2005 Northwest Pear Research Review
Washington Tree Fruit Research Commission
Winter Pear Control Committee
17-18 February 2005
Hood River - Best Western

Page	Time	PI	Project Title	Funding length
	8:00	McFerson/Ing	Introduction	
2	8:15	Mielke	Rootstock evaluations at Hood River	LT
5		Mielke	Northwest multi-site rootstock trials	LT
9	8:30	Auvil	Rootstock and deficit irrigation trial	**03-04**
16	8:45	Elfving	Branch induction with bioregulators	**02-04**
22	9:00	Drake	Quality and condition of winter pears	**02-04**
26	9:15	Xiao	Phacidiopycnis rot of pears	**02-04**
35	9:20	Hilton	IPM programs for codling moth control	**04**
37	9:25	Unruh	Effects of new pesticides on natural enemies of pears	**02-04**
38	9:30	Horton	Biology and management of pear pests	04-06
44	9:35	Jones	Importance of dispersal in biological control	04-06
48	9:40	Dunley	Areawide organic pest management	03-05
54	9:45	Dunley	Biological control in organic and super-soft orchards	03-05
59	9:50	Lacey	Spinosad and granulovirus effects of codling moth	04-05
65	9:55	Proebsting	Propagation of pear rootstocks	03-05
69	10:00	Randall	MCP interaction with fumigants to control decay	03-05
75	10:30	Mattheis	Harvest and postharvest practices for optimum quality	04-06
82	10:45	Bai	MCP and coatings to improve storage	04-06
89	11:00	Sharrock	Ethylene ripening by unconventional means	**04**
96	11:15	Anderson	Red peel-on processed winter pears	**04**
103	11:30	Ing	Coordination - varieties	
106	1:30	Johnson	Integrated fire blight management	04-06
112	1:45	Johnson	Survival of *Erwinia amylovora* on pear fruit	04-06
118	2:00	Spotts	New approaches to decay control of pear	
123	2:15	Stotz	Ethylene induced resistance to botrytis	
129	2:30	Kupferman	Managing storage scald in Anjou pears	04-06
134	2:45	Sugar	Storage decay research	LT
	denotes final report			

FINAL REPORT
WTFRC Project #: PR-01-92

Project title: Evaluation of Pear Rootstocks

PI: Eugene A. Mielke

Organization: OSU – Mid-Columbia Agricultural Research and Extension Center

Cooperator: Bill Proebsting, Horticulture Department
Joseph Postman, USDA, Corvallis, Oregon
Richard Bell, USDA, Kerneysville, West Virginia

Research Technician: Kathleen McFarland

Objectives: To develop a rootstock that is precocious, high yielding with high quality fruit, and has some dwarfing characteristics. This would result in high-density orchards, that inputs are efficiently managed from the ground or platforms, and are friendlier to the environment. Ideally the rootstock should be resistant (or at least tolerant) to the pests and diseases that plague Northwest orchards.

1. Identify rootstocks that induce dwarfing characteristics, precocity, high production, and high fruit quality under varying soil and climatic conditions in the Northwest utilizing conventional rootstocks, interstems, and newly available rootstock material.

2. Determine the fireblight sensitivity of the new rootstocks.

Significant findings:

- On d'Anjou, Bartlett, Golden Russet Bosc, and Comice trees on 708-12 rootstocks were larger than the non-interstem control.

- Trees with 708-36 rootstocks continue to produce more fruit; however, production is more variable between 708-36 rootstocks.

- More variability between trees with 708, Fox 11, or Fox 16 rootstocks was noted in tree growth and production.

- Pyrodwarf, Pyro II, and OHxF 97 rootstocks had no effect on 5-year-old Bartlett, Comice, and Concorde pears.

- 3-year-old Columbia Red d'Anjou trees with 708-36 rootstocks were significantly larger than trees with OHxF 97 rootstocks.

Methods:

Objective 1: Identify rootstocks that induce dwarfing characteristics, precocity, production, and fruit quality under varying soil and climatic conditions in the Northwest utilizing conventional rootstocks, interstems, and newly available rootstock material.

Maintain the following plantings. Evaluate each plot annually for growth, flowering, productivity, and winter survival. Evaluate fruit for yield, size, and quality.

a. 1996 Interstems – terminate 2005.

b. 1998 English 708 and French OH11 trial – terminate 2007.

c. 2000 Pyrodwarf and Pyro 2-33 trial – terminate 2009.

d. Pyrodwarf/Pyro II trial – terminate 2009

e. 2001 Fox/708 trial – terminate 2010

f. 2001 Pyronia trial – terminate 2010

g. Continue propagation of three Russian rootstocks for a future trial – terminate after 10 years. (Material should be released from quarantine this year.)

h. Grossly evaluate the remainder of the Horner rootstock series. Three hundred of the Horner selections are currently at Fowler Nursery and will be planted in 2004. The remainder, propagated in the summer 2002, shipped to Fowler spring 2003 to be budded and returned for field planting in 2005. Repropagated selections will be planted in 2006. Each set of selections would be evaluated for 5 years. Based on a set of desirable characteristics selections will be identified for further development. Initial evaluation to terminate 2008-2010.

i. Begin propagation of *P. betulifolia* (Shaanxi, China), *P. ussuriensis* (Khabarovsk, Russia), and *P. salicifolia* (Arpa Gorge, Armenia) for future trial – terminate after 10 years.

Results and discussion:

1996 Interstems: In the 9-year trial of d'Anjou, Bartlett, Bosc, and Columbia Red d'Anjou with five OHxF rootstocks with four OHxF interstems and a non-interstem control, the main effect of fruit size and production is contributed by the rootstock with slight modification by the interstem. Interstem does affect tree shape and growth habit.

1999 English 708 and OH11 Rootstock Trials: In the 6-year-old green d'Anjou, Bartlett, Golden Russet Bosc, and Comice trial with 708-2, 708-12, 708-36, OH11, and Bartlett seedling rootstocks, Bartlett and Bosc trees with 708-12 rootstocks were significantly taller, and had the largest diameters and TCSAs. D'Anjou and Comice tree size was not affected by rootstock. D'Anjou and Bartlett trees with 708-36 produced the most fruit and greatest yield, producing almost

twice as much as trees with seedling Bartlett rootstocks; however, yields on 708-36 were more variable. Fruit size was not affected by rootstock or crop load.

2000 Pyrodwarf and Pyro II Rootstock Trial: In a 5-year-old Bartlett, Comice, and Concorde trial with Pyrodwarf, Pyrodwarf II, and OHxF 97 rootstocks, rootstock did not significantly TCSA, yield or tree size. This contradicts observed results in larger grower trials.

2001 Hanners Rootstock Trial: In a 4-year-old d'Anjou and Bartlett trial with 708-2, 708-12, 708-36, Fox 11, Fox 16, OHxF 40, and OHxF 87 rootstocks, rootstock significantly affected TCSA in d'Anjou and Bartlett. D'Anjou trees with Fox 16 rootstocks were significantly larger than trees with 708-12 rootstocks. Bartlett trees with Fox 11 rootstocks had significantly larger TCSA than did trees with 708-36 rootstocks, which were 33% smaller. D'Anjou and Bartlett trees with 708-36 rootstocks produce more fruit than trees with any other rootstock. The possibility exists that trees on 708-36 rootstocks may be becoming biennial in yield. Fruit size was not affected by either rootstock or crop load

.*2001 Pyronia and 708-36 Rootstock Trial:* In a 4-year-old d'Anjou and Columbia Red d'Anjou trial with Pyronia (*P. pyronia sp.*), 708-36, OHxF 87, and OHxF 97 rootstocks, rootstock had no significant size effect in d'Anjou. Columbia Red d'Anjou trees with 708-36 rootstocks had a significantly larger TCSA and were significantly taller and had a larger branch spread than trees with OHxF 97 rootstocks. This trial has become significantly influenced by the roots from an adjoining Port Orford cedar windbreak.

Horner Mother Block Evaluation: Liners from the first 289 of the Horner selections were established in 4.5' x 5' x 16' double row spacing. The goal was to plant two trees of each selection; however, growth and budding losses reduced some selections to a single tree. Where tree numbers were reduced below two, materials were to be repropagated for the 2006 planting. In spring 2005, 152 additional selections will be shipped from Fowler and established in Hood River. Approximately 50 selections that were repropagated will be shipped from Fowler Nursery for planting in spring 2006. These trees will be initially evaluated for 5 years for dwarfing character, precocity, productivity, and fruit size. The goal will be to reduce the collection to the most desirable 15 to 20 selections for further evaluation.

P. betulifolia (Shaanxi, China): A limited number of seedlings of previously collected Shaanxi seeds were available from Joseph Postman at the USDA Germplasm Repository in Corvallis. These were sent to Fowler Nursery, and will be included in the Hood River Northwest Pear rootstock trial to be planted in 2006.

Russian Rootstocks: The Russian three rootstocks will be released this year. The intent is for Bill Proebsting to increase the rootstock lines to permit initial tests in Hood River.

FINAL REPORT
WTFRCP Project #: PR-01-90

Project Title: Northwest Pear Rootstock Trial

PI: Eugene A. Mielke

Organization: OSU – Mid-Columbia Agricultural Research and Extension Center

Co-PIs and affiliations: Dana Faubion, WSU, Extension Service, Yakima
Tim Smith, WSU, Extension Service, Wenatchee

Cooperators: Bill Proebsting, OSU, Horticulture, Corvallis
Jim McFerson, WTFRC, Wenatchee
Tom Auvil, WTFRC, Wenatchee
Richard Bell, USDA, Kerneysville, WV

Research Technician: Kathleen McFarland, OSU, MCAREC

Objectives: To develop a rootstock that is precocious, high yielding with high quality fruit, and has some dwarfing characteristics. This would result in high density orchards, that inputs are efficiently managed from the ground or platforms, and are friendlier to the environment. Ideally the rootstock should be resistant (or at least tolerant) to the pests and diseases that plague Northwest orchards.

1. Identify rootstocks that induce dwarfing characteristics, precocity, high production, and high fruit quality under varying soil and climatic conditions in the Northwest utilizing conventional rootstocks, interstems, and newly available rootstock material.

2. Determine the climatic adaptability of Concorde and Taylor's Gold with three rootstocks in the Pacific Northwest.

Significant findings:

- Bosc on 708-36, Fox, and Pyro 2-33 rootstocks suffered the greatest losses to a suspected early freeze.
- Differences in TCSA response to rootstock occurred between the two d'Anjou plantings.
- D'Anjou trees with Fox 16 or Pyrodwarf II roots, Bosc trees with Fox 16 roots, and Bartlett trees with 708-36 roots had the smallest TCSA.
- Rootstock did not significantly affect D'Anjou canopy volume.
- No significant differences were observed in the Taylor's Gold and Concorde rootstock trial.

Methods:

Objective 1: Assess rootstocks for dwarfing and semi-dwarfing characteristics, precocity, yield, and fruit quality under varying soil and climatic conditions in the Northwest utilizing conventional rootstocks and newly available rootstock material.

Maintain d'Anjou plantings in Hood River and Cashmere, a Bartlett plantings in Parker, and a Golden Russet Bosc plantings in Tonasket. The following trials will be maintained or established:

1. Maintain phase I planting of the Northwest pear rootstock trial. Rootstocks include: Pyrodwarf, Pyro II (2/33), Fox 10, Fox 11, 708-36, OHxF 87, OHxF 40 – planted in 2002, terminate 2011.

2. Prepare site for d'Anjou planting as second part of Northwest pear rootstock trial. Rootstocks will include: Brossier (28-152), Retuzier (OH11), Horner (H-4, H-10, & H-51), BM-200 (Australia), INRA P-2532, *Pyrus heterofolia*, and OHxF 87. – plant 2005, terminate 2014.

3. Continue to develop the rootstocks for the 2006 planting.

The experimental design will be a randomized complete block design with 10 blocks. The spacing will be wide enough to minimize the chance of tree interactions within the life of the experiment (10 years), but will need to fit within the grower's orchard. Pollenizers will account for 20% of the trees. Pruning and training will be consistent with industry standards except we will utilize a support system.

Data to be collected annually will include: 1) Trunk cross sectional area (25 cm above bud union); 2) Canopy height, canopy spread (2 directions); 3) Flower clusters and fruit set (whole trees 1st five years); 4) Yield; and 5) Fruit size and grade.

Additional data to be collected: 1) Planting time root system rating (1 to 5, poor to excellent), and TCSA; 2) Any observations as to insect or disease preference (we are not going to scout the blocks every week); and 3) Reason(s) for tree loss, if any.

Objective 2: Determine the adaptability of Concorde and Taylor's Gold with three rootstocks in the Pacific Northwest.

Establish Taylor's Gold and Concorde pears on three rootstocks. Establish them in 2004 in conjunction with the rootstock trials as listed in objective 1. The procedures and data to be collected are the same as described above.

Results and discussion:

Rootstock played a significant role in tree survival (Table 1). Bosc and Bartlett trees on 708-36, Fox, or Pyro 2-33 rootstocks suffered up to 50% loss due to a suspected early freeze.

Table 1. Effect of rootstock on tree survival in 3-year-old trees.

	Percent survival[z]				
Rootstock	Cashmere D'Anjou	Hood River D'Anjou	Tonasket Bosc	Yakima Bartlett	Cashmere Bartlett
708-36	80	100	56 bc	90 ab	-
Fox 11	90	100	44 c	100 a	-
Fox 16	90	-	80 ab	80 b	-
Pyro 2-33	90	100	43 c	100 a	100
Pyrodwarf	90	100	100 a	100 a	100
OHxF 40	90	100	100 a	100 a	-
OHxF 87	90	100	100 a	100 a	100
OHxF 97	-	100	-	-	-
Nellis	-	100	-	-	-

[z] Means within a column followed by the same letter are not significantly different at p=0.05 – Tukey's test.

D'Anjou trees with Fox 16 or Pyrodwarf II rootstocks had the smallest trunk cross sectional area (TCSA) (Table 2). Bosc trees with OHxF rootstocks had significantly larger TCSAs than Bosc trees on other rootstocks. In the Yakima Bartlett planting trees with 708-36 rootstocks had the smallest TCSA.

Table 2. Effect of rootstock on canopy volume and TCSA in 3-year-old trees.

	Trunk Cross Sectional Area (cm2)[z]				
Rootstock	Cashmere D'Anjou	Hood River D'Anjou	Tonasket Bosc	Yakima Bartlett	Cashmere Bartlett
708-36	19.7 b	26.9 ab	21.9 b	5.6 d	-
Fox 11	20.0 b	24.0 ab	20.0 b	7.5 bc	-
Fox 16	18.1 b	-	18.1 b	7.3 cd	-
Pyro 2-33	18.4 b	22.3 b	19.3 b	9.0 abc	19.1
Pyrodwarf	21.6 b	28.7 a	21.6 b	10.2 a	19.6
OHxF 40	26.6 a	25.9 ab	26.6 a	9.3 ab	-
OHxF 87	27.7 a	26.1 ab	27.7 a	9.2 ab	20.9
OHxF 97	-	26.9 ab	-	-	-
Nellis	-	26.0 ab	-	-	-

[z] Means within a column followed by the same letter are not significantly different at p=0.05 – Tukey's test.

Trees with Pyrodwarf II rootstocks again produced the greatest increase in TCSA between 2003 and 2004 for the two d'Anjou and the Bartlett plantings (Table 3). The greatest increase in TCSA in the Bosc trial occurred with the OHxF rootstocks.

Table 3. Effect of rootstock on the percent growth increase in TCSA of 3-year-old trees.

	Growth Increase in TCSA (%)[z]				
Rootstock	Cashmere D'Anjou	Hood River D'Anjou	Tonasket Bosc	Yakima Bartlett	Cashmere Bartlett
708-36	92.7 b	133.3 b	116.4 b	76.1 c	-
Fox 11	113.8 b	116.4 b	109.0 b	78.1 c	-
Fox 16	122.7 ab	-	98.0 b	79.1 c	-
Pyro 2-33	188.5 a	160.7 a	105.1 b	113.9 ab	129.2 b
Pyrodwarf	98.1 b	135.5 ab	115.4 b	127.6 a	166.3 a
OHxF 40	112.1 b	117.6 b	145.5 a	104.3 b	-
OHxF 87	154.2 ab	134.7 ab	155.6 a	108.4 ab	172.5 a
OHxF 97	-	135.2 ab	-	-	-
Nellis	-	134.9 ab	-	-	-

[z] Means within a column followed by the same letter are not significantly different at p=0.05 – Tukey's test.

Minor yields occurred in 2004 (Table 4). Hood Rive d'Anjou trees with OHxF 40 rootstocks and Tonasket Bosc trees with Pyro 2-33 rootstocks produced the greatest yields.

Table 4. Effect of rootstock on yield in 3-year-old trees.

	Yield (pound per tree)[z]				
Rootstock	Cashmere D'Anjou	Hood River D'Anjou	Tonasket Bosc	Yakima Bartlett	Cashmere Bartlett
708-36	0.0	0.4 abc	0.5 b	0.0	-
Fox 11	0.0	0.9 ab	2.8 b	0.0	-
Fox 16	0.0	-	0.0 b	0.0	-
Pyro 2-33	0.0	0.1 bc	2.2 b	0.0	0.0
Pyrodwarf	0.0	0.0 c	0.1 b	0.0	0.0
OHxF 40	0.0	1.2 a	0.5 b	0.0	-
OHxF 87	0.0	0.4 abc	7.9 a	0.0	0.0
OHxF 97	-	0.5 abc	-	-	-
Nellis	-	0.0 c	-	-	-

[z] Means within a column followed by the same letter are not significantly different at p=0.05 – Tukey's test.

Final Report

Project title: Pear Rootstock and Deficit Irrigation Trial

PI: Tom Auvil
Organization: Washington Tree Fruit Research Commission

Cooperator: Randy Smith, Cashmere

Advisory Committee: Bob Gix, Blue Star
Randy Smith, Cashmere
Fred Valentine, Stemilt Growers, Inc.
Tim Smith, WSU Cooperative Extension
Chris Peters, WTFRC Commissioner

Objectives:

- Compare performance of OHxF 40, 69, 87, 97 with scion varieties Bartlett, Golden Russet Bosc and Anjou.
- Compare performance using three irrigation treatments targeting soil moisture levels of 70, 80 and 90% of field capacity.

Overview:

The project was initiated in 1992 and planted in 1996 to investigate two issues: 1) impacts of irrigation management, and 2) if any of the rootstocks offer an economic advantage. The trial site was thoroughly treated for replant issues (pH, compaction, fumigation sulfur). Soil type is loam / silt loam. The irrigation system is microsprinkler, with one maxijet per tree. The first year had irrigation challenges followed by very heavy snow which stripped limbs off the tree. The block had bloom in 1999 (fourth leaf) which were killed by a late spring freeze. The first harvest was in 2000, the fifth leaf. These extraordinary events impeded the ability to evaluate precocity of rootstocks. Today, the block has a very uniform canopy capable of sustaining 40 to 50 bin per acre yields of 90 count and larger fruit.

Significant findings: Rootstock scion performance

General Conlusions:

- Planting densities of 400 to 600 trees per acre, trained in an axe format (central leader, with disciplined removal of large limbs) can maintain production of superior yields of target fruit.
- The rootstock effect is most pronounced in the first five years of the planting and diminishes as the canopy fills its allotted space.
- Axe planting systems can improve production efficiencies by being able to utilizing newer spray application technologies and mobile work platforms.
- Higher density plantings can produce significant yields in the fourth leaf.
- OHxF rootstocks 40, 69, 87, 97 with Bosc, Bartlett and Anjou are successful in high density axe systems.

Rootstock specific conclusions:

- OHxF 87:
  1. is more precocious than the other stocks.
  2. should be planted at higher densities than other rootstocks.

3. can over-crop and become stunted.
4. can produce smaller fruit. Management practices (pruning, thinning and nutrition) can mitigate fruit size issues.

- OHxF 97:
  1. is more vigorous
  2. is more suitable to lower vigor sites and/or lower vigor cultivars.

Scion specific conclusions

Anjou:

- Central leaders of young trees on OHxF 87 can crop heavily, inducing the central leader to bend out of position or break. Fruit thinning can minimize the problem.
- Fruit tends to set in doubles and triples with OHxF 87. Detail pruning on well developed spur systems and additional fertilizer may be needed to grow large fruit.
- OHxF 87 with vigorous varieties such as Anjou may come into commercial production one to two years earlier than OHxF 69 or 97.

Table 1: Anjou production values by rootstock for 2000 crop

2000	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
69	932b	1898b	90ab	58b	119b	20b	59c	0.45b
87	1840a	3998a	96a	115a	250a	41a	61b	0.85a
97	865b	1697b	87b	54b	106b	19b	65a	0.38b

Table 2: Anjou production values by rootstock for 2001 crop

2001	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
69	1815b	3344ns	81ns	113b	209ns	40b	73ns	0.71ns
87	1934a	3762	85	121a	235	43a	71	0.77
97	1948a	3710	84	122a	232	43a	79	0.70

Table 3: Anjou production values by rootstock for 2002 crop

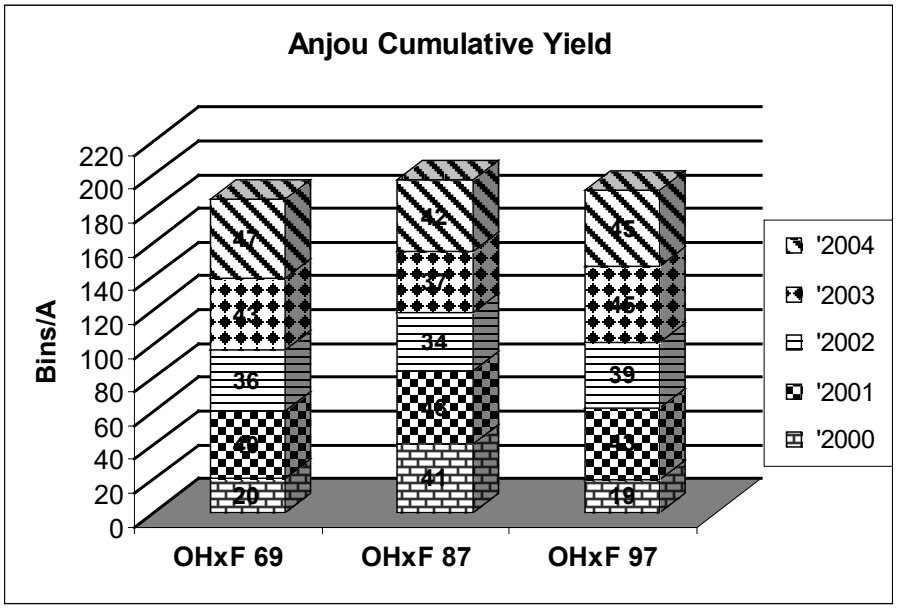
2002	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
69	1487ns	2811ns	83ns	93ns	176ns	36ns	86ns	0.49ns
87	1406	2616	82	88	164	34	82	0.48
97	1600	3003	82	100	188	39	87	0.52

Table 4: Anjou production values by rootstock for 2003 crop

2003	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
69	1904ns	3046ns	70ns	123ns	196ns	43ns	101ns	0.55ns
87	1690	2745	72	106	172	37	97	0.50
97	1951	3190	72	126	206	45	108	0.53

Table 5: Anjou production values by rootstock for 2004 crop

2004	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
69	2109ns	3558ns	74ns	132ns	222ns	47ns	120ns	0.50ns
87	1902	3137	73	119	209	42	112	0.48
97	2017	3343	73	126	196	45	128	0.45



Bosc:

- The best performing rootstocks are OHxF 69 and 87.
- OHxF 40 is not performing well with Bosc.
- Fruit size is excellent (peak size 70's) across all rootstocks.

Table 6: Bosc production values by rootstock for 2000 crop

2000	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	858ns	1823b	93b	54ns	114b	25ns	42ns	0.58b
69	1192	2550ab	94b	74	159ab	35	46	0.74a
87	1171	2681a	101a	73	168a	34	45	0.74a
97	1121	2322ab	91b	70	145ab	33	48	0.66ab

Table 7: Bosc production values by rootstock for 2001 crop

2001	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1055b	1810ns	75ns	66b	113ns	31b	56ns	0.53b
69	1414a	2366	74	88a	148	41a	58	0.69a
87	1323ab	2288	76	83ab	143	39ab	58	0.65ab
97	1269ab	2070	72	79ab	129	37ab	63	0.57ab

Table 8: Bosc production values by rootstock for 2002 crop

2002	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	933b	1620b	76ns	58b	101b	27b	74ns	0.36b
69	1205a	2073a	76	75a	129a	35a	73	0.47a
87	1254a	2179a	77	78a	136a	37a	72	0.50a
97	1010b	1600b	70	63b	100b	30b	80	0.36b

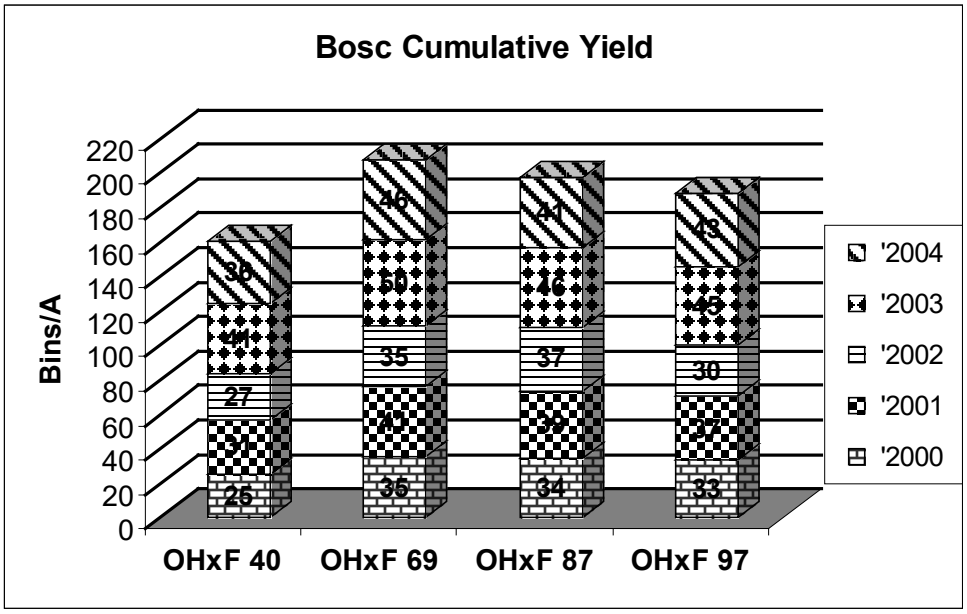
Table 9: Bosc production values by rootstock for 2003 crop

2003	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1377b	2354ns	75a	86b	147ns	41b	83ns	0.47ns
69	1714a	2739	70b	107a	171	50a	84	0.58

87	1548ab	2492	71b	97ab	156	46ab	85	0.52
97	1537ab	2519	72b	96ab	157	45ab	96	0.46

Table 10: Bosc production values by rootstock for 2004 crop

2004	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1226ns	2369ns	86ns	77ns	148ns	36ns	115ns	0.30ns
69	1554	2887	82	97	180	46	99	0.44
87	1395	2648	84	87	166	41	104	0.38
97	1462	2680	81	91	168	43	101	0.41



Bartlett

- No rootstock shows significantly superior performance, but OHxF 40 and 69 produces more pounds, better fruit numbers and better fruit size.
- OHxF 87 consistently has smaller fruit.

Table 11: Bartlett production values by rootstock for 2000 crop

2000	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1339ns	3336ns	110ab	84ns	208ns	39ns	45a	0.84ns
69	1384	3256	104b	87	203	41	49a	0.81
87	1060	2762	115a	66	173	31	37b	0.82
97	1069	2661	110ab	67	166	31	43ab	0.71

Table 12: Bartlett production values by rootstock for 2001 crop

2001	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1437ns	2351ns	72ns	90ns	147ns	42ns	62a	0.66ab
69	1474	2424	72	92	151	43	63a	0.67ab
87	1231	2226	80	77	139	36	48b	0.73a
97	1159	1981	76	72	124	34	56ab	0.58b

Table 13: Bartlett production values by rootstock for 2002 crop

2002	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1330ns	2520ns	84ab	86ns	163ns	41ns	70a	0.56ns

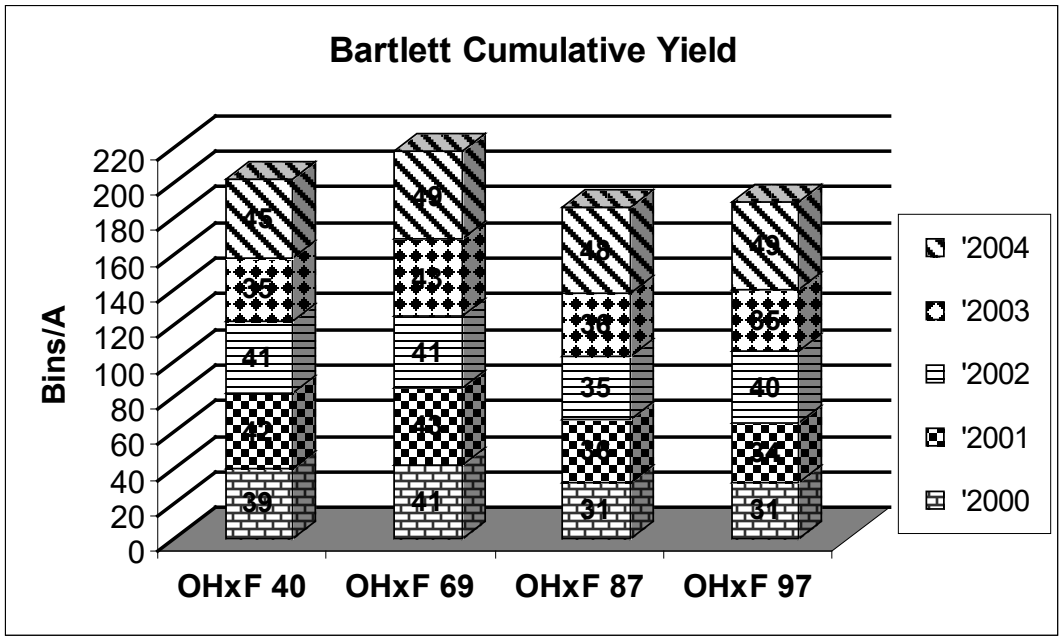
69	1407	2676	84ab	88	167	41	74a	0.55
87	1201	2622	96a	75	164	35	57b	0.60
97	1372	2481	80b	86	155	40	66a	0.59

Table 14: Bartlett production values by rootstock for 2003 crop

2003	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1194ns	2260ns	81ns	75ns	141ns	35ns	76ab	0.44ns
69	1472	2329	70	92	146	43	82a	0.51
87	1224	2348	85	77	147	36	67b	0.52
97	1175	1841	69	73	115	35	76ab	0.48

Table 15: Bartlett production values by rootstock for 2004 crop

2004	Pounds	PearNo	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa
40	1533ns	2380ns	68b	96ns	149ns	45ns	89ab	0.49ns
69	1654	2497	66b	103	156	49	94a	0.5
87	1621	2780	76a	101	174	48	79b	0.58
97	1658	2574	68b	104	161	49	89a	0.53



Significant findings: Irrigation

- In 2003, fruit size declined with volume of irrigation applied; a similar, but weaker trend was observed in 2004.
- Fruit sugars were significantly higher in fruit from trees grown with less water in both seasons.

- Irrigation technology is available to improve application efficiency. Very uniform application of water allows more manipulation of soil moisture levels with less risk to the crop.
- With excellent irrigation application efficiency, (no rain shadows or wet spots) pears may be grown successfully with less than 24 inches of irrigation per acre compared to standard applications of 30 to 40 inches.

Table 16: Anjou production values by irrigation treatment for 2003

2003	Pounds	# Pears	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa	% Cork	% Brix	Firmness
70%	1654ns	3149ns	84a	105ns	199ns	37ns	99ns	0.48ns	13ns	14.3a	14.8ns
80%	1552	2734	78ab	99	174	35	97	0.47	18	14.1a	15.2
90%	1848	2993	71b	118	191	42	102	0.53	14	13.3b	15.2

Table 17: Anjou production values by irrigation treatment for 2004

2004	Pounds	# Pears	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa	% Cork	% Brix	Firmness
70%	2083ns	3743ns	80a	130ns	234ns	46ns	111ns	0.53ns	1.2ns	13.9a	16.0ns
80%	2263	3877	75ab	141	242	50	115	0.56	3.3	14.1a	15.7
90%	2009	3346	73b	125	209	44	120	0.48	0.83	12.7b	15.9

Table 18: Bosc production values by irrigation treatment for 2003

2003	Pounds	# Pears	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa	% Brix	Firmness
70%	1551ns	2998a	85a	97ns	187a	46ns	84ns	0.52ns	13.9a	16.4a
80%	1660	2937a	78b	104	184a	49	90	0.53	12.8b	16.0a
90%	1544	2526b	72c	97	158b	45	87	0.51	12.2c	15.2b

Table 19: Bosc production values by irrigation treatment for 2004

2004	Pounds	# Pears	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa	% Brix	Firmness
70%	1434ns	2789ns	86a	90ns	174ns	42ns	103ns	0.40ns	13.1a	15.5a
80%	1428	2612	80b	89	163	42	108	0.38	12.0b	14.7ab
90%	1409	2646	83ab	88	165	41	105	0.38	12.1b	14.3b

Table 20: Bartlett production values by irrigation treatment for 2003

2003	Pounds	# Pears	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa	% Brix	Firmness
70%	1063b	2100b	87a	66b	131b	31b	71ns	0.43a	13.1a	18.4a
80%	1305a	2475a	84ab	82a	155a	38a	73	0.51b	12.4b	17.8b
90%	1266a	2194ab	76b	79a	137ab	37a	75	0.48ab	11.9c	17.5b

Table 21: Bartlett production values by irrigation treatment for 2004

2004	Pounds	# Pears	BoxSize	Lbs/Tree	Pear/Tree	Bin/A	Tcsa(cm^2)	Kg/tcsa	% Brix	Firmness
70%	1527ns	2437ns	71ns	95ns	152ns	45ns	83b	0.52ns	12.4a	17.4ns
80%	1556	2391	67	97	149	46	87ab	0.51	11.6b	16.8
90%	1617	2557	70	101	160	48	88a	0.52	10.9b	17.1

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Mike Davis and the Randy Smith farm crew have made this project possible.
WTFRC staff: Felipe Castillo, Mark Aldrich, Sandy Stone and Tory Schmidt have contributed to this project.

FINAL REPORT
WTFRC Project #PR-02-228 **WSU Project #13C-36556-7298**

Project title: Branch induction in pear trees with bioregulators

PI: Don C. Elfving, Horticulturist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Cooperator: Dwayne Visser, Agricultural Research Technologist II, WSU-TFREC, Wenatchee, WA

Contract administrator: Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691

Objectives:
1. Determine the effectiveness of cyclanilide for induction of lateral branch development in young, vigorous pear trees under orchard conditions.
2. Determine whether the effects of cyclanilide are enhanced by applying the material in combination with other bioregulator products that affect apical dominance, such as proprietary cytokinin/gibberellin mixtures.
3. Establish a trial to determine whether chemically induced branching in pear trees results in improved orchard production.

Significant findings:
• Cyclanilide is effective for induction of lateral branching in pear trees. Regardless of the method of application, the amount of active ingredient required to produce substantial branching is much less than needed for apple or sweet cherry.

• Cyclanilide produces lateral branching in the commercial pear cultivars 'Bartlett', 'Bosc', 'd'Anjou', 'Kalle' (Clapp's Favorite), and the ornamental pear cultivar 'Bradford'. Rootstock appears to have no effect on product efficacy on the scion cultivar.

• Sprays of cyclanilide at 5-20 ppm (mg/liter) produce excellent branching when applied after shoot growth has begun; comparable branching in apple or sweet cherry requires spray concentrations of 50-150 ppm.

• Cyclanilide applied in paint directly to pear buds on 1-year-old wood in early spring prior to budbreak has little effect on stimulating the treated buds to become active and develop into either lateral shoots or spurs the year of treatment but appears to translocate to the tips of the new growth, where it induces substantial lateral bud activity in a second growth flush that season.

• Cyclanilide demonstrates this translocated effect when applied to buds on last year's shoots, when sprayed on trunks or when applied as a trunk drench in either the fall after growth has stopped or in spring before budbreak.

• This translocated effect has only a small influence on the dormant buds that break in spring following a trunk spray or trunk drench treatment but produces a lot of secondary branching when the first growth flush produces a second flush in late June or early July. This actual interruption of apical

dominance and appearance of new branches can occur up to 8 months after the cyclanlide is applied as a trunk spray or trunk drench.

• Trunk drenching young 'Bosc' trees in fall or spring with as little as 50 mg (0.002 oz. active ingredient) of cyclanilide per tree increased lateral branching throughout the canopy the following season by several-fold.

• When cyclanilide increases branching in pear trees, bloom the following year is reduced because of the increased vegetative growth activity.

