician; benefits at 30% (funds are for same technician supported previously on this project)

OBJECTIVES

Our original objectives were:

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

Objective 1 has been addressed in 2010 and will be readdressed in 2011 to confirm the trends observed in 2010. Objective 2 will be addressed in 2011 and objective 3 was addressed and completed in 2010.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- Submitted proposal to BARD for continuation of funding: "Optimization and field-testing of synthetic sex attractants for two psyllid pests of pears"; $319,000 for 3 years (decision in May 2011).
- Determined that there is a seasonality in attraction of winterform males to 13-MeC27 both in the field and in laboratory bioassays which seems to coincide with females reaching reproductive maturity and being mated in the field.
- Identified 3 chemicals that were predominant in summerform female extract compared to male extract (13-methylheptacosane, 13-MeC27; 2-methylheptacosane, 2-MeC27; and 3-methylheptacosane, 3-MeC27).
- Demonstrated attraction of males but not females to 13-MeC27 and to a blend of 13-MeC27+2-MeC27+3-MeC27.
- Demonstrated that summerform males are as attracted to 13-MeC27 as to blend of chemicals, and that 13-MeC27 and the blend are as attractive to males as an extract of females.
- Demonstrated in the field that summerform males are attracted to traps baited with 13-MeC27 and to traps baited with the blend of chemicals compared to control traps.
- Demonstrated in the field that summerform females are not attracted by either 13-MeC27 or the blend of chemicals in either laboratory or field assays.

METHODS

Seasonality of attractiveness of 13-methylheptacosane. 13-methylheptacosane was loaded into gray rubber septa and pinned to the center of sticky traps composed of sections of nylon mesh and covered with tanglefoot. Simultaneously, psylla were collected from the orchard and immediately assayed in the olfactometer. The seasonality of attractiveness was assessed from end of January to early April.

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

RESULTS AND DISCUSSION

Seasonality of attractiveness of 13-methylheptacosane. Attraction by winterform males to synthetic 13-MeC27 occurred from early to late February and was consistent between laboratory (**Figure 1**; filled bars indicate significant preference) and field (**Figure 2**; asterisks indicate significant preference) assays. Females were not attracted to 13-MeC27 baited traps in the field (**Figure 3**). Beginning in March, males were no longer attracted to 13-MeC27 in laboratory or field assays (**Figure 1 and 2**), coinciding with females reaching reproductive maturity in the field (**Figure 4** dashed line) and being mated in the field (**Figure 4**). Furthermore, males assayed to live females in olfactometer tests on March 2nd, 2010 were not attracted to females when paired with a blank. Similar experiments will be conducted in early 2011 to confirm this trend.

Summerform response to sex attractants. Chemical analyses of whole-body washes of summerform psylla revealed that 13-MeC27, 2-MeC27, and 3-MeC27 were found to be considerably more abundant in females than males. Females did not respond to either 13-MeC27 or to the blend of chemicals, i.e. 13-MeC27+2-MeC27+3-MeC27 (**Figure 5**). On the other hand, males were attracted to both 13-MeC27 and to the blend, with no statistical difference between 13-MeC27 and the blend when presented in pair (**Figure 6: upper panel A**; filled bars and asterisks indicate significant preference). We then compared male attraction to 13-MeC27 and the blend vs. an extract of females. Males did not show a preference for 13-MeC27 when paired with the extract of females. Similarly, males did not show a preference for the blend when paired with the extract of females (**Figure 6: middle panel B)**. We also assessed the effect of chirality of 13-MeC27, i.e. (*R*)-13-MeC27 and (*S*)-13-MeC27 enantiomers, on male attraction. More males were attracted to the racemic blend containing both enantiomers than to the (*R*)-13-MeC27 or the (*S*)-13-MeC27 enantiomers (**Figure 6: lower panel C)**. Males did not show a preference for either enantiomer when presented in pair. Finally, in field assays, more males were caught on traps baited with 13-MeC27 alone and on traps baited with the blend than on unbaited traps, with no significant difference in trap catches between 13-MeC27- and blend-baited traps (**Figure 7**). Females were not attracted to 13-MeC27- or blend-baited traps compared to the control traps (**Figure 7**). These results suggest that 13-MeC27 is also a sex attractant pheromone for pear psylla males of the summerform.

PUBLICATIONS

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

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

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

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

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

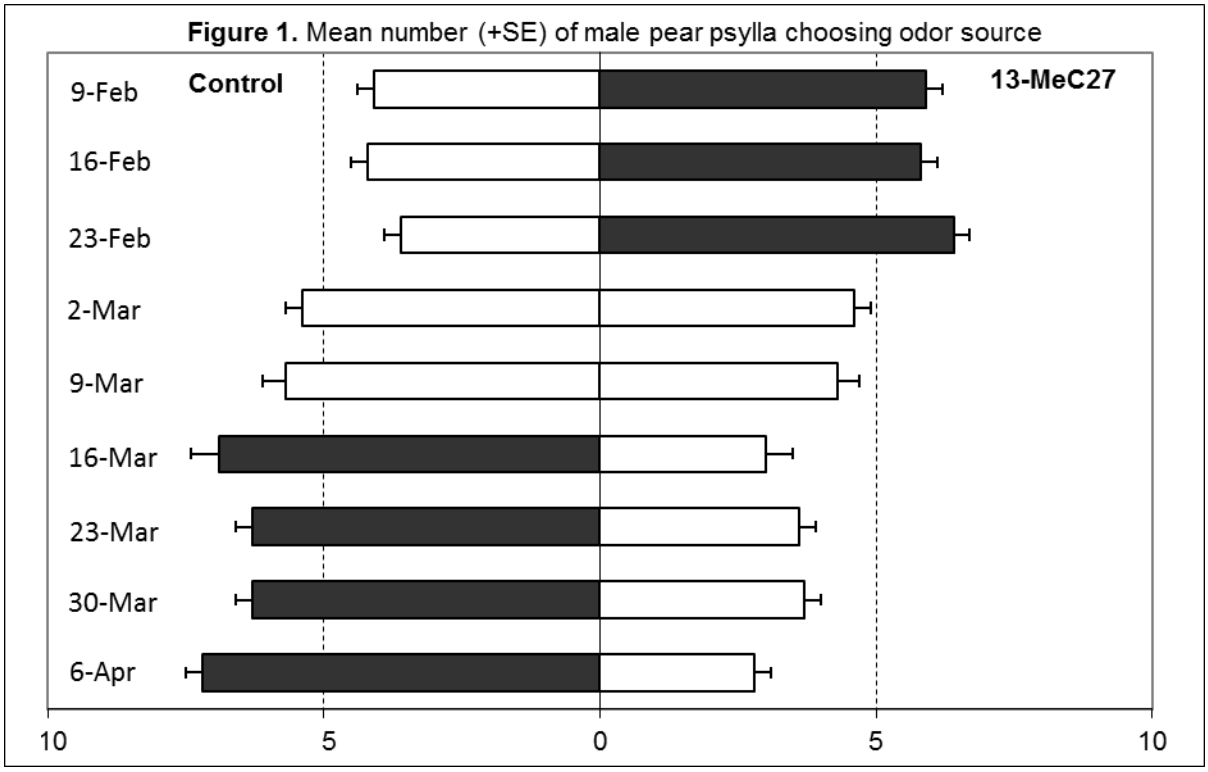


Figure 1. Mean number (+SE) of male pear psylla choosing odor source
Control
13-MeC27
9-Feb
16-Feb
23-Feb
2-Mar
9-Mar
16-Mar
23-Mar
30-Mar
6-Apr
10
5
0
5
10

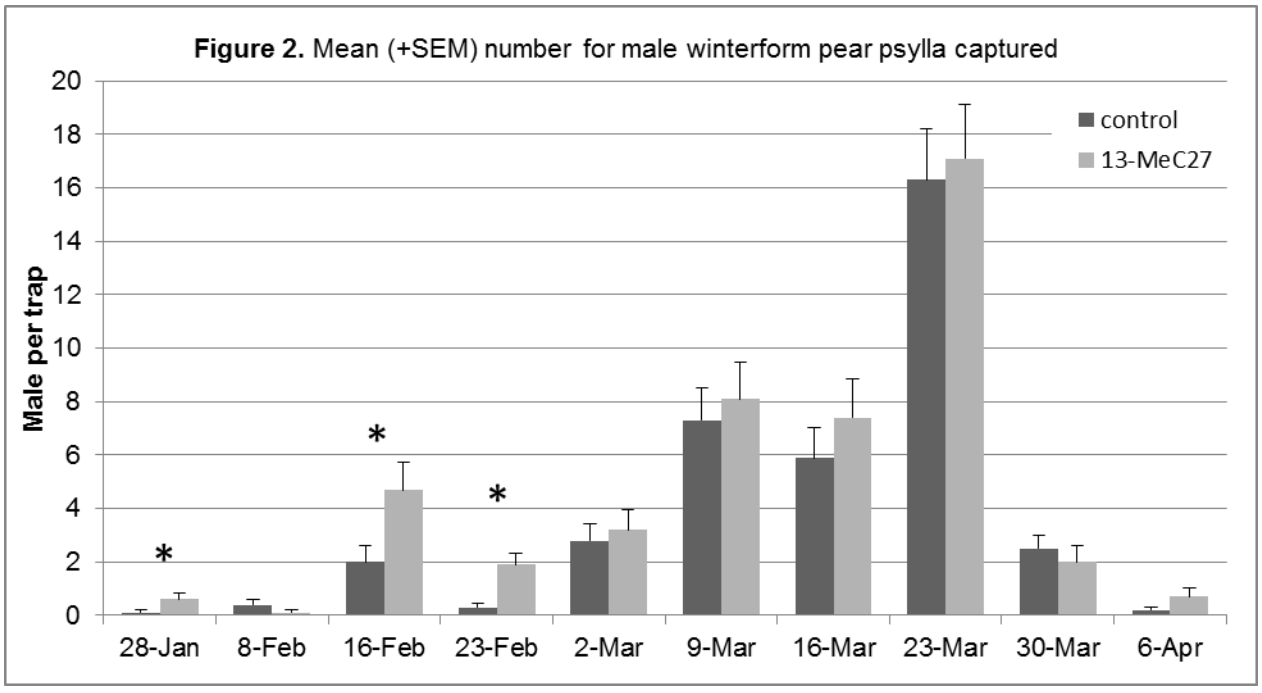
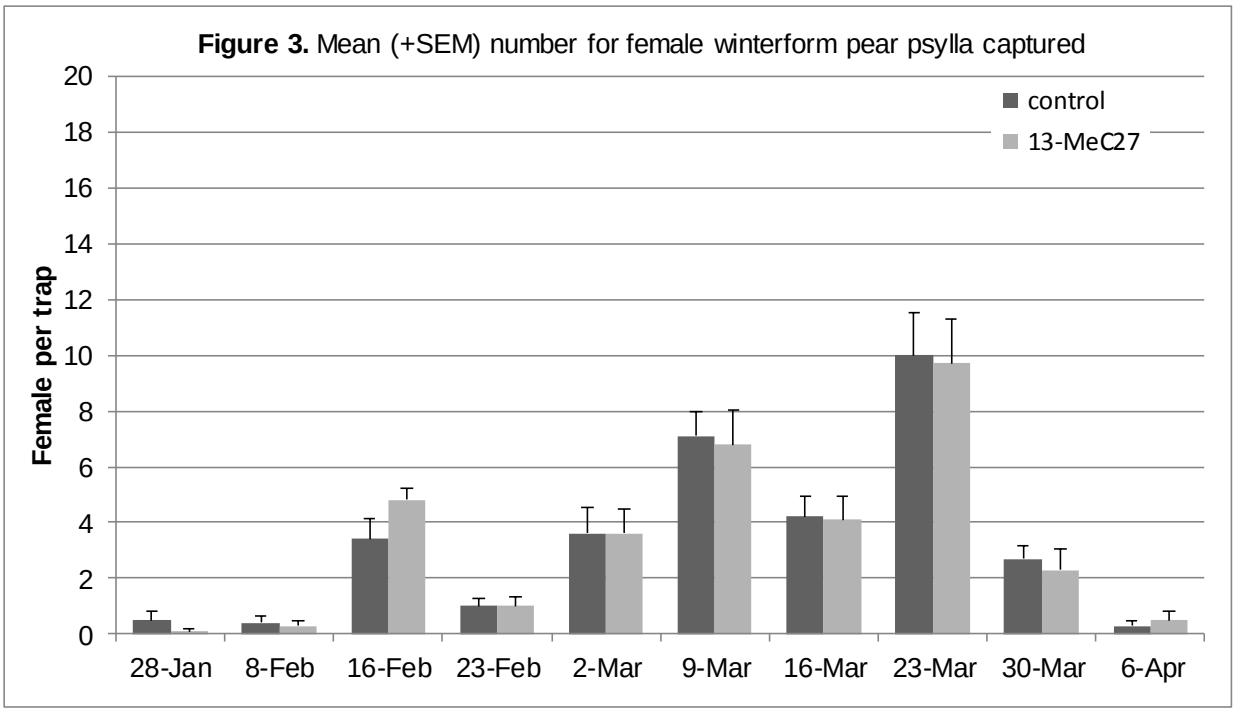
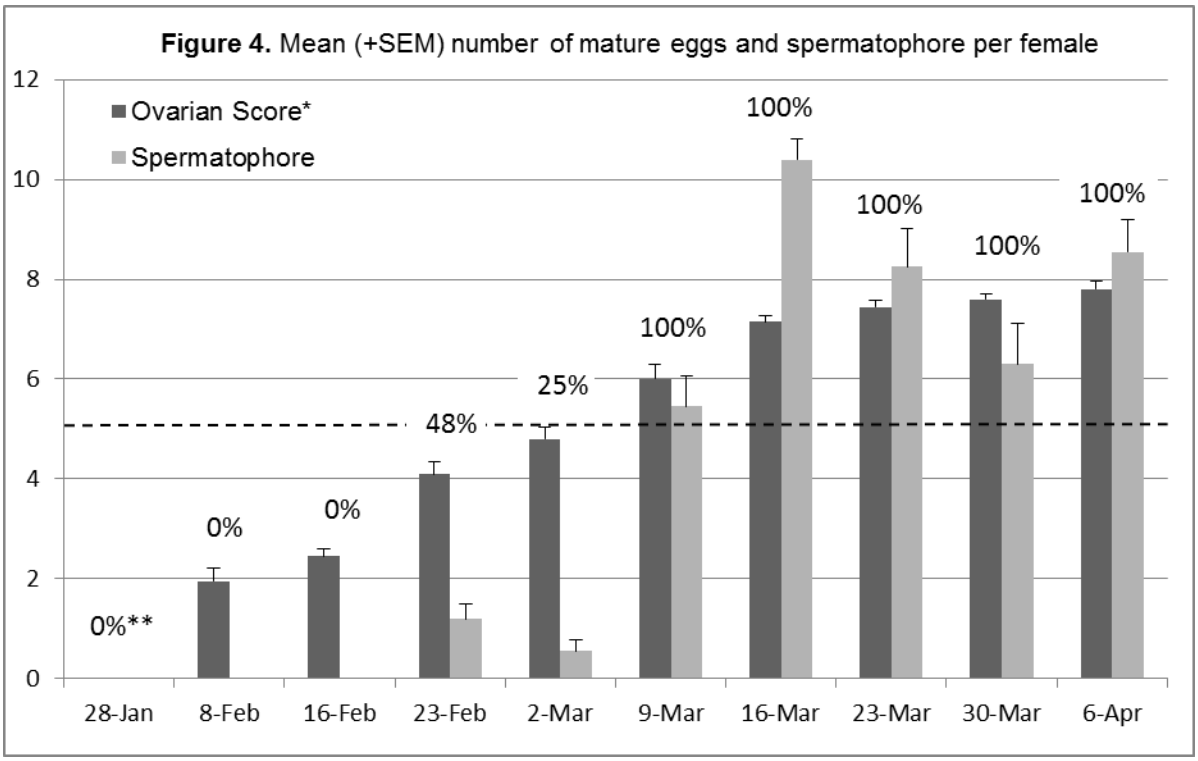


Figure 2. Mean (+SEM) number for male winterform pear psylla captured
Male per trap
control
13-MeC27
20
18
16
14
12
10
8
6
4
2
0
28-Jan
8-Feb
16-Feb
23-Feb
2-Mar
9-Mar
16-Mar
23-Mar
30-Mar
6-Apr





*Dashed line indicates the ovarian score (5) at which females become attractive to males in olfactometer assays (Horton et al. 2007).
**Percent mated females

Figure 5. Mean (+SEM) number of female pear psylla choosing odor source

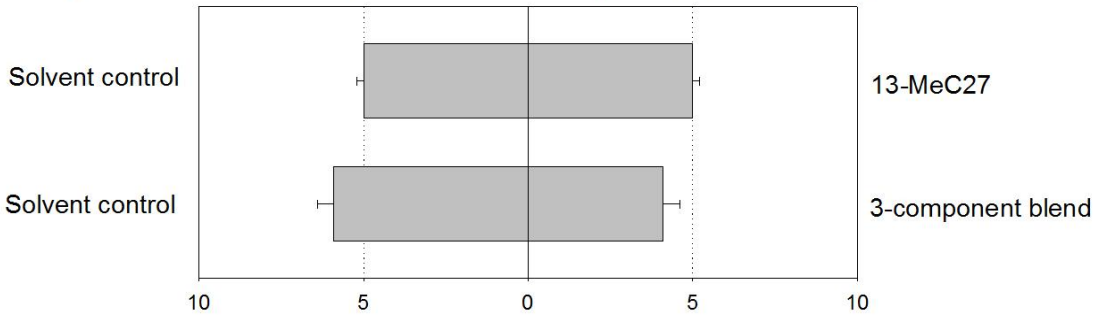
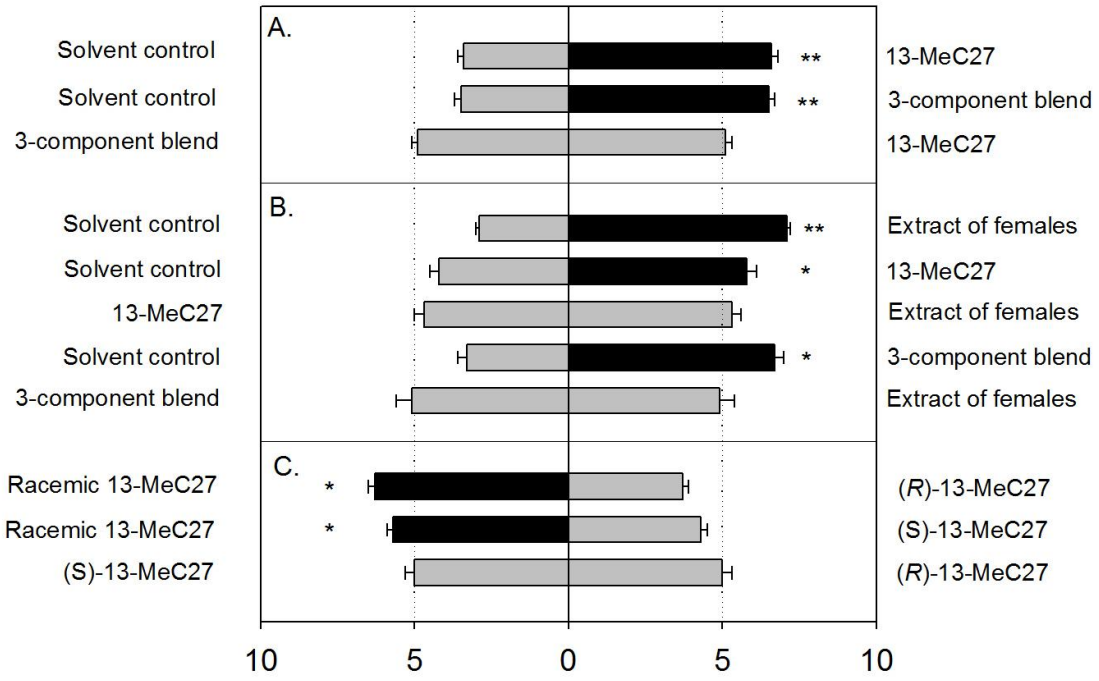
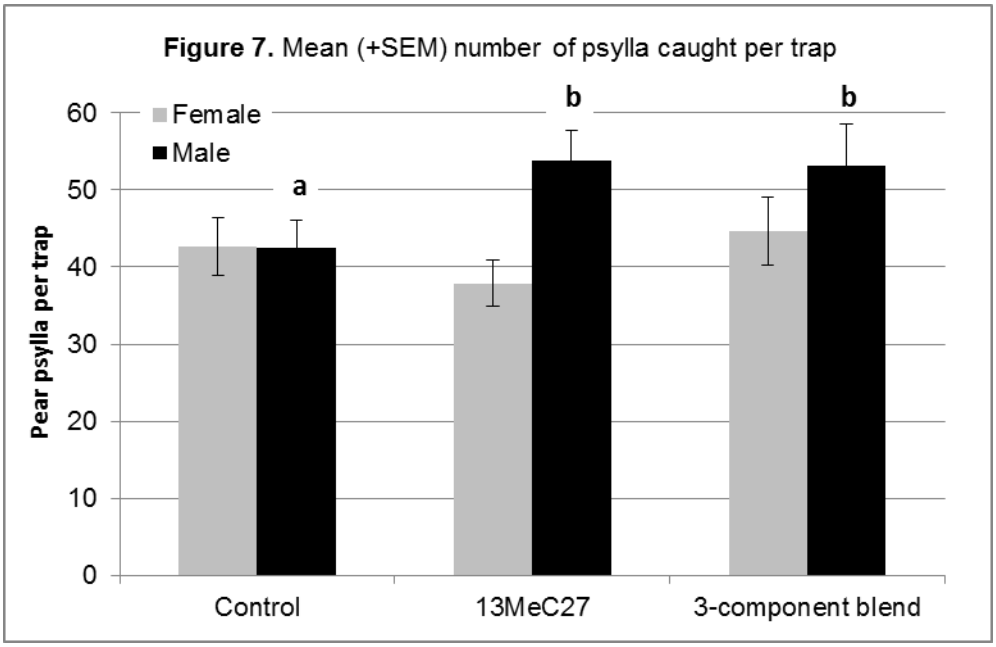