• In one trial, cyclanilide, Promalin, or the combination applied once to third-leaf 'Kalle' trees (2002) resulted in improved branching from cyclanilide but little effect from Promalin and produced a reduction in bloom the next season (2003, first year of flowering, little or no yield). The second year after treatment (2004), flowering was slightly improved by Promalin, but yield was not affected by any branching treatment.

Methods:
Over the three-year period of this project, trials were established in non-cropping pear trees to determine effects of various bioregulator products on both growth and fruiting behavior. All trials employed single-tree plots in randomized complete block designs.

Results and discussion:
During the course of this project, progress was made on all objectives. The following results and conclusions have been obtained during the three years of this project:

A. Dormant-season or green-tip applications of cyclanilide directly to pear buds.

1. Cyclanilide at up to 5,000 ppm was painted onto buds on 1-year-old wood of 'Kalle' and 'Bosc' pear trees before budbreak or at green-tip (2002). Painting buds had virtually no effect on inducing treated buds to become active and develop into shoots or spurs. However, in all cases, when a second flush of vegetative growth began in early July the painted treatments induced a substantial increase in budbreak and lateral-shoot development from newly formed buds on the first vegetative flush that season. This "translocated" effect occurred 3-4 months after the cyclanilide was applied. Further tests were carried out to evaluate the potential for the "translocated" effect to be exploited in a much more labor efficient way.
2. Flowering the second season following cyclanilide treatments (2003) was reduced due to increased vegetative development the previous year. In the next season (2004), there were no treatment effects on flowering.

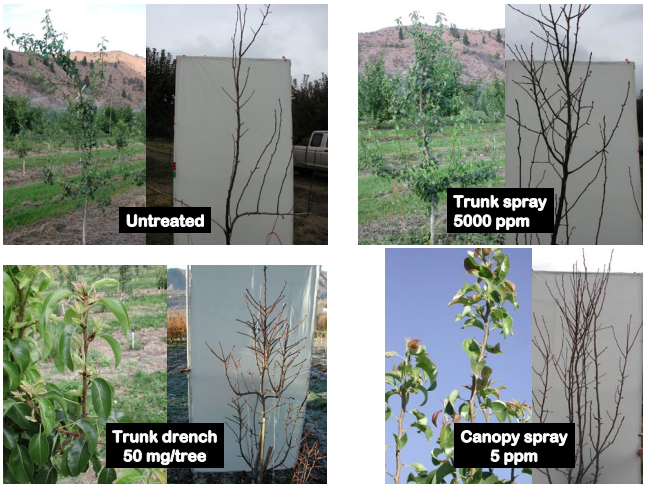
B. Dormant-season applications to trunks and soil of young pear trees.

1. Cyclanilide at up to 15,000 ppm sprayed onto trunks of young 'Bosc' and 'Kalle' trees in either October 2002 or early March 2003 produced little effect on the development of shoots in the first flush of growth in 2003 that originated from buds formed the previous year.
2. Despite the minimal effect on the first shoot growth flush, trunk-spray treatments substantially increased secondary branching in the second vegetative flush in 2003, in direct proportion to the amount of cyclanilide applied.
3. Trunk spray treatments were effective for inducing second-flush branching up to 8 months after the treatments were applied.

4. Again, flowering was reduced the year following the observation of growth effects (2004) due to increased vegetative activity in treated trees the previous year.
5. Based on the results of the trunk spray trials, a new trial was established in 2004 on second-leaf 'Bosc' trees. In this trial, 50, 100 or 150 mg of cyclanilide was applied as a trunk drench in 50 ml water per tree in either October 2003 or March 2004.
6. Branch development followed a similar pattern to that reported here for trunk spray treatments, in that there was little effect on the first vegetative flush but a very large increase in lateral branching in the second flush. The 50-mg/tree treatment proved to be more than adequate for branching; the 100- and 150-mg treatments produced excessive lateral-bud activity and poor shoot development.

C. Cyclanilide and Promalin sprays during the growing season.

1. Sprays of cyclanilide at 5-20 ppm were found to be very effective on 'Bosc' and 'Kalle' pear for inducing lateral branching in the second vegetative flush when applied in late May to late June, while the first vegetative growth flush was active but before the second flush had begun.
2. Promalin was effective on 'Bosc' but less so on 'Kalle' for inducing branching. There was no benefit on branch development from combining the two products.
3. Cyclanilide and Promalin spray treatments resulted in reduced flowering the following year in both cultivars due to increased vegetative activity.
4. In one study where cyclanilide and/or Promalin was sprayed on third-leaf 'Kalle' trees, there was no effect on flowering or yield two years later (first crop).



Summary:
Cyclanilide is very effective for branch induction in pear trees at low concentrations or amounts per tree. This highly sensitive response has made possible the discovery and initial exploration of the so-called "translocated" effect on branching, in which a cyclanilide application made even several months before shoot growth begins produces its effect on lateral branch development months later, after the first growth flush is complete. Interestingly, no matter how cyclanilide is applied, it has its major branching effect on the second vegetative flush; a second growth flush is typical in young, nonfruiting pear trees in Washington and begins normally in late June or early July. The mechanism by which cyclanilide itself or a metabolite is translocated and becomes effective for interrupting apical dominance several months later is unknown. However, this kind of response offers the potential for a very labor efficient method for treatment, should this product become registered for use. Applying cyclanilide as a trunk drench in fall or spring is fast, efficient, occurs when other work requirements are reduced, and requires no follow-up for the branching effect to occur. Important questions that still remain unanswered include how much cyclanilide is actually needed per tree to produce an optimal response, does the amount required depend on tree age or tree size, and what are the effects of repeat treatments in successive years on canopy development and the onset of production.

Promalin can be used for branching of pear trees; its effect is less strong than cyclanilide and there is no benefit on lateral branching by combining the two products together in a tank mix. Both products reduce flowering in the next growing season after treatment effects are observed. The significant increase in vegetative growth activity and stimulation of lateral buds appears to be the principal reason for this observation. There is no evidence to suggest that cyclanilide inhibits flowering directly.

In one trial in which both cyclanilide and Promalin were applied as sprays to third-leaf 'Kalle' pear trees, no effects were observed on flowering or production two years later (the first cropping year). This absence of an effect may be due in part to the fact that a single spray in one growing season only affected a portion of the total canopy volume. It may be necessary to apply repeat treatments during the canopy development phase of tree growth to produce a canopy capable of greater production. The potential for such branch induction treatments to reduce the need for heavy pruning, especially the heading-back pruning so typical in young, vigorous pear trees, may also allow a "calmer" tree to enter the transition to fruiting more rapidly. Studies should be carried out to assess the longer term effects of a systematic lateral-branch induction program on flowering and fruiting behavior in young pear trees.

Acknowledgments:
The assistance and support of the following persons and organizations are gratefully acknowledged: Jeff Cawood, Mike Cawood, Dick Cox, John Griffith, Daryl Harnden, Jeff Henry, Jeff Holmer, Dr. Chris Ishida, Barbara Lorenz, Chris Olsen, Dwayne Visser, Bayer Environmental Science, Cawood Orchards, Cox Orchards, Griffith Orchards, Holmer Orchards, Valent BioSciences, and Washington Tree Fruit Research Commission.

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Summary of total project costs:

Project duration: Three years
Total project costs: $31,512

FINAL REPORT

WTFRC Project No: PH-02-227 **ARS Project No.** 5350-43000-004-003T

Project Title: Quality and condition of winter pears as influenced by harvesting, handling, packing and storage

Principal Investigator: Stephen R Drake, Research Horticulturist
Organization: USDA, ARS, TFRL, Wenatchee, WA

Contract Administrator: Janet Tsukahira
USDA, ARS, PWA
800 Buchanan St.
Albany, CA 94710
JTsukahira@ars.pw.gov
510-550-6019

Objectives:

1. Determine time and type of atmosphere (oxygen, carbon dioxide, temperature) establishment in conjunction with different maturity levels to optimize storage of 'd'Anjou', 'Bartlett', 'Bosc' and 'Concord' pears. Emphasis on packed pears in both controlled atmosphere and modified atmosphere will be addressed.

2. Investigate alternative packing materials (paper type, pear floats) to aid in maintaining pear quality and the relationship of these alternative materials to various storage environments.

Significant findings:

- 'd'Anjou' pears wrapped in paper containing DPA (1000 ppm) not as scald resistant as pears wrapped in paper with ethoxyquin (1000 ppm).
- Paper impregnated with organic oils (3.0 %) do not prevent scald in 'd'Anjou' pears.
- 'Bartlett' pears in modified atmosphere packages maintain good quality and compare favorably with pears in controlled atmosphere storage.
- Ethoxyquin and Scholar can be used in combination as a pre-storage drench with no adverse reaction in fruit quality after long term storage.
- Quality was similar between pears packed in boxes (paper wrap + liner), or poly bags after long term storage.
- 'Concorde' pears are good candidates for 90 days of RA storage or 180 days of CA storage.

Results and Discussion:

'd'Anjou' pears packed after harvest, stored in regular atmosphere (RA) or controlled atmosphere (CA) storage for periods not exceeding 120 days, and wrapped in paper containing either 3 to 9% oil with Copper & Ethoxyquin (C&E) or Biox A or E maintained good quality. Storage of pears in paper containing diphenylamine (DPA) produced acceptable scores for appearance and finish, but some scald should be

anticipated. Use of DPA in the paper wrap beyond 120 days of storage resulted in excessive scald damage. Organic oils (lemon, clove, citronella) in pear wraps may produce some benefit for quality retention, but only for short storage periods. Quality of pears in wraps containing organic oils was approximately equivalent to use of dry paper but did not produce the quality of the standard industry wrap (3% oil + C&E). If pears are to be held in long-term CA storage (210 days), only paper wraps containing 3 or 6% oil + C&E should be considered for scald control. After loose storage in bins, the best quality pears were wrapped in paper containing 3% oil + C&E. Pears in paper containing DPA or DPA + Cu displayed excessive amounts of scald.

Commercially mature 'Bartlett' pears for were obtained from local commercial packing facilities (Blue Bird, Inc., Peshastin, WA and Blue Star, Inc., Cashmere, WA). In one study (Blue Star, Inc.), pears were packed in modified atmosphere bags and placed in boxes or packed normally with an individual paper wrap around each pear plus a polyethylene liner in the box. Boxed pears from both types of packaging were stored in RA storage at 33 F, for 30 or 90 days. In a second study (Blue Bird, Inc.), pears were packed normally and stored in both RA and CA storage for 45 or 90 days, or packed in modified atmosphere bags and stored in RA at 33 F. After 45 days, normally packed pears from both RA and CA were removed from storage, placed in modified atmosphere bags and returned to RA storage for an additional 45 days. Pears stored in modified atmosphere bags were superior in quality to normally packed pears stored only in RA storage and equal in quality to pears stored in CA for 90 days. The quality of pears held in modified atmosphere bags under CA conditions deteriorates after short periods of time (<45 days). Pears in modified atmosphere bags should be stored only in RA. After 90 days of RA storage the atmosphere in the MAP averaged 5 % oxygen and 5 % carbon dioxide.

Neither Scholar nor Ethoxyquin, applied as a pre-storage drench, influenced the peel color and firmness of pears stored for 4 months in CA. This lack of difference in color and firmness was evident in both packed pears and pears in poly bags after 90 days additional RA storage. Use of Ethoxyquin + Scholar combined reduced the green color (hue) in packed pears. This color difference for pears treated with Ethoxyquin + Scholar was greater than one color unit when compared to control fruit and would be visible to the consumer. Treating pears with Ethoxyquin alone resulted in some color loss, but differences were not significant. Time in RA storage, after bin storage in CA, resulted in lighter color, loss of green color and loss of firmness for pears packed in boxes or poly bags regardless of bin treatment. This change in color and loss of firmness was very pronounced between 30 and 60 days of storage for packed pears and to a lesser extent after 90 days of storage. Color and firmness values were similar between pears in packed boxes and pears in poly bags after 90 days of storage. Pears ripened for 7 days lost similar color and firmness in both packed boxes and poly bags. Pears in packed boxes or poly bags had an excellent firmness level (3 lbs., or less) for eating after 7 days.

'Concorde' pears were harvested at multiple maturities from three growers, stored in RA or CA and quality evaluated. 'Concorde' pears can be harvested (14/15 lbs) over a period of 14 days with no quality loss and be good candidates for either RA or CA storage. A 14-day delay in harvest resulted in an increase of one-box size. Regardless of harvest, 'Concorde'

pears can be stored in RA for periods not to exceed 90 days. RA storage beyond 90 days resulted in reduced finish, reduced pedicel condition and enhanced internal breakdown. Early harvest should be considered when RA storage is expected to exceed 90 days; however astringency (taste) may develop. Regardless of harvest, 'Concorde' pears can be stored for 180 days in CA with no quality loss, particularly if the CA is maintained at 1.5% oxygen and 1.0% carbon dioxide. Internal breakdown can be a major problem in CA if the carbon dioxide exceeds 1.0%. Low oxygen (<1.5%) is not recommended for 'Concorde' pears due to internal breakdown.

DO NOT MIX ETHOXYQUIN WITH CAPTEX = BURN

BUDGET:

Project title: Quality and condition of winter pears as influenced by harvesting, handling, packing and storage
PI: Stephen R. Drake
Project duration: 2002-2004
Current year: 2004-2005
Project total (3 years): $112,948

Year	**Year 1 (2002)**	**Year 2 (2003)**	**Year 3 (2004)**
Salaries	$29,178	$31,500	$13,700
Benefits	12,460	13,500	4,110
Supplies	5,000	1,500	2,000
Miscellaneous	100		
Total	$46,638	$46,500	$19,810
Total for all years: $112,948			

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Sincere appreciation is expressed to: Blue Bird, Blue Star, Stemilt, Independent, Hi Up and McDougal packing, for their cooperation, suggestions and interest in this project.

FINAL REPORT **YEAR 3/3**

WTFRC Project # PR-04-435 **WSU Project # 13C-3661-8367**

Project title: Phacidiopycnis Rot of Pears

PI: Chang-Lin Xiao, Assistant Plant Pathologist
Organization: WSU-TFREC, 1100 N. Western Avenue, Wenatchee WA 98801
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Cooperators: Dana Faubion, WSU Extension, Yakima

Objectives in 2002:
1. Conduct packinghouse surveys to determine the occurrence of Phacidiopycnis rot and rots caused by other pathogens in storage.
2. Test *in vitro* sensitivity of the fungus, *Phacidiopycnis piri,* to various fungicides in order to develop a fungicide program for control of this disease.
3. Evaluate the effectiveness and timing of postharvest treatments with fungicides (thiabendazole, TBZ and fludioxonil, Scholar) and biocontrol agents for control of Phacidiopycnis fruit rot.

Objectives in 2003:
1. Determine when *Phacidiopycnis piri* inoculum is available for fruit infection in the orchard.
2. Determine when fruits are infected in the orchard by the fungus *P. piri.*
3. Evaluate effects of selected fungicides on the fungus *P. piri.*
4. Evaluate effectiveness of fungicides and biocontrol agents to control Phacidiopycnis rot.

Objectives in 2004:
1. Determine when *Phacidiopycnis piri* inoculum is available for fruit infection in the orchard.
2. Determine seasonal susceptibility of pear fruit to infection by *P. piri* in the orchard.
3. Determine non-target effects of preharvest fungicides on *P. piri* in the orchard.
4. Evaluate effectiveness of pre- and postharvest treatments with fungicides in controlling Phacidiopycnis rot.

Significant findings during the past three years:
- A 2-year survey indicated that gray mold, Phacidiopycnis rot and blue mold are the three major postharvest diseases in d'Anjou pears grown in central Washington. In addition to gray mold and blue mold, Phacidiopycnis rot should be included as one of the targets for control of postharvest diseases in d'Anjou pears in the region.
- In addition to d'Anjou pears, Phacidiopycnis rot has also been found on Bosc and Comice pears.
- Phacidiopycnis rot causes three types of symptoms on pears: stem-end rot, calyx-end rot and wound-associated rot originating from infection of stem, calyx and skin wound of the fruit, respectively. Latent infection of stem and calyx of the fruit by the Phacidiopycnis fungus occurs in the orchard and symptoms develop during storage.
- *P. piri* was found to be associated with a canker and twig dieback disease of pear trees and widespread in pear-producing areas in the Pacific Northwest.
- The fruiting bodies (pycnidia) of *P. piri* containing viable conidia were available throughout the pear growing season, indicating that inoculum is likely not a limiting factor for fruit infection and rainfall or irrigation is more important to fruit infection since conidia of the fungus are water dispersed.

- None of the six fungicides (Flint, Ziram, Vangard, Procure and Dithane) were able to eliminate *P. piri* in twigs that were inoculated with the fungus two weeks before applications of fungicides, and pycnidia containing viable conidia were produced on majority of fungicide-treated twigs, indicating that once twigs infected by *P. piri*, fungicides are generally not effective to control the fungus in diseased twigs.
- It appeared that d'Anjou pear fruit near harvest were more susceptible to infection by *P. piri*, but infection can also occur during early growing season when environmental conditions were met.
- Sensitivity of *P. piri* to various classes of fungicides was tested in vitro. Captan, Dithane, Procure and Ziram at the label and $1/10^{th}$ label rates, and TBZ and Scholar at the label, $1/10^{th}$ and $1/100^{th}$ label rates were effective in inhibiting mycelial growth of *P. piri*. Flint was not effective in inhibiting mycelial growth. Captan, Dithane, Flint, Scholar, TBZ, Vangard and Ziram at the label and $1/10^{th}$ label rates were effective in inhibiting conidial germination of *P. piri*. Procure was effective at the label rate but less effective at lower than $1/10^{th}$ the label rate. Information on sensitivity to various pre- and postharvest fungicides helps us develop fungicide programs for control of Phacidiopycnis rot.
- Ziram applied at 2 weeks before harvest significantly reduced both incidence and severity (size of the decay) of Phacidiopycnis rot that originated from infection of wounds on the fruit surface, but did not provide a satisfactory control.
- TBZ, Scholar and BioSave were very effective to control Phacidiopycnis rot originating from infection of wounds by *P. piri*. *Cryptococcus laurentii* strain 87-108 reduced Phacidiopycnis rot by 40-50% compared with the non-treated control. Aspire was not effective in controlling Phacidiopycnis rot in this experiment.
- Fruit dipped in TBZ one day after inoculation had either no or a very low percentage of Phacidiopycnis stem-end and calyx-end rot after six months of storage, indicating that TBZ drench after harvest may be effective in reducing the infection in stem and calyx that established near harvest.
- Inoculated fruit developed more Phacidiopycnis stem-end and calyx-end rot as the time of TBZ application was delayed. This indicates that TBZ applied on the packing line is likely not effective to eliminate established infection in pear stem and calyx of non-drenched fruit (fruit not treated with TBZ shortly after harvest) that had been stored for a period of time before packing.

Methods:

In 2001 and 2002, decayed fruit were collected during repacking and repackaging operations during March to May. Approximately 60 decayed fruit from each grower lot were randomly sampled. Decayed fruit were categorized by casual agents through visually examining symptoms, presence of sporulation of the pathogen or isolating from the diseased tissue.

Inoculum availability of *P. piri* was monitored in two commercial orchards from early spring to harvest during the pear growing seasons in 2002 and 2003 and in one orchard in 2004. Samples of dying or dead bark and dead fruit spurs were sampled and examined for the presence of fruiting bodies (pycnidia or apothecia) of the fungus.

To determine susceptibility of pear fruit to infection by *P. piri* during the pear growing season, pear flowers during bloom and fruit at different growth stages were inoculated with the fungus in 2003 and 2004. Fruit were harvested and stored in air for decay evaluation.

To evaluate the efficacy of postharvest treatments with fungicides and biocontrol agents for control of Phacidiopycnis rot, surface-disinfested pear fruit were wounded and inoculated. Three biocontrol agents, the *Cryptococcus laurentii* strain 87-108, BioSave, and Aspire, and two fungicides, thiabendazole (Mertect) and fludioxonil (Scholar), were tested. After inoculation, fruit were tray-packed and stored at 32°F in air.

To evaluate effectiveness of preharvest fungicides in controlling Phacidiopycnis rot originating from stem and calyx infections, fruit were inoculated with spore suspensions of the fungus during the pear growing season. Part of the inoculated fruit was sprayed with Ziram (at 14 days before harvest), and the rest was not sprayed with Ziram. All fruit were harvested and stored at 32˚F in air for decay evaluation.

Experiments were conducted to evaluate whether postharvest treatments with TBZ can eliminate or reduce Phacidiopycnis rot originating from infections by the fungus of the stem and calyx of the fruit. The stem and calyx ends of the fruit were inoculated with spore suspensions of the pathogen. Fruit were kept in moist containers at room temperature overnight. Fruit were then stored in RA. Part of the inoculated fruit was treated with TBZ at 1, 10, 20, and 30 days after inoculation. Fruit were evaluated periodically for decay development.

In 2003 and 2004, experiments were conducted in two orchards to evaluate the effectiveness of five fungicides belonging to different classes in inhibiting production of pycnidia (fruiting bodies) of the fungus and the effects on survival of the fungus in diseased twigs.

Results and discussion:

Prevalence and incidence of Phacidiopycnis rot

A 2-year survey conducted in 2001 and 2002 indicated that gray mold caused by *Botrytis cinerea*, Phacidiopycnis rot caused by *Potebniamyces piri* (anamorph *Phacidiopycnis piri*) and blue mold account for an average of 60, 18 and 12% of the decay in the field bins (not drenched) (Table 1), respectively, and 25, 30 and 26% of the total decay on packed fruit in cardboard boxes, respectively (Table 2). The results indicate that gray mold, Phacidiopycnis rot and blue mold are the three major postharvest diseases on d'Anjou pears grown in Washington State, and decay control should target these three diseases in the region.

Table 1. Occurrence of postharvest diseases in the field bins sampled from mid-November 2001 to early January 2002.

Sampling period [1]	Phacidiopycnis rot	Gray mold	Blue mold	Mucor rot	Bull's eye rot[2]	Sprinkler rot[3]	Alternaria rot	Sphaeropsis rot
November	11.7	51.9	20.4	1.0	0	0.2	4.5	0
December	24.9	63.3	4.9	0.1	0	2.4	0.4	1.9
January	18.5	63.9	10.7	0	1.0	0.8	1.0	2.3
Mean	18.4	59.7	12.0	0.4	0.3	1.1	1.7	1.4

[1] Pears were stored in the field bins before packing. Samples were collected when packing was in operation. Ten, 13, and 10 samples of approximately 60 decayed fruit each were collected in November, December 2001, and January 2002, respectively. Each sample represents the fruit from one unique orchard.

[2] Bull's eye rot is caused by *Neofabraea* spp.

[3] Sprinkler rot is caused by *Phytophthora cactorum.*

Table 2. Occurrence of Postharvest diseases on packed fruit in the cardboard boxes sampled from March to May in 2001 and 2002[1]

Year	Production system	Phacidiopycnis rot	Gray mold	Blue mold	Mucor rot	Bull's eye rot[2]	Sphaeropsis rot	Others[3]
2001	Conventional	34.1	10.3	33.6	19.3	0.8	ND[4]	1.8
	Organic	22.8	35.7	23.5	1.4	7.4	ND	9.2
2002	Conventional	19.6	26.8	37.4	8.1	2.2	2.0	3.8
	Organic	42.2	25.7	8.2	0.5	7.4	4.5	11.5

[1] Pears were stored in cardboard boxes after packing. Samples were collected when repacking or repackaging was in operation. In 2001, 26 samples (6 organic and 20 conventional) of approximately 60 decayed fruit each

were collected. In 2002, 39 samples (13 organic and 26 conventional) of approximately 60 decayed fruit each were collected. Each sample represents the fruit from one unique orchard.
[2] Bull's eye rot is caused by *Neofabraea* spp.
[3] Include Alternaria rot, Aureobasidium rot, Cladosporium rot, and other minor fruit rots caused by unidentified fungi and yeasts.
[4] ND=not determined.

Inoculum availability of *Phacidiopycnis piri* in the orchard.

Inoculum availability was monitored in two commercial orchards in 2003 and one in 2004. Results from the two years were similar. At each sampling time during the pear growing season in 2004, all sampled trees had viable pycnidia present on either bark or fruit spurs and 60-100% trees had pycnidia of the fungus containing fresh conidia inside (Table 3). From April to September, 65-72% of the bark samples and 16-31% of the spur samples had viable pycnidia; 19-54% of sampled bark and 8-22% of sampled spurs had pycnidia containing fresh spores. The data indicate that viable inoculum of *P. piri* appeared to be available during the pear growing season from April to September. This may also indicate that inoculum is likely not a limiting factor for fruit infection and that rainfall or irrigation is more important to fruit infection since spores of *P. piri* are water dispersed.

Table 3. Inoculum availability of *Phacidiopycnis piri* in a commercial Anjou pear orchards in 2004.

Date	Type of samples[a]	% Trees with pycnidia	% Trees with viable pycnidia	% Trees with fresh spores in pycnidia	% Samples with pycnidia	% Samples with viable pycnidia	% Samples with fresh spores in pycnidia	% Samples with viable apothecia
Apr-04	Spurs	90	80	70	18	17	14	0
	Bark	100	100	100	67	66	54	7
Jun-04	Spurs	100	100	70	34	31	17	1
	Bark	100	100	100	73	69	19	11
Jul-04	Spurs	100	100	90	34	29	22	1
	Bark	100	100	90	79	72	38	13
Sep-04	Spurs	90	70	60	24	16	8	2
	Bark	100	100	100	70	65	32	8

[a] At each sampling time, 10 trees were arbitrarily selected, and 10 samples each of dying or dead bark and dead fruit spurs were collected from each of 10 selected trees. The trees were at least two rows apart between rows and five trees apart within a row.

Seasonal susceptibility of Anjou pear fruit to infection by *P. piri* in the orchard.

In 2003, inoculation of fruit was conducted five times during the growing season. All inoculated fruit and fruit from the non-inoculated controls were harvested and stored in air at 32°F. The results indicate that d'Anjou fruit near harvest were more susceptible to infection at stem and calyx by *P. piri* (Fig. 1). The results also indicate that because *P. piri* causes latent infection and decay develops slowly during storage, Phacidiopycnis rot likely occurs more commonly on fruit after an extended period of storage. The experiment was repeated in 2004. At the time of submitting this report, fruit is still in storage for decay development. Results will be forthcoming later when experiments have been terminated.

Sensitivity of *Phacidiopycnis piri* isolates to selected fungicides.

Fungicide sensitivity data of representative isolates from the Wenatchee River Valley, the Hood River area, and the Medford area have been presented in previous reports. Sensitivity of mycelial growth and conidial germination has been tested. Here is a summary of the results. Captan, Dithane, Procure and Ziram at the label and $1/10^{th}$ label rates, and TBZ and Scholar at the label, $1/10^{th}$ and $1/100^{th}$ label rates were effective in inhibiting mycelial growth of *P. piri*. Flint was not effective in

inhibiting mycelial growth. Captan, Dithane, Flint, Scholar, TBZ, Vangard and Ziram at the label and 1/10th label rates were effective in inhibiting conidial germination of *P. piri*. Procure was effective at the label rate but less effective at lower than 1/10th the label rate.

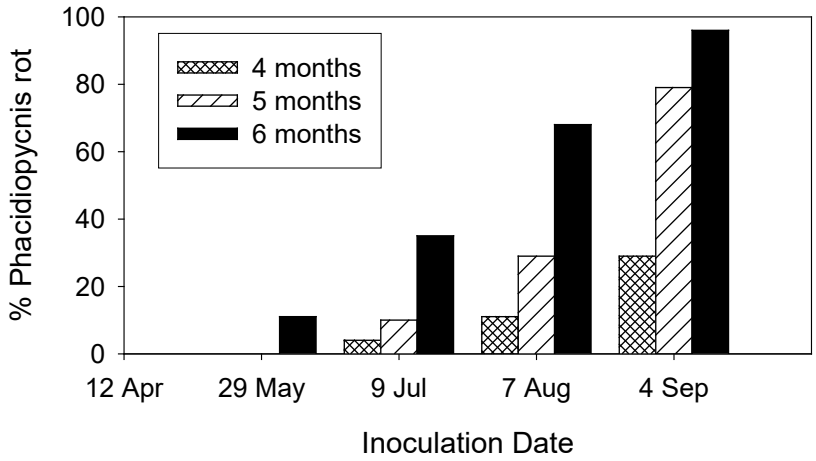


Fig.1. Susceptibility of d'Anjou fruit at different growth stages to Phacidiopycnis rot. Fruit were inoculated 5 times during the 2003 season and decay development was evaluated in 2004 (4, 5 and 6 months after the 2003 harvest).

Control of Phacidiopycnis rot originating from wound infections by *P. piri*.

Preharvest applications of fungicides to control Phacidiopycnis rot originating from infection of wounds on the fruit surface.

The trial was conducted in 2003. The data were presented in a previous report. Findings are summarized as follows. Ziram applied at 2 weeks before harvest significantly reduced both incidence and severity (size of the decay) of Phacidiopycnis rot that originated from infection of wounds on the fruit surface, but did not provide a satisfactory control (80% inoculated fruit developed Phacidiopycnis rot). Elevate and Procure applied at 2 weeks before harvest did not provide a satisfactory control in comparison with the non-treated treatment. Future research is needed to screen more effective fungicides and test effects of timing of applications on efficacy. Phacidiopycnis rot has the ability to spread from decayed fruit to the surrounding sound fruit in storage. Reducing decay incidence and severity (size of the decay) of Phacidiopycnis rot by preharvest fungicides would be beneficial in reducing secondary infections during the long-term storage of pears in the field bins.

Postharvest applications of fungicides and biocontrol agents to control Phacidiopycnis rot originating from infection of wounds on the fruit surface.

Experiments were conducted in 2002-03 and 2003-04. The first decay evaluation was done at two months after fruit inoculation. Scholar, TBZ and Biosave were effective in controlling Phacidiopycnis rot originating from infection of wounds of surface of the fruit (Fig. 2). No decay developed on fruit treated with TBZ and Scholar. *Cryptococcus laurentii* strain 87-108 reduced Phacidiopycnis rot by about 40-50% compared with the non-treated control. Aspire was not effective in controlling Phacidiopycnis rot in this experiment. The results indicate that a postharvest treatment of TBZ, Scholar or BioSave applied shortly after harvest would be effective in controlling Phacidiopycnis rot originating from wound infections by the fungus *P. piri*.

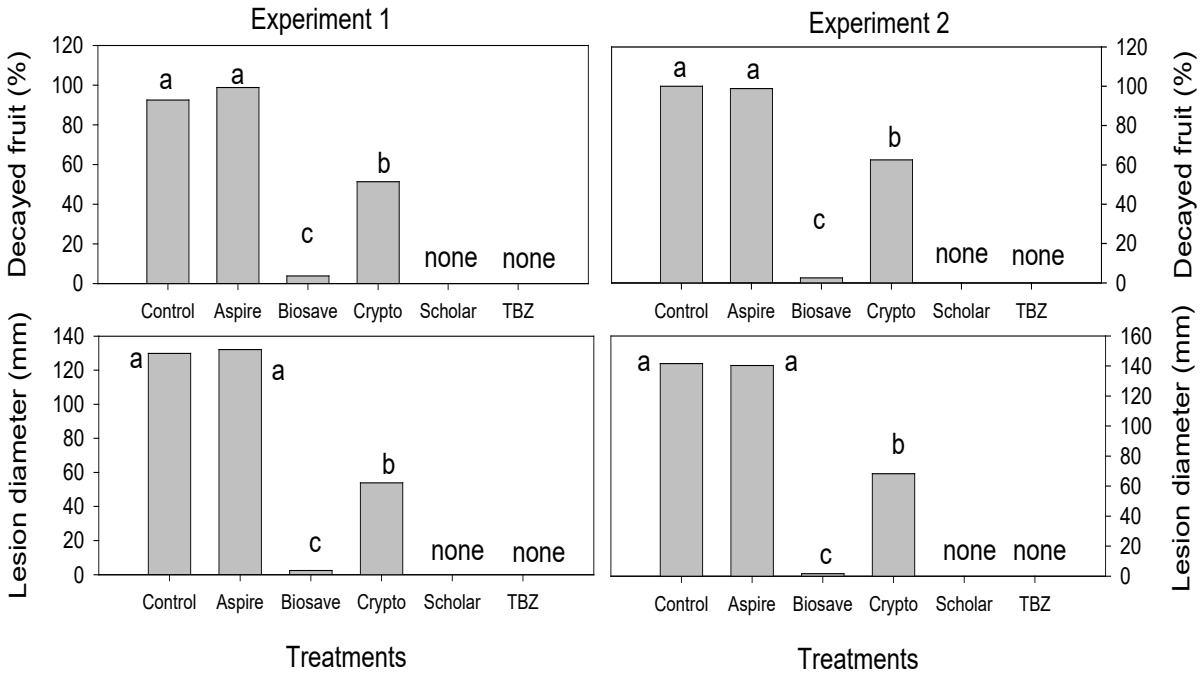


Fig. 2. Effectiveness of postharvest treatments with TBZ and three biocontrol agents, the *Cryptococcus laurentii* strain 87-108 (Crypto), BioSave and Aspire in controlling Phacidiopycnis rot originating from wound infections by *Phacidiopycnis piri* in 2003-04. Data of decay incidence after 3 months of storage are presented.

Effects of preharvest Ziram on Phacidiopycnis rot originating from infections of stem and calyx of the fruit.

The experiment was conducted in a research block. Fruit were inoculated with conidial suspensions of *P. piri* at three weeks before harvest, and Ziram was applied at two weeks before harvest. All fruit were harvested and stored in RA. Phacidiopycnis rot symptoms (stem-end rot or calyx-end rot) developed after three months of storage. By six and a half months after harvest, 66% of inoculated fruit that were not treated with fungicides before harvest developed Phacidiopycnis rot originating from infection at either stem or calyx of the fruit (Table 4). Ziram applied at 14 days before harvest reduced Phacidiopycnis rot by 32% compared with the non-fungicide treated control.

The experiment conducted in 2004 is still in progress for decay evaluation and results will be forthcoming after the experiment is completed.

Table 4. Effects of a preharvest application of ziram on Phacidiopycnis rot originating from infection of stem and calyx of the fruit.

Treatment	% of the fruit that developed Phacidiopycnis rot			
	12 Jan 2004 (4 months in storage)	11 Feb 2004 (5 months in storage)	11 Mar 2004 (6 months in storage)	26 Mar 2004 (6.5 months in storage)
No fungicides	3	25	54	66
Ziram at 14 days before harvest	3	16	36	45

Effects of timing of postharvest TBZ on Phacidiopycnis rot originating from infections of stem and calyx of the fruit.

Experiments were conducted in the 2002-03 and 2003-04 storage seasons. Results from the 2-year experiments were very similar. The results from one experiment conducted in 2003-04 are presented in Fig. 3. Inoculated fruit treated with TBZ at 1, 10 and 20 days after inoculation had significantly lower percentages of Phacidiopycnis stem-end rot compared with those that were either not treated with postharvest fungicides or treated with TBZ 30 days after inoculation. Significantly lower percentages of fruit treated with TBZ within 20 days after inoculation developed Phacidiopycnis calyx-end rot compared with the non-treated control. But TBZ applied at 30 days after inoculation did not significantly reduce Phacidiopycnis calyx-end rot compared with the nontreated control. The results from this study suggest that a postharvest drench with Mertect (TBZ) may be effective to reduce newly established infection in stem and calyx of the fruit. However, TBZ applied on the packing line is likely not effective to eradicate established infection in pear stem and calyx of non-drenched fruit that had been stored for a period of time before packing.

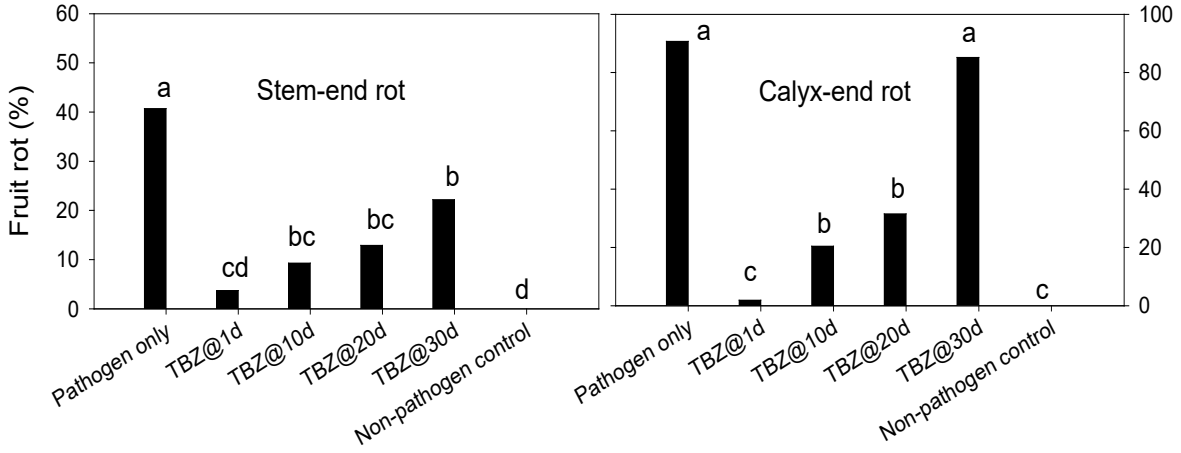


Fig. 3. Effects of timing of a postharvest treatment with thiabendazole on Phacidiopycnis stem-end and calyx-end rot in 2003-04. Inoculated fruit were treated with TBZ at 1, 10, 20, and 30 days after inoculation. Data represent the decay after five months of storage. Bars with different above them are significantly different.