Figure 6. Mean (+SEM) number of male pear psylla choosing odor source





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

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number:

Project Title: Ripening capacity and decay control in Winter Pears

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

Cooperators: E.J. Mitcham, E.A. Kupferman

Budget: **Year 1**: 33,590 **Year 2**: **33,590**

Other funding sources: None

WTFRC collaborative expenses: None

Budget
Organization Name: Oregon Agricultural Research Foundation
Contract Administrator: Cynthia Cox
Telephone: 541-737-4066 **Email address:** cynthia.cox@oregonstate.edu

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

OBJECTIVES

This project has two overall objectives:

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

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

In the Pear Research Priority Survey of October, 2009, in response to the question regarding where the pear industry could most increase grower return, the two most frequent responses were: (1) increasing per capita consumption, and (2) decreasing postharvest losses. Research in the first objective of this proposal should contribute to increased consumption by facilitating the earlier marketing of winter pears with the capacity to ripen to a buttery-juicy texture. Research in the second objective specifically addresses postharvest decay.

SIGNIFICANT FINDINGS

Objective 1 (Ripening Capacity):

- The duration of temperature conditioning at 31 °F needed by 'Anjou' pears to develop ripening capacity decreased with advancing harvest maturity in a linear fashion. Conditioning time can be calculated based on the harvest date relative to the orchard block reaching 15 lbf average fruit firmness.
- The most efficient temperature for inducing ripening capacity in 'Anjou' and 'Comice' pears was 50 °F.
- Further temperature conditioning was needed for 'Anjou' pears to develop ripening capacity after 24 or 48 hours in ethylene, but not after 72 hours. After 24 or 48 hours in ethylene, conditioning can be completed faster at temperatures up to 50 °F than at 31 °F.
- Smaller pears softened faster in response to ethylene treatment than did larger pears.
- Ethylene-temperature combinations that can induce ripening capacity result in fruit with shipping firmness greater than 8 lbf, with most greater than 10 lbf.
- The storage potential at 31 °F of 'Anjou' and 'Comice' pears after conditioning decreases with increasing time in ethylene, warmer post-ethylene conditioning temperatures, and later harvest.

Objective 2 (Postharvest Decay):

- Delay in application of Scholar fungicide until 6 weeks after harvest compromised efficacy.
- Efficacy of Bio-Save 10 as a postharvest linespray was compromised when application was delayed until 3 or more weeks after harvest.
- Calcium chloride summer sprays resulted in strong reduction in decays caused by *Cladosporium* and *Alternaria* fungi, but not in decay caused by *Botrytis* (gray mold).
- Pristine fungicide applied one week pre-harvest reduced all types of natural infection in these experiments, while Luna Sensation was effective in reducing *Botrytis* infection but not *Cladosporium* / *Alternaria* infections.
- Potential organic decay control programs with yeast orchard sprays followed by a Bio-Save 10 linespray was did not provide significant decay reduction.

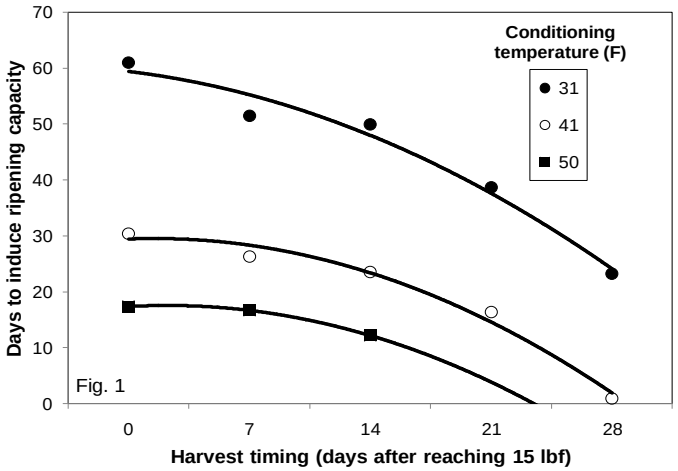
METHODS

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

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

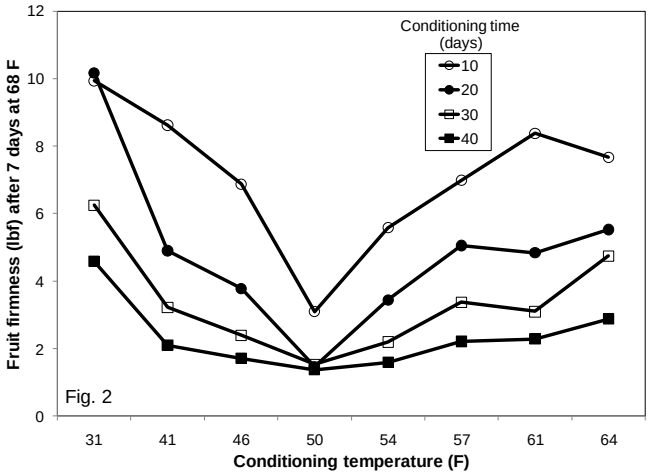
RESULTS & DISCUSSION

Objective 1 (Ripening Capacity):



The relationship between fruit maturity at harvest and the duration of postharvest conditioning required to induce ripening capacity in 'Anjou' pears was studied at Medford and Hood River. The combined results (Fig. 1) indicate a linear decrease in conditioning time with advancing harvest maturity, and a sharp decrease in conditioning time associated with warmer conditioning. The conditioning time at any of these temperatures can be calculated from the equation for the line describing the relationship.

A range of potential conditioning temperatures for 'Anjou' pear were studied in 2009 and 2010; combined results show that the most efficient temperature for inducing ripening capacity among the temperatures studied was 50 °F (Fig. 2).



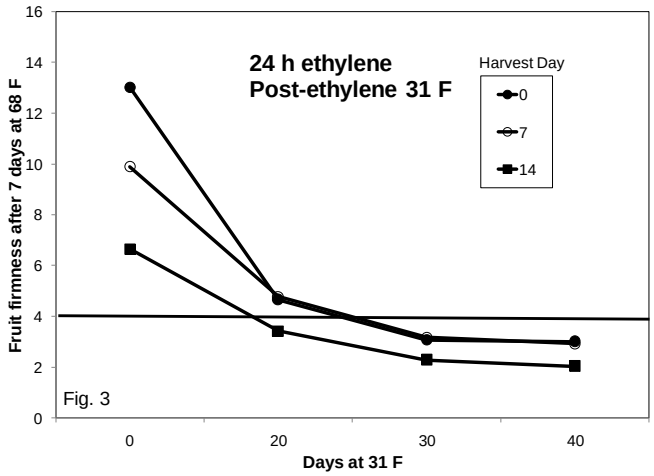


Fig. 3

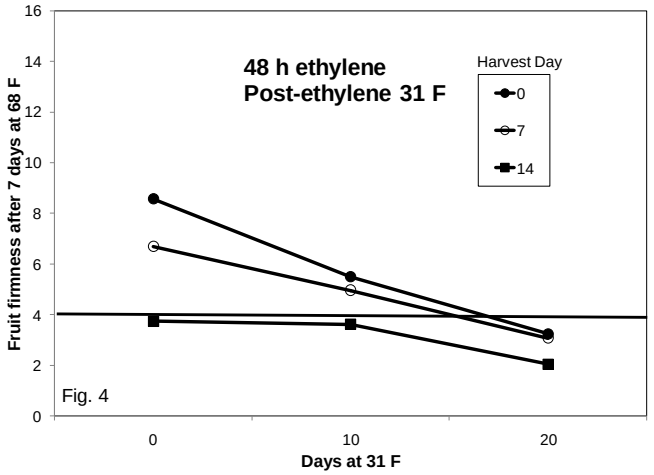


Fig. 4

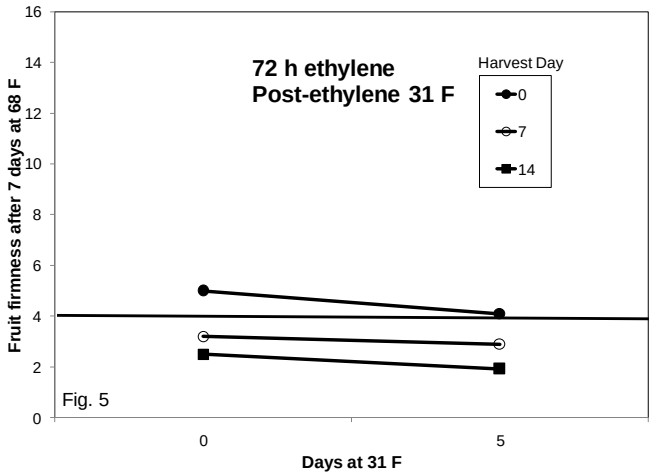
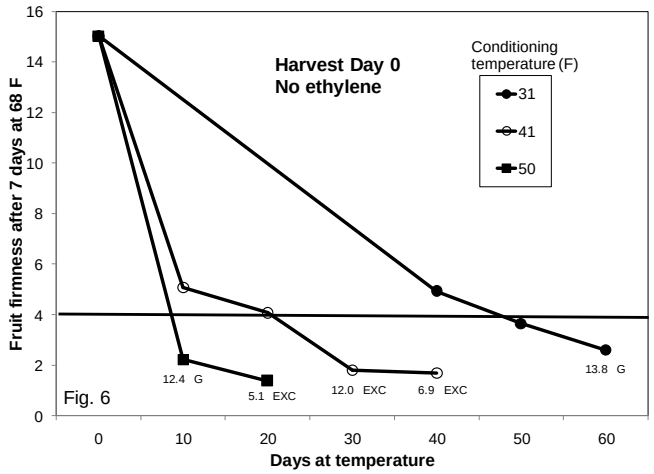


Fig. 5

'Anjou' pears harvested at 15 lbf and conditioned for 24 hours in 100 ppm ethylene at 68 °F needed an additional 30 days to develop the capacity to ripen to a buttery, juicy texture. 'Anjou' pears harvested 7 days later needed the same duration of conditioning to achieve ripening capacity, while those harvested 14 days later needed approximately 20 days (Fig. 3). After 48 hours in ethylene, 'Anjou' pears from the first harvest and those harvested 7 days later needed approximately 20 additional chilling days at 31 °F, while those harvested 14 days later reached were capable of reaching a firmness of 4 lbf after 7 days at 68 °F without further chilling (Fig. 4).

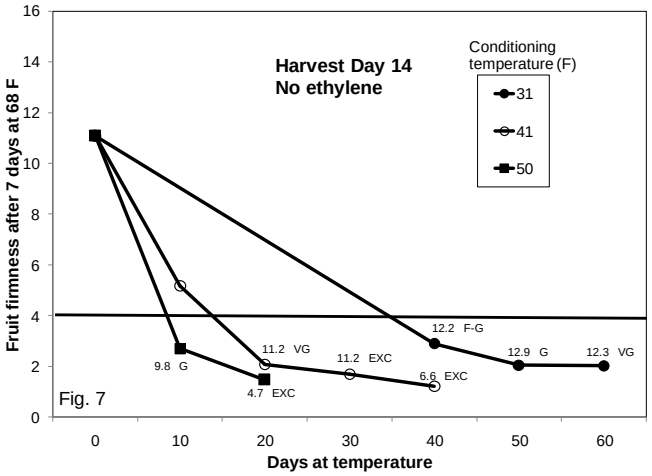
After 72 hours in ethylene, 'Anjou' pears softened to nearly 4 lbf (used herein as indicating the onset of a buttery-juicy texture), although re-cooling and cooling during shipment would likely improve the softening capacity during ripening (Fig. 5).

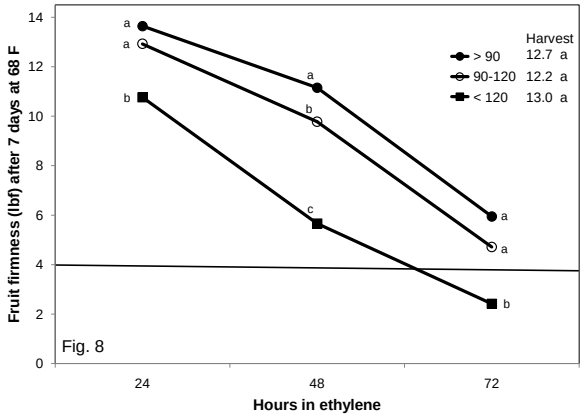
The ripening capacity of 'Anjou' pears was studied using pears from harvest days 0 (first day of average firmness in the orchard below 15.0 lbf), 7, and 14, exposed to ethylene for 24, 48, or 72, and temperature conditioned at 31, 41, or 50 °F for varying lengths of time. Following conditioning, the fruit were allowed to ripen for 7 days at 68 °F, then firmness and eating quality were measured. As examples, data from harvest days 0 and 14, without ethylene, stored at the three temperatures are shown in Figs. 6 and 7. The firmness after 7 days is used as the shipping firmness. Fruit from Harvest Day 0 needed approximately 60 days to achieve ripening capacity at 31 °F, 30 days at 41 °F, or 10 days at 50 °F. The corresponding shipping firmness was greater than 12 lbf, and the eating quality was rated as "good" or better (Fig. 6). In fruit from Harvest Day 14, ripening capacity was achieved after 40, 20, or 10 days at 31, 41, or 50 °F, respectively, with the lowest shipping firmness of 9.8 lbf (10 days at 50 °F) and the minimal eating quality of "fair-good" (40 days at 31 °F).



'Comice' pears of three size categories (larger than 90, 90-120, and smaller than 120) were harvested and fruit firmness was measured after the fruit were exposed to ethylene for 24, 48, and 72 hours. While the differences in firmness in response to ethylene was slight among fruit larger than 120, fruit in the smallest size category appeared to respond to ethylene more quickly, and softened to a greater extent than did the larger fruit (Fig. 8).

The firmness of 'Anjou' and 'Comice' pears after a range of durations of ethylene treatments and post-ethylene conditioning temperatures indicates the potential storage life after the fruit have experienced various conditioning strategies. The storage potential at 31 °F of 'Anjou' and 'Comice' pears after conditioning decreases with increasing time in ethylene, warmer post-ethylene conditioning temperatures, and later harvest. Specific post-conditioning storage life data are being evaluated.





Objective 2 (Postharvest Decay):

The integration of orchard treatments with postharvest treatments as key elements of a comprehensive decay control strategy may be critical to reducing economic losses due to postharvest decay. Relying solely on postharvest treatments, the ability to control infections by decay fungi at wounds made at harvest was largely lost when postharvest treatment with Scholar fungicide was delayed until 6 weeks or more after harvest, and when postharvest treatment with Bio-Save 10 biocontrol agent was delayed until 3 weeks or more after harvest (Fig. 9). In other words, the treatment materials that may be effective when applied promptly after harvest may be of little value for decay control if applied a few weeks later, even if the fruit are kept cold between harvest and treatment. The effectiveness of control treatments depends on the mode of action of the material; diminished effectiveness likely indicates that the infection has advanced beyond the ability of the control material to influence the pathogen.

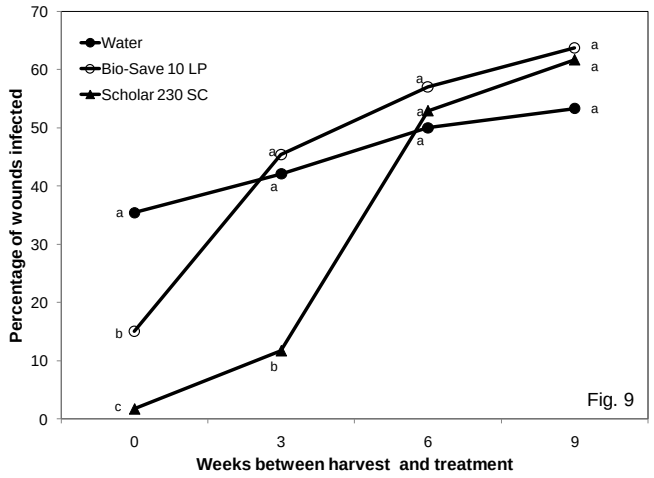


Fig. 9

This research was organized to address the common industry situation in which a large portion of the winter pear crop intended for mid-to-long-term storage may not receive postharvest fungicide treatment promptly after harvest, and thus postharvest fungicide treatment may be inadequate. The application of fungicides prior to harvest, with and without summer spray programs to enhance fruit calcium content was tested with reference to the timing of postharvest Scholar or Bio-Save applications.

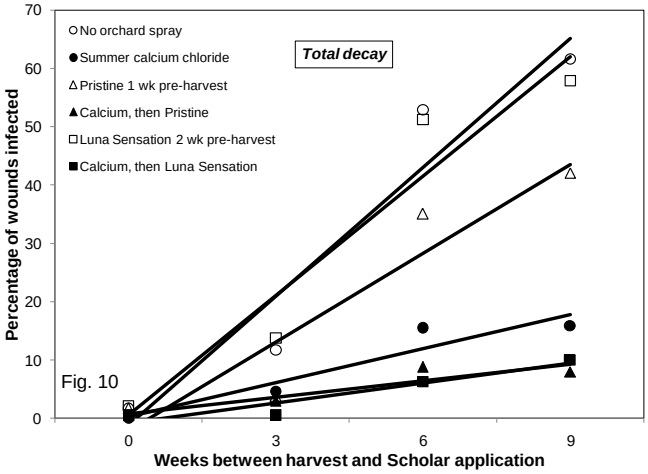


Fig. 10

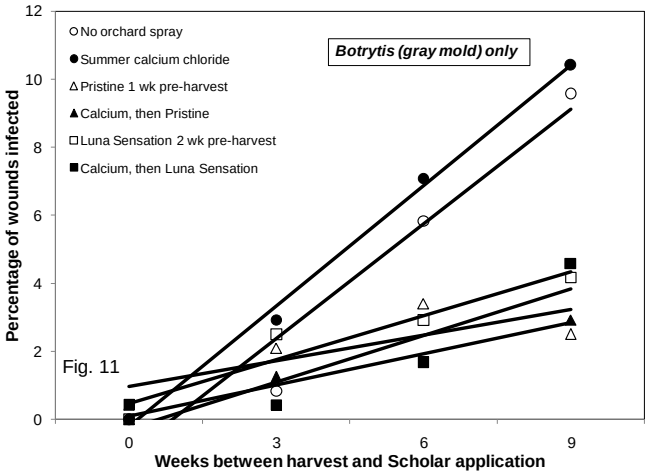


Fig. 11

The effectiveness of orchard fungicide treatments can depend on the specific fungi that cause decay in a specific orchard-packinghouse system. Natural decay infections in our 2010 experiments were dominated by *Cladosporium* and *Alternaria* fungi, as in most years, but there was also a lesser but significant amount of decay caused by *Botrytis cinerea* (gray mold). Pristine fungicide applied one week pre-harvest was effective when assessing the total decay incidence, while Luna Sensation applied two weeks pre-harvest did not appear to be effective (Fig. 10). However, when only the *Botrytis* infections

were considered, Luna Sensation treatments reduced decay (Fig. 11). It is also notable that calcium chloride summer treatments can be highly effective in reducing decay by fungi such as *Cladosporium* and *Alternaria* (Fig. 10), but were not effective in controlling Botrytis (Fig. 11).