Evaluation of Penbotec for control of Phacidiopycnis rot originating from wound infections.
In 2004, an experiment was conducted to evaluate Penbotec for control of Phacidiopycnis rot resulting from infection of wounds on surface of the fruit. The purpose was to determine whether a postharvest drench with Penbotec is effective to control wound infections. The experiment is still in progress at the time of submitting this research report. Results from this experiment will be forthcoming.

Effects of fungicides on production of fruiting bodies and survival of *P. piri* in diseased twigs.
In both 2003 and 2004, five fungicides belonging to different classes were evaluated for the effectiveness in inhibiting production of pycnidia (fruiting bodies) of the fungus and the effects on survival of the fungus in diseased twigs. The purpose of this experiment was to determine if *P. piri* has established in twigs or bark tissues, whether fungicide treatments in the orchard can control the

fungus in diseased twigs and inhibit production of fruiting bodies (pycnidia) of the fungus in diseased twigs. We found that none of these five fungicides were able to eliminate the fungus in diseased twigs and majority of fungicide-treated twigs were able to produce pycnidia containing viable conidia (Table 5). The results suggest that once twigs infected by *P. piri*, fungicides are generally not effective to control the fungus in diseased twigs.

Table 5. Effects of fungicides on the production of pycnidia and survival of *P. piri* in diseased twigs in 2004.

Orchard	Treatment[1]	% Twigs with viable pycnidia	% Twigs from which *P. piri* was recovered
ST 7	Nontreated	81.3	100
	Flint	87.5	100
	Ziram	93.8	100
	Vangard	81.3	100
	Procure	92.9	100
	Dithane	93.3	100
TFREC 8	Nontreated	87.5	100
	Flint	81.3	100
	Ziram	100.0	100
	Vangard	85.7	100
	Procure	81.3	100
	Dithane	87.5	100

[1] All twigs were freeze-treated and inoculated with mycelial plugs of *P. piri* on April 22. Twigs in the fungicide treatments were applied with fungicides at 2 and 4 weeks after inoculation. All twigs were removed from trees at 6 weeks after inoculation and subjected to examination for the presence of pycnidia and isolation of the fungus from diseased tissues.

Acknowledgments

I thank Robin Boal, Qingchun Liu, Yong-Ki Kim, Debbie Corey and Cindy Kohn for technical assistance; Bob Spotts and David Sugar for assistance in sampling in Oregon; and the Winter Pear Control Committee, the Washington Tree Fruit Research Commission and the WSU Agricultural Research Center Graduate Research Assistant Enhancement Program for support of this research.

Budget:

Project title: Phacidiopycnis Rot of Pears
PI: Chang-Lin Xiao
Proposed project duration: 2002-2004
Current year: 2004
Project total (3 years): $62,281
Current year request: $25,081

Current year breakdown:

Item	2002	2003	2004
Salaries [1]		14,000	14,560
Benefits (18%)		2,520	2,621
Wages [2]	8,000	2,500	2,500
Benefits (16%)	1,280	400	400
Supplies [3]	2,500	4,000	4,000
Travel [3]	1,000	1,000	1,000
Total	12,780	24,420	25,081

[1] Salary for a 0.49 FTE technical helper (Associate in Research).

[2] Wages for time-slip in summer and during harvest.

[3] Culture media, chemicals, Petri dish plates, cryogenic vials and other supplies for ultra-low temperature storage of fungal isolates, and fungicides. Cost of fruit bought from commercial orchards. Cell phone charges are allowed.

[4] We will be using a leased vehicle.

FINAL REPORT

Project Title: Codling moth control using CpGV—with and without mating disruption.

PI: Richard Hilton, Entomologist
Philip VanBuskirk, Professor
Oregon State University, Southern Oregon Research and Extension Center

Funding in 2004-05: 7,500

Significant findings:
There was no significant difference in codling moth control between *Cydia pmonella* granulosis virus, or CpGV, applied every one and a half weeks and conventional codling moth treatments (Imidan or Calypso) applied every three weeks.

The addition of CpGV to mating disruption significantly improved control of codling moth. However, there were few significant differences in the level of codling moth damage at harvest between the CpGV applied every three weeks versus every week and a half.

Results and Discussion:

Tests conducted during 2003 and 2004 in southern Oregon with CpGV products indicate that the formulations tested can provide a high degree of codling moth control when applied frequently. In the trial where mating disruption was not used, the CpGV treatment applied every week and a half resulted in control of codling moth that was significantly better than the check and not different from Imidan at 5 lb/ac or Calypso at 6 fl oz/ac applied on a three week interval (Table 1).

In comparing the results of the two trials conducted in 2004, one without mating disruption (Table 1) and one with mating disruption (Table 2), it appeared that addition of mating disruption may have provided little additional benefit to the overall control of codling moth. The fact that the block where mating disruption was employed was fairly small (1.2 ac.) and that codling moth pressure was extremely high likely resulted in the very poor performance of mating disruption alone, particularly when the codling moth population built up in the second generation. Given the differences between the two blocks which were used in these studies (e.g. Bartletts vs Packham's) it is not possible to make direct statistical comparisons but as the codling moth pressure was uniformly high it is notable that in both studies application of CpGV every week and a half resulted in very similar levels of codling moth damage, indicating that the use of mating disruption did not increase the level of control much beyond that provided by the CpGV alone.

One finding of interest was the lack of large differences between the CpGV applied every three weeks versus every one and a half weeks in the block with mating disruption. While there was a consistent trend to higher levels of damage with the three week interval, the differences were not statistically significant in any of the Bartlett harvest data, although larval entries were 30 % higher in the three week interval versus the shorter interval. In Anjous, harvested two and a half weeks later, the number of stings was significantly higher and both larval entries and exits were substantially increased in the longer interval treatment schedule. Taking the Bartlett and Anjou data together it does seem that the three week interval resulted in a gap in control which allowed a window for larval entry late in the season. Further testing is needed to determine whether the presence of mating disruption in the block could have played a role in limiting the differences between the two treatment intervals, particularly early in the season, during the first generation of codling moth, prior to the late season increase in the codling moth population.

Table 1. Control of codling moth in Packham's pear (in a block without mating disruption) under different treatment programs.

Treatment	% CM infestation at harvest (Aug. 16)		
	Stings	Larval Entries	Exits
Check	21.3 b	32.5 b	31.0 b
Cyd-X at 3 fl oz/A with Nu-Film 17 at 16 fl oz/A Applied every 10-11 days	68.0 c	6.75 a	3.0 a
Calypso at 6 fl oz/A Applied every 21 days	14.5 ab	6.0 a	2.0 a
Imidan at 5 lbs/A Applied every 21 days	12.0 a	5.5 a	1.5 a

Table 2. Control of codling moth in a block of Bartletts and Anjous with mating disruption and CpGV treatments.

Treatment	% CM infestation in Bartletts at harvest (Aug. 16)		
	Stings	Larval Entries	Exits
Check—with mating disruption and no CpGV	40.7 a	31.0 b	12.7 b
Cyd-X at 3 fl oz/A with Nu-Film 17 at 16 fl oz/A Applied every 10-11 days	67.8 b	7.5 a	1.0 a
Cyd-X at 3 fl oz/A with Nu-Film 17 at 16 fl oz/A Applied every 21 days	69.3 b	10.5 a	1.0 a

Treatment	% CM infestation in Anjous at harvest (Sept. 3)		
	Stings	Larval Entries	Exits
Check	18.0 a	35.0 b	9.7 b
Cyd-X at 3 fl oz/A with Nu-Film 17 at 16 fl oz/A Applied every 10-11 days	32.5 b	4.75 a	0.3 a
Cyd-X at 3 fl oz/A with Nu-Film 17 at 16 fl oz/A Applied every 21 days	48.0 c	9.25 a	1.25 a

FINAL REPORT

Project Title: Effects of new pesticides on natural enemies of pears

PI: Tom Unruh

No report submitted

CONTINUING PROJECT REPORT **Year 1/3**
WTFRC Project # PR-04-431

PROJECT TITLE: Biology and Management of Pear Pests
PI: David Horton
USDA-ARS, Yakima Agric. Research Lab., Wapato, WA
Horton@yarl.ars.usda.gov
COOPERATORS: Tom Unruh
Vince Jones
CONTRACT ADMIN: Janet Tsukahira, jtsukahira@pw.ars.usda.gov, (510) 559-6019

OBJECTIVES:
Project objectives are to determine if the predatory insect and spider fauna associated with orchard floor vegetation contributes to biological control in the pear tree, emphasizing biological control of pear psylla. This objective encompasses 3 questions to be addressed in this project:

1. Do predator species that are common in orchard floor vegetation also occur in the pear tree?
2. Which predator species that occur in trees and in orchard floor vegetation also feed on psylla?
3. How much movement is there between the orchard floor and the tree canopy by predators?

We used a cover crop composed of vetch, winter wheat, winter peas, and crimson clover to produce arthropod densities in the orchard floor sufficiently high to address project objectives. Control plots (grass understory) were included for comparison in some studies.

Activities for 2005:

1. Use egg white marker to mark ground cover arthropods (with V. Jones), and monitor dispersal between orchard floor vegetation and the tree canopy. Achieve better taxonomic resolution for marked lacewings and ladybeetles (shown in 2004 to move from floor vegetation into tree). See if marked predators are picked up on trees outside of the sprayed plots. Test whether it is possible to prompt predator movement into trees from the cover crop by killing the cover crop.

2. Develop soy milk marker for marking tree canopy arthropods, and monitor the reverse (i.e., from tree to orchard floor vegetation) movement (with V. Jones).

3. Address problems (see below) associated with using ELISA and PCR to detect pear psylla proteins or DNA in guts of predators (with T. Unruh), and continue with feeding assays.

SIGNIFICANT FINDINGS:

1. **Psylla and predator numbers in cover crop plots versus control plots**
   - Densities of adult psylla highest in cover crop plots (results agree with 2003 data);
   - Despite this, densities of psylla eggs and nymphs were not higher in the cover crop plots (agrees with 2003 data), and in fact counts were smaller in the cover crop plots;
   - Predator counts in trees were highest in cover crop plots (agrees with 2003 data), which may help explain the drop in psylla immatures in these plots;
   - Predator counts on orchard floor were substantially higher in cover crop plots than control plots (agrees with 2003 data).
2. **Composition and habitat preferences of various predator communities**
   - Five ladybeetle species were common in the pear orchard; 2 species showed strong habitat preferences (tree vs orchard floor vegetation);
   - Five green lacewing species were common in the pear orchard; 3 species showed strong habitat preferences;
   - A summary table for true bug, ladybeetle, and green lacewing taxa was developed to show in which habitats adults and immatures were collected (tree vs orchard floor vegetation), and to show which species are known to feed on pear psylla.

3. **ELISA and PCR to detect psylla proteins in guts of predators (with T. Unruh).** Objectives are (1) to use ELISA and PCR on ground-collected predators to determine whether they have fed on pear psylla (which would be evidence for dispersal between tree and orchard floor by predator); and (2) to rank predators as to their importance as psylla predators.
    - Predators were collected from orchard floor vegetation and the tree canopy over the duration of the summer, and have been placed in a freezer for storage;
    - We discovered that psylla honeydew produces positive ELISA and PCR scores, which potentially could lead to inflated estimates of psylla predation rates (i.e., predators that have not fed on psylla but that had contacted honeydew could score positive). The problem is being addressed.
4. **Marking of cover crop arthropods to monitor dispersal (with V. Jones)**
    - Protocols were developed for using egg whites to mark arthropods in cover crops;
    - Marking studies were conducted over a 5-week period; 1700 arthropods collected from tree and cover crop have so far been assayed using ELISA to detect marker; approximately 2/3 of total collected specimens remain to be assayed;
    - Between 0 and 80% (depending upon predator species) of predators collected in the tree canopy had the cover crop marker, which is strong evidence that several predator species move between orchard floor vegetation and the tree canopy.

METHODS FOR 2005:

Marker studies (with V. Jones). The same 3 cover crop plots that were used in the 2004 study will be used in 2005. The plots were replanted in autumn to a mix of winter peas, hairy vetch, red clover, and winter wheat. Each plot is 4 aisles wide x 60-80 feet long. The three plots will be sprayed with 20-30 gallons of marker (20% egg white in water) per plot. The solution is applied using a 25 gallon, 12 volt sprayer with 10 foot boom, attached to an ATV. A fourth plot (not used in 2004) will be used to develop protocols for spraying the tree canopy with a soy milk marker, for eventual use in monitoring movement between the tree canopy and the orchard floor.

Arthropods will be collected using the same methods as developed in 2004. A stiff rubber hose is used to dislodge the arthropods from vegetation onto sticky cards. The arthropods are collected from sticky cards using wooden toothpicks, and put individually into microcentrifuge tubes for storage, until they can be assayed for presence of the marker using ELISA (by V. Jones).

In 2004, plots were sprayed once per week for 5 weeks. Arthropods were collected from the tree and from the cover crop twice per week. We will use a similar protocol in 2005. Changes in 2005 will include better taxonomic detail for the green lacewings and ladybeetles, to resolve just what species of green lacewings (or, ladybeetles) actually move between orchard floor and tree habitats. Second, in 2004 only the trees within the cover crop plots were sampled. In 2005, we will also sample trees 5 aisles distant from the sprayed plots, to determine if predator movement of this distance can be detected. Third, we have tentative plans to treat one cover crop plot with herbicide, to determine if destruction of the cover crop prompts predators to increase their rates of movement into the tree canopy. This study may be delayed until 2006, depending upon availability of time in 2005.

ELISA and PCR to detect psylla in predator guts (with T. Unruh). Unanticipated concerns that contact with psylla honeydew may produce false positives in predators are being addressed by allowing predators to walk on honeydew-loaded leaves or by dabbing predators with honeydew, and then washing them before the ELISA or PCR is done. Preliminary results indicate that predators are generally "waterproof" and that honeydew does not stick to them. Results indicated that ELISA readings are equally negative in honeydew painted predators that either were or were not then washed. Early in 2005 we will complete these studies and include PCR assays with and without washing the specimens. We will also test factors associated with honeydew that may make it test positive, including honeydew age and presence of cast psylla skins in the honeydew. After we prove that we are not getting signals from externally applied honeydew, the field samples from 2004 (in freezer) and 2005 will be evaluated. Finally, it is possible that some predators may ingest honeydew (as seen in parasitic wasps). We will assay

our important predators by allowing access to droplets of honeydew, and then determining if predator feeding on honeydew leads to positive scores with ELISA or PCR. These tests will allow unbiased interpretation of results from field-collected predators.

RESULTS AND DISCUSSION (2004):

1. Psylla and predator numbers in cover crop plots versus control plots

Predator densities were considerably higher in the ground cover of the cover crop plots than the control (grass) plots (**Fig. 1**). The effect carried over to some extent in the tree canopy (**Fig. 2**). Both results are very similar to 2003 results. Adult psylla were (as in 2003) more numerous in the cover crop plots than the control plots (**Fig. 3**), possibly due to unknown microclimatic differences between cover crop and control plots, or to increased nitrogen levels in trees in the cover crop plots due to presence of legumes. Despite the higher adult densities, psylla egg and nymph densities were not higher in the cover crop plots (again, similar to 2003), and nymphs actually showed a numerical decline in the cover crop plots relative to the control plots (**Figs. 4-5**). The decline perhaps can be attributed to the higher densities of predators in the cover crop plots (**Fig. 2**).

Fig. 1: Ground cover (number predators per 10 sweeps)

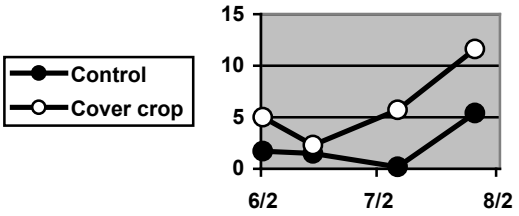


Fig. 2: Tree (number predators per beat tray)

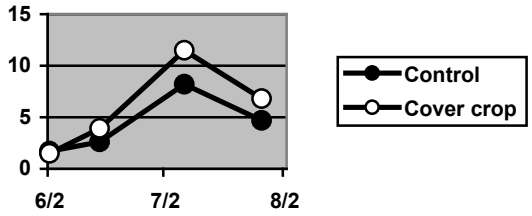


Fig. 3: Adult psylla per tray

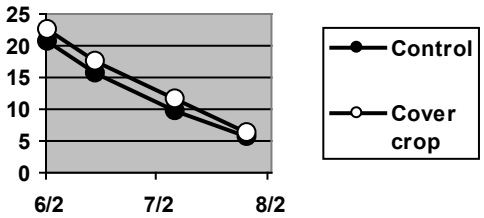


Fig. 4: Psylla eggs per leaf

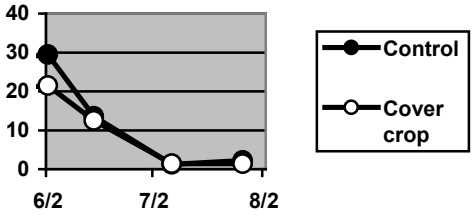
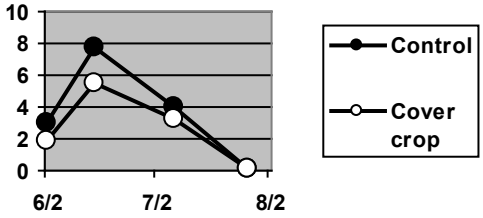


Fig. 5: Psylla nymphs per leaf



2. Composition and habitat preferences of various predator communities

The ladybeetle community (excluding small beetles such as *Stethorus*) comprised 5 common species. Based upon numbers collected in the two habitats, I assigned a crude index of preference (orchard floor vegetation vs tree) to each species (**Table 1**). Three species (*C. transversoguttata*, *C. septempunctata*, and *H. lateralis*) occurred fairly regularly in both habitats.

The other species preferred orchard floor vegetation (*H. convergens*) or tree canopy (*H. axyridis*). For green lacewings, 3 species showed habitat preferences (**Table 1**); preferences were very strong in 2 of these species. *Chrysoperla plorabunda* was very common in both habitats.

Table 1. Habitat preferences (tree vs orchard floor vegetation) of most common ladybeetle (excluding small-bodied species such as *Stethorus*) and green lacewing species. Preferences categorized as "Only" (indicates rarely or never found in other habitat), "Preferred" (indicates preference for habitat, but sometimes found in other habitat), or "Both" (occurred in both habitats). N indicates number of specimens collected.

	TREE			ORCHARD FLOOR			
	Only	Preferred	Both	Preferred	Only		N
LADYBEETLES							
Coccinella transversoguttata			■				73
Coccinella septempunctata			■				16
Harmonia axyridis		■					30
Hippodamia convergens				■			99
Hyperaspis lateralis			■				138
GREEN LACEWINGS							
Chrysopa nigricornis	■						35
Chrysopa oculata					■		104
Chrysopa coloradensis				■			10
Chrysoperla plorabunda			■				121
Eremochrysa sp.			■				10

Table 2. Summary of my collections for most common true bug predators, ladybeetles, and green lacewings; Moxee 2003-2004. Gray fill indicates species collected from that habitat (orchard floor vegetation or tree canopy); cross-hatching indicates species collected from that habitat, but only very rarely. Right-most column indicates whether predator will feed on psylla (from my rearing studies).

	Adults collected from:			Immatures collected from:		Feed on pear psylla?
	Orchard floor veg.	Tree		Orchard floor veg.	Tree	
TRUE BUGS						
Deraeocoris brevis	■	■			■	Y
Anthocoris tomentosus	■	■			■	Y
Anthocoris antevolens	▨	■			■	Y
Orius tristicolor	■	■		■	■	Y
Campylomma verbasci		■			■	Y
Geocoris sp.	■	▨		■		?
Nabis sp.	■	■		■		Y
LADYBEETLES						
Coccinella novemnotata				▨		?
Coccinella transversoguttata	■	■		■		?
Coccinella septempunctata	■	■		■		?
Harmonia axyridis		■		▨	■	Y
Hippodamia convergens	■	▨		■		Y
Hyperaspis lateralis	■	■				?
Stethorus sp.	■	■			■	N?
GREEN LACEWINGS						
Chrysopa nigricornis		■			■	Y
Chrysopa oculata	■			■		?
Chrysopa coloradensis	■	▨				?
Chrysoperla plorabunda	■	■		■	■	Y
Eremochrysa sp.	■	■				?

I have summarized in **Table 2** data from 2003 and 2004 for true bugs, green lacewings, and ladybeetles, indicating whether I collected adults and immatures, listing the habitat from which they were collected, and showing whether each species is known (from my rearing studies) to feed on pear psylla. A number of species were found as adults in both habitats; fewer species were collected from both habitats as immatures. The presence of immatures in a given habitat suggests that the predator used the habitat for reproductive activities. For many species, it still remains to be determined whether they potentially are important psylla predators.

3. ELISA and PCR to detect psylla proteins in guts of predators (with T. Unruh)

Predators were collected from both orchard floor vegetation and the tree canopy, to determine with ELISA and PCR whether they had been feeding on pear psylla. The data would help fill in some of the blanks in **Table 2**, but could also be used as an additional method to determine whether certain predator species move between habitats (i.e., as would be suggested if the psylla protein showed up in predators collected from the orchard floor). However, as noted above we are encountering some problems with the assays, as there is evidence that psylla honeydew hypothetically could lead to erroneous positive scores in insects that have not in fact fed on psylla. We are addressing the problem. Samples for 2004 will remain in the freezer until the problem is resolved.

4. Marking of cover crop arthropods to monitor dispersal (with V. Jones)

Protocols for the 2004 studies are summarized in **Table 3**, and consisted of an application of egg white made to cover crops (on Mondays), followed each Tuesday and Friday with vegetation samples (to confirm presence of the marker), arthropod samples from trees, and arthropod samples from the sprayed cover crops. A third vegetation sample was taken each Monday (following a weekend of irrigation) to determine if the marker disappeared following irrigation. The entire process was done on 5 occasions, beginning 5 July and ending the week of 9 August. Negative controls (i.e., arthropods known to be free of the marker) were collected from a stand of alfalfa ca. 500 m from the study orchard, and from a second pear orchard several miles away from the study orchard.

Negative controls were uniformly free of the marker (data not shown). Arthropod samples from the first 2 weeks (of 5 weeks total) of sampling have been processed by V. Jones for presence of the marker. Results (**Table 4**) are based upon 1716 assayed specimens, from the first 2 weeks of the 5 week study. The remaining specimens (approximately 60% of total collected) will be assayed this winter. Almost 100% of arthropods collected from the cover crop scored positive for the marker (**Table 4**). Of more interest, a fairly large proportion (0-80%, depending upon species) of predators and one pest species (*Lygus*) were marked, strongly supporting our hypothesis that there is substantial movement by certain arthropod species between the orchard floor and the tree canopy. These are quite striking results, and demonstrate the value of this new technology.

Table 3. Schedule of events in 2004 marking study; Moxee farm. Entire process was repeated 5 times between early July and early August.

Weekend	Monday	Tuesday	Wednesday	Thursday	Friday	Weekend
Irrigation	3rd vegetation sample*	1st vegetation sample			2nd vegetation sample	Irrigation
	Spray cover crop	Arthropod sample (tree)			Arthropod sample (tree)	
		Arthropod sample (cover crop)			Arthropod sample (cover crop)	

* Vegetation collected post-irrigation, but before Monday application of marker.

Table 4. Results of ELISA to detect egg white marker on predators and one pest species (*Lygus*) collected from cover crop or tree canopy; 4 collection dates July 6-16. Done in collaboration with V. Jones.

Arthropod	Number assayed	Number positive for marker	% positive for marker
COVER CROP			
Anthocoris tomentosus	61	57	93.4
Green lacewings*	20	20	100
Ladybeetles*	238	238	100
Damsel bugs (*Nabis*)	24	24	100
Deraeocoris brevis	70	69	98.6
Geocoris sp.	61	59	96.7
Orius tristicolor	318	310	97.5
Spiders	192	186	96.9
Lygus (pest)	249	240	96.4
Total for cover crop	**1233**	**1203**	**97.6**
TREE CANOPY			
Anthocoris tomentosus	248	53	21.4
Green lacewings*	12	7	58.3
Ladybeetles*	15	12	80.0
Deraeocoris brevis	98	19	19.4
Damsel bugs (*Nabis*)	2	0	0
Orius tristicolor	3	1	33.3
Spiders	96	14	14.6
Lygus (pest)	9	5	55.6
Total for tree canopy	**483**	**111**	**23.0**

*These are species' complexes (see Table 1); taxonomic resolution of marked lacewings and ladybeetles to be improved in 2005.

BUDGET:

Project title: Biology and Management of Pear Pests
PI: David Horton
Project duration: 2004-2006
Current year: 2005
Project total (3 years): $71,370
Current year request: $23,790

Item	Year 1 (2004)	**Year 2 (2005)**	Year 3 (2006)
Salaries[1]	10,000	**10,300**	10,600
Benefits (30%)	3,000	**3,090**	3,180
Time slip[2]	10,400	**10,400**	10,400
Total	23,400	**23,790**	24,180

[1] 33% GS-5 term technician. The technician will assist with sampling, collecting predators, sorting predators for shipment to V. Jones, and will help with the honeydew/ELISA studies.
[2] 130 days at $10.00 per hour. The person will assist with collecting and sorting predators.

Continuing Project **Year 1/3**
WTFRC Project #AE-04-428 **WSU Project #13C-3643-8366**

Project title: The importance of dispersal in biological control and IPM

PI: Vincent P. Jones, Associate Entomologist
Organization: WSU Tree-Fruit Research and Extension Center
Address, phone, e-mail: 1100 N. Western Avenue, Wenatchee, WA 98801; (509) 663-8181 ext. 273; vpjones@wsu.edu
Co-PIs and affiliations: Jay F. Brunner, WSU-TFREC
Tom Unruh, USDA-ARS, Wapato
Dave Horton, USDA-ARS, Wapato

Contract administrator: Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691

Objectives:
1. Determine the contribution of the ground cover to natural enemy populations and biological control that occur in pear.
2. Examine the area of influence ("active space") of a rose patch used to bolster parasitism of leafrollers.
3. Examine the movement of pests from areas of high population density to surrounding managed areas.

Significant findings:

- The marking procedure used for the ground cover worked exceedingly well; 97.6% of all insects collected in the ground cover were marked. In the canopy above the ground cover, we found an average of 23% of the predators were marked as having visited the ground cover.
- Our *Colpoclypeus florus* collections were very sparse this year due to low overwintering populations and overspray from the adjacent orchard.
- OBLR movement from post-harvest cherries appears to contribute significantly to the OBLR populations in adjacent apple blocks.

Objective 1. This year we set up plots at USDA's Moxee Experimental Farm to test whether we could detect the movement of pear psylla predators from the ground cover to the canopy of pear psylla infested trees. The various ground covers were planted in early spring, and the egg marker sprays began 10 June after predators were first detected and ended 9 August after plots became overgrown with lamb's-quarter. Applications of 20% egg solution at 20-25 gallons per plot were made at roughly weekly intervals with a weed sprayer mounted on a four-wheeler. Only the ground cover was treated.

Insect collections were made by beating foliage from the ground cover or trees over a sticky panel and picking off the insects thought to be psylla predators. Each insect was identified, placed separately into a micro-centrifuge tube, and tested for the presence of the egg marker.

Results*:* At the time of this report, we have processed about half of the samples. To this point, our data has been extremely clean, with 97.6% of the predator samples collected from the ground cover scoring positive. This means that the marker and the application method (using a four-wheeler mounted weed sprayer) are extremely efficient, which will reduce the possibility of false negatives (i.e., an insect originating in the ground cover and collected in the canopy but scoring negative for the mark) essentially to less than 2.5%.

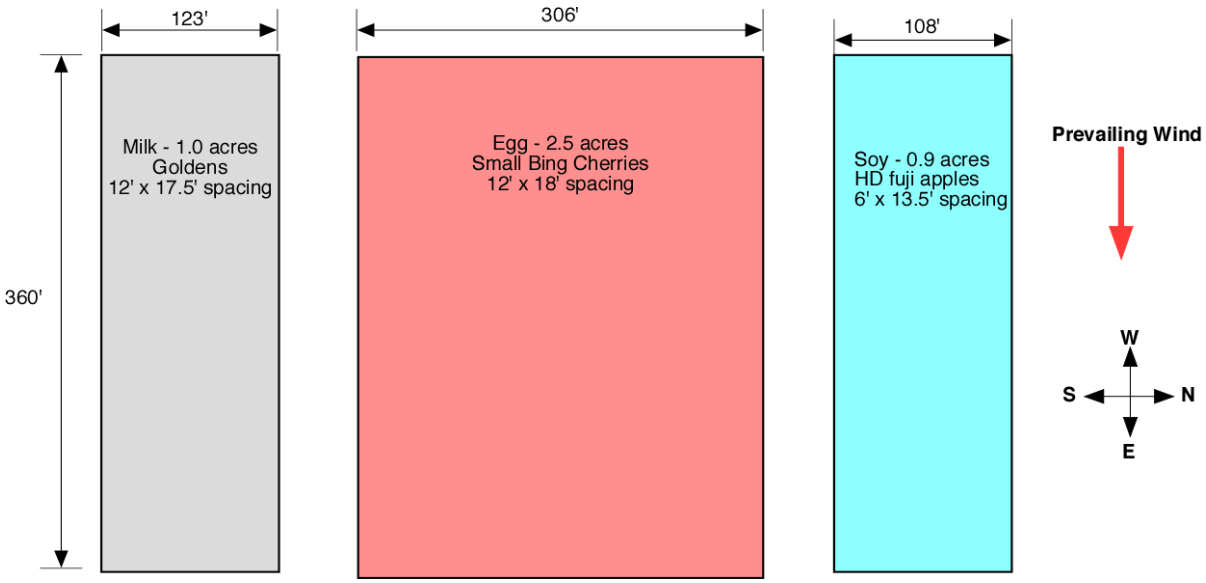
We found that 23% of all psylla predators collected in the canopy, across all dates, scored positive for egg. For the dates we have completed, *Anthocoris, Deraeocoris*, and spiders were the most abundant in the tree canopy and were marked 21.4, 19.4, and 14.6% of the time, respectively. There were small numbers of lacewings, coccinellids, *Lygus*, Nabids, and *Orius* collected, all of which had at least one representative marked; but until more samples are processed, percentages could be very misleading as to the movement from the ground cover. Even so, 80% of the coccinellids were marked, and 58.3% of the lacewings were marked. We will have more data processed by the presentations this winter.

Objective 2. We treated four rose patches with marker in the early spring to determine movement patterns of the OBLR parasitoid, *C. florus*, from the rose patches to the adjacent orchards. Unfortunately, this past year was a very poor season for *C. florus* and there were virtually no parasitoids collected from any of these orchards in the spring, even when we used sentinel larvae. In addition, at two of the sites, the grower applied leafroller sprays to the adjacent orchards in the spring, further affecting the parasitoids.

In the fall (when *C. florus* is at peak population levels), we placed sentinel larvae in an orchard adjacent to a treated rose patch and were able to find parasitoids in ≈80% of the leafroller retreats. These adults are currently being checked for markers, and that information will be available at the research review.

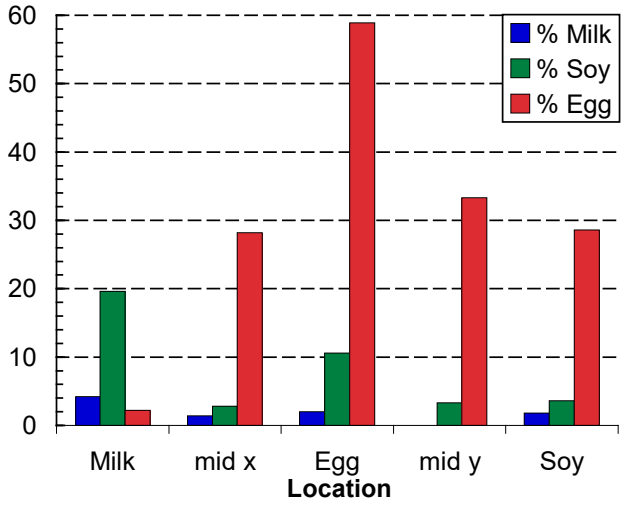
Objective 3. This year we ran a test to examine the dispersal of OBLR from cherries (after harvest) to adjacent apple blocks. The test was set up 29 July at a large orchard in Mattawa, WA. The trial consisted of 3 blocks: a central Bing cherry block, a block of large Goldens on the south side of the cherries, and a high density trellised Fuji planting on the north side of the cherry block (below). We treated the cherries with egg whites (10%), the Goldens with milk (20% + 0.5% glycerol + water softener), and the Fujis with soy milk (20%). All treatments were applied using an airblast sprayer at ≈150 gpa.

Four transects were set up with traps spaced at ≈50 feet apart in the Goldens and Bings and 41 feet apart in the high density Fuji planting. Over the four transects, a total of 15 traps were placed in the two smaller apple blocks, 5 in each of the untreated areas between the treated areas, and 30 in the egg treated area. For convenience, the untreated areas are named mid-X (between the



Goldens and Bings) and mid-Y (between the Bings and Fujis). Each was roughly 50 feet wide.

Results:
We captured an average of 6 OBLR moths per trap over the first 11 days after the markers were applied. The Golden block contributed little to the number of moths caught in either of the other two blocks. The moths originating in the Fuji block were picked up about 20% of the time in the Goldens and 10% of the time in the Bings (figure right). The largest number of insects that were marked came from the Bings. This location is twice as large as the other two plots but, even so, the percentage of insects marked with egg in the Fujis (where soy was sprayed) was ≈28% or 7-fold higher than the recovery of soy-marked moths in the same area. Our data clearly suggest that the Bings were a major source of OBLR for the adjacent apple blocks.



The relatively low level of recovery of milk and soy-marked insects is likely the result of the grower applying Raynox in both blocks of apples 3 days after the markers were sprayed. In the lab, we have found Raynox to interfere with our ability to detect the mark. In the field, we think that it may "seal" the marker onto the leaves and reduce the probability that insects that walk on the residue will acquire the mark. The egg section was not treated with the Raynox so insects flying into that area and walking across the foliage would acquire the egg mark in much greater numbers than the other two areas where insects walking on the foliage would only have been able to acquire the mark for the 3-day period before the Raynox was sprayed.

Next year we will make sure that none of the blocks are treated with Raynox over the test period, and we also plan to investigate areas where abandoned (or untreated) blocks are adjacent to managed blocks. We will test for both CM and LR movement patterns, depending on the populations in each block.

Budget:

Project title: The importance of dispersal in biological control and IPM
PI: Vincent P. Jones
Project duration: 2004-2006 (3 years)
Current year: 2005
Project total (3 years): **$**138,070
Current year request: **$**46,134

Item	Year 1 (2004)	**Year 2 (2005)**	Year 3 (2006)
Salaries[1]	20,487	**21,306**	22,158
Benefits (30%-yr 1; 34% yrs 2-3) [2]	6,146	**7,244**	7,534
Wages[3]	11,000	**11,000**	10,000
Benefits (16%-yr 1; 11% yrs 2-3)	1,760	**1,210**	1,100
Supplies[4]	3,200	**3,200**	3,200
Travel[5]	3,200	**3,200**	3,200
Total	45,793	**47,160**	47,192

[1] Callie Eastburn, Associate in Research (.50 FTE).
[2] Benefits – increase from year 1 to years 2 and 3 is due to the increase in health insurance benefit contributions WSU makes on behalf of the employee.
[3] Time-slip employees.
[4] Lab supplies. Cell phone charges are allowed.
[5] Travel to research plots.

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project #PR-03-341 **WSU Project #13C-3643-4385**

Project title: Development of areawide organic insect pest management in pear orchards

PI: John E. Dunley, Associate Entomologist

Organization: WSU Tree Fruit Research and Extension Center

Co-PIs and affiliations: Tara M. Madsen, Associate in Research, WSU-TFREC;
Bruce Greenfield, Agricultural Research Technologist III, WSU-TFREC

Cooperators: Peshastin Creek Growers Association

Contract administrator: Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691

Objectives:

1. Replace conventional pest management practices with organic practices.
   a. Organic insect pest management is the primary management strategy.
   b. Insect growth regulators (or other selective materials) are used where organic practices do not provide acceptable control.
2. Document the effects of different pest management strategies (organic, soft, conventional) on pest densities and crop damage.
3. Document the effects of different pest management strategies (organic, soft, conventional) on densities of natural enemy species.
4. Document costs of different pest management strategies (organic, soft, conventional) on costs of pest control programs.

Significant findings:

- Organic and soft pest management strategies worked very well on an areawide basis.
- Pear psylla management was good, despite high pressure relative to 2003.
- Spider mite and grape mealybug densities were low in 2004, as occurred in 2003.
- Codling moth control was very good; pressure was greatly reduced from 2003.
- Organic pest management is limited by the control of pear rust mite.
- Natural enemy populations did not increase significantly until late season, as occurred in 2003.
- Costs were variable, and differences in programs were again not statistically significant.
- Communication of monitoring data was increased via the Web.
  - Sampling frequency, area, and precision were increased.
- More organic and soft tactics were incorporated into conventional programs.

Methods:

The project area is comprised of pear orchards in the Peshastin Creek valley of central Washington State. Using GPS mapping we determined that, of the approximately 300 acres of tree fruit in the valley, 230 acres were actively sampled in the 2003 season. Sampling areas were identified in March with the input of the growers; each sample site was identified to correspond to actual management blocks rather than to areas arbitrarily defined by research. We established 41 sample sites, doubling both the sampling precision and the coverage over the same area as was sampled in 2002. A portable GPS

unit was used to plot block boundaries, and GIS maps were used in conjunction with aerial photos during sampling.

Orchard management types were defined as Organic, which used certified organic management practices; Soft, which used organic techniques when possible but allowed the use of IGRs and other selective pesticides; and Conventional, where organophosphates and other non-selective insecticides (e.g., Agri-Mek) were used. While Conventional orchards are not part of the Peshastin Creek Areawide Organic Project, they are included in our research sampling for comparison. Of the acreage sampled, 38 acres were Conventional, 100 acres were Soft, and 91 acres were Organic.

Insect pest and natural enemy populations were sampled weekly. Sampling for adult pear psylla and predators began in late March as the psylla population began increasing due to migration from the surrounding vegetation into the orchard. Beating trays were used to sample adult psylla and natural enemies; 25-tray samples were made per sampling block, distributed throughout. Sampling for pear psylla eggs and nymphs, as well as other small pests such as twospotted spider mite, European red mite, pear rust mite and grape mealybug, was initiated in early April. During the first month of sampling, before foliar expansion, 10 fruit spurs were collected from each block and examined under magnification in the lab. Between 13 May and 5 June, 50 leaves from developing fruit spurs were collected from each block. Beginning on 11 June, summer sucker growth had progressed enough to allow two separate samples of 50 leaves to be collected from each block, one from shoots in the upper canopy and one from the lower canopy. Five leaves were pulled from each shoot to obtain a broad distribution of leaf ages: the basal, the terminal, and three mid-shoot leaves. All leaf samples were brought to the lab, and a leaf-brushing machine was used to brush insects and eggs onto a glass plate. Pests were counted over half the surface area of the plate, although rust mites were counted over 5% of the surface.

Codling moth monitoring began at the end of April. A delta trap with pheromone lure was hung in each block in the upper third of a tree (at least one trap per 10 acre). In blocks where mating disruption was being used to manage codling moth 10X lures were used, and 1-mg lures were used in those blocks not using mating disruption. Lures were changed initially every four weeks and then every three weeks as temperatures increased. All traps were checked at least once per week. Temperature recorders were deployed at 12 locations through the valley. Temperature data were imported into a multi-pest degree-day calculator and used to estimate codling moth degree-days, based on a codling moth degree-day model.

Sampling for adult pear psylla, immature and small pests, and predators continued until harvest (the beginning of September), and codling moth was then monitored for an additional three weeks to ensure complete coverage of the flight. Fruit damage was assessed twice during the season, and pack-out records will be used to make a final evaluation of insect damage. In the first two damage evaluations we examined fruit for codling moth damage. Pack-out records will evaluate damage from other insects, as well as general fruit quality.

Codling moth damage evaluations were conducted each generation. For each sampling block, 50 trees were randomly chosen (border trees were excluded), and 10 fruits from the lower canopy and 10 fruits from the upper canopy of each tree were examined (1000 fruits sampled per block). The first damage evaluation was timed to fall between approximately 900 and 1100 codling moth degree-days, at which point first generation codling moth larvae were developing and damage was visible. The second codling moth damage evaluation was done immediately before harvest, when damage from the second generation was visible.

Results were analyzed by analysis of variance. Data were transformed to achieve normality using Box-Cox (x+1) transformations. Tests of mean separations were conducted using Fisher's

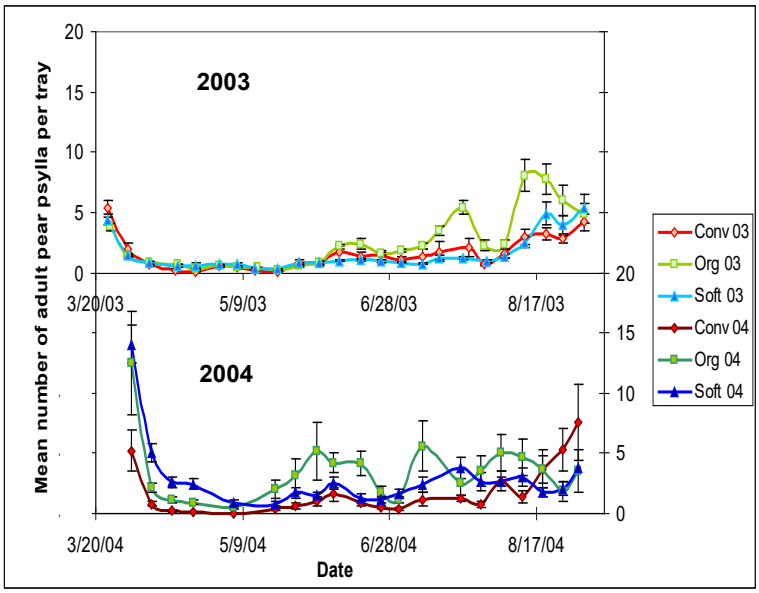
Protected Least Squares Differences, both within sample dates and averaged over specific time periods (prebloom, first generation, etc.).

Project growers provided their spray records for the season at harvest. These records were used to verify the assignment of blocks into the three management categories. Spray records will also be used to calculate cost per acre of the different management types. To improve the communication between our lab and members of the Peshastin Creek Growers Association and to provide the growers with a timely monitoring service, the following website was established: (http://entomology.tfrec.wsu.edu/pearent/pcg.htm). Clickable maps (http://entomology.tfrec.wsu.edu/pearent/pcg%20map.htm) indicating the sampling areas were linked to charts showing pest monitoring information, which was updated weekly. Other information, including notes about the project, sampling, and management recommendations, was included.

Results and discussion:

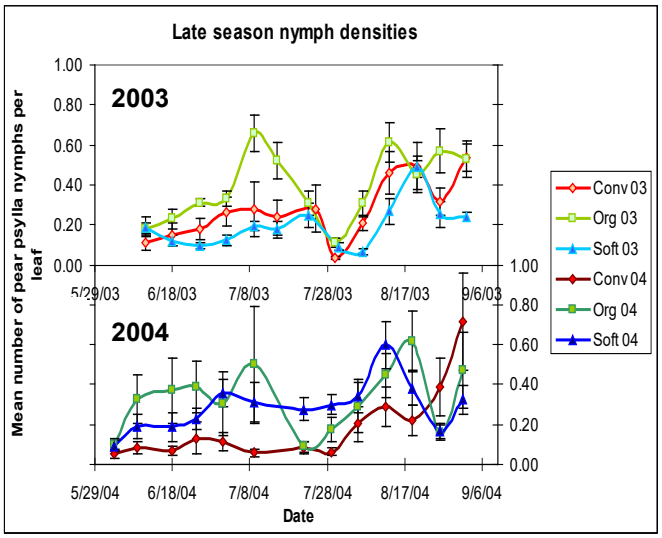
Results from the third year of implementation (second year as a funded project) of areawide organic and soft pest management were again positive. Densities of pear psylla, historically the key pest in the Peshastin Creek area, were much the same in all three programs in 2004 relative to 2003 but were still greatly reduced from 2002. No particular patterns among management programs were seen in densities of adult pear psylla in 2004 (Fig. 1).

Fig. 1. Adult pear psylla densities, 2003 and 2004.



Densities of pear psylla nymphs were higher in the Organic blocks than Soft blocks in the early season, although neither was different from Conventional blocks (Fig. 2). There was an increase in pear psylla nymphs near harvest, as was found in 2003; however, this year the increase occurred in all three programs.

Fig. 2. Pear psylla nymph densities, late season 2003 and 2004.



The successful management of high codling moth densities in 2003 led to reduced pressure in 2004 (Fig. 3). Codling moth density remained very low throughout the 2004 season, and fruit damage was very low in all programs (Table 1). This success is expected to continue into 2005, and codling moth control tactics are likely to be reduced next season.

Figure 3. Mean codling moth trap by management program, 2003 and 2004. Codling moth density throughout the project area was greatly reduced in 2004.

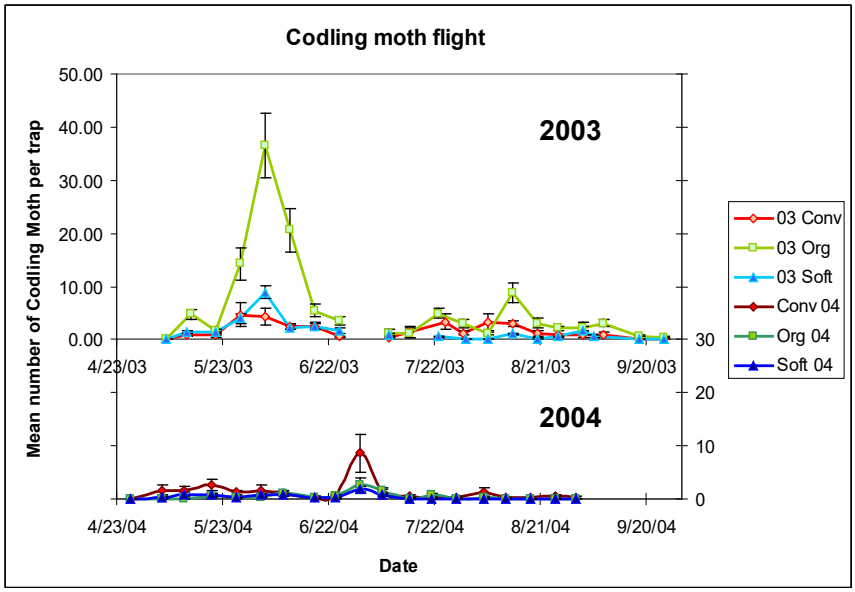


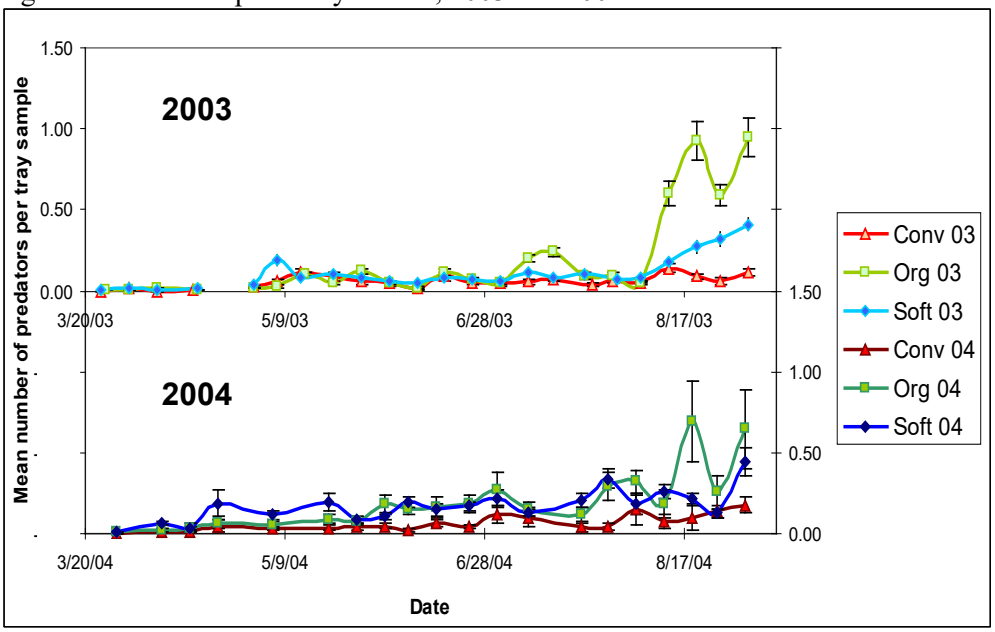
Table 1. Percent codling moth-infested fruit at harvest, 2003 and 2004. The relationship between codling moth trap catch (for each program and for each generation) and codling moth fruit infestation.

	2003	2004
Conventional	0.54%	0.01%
Soft	0.01%	0.03%
Organic	0.73%	0.10%

Twospotted spider mite densities were very low throughout the Peshastin Creek Valley and have yet to present a problem. Grape mealybug densities were also very low in 2004, although it was found more frequently than 2003. Pear rust mite was less problematic than in 2003; however, limitations in control options will continue to maintain this pest as a significant threat.

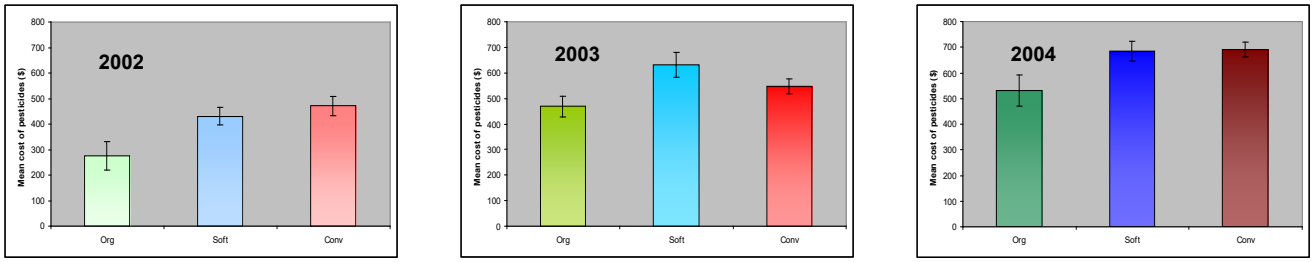
Predatory insect densities followed very much the same patterns in 2004 as were found in 2003, although overall densities were somewhat increased in all programs (Fig. 4). Predator densities were lowest throughout the season in the Conventional program. Densities were also low in the Soft and Organic blocks; however, there again was a significant increase in late season predator populations in the Organic blocks. The increase in both years followed the late season increase in pear psylla in those programs.

Fig. 4. Densities of predatory insects, 2003 and 2004.



Program costs over the three years of the project have shown no significant differences among program costs (Fig. 5). Additionally, there were no differences among programs in numbers of applications or numbers of trips through the orchard, either in 2003 or 2004.

Figure 5. Mean insecticide costs by program, 2002-05.



In summary, the implementation of organic and near-organic pest management on an areawide basis has been successful, relative to conventional programs, for three years. While pests can be controlled using available chemical tools (with perhaps the exception of pear rust mites), an increase in biological control has not yet been observed. The late season increase in predators seen in Organic blocks may be an indicator that biocontrol may take several years to establish. Alternatively, the correlation with pear psylla increase this year may mean that next year the predators will again decrease as pear psylla is controlled in the early season. Further study is necessary to address this critical issue. Nevertheless, this project demonstrates that insect pest management is not a barrier to implementing organic practices.

Budget:

Project title: Development of areawide organic insect pest management in pear orchards
PI: John Dunley
Project duration: 2003-2005
Current year: 2005
Project total (3 years): $80,718
Current year request: $27,441

Item	Year 1 (2003)	Year 2 (2004)	**Year 3 (2005)**
Salaries[1]	8,150	9,155	**9,210**
Benefits (27%-yrs 1 and 2; 34%-yr 3)	2,200	2,472	**3,131**
Wages[2]	11,000	11,000	**11,000**
Benefits (16% - yrs 1 and 2; 11% - yr 3)*	1,760	1,760	**1,210**
Supplies[3]	1,350	1,350	**1,350**
Travel[4]	1,540	1,540	**1,540**
Total	26,000	27,277	**27,441**

[1] A portion of the salary for Bruce Greenfield's Agricultural Research Technologist position.
[2] Time-slip wages.
[3] Supplies include pheromone traps and liners, beating trays, opti-visors. Cell phone charges are allowed under this grant.
[4] Travel: local travel to research plots only.

*Benefit rate increase is due to the increase in health insurance benefit that WSU contributes on behalf of the employee.

CONTINUING REPORT **YEAR 2/3**
WTFRC Project #PR-03-342 **WSU Project #13C-3643-4431**

Project title: Biological control in areawide organic and "super-soft" pear orchards
PI: John E. Dunley, Associate Entomologist
Organization: WSU Tree Fruit Research and Extension Center
Co-PIs and affiliation: Tara M. Madsen, Associate in Research, WSU-TFREC; Bruce Greenfield, Agricultural Research Technologist III, WSU-TFREC, Wenatchee, WA

Contract administrator: Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691

Objectives:
1. Examine the effects of endemic biocontrol in areawide organic pear pest management using direct measurements by exclusion and inclusion cages.
2. Examine the effects of endemic biocontrol in areawide organic pear pest management using indirect measurements for determining predator densities.
3. Determine the potential natural enemies in vegetation surrounding the areawide organic pear project.

Significant findings:
- The cumulative totals of predators collected from Conventional, Soft or Organic environments (orchard plus surrounding vegetation) were not significantly different.
- Significantly more biocontrol agents were present in native vegetation adjacent to orchards.
  - Organic orchards had significantly more natural enemies in surrounding vegetation, followed by Soft orchards, then Conventional.
- There were no significant differences between natural enemy densities within orchards under the three IPM regimes, although Soft blocks generally contained more natural enemies.
- More natural enemies occurred 5 m into Soft orchards than into Organic orchards, followed by Conventional orchards.
  - No differences occurred 25 m and 50 m into the orchards.
- Natural enemy densities increased in the late season in all orchard programs.
  - Increase was greatest in Organic orchards.
  - Increase was correlated to an increase in pear psylla densities.
- Predation of sentinel prey was not significantly different among programs.
  - Predation was significantly higher outside of orchards than within.

Methods:
To monitor predator densities in pear orchards and surrounding vegetation and examine biological control of pear orchard pests, nine transects were established in the Peshastin Creek valley in central Washington State. Within the entire project, natural enemy populations in the pear orchards were monitored weekly, beginning in late March and continuing into September, across the entire project area in 2003 and 2004. Monitoring was conducted in 41 plots, from two to ten acres each (5.5 acres on average). Beating trays were used to sample natural enemies at a rate of 25 trays per block. Results were analyzed by analysis of variance. Data were transformed to achieve normality using Box-Cox (x+1) transformations. Tests of mean separations were conducted using Fisher's Protected Least Squares Differences.

Direct measurements of biological control were conducted in a subset of orchards from within the project. In 2003, three transects each were established using pear orchards under Organic, Soft, and Conventional type management. Conventional orchards had no restrictions on insecticides

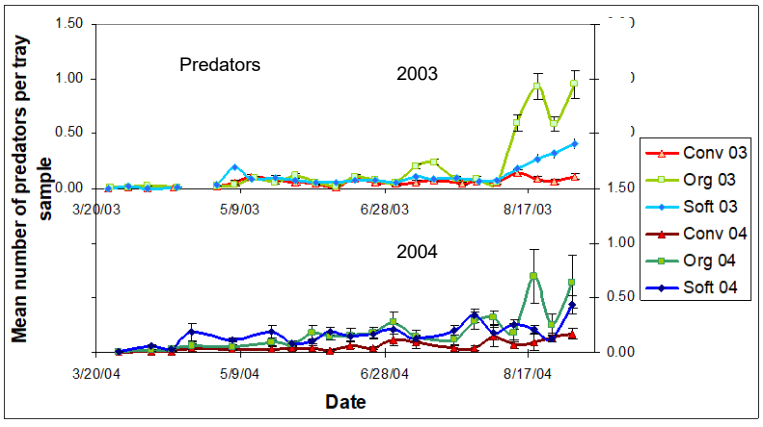
used, Soft orchards used primarily organic materials and insect growth regulators for pest management, and Organic orchards were certified organic and thus strictly limited to organic insecticides. Management decisions by the cooperating orchardists in 2004 caused a shift of one orchard from Organic to Soft, resulting in three Conventional, four Soft, and only two Organic transects. Each transect was 75 m long, extending 25 m into the surrounding vegetation from the first orchard row and 50 m into the orchard. Sampling points in the native vegetation were located at 10 m and 25 m from the 0 point (the orchard edge), and into the orchard at 5 m, 25 m and 50 m. Where access roads or canals separated native vegetation from the orchard margin, the 10-m sample point was adjusted into the nearest vegetation. The 5-m point in the orchard was located at the second row of trees, between 4 m and 7 m from the border depending on orchard spacing. Beating tray samples were taken at five locations per transect, on a weekly to bi-weekly basis. All predatory (and potentially predacious) insects were noted, and unknown insects were collected and brought to the lab pending identification.

Exclusion cages were used to determine direct predation of sentinel prey by natural enemies. Exclusion sleeve cages were constructed of 125-count silkscreen cloth to prevent predation of sentinel pear psylla in control treatments along transects; sentinel prey exposed to predation served as the experimental treatment. Several prey items were examined in 2003 for use as sentinel prey, including pear psylla nymphs, *Sitotroga* eggs, and *Ephestia kuehniella* eggs. The trials using *Ephestia* eggs proved the most repeatable; thus in 2004 this was the chosen prey.

Small cards (1.5 x 0.75 in.) holding flash-frozen *Ephestia* eggs were exposed to predators at each of the five sampling points, three in the orchard canopy and two in the surrounding vegetation. Cards at each of the sample points in the orchard were placed in the canopy 1.5 m high on a main scaffold, and three-foot stakes with wire hoops on them held the cages suspended in the vegetation at points in the surrounding vegetation. Four replications of cards were used at each distance on each transect. Additionally, four caged cards were placed at each sample location to exclude predators and provide an estimate of natural egg mortality. Eggs were monitored for mortality at 24, 48 and 72 hours of exposure. Data were analyzed after applying Schneider-Orelli's correction for control mortality.

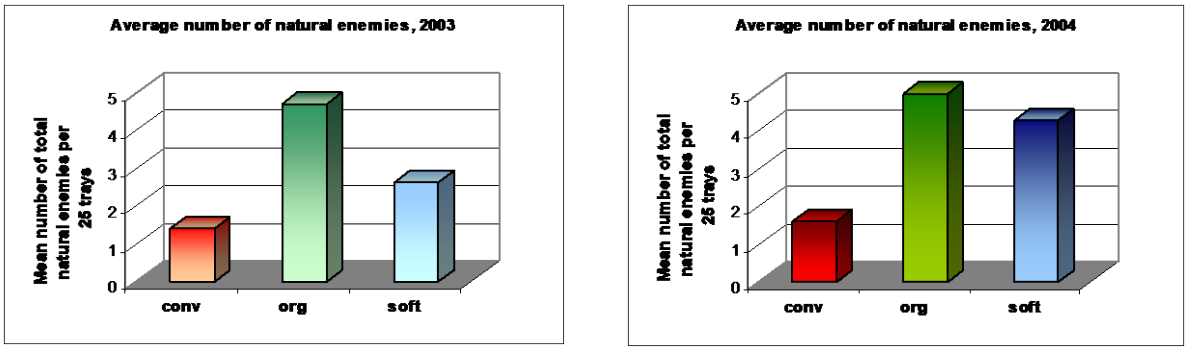
Results and discussion:
Results from the large scale sampling of the areawide project show that in both 2003 and 2004 natural enemy densities were low, and the Soft and Organic orchards had significantly greater overall numbers than the Conventional (Fig. 1). In 2003, these differences did not develop until late summer (August). In 2004, the Soft orchards supported significantly more natural enemies than the Conventional orchards from the beginning of the season, and the Organic levels rose above the Conventional in June.

Figure 1. Predator densities in the Peshastin Creek Areawide Project, 2003 and 2004.



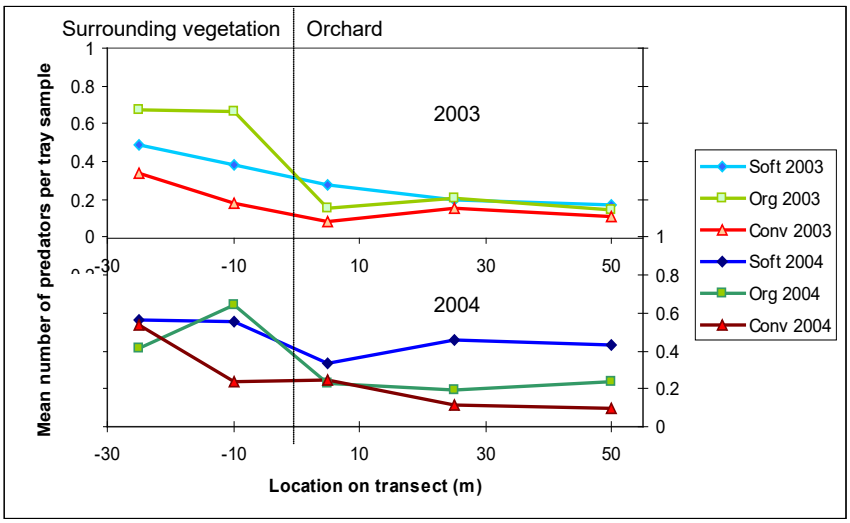
Averaged over each season, predator densities were greatest in the Organic blocks (Fig. 2). Mean predator densities remained the same between 2003 and 2004 in the Organic and Conventional treatments, whereas the Soft program had a significant increase in predator numbers. In general, predator numbers correspond with increases in pear psylla densities (also see the Areawide Organic Project Report for 2004), suggesting that prey density affects the density of natural enemies at least as much as the spray programs in use. The low economic threshold for control of pear psylla may constrain predator densities from reaching much higher levels despite the use of selective pesticides. Thus, while predators may augment pear psylla control programs, it is unlikely that biological control will replace insecticide use.

Figure 2. Mean natural enemy densities by program, 2003 and 2004.



An uneven distribution of predators was found in the transect samples across each of the management programs (Fig. 3). Samples from surrounding vegetation were consistently higher than those within orchards. In 2003, the management types did not harbor levels of predators significantly different from each other overall. In the surrounding vegetation, levels were highest in organic treatment and lowest in conventional; in the orchard, the Soft treatment had higher levels of predators than Conventional treatment, with Organic intermediate. In 2004, the Soft treatment had more predators overall than the Conventional treatment, with Organic intermediate. The samples from surrounding vegetation showed no differences between treatments; in the orchard, levels in the Soft treatment were significantly higher than in the other treatments.

Figure 3. Predator densities along sample transects, 2003 and 2004.

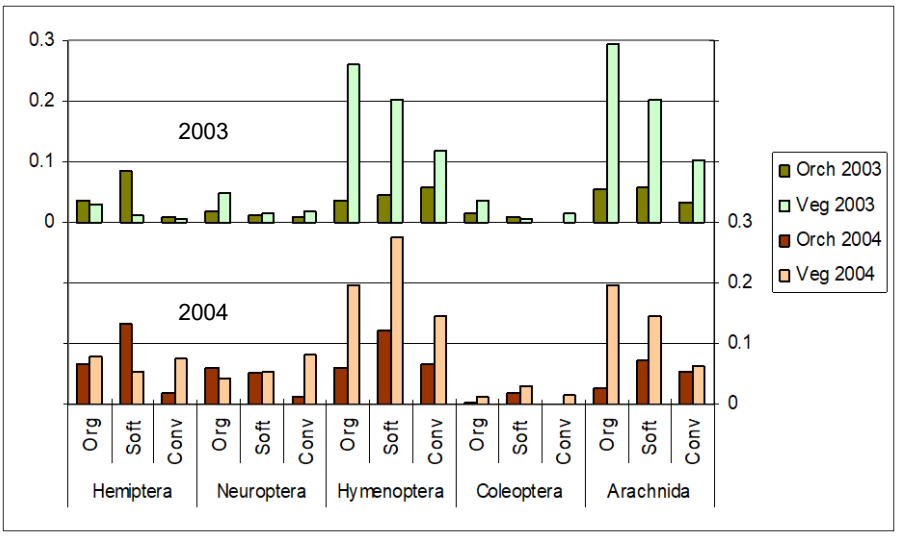


The predators which comprise the communities along the transects tend to vary by habitat type (Fig. 4). The dominant predators in the orchard were green lacewings, *Deraeocoris*,

Campylomma, Anthacorids, *Trechnites* and ladybird beetles, while the surrounding vegetation supported more *Geocoris*, *Nabidae*, ants and spiders. An examination at the level of order shows Hymenoptera and Arachnida to be the only groups that show consistent differences in densities between surrounding vegetation and orchard, with each higher in the surrounding vegetation among all program treatments.

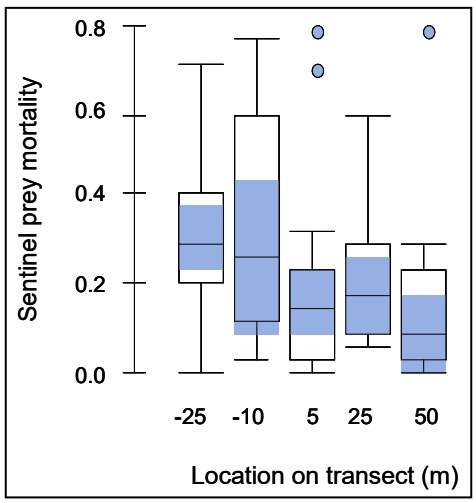
In the entire Areawide Project, spiders, *Deraeocoris, Trechnites, Campylomma* and lacewings were the most common predators within the orchards, although ants were also common in 2004. All other predators made up no more than 6% of the total natural enemy population.

Figure 4. Predator composition by order (% of total) for 2003 and 2004 along sample transects.



Results from the sentinel prey predation study in 2004 were consistent with results from transect monitoring, tending to show greater predation in the surrounding vegetation. Levels of potential predation, based on mortality of sentinel prey over 24 to 96 hours, tended to be higher in surrounding vegetation than in orchard habitat, and did not vary consistently by distance within a habitat type. In general, predation levels were relatively low.

Fig. 5. Box plots of predation on sentinel *Ephestia* eggs along transects, combining all treatments.



Preliminary results show that although low predator densities were present in all programs, predators appear to be more numerous in orchards under Organic and Soft pest management. In all programs predator density was higher outside the orchards, as expected. The species composition also tended to be different inside and outside the orchards. Early data on predation levels suggest that distance from untreated native vegetation significantly reduced predation. The study will be continued in 2005.

Budget:

Project title: Biological control in areawide organic and "super-soft" pear orchards
PI: John E. Dunley
Project duration: 2003-2005
Current year: 2005
Project total (3 years): $99,354
Current year request: $35,399

Item	Year 1 (2003)	Year 2 (2004)	**Year 3 (2005)**
Salaries[1]	16,300	18,310	**19,775**
*Benefits (27%-years 1 and 2; 34%-year 3)	4,401	4,944	**6,724**
Wages[2]	6,000	6,000	**6,000**
Benefits (16%-years 1 and 2; 11%-year 3)	960	960	**660**
Supplies[3]	2,300	700	**700**
Travel[4]	1,540	1,540	**1,540**
Total	31,501	32,454	**35,399**

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

*Increase in benefits is due to increased health insurance cost WSU contributes on behalf of the employee.

CONTINUING PROJECT REPORT **YEAR 1/2**

Organization Project # 5352 22000 015 13D

PROJECT TITLE: Impact of spinosad and granulovirus on codling moth and nontarget organisms

PI: Lawrence A. Lacey

Co-PI: Steven Arthurs

Cooperators: David Horton and Thomas Unruh

ORGANIZATIONS: USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA

Contract Administrator: Janet Tsukahira

OBJECTIVES:
1. Compare the efficacy of Entrust (spinosad) and codling moth granulovirus (CpGV) at recommended label rates and application frequencies for codling moth control.
2. Determine the impact of such applications on the population density and diversity of beneficial insects and other nontarget organisms in the orchard agroecosystem.

SIGNIFICANT FINDINGS – 2004:
- Entrust was effective at suppressing codling moth populations in apple and pear (e.g. < 2% fruit damage in a heavily infested Bartlett pear in an experimental orchard).
- CpGV was less effective than spinosad at preventing fruit damage, but killed the majority of CM larvae that reached the fruit. CpGV appeared to be more effective against the first larval generation of CM.
- Although sample processing is incomplete, we have yet to document significant a deleterious impact of spinosad on non-target arthropods in pear.
- The predatory mirid *Deraeocoris* spp. and lacewings *Chrysoperla* spp. were frequently found in Entrust-treated plots and showed few direct effects of the treatment.
- No evidence for phytophagous mite resurgence (2 species) resulting from spinosad use.
- Sweep net samples suggested several epigeal taxa were not affected by spinosad treatments.

METHODS

Efficacy of CpGV and spinosad on codling moth

In 2004 we will compare the efficacy of CpGV and Entrust in replicated blocks in an experimental (Moxee). In all trials fruit damage will be assessed (mid season and harvest). Wormy fruit will be returned to the laboratory and dissected to assess larval mortality. Moth activity will be recorded with pheromone traps.

Trials will be conducted in Bartlett pear at the USDA experimental orchard near Moxee, WA. Treatments will be Entrust formulation of spinosad (Dow Agrosciences) and the Cyd-X virus formulation (Certis, USA) applied in a full-season for program for codling moth and an untreated control. The study is a complete randomized block design with 5 replicates for each treatment (12-16 trees per block). Entrust and Cyd-X are applied within recommended label rates (3 oz/ac. for both) plus sticker (NuFilm17 @ 8 oz/ac.) at volume application rate of 100 gpa. Applications are made using an ATV-mounted airblast sprayer built for experimental purposes. A large tarp will be used to minimize contamination between plots and spraying will occur early morning during calm conditions. Applications are made at approximately 8 day intervals throughout the season in accordance with pheromone trap catches and the WSU phenology model (Beers et al. 1993).

Non-target effects of spinosad and CpGV

In the Moxee trial, predatory bugs, lady beetles and lacewings will be monitored every 1-2 weeks by taking beating tray samples from the four center trees in each plot. Leaf and shoot samples will be taken to monitor aphids and mites. Sweep net samples will be used to census leafhoppers and other non-target taxa on the orchard floor during spraying periods. Yellow sticky cards will be used to monitor flying insects in the centre of treated plots. In addition collections of pear psylla nymphs will be made twice (mid and late season) to estimate levels of parasitism by *Trechnites insidiosus* or other parasitoids. Cardboard bands were placed around trees at the end of the season will be used to capture predators searching for overwintering locations.

RESULTS AND DISCUSSION

Similar studies (see methods) were conducted 2004.

Efficacy of CpGV and spinosad on codling moth

Entrust worked well at suppressing codling moth populations. At Moxee there was < 2% CM fruit damage at harvest, compared with 15% in controls (Figure 1). Cyd-X was less effective at protecting fruit (12% damage), but the majority of larvae (62% ± 3.7) were killed based on dissections of injured fruit. A partial third CM generation may have been partially responsible. Although we sprayed, there were not enough CM stings or entries in fruit to reliably compare these treatments after the first larval generation.

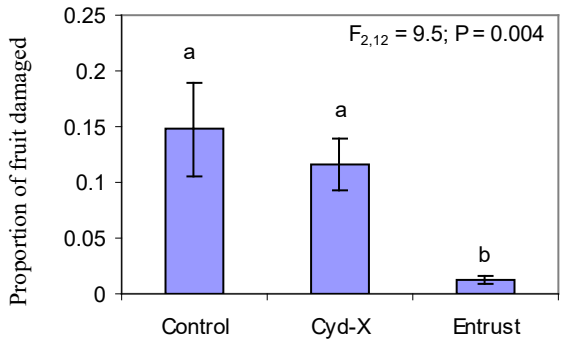


Figure 1. Fruit damage at harvest. Treatments were applied for CM control at approx. 8-day intervals in 12-16 tree plots. (Bartlett pear, Moxee experimental station).

Trials were conducted in blocks in commercial orchards where formulations of spinosad (Entrust or Success) and CpGV (Cyd-X or Carpovirusine) were used operationally. Growers applied Entrust and CpGV in separate blocks (2 replicate blocks per treatment each approximately 1 acre) in mixed pear (Mellow) and Delicious (Knutson). Application rate and frequency were in accordance with label recommendations and localized pest pressure determined on site. Blocks were sprayed within 2 days of each other. Because spinosad is restricted for resistance management (9 oz/ac season for Entrust, 29 oz/acre for Success), in the second larval generation it was either alternated with the virus plots (Mellow) or replaced with an IGR (Intrepid) in the Delicious blocks (Knutson). In a mixed 6 acre Golden Delicious/Granny Smith orchard another grower (Ing) replaced part of his OP program (Imidan) with the Carpovirusine formulation of CpGV.

Data from commercial orchards are shown in Tables 1-2. In Delicious, Success was more effective at protecting fruit compared with CpGV in the 1st generation; although the vast majority of CM were killed in the virus plots (85-100%) indicating the virus was highly effective at population suppression. Virus was less effective against second generation larvae (63-69% mortality), although at harvest fruit damage was similar between virus and Intrepid blocks. Carpovirusine applied at 10-d intervals killed 91-93% CM larvae in the 1st and 2nd generation respectively, but was not as effective as Imidan at protecting fruit (Table 3). Data from an additional site (Mellow) still needs to be collated.