Potential organic decay control strategies were evaluated, involving two yeast-based products applied before harvest and the bacterial-based biocontrol product Bio-Save 10 applied as a postharvest line. In general, the biocontrol programs based on either yeast followed by Bio-Save performed similarly to the check in decay control (Fig. 12), while the most effective fungicide program (Pristine followed by Scholar) was highly effective when applied promptly after harvest.

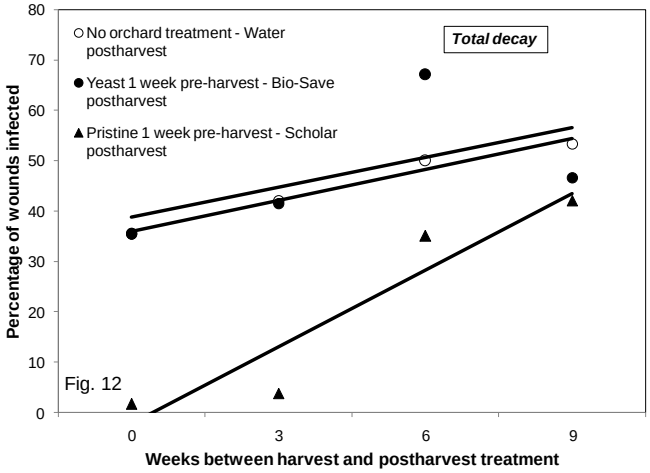


Fig. 12

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-10-104

Project Title: Physiological genomics of pear ripening

PI	Amit Dhingra	**Co-PI**:	Todd Einhorn
Organization:	Washington State University	**Organization**:	Oregon State University
Telephone:	509 335 3625	**Telephone**:	541.386.2030 ext. 13
Email:	adhingra@wsu.edu	**Email**:	Todd.Einhorn@oregonstate.edu
Address:	Johnson 46	**Address:**	3005 Experiment State Drive
City:	Pullman	**City:**	Hood River
State/Zip:	WA 99164	**State/Zip:**	OR 97031

Cooperators: Tim Smith, WSU, Gene Kupferman, WSU, Chris Hendrickson, Graduate Student, WSU and Kate Evans, WSU

Total Project Request: **Year 1**: 48,062 **Year 2**: **64,785** **Year 3**: 56,575

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1 Amit Dhingra
Organization Name: WSU **Contract Administrator:** ML. Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

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

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

Budget 2 Todd Einhorn

Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton

Telephone: 541 737-3228 **Email address:** dorothy.beaton@oregonstate.edu

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

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

OBJECTIVES

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

We will study how all the known ethylene genes work especially in relation to the cold-induced ripening master switch gene.

Another intriguing question to be addressed: what duration of cold-treatment triggers ethylene burst and corresponding expression of the genes involved in ripening?

We will use summer pear – Green and Red Bartlett; winter Pear – Green Anjou, Bosc, Seckle and Comice pear will be picked before maturity and stored at 42 +/- 2 deg F for varying amount of days and activity of the gene studied to establish a relationship between the gene and the ripening.

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

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

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

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

Objectives of the preceding year were to test activity of genes involved in ethylene perception and production in pear, as they correlate to the activity of the proposed cold-induced 'master switch' gene in winter pear. Pear conditioning trials (using the methods developed by Kupferman and Sugar) were performed in the Einhorn lab at the MCAREC (*1*). From these samples, we are currently examining gene-activity related to ethylene signaling, production, calcium-signaling, cold-signaling, and other genes related to phytohormone production. Findings of this work will provide critical information regarding the role of the proposed master switch gene, and other genes in facilitating ripening. Following its completion, we expect to identify the duration and severity of cold-treatment that triggers an increase in activity of genes correlated with ripening fruit.

For 2011, objectives are to repeat the pear conditioning trials with greater replication with both winter and summer pears to identify differences in activity of the genes currently being investigated. Samples will be subjected to the conditioning procedures employed in 2010. A full comparative analysis of activity of these genes between the treated summer and winter pears will be completed by December 15th, 2011. Additionally, we expect to gain significant insight into the possible functional role of the proposed master switch gene as well as a more detailed and complete model of cold-induced ripening in winter pear cultivars.

SIGNIFICANT FINDINGS

- **Using modified pear conditioning methods of Kupferman and Sugar, we produced fruit at a full range of ripening stages. D'Anjou pears exposed to the ethylene treatment for 24, or 48 hrs. were less firm then pears that did not receive the treatment at all temperatures of storage.**
- **Over 170 genes were** shortlisted **which can provide insight into the complete mechanism of cold-induced ripening, and the possible functional role of the proposed master switch gene.**
- **The MADS-Rin** (a gene that inhibits the second burst of ethylene as in pears)**, germin-like protein 1 and 2, ACS1A, and ACS1B** (involved in ethylene generation) **genes have been identified in winter pear, which are reported to be required for climacteric fruit transition into the ethylene 'burst' phase of ripening.**
- DNA –based anchors (DNA primers) **for the genes being analyzed were designed, obtained, and** tested to be **effective for subsequent activity analysis (being performed now).**

METHODS

Fruit were obtained from cooperating orchards in and around the Hood River Valley once they had attained appropriate harvest maturity, identified by pressure values between 14-15 lb. Fruit were immediately provided one of three storage temperature treatments (31, 42, or 50° F), and 3 durations of exposure to 100 ppm ethylene gas at 68 °F (0, 24, or 48 hrs.) (Figure 1), as described in Sugar and Einhorn (2011). Each treatment was replicated four times. Pear samples were collected from each treatment following 0, 10, 20, and 30 days of cold storage. Peel and core tissue was taken from each fruit sample, after being tested for firmness with a Fruit Texture Analyzer (FTA). A subsample of 30 pears each were then placed into storage at 68° F (± 1 °F) for seven days to allow for ripening. Firmness was determined on the 7th day, and immediately followed by peel and core tissue sampling. Upon isolation of the peel and core tissue, samples were immediately frozen in liquid nitrogen, then stored at -80°C until further processed. We are extracting the cellular "message" of these genes (RNA) from each sample and comparing gene-activity quantitatively between summer and winter pears using a quantitative real-time PCR (qPCR) procedure.

RESULTS & DISCUSSION

Work over the previous year has established the experimental foundation from which we can begin to resolve the mechanisms underlying cold-induced ripening in winter pear. Sample fruit comprised a wide range of ripening stages (Figure 1), allowing us to capture short-lived events of gene-activity that may govern the fruits' development of ripening capacity. We have identified over 125 genes from multiple processes (Figure 3) with a potential role in ripening based on extensive review of current literature, and have found these genes present in winter pear. Critically, we have found the few genes reported essential to climacteric fruit for transition into the ripening-associated ethylene burst to be present in winter pear. These genes include the germin-like proteins (1 and 2), ACS1A, ACS1B, and MADS-Rin (*2, 3, 4, and 5*). Presence of these genes was tested by a procedure which amplifies specific genes out of the large pool of DNA found inside each plant cell, called PCR. We have designed DNA anchors for these genes called primers, and tested their gene-target efficacy using the PCR procedure.

We are now well-positioned to complete comparative analysis of activity among genes involved in pear ripening in the coming days. By expanding the sample count and including summer pear cultivar(s) in the repeated conditioning trials this year, we will gain detailed insight into the events leading to, during and following the ethylene burst associated with ripening in climacteric

fruits. This comparative analysis will allow greater understanding of the role of the proposed master switch gene in both winter and summer pears. We also will be able to provide improved gene-based and physiology integrated model of ripening among pear cultivars. This model will integrate the roles of the ethylene perception, production and cold-signaling with not only the master switch gene, but genes associated with factors known to affect the sequence of ripening events in climacteric fruit (such as calcium and auxin, and jasmonate content) (*6*,*7*).

Results of this work and the model we aim to produce upon its completion have significant economic impacts. One of the primary concerns for the pear industry of the PNW remains: delivery of a consistent, high-quality crop to market in a timely manner. In parallel, pear consumption per capita has trended downward. Minimizing crop damage and spoilage along the pear production, storage and transportation pipeline is a powerful means to prevent loss of income from growers, packhouses and end-point retailers encourage greater consumption, and encourage greater consumption. Losses from Washington packinghouses alone can exceed $2.0M annually (*8*). These challenges emphasize the importance of gaining a detailed, fundamental understanding of the forces and mechanisms underlying impaired ripening in some pear cultivars. With this knowledge, procedures within the existing pipeline of production-storage-transport can be modified, to improve delivery of consistent, high-quality fruit to market. With the broad analysis of gene-activity, we also can provide gene-activlity lined information optimized to both summer and winter pear cultivars to provide growers with a precise time in which fruit can be harvested to ensure optimal fruit quality entering, and leaving storage. Such a product is available in the market and is sold by NSure (http://nsure.eu/default.asp)