Table 1. Efficacy of Cyd-X versus Success (spinosad); 4 ×1 acre blocks Delicious. Success was replaced with Intrepid in the second generation (Knutson, Mattawa).

Treatment	Block	Aug 3rd (450 fruit/block)		Sept 9th (1080 fruit/block)	
		% CM fruit injury	% larval mortality	% CM fruit injury	% larval mortality
Cyd-X/	#1	1.8	100 (n=12)	2.0	63.1 (n=111)
Carpovirusine	#2	4.9	85 (n=27)	5.0	68.7 (n=147)
Success/	#1	0.4	100 (n=2)	3.0	82.3 (n=79)
Intrepid	#2	1.1	60 (n=5)	5.1	81.6 (n=125)

Table 2. Efficacy of Carpovirusine versus Imidan. Approx 6 acres Golden Delicious and Granny Smith, unreplicated (Ing, Hood River).

Treatment	17 July (n = 1056 fruit)		Oct. 4th/5th (n = 3357 fruit)	
	% CM fruit injury	% larval mortality	% CM fruit injury	% larval mortality
Carpovirusine @ 10d	4.5	91.8	6.3	92.9
Imidan @ 20d	0.25	100 (2fruit)	0.33	100

Non-target effects of spinosad and CpGV

Although data from beating trays are not yet analyzed, at Moxee the predatory mirid *Deraeocoris* spp. and lacewings (*Crysoperla* spp.) were frequently found in Entrust-treated plots and showed few direct effects of the treatment. A mid-season outbreak of apple aphid (*Aphis pomi*) started in one of the Entrust-plots, but was both bought under control by beneficials.

Population trends for leafhoppers and several other taxa monitored in sweep net samples in spinosad, virus and untreated plots are in Figure 2. Despite being sampled in the center of plots, there were no clear differences for leafhoppers (predominantly *Dikraneura* and *Cratagallia* spp.) or soil/fungus gnats (Diptera: Mycetophilidae, Sciaridae and Ephydridae). Spider and adults syrphids were also recorded, but may not have been reliably captured.

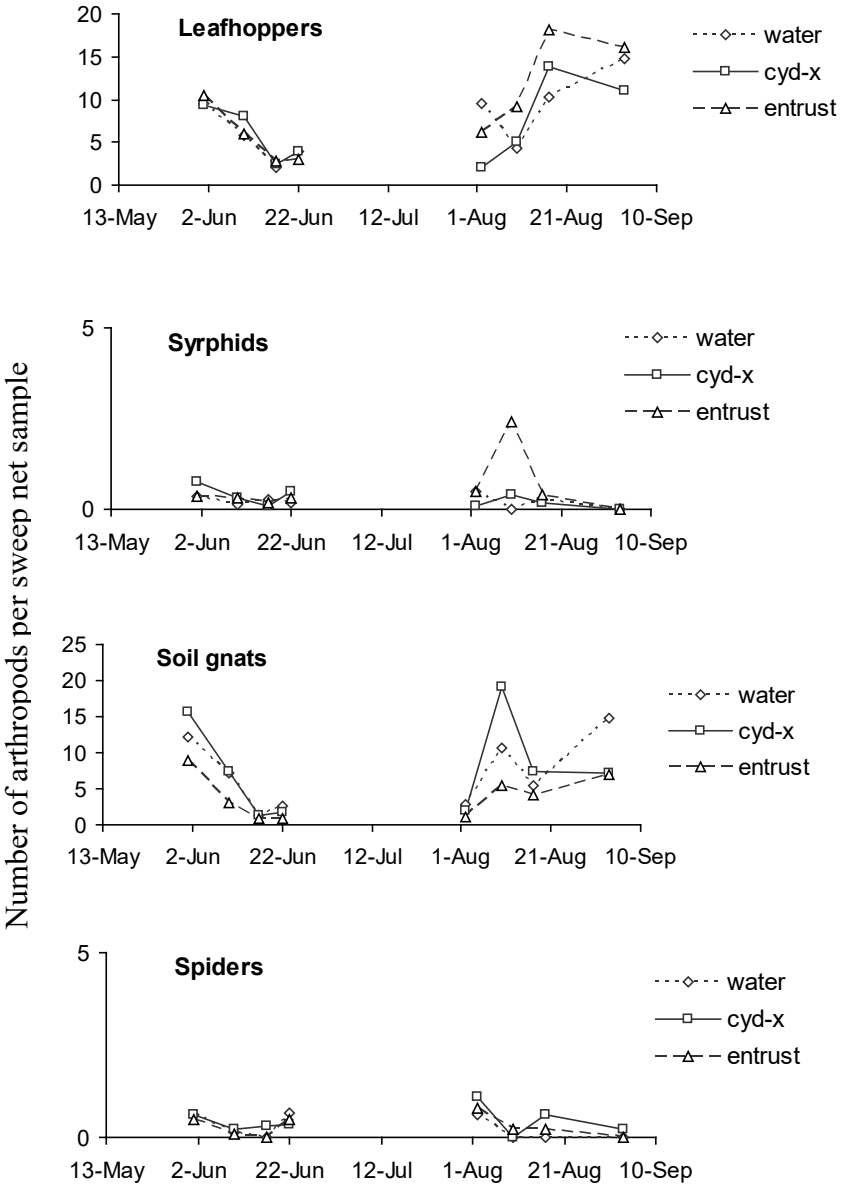


Figure 2. Non-target taxa monitored in sweep net samples. Treatments were applied at approx. 8-day intervals in replicated 12-16 tree plots. (Bartlett pear, Moxee experimental station).

Because of anacedotal reports of pest resurgence (aphids and mites) in plots treated with spinosad in 2003 and supporting literature on spinosad's toxicity to certain beneficials, we compared populations of phytophagous mites in spinosad-treated plots with CpGV and untreated plots. Statistical analysis showed no difference in abundance of pear rust mite (PRM) *Epitremerus pyri* Nalepa and pearleaf blister mite, *Phytoptus pyri* Pagenstecher in spinosad–treated versus other plots. Mite populations are shown for Moxee experimental orchard (Figure 3) although similar observations were made at a commercial orchard, where late season PRM abundance was statistically similar between virus-treated and Entrust-treated plots in Anjou pears (data not shown). Full details of non-target studies will be provided in the final report (see *Proposed schedule of accomplishments*).

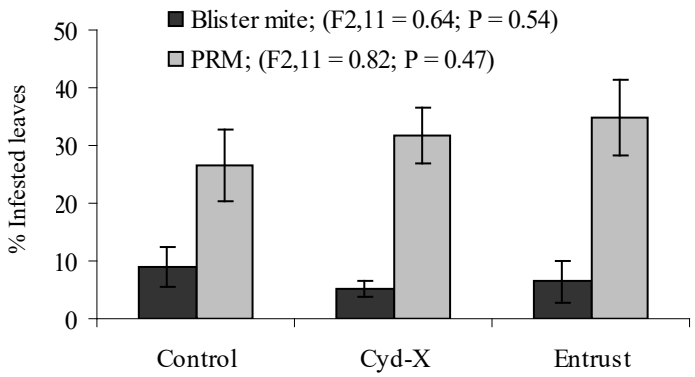


Figure 3. Tests for late season mite resurgence in replicated blocks (Moxee, Bartlett pear). N = 12 trees per treatment

PROPOSED SCHEDULE OF ACCOMPLISHMENTS

In year 1 (2004) we evaluated spinosad and CpGV for CM control in our experimental farm (Moxee) and at several commercial orchards. However, because of spinosad's wide-spread use and potential for disruption of biological control in orchards, we will conduct additional supporting studies in 2005.

Data collected on non-target arthropods during the 2004 season will be processed and analyzed (Jan through April 2005). Insects collected on leaves and yellow sticky cards still need to be identified. Additionally pear psylla nymphs (collected from each plot and frozen) will be dissected to compare rates of parasitism by *Trechnites insidiosus* and other parasitoids among spinosad, CpGV and untreated plots. Mounting documentation indicates there are deleterious effects of spinosad on parasitoids (Hill and Foster, 2000; Consoli et al., 2001; Mason et al., 2002; Williams et al., 2003).

The efficacy and non-target data will be conducted (May through October 2005) using the procedures outlined. Full details from both years will be reported in the trade and scientific press.

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Beers, E.H., Brunner, J.F., Willett, M.J. and Warner, G.M. 1993."Orchard Pest Management: A Resource Book for the Pacific Northwest" Good Fruit Grower, Yakima, WA

Consoli, F.L., Botelho, P.S.M., and Parra, J.R..P. 2001. Selectivity of insecticides to the egg parasitoid *Trichogramma galloi* Zucchi, (Hym. Trichogrammatidae). J. Appl. Entomol. 125: 37-43.

Hill, T.A. and Foster, R.E. 2000. Effect of insecticides on the diamondback moth (Lepidoptera : Plutellidae) and its parasitoid *Diadegma insulare* (Hymenoptera : Ichneumonidae). J. Econ. Entomol. 93: 763-768.

Mason, P.G., Erlandson, M.A., Elliott, R.H., et al. 2002. Potential impact of spinosad on parasitoids of *Mamestra configurata* (Lep: Noctuidae). Can. Entomol. 134: 59-68.

Williams, T., Valle, J., and Viñuela, E. 2003. Is the naturally derived insecticide spinosad(R) compatible with insect natural enemies? Biocontrol Science and Technology, 13: 459-475.

BUDGET

Title: Impact of Entrust (Spinosad) and codling moth granulovirus on codling moth, beneficials and other nontarget organisms in apple and pear.

PI: Lawrence A. Lacey[1]

Project duration: 2004-2005 (2 years)

Current year: 2005

Project total: $30,000

Current year request $15,000

	2004	2005
Salaries and wages (includes benefits)		
Technician, partial support for GS-4	10,000	10,000
Summer help, GS-3, 1 FTE (3 mos.)	3,500	3,500
Subtotal	$13,500	$13,500
chemicals, plasticware, misc. materials	1,500	1,500
Subtotal	1,500	1,500
Total	$15,000	$15,000

CONTINUING PROJECT REPORT **YEAR 2/3**

WTFRC Project PR-03-339 Agricultural Research Foundation #3740

Project Title: Introduction and propagation of pear rootstocks

PI: Dr. William M. Proebsting
Department of Horticulture
Oregon State University
Corvallis, OR 97331-7304

Cooperator: Steve Castognoli
Mid-Columbia Research & Extension Center
Hood River, OR

Objectives: This project conducts research in propagation of pear to: 1) help the flow of clonal rootstocks, from research programs towards commercial propagation, and 2) improve propagation of these clones.

Significant Findings:

General. This program propagates small quantities of high quality liners. 1) In most cases, these clones are not readily available from nurseries. 2) Nurseries are generally not interested in these clones at such an early, unproven stage of development. 3) Our liners, both micropropagated and cuttings, have grown extremely well in all situations, demonstrating that both are suitable methods for propagating pear rootstocks.

1) **Horner series**. About 450 clones were tested for rooting of softwood cuttings in the following sequence:
● Cuttings from 294 clones were propagated at Corvallis in July, 2001. Two or more liners of each were sent to Fowler Nursery in February, 2002 for grafting to be returned to Hood River for testing.
● The remainder of the Horner series, 148 clones, were propagated in July, 2002. Liners of about 130 were sent to Fowler in February, 2003.
• 77 Horner clones were re-propagated in July, 2003. Liners of 64 were sent to Fowler in February, 2004.
• Horner 4 and Horner 51 were initiated in tissue culture. Horner 4 is propagating well, whereas Horner 51 gradually died out.

2) **Russian clones**.
• In February, 2002, we received budwood from three clonal rootstocks, Q29857, Q29858, Q29859, from Russia. These were initiated into tissue culture. Q29859 has multiplied very quickly, whereas the others are progressing slowly. APHIS has just released these clones (12/04).

3) **Rootstock collection.**
• 20 pear rootstocks are currently in tissue culture at OSU awaiting requests for liners for research or transfer of cultures to nurseries.

4) **Rooted scions.**
• Rooting of ‘Comice’ and ‘Red Anjou’ softwood cuttings was tested and shoots were established in tissue culture.

Methods:

Softwood cuttings. Horner series. Cuttings were collected from the original seedlings growing at Hood River. These trees were pruned hard to induce vigorous shoot growth. All available cuttings from each stock plant were collected on July 14. Cuttings were prepared by removing the expanding shoot tips and then making 10" cuttings, except for dwarf clones for which 6" cuttings were made. The cutting bases were dipped for 5 sec in 100 mM IBA dissolved in 0.25 M KOH and planted in medium (perlite:peat, 3:1) in bands 2 ¼" squares by 5" deep at 22°C. The mist conditions were: 0700-0900 hours, 24 min interval, 0900 to 1000, 16 min interval, 1000 to 1700, 8 min interval, 1700 to 1900, 16 min interval and 1900 to 2000, 24 min interval. All mist applications were 10 sec duration.

'Comice' and 'Red Anjou' cuttings were collected from an orchard near Parkdale on July 14 and treated as above.

During the last week of August, the cuttings were removed from their containers, evaluated and well-rooted cuttings transplanted to a raised bed adjacent to the greenhouse.

Micropropagation. Cultures were established using vigorous shoot tips collected during active growth. These shoots were surface sterilized in 10% bleach solution and planted in individual tubes containing DKW medium consisting of 0.8% agar, 3% sucrose plus DKW salts and vitamins. Shoots which were sterile and still actively growing were transferred to a multiplication medium consisting of DKW medium plus 1 ppm benzylaminopurine (BAP). Every 4-6 weeks, shoot clumps were divided into single shoots and re-cultured on multiplication medium.

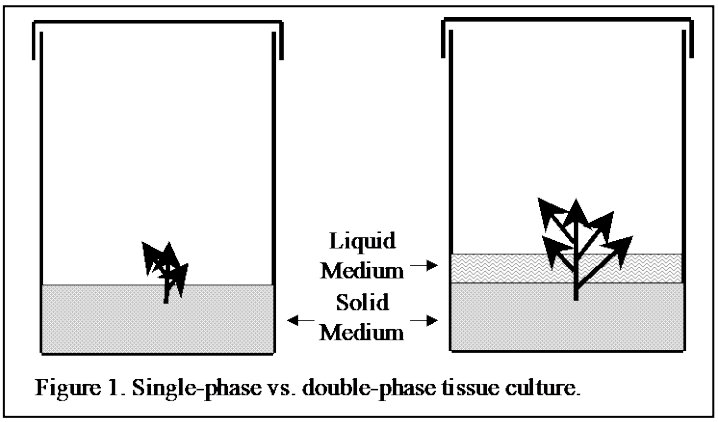

Figure 1. Single-phase vs. double-phase tissue culture.

When liquid medium is used in double-phase culture, enough liquid is added, about 25 ml, to nearly cover shoots that had just been divided and transferred (Figure 1).

When a sufficient number of shoots are available, the surplus is treated with indolebutyric acid (IBA) to stimulate rooting. Rooted shoots are transplanted into clean potting medium, grown under intermittent mist for two weeks and then transferred to the greenhouse. In the greenhouse, the shoots are grown to liner size and transferred to other research programs.

For transfer to commercial micropropagators, shoot cultures are sealed in sterile, plastic pouches containing a small amount of DKW solid medium and mailed to the nursery.

Results and Discussion:

1) **Horner series.** The production characteristics of this group of over 400 open-pollinated 'Old Home x Farmingdale' seedlings are being evaluated. Further testing was warranted when small, preliminary studies found some promising rootstocks.

In this situation, tissue culture of 400 clones is inappropriate. Because these are seedlings, however, and have been maintained as small, heavily-pruned trees, rooting potential of softwood cuttings of each clone should be near its maximum. Furthermore, since only 2-5 liners of each clone were required for the rootstock trial, low rooting percentage is not a serious obstacle.

In 2001 and 2002, 451 clones were tested, some of them re-tests in 2002. 427 clones made the two liner minimum. In February, 2002 and 2003, the liners were shipped to Fowler Nursery, Newcastle, CA. Both sets of liners grew very well and were summer budded.

In 2003, we re-tested 77 clones that either rooted poorly the first time or were lost in the nursery. 64 of these made the two liner minimum and will be shipped to Fowler in February, 2004.

Two promising rootstocks, **Horner 4** and **Horner 51** were initiated in tissue culture in July, 2003. Horner 4 is growing very well, Horner 51 has been difficult and will be reestablished. Liner production can begin when more information about these rootstocks is required.

2) **Russian rootstocks.** Several years ago, three clonal pear rootstocks were imported from Russia by Californians Larry Rogers and Jim LaRue. They are purportedly dwarfing. APHIS was willing to make these available for preliminary propagation. With the assistance of Gene Milbrath, Oregon Department of Agriculture, to obtain the necessary paperwork, APHIS sent me budwood in February, 2002. We budded it on seedlings in the greenhouse and initiated cultures in late April, 2002.

Table 1. Pear rootstock clones in tissue culture at OSU, December, 2004.

517-9	OH11
708-2	OHxF 40
708-12	OHxF 51
708-13	OHxF 87
708-36	OHxF 97
96FI11	Pyronia
96FI12	Q29857
Fox 11	Q29858
Fox 16	Q29859
96FI15	Horner 4

Q29857 and Q29858 are multiplying very slowly, but have just been put in double-phase culture, which should stimulate growth. APHIS released these from quarantine in December, 2004. We will produce liners as quickly as possible.

3) **Micropropagation.** As we have provided rootstock liners for testing, we have maintained a small number of each clone in culture. We now have 20 clones in culture. If a rootstock merits further testing or commercial propagation, these established cultures will enable us to respond quickly.

4) **Rooted scions.** Gene Mielke is studying a syndrome of problems in the Hood River area that may involve scion/rootstock interaction. At his request, we attempted rooting of softwood cuttings of ‘Comice’ and ‘Red Anjou’ to provide own-rooted controls for plots. The problem with this material is that it is physiologically mature and therefore difficult to root. Rooting was about 13%, yielding 33 ‘Comice’ and 24 ‘Red Anjou’ cuttings. These were planted in raised beds for growing on. They grew well in 2004 and will be sent to Hood River in Spring, 2005.

We also placed shoots of these varieties in tissue culture. This could be useful for plant production directly from micropropagation or micropropagated plants could serve as stock plants to produce cuttings with higher rooting potential than those from orchard trees. ‘Comice’ is growing well in culture, especially for a scion clone, whereas ‘Red Anjou’ is struggling.

Budget:

Project Duration: 2003-05
Current Year: 2005
Project Total: $69,690

Year	2003	2004	2005
Total			$23,896

Details

	2003	2004	2005
Salary, Faculty Research Assistant[1]	$11,373	$12,299	$11,414 (0.4 FTE)
OPE	6,028 (50%)	6,764 (55%)	7,305 (64%)
Student Wages[2]	1900	1900	1,000
OPE ($3.12/mo.)	95	75	37
Services and Supplies[3]	4,000	4,000	500
Travel[4]	500	500	0
Total	23,896	25,538	20,256

[1]Luigi Meneghelli, Research Assistant
[2]Undergraduates maintain most of the cultures and field plots
[3]Tissue culture and greenhouse supplies
[4]Travel to plots at the Lewis-Brown Farm

Other support requested:
Oregon Hazelnut Commission, $20,256, decision March, 2005.

USDA NRI- collaboration on proposal "Mismatch Repair in Arabidopsis and Tomato." Requesting 0.20 FTE for Luigi Meneghelli's salary. Decision June, 2005.

CONTINUING PROJECT **YEAR 2/3**
WTFRC Project # PR-03-340

Project Title: MCP interaction with fumigants to control decay.

PI: Paul Randall, Peter Sholberg, AAFC-PARC, Summerland, British Columbia

Cooperator: Robert Spotts, Professor, Oregon State University, Hood River, Oregon

Objectives:
1. Determine the best combination of 1-MCP and hexanal to reduce storage decay and improve the aroma on stored pears.

2. Identify the optimal time to apply hexanal to assist the ripening of 1-MCP treated pears.

3. Test other fumigants (acetic acid, propionic acid) in combination with 1-MCP.

4. Conduct 1-MCP/hexanal trials at Hood River, Oregon (Robert Spotts) and PARC, Summerland, BC.

Significant Findings:
1. There was no negative interaction between hexanal and 1-MCP

2. The quality of the d'Anjou pears was not significantly changed by being treated with hexanal and or 1-MCP at 15°C (59°F) for 18 hours.

3. The treatments with hexanal, 1-MCP or combination treatments had less decay than those which were not fumigated.

4. Use of acetic acid (2mg/l) is very effective on *Penicillium* spp., but will burn d'Anjou pears if fumigated longer than 30 minutes.

METHODS

1. LARGE SCALE TRIALS

A. HOOD RIVER
d'Anjou pears were harvested on the day of the fumigation. A *Botrytis cinerea* spore suspension (1×10^4 CFU/ml), the causal agent of gray mold, was sprayed on the fruit using an airbrush (Paache Airbrush Company, Harwood Heights, IL). The fruit was immediately placed in to a 0.35 m^3 (12.4 ft^3) chamber and air cooled to 15^0C (59°F). The pears were then fumigated with 944 ppm (4 mg/l) of hexanal for 18 hours or with 300 ppb (0.270g) of 1-Methylcyclopropene (1-MCP) for 18 hours or a combination of hexanal and 1-MCP (see Table 1 for detailed description of the various treatments). 1-MCP was generated from Ethylbloc powder provided by Agrofresh, Inc, a subsidiary of the Rohm and Haas Company. Upon completion of the treatment, the pears were hand packed into polylined wooden boxes and placed in the cold room at -1^0C (30 °F). One box per replicate was used for quality analysis (fruit firmness and soluble solids checked after treatment, then 4, 6, 8 months after storage). It is important to note that the fruit for quality analysis was not inoculated with *B. cinerea* spores. All the replicates will be evaluated for post harvest decay and quality at the end of 8 months.

Table 1. Description of the various treatments.

Treatment	Description
Standard (Pick & cool)	d'Anjou pears, inoculated with *B. cinerea*, then placed immediately into a cold room at -1^{0}C (30^{o}F).
Control (No Hexanal, no MCP)	d'Anjou pears, inoculated with *B. cinerea*, left at 15^{0}C (59 ^{o}F) for 18 hours, then placed in a cold room at -1^{0}C (30 ^{o}F).
Hexanal only (4 mg/l (944 ppm)).	d'Anjou pears, inoculated with *B. cinerea*, placed at 15^{0}C (59 ^{o}F) and treated with 4 mg/l hexanal for 18 hours, and then stored in a cold room at -1^{0}C (30 ^{o}F).
1-MCP (300 ppb)	d'Anjou pears, inoculated with *B. cinerea*, placed at 15^{0}C (59 ^{o}F), treated with 1-MCP, and then stored in the cold room at -1^{0}C (30 ^{o}F).
1-MCP (300ppb) + Hexanal (4 mg/l).	d'Anjour pears, inoculated with *B. cinerea*, placed at 15^{0}C (59 ^{o}F) and, treated with 1-MCP and Hexanal for 18 hours, and then stored in a cold room at -1^{0}C (30 ^{o}F).

B. PARC

d'Anjou pears were harvested on the day of the fumigation. A *B. cinerea* spore suspension (1 x 10^4 CFU/ml) was sprayed on the fruit using a Paache airbrush. The fruit was placed in to a 1 m^3 (35.3 ft^3) chamber and air cooled to 15^{0}C (59^{o}F). The pears were then fumigated with 944 ppm (4 mg/l) of hexanal for 18 hours or with 30 ppb (0.048g) of 1-MPC for 18 hours or combination of hexanal and 1-MCP (see Table 2 for detailed description of the various treatments). Upon completion of the treatment, the pears were hand packed into polylined cardboard boxes with top pad and lid, and placed in the cold room at 1^{o}C (34 ^{o}F). One box per replicate was used for quality analysis (fruit firmness and soluble solids checked after treatment, then 2, 4, 6, and 8 months after storage). All the replicates will be evaluated for post harvest decay and quality at the end of 8 months.

Table 2. Description of the various treatments.

Treatment	Description
Standard (Pick & cool)	d'Anjou pears, inoculated with *B. cinerea*, then placed immediately into a cold room at 1^{o}C (34^{o}F).
Control (No Hexanal, no MCP)	d'Anjou pears inoculated with *B. cinerea*, left at 15^{0}C (59 ^{o}F) for duration of treatments (18 hours), then placed in cold room at 1^{0}C (34 ^{o}F).
Hexanal only (944 ppm (4 mg/l)).	d'Anjou pears, inoculated with *B. cinerea*, placed at 15^{0}C (59 ^{o}F) and treated with 4 mg/l hexanal for 24 hours, and then stored in cold room at 1^{o}C (34 ^{o}F).
1-MCP (30 ppb (0.048g)	d'Anjou pears, inoculated with *B. cinerea*, placed at 15^{0}C (59 ^{o}F), treated with 1-MCP, and then stored in the cold room at 1^{0}C (34 ^{o}F).
1-MCP (30 ppb) + Hexanal (4 mg/l).	d'Anjou pears, inoculated with *B. cinerea*, placed at 15^{0}C (59 ^{o}F) and, treated with 1-MCP and hexanal for 18 hours, and then stored in cold room at 1^{o}C (34 ^{o}F).

2. Quality Analysis (Methods)

A. Fruit Firmness: The various treatments were checked for fruit firmness with a pressure tester (Lake City Technical Products, Model Ept-1, Kelowna, BC) equipped with a 7.9 mm (0.029 inches) tip. Ten fruit per treatment were tested. Fruit from cold storage were left at room temperature (20^{o}C (68^{o}F)) for 4 hours, prior to checking firmess.

B. Soluble Solids: An AO Scientific Instruments (Buffalo NY), digital refractometer ABBE MARK II was used to determine percent soluble solids. The various treatments were tested immediately after fumigation. Fruit firmness will be tested after the pears have been in storage for 4, 6 and 8 months.

3. Use of Hexanal to assist in ripening and improve aroma on 1-MCP Treated Pears.

Methods
Fifty boxes of d'Anjou pears were harvested, and divided into five treatments, to be fumigated with 0, 30, 50, 100, and 130 ppb of 1-MCP for 18 hours. The pears were not inoculated with fungal spores. Upon completion of the treatments, the pears were hand packed into polylined cardboard boxes with top pad and lid, and placed in the cold room at 1^0C (34 ^{o}F).

After a minimum of 4 months storage, the treated fruit will be removed from cold storage and placed at 20^{o}C (68^{o}F). After one day when the fruit has reached room temp (20^{o}C (68^{o}F)) the treated fruit will be subdivided and treated with two rates of hexanal. After one week the fruit will be assessed for QA (fruit firmness and soluble solids), aroma and decay.

4. Testing of Other Fumigants.

Methods
Small scale efficacy tests. Tests to determine efficacy of other fumigants and phytotoxicity[1] (browning of the fruit), were done by inoculating with a set number of spores (1 x 10^4 CFU/ml) of a decay-causing fungus over the fruit surface, and allowing the inoculum to dry. The inoculated pears were placed in a 26 l (6.9 gal US) chamber. The pears were fumigated with various levels of a fumigant (408 to 4864 ppm) (1 to 8 mg/l) for various periods of time (30 to 90 minutes). The relative humidity was adjusted to 70% if necessary, by evaporating water into the chamber. Food Grade Acetic Acid was evaporated by heating with a small electric heater. The acetic acid concentration was monitored by withdrawing a 1 ml sample of air from the chamber with a 1 ml syringe shortly after the start and at regular intervals during fumigation. The gas sample was injected into a Model 910 gas chromatograph (GC) (Questron Technologies Corp. Mississauga, Ontario) and within approx. 1 minute the concentration in the chamber was known. The GC was outfitted with an flame ionization detector (FID) and fused silica capillary column (Zebron ZB-FFAP, Phenomenex, Torrance, CA). At the end of the fumigation, the chamber was vented and the fruit removed. The fruit was then wounded and placed at 20^0C (68^{o}F) for 5 days when decay and phytotoxicity were recorded.

5. Statistical analysis (Methods).
The data was analyzed using the general linear model procedure (SAS Institute, Cary, NC) and the means were separated with Waller-Duncan K-ratio T test (K=100 which approximates p=0.05).

Results and Discussion:

1. Large Scale Trials
The results of the large scale trial in 2003/2004 (Figure 1) were variable but it was possible to obtain significant differences between the amount of clean fruit and the degree of *Penicillium* rot (blue mold). The fruit treated with hexanal, MCP or a combination of both had significantly more mold free fruit (69 to 77%) then the control or standard (37-41% mold free). The hexanal, MCP treatments also had significantly less *Penicillium* rot than for the control and the standard. The 2004/05 large scale trials at Hood River and PARC are to be evaluated after a minimum of six months storage (March 2005) or up to a maximum of eight months storage (May 2005), depending on pear quality.

[1] Phytotoxicity or damage to the fruit is evidenced by blackening starting at the lenticels and spreading over the fruit surface.

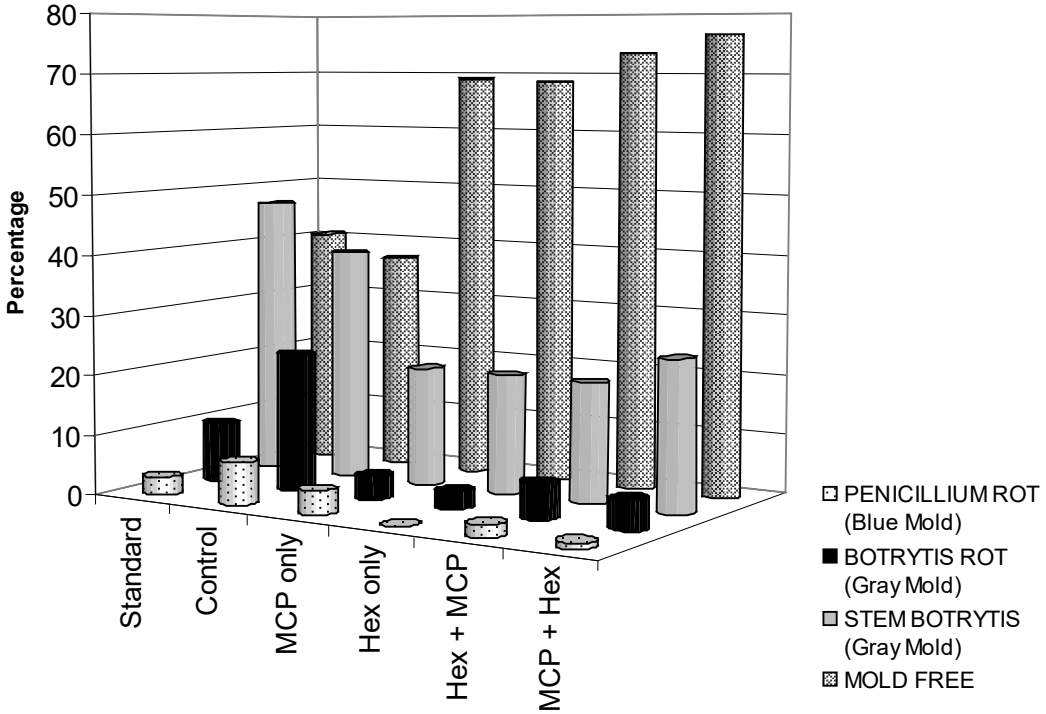

Figure 1. Percentage of mold free or decayed fruit after treatment with hexanal and or 1-MCP after 4 months of storage at 1ºC (34ºF).

2. Quality Analysis

The control fruit had the lowest fruit firmness after 51, 82, 113 and 120 at 1ºC (34ºF) (see Table 3). All the other treatments had higher values. It is unclear if 20 ppb of 1-MCP aided in keeping d'Anjou pears firmer, because the treatments containing MCP were no better than the fruit cooled in the standard way (Figure 2). It could be that the amount of 1-MCP used was too low or the pressure of the fruit was below the level where rate of 1-MCP used, would be effective in maintaining pear firmness (Mattheis 2004).

Table 3. Anjou quality (Firmness) in lbs 2003/2004. (Standard Deviation in brackets)

Treatments	**Days in Storage**				
	0 Days (01 Oct 03)	**51 Days 21 Nov 03**	**82 Days 22 Dec 03**	**113 Days 22 Jan 04**	**120 Days (29 Jan 04)**
Standard	11.44 (0.85)	11.17 (1.00)	10.70 (1.05)	9.59 (1.20)	3.26 (0.30)
Control	11.35 (0.90)	10.30 (1.30)	7.29 (1.20)	4.15 (0.50)	2.54 (0.50)
Hexanal	12.08 (0.90)	11.58 (1.05)	10.50 (1.25)	8.35 (1.60)	3.28 (0.60)
MCP	11.52 (0.80)	10.99 (0.85)	9.94 (1.15)	7.07 (1.50)	2.60 (0.40)
MCP + Hexanal	11.12 (1.00)	11.43 (0.90)	10.06 (0.85)	8.74 (0.90)	2.57 (0.40)
Hexanal + MCP	11.71 (0.80)	11.10 (1.00)	10.91 (1.10)	9.22 (1.89)	3.51 (0.80)

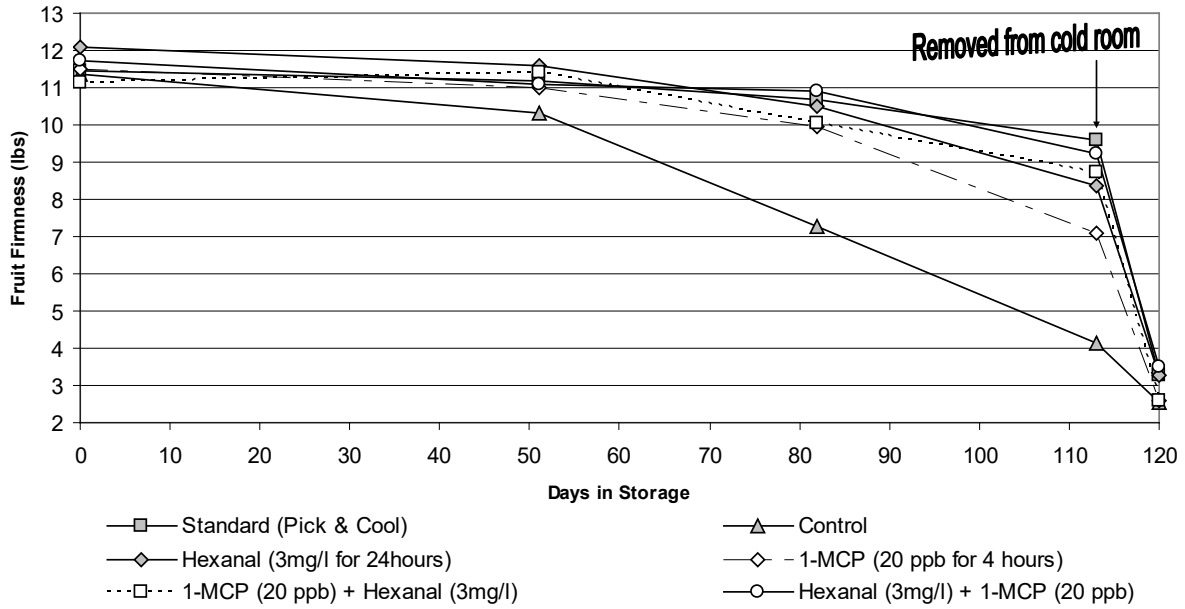

Figure 2. d'Anjou pear fruit firmness over 120 days in air storage at 1°C (34°F) (PARC 2003/04)

3. Use of Hexanal to assist in ripening and improve aroma on 1-MCP treated pears.
The second part of this experiment is to be conducted at the end of January 2005, after the 1-MCP treated fruit has been in storage for a minimum of 4 months. The result will be presented at the Northwest Pear Research Review on 17 February 2005.

4. Testing Other Fumigants
Acetic Acid
Previous work has shown that acetic acid vapors are very effective against *Botrytis* and *Penicillium* spores on fruit surfaces. The level of acetic acid vapor required to reduce these pathogens on d'Anjou pears and not damage the fruit surface is minimal. A number of trials were conducted at 25°C to determine acetic acid effectiveness and its phototoxic effect on d'Anjou pears (Table 4).

Table 4. Acetic Acid fumigation rate vs time duration of the fumigation

	90 Minutes		60 Minutes		45 Minutes		30 Minutes	
mg/l	% Decay	% Phyto	% Decay	% Phyto	% Decay	% Phyto	% Decay	% Phyto
0	98	0	100	0	100	0	100	0
1	Not tested	Not tested	Not tested	Not tested	80	0	Not tested	Not tested
1.5	Not tested	Not tested	Not tested	Not tested	**10**	**0**	Not tested	Not tested
2	0	10	0	5	**0**	**1**	**0**	**0**
3	Not tested	Not tested	Not tested	Not tested	**0**	**10**	Not tested	Not tested
4	0	20	0	10	Not tested	Not tested	0	5
6	0	40	0	40	Not tested	Not tested	0	10
8	0	75	0	75	Not tested	Not tested	0	20

The rate of acetic acid required to control Blue mold caused by *P. expansum* is 2 mg/l for 30 minutes or for 90% control, 1.5 mg/l for 45 minutes. These fumigation durations are likely too short for fumigation for large volumes of fruit (over 100 bins) but could be used to fumigate smaller numbers of bins such as in a 40 ft container where the vapour could be vented in 15-30 minutes. Furthermore larger volumes could be fumigated where fruit damage is not a major concern such as in processing pears.

Proposed Research for next year

1. Repeat the large scale hexanal/1-MCP experiments at Hood River, Oregon and PARC, Summerland.

2. Conduct more trials using hexanal as a post storage fumigant to assist in decay control and to improve the ripening and aroma of 1-MCP treated pears.

Budget:
Project Title: MCP interaction with fumigants to control decay.
PI: Paul Randall, Peter Sholberg
Project Duration: 2003-2005
Project Total (3 years) $30,000

Item	**Year 1** 2003	**Year 2** 2004	**Year 3** 2005
Salary	7,000	7,000	7,000
Benefits	1,800	1,800	1,800
Materials and supplies	600	600	600
Travel	600	600	600
Total	**10,000**	**10,000**	**10,000**

In year 2 & 3 funds to be matched by the Matching Investment Initiative Program of Agriculture and Agri-Food Canada

Literature Cited
Mattheis, J., Chen, P., and Roberts, R. 2004. Manipulation of Pear Fruit Ripening by Control of Ethylene Action. Northwest Pear Research Review 2004, p77-84

CONTINUING PROJECT REPORT **YEAR 1/3**
WPPC & WTFRC Project #: PR-04-433 **ARS Project #:** 5350-43000-004-06T

Project title: Management of Harvest and Postharvest Practices to Promote Optimum Quality of Fresh Pears

PI: Jim Mattheis
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Objectives:

1. Identify additional indicators of physiological and/or horticultural maturity that are indicative of storability.

2. Identify protocols for 1-MCP use that ensure predictable ripening.

3. Characterize how pear fruit ripening and development of disorders are impacted by prolonged storage at the low O_2 limit.

The goals of this project are to identify indices of maturity in addition to firmness that can be used to estimate storability, and to determine how fruit ripening and development of disorders can be manipulated by newly available postharvest technologies to maximize postharvest life while maintaining optimum dessert quality and appearance. Accomplishment of these goals would enhance profitability for the pear industry by enabling better control over harvest management, particularly if harvest can be delayed to achieve larger fruit size. More efficient use of new and existing postharvest technologies could extend the marketing period for fresh pears and also reduce losses due to physiological disorders during and after storage.

Significant Findings:

- Changes in production of several volatile compounds by freshly harvested 'Bartlett' pears correlates with optimum harvest date based on firmness at harvest and storability.

- Ripening capacity of 1-MCP treated Anjou pears increases with CA O_2 concentration and CA storage duration.

- All lots (10 each) of 1-MCP treated 'Bartlett' and 'Anjou' pears ripened following long-term storage in air or CA plus a warm pre-conditioning period.
- Efficacy of 1-MCP can be reduced by sufficient ethylene or CO_2 present during treatment at harvest or after storage.

- Exposure to nitric oxide has not consistently modulated ripening of 'Bartlett' or 'Anjou' pears.

- The low oxygen limit, defined by chlorophyll fluorescence, for Bartlett and Anjou pears used in current year studies was 0.5 and 0.4 %, respectively.

- Impacts of Bartlett and Anjou storage at the low oxygen limit are cultivar specific and dependent on storage duration.

Methods:

Maturity: Fruit are sampled in commercial orchards starting several weeks prior to anticipated harvest. Samples will be analyzed at harvest and after storage in air or CA to determine relationships between maturity at harvest and storage performance. Analyses of fruit quality components (firmness, color, starch, soluble solids, titratable acidity) along with indicators of physiological development (production of ethylene and other volatiles, respiration rate) will be assessed using standard methods. The Mohr Digitest instrument will be used to assess firmness and other physical characteristics. Ethylene and other organic volatiles will be measured using gas chromatography (GC). Ethylene responsive stickers provided by Robert Klein will also be evaluated. Volatiles will be sampled using Tenax traps and analyzed by GC-mass spectrometry. Nitric oxide (NO) will be measured by monitoring chemiluminescence of the reaction products of NO and ozone. Our objective is to identify changes in the production of volatile compound(s) that are coincident with maturation, and then to determine if analyses other than GC or chemiluminescent analyses can be used to detect these volatile signals. The starch iodine test will be used to assess starch metabolism. Color will be assessed visually as well as with a colorimeter. Soluble solids content and titratable acidity will be determined with a refractometer and a titrator, respectively.
Seasonal variability can have significant impacts on fruit development, and based on similar studies conducted with apples, 3 years will be required to confirm the utility of other indicators of fruit maturity.

1-MCP: Previous research has demonstrated the utility of postharvest applications of 1-MCP for slowing pear fruit ripening. A number of factors including 1-MCP rate, interval between harvest and treatment, post-treatment storage conditions including temperature and CA gas composition impact the magnitude and duration of 1-MCP responses. While consistently effective when applied under experimental conditions, a number of treatment failures have occurred when applied in commercial rooms. It is known from research with apples that ethylene and/or CO_2 present in sufficient concentrations during 1-MCP treatment can reduce or prevent treatment efficacy. One objective is to evaluate whether the same is true for pears, and what amounts of ethylene and CO_2 are critical for influencing 1-MCP efficacy. The range of maturity over which 1-MCP treatments applied at harvest is effective will continue to be evaluated using fruit from sequential harvests as described above. Reliable ripening after shipment is a key factor currently limiting commercialization of 1-MCP. Treatment of partially ripe fruit reduces the duration of 1-MCP responses to days or weeks, however, fruit treated when too ripe does not respond. Defining what is 'too ripe' for different cultivars in terms of measurable parameters (firmness, color, ethylene production) is one of the goals of this

project. Another goal is to identify similar markers to aid in conduct of preconditioning protocols for fruit treated at harvest and held at warmer than typical storage temperatures prior to shipment. These studies will continue to attempt to identify measurable fruit characteristics that are indicative of further ripening in response to defined periods of holding at relatively high temperatures.
Studies to further characterize the relationship between 1-MCP application rate and timing for d'Anjou will focus on the relationship between superficial scald control and effects on ripening. Previous research indicates treatments at 300 ppb or higher delayed 2 to 4 weeks after harvest do not provide complete control of superficial scald. Continued studies examining low rates and timing of 1-MCP application will focus on achieving scald control with less impact on ripening.
These studies will be conducted using commercial SmartFresh® material obtained from AgroFresh, Inc. Treatments will be applied in small chambers at the USDA, ARS TFRL or in storage rooms at the Stemilt RCA facility.

Low oxygen tolerance: The effects low oxygen storage on ripening of climacteric fruit increase as O_2 concentration decreases. There is a critical O_2 concentration below which fruit metabolism becomes anaerobic and significant quality loss occurs due to accumulation of ethanol and other anaerobic byproducts. The ability to monitor fruit response to O_2 concentration provides a means to store fruit at very low O_2 concentrations while avoiding the risk of anaerobic metabolism. For apples, control of superficial scald can also be obtained when fruit are stored in O_2 below 1%. Preliminary work with 'Bartlett' and 'd'Anjou' pears indicates fruit are tolerant of O_2 well below 1%, and a portion of this project would be focused on characterizing responses of pears stored near the low O_2 limit. Fruit response to low O_2 would be monitored using instrumentation to measure chlorophyll fluorescence of fruit peel recently commercialized by Satlantic, Inc., Halifax, NS, Canada. Studies would be conducted where maturity, 1-MCP treatment and low O_2 treatments are integrated to determine what interactions between these variables exist, and what benefits for postharvest management are obtainable through the use of 1-MCP and/or low O_2 storage.

Results and Discussion:
Maturity Indicators of Bartlett pears: Firmness of Bartlett pears collected over a 5 week period averaged 19.6, 19.1 and 19 lbs the first 3 weeks then decreased to 16.1 and 15.8 at weeks 4 and 5. Changes in fruit crispness had a pattern similar to firmness, and changes in Mohr Digitest measurements did not precede the drop in firmness. Ethylene was detected only at week 4, and starch clearing was evident at weeks 4 and 5. Ethylene responsive stickers were not yet available. Of the 48 organic compounds detected through the harvest period, production of butyl acetate, pentyl acetate, and hexyl acetate increased significantly between weeks 2 and 3. Nitric oxide production was detected only at weeks 4 and 5. Of the physiological and quality parameters examined only changes in volatile production preceded the drop in fruit firmness. If consistent between seasons and orchards, this change in production of specific volatiles may be useful as an additional index of maturity. Results of a similar study using Anjou pears will be reported at the research review.

Table 1. Indicators of Bartlett fruit development at harvest. Nd: not detected; *: nmoles/kg/m^3

	August 11	August 18	August 25	September 1	September 8
Firmness lbs	19.6	19.1	19.0	16.1	15.8
Starch (1-6)	1	1	1	1.5	4.5
Ethylene (ppm)	nd	nd	nd	0.02	nd
Butyl acetate*	13.3	13	848	266	216

Pentyl acetate*	1.6	1.7	87	10.5	1
Hexyl acetate*	3.0	4.4	1879	314	0.14

Anjou 1-MCP CA: Anjou pears treated with 1-MCP at harvest were stored at 33 ^{o}F in CA with 0.5% CO_2 and up to 5% O_2. After 6 and 9 months plus 7 days at 68 ^{o}F, peel color rating (1=green, 5=yellow) increased with O_2 concentration but 1-MCP treated fruit remained greener than controls. Softening increased with increased O_2 concentration after 6 and 9 months, however, after 6 months, 1-MCP treated fruit did not soften to 6lbs or less in 7 days. After 9 months, 1-MCP treated fruit stored at 3 or 5% O_2 softened to 3.8 and 3.4 lbs, respectively. Fruit treated with 1-MCP did not develop scald regardless of storage environment and decay incidence also was lower in treated fruit regardless of O_2 concentration.

Table 2. Quality of 1-MCP treated Anjou pears after storage. Fruit treated with 300 ppb 1-MCP at harvest.

Month	O_2 %	Color d0	Color d7	Lbs	Scald (%)	Decay (%)
3	1	1	1	13	0	0
	3	1	1	12.9	0	0
	5	1.2	1.2	12.7	0	0
6	1	1	1	12.3	0	0
	3	1.3	1.6	10.6	0	0
	5	1.4	1.7	9.5	0	0
9	1	1.1	1.4	12.4	0	0
	3	2.0	2.9	3.8	0	0
	5	1.7	3.2	3.4	0	0

Impact of Ethylene and CO_2 on 1-MCP efficacy: Bartlett pears were treated with 300 ppb 1-MCP with up to 1000 ppm ethylene or up to 4% CO_2 present during treatment. The presence of 1 or more ppm ethylene was sufficient to completely inhibit efficacy of 1-MCP. A CO_2 concentration of 4% during 1-MCP treatment prevented typical 1-MCP responses while CO_2 at 2% had no effect.

Table 3. Bartlett pear quality after 2 months storage in air plus 7 days at 68 ^{o}F. Fruit treated with 300 ppb at harvest with 0, 1, 10, 100, or 1000 ppm ethylene.

Treatment	Ethylene	Peel color	Titratable acid %	Lbs	Scuffing %
Control	0	5	0.250	2.5	0
1-MCP	0	2.8	0.332	18.2	0
1-MCP	1	4.8	0.244	2.1	0
1-MCP	10	5	0.202	2.1	12
1-MCP	100	5	0.227	2.1	11
1-MCP	1000	5	0.193	2.0	11
	CO_2				
1-MCP	0.5	2.7	0.341	17.5	0
1-MCP	1	2.8	0.325	17.2	0
1-MCP	2	3.6	0.313	15.2	0
1-MCP	4	5	0.222	2.1	100

Peel color: 1=green, 6=yellow

Delayed 1-MCP treatment of Bartlett pears: Fruit were treated with 1-MCP either at harvest, the day prior to removal from CA, or after removal from CA. After 2 months storage, delayed 1-MCP treatments slowed but did not prevent ripening. Treatment with 1-MCP after 4 months storage was not effective. Ethylene produced by fruit accumulated to 18 ppm during the 1-MCP treatment after 4 months.

Table 4. Bartlett fruit quality after storage. 1-MCP applied at harvest or prior to or after removal from CA.

Month	Days at 68 °F	Treatment	Color (1-5)	lbs
2	4	Control	2.5	3.6
		1-MCP at harvest	1.1	18.9
		1-MCP during CA	1.7	6.1
4	4	Control	3.6	3.5
		1-MCP at harvest	1.8	16.5
		1-MCP during CA	3.8	3.5
		1-MCP after CA	3.7	3.4

Responses of Bartlett and Anjou pear stored at the low O_2 limit as defined by chlorophyll fluorescence: The O_2 concentration at which changes in peel chlorophyll fluorescence of Bartlett and Anjou pears (3 lots each) occurred were 0.2 and 0.3% O_2, respectively Fruit were stored in CA with 0.5% CO_2 with 1.5 (control) or 0.4 O_2 for Bartlett and 0.5% O_2 for Anjou. O_2 concentration was raised to 1.5% for some fruit stored at the low O_2 setpoint at 2 month intervals. Responses of both cultivars varied between lots and with storage duration. Bartlett fruit stored at 0.5% O_2 were slightly greener than fruit stored at 1.5% O_2 when fruit was removed from CA. Incidence of core browning, senescent scald, and internal breakdown were reduced by storage at 0.5% O_2. Anjou fruit stored at 0.4% O_2 degreened slower, did not develop scald, softened slower but developed more peel speckling compared to fruit stored at 1.5% O_2. Increasing O_2 concentration to 1.5% after 2 months reduced but did not prevent subsequent development of speckling (data not shown).

Table 5. Bartlett fruit quality after storage. Fruit were held at 68 °F for 7 days prior to analysis. Values are means for 3 lots.

Month	Trt	Color d0	TA %	Core B %	Sen. Scld %	IB %	Lbs d7	Decay %
2	air	2.7	0.314	42	0	0	2.3	2
	1.5 O_2	1.4	0.351	0	0	0	2.0	0
	0.4 O_2	1.1	0.354	0	0	0	2.0	0
4	Air	4	-	-	40	-	-	72
	1.5 O_2	2.8	0.313	0	0	37	1.4	4
	0.4 O_2	1.9	0.347	0	0	9	1.9	2
6	Air	-	-	-	94	-	-	41
	1.5 O_2	3.5	0.268	15	5	29	1.8	59
	0.4 O_2	3.0	0.301	4	0	4	2.0	44

Trt: treatment; Color: 1=green, 5=yellow; Core B: core browning incidence; sen scld: senescent scald incidence; IB: internal browning and/or breakdown; decay: decay incidence

Table 6. Anjou fruit quality after storage. Fruit were held at 68 ^{o}F for 7 days prior to analysis. Values are means for 3 lots.

Month	Trt	Color d0	Color d7	TA %	Speckling %	Scald %	lbs	Decay %
2	RA	1.5	2.6	0.248	0	0	1.9	0
	1.5 O_2	1.4	1.8	0.264	0	0	3.4	0
	0.5 O_2	1.2	1.9	0.274	0	0	5.8	0
4	RA	2.5	3.6	0.221	0	0	2.1	4
	1.5 O_2	1.2	2.3	0.247	0	0	1.9	2
	0.5 O_2	1.0	1.9	0.243	0	0	2.9	0
6	RA	3.1	3.8	0.185	0	39	2.6	59
	1.5 O_2	1.4	2.5	0.223	4	2	1.5	6
	0.5 O_2	1	2.0	0.232	15	0	2.4	4
8	RA	3.7	4	0.156	0	91	3.4	72
	1.5 O_2	1.7	2.7	0.206	0	12	1.8	20
	0.5 O_2	1.4	2.3	0.206	37	0	2.2	13

Trt: treatment; Color: 1=green, 5=yellow; TA: titratable acidity: speckling: peel speckling incidence; scald: superficial scald incidence; decay: decay incidence

Budget

Project title: Management of Harvest Maturity, Postharvest Treatments and Storage Environment for Optimum Quality of Fresh Pears
PI: J. Mattheis
Project duration: 2004-2006
Current year: 2005
Project total (3 years): $196,013
Current year request: $66,054

Item	Year 1 (2004)	**Year 2 (2005)**	Year 3(2006)
Salaries*	44,233	**49,042**	52,197
Benefits (%)	13,270	**14,712**	15,659
Supplies	2,300	**2,300**	2,300
Total	59,803	**66,054**	70,156

*Salaries: 2004: GS-9 biological science tech., 2005-6: GS-11 Postdoctoral Research Associate.

CONTINUING PROJECT REPORT **YEAR 1/3**

Project title: MCP and edible coating to improve storage life and marketing quality of pears
PI: Jinhe Bai
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Cooperator(s): Dr. Robert A. Spotts, Oregon State University, Mid-Columbia Agricultural Research and Extension Center
Dr. James P. Mattheis, USDA/ARS, Tree Fruit Research Lab.

Objectives (2004)

1. Develop preconditioning regime for promoting normal ripening capacity of MCP-treated 'd'Anjou' pears after storage.
2. Identify the critical dosage of MCP for control of superficial scald without inhibiting normal ripening of 'd'Anjou' pears.
3. Develop edible coatings to maintain green life of 'd'Anjou' pears and other pear cultivars during preconditioning and marketing periods.

Objectives (2005)

1. To understand influence of maturity of 'd'Anjou' pears on response of MCP treatment, especially focusing into ripening capacity and superficial scald incidence of fruit.
2. Develop thermofogging technology of DPA and Ethoxyquin to prevent superficial scald of 'd'Anjou' pears.
3. Develop edible coatings to maintain marketing quality of pears.

Significant findings:

- MCP treatment maintained quality of 'Bartlett' pears for two months longer than control fruits in both RA and CA conditions. We developed preconditioning regime for MCP-treated 'Bartlett' pears which ripened and had longer marketing life in comparison with control.
- MCP treatment completely controlled superficial scald of 'd'Anjou' pears during up to 9 months of CA storage. By preconditioning, MCP-treated and 9-month CA stored 'd'Anjou' pears ripened properly.
- Developed candelilla coatings for 'd'Anjou' pears. Candelilla coated fruit showed natural shininess.
- Developed an intergraded MCP and Ethoxyquin treatment system for 'd'Anjou' pears. The method extended storage life without inhibiting normal ripening capacity.

Methods:

1. Preconditioning regime:
   1) MCP treatment: 'Bartlett' and 'd'Anjou' pears were harvested at commercial maturity. Thirty six boxes of fruit were treated with 300 ppb MCP in an air-tight room at 68°C for 24 hours within 2 days after harvest. Treated fruit were stored in regular air (RA) or controlled atmosphere (CA, 1.5 % O_2 / 0.5 % CO_2) at 30 °F.
   2) Preconditioning: For 'Bartlett' after 2 and 4 months of RA storage and 4 and 6 months of CA storage, For 'd'Anjou' after 3 and 6 months of RA storage and 6 and 9 months of CA storage, fruit were transferred from the cold storage to dark preconditioning rooms at 50, 59 or 68 °F for 5, 10 or 20 days, respectively. Then fruit were transferred to 68 °F for 0, 7 and 14 days to simulate marketing life (shelf life).

3) Evaluation of ripening capacity of pears: for 'Bartlett' pears, ripen is identified when flesh firmness decreases to < 6 lb and extractable juice reduced to < 55 mL/100 g F.W. For 'd'Anjou' the standard is 6 lb and <60 mL 100 g-1 F.W.

2. Intergraded MCP and Ethoxyquin treatment system:
   1) MCP treatment: Forty five boxes of 'd'Anjou' pears were treated with 25 ppb MCP in an air-tight room at 68°C for 24 hours directly after harvest. Treated and non-treated fruit were stored in regular air (RA) at 30 °F for up to 5 months.
   2) Ethoxyquin treatment: MCP treated or non-treated fruit were drenched with 1000 ppm ethoxyquin 1, 7, 30, or 70 days after harvest, and non-drenched fruit were left as control.
   3) Evaluation of superficial scald, and ripening capacity: After 3, 4, and 5 moths of storage, fruit were transferred to 68 °F marketing temperature, and the quality factors were analyzed.
3. Natural coatings for 'd'Anjou', 'Concorde' and 'Bartlett': carnauba, candelilla, and resin emulsions or solutions were formulated and tested on pears. Fruit quality, surface shininess and internal gases were analyzed.
4. Thermofogging: use thermofogging technology to apply Ethoxyquin (trade mark: Xedaquin) with dosages of 60-120 g/MT or DPA (Trade mark: Xedamine) with dosages of 20-40 g/MT. After 4-6 months of storage, evaluate the superficial scald and phytotoxicity.

Results and Discussion

1. 'Bartlett' pears treated with 300 ppb MCP at 68 °F for 24 h shortly after harvest, and were stored at 30 °F in either RA or CA. After 2 and 4 months of RA storage, or 4 and 6 months of CA storage, fruit were preconditioned at 50, 59 or 68 °F for 5, 10 or 20 days, respectively. Preconditioned fruit were then transferred to 68 °F for 14 days to simulate marketing conditions. Control fruit stored in RA for 2 months or CA for 4 months ripened within 7 days but suffered severe internal breakdown within 14 days. However, the fruit stored in RA for 4 months or CA for 6 months suffered internal breakdown within 7 days after transfer to 68 °F. Fruit treated with MCP and followed by appropriate preconditioning after storage ripened without physiological disorder within 7 days after transferring to 68 °F. The best preconditioning combinations of temperature and duration were 68 °F for 10 days or 50 °F for 20 days if the fruit had been stored in RA for 2 months or CA for 4 months and 59 °F for 10 days if the fruit had been in RA for 4 months or CA for 6 months (Table 1). In these combinations, fruit maintained > 13 lb of flesh firmness, which could be shipped and distributed without risking mechanical damages, and softened to < 6 lb (eating quality) within 7 days of marketing conditions (Table 1).
2. 'd'Anjou' pears were treated with 300 ppb MCP at 68 °F for 24 h shortly after harvest, and were stored at 30 °F in either RA or CA. After 3 and 6 months of RA storage, or 6 and 9 months of CA storage, fruit were preconditioned at 50, 59 or 68 °F for 5, 10 or 20 days, respectively. Preconditioned fruit and control fruit (without MCP treatment and without preconditioning) were then transferred to 68 °F for 14 days to simulate marketing conditions. Physiological disorder, flesh firmness (FF), extractable juice (EJ), titratable acidity (TA) and soluble solids (SS) were monitored during the marketing period. Control fruit suffered severe superficial scald regardless of storage atmosphere and time upon transfer to 68 °F within 7 days. For the fruit treated by MCP, and stored in RA for 3 or 6 months, or in CA for 6 months, all of the preconditioning combinations of durations and temperatures did not initiate ripening, therefore, no significant softening of fruit was observed during subsequent marketing period of 14 days at 68 °F. However, for the fruit treated by MCP, stored for 9 months in CA, and preconditioned for 20 days at 59 or 68 °F, ripening was initiated. With the preconditioning of 20 days at 68 °F, fruit reached eating quality within marketing period

of 2 weeks at 68 °F without superficial scald. In contract with non-MCP-treated fruit which suffered 93% incidence of superficial scald during ripening (Table 2).

3. Previous experiment showed that the optimum dosage of MCP for 'd'Anjou' pears is some where between 20 and 30 ppb. In this research, 'd'Anjou' pears, pretreated or non-treated with 25 ppb MCP, were stored in RA for up to 5 months at 30 °F. After 1, 7, 30 or 70 days of storage sub-samples were pulled out, immediately drenched with 1000 ppm Ethoxyquin and then were returned to the storage again. Firmness, titratable acidity (TA), soluble solids content (SSC), alfa-farnesense and CT were analyzed after 3-5 months of storage. MCP or Ethoxyquin (1000 ppm) applications immediately after harvest prevented scald for 3 months. However, the applications of MCP + Ethoxyquin prevented scald for 4 months or longer. The result indicates that a MCP application directly after harvest can allow a delayed and low-dosage application of Ethoxyquin (Fig. 1).
4. Seven experimental coatings with different gas permeability were applied on 2-4 months stored pears of 'd'Anjou', 'Concorde' and 'Bartlett'. The coated or non-coated fruit were held at 68 °F for up to 2 weeks. The gas concentration inside the fruit for the various coatings ranged from 3-20% CO_2 and 15-1%O_2 (Table 3). The coatings with intermediate gas permeance (5-10% carnauba or candelilla) gave intermediate values of CO_2 and O_2 in the fruit. The coatings with lowest permeance (carnauba 20%) caused high internal CO_2, and low O_2, resulting in anaerobic fermentation in pears. Candelilla coated pears showed lowest gloss and provide a more natural appearance (Table 4).

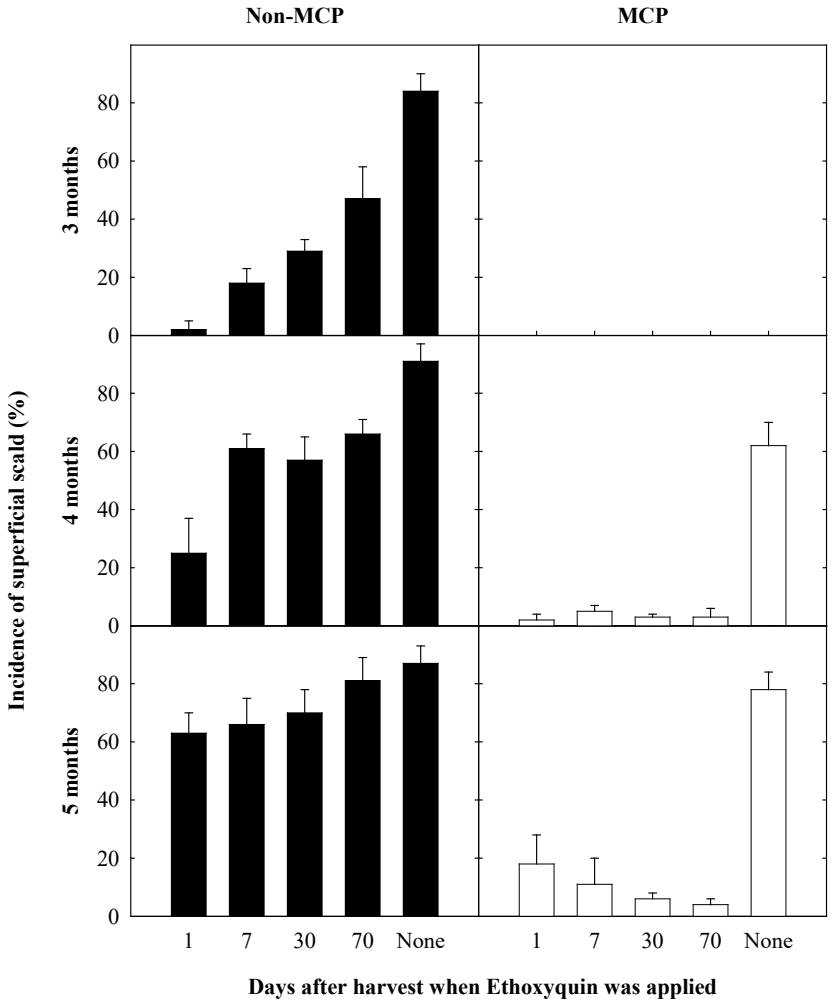

Fig. 1. Effect of MCP and Ethoxyquin on superficial scald of 'd'Anjou' pears. Fruit were treated or untreated with 25 ppb MCP for 24 h immediately after harvest and then stored at 30° F for up to 5 months. Ethoxyquin drench (1000 ppm) was applied after 1, 7, 30 or 70 days of storage.

Table 1. Incidence (%) of internal breakdown and over all quality of 'Bartlett' pears during marketing at 68 °F. Fruit treated with MCP and non-treated control were stored in regular air (RA) for 2 or 4 months, or controlled atmosphere (CA) for 4 or 6 months at 30 °F, and subsequently preconditioned at 50, 59 or 68 °F for 5, 10 or 20 days prior to transferring to marketing at 68 °F.

Treatment	Storage		Preconditioning		Disorder and over all quality during marketing at 68 °F					
					Internal breakdown (%)			Over all quality		
	Atmos-phere	Months	Temperature (°F)	Days	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control	RA	2		0			57 ± 12	S [z]	R	D
1-MCP	RA	2	50	5				S	S	U
				10				S	U	R
				20				S	R	R
			59	5				S	U	R
				10				S	U	R
				20			77 ± 5	U	R	D
			68	5				S	U	R
				10				S	R	R
				20		73 ± 12	100 ± 0	R	D	D
Control	RA	4		0		97 ± 5	100 ± 0	S	D	D
1-MCP	RA	4	50	5				S	U	R
				10			50 ± 8	S	U	D
				20		33 ± 16	67 ± 12	U	D	D
			59	5				S	U	R
				10			63 ± 5	S	R	D
				20		77 ± 8	100 ± 0	R	D	D
			68	5			37 ± 17	S	U	D
				10			63 ± 12	U	R	D
				20	77 ± 12	93 ± 5	100 ± 0	D	D	D
Control	CA	4		0			33 ± 12	S	R	D
1-MCP	CA	4	50	5				S	S	U
				10				S	U	R
				20				S	R	R
			59	5				S	S	R
				10				S	U	R
				20			53 ± 16	U	R	D
			68	5				S	S	R
				10				S	R	R
				20		70 ± 0	100 ± 0	R	D	D
Control	CA	6		0		47 ± 12	100 ± 0	S	D	D
1-MCP	CA	6	50	5				S	U	R
				10			63 ± 5	S	U	D
				20		57 ± 12	100 ± 0	U	D	D
			59	5				S	U	R
				10			63 ± 5	S	R	D
				20		37 ± 5	67 ± 12	R	D	D
			68	5			47 ± 17	R	U	D
				10			77 ± 5	U	R	D
				20	37 ± 16	67 ± 5	100 ± 0	D	D	D

[z] S: Shippable firmness with FF > 13 lb; U: unripe with FF = 6 - 13 lb; R: ripe with FF < 6 lb; and D: disorder (internal breakdown) > 10%.

Table 2. Incidence (%) of superficial scald and over all quality of 'Bartlett' pears during marketing at 68 °F. Fruit treated with MCP and non-treated control were stored in regular air (RA) for 3 or 6 months, or controlled atmosphere (CA) for 6 or 9 months at 30 °F, and subsequently preconditioned at 50, 59 or 68 °F for 5, 10 or 20 days prior to transferring to marketing at 68 °F.

Treatment	Storage		Preconditioning		Disorder and over all quality during marketing at 68 °F					
	Atmos-phere	Months	Temperature (°F)	Days	Superficial scald (%)			Over all quality		
					Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control	RA	3		0		43 ± 18	62 ± 7	S [z]	D	D
1-MCP	RA	3	50	5				S	S	S
				10				S	S	S
				20				S	S	S
			59	5				S	S	S
				10				S	S	S
				20				S	S	S
			68	5				S	S	S
				10				S	S	S
				20				S	S	S
Control	RA	6		0		100 ± 0	100 ± 0	S	D	D
1-MCP	RA	6	50	5				S	S	S
				10				S	S	S
				20				S	S	S
			59	5				S	S	S
				10				S	S	S
				20				S	S	U
			68	5				S	S	S
				10				S	S	S
				20				S	S	U
Control	CA	6		0		60 ± 11	73 ± 9	S	D	D
1-MCP	CA	6	50	5				S	S	S
				10				S	S	S
				20				S	S	S
			59	5				S	S	S
				10				S	S	S
				20				S	S	S
			68	5				S	S	S
				10				S	S	S
				20				S	S	S
Control	CA	9		0		93 ± 6	100 ± 0	S	D	D
1-MCP	CA	9	50	5				S	S	S
				10				S	S	U
				20				S	S	S
			59	5				S	S	S
				10				S	S	S
				20				S	U	U
			68	5				S	S	S
				10				S	S	S
				20				S	U	R

[z] **S: Shippable firmness with FF > 10 lb; U: unripe with FF = 6 - 10 lb; R: ripe with FF < 6 lb; and D: disorder (Superficial scald) > 10%.**

Table 3. Internal CO_2 and O_2 (%) of pears at 68°F stored 7 days after application of different coating.

Carnauba concentration	d'Anjou		Bartlett		Concorde	
(%)	CO_2	O_2	CO_2	O_2	CO_2	O_2
0	2	19	3	18	2	16
2	3	15	4	13	4	12
5	7	11	8	10	10	9
10	13	6	15	3	12	7
20	16	2	20	1	17	2

Table 4. Gloss, weight loss, and firmness of 'd'Anjou' pears coated with different formulations after 7 days at 68 °F

Coating	Gloss (GU)	Weight loss (%)	Firmness (N)
Non-coated	5.8	3.6	11
Candelilla 5%	6.9	2.1	22
Candelilla 10%	7.5	1.7	25
Carnauba 5%	9.7	1.8	19
Carnauba 10%	10.9	1.4	27
Shellac 5%	11.1	2.5	26
Shellac 10%	13.4	2.2	33

Budget

Project title: MCP and edible coating to improve storage life and marketing quality of pears
PI: Jinhe Bai
Project duration: 2004-2006
Current year: 2005
Project total (3 years): $79,200
Current year request: $29,700

Item	Year 1 (2004)	**Year 2 (2005)**	Year 3(2006)
Salaries		**9,865**	15,000
Benefits (49%)		**4,835**	7,350
Wages			
Benefits (%)			
Equipment	15,000	**14,700**	11,750
Supplies			
Travel		**300**	400
Miscellaneous			
Total	15,000	**29,700**	34,500

CONTINUING PROJECT REPORT **YEAR 2/3**

Project title:	Ethylene ripening of pears by unconventional means
PI:	Dr Keith Sharrock
Organization:	The Horticulture and Food Research Institute of New Zealand (HortResearch)
Address:	Private Bag 3123, Hamilton, New Zealand
Phone:	64 7 858 4789
E-mail:	ksharrock@hortresearch.co.nz
CO-PI:	Dr Ron Henzell (HortResearch)
Cooperator:	Dr Eugene Kupferman (WSU Wenatchee)

OVERALL PROJECT GOAL

This project aims to test the potential of unconventional approaches to ethylene conditioning to expand the market window for winter pears, particularly Green Anjou. This has involved firstly confirming the reported need over the first month of storage for more prolonged and elevated exposures to ethylene than are practical using conventional conditioning methods. That knowledge will then be applied in testing the usefulness of our Ethylene Release Capsules (ERCs) as a viable alternate means of achieving optimal conditioning without requiring expensive conditioning facilities.

OBJECTIVES FOR 2004:

- Determine the influence of ethylene concentration during conditioning at 7ºC and 20ºC on subsequent softening and aroma production by Green Anjou (in USA) and Comice (in NZ) after one and 3.5 weeks of cold storage.
- Identify appropriate levels of ventilation for 20 kg box and clamshell applications that prevent detrimental modification of respiratory gas levels while permitting enclosed ERCs to promote ripening of Green Anjou and Comice.

OBJECTIVES FOR 2005:

- Continue to determine the influence of ethylene concentration and length of conditioning period at 20ºC on subsequent softening and aroma production by Comice (in NZ) after one and 3 weeks of cold storage.
- Test the use of ERCs for pre-conditioning Green Anjou in boxes immediately prior to and during the first day of transport to the East Coast, to be compared in terms of eating quality and cosmetic attributes with fruit given the current industry standard pre-conditioning, after all have been further ripened to a similar extent upon arrival.

The above objectives for 2005 differ from those proposed last year in the following respects:

1. Green Anjou has been removed from the first objective, since our tests over the past two years on that variety have yielded sufficient information to permit more applied testing (see below). Tests on Comice in Year 1 were less conclusive, so continued laboratory testing in Year 3 will be required on that variety.
2. Green Anjou studies have been advanced to include a more complete test of their practical application. Taste panel assessments of the effects of continuous exposure to ethylene during ripening have been deferred in favour of the more geographically demanding and therefore necessarily less formal taste comparison of alternative pre-conditioning strategies after trucking across America.

Significant findings: Based on two season's work on conditioning Green Anjou, the following interim results and conclusions seem significant. Firm conclusions for Comice require repeat studies.

Effects of ethylene concentration

- Levels as low as 2 ppm ethylene produced definite stimulation of ripening (based on both firmness and aroma).
- Full softening was triggered by lower levels of ethylene than those required to trigger full aroma production. Effects on softening plateaued at about 10 ppm but to trigger full aroma potential required >100 ppm (for 7 day 20°C conditioning).
- Higher levels of ethylene during conditioning resulted in a greater proportion of the fruit becoming autocatalytic (producing their own ethylene) after subsequent ripening.

Effects of temperature during conditioning

- Ethylene conditioning at 7°C had a significant positive effect on fruit capacity to subsequently soften and produce aroma (particularly the latter). However, conditioning at 20°C was markedly more effective than conditioning at 7°C in both respects.

Effects of length of conditioning period

- Longer (6-7 day) ethylene conditioning generally had a greater effect on capacity to soften, and always had a greater effect on aroma, than shorter (3 day) exposures, which required higher levels of ethylene to trigger the same responses.

Influence of period in cold storage

- Anjou and Comice became progressively less dependent on external ethylene with increased time in cold storage, and increasingly capable of producing their own ethylene.
- By 3 weeks (Comice) and 5 weeks (Anjou) after harvest, ethylene conditioning no longer increased the capacity to soften, but still enhanced aroma production potential.