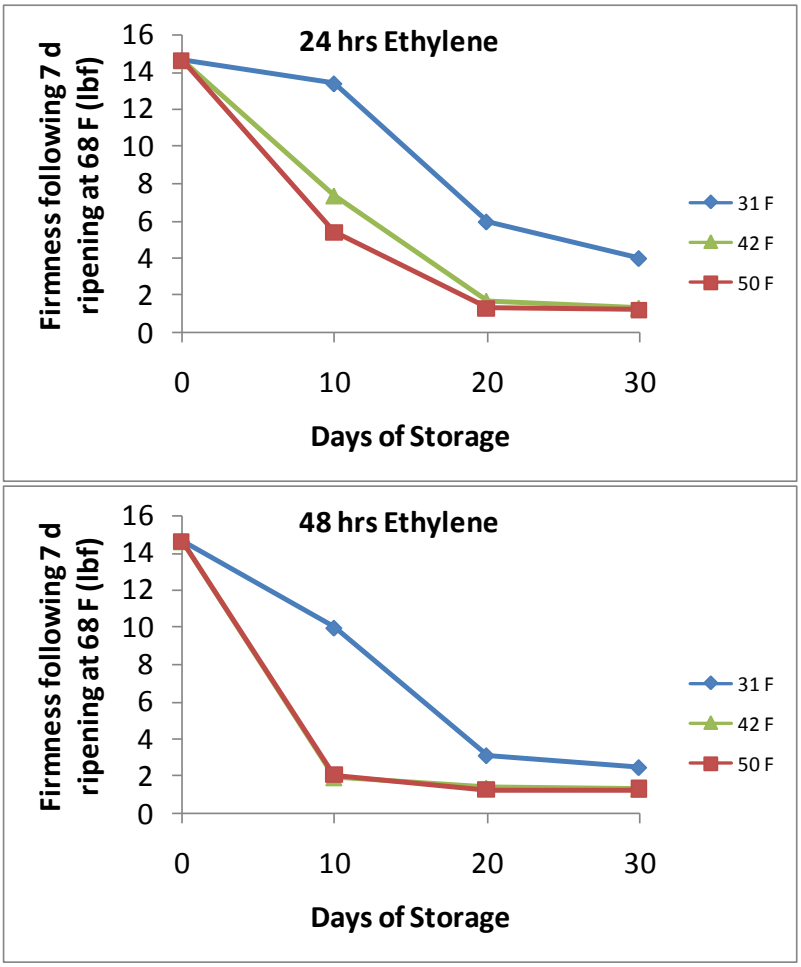


Figure 1. Fruit firmness of 'd'Anjou' pears following 0, 10, 20, 30 days storage at 31, 42 and 50 F, plus 7 days ripening treatment at room temperature. Fruit were either provided 0 hrs (top), 24 hrs (center), or 48 hrs (lower) of 100 ppm ethylene.

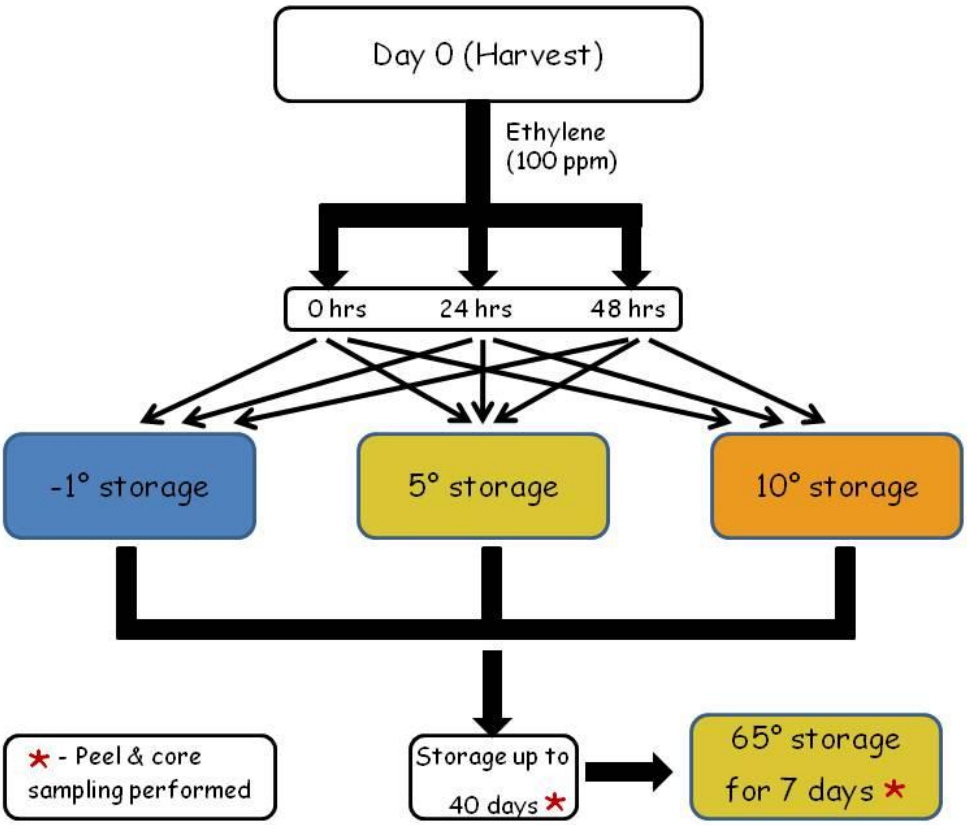


Figure 2. Schedule of pear conditioning treatments and sample collection performed at MCAREC.

A	B	C	D	E	F	G	H
C2H4 perception	**C2H4-signaling**	**C2H4-prod.**	**Cold-signaling**	**Ca(2+)-signaling**	**Auxin-prod.**	**Auxin-signaling**	**Jasmonate, ABA & stress**
ETR1a	EIL2	ACS1a	HOS1	calmodulin	NIT1	PIN1	catalase1-like
ETR1b	CTR1-like	ACS1b	XBAT32-like	CDPK1	AMI1	PIN2	PIP1
ERS1a	EIN2	ACS2a	CBF2/AP2D7	CDPK2		PIN5	MT1
ERS1b	EIN3/EIL1	ACS2b	ICE1	CIPK1		ABP1/GLP2	MT2
ETR5	EIN5/XRN4	ACS3	FRY1	CIPK2		ABP2/GLP1	AMT1
RTE1	EIL3	ACS4	ICE2	CBL1		ARF1	PDX1.1
RAN1	ERFLP1-like	ACS5	HOS2	CBL3		ARF2	PDX1.3
ASP1	ERF1a	ACO1	CBF1/DREB1b	MIP (*proposed*		ARF5	PDX2
	ERF1b	ACO2	CBF3/DREB1a	*master switch*)		ARF8	PDX3/PPOX1
	ERF2a	AdoMet-ase1	DRIP1-1			TASIR-ARF	LEA14-1
	ERF2b	MTK1	DREB2a			ARP1	ABA1
	ERF3a	MTAN1-like	DREB2b			GNOM1	ABA2
	ERF3b	ACD1	GRRB1			BIG	ABI1
	IAA29	MADS-Rin like	AHS1			RUB1	ABI2
	MKK2	MADS1-like				RCE1	ABI3-like
	MKK9	MADS8-like				SKP1	ABI4
	MPK3	MADS9-like				TIR1	ABI5
	MPK6					Cullin1	AIP2
	PcRbo-HD1					AXR1	AOX1b
	PcRbo-HF1					RBX1	AFP2
	PcPLD-Gamma1					IAA3	CPI1
	Cullin3					mybR3R2-1	NME1
	EOL1					GH3-1	UBI1-like
	ETP1					GH3-17	OMT1
	EBF1						LRRB1
							mybS3
							AC1
							AC2
							OPCL1

Figure 3. Signaling pathways and relationships with each other and ripening in pear, with corresponding genes which are being comparatively analyzed in our current work.

References

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CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-10-103

Project Title: Systems approach for ensuring superior pear fruit quality

PI	Amit Dhingra	**Co-PI**:	Todd Einhorn
Organization:	Washington State University	**Organization**:	Oregon State University
Telephone:	509 335 3625	**Telephone**:	541.386.2030 ext. 13
Email:	adhingra@wsu.edu	**Email**:	Todd.Einhorn@oregonstate.edu
Address:	Johnson 46	Address:	3005 Experiment State Drive
City:	Pullman	City:	Hood River
State/Zip:	WA 99164	State/Zip:	OR 97031
Co-PI:	Qin Zhang	**Co-PI**:	Kate Evans
Organization:	Washington State University	**Organization**:	Washington State University
Telephone:	509-786-9360	**Telephone**:	509-663-8181
Email:	qinzhang@wsu.edu	**Email**:	Kate_evans@wsu.edu
Address:	24106 N. Bunn Rd	Address:	1100 N. Western Ave
City:	Prosser	City:	Wenatchee
State/Zip:	WA 99350-8694	State/Zip:	WA 98801-1230

Cooperators: WSU - Matthew Whiting, Don Elfving, Tim Smith, Ananth Kalyanaraman, Carolyn Ross, Shyam Sablani, Karen Killinger; Marie-Helene Simard (Pear Breeder at INRA), Yves Lespinasse, Charles-Eric Durel, Elisabeth Chevreau, INRA at Angers, France; Richard Bell, USDA; Riccardo Velasco, IASMA, Italy; Gavin Ross, Plant and Food Systems, NZ, Toshiya Yamamoto, Japan, Stefano Tartarini, Italy, Josh Koempel, Nate Squire and Ray Schmitten.

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

Other Funding sources: None

WTFRC Collaborative expenses: None

Budget 1 Amit Dhingra
Organization Name: WSU **Contract Administrator:** ML. Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	2010	2011	2012
Salaries[1]	55,002	46,765	43,871
Benefits	10,523	3,337	10,143
Wages	7,546	7,847	8,160
Benefits	724	753	783
Supplies	8,000	8,000	8,000
Travel	5,000	9,000	2,000
Consumer panel			5000
Miscellaneous – 454 sequencing		11,000	11,000
Total	$86,795	**$86,702**	$88,957

Footnotes: [1] Salaries for agriculture research assistant (PhD-12 months) and agriculture research assistant (MS-9 months @ 65% of 0.50FTE) and visiting scholar for performing physiological and genomic profiling, all molecular work; sanitization platform and robotics respectively. The increase in salaries for years two and three reflects a 4 % rate increase**.**

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

Item	2010	2011	2012
Salaries[1]	21,662	22,529	23,430
Benefits[2]	2,484	2,608	2,738
Wages[3]	2,000	2,000	2,000
Benefits[4]	170	170	170
Equipment			
Supplies			
Travel[5]	750	750	750
Miscellaneous			
Total	$27,066	**$28,057**	$29,088

Footnotes:
[1]Salary is for a 0.49 FTE M.S. candidate calculated based on a 1.0 FTE salary rate of $44,208.
[2]MS OPE rate is $567/term * 4 terms/academic year
[3]Hourly wages for time-slip labor (~200 hours @ $9/hour) to assist with data collection and cultural practices
[4]Benefit rate for part-time employee is 8.5 %
[5]Travel includes transportation to off-station sites in OR, and one trip per year to WA sites at 0.59 cents/mile

OBJECTIVES

Summary Statement: This project represents multiple activities that have been initiated in year 1 and are currently ongoing. This endeavor has catalyzed the formation of a global pear research and industry consortium to address the basic issue of declining pear consumption. Over 68 scientists from US, Europe and South America representing diverse disciplines are working together to submit a SCRI research and planning grant in 2011 followed by a full proposal in January 2012.

Objective 1: (Year 1-3) Training systems: Evaluate, devise, and plant efficient orchard systems that are amenable to mechanized pruning and harvest using labor assist platforms. These will be located on both research station and grower cooperator sites.

1a.Develop cropload indices for the optimum productivity of target fruit.

1b.Plant progressive, high-density pear systems using both the physiological thresholds identified from objective 1a, and experience gained from recent high-density PNW pear plantings.

1c.Identify genotypic sources of dwarfing in rootstocks and collate information from Co-PIs project on potential rootstocks for pear.

1d.Assess the potential of mechanized pruning in high density, vertical trellis or inclined UFO pear orchards.

Most of the objectives in this section are currently under progress.

Objective 1b: A replicated block of Bartlett, Starkrimson grafted on to OHF 87 was planted in 2010 in Koempel block to the UFO training style. In the growing period, several offshoots of uniform growth (6-8 inches) were observed. The trees had experienced a freeze so the growth of these trees was not as expected. We plan to observe the growth and development of this block in the coming season.

Objective 1C: There is no pear rootstock breeding program in the US despite the fact that there is a dire need for such genetic material if pears are to be grown at high density with a manageable tree size. At the recently concluded International Pear Symposium, formal collaborations were set up with colleagues from IRTA, Spain, INRA, France, University of Bologna, Italy and INTA, Argentina. These groups have invested several decades in developing disease resistant, precocious and dwarfing rootstocks. Work is underway to import these rootstocks so that they can be tested in the PNW environment. Kate Evans is in the process of tracking pear rootstock material developed at East Malling Research and it will be included in our selection. These materials are not currently available in the US to the best of our knowledge.

Objective 2: (Year 1 and 2) Vigor Control: Assess the effectiveness of vigor-retarding mechanical and chemical techniques such as limb angle manipulation, application of ABA, apogee and treehold by understanding the underlying gene function. Examine their influence on fruit and shoot growth, return bloom, fruit set, and develop timing and rate recommendations.

2a. Identify optimal limb orientation on vigor (shoot growth) precocity, fruit size and fruit quality in planar trellis systems.

2b. Perform a comparative analysis (physiological and gene-level) on the effect of vigor control chemistries on apple and pear.

2c. Assess different chemistries for vigor control and develop timing and rate recommendations for effective vigor control in pear.

SIGNIFICANT FINDINGS

Vigor Control:

- Abscisic acid (ABA) did not consistently reduce the length of ‘d’Anjou’, ‘Starkrimson’ or ‘Bosc’ shoots when applied at 250, 500 or 1,000 ppm.
- Apogee markedly reduced shoot length between 30 and 60 % of untreated control ‘Bosc’, ‘d’Anjou’ and ‘Starkrimson’ shoots when applied either once in the upper Hood River Valley, or twice in the lower Hood River Valley at a rate of 250 ppm.
- Apogee did not affect fruit set, or fruit size of ‘d’Anjou’ at either site. ‘Bosc’ fruit size was significantly reduced for Apogee treated limbs, though fruit size peaked on 80.

- Limb angle influenced total number of new shoots, length of new shoots and total shoot growth of 'd'Anjou' and 'Bartlett' trees. Limbs trained at an angle 45 ° from horizontal produced the most vigorous growth. Limbs trained to 30 or 0 ° from the horizontal resulted in significantly less growth. Bartlett flowering and fruit set was highest for 45°, intermediate for 30° and lowest [50 % of 45°] for 0°.

Methods:

Vigor Control:

1. Chemical. Entire primary scaffold limbs of 'Starkrimson', 'Golden Russet Bosc' and 'd'Anjou' [two sites for 'd'Anjou'; one lower and one upper Hood River valley] were treated with ABA and Apogee beginning ~ 20 days after full bloom [DAFB] when shoot elongation was < 4 inches. Treatments were: 1) Control [water + surfactant], 2-4) 250, 500, and 1,000 ppm ABA, 5) 250 ppm P-Ca , and 6) 125 ppm P-Ca at ~ 4 inches shoot elongation + 250 ppm P-Ca 30 days later. Treatment 6 was only applied to 'GR Bosc' and one site of 'd'Anjou'. For each treatment, five or six replicate scaffolds [depending on site] were treated. Four newly emerged shoots per scaffold were measured weekly. Following bud set in late summer, all shoots on the scaffold were assessed for growth.
2. Mechanical. All primary shoots were removed from four-year-old, eight tier central leader trellised trees in spring of 2009 using Dutch-cuts. Three limb angle treatments (0, 30 and 45° from horizontal) were established once new shoots emerged. For each treatment, five single tree replicates, each with 16 shoots (2 per tier), were trained to their respective angles. The experiment was applied to both 'd'Anjou' and 'Bartlett' trees. Total length of each primary shoot, and all new shoots (watersprouts and lateral branches) initiating from the primaries were measured in 2009 and 2010. Number of flowers and fruit borne in 2010 ('Bartlett' only) were counted. Bloom and fruit set will be assessed in 2011.

Results and Discussion:

Vigor Control

1. Chemical. ABA reduced shoot growth rate via depressed rates of photosynthesis. Photosynthesis was reduced in a rate-dependent manner, indirectly via stomatal closure, but the compound was metabolized rapidly and the effect was transient lasting < two weeks. ABA reduced photosynthesis within 1 hour of application. However, shoot growth was only reduced for roughly one week resulting in a lack of consistent growth regulation. In addition, 1,000 ppm was phytotoxic to leaves.

   In the case of 'd'Anjou' Apogee resulted in good vigor control without adversely impacting fruit size (Fig 1, Table A). In fact, Apogee provided strong control over shoot growth on all varieties tested (Fig 1). Lower valley 'd'Anjou' shoot growth resumed at rates exceeding control limbs roughly one month following early spring application. A second application of Apogee improved control, but the magnitude was cultivar dependent (Fig 1). 'Bosc' fruit growth was significantly affected by Apogee (Table B), as previously shown. 'Starkrimson' scaffolds did not have sufficient fruit to adequately assess fruit growth. Return bloom will be assessed in limbs treated in 2010. All experiments will be repeated in 2011, and future studies will focus on the role of Apogee on non-fruiting shoots.
2. Mechanical. Limb angles at both 45 and 30° from the horizontal increased leaf canopy and light interception. Light interception is positively and linearly related to tree fruit yield. Further, fruit bud development appears greatest on 45 and 30° limbs, and will be characterized via flower, fruit set and fruit size measurements in 2011.

   Figures and Tables:

   Table A. Effect of ABA and Apogee on 'd'Anjou' shoot and fruit relations. Treatments were made on entire primary scaffolds selected for uniformity in size and potential cropload.

Treatment	no.shoots	avg. shoot length (cm)	ttl. shoot length (cm)	no.fruit	yield (kg)	avg. fruit sz. (g)
Control	61.2	37.1 a	2261	35	8.9	255.9 ab
250 ppm ABA	58.2	38.9 a	2256	29.8	6.8	234.7 ab
500 ppm ABA	60.4	38.6 a	2452	29.2	6.8	235.3 ab
1,000 ppm ABA	66.6	38.4 a	2561	39.8	9.1	223.8 b
250 ppm P-Ca 1x	55	33.4 ab	1797	28.2	7.5	255.7 ab
250 ppm P-Ca 2x	54.2	25.9 b	1438	38.6	10	270.1 a

Table B. Effect of ABA and Apogee on 'Bosc' shoot and fruit relations. Treatments were made on entire primary scaffolds selected for uniformity in size and potential cropload.

Treatment	no.shoots	avg. shoot length (cm)	ttl. shoot length (cm)	no.fruit	Yield (kg)	avg. fruit sz. (g)
Control	11.8 c	45.4 a	536.3	10.8 ab	3.4 ab	319.9 a
250 ppm ABA	12.4 bc	34.9 ab	423.2	7.8 b	2.4 bc	303.2 ab
500 ppm ABA	10.2 c	46.2 a	441.1	6 b	1.8 c	313.6 ab
1,000 ppm ABA	12.8 bc	38.4 ab	476.5	6.2 b	2 bc	323.9 a
250 ppm P-Ca 1x	18.2 a	25.7 bc	460.2	8.2 b	2.6 abc	316.9 a
250 ppm P-Ca 2x	17.2 ab	21.9 c	385.6	13.4 a	3.8 a	288.2 b

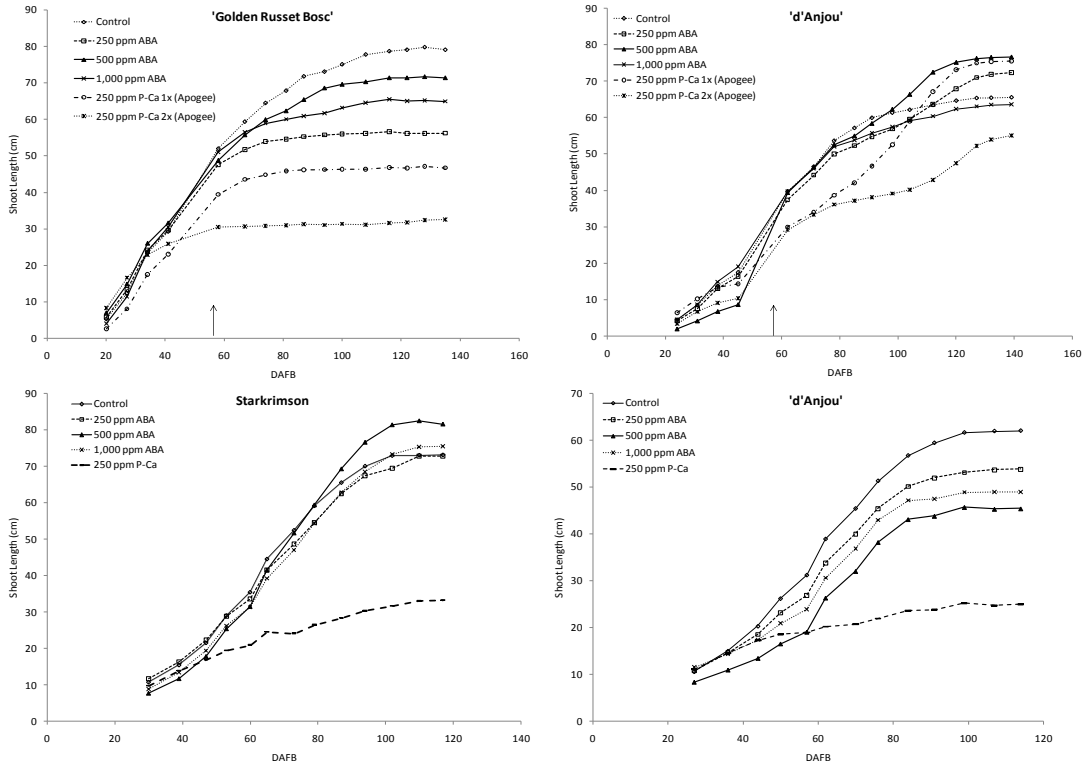


Figure 1. Effect of ABA and Apogee on shoot growth [length (cm)] of 'GR Bosc' [top left], 'd'Anjou lower Hood River Valley [top right], Starkrimson [bottom left] and 'd'Anjou' upper Hood River valley [bottom right]. The arrow in the top two panes signifies 2nd application.