Usefulness of ERCs as an method of conditioning

- ERC prototypes were capable of producing and maintaining levels of ethylene sufficient to simply and effectively condition early season Anjou pears in a range of packaging, including clamshells, Euro-boxes and bushel boxes, using conventional perforated apple box liners.

Methods

Influence of ethylene concentration, exposure period and temperature during conditioning

Methods of supplying ethylene and creating the conditioning environment have evolved considerably during the course of this project, to capitalize on recent advances in our ERC technology. The first season's tests on Anjou and Comice involved sealing pears in air-tight jars in order to expose them to the desired range of ethylene concentrations at different temperatures. Each jar had to be flushed briefly with fresh air each day before being resealed, re-equilibrated to the incubation temperature and then reloaded with the original quantity of ethylene; all extremely labor-intensive.

In this last season a less laborious approach permitted experiments to be scaled up, providing more adequate replication. The key was the availability of three new models of a prototype ERC designed to expose fruit in HortResearch clamshells to around 5, 50 and 200 ppm ethylene for several days. These clamshells had previously been shown to be adequately ventilated to permit pears to ripen normally, and were used commercially in conjunction with ripeSense™. The latter aroma-sensing labels were used to compare aroma production by fruit in the clamshells during post-conditioning ripening at 20°C following removal of the ERCs. The new testing protocol therefore replicated a possible application of the ERCs in conjunction with ripeSense™ packs. This approach will be used in further studies of the Comice conditioning requirements over the next two seasons in New Zealand.

Between-fruit interactions can be problematic when four fruit are treated and subsequently ripened in the same clamshell. About 15% of the Anjou used in 2004 were affected by externally invisible cork pit, causing them to soften prematurely and to produce ethylene in quantities sufficient to also affect other fruit in the same clamshell. This compromised some of the 2004 Anjou data, particularly that relating to the first conditioning period, begun at one week after harvest. The effects of the problem were reduced in the second set of conditioning tests, begun at three weeks after harvest, by opening most of the clamshells after the conditioning period. Aroma production was monitored by transferring eight fruit from each treatment individually into separate clamshells containing ripeSense™ labels. Future trials should ideally exclude any fruit exhibiting cork pit.

Conditioning pears in boxes using Ethylene Release Capsules Green Anjou (80-90 ct), harvested at commercial maturity on 1-2 September and kept in commercial cold storage until 20 September, were loaded into Euro-boxes containing either standard plix trays (2 layers of 24 fruit) or 12 HortResearch 4-pack clamshells. Two ERCs (highest release "green" type) were added to each box (except those intended as controls) while the fruit were still cold. All boxes, including controls without ERCs, were then enclosed in one of three different types of liner film; (i) standard apple liner with the normal distribution of ¼ inch holes; (ii) micro-perforated PVC (Resinite MX-P Micro 76, AEP Industries, UK); (iii) LifeSpan modified atmosphere membrane. Each box was incubated at 20°C without opening for six days, while the internal levels of ethylene, oxygen and CO_2 were monitored. The covering films were removed after six days conditioning and samples of the fruit were compared in terms of firmness and eating quality during the following six days.

In-transit conditioning tests proposed for 2005 Green Anjou at 1 and 3 weeks from harvest will be pre-conditioned by placing ERCs in various types of packaging, including clamshells, lined Euros and bushel boxes, for 1, 3 and 5 days while the fruit are held at 20°C prior to refrigerated transport. Further samples of the same batches of fruit in unlined Euros and bushel boxes will be given the current industry-standard conditioning treatment (warmed for 1 day, exposed to ethylene at 100 ppm for one day and then thoroughly vented). Control fruit will be in identical types of packaging but not given any ethylene treatment. Controls and all the treatments will then be loaded, while still warm and with any ERCs still in place, into a refrigerated truck for shipment to an East Coast destination. Ethylene-sensitive vegetables will be included in adjacent boxes to assess the damage potential of ethylene leakage from boxes containing ERCs. Upon arrival, the packaged pears will be returned to 20°C to permit further ripening. Sampling over the following week at the destination will determine the time taken for fruit of each treatment to ripen to a standard firmness (say 2.5 lb), at which point the remainder of that treatment will be rapidly chilled to a holding temperature of around 0°C. Once fruit of all the treatments (apart from the no-ethylene controls) have reached the standard firmness level and are chilled to the holding temperature, they will be further compared with respect to eating quality attributes, including aroma, and thoroughly inspected for transit-related damage (e.g. bruising, scuffing etc). A local consumer taste panel will be employed to provide unbiased comparison of the effects of our various conditioning treatments on fruit eating quality after delivery across America.

Results and Discussion

Influence of ethylene concentration, exposure period and temperature during conditioning

Comice in New Zealand

Comice were harvested at commercial maturity on 2 March in the Waikato, New Zealand, to be subjected to a similar protocol of conditioning tests to that used on Anjou six months earlier in Oregon (covered in our 2004 report).

Potential to soften and capacity to produce aroma both increased with increasing ethylene during conditioning (Fig. 1). Raising the ethylene concentration above 20 ppm, or extending conditioning from three to seven days, had little impact on the potential to soften and produce aroma (Fig. 1).

Attempts to repeat these conditioning treatments on Comice at three weeks after harvest were seriously compromised by the early onset of endogenous ethylene production, which resulted in all the fruit softening at similar rates, including those intended to be ethylene-free controls. The "control" jars containing fruit with no added ethylene were found to have accumulated 20 ppm ethylene after 16 hours, whereas two weeks earlier the equivalent test could not detect any ethylene produced by the fruit. It is likely that the shorter than expected chilling period required can be attributed to these Comice being more mature than those tested in literature studies. A planned repeat study in 2005 will use fruit harvested much earlier in the season. Special care will also be taken to ensure that the fruit are not exposed to ethylene during storage in the cold room.

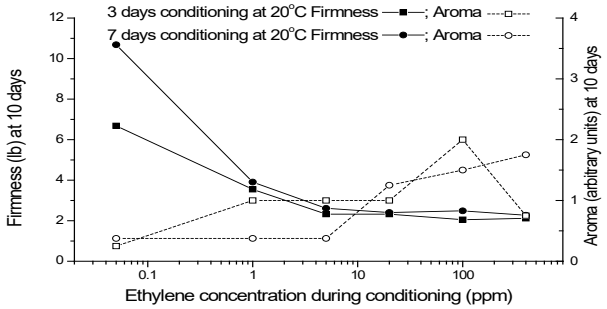

Figure 1. Effects of concentration of ethylene and length of conditioning period, commencing 1 week after

Green Anjou in Wenatchee

Levels of ethylene maintained in the clamshells by the three types of ERC are shown in Figure 2A. The highest release type needed to be replaced after 2-3 days in order to maintain ethylene at around 200 ppm in the clamshell, but the two lower release models clearly had the potential to maintain 5 and 50 ppm levels for much longer than the 6-day conditioning period monitored. Ethylene levels were also monitored in clamshells with fruit during the post-conditioning ripening after the ERCs had been removed. These levels were initially all very low or undetectable, but in some cases had increased significantly by the end of the ripening period. Stimulation of autocatalytic ethylene production by the fruit was greater following the conditioning at three weeks than at one week after harvest, and most accentuated by conditioning at 20°C for 7 days (Fig. 2B).

Increased ethylene concentrations during conditioning of Green Anjou fruit at one and three weeks after harvest had greater effects on subsequent rates of softening if conditioning was performed at 20°C than at 7°C (Fig. 3B vs. 3A and 3D vs. 3C). Conditioning at 7°C at concentrations >5 ppm nonetheless had a significant effect on subsequent rates of softening and aroma production (Fig 3C). At 20°C the effects of increasing ethylene concentration on softening rates began to flatten out at levels above 10 ppm whereas the effects on aroma production continued to increase substantially until some point between 60 and 200 ppm (Figs 3B & 3D). Increasing the conditioning period at 20°C from 3 to 6 days had little effect on the rate of subsequent softening but had a marked impact on the capacity of the fruit to produce aroma as they ripened (Figs 3B & 3D). This is in keeping with our conclusions from the previous season, and with an earlier study[1] that concluded that, during the first 4 weeks in storage, Anjou need at least 4 days of 100 ppm ethylene to trigger the capacity to fully ripen.

Aroma production shown in Figure 3, expressed in arbitrary units relative to a ripeSense™ color scale, of fruit conditioned at one week should not be compared in absolute terms with those of fruit conditioned at three weeks after harvest. In the former case the sensor responded to aroma from four

[1] Facteau, T.J. and Mielke, E.A. 1998 *Acta Hortic.,* **475:** 567-574

fruit within a clamshell, whereas in the latter case fruit were assessed individually, using a more sensitive sensor, to minimize the impact of fruit with cork pit. Fruit with cork pit typically began to produce their own ethylene at an abnormally early stage in storage, causing accelerated softening and aroma production, both in themselves and in adjacent fruit in the same container.

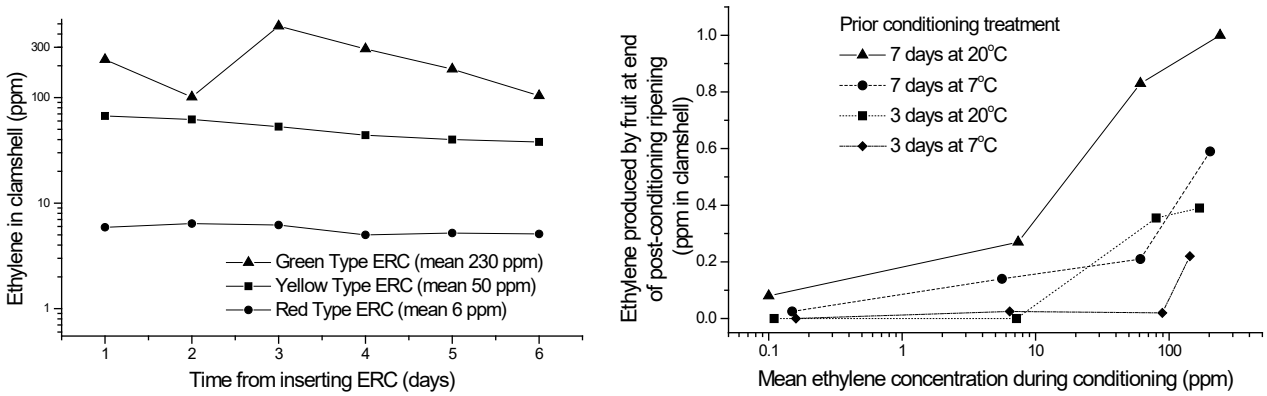

Figure 2: Ethylene concentrations inside representative clamshells **A:** containing one of each of the three different types of ERC, plus four pears, during conditioning. (The green type needed to be replaced after 2-3 days in order to maintain an ethylene level of >100 ppm for the entire 6-day period.) **B:** at the end of the post-conditioning ripening at 20°C following removal of the ERCs.

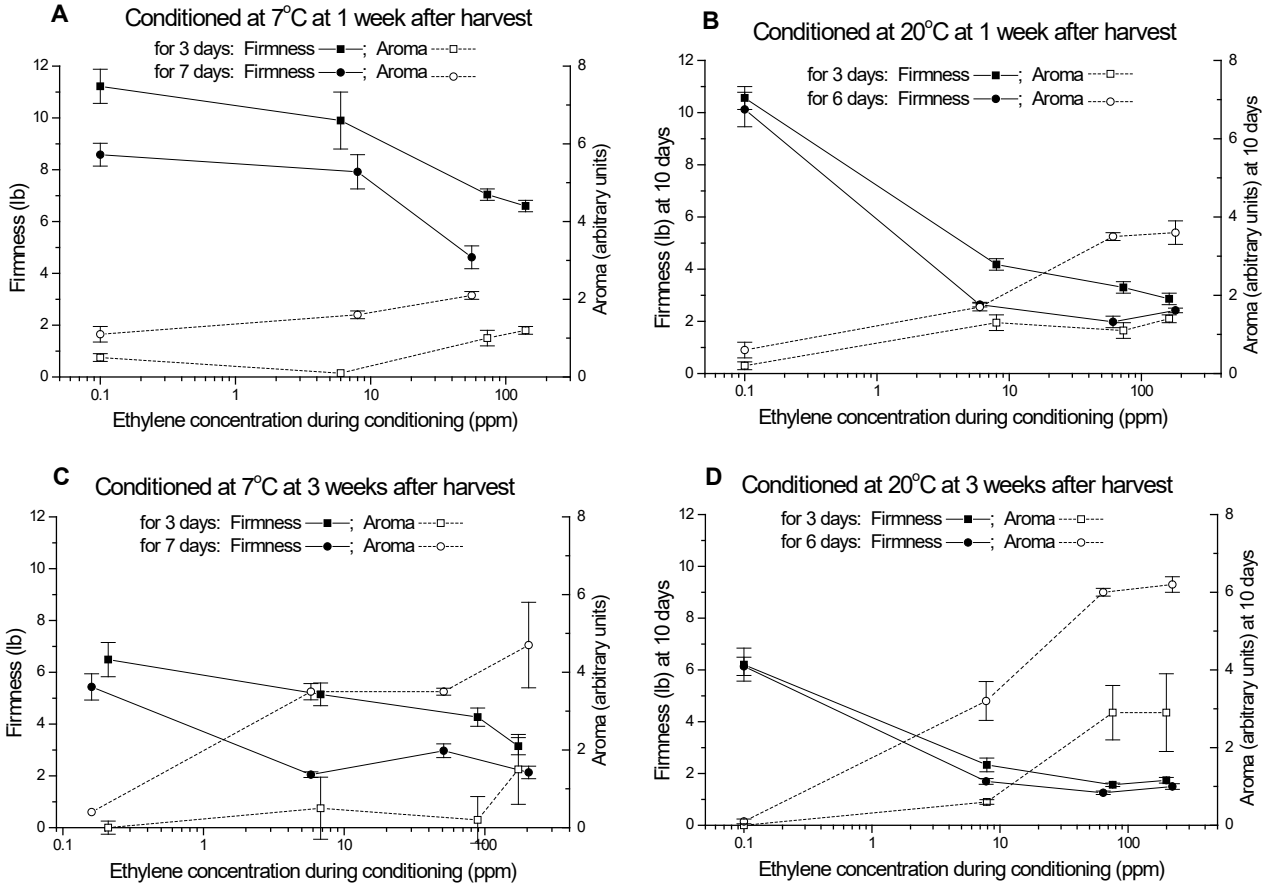

Figure 3: Effects of concentration of ethylene and length of conditioning period, commencing one and three weeks after harvest, on firmness and aroma of Comice. Fruit conditioned at 20°C and subsequently ripened at 20°C were assessed at 10 days after the start of conditioning; fruit conditioned at 7°C for 3 and 7 days were assessed after 10 and 8 days (respectively) of post-conditioning ripening at 20°C.

Conditioning pears in boxes using Ethylene Release Capsules

Anjou pears removed from cold storage at 20 days after harvest, conditioned for six days in two-layer Euro-boxes at 20°C also containing two ERCs, were soft, juicy and good eating after two further days of ripening in open boxes, whereas control fruit (without ERCs but otherwise treated identically) remained hard and inedible (Fig. 4). To restrict the rate of ethylene loss, each box was wrapped with one of three potential liner or shroud films. These films differed significantly with respect to their capacities to retain ethylene while permitting escape of respiratory CO_2 (Fig. 4A). However, even the most leaky (standard apple liner with ¼ inch holes) retained sufficient ethylene to trigger significant softening, relative to the controls (Fig. 4B).

Greater softening of the controls (no ERCs) wrapped in LifeSpan than in the other films (Fig. 4B) was probably associated with the higher levels of ethylene that accumulated inside the LifeSpan-wrapped controls, largely attributable to a few cork-pitted fruit. In apparent contrast, the fruit wrapped in LifeSpan film that were intentionally treated with ethylene were firmer than those exposed to ethylene inside more permeable films. This difference was probably due to the ripening retarding effects of CO_2 that accumulated to the highest levels inside the LifeSpan film. (Fig 4A).

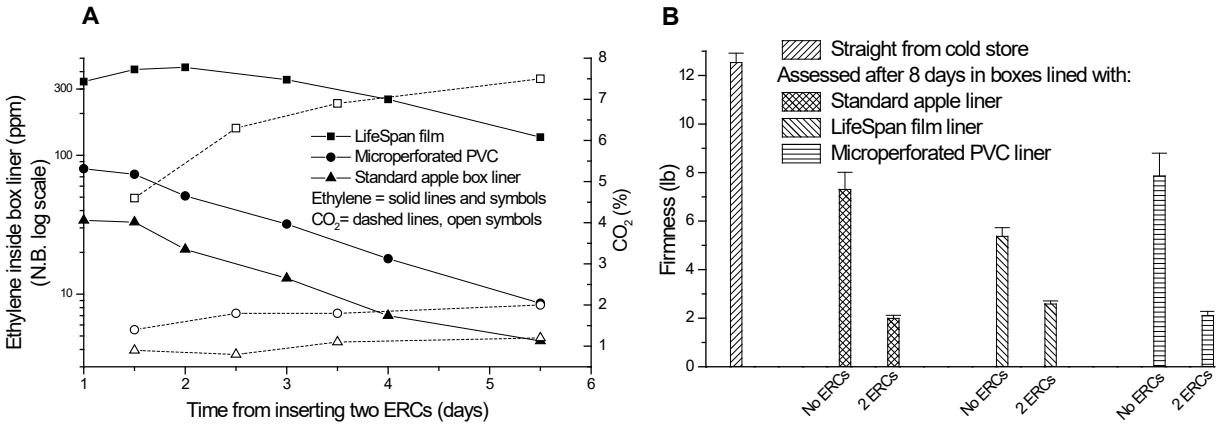

Figure 4: A: Ethylene and carbon dioxide levels during conditioning of pears inside two layer Euro-boxes wrapped as indicated and containing two high-release-rate "green" ERCs. **B:** Effects of conditioning for six days in film-wrapped boxes containing ERCs, producing the ethylene levels shown in A, on fruit firmness relative to control fruit incubated without ERCs in identical boxes.

The use of either conventional plix trays or clamshells to support the fruit within the wrapped Euro-boxes had no detectable effect on the efficiency of the conditioning by ERCs, which were placed amongst rather than inside the clamshells.

The challenge of obtaining Anjou of good eating quality early in the storage season was appreciated by our industry associates, who asked us to use our ERCs to condition nine boxes of Green Anjou for shipping to Los Angeles for sampling by attendees of the Produce Marketing Association annual meeting in mid-October 04. We simply placed two or three ERCs amongst the fruit in each box, inside standard apple liners, and allowed the boxes to warm up to 20°C. After four days the fruit had been conditioned down to 7 lb firmness, ready for chilling and trucking to L.A. Evidently it ripened fully upon arrival and developed sufficient flavor and aroma for use in the ripeSense™ display.

Budget

Project title: Ethylene ripening of pears by unconventional means
PI: Dr Keith Sharrock
Project duration: 2003-2005
Current year: 2005
Project total (3 yrs): US$139,700
Current year request $59,800

Item	Year 1 (2003)	Year 2 (2004)	**Year 3 (2005)**
R&D Fees[1]	26,240	32,000	**30,200**
Equipment[2]	400	7,000	**4,000**
Supplies [3]	500	1,500	**8,000**
Travel [4]	2,860	7,200	**10,000**
Accommodation in US [5]		1,600	**2,000**
Cool store fees		600	**600**
Informal taste panel[6]			**5,000**
Total	30,000	49,900	**59,800**

[1] These fees pay for each of the two PIs' salaries and benefits for six weeks full time in Year 3. New Zealand Crown Research Institutes (CRIs) are incorporated companies owned by the NZ government but operate uniquely in that all funding, including that required for staff salaries, is contestable and obtained in competition with private companies and publicly owned research entities. As it is a "for-profit" company, HortResearch's R&D fees must at least cover the full cost of the R&D, including organizational overheads. Otherwise it would be cross-subsidizing research for international clients at the expense of other New Zealand research, which CRIs are not allowed to do. As a special concession, HortResearch will forego the normal requirement for a project to return a profit from the funding requested. It is expected that New Zealand's internationally competitive salaries and cost structures more than compensate for these unique requirements for a government–owned research institute to operate as a for-profit company.

[2] Includes a portable device for monitoring ethylene levels, as a more convenient alternative to the gas chromatograph currently used.

[3] Includes production of prototype ERCs and clamshells, purchase of ethylene, fruit and packaging, and the cost of trucking fruit across America.

[4] Includes two return economy class airfares to Auckland-Wenatchee in 2005; two economy return airfares from Wenatchee to an East Coast fruit delivery destination, car rental in the US for one month; and a food allowance ($25/day) while in the US.

[5] Utilizing on site accommodation (trailer home) at WSU Wenatchee for two weeks in 2005, including power and gas, and motel accommodation for two for two weeks at the East Coast delivery destination.

[6] We envisage employing a local consumer taste panel to provide unbiased comparison of the effects of our various conditioning treatments on fruit eating quality after delivery across America.

FINAL REPORT

Project Title:	Red Peel-on Processed Winter Pears
P.I..	David Anderson Green & Green, Inc.
Co-PI(s) and affiliations:	Yanyun Zhao, Oregon State University Dr. Eric Wilhelmsen, Green & Green, Inc.
Cooperators:	Greg Sarley, Harry & David Stores Peter Truitt, Truitt Bros. Inc. Bill Eckart, Fruit Growers League, Jackson Co.

Objectives:

1. Stable red peel color in processed winter pears
2. Commercially viable infusion process development for red and green pears

Significant Findings:

1. Stable red peel color
    a. Anthocyanin is the primary pigment in red pears, concentrations vary greatly by variety.
    b. The literature search found much prior art on anthocyanin color retention, which has proven a challenging problem over the years.
    c. The red pigment layer in pears is very thin and fragile relative to the green pigment layer in pears.
    d. Wax removal and subcutaneous damage both were required for color stabilization in both green and red pigmented pears.
    e. Cation complexes were shown to create many color hues when a process similar to the previously developed green color fixing was employed to fix pigment color in red pears.
    f. Off-color hues from pigment/cation complexes were shown to be color stable. Many pigment complexes with different colors were observed. Many were thermally stable under certain conditions.

2. Commercial process development for red and green color fixing
    a. Existing process technology did not accomplish wax removal/ion penetration at commercial volumes.
    b. Controlled abrasion under proper conditions using a “sand blasting” technique was successfully developed and a provisional patent filed with the assignment of rights to Pear Bureau North West.
    c. The Truitt Bros. commercial production trial of Bosc, yellow Bartlett and green Anjou yielded small lots of peel-on chunked winter pears.
    d. Formulated peel-on glass-packed products were developed for possible line extensions to the Harry and Davids peeled Comice products.
    e. Frozen versions were presented to Schwann’s for product ideation work

Background

In a prior study funded by the USDA, Green & Green, Inc. (G&G) did consumer studies and process technical development work that showed that smaller, low value winter pears could be used in

formulated products sold in clear plastic and glass packaging and potentially generate new revenue to the grower. The bright peel colors and unique winter pear flavors were key drivers of this potential new demand. Follow-up work funded by the Fruit Growers League of Jackson Count developed bench-top technology to hold green peel color during thermal processing to augment G&G technology. The Fruit Growers League owns exclusive rights to this patented work.

Prototypes of these winter pear products gained acceptance with Harry and Davids marketing staff. Their tests indicated good product demand at the price points necessary for good profitability. In a separately funded, but related product commercialization project, G&G has co-operated with Truitt Bros. cannery staff to ship commercial quantities of the winter pear products to Harry and Davids' stores to test product demand and evaluate the shelf life of the new G&G processing technology

A four color (yellow, russeted, green & red) mix of peel-on winter pears was preferred in the consumer work. Yellow and russeted skinned pears have been produced as peel-on chunks at commercial levels, but no red technology existed and the green skin bench-top process was too slow to support commercial production of red and green peel-on chunk production. The purpose of this study was to utilize the cationic infusion technology to hold red peel colors during processing and to do so at commercial volumes for both red and green peel-on winter pear chunks.

Methods

Red Color Retention

OSU professor Yanyun Zhao did prior work funded by G&G and the ODA to optimize green peel color retention during thermal processing using zinc cations. In this study conducted from July 2004 through the present, they have explored using cations to fix red colors in a manner similar to the green peel-on bench-top process. Research on cherries over many years has led to unsatisfactory natural red processed colors, this was a warning that this was a challenging undertaking.

Our work using cations has not yielded the appropriate red color needed, but has generated many color hues in pear skins that show promise. This creative approach of generating insoluable pigment complexes tied together using cations is limited by the inability to expose the pigmented layer to the cation being used in the treatment and any native or supplemental pigments. This is due to thinness of the red pigment layer and the durability of the waxy coat on the red and green skinned winter pear.

A complete review of the work Dr. Zhao and her doctoral candidate Thao Ngo appears in attachment I.

We are presently evaluating the best approach to completing the red peel color retention work. Thao Ngo will continue his research into creating an acceptable red peel color. Additional funding may be required once we fully evaluate the combined possibilities of the red color retention results combined with the cation infusion technology.

Cation InfusionTtechnology

For red and green color retention to work using cation fixing, the cations need to penetrate red and green peel structures prior to thermal processing. Work by G&G showed that standard infusion technologies and chemical stripping methods for wax were unsuccessful. Manual abrasion methods used on the bench-top were too time consuming. Work by Dr. Eric Wilhelmsen and others showed

that by modifying standard sand blasting equipment and using food grade abrasives, it was possible to strip the cold hard wax off the softer cuticle in a way that allowed the pigment to be fixed. The provisional patent filing is shown in attachment II.

In this methodology the fruit is impacted by grains of sugar or ice at a velocity that breaks the wax off, but only lightly damages the underlying cuticle. Subsequent treatment of the whole pear with the previously described antioxidant/cationic dips prior to processing yields stable peel colors after rotary retorting the pears in #10 tin lined cans.

Results and Discussion

The infusion technology developed under this work allowed us to make a short cannery run to attempt green peel-on production. The test was done at Truitt Bros. in their Front Street facility. The test was negatively impacted by the high humidity of the cannery that broke down the crystalline structure of the sugar used as an abrasive. Only small quantities of green skinned chunks were produced with uneven color quality. The commercial samples of peel-on Bosc, Bartlett and Anjou peel-on pears were then used to formulate prototypes for Harry and David stores. These prototypes are currently being evaluated for addition to the product line.

Path Forward

The primary goal of demand-side research is to create new products with significant consumer demand. This will then create production demand and improved agricultural raw material pricing. Toward this goal, we have succeeded in getting the first winter pear in glass items into commercial distribution through the Harry and David specialty retail outlets. We must:

1. Track sales and product quality in the initial retail launch of the first items and make production adjustments as necessary.
2. Complete the installation of the scaled up production facility for winter pears in glass at the Truitt Bros. cannery in Salem Oregon.
3. Expand canned Comice production at Truitt Bros. to meet glass repack demand.
4. Start-up production of the initial winter pears in glass items at Truitt Bros. Expand distribution of the initial winter pear product line.
5. Test additional ion/pigment combinations to find an acceptable red color that is uses technology developed in this study to remain fixed during the canning process
6. Produce three color peel-on winter pears in glass to test sales potential in a live test market.
7. Add red colored peel-on winter pears to the items developed in #6 above and evaluate consumer demand.
8. Produce test volumes of products using remanufactured canned peel-on winter pears for frozen items.
9. Utilize the Truitt Bros. remanufacturing line to produce clear plastic packaged winter pears items.

Acknowledgements

This project to stimulate consumer demand for processed winter pears has been a two year effort involving several independent projects and many individuals. We wish to thank the Winter Pear Control Committee for their support in this phase. We also wish to recognize the exceptional efforts of Jerry Gardner at the ODA and Jeff Clawson at OSU. Truitt Bros. Inc. and Bear Creek Orchards also deserve acknowledgement for the business risk they have taken to evaluate the consumer demand for processed winter pear products.

Attachment I Red research color retention findings detail

PROJECT REPORT

CONFIDENTIAL

To

Dennis Anderson
Green & Green, Inc.
7623 NW McDonald Pl.
Corvallis, OR 97330

From

Thao Ngo and Yanyun Zhao
Dept. of Food Science & Technology
100 Wiegand Hall
Corvallis, OR 97331-6602

Phone: 541 -737-9151
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E-mail: Yanyun.zhao@oregonstate.edu

January 8, 2005

IV. CONCLUSION

Major pigments responsible for the red pigmentation in winter pears are anthocyanins. They are very water-soluble, thermal sensitive and usually destroyed during severe thermal treatment. By adjusting the composition of the canning solution, e.g. adding sugar and/or acids, natural red pigmentation can be retained. However, due to their highly water-soluble nature, pear's red pigments can not be fixed onto peels, but wear off from the peels during thermal treatment and storage of processed products. Using divalent metal ions such as tin (stannous chloride) helps to create new pigments that are water-insoluble and well retained onto peels. However, this can cause a shift in hues of product color from red to purple. More works are required for understanding the formation of different pigment complex between anthocyanins and different metal ions, thus obtaining and retaining desirable red pigments in creating red water-insoluble pigmentation in pear peels.

V. FURTHER RESEARCH FOR COLOR STABILIZATION OF CANNED RED PEARS

Based on our current findings, here are several proposed approaches for our future studies.

- Selecting fruit varieties that have relatively high anthocyanin content. This may include Max-Red Bartlett, Rosi-Red Bartlett, and Red Bartlett, P.I. 258948, and Starkrimson as shown in Table 2.
- Formulating the soaking solution for retaining the hue of the natural color of red pears. We believe that, besides cyanidin from red pears, if we have right metal ions in right proportion with other phenolic or non-phenolic compounds, red complexes should be able to form and to be retained;
- Trying on different chemicals to add in soaking or canning solutions that can serve as bonding agents to attach red large created pigments to the cell wall in pear peels.

Attachment II Pear Surface Preparation Provisional Patent

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APPL NO.	FILING OR 371 (c) DATE	ART UNIT	FIL FEE REC'D	ATTY.DOCKET NO	DRAWINGS	TOT CLMS	IND CLMS
60/633,786	12/07/2004		80				

Green & Green, Inc.
7625 NW McDonald Place
Corvallis, OR 97330

CONFIRMATION NO. 3979

FILING RECEIPT

OC000000014815555

Date Mailed: 12/23/2004

Receipt is acknowledged of this provisional Patent Application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Filing Receipt Corrections, facsimile number 703-746-9195. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Dennis C. Anderson, Corvallis, OR;
Pear Bureau Northwest, Milwaukie, OR;

Power of Attorney: None

If Required, Foreign Filing License Granted: 12/22/2004

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US60/633,786

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

** **SMALL ENTITY** **

Title

Fruit surface preparation for thermal processing with color retention

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LICENSE FOR FOREIGN FILING UNDER

CONTINUING REPORT **YEAR 1/2**

WINTER PEAR CONTROL COMMITTEE

GEORGE ING, WPCC RESEARCH CO-ORDINATOR

OBJECTIVES:

1.Regional, National and International interactions with activities, programs, administrators, industry people.

2. Sustaining office activities.

3. Evaluating foreign varieties.

2004 ACTIVITIES:

1. PROGRAM MAINTENANCE: office, local travel, phone, fax, e mail, lodging, mileage,personal contacts. Attended affiliated meetings. Handling inquiries regarding varieties, cultural practices, research. Hosting visitors. Interacting with research and extension personnel. Interacting with growers and handlers· Initiating and progressing projects. Communication with other entities. Visited pear industry people in Wenatchee and Okanogan.

2. INTERNATIONAL:

1. Attended International Pear Symposium in South Africa, Feb.2004. Prepared 58 page report with prose, colored and black and white photos. Prepared slide talk. Wrote articles for Goodfruit Grower. Provided photos to GFG.

2. Placed grafts of 2 additional varieties from Italy. There are now 6 varieties from two sources in Italy, 3 varieties from France, one from Turkey in the post quarantine plot in Hood River. Plot also has a cherry and an apple.

3. Continued dialogue with germplasm, plant quarantine, breeding and other personnel regarding varieties. Dialogue with a nursery interested in testing materials. Receipt of scionwood is pending for two varieties from Hungary. Some dialogue regarding rootstocks.

4. Continued interaction with a private breeding program in Italy which appears to have one or two pears of interest. Two trees of Rubens, an apple from that program, should have fruit in 2005.

3. LOCAL VARIETY EVALUATION

1. The variety Deveci (Dee-veggie), imported from Turkey in 1998, had about 40 pears in 2003. Those were harvested at four intervals one week apart starting at early Anjou timing. Pears from all harvest dates were removed from cold storage at various intervals starting in mid December. None ripened. In mid March, somewhat desperate, some were cut and found to be quite edible despite firmness.

When first encountered in Turkey in 1997 pears removed direct from cold storage in mid May had good flavor and texture thus this may be a non softening variety intended for late season marketing. In 2004 there was half a bin of fruit again harvested at intervals one week apart. Fruit from different harvest dates has been removed from storage starting in early January for evaluation.

During its third crop, in 2004, Deveci fruit is pear shaped, attractive, smooth, green turning yellowish after extended time on the tree and in storage. Tree growth character is good with good leaf surface and bearing habit. Bloom time is approximately Bartlett. Fruit was of various sizes but it is evident that with thinning fruit can be large.

2, Angelys. A late season variety from France intended to replace Passa Crassane which was wiped out by fire blight. Angelys has the same affliction as Taylors Gold Comice in that it is a russetted pear but not always. However, it is being grown adjacent to a few trees of Taylors Gold and in 2003 and 2004 had more russett.

There were about 12 pears in 2003 and those were ripened over time starting in mid March and continuing to mid April. When ripened, fruit surface is quite attractive as the russet is brighter. Fruit quality is also good; not as good as comice or a good Bartlett but better than most other pears. Flesh is white, sweet and smooth.

In 2004 we harvested about 2 boxes of Angelys with 3 harvest dates, Sept. 21, Sept. 28 and October 4. Fruit had much more russet in 2004 with many pears fully russetted. When fully russetted, Angelys is very attractive. It is a comice shaped pear of good size and growth habit. Trees bear early. Fruits have been removed from cold storage starting in early January for ripening and evaluation.

We understand that Angelys continues to have some plantings in France. It has resistance to fire blight per the French.

3. Bautommne. Another pear from France. This pear was seen by Gene Mielke and George Ing while traveling in Europe in November 1989. Pears left in the automobile ripened and were quite edible. The pear in France was light green in color. This variety, from the French federal breeding program, has not been planted because it has fire blight susceptibility.

In 2004 we had first fruits, about 20, also harvested over intervals one week apart. The pear is large, with shape perhaps between Anjou and boss. Pears were removed from storage for ripening starting in early January. The problem with this pear appears to be its lacy russetting with greenish/yellowish background. While the appearance is not offensive, it does not meet the criteria of being solid in coloring. Tree growth is quite satisfactory.

4. Bauroutard. Another pear from the French breeding program that has not been planted because it also has some fire blight susceptibility. The few fruits in 2004 were fully russetted, more bronze than tan. Fruits are comice shaped, large. One late harvested fruit (Oct. 4) was ripened after household refrigeration for 3 weeks. Its quality was satisfactory. Others will be ripened. This pear was also given to Gene and George in 1989 in France and ripened in the automobile. It, too, was of satisfactory quality.

5. Pears from the Italian breeding program at Forli (Norma, Carmen, Turandot), are proving to be vigorous and difficult to flower. Some have reddish wood which is good. If we do not have flowers in 2005 we will resort to severely manipulating vigor or limb position to induce flowering. Those varieties are reportedly early season, prior to or with Bartlett and, when exhibited at the International pear Symposium in Ferrara, Italy in Sept. of 2000 were smaller sized fruits, often with a red cheek, unusual for the climate.

The question could be asked as to why test a small early season pear when smaller pears are more

difficult to sell (seckel and forelle may be exceptions) and if the pear is early it will be grown in California. Testing rationale is that we do not know where the fruit will fit seasonally in Northwestern U.S., the red color aspect is interesting and sometimes size can be manipulated; gala apple, originally a small fruit, as an example. Furthermore, some of the rationale is to have connections with breeding programs and to test some materials as a part of those interactions.

6. Other Varieties. Many varieties were exhibited during the International Pear Symposium in South Africa, early Feb. of 2004. There were none with unusual possibilities, in my opinion. The varieties Flamingo and Rosemarie became commercial in South Africa but are dropping in volume and interest. Those, with Forelle parentage, are small attractive pears. Others exhibited had less appeal. There were, however, tan pears and green pears and fully red pears; none with names and all of a small to moderate size.

We traveled with people from Portugal where the principal variety is Rocha, sold in western Europe and to some extent in Eastern Canada. Thus there have been periodic inquiries regarding Rocha as a U.S. variety. Rocha is a smallish green pear with some russet that has good dessert quality. I am pessimistic that Rocha can be grown profitably in the U.S. Northwest because of its size and russetting, at least in photos of the fruit as grown in Portugal.