Objective 3: Fruit Quality

3a. Study the impact of cuticle or fruit skin on fruit quality

3b. Understand cork spot and russet using microscopy and genomic profiling under physiologically inductive conditions

3c. Test the impact of chlorophyll stabilizing chemistries on scuffing and fruit quality

SIGNIFICANT FINDINGS

1. Freeze fracture methods is an efficient method for determining cuticle structure
2. Chlorophyll stabilizing chemistry shows a dose response curve in Anjou pears maintaining at harvest firmness after storage and having reduced brix after being in CA storage for 3 months.

METHODS

Cuticle measurements were performed with various microscopic methods at the Franceschi Microscopy facility at WSU. To determine the best method, peel samples were collected from store procured samples. During this growing season, pears were harvested and peel tissues frozen for subsequent analysis in the coming days.

Corked pears were kindly provided by Bob Gix. Corked and non-cork spot tissues have been harvested and frozen in liquid nitrogen for subsequent analysis during the coming months.

Chlorophyll stabilizing chemistry (CSC) was spray applied 15 or 30 days prior to harvest in Bartlett and Anjou Blocks (Schmitten and Koempel). Pears were scored for pressure, and peel tissues harvested for cuticle and pigment measurements on the day of harvest, two weeks prior to harvest, at harvest and on the day of release from CA storage.

RESULTS & DISCUSSION

The chlorophyll stabilizing chemistry shows an affect in d'Anjou pears which is a repeat of what was observed last year. Lower brix levels after CA storage can be exploited for delivering better pears. Since this chemistry does not interfere with the ethylene pathway it may provide an alternative to MCP in maintaining firmness in pears.

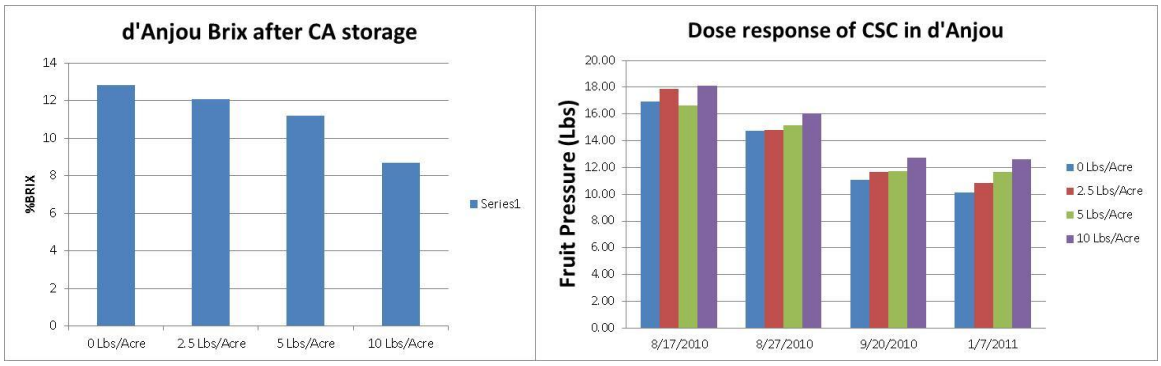


Figure 2: Brix for d'Anjou pears was measured after 3 months in CA storage. Chlorophyll stabilizing chemistry shows a clear dose response in maintaining fruit pressure similar to at harvest levels (right panel). Pears were stored in McDougal and Sons CA storage rooms.

Objective 4. (Year 1-3) Evaluate alternative fruit sanitization platforms like UV or gamma rays in lab settings. These platforms could allow direct warehousing of fruit eliminating the washing step. Lack of moisture and damage on the line could extend storage times. Non-abrasive methods could reduce incidence of scuffing.

4a. Test alternate fruit sanitization methods to reduce pathogen load.

4b. Identify alternate methods of processing fruit on processing lines to prevent skin damage.

4c. perform a consumer preference study to assess consumer experience with alternately sanitized or processed pears.

Objective 4a: A customized table top UV-C test system was designed and manufactured by Reyco Systems, Meridian, ID. The system consists of four 16" UVC emitters (Steril-Aire, Inc.) mounted in an adjustable hood assembly above a small motor driven roller conveyor with variable speed. The system allows rotation of pears during the treatment exposing entire surface to UV light. Preliminary experiments were conducted to test UV light uniformity in the chamber. The effectiveness of UV

system was tested on general *E Coli* in petri dishes. We plan to treat pathogen inoculated pears in UV chamber for different residence time and different UV intensity.

SIGNIFICANT FINDINGS

A table top UV system is procured. UVC treatment was effective on generic E. Coli.

METHODS

Petri dishes inoculated with 10^8 *E Coli* were exposed to UVC emitters at 8-10 cm heights for 2 minutes.

RESULTS & DISCUSSION

A treatment time of 2 minutes was able to completely inactivate *E. Coli* load of 10^8. Knowledge of microbial inactivation as a result of UV treatment on the will assist in commercial UV system design to control fruit rot. Development of alternate method for controlling of postharvest decay will significantly reduce the economic losses for pear growers and traders.

Objective 4b: Assessment of Pear Quality on Packing Line (Before and After Singulator)

SIGNIFICANT FINDINGS

Pear appearance does not seem to be affected by processing on the singulator. However, these pears were treated with MCP. The experiments needs to be repeated with non-MCP treated pears and with a larger sample set.

METHODS

To determine the effect of current packing line (mainly the impact and friction of the fruits on singulators) on pear quality, four sets (Anjou and Bartletts) of 50 pear samples were randomly collected before and after the line singulators On September 30, 2010 and brought to the laboratory for quality tracking and evaluation. This quality assessment was basically a quality deterioration tracking. In this assessment the four sets of 50 pear sample of different variety was divided in eight 25 pears groups: for each variety, two 25-pear groups were collected before entering the singulator and another two groups collected after the singulator. One group each of both varieties collected from before and after the singulator was placed on shelf under regular room temperature and another half groups were stored in a refrigerator as summarized in Table 1.

Table 1. Treatment of Pear Samples Groups

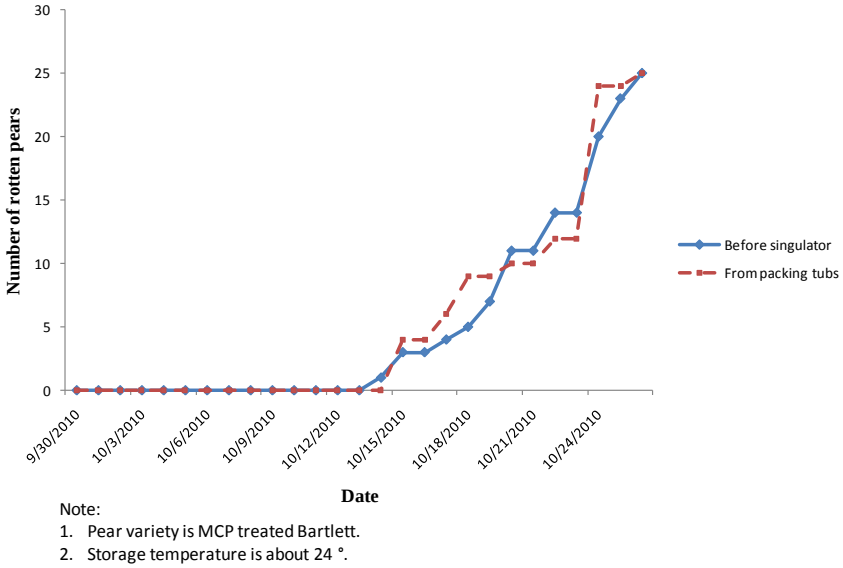
Group	Variety	Collecting location	Storage
A	Anjou	Before singulator	Room temperature (24 °C)
B	Anjou	After singulator	Room temperature (24 °C)
C	Bartletts	Before singulator	Room temperature (24 °C)
D	Bartletts	After singulator	Room temperature (24 °C)
E	Anjou	Before singulator	In refrigerator (4 °C)
F	Anjou	After singulator	In refrigerator (4 °C)
G	Bartletts	Before singulator	In refrigerator (4 °C)
H	Bartletts	After singulator	In refrigerator (4 °C)

All samples were visually observed daily for bruise spot(s), a measure of the fruit appearance.

RESULTS & DISCUSSION

From the observation, it was found that Anjou pears (groups A and B) could keep the good appearance quality much longer than Bartletts pears (groups C and D) as already known. For Anjou pears, 4 pears with limited brown spots in group B (collected after the singulator) compared to only 1 in group A (collected before the singulator) were observed up to date (January 7, 2011). For Bartletts apples, a few brown spots (treated as rotten fruits) were observed starting on Day 14 (collected before the singulator) and Day 15 (collected after the singulator) as summarized in Figure 2. From the results obtained from appearance deterioration assessment, there is not enough evidence that singulator was the major damaging source to pear appearance.

Caveat: Since this study was based on very small number of samples, it was not sufficient to provide a statistical sound conclusion, a large scale assessment would be necessary to confirm this finding. NON-MCP treated pears also need to be tested before making any sound conclusions.



Note:
1. Pear variety is MCP treated Bartlett.
2. Storage temperature is about 24 °.

Figure 2 Brown spot(s) tracking on sample collected either before or after the singulator in a packaging line