AMOUNT FUNDED 2004 $ 6,000

REQUESTED 2005$ 6,000

CONTINUING PROJECT REPORT **YEAR 1/3**

Project title: Integrated management of fire blight of pear and apple
PI: Kenneth B. Johnson
Organization: Dept. Botany & Plant Pathology, Oregon State University, Corvallis, OR
Co-PI(s) and affiliation(s): Virginia Stockwell (OSU, Corvallis)
Cooperator(s): David Sugar (OSU, Medford), Joyce Loper (USDA-ARS, Corvallis)
Contract Administrator: Dorothy.Beaton@orst.edu,
OSU Agric. Research Foundation, (541)737-3228

Objectives:
In 2004*:*

1. Evaluate new products for fire blight suppression.
2. Field-test mixtures of beneficial bacteria optimized for compatibility of their mechanisms of suppression.
3. Evaluate potential for epiphytic growth of *Erwinia amylovora* on common flowers frequented by honey bees but which are not hosts of fire blight.

Proposed In 2005*:*

1. Field-test an optimized beneficial bacteria strategy in combination with oxytetracycline.

Significant findings:
Objective 1 and 2: Three inoculated field trials (one in pear, two in apple) were conducted to evaluate products for suppression of blossom blight. Disease pressure was severe in two of the trials, and light in the third.

- In general, as stand alone treatments, biological products (BlightBan A506, BlightBan C9-1, and Bloomtime) provided poor to moderate levels of disease suppression.
- Among biological treatments, the mixture of *Pseudomonas fluorescens* strain A506 *AprX*- and *Pantoea agglomerans* strain C9-1 continued to provide 50 to 60% disease control (relative to treating with water only).
- Oxytetracycline products provided 35 to 60% disease control with no apparent differences in the Mycoshield and Fireman formulations.
- The efficacy of BlightBan A506 was enhanced by a tank mix with the iron chelate, FeEDDHA (Sequestrene 138). Disease suppression with this tank mix was similar to that obtained with an oxytetracycline product.
- In two severe disease situations, an early treatment with BlightBan A506 plus Sequestrene 138 followed by a Mycoshield treatment after full bloom resulted in disease reductions of 74 and 83%; two applications of streptomycin targeted at a strep-sensitive pathogen provided 88 and 67% control.

Objective 3: As we have observed previously, the fire blight pathogen grew significantly better on non-disease host plants of the rose family - peach, cherry, plum, blackberry, and serviceberry – than on flowers of other common landscape plants**. In field trials**, high populations of *E. amylovora* developed on flowers of Himalayan blackberry and of sweet cherry. Flowers of scotch broom supported moderate populations of the fire blight pathogen, but flowers of big leaf maple were poor supporters of pathogen growth.

Methods:
Objectives 1 and 2. New chemical and biological agents with potential to control fire blight were tested (see results section). This experiment was conducted in a Bartlett pear, Golden Delicious and Rome Beauty apple orchards at the Botany and Plant Pathology Experimental Farm near Corvallis Oregon. (A fourth trial was conducted in a Bartlett pear block in Medford, OR, but disease failed to develop in this plot). Experimental treatments were arranged in randomized block designs with 4 replications of individual trees. Treatments included alternative products, a water-treated control and standard antibiotic products (streptomycin and oxytetracycline). Treatments timings were varied according to properties of the product, but generally, two application of each product were made. Products were applied to near run-off a hand-directed backpack sprayers. Freeze-dried inoculum of the fire blight pathogen (strain *Ea*153nal, streptomycin sensitive) was applied near full bloom Beginning in mid-May and ending in July, incidence of fire blight was evaluated weekly by counting and removing the diseased blossom clusters on each tree.

Objective 3. In a growth chamber, flower-bearing branches were collected from non-host species, inoculated with freeze-dried cells of *E. amylovora* strain 153Nal, and incubated for 96 hours (15°C). Growth of *Ea*153Nal was monitored by washing the flowers dilution plating onto selective media. Eight blossoms were washed per non-host replicate; 2-4 replicates of each non-host species were conducted. Population sizes among various flower types were standardized by computing relative growth rate (% increase in bacterial populations per hour). Field inoculations of *E. amylovora* onto non-host flowers were conducted with four non-disease host species: sweet cherry, Himalayan blackberry, Big leaf maple, and scotch broom. Field sites were selected in the vicinity of Corvallis area. At the appropriate bloom stage (~50-70% bloom), suspensions of Ea153S (1 x 10^7 CFU/ml) were onto blossoms to near runoff with a hand-held, adjustable trigger sprayer. Flowers were sampled from the field sites at 0, 4 and 8 days after inoculation to measure the population size of *Ea*153S and associated indigenous epiphytes on the surfaces of pistils

Results: Objectives 1 and 2

Golden Delicious Apple Trees used in the study were moderately sized with an average of ca. 970 blossom clusters per tree. Disease pressure was high; perhaps due in part to intense secondary bloom that opened on trees after treatments were applied. Symptoms of fire blight developed on 32% of the blossom clusters on inoculated trees treated with water. The treatments BlightBan A506, BlightBan C9-1 alone, BlightBan C9-1 combined with BlightBan A506, and Bloomtime did not significantly ($P \leq 0.05$) reduce the incidence of fire blight compared to treatment of trees with water. The commercial antibiotic formulations and most combinations of BlightBan A506 with iron significantly ($P \leq 0.05$) reduced the incidence of fire blight in this trial on Golden Delicious apple. Mycoshield and Agri-mycin 17 provided moderate disease control and reduced incidence of fire blight by 50% compared to water-treated trees. Fewest number of diseased blossom clusters were recorded on trees treated with the combination of BlightBan A506 with Sequestrene 138 followed by Sequestrene 138 and Mycoshield; the incidence of fire blight on these trees was 79% lower than that on water-treated trees.

		Date treatment applied*							
Treatment	Rate/100 gallons water	12 Apr 30% bloom	16 Apr 70% bloom	22 Apr post-pathogen	26 Apr petal fall	Mean # blighted clusters per tree		Mean % clusters blighted	
BlightBan A506	18 oz	X[§]	X	---[§]	---	342	A**	36.6	A***
Water control	**------**	**X**	**X**	---	---	**316**	**AB**	**32.1**	**AB**
BlightBan C9-1 (10^8 CFU/ml)	11 oz	X	X	---	---	273	ABC	27.4	ABC

BlightBan A506 &	17 oz	X	X	---	---				
Seq. 138 (post/pf)	16 oz	---	---	X	X	257	ABCD	26.6	ABC
BlightBan C9-1 and A506 ($2X10^7$ CFU/ml)	13 oz	X	X	---	---	208	BCDE	23.2	BCD
Bloomtime ($1 X 10^7$ CFU/ml)	22 oz	X	X	---	---	198	CDE	22.5	BCD
BlightBan A506 &	17 oz	X	X	---					
Seq. 330 (70/post)	16 oz	---	X	X	---	164	CDEF	15.4	CDEF
BlightBan A506 &	17 oz	X	X	---					
Seq. 138 (70/post)	16 oz	---	X	X	---	159	DEF	14.3	DEF
Mycoshield	16 oz.	---	X	X	---	130	EF	14.8	DEF
Agri-mycin 17	8 oz	---	X	X	---	104	EF	16.3	CDE
BlightBan A506 &	17 oz	X	X	---					
Seq. 138 (30/70)	16 oz	X	X	---	---	104	EF	10.3	EF
BlightBan A506 &	17 oz	X	X	---					
Seq. 138 (80/post)	16 oz	---	X	X					
& Mycoshield	16 oz	---	---	X	---	56	F	6.8	F

Bartlett Pear. Blossom cluster density on the Bartlett pear trees averaged 1,200 clusters per tree. Disease pressure was high; symptoms of fire blight developed on an average of 28% of the blossom clusters on inoculated water-treated trees. Based on analysis of mean strikes per tree, all treatments evaluated resulted in significantly ($P \leq 0.05$) fewer diseased blossom clusters compared to water-treated inoculated controls. Analysis of variance of arcsine-square root transformed disease incidence revealed that antibiotic treatments, some combinations of BlightBanA506 with FeEDDHA, and treatments of C9-1S combined with BlightBan A506 or A506 $AprX^-$ significantly ($P \leq 0.05$) reduced the incidence of disease compared to that observed on water-treated inoculated control trees.

The commercial antibiotic Mycoshield provided moderate control of fire blight (40% reduction in disease incidence). The level of disease control by the formulation of oxytetracycline called Fireman was not statistically different from Mycoshield in this field trial. The antibiotic treatment Agri-mycin 17 provided excellent control of fire blight (89% reduction in disease incidence compared to inoculated water-treated trees). The only treatment that was statistically similar to control provided by Agri-mycin 17 was the combination of BlightBan A506 with Sequestrene 138 followed by a post-inoculation application of Mycoshield combined with Sequestrene 138. This combination of BlightBan A506, Sequestrene 138, and Mycoshield provided significantly better control of fire blight compared to the individual treatments BlightBan A506 with Sequestrene 138 applied at mid and late bloom and Mycoshield alone.

		Date treatment applied*							
Treatment	Rate/100 gallons water	31 Mar 30% bloom	3 Apr 80% bloom	5 Apr post-pathogen	12Apr petal fall	Mean # blighted clusters/ tree		Mean % clusters blighted	
Water control	**------**	**X**§	**X**	---§	---	**318**	**A****	**28**	**A****
Fireman	16 oz.	---	X	X	---	222	B	20	ABC
BlightBan A506	17 oz	X	X	---	---	214	B	20	ABC
BlightBan A506 &	17 oz	X	X	---					
Seq. 138 (80/post)	16 oz	---	X	X	---	214	B	26	AB
BlightBan A506 &	17 oz	X	X	---	---				
Seq. 138 (post/pf)	16 oz	---	---	X	X	203	B	14	CD

Mycoshield	16 oz.	---	X	X	---	190	B	17	BC
BlightBan A506 &	17 oz	X	X	---					
Seq. 330 (80/post)	16 oz	---	X	X	---	196	B	19	ABC
BlightBan A506 &	17 oz	X	X	---					
Seq. 138 (30/70)	16 oz	X	X	---	---	182	B	14	CD
C9-1S and	total 10^8								
A506 AprX$^-$	CFU/ml	X	X	---	---	166	B	14	CD
BlightBan C9-1 and									
A506	13 oz	X	X	---	---	165	B	16	BCD
BlightBan A506 &	17 oz	X	X	---					
Seq. 138 (80/post) &	16 oz	---	X	X					
Mycoshield	16 oz	---	---	X	---	83	C	8	DE
Agri-mycin 17	8 oz	---	X	X	---	28	C	3	E

Rome Beauty Apple. Trees used in the study were large with an average of 556 blossom clusters per tree. For an inoculated experiment, disease pressure was light with symptoms of fire blight developing on 4% of blossom clusters on water-treated trees. Based on analysis of mean strikes per tree, all treatments resulted in significantly ($P \leq 0.05$) fewer diseased blossom clusters compared to water treated controls. Agri-mycin 17 applied near full bloom and again following the pathogen inoculation provided outstanding disease control (80% fewer strikes than water-treatment). All other treatments provided intermediate and statistically similar levels of disease suppression; however, for both dependent variables, the level of suppression provided by the mixture of A506 AprX- and C9-1S was statistically similar to the level of protection provided by Agri-mycin 17. For mean percent of clusters blighted, Fireman and the combination of BlightBan A506 plus Sequestrene 138 also showed levels of protections that were statistically similar to Agri-mycin 17.

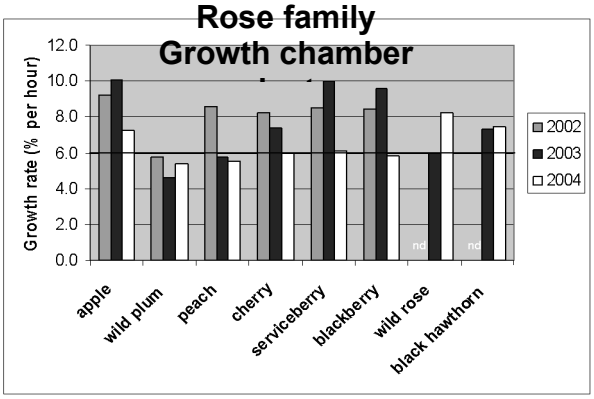
		Date treatment applied*				
Treatment	Rate per 100 gallons water	16 Apr 30% bloom	3 April 85% bloom	26 Apr post-pathogen	Mean numbeı of blighted clusters/ tree	Mean % of clusters blighted
Water control	----------	X§	X		21 A**	3.8 A
BlightBan A506	5 oz.	X	X		13 B	2.3 B
P. fluorescens A506 AprX-	Fresh cells	X	X		12 B	2.2 B
BlightBan A506 &	5 oz. &	X	X			
Sequestrene 138	16 oz.		X	X		
followed by Mycoshield				X	12 B	2.2 B
Fireman (200 ppm)	16 oz.		X	X	12 B	2.0 BC
P. agglomerans C9-1S	Fresh cells	X	X		11 B	2.1 B
Mycoshield (200 ppm)	16 oz.		X	X	10 B	2.2 B
BlightBan A506 &	5 oz. &	X	X			
Sequestrene 138	16 oz.		X	X	10 B	1.8 BC
A506 AprX- & C9-1S	Fresh cells	X	X		9 BC	1.8 BC
Agri-mycin 17 (100 ppm)	8 oz.	X	X	X	4. C	0.8 C

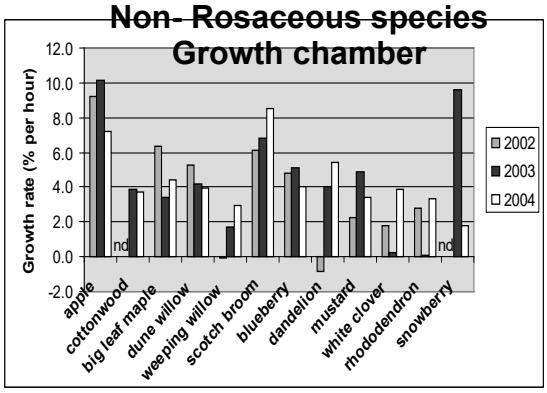
**Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at P = 0.05

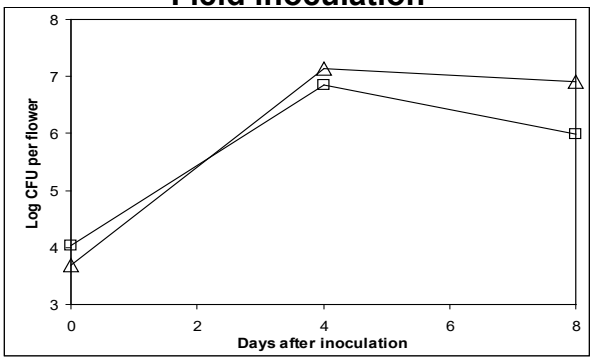
**Arcsine of square root transformed disease incidence followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$.

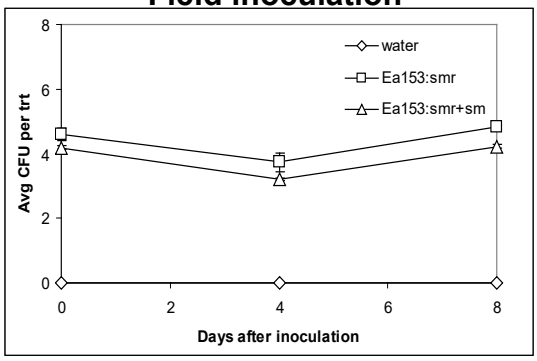
§ X indicates material sprayed, --- indicates material not applied on a specific date.

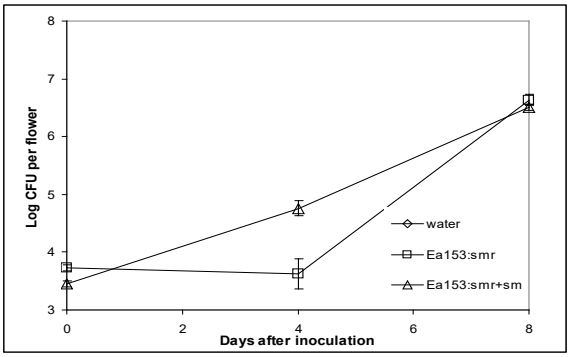
Objective 3. As we have observed previously, the fire blight pathogen grew significantly better on non-disease host plants of the rose family - peach, cherry, plum, blackberry, and serviceberry – than on flowers of other common landscape plants**. In field trials**, high populations of *E. amylovora* developed on flowers of Himalayan blackberry and of sweet cherry. Flowers of scotch broom supported moderate populations of the fire blight pathogen, but flowers of big leaf maple were poor supporters of pathogen growth**.**

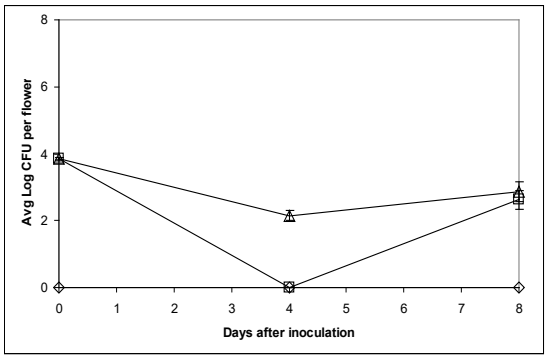












Implications: Because vectors of *E. amylovora*, principally bees, visit many kinds of flowers in the landscape, the observations suggest that epiphytic sources of inoculum of *E. amylovora* could become broadly dispersed within a valley/region as a season transitions (e.g., from pear to apple, low to higher elevation, primary to secondary bloom). In other words, hold-over cankers are sources of inoculum, but rosaceous flowers of host and non-host species may surpass overwintering cankers in importance as the season progresses. A practical consequence of this phenomenon is that action thresholds within temperature-based disease warning models may trigger too many treatments in

early spring (owing to the rarity of inoculum) and too few treatments later in the season (as epiphytic sources of the pathogen increase). Fire blight warning models (e.g., CougarBlight) use cultivar and disease history to adjust thresholds that trigger fire blight control actions. Time of season also could be a consideration in determining the risk of blight outbreaks.

Proposal for 2004:

Justification: The goals of this project are to understand to the biology and epidemiology of the fire blight pathogen, to develop and refine control methods for fire blight of pear and apple, and to integrate these technologies into commercial orchard management. In this context, we have evaluated many potential products for blight suppression with an emphasis on beneficial bacteria. Based on recent results, we have begun to investigate combinations of beneficial bacteria with chemical products. This last year, we were encouraged that blossom blight suppression was enhanced by combining an early treatment with BlightBan A506 plus Sequestrene 138 with a later Mycoshield treatment at full bloom (Tables 1&2). Importantly, these results were obtained in orchards where where fire blight was severe. Consequently, we have one objective this year: **Field-test an optimized beneficial bacteria strategy in combination with oxytetracycline.**

Approach: We will test the efficacy of treatment with beneficial bacteria followed by oxytetracycline. This treatment combination fits well with the strategy to apply beneficial bacteria early in bloom and then follow with an antibiotic application if warning models forecast moderate to high fire blight risk.

Several treatments will be evaluated and will represent variations of the overall objective. In these variations we will include discoveries we have made to enhance the effectiveness of antagonist mixtures (i.e., use of mixtures of beneficial bacteria, use of a mutant strain of A506 (A506 Ecp- or AprX-) in combination with C9-1, and use of an iron chelate to induce A506 to produce its antibiotic). The treatments will be evaluated in 3 to 4 orchards following methods described above. Standard antibiotic products (streptomycin and oxytetracycline) will be included as controls.

Budget:

Proposed duration of objective: 2 years

Year	Last Year (2004)	**Current request 2005**	Next year (2006)
Total	16,130	**7,680**	7,984 **(final year)**

Budget specifics

Item	Last Year (2004)	Current Request	Next year (2006)
Salaries	9,000	**4,000**	4,200
Benefits (52%)	4,680	**2,080**	2,184
Supplies	1,000	**300**	300
Travel (Medford)	450	**300**	300
Plot Maintenance	1,000	**1,000**	1,000
Total	16,130	**7,680**	7,984

2005 Salary is 1.5 months of a senior faculty research assistant

Support we receive currently from other funding sources:

OSU AES, NWHC/USDA-FAS: Survival of *E. amylovora* on pear fruit 2004-2006 $112,000
USDA-NRICGP: 2004-2006, $297,415 (Research on avirulent *E. amylovora*).
Occasional grants-in-aid of research from chemical companies.

CONTINUING REPORT Project Report 2004 YEAR 2/3

Project title: Survival of *Erwinia amylovora* on pear fruit

PI: Kenneth B. Johnson
Organization: Dept. Botany & Plant Pathology, Oregon State University, Corvallis, OR
Cooperator(s): Larry Pusey (USDA-ARS, Wenatchee), Virginia Stockwell (OSU, Corvallis), David Sugar (OSU, Medford), Joyce Loper (USDA-ARS, Corvallis), Rodney Roberts (USDA-ARS Wenatchee), Washington State and Oregon State University Extension Personnel.

Objectives:

In 2004 (all ongoing for 2005):

1. Estimate incidence of contamination of d'Anjou pear fruit cultivated in four growing districts in the Pacific Northwest with *Erwinia amylovora*.
2. Evaluate capacity of *Erwinia amylovora* to colonize or persist on pear fruit surfaces.
3. Evaluate internal fruit contamination by *Erwinia amylovora* on trees that were diseased in the spring and remained diseased through the summer until harvest.
4. Evaluate internal and external survival of *Erwinia amylovora* on wounded fruit in cold storage.

Significant findings:

- A survey of commercial d'Anjou pear fruit orchards from four PNW growing areas was conducted to estimate the incidence of contamination of fruit with *E. amylovora*. 100 fruit were sampled from each of 14 apparently disease-free orchards and from each of 7 orchards with disease in or adjacent to the orchard. Bacteria (e.g., *Pseudomonas* and *Pantoea* spp.) were recovered occasionally from fruit washings, but *E. amylovora* was not detected.

- We monitored survival of *E. amylovora* on developing pear and apple fruits in orchards. Cells of E. *amylovora* (freeze-dried or from ooze produced on immature fruits) were sprayed onto whole fruits or painted directly onto the calyx ends of fruit. Recovery of *E. amylovora* directly after spraying/painting was 100% at populations of 10^5 to 10^8 viable cells fruit. *E. amylovora p*opulations declined rapidly, with less than half of the cells remaining viable after 2 days. By 14 and 35 days after inoculation, fruit on which *E. amylovora* could be detected had declined to 20 and 6 %, respectively. At 56 days after inoculation, 4 viable cells of *E. amylovora* were recovered from one sampled fruit that had been painted with ooze. Fire blight symptoms were never observed on inoculated trees or fruit.

- Five d'Anjou pear trees in Medford, OR and in Wenatchee, WA were inoculated with *E. amylovora* during bloom, and fire blight was maintained on the trees throughout the summer. At harvest, 100 fruit were sampled from the diseased trees and examined for external and internal contamination by *E. amylovora.* External fruit washings were negative for the detection of the pathogen, and after 6 weeks of storage, were negative for detection from internal core tissues as determined by an enrichment broth recovery method. A second detection method, polymerase chain reaction (PCR), also was employed; processing of core samples by this method is ongoing.

- We monitored postharvest survival *E. amylovora* pear and apple fruit. Cells of the pathogen were applied to the fruit skin, and then a small puncture wound was made at the site of cell placement. Fruit were processed through an SOPP dump tank and stored at 0-4ºC. Prior to dump tank submersion, populations of *E. amylovora* were between 10^1 and 10^3 viable cells per fruit. After 2 weeks of storage, an average of 10 viable cells were recovered from 8% of fruit; after 4 and 7 weeks of storage, E. *amylovora* had declined to undetectable levels.

Justification: Export of winter pears grown in the Pacific Northwest into countries where fire blight does not occur is restricted by phytosanitary concerns over the possible contamination of fruit with the fire blight bacterium, *Erwinia amylovora*. Similar concerns have been applied to apples, but extensive research and risk assessment analyses have concluded that introduction and successful establishment of *E. amylovora* into a new geographic region via commercial shipments of apple fruit is very unlikely. Roberts (USDA- ARS, Wenatchee) et al. (1998) listed three reasons for this low likelihood: 1) viable cells of *E. amylovora* are detected on mature apple fruit only rarely, 2) *E. amylovora* has a low epiphytic fitness on apple fruit, and 3) a pathway that demonstrates successful infection of susceptible host material via fruit borne inoculum has never been documented (this last reason is true for both apple and pear).

The purpose of this proposal is to investigate if the first two reasons cited by Roberts also hold true for pear. Consequently, the objectives of this proposal will 1) determine the frequency which viable cells of *E. amylovora* can be detected on mature fruit of D'Anjou pear in growing districts of Washington and Oregon, and 2) evaluate in experiments the capacity of *Erwinia amylovora* to colonize or persist on pear fruit surfaces; apple fruit will be used as a comparative control in these experiments. Results of this study are expected to form a foundation for assessing the risk of introduction of fire blight into a new geographic region via shipments of commercial pear fruit.

Methods:

Objective 1. 22 d'Anjou pear orchards in the Rogue, Hood River, Yakima, Wenatchee and Okanogan valleys of the Pacific Northwest were surveyed within one week of commercial harvest. For 14 of the surveyed orchards, no visible fire blight infections were present whereas 7 orchards had visible fire blight strikes within the orchard or in a orchard block immediately adjacent to the sampled orchard. 100 healthy fruit were selected from each orchard; 4 from each of 25 randomly selected trees. Each fruit was placed individually into a labeled, quart-size, re-sealable plastic bag. The fruit were placed in an ice cooler and transported to the laboratory for processing. In a sterile transfer hood, 50 ml of sterile washing buffer (10 mM phosphate buffer, pH 7.1) will be added to each pear fruit and the bag resealed. After vigorous massage of the fruit, each bag was sonicated for 2 minutes. A sample of the buffer wash of each fruit was spread onto selective media (Miller-Schroth medium) and incubated at 25°C. Positive and negative controls were processed simultaneously to ensure quality of the isolation protocol. The remainder of the wash buffer was passed through a 0.2µm filter membrane (Pall life sciences, 45mm diameter) to capture the remaining bacteria in the suspension. The filter was laced on the surface of Miller-Schroth medium amended with 50µg/ml cycloheximide and incubated for 7 days at room temperature.

Objective 2. Field trials were conducted on d'Anjou pear (Southern Oregon Research and Extension center near Medford, OR), and Bosc pear and Braeburn apple (Botany and Plant Pathology Experimental Farm near Corvallis, OR) fruit to determine survival of *E. amylovora* on fruit surfaces. The treatments were freeze-dried cells and cells in fresh ooze from immature pear. Treatments were: 1) Freeze-dried cells of the pathogen suspended in water at 10^7 CFU/ml and sprayed, or 2) ooze painted directly onto the calyx end of developing fruits. Inoculation times were May and July. Three fruit from each tree (15 total per treatment per inoculation timing) were harvested at 1 hour, and 3, 7, 14, and 35 (and 56 for Bosc pear) days after inoculation and placed individually into labeled, quart-size, re-sealable plastic bags. The bags of fruit were placed in an ice cooler, transported to the laboratory, and processed as described under Objective 1.

Objective 3. Experimental plots were established in d'Anjou orchards located in Medford, OR and Wenatchee, WA. During bloom, flower clusters on two branches of each tree were inoculated with E.

amylovora, and fire blight was allowed to develop over the summer. At harvest, 20 mature fruit per treatment tree (5 fruit per non-inoculated tree) were sampled and placed in re-closable plastic bags, packed into coolers and transported to the lab. Fruit were assayed for the external presence of *E. amylovora* following methods described under Objective 1. After 6 weeks storage at 0-4ºC, the fruit were surface disinfested in a 10% bleach dip followed by removal of the stem and calyx regions. A flamed no. 9 cork borer was used to remove the entire core and part of the cortex tissue. The sampled core were placed in a re-sealable (2 ml thick) plastic bag, bathed in 5 ml sterile phosphate buffer (pH 7.0) and gently macerated with a rubber mallet. Two 1 ml samples of the macerated core were frozen (-80ºC) in 50% (V/V) glycerol, and 30 ml of nutrient yeast extract broth was added to the remaining macerate. After incubation at room temperature for 24 hr, three 0.1ml samples per core were spread on modified Miller-Schroth medium and examined for orange colonies after 7 days. Subsequently, nested PCR analysis will be performed on the 1 ml frozen buffer/macerate samples from each fruit following an isopropanol extraction procedure. Primer used in the PCR reaction will be specific for pEA29, a plasmid that is ubiquitous in and specific to *E. amylovora*.

Objective 4. At harvest, 300 d'Anjou and Comice pear (Medford, OR) and Braeburn apple (Corvallis, OR) fruit were picked and packaged in coolers and transported to the lab. In the lab, fruit were surface disinfested in 1 % SOPP (sodium ortho-phenylphenate), air dried, and inoculated with a 10 µl drop of re-suspended freeze-dried cells of *E. amylovora* on fruit surfaces. Inoculum concentrations were: no cells (0), 10 cells, 100 cells, and 1000 cells per fruit. Each treatment was applied to 3 repetitions of 25 fruit (75 fruit per treatment per cultivar). Once the inoculum was air dry, a wound was introduced over the inoculum with a small finishing nail (4D, finishing) secured to a wooden block. Fruit were incubated at room temperature for 24 hours, followed by a dump tank treatment in 1 % SOPP and placed in 4ºC cold room for up to 49 days. Sampling intervals included, day 0, 7, 14, 28, and 49 days. A sub-sample of 3 fruit per cultivar per inoculum treatment was processed prior to the SOPP dump tank to verify the initial pathogen populations. On each sampling date, 15 fruit per cultivar (5 fruit per repetition) per inoculum treatment were removed from 4ºC and, at the wound site, a 1 cm diameter by 5 mm sample of tissue was removed and placed in 4 ml's of sterile phosphate buffer (pH 7.0). A flamed glass rod macerated the tissue which was passed through a 0.2 µm (Pall life sciences, 47mm diameter) filter and the filter was placed on the surface of Miller-Schroth medium amended with 75µg/ml nalidixic acid for the selective recovery of E. amylovora. After 7 days of incubation at room temperature, plates were examined for orange bacterial colonies and enumerated.

Results and discussion:

Objective 1. A total of 2100 fruit were sampled from 14 apparently disease-free orchards and 7 orchards with disease in or adjacent to the plot. Bacteria (e.g., *Pseudomonas* and *Pantoea* spp.) were recovered occasionally from fruit washings, but *E. amylovora* was not recovered from any commercial sample (Table 1).

Table 1. Incidence of *E. amylovora* on d'Anjou pear fruit with from commercial orchards in 2004.

Orchard location[x]	Harvest date	No. fruit	Control Ea153N	*Erwinia amylovora*	*Pseudomonas* spp.[z]	Disease in orchard	Other spp.
Hood River, OR	8/31	100	2/2 [y]	0%	11%	Yes	100%
Hood River, OR	8/31	100	2/2	0	15	No	100
Hood River, OR	8/31	100	2/2	0	17	No	100
Hood River, OR	9/02	100	2/2	0	25	No	100
Hood River, OR	9/02	100	2/2	0	5	No	100

Table 1. Incidence of *E. amylovora* on d'Anjou pear fruit with from commercial orchards in 2004 (cont.).

Yakima, WA	9/06	100	2/2	0	19	No	100
Yakima, WA	9/06	100	2/2	0	18	No	100
Yakima, WA	9/06	100	2/2	0	26	No	100
Yakima, WA	9/06	100	2/2	0	15	No	100
Medford, OR	9/01	100	2/2	0	35	No	100
Medford, OR	9/01	100	2/2	0	9	Yes	100
Wenatchee, WA	8/30	100	2/2	0	23	Yes	100
Wenatchee, WA	8/30	100	2/2	0	0	Yes	100
Wenatchee, WA	8/30	100	2/2	0	11	Yes	100
Wenatchee, WA	8/30	100	2/2	0	13	Yes	100
Wenatchee, WA	8/30	100	2/2	0	4	No	100
Wenatchee, WA	8/30	100	2/2	0	19	No	100
Wenatchee, WA	8/30	100	2/2	0	1	Yes	100
Malott, WA	9/06	100	2/2	0	30	No	100
Malott, WA	9/06	100	2/2	0	3	No	100
Malott, WA	9/06	100	2/2	0	18	No	100

[y]Orchard fruits were randomly sampled at each location; 100 fruit from each commercial orchard.
[y] Number positive for detection *Ea*153N in control samples processed with the fruit sampled from each orchard.
[z]Based on fluorescence on King's medium B.

Objective 2.

Erwinia amylovora strains 153N and 153 were inoculated onto d'Anjou and Bosc pear and Braeburn apple fruit as re-suspended freeze-dried cells sprayed directly onto fruit, or as fresh ooze cells harvested from immature fruit and painted onto the calyx end of fruit. Trees used in the study averaged 50 to 200 fruit per tree. During the experiments, daily maximum temperature averaged 82ºF (max. temp. 105ºF). Recovery of *E. amylovora* directly after spraying or painting was 100% (n = 270) at populations of 10^5 to 10^8 CFU/fruit. Population size and incidence of recovery declined rapidly, with less than half the population remaining after 2 days (Tables 1-3). By 14 and 35 days after inoculation, fruit on which *E. amylovora* could be detected had declined to 20 and 6 %, respectively. At 56 days after inoculation, 4 viable cells of *E. amylovora* were recovered from one sampled fruit that had been painted with ooze. Fire blight symptoms were never observed on inoculated trees or fruit.

Table 1. Population size of recovered *E. amylovora* from inoculated d'Anjou pear fruit in 2004

			Day after inoculation [x]				
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	5/24 – 6/28	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	5/24 – 6/28	4.5E+06	2.1E+02	0.0E+00	0.0	0.0
Ea153 Freeze-dried	10^7	5/24 – 6/28	7.7E+05	8.1E+03	4.7E+02	0.0	0.0
Ea153N Ooze	10^9	5/24 – 6/28	3.8E+07	2.9E+04	1.8E+04	0.0	0.0
Check	-	7/20 – 8/24	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/20 – 8-24	2.9E+06	0.0	9.0E+03	0.0	0.0
Ea153 Freeze-dried	10^7	7/20 – 8/24	1.2E+06	6.7E+03	5.3E+01	0.0	0.0
Ea153N Ooze	10^9	7/20 – 8/24	1.6E+08	2.3E+06	1.9E+04	2.6E+04	0.0

[x]Average colony forming units are represented as the mean of 15 fruit per treatment.
[y]Treatments include *Ea*153N and *Ea1*53 wild-type inoculated as freeze-dried cells or ooze cells from immature pear.
[z]Colony forming units (CFU) of inoculum concentration applied to fruit.

Table 2. Population of recovered *Erwinia amylovora* from inoculated Bosc pear fruit in 2004

			Days after inoculation[x]				
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	5/25 – 6/29	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	5/25 – 6/29	3.9E+06	3.0E+03	4.1E+01	1.3E-01	8.7E-01
a153 Freeze-dried	10^7	5/25 – 6/29	1.1E+06	1.4E+04	1.8E+03	0.0	3.7E+00
Ea153N Ooze	10^9	5/25 – 6/29	2.6E+07	1.3E+07	4.5E+04	2.6E+04	8.7E+01
Check	-	7/19 – 8/23	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/19 – 8/23	7.6E+06	9.6E+04	6.7E+04	2.1E+02	0.0
Ea153 Freeze-dried	10^7	7/19 – 8/23	2.6E+06	3.0E+05	6.1E+04	1.7E+02	0.0
Ea153N Ooze	10^9	7/19 – 8/23	3.3E+07	1.1E+07	7.2E+04	2.5E+04	0.0

[x]Average colony forming units are represented as the mean of 15 fruit per treatment.
[y]Treatments include *Ea*153N and *Ea1*53 wild-type inoculated as freeze-dried cells or ooze cells from immature pear.
[z]Colony forming units (CFU) of inoculum concentration applied to fruit.

Table 3. Population of recovered *Erwinia amylovora* from inoculated Gala apple fruit in 2004

			Days after inoculation[x]				
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	5/25 – 6/29	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	5/25 – 6/29	4.4E+05	1.1E+04	6.2E+02	0.0	0.0
Ea153 Freeze-dried	10^7	5/25 – 6/29	4.3E+05	9.4E+03	1.0E+03	0.0	0.0
Ea153N Ooze	10^9	5/25 – 6/29	2.8E+07	2.0E+05	2.7E+04	0.0	0.0
Check	-	7/19 – 8/23	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/19 – 8/23	9.6E+06	3.4E+03	3.5E+03	5.3E+01	0.0
Ea153 Freeze-dried	10^7	7/19 – 8/23	2.8E+06	1.1E+04	1.6E+02	4.5E+01	0.0
Ea153N Ooze	10^9	7/19 – 8/23	2.3E+08	5.9E+06	5.0E+04	1.3E+04	0.0

[x]Average colony forming units are represented as the mean of 15 fruit per treatment.
[y]Treatments include *Ea*153N and *Ea1*53 wild-type inoculated as freeze-dried cells or ooze cells from immature pear.
[z]Colony forming units (CFU) of inoculum concentration applied to fruit.

Objective 3.
For both the Medford and Wenatchee experiments, 100 fruit were sampled from the diseased trees at harvest and analyzed for external and internal contamination by *E. amylovora* 153N. External fruit washings were negative for the detection of the pathogen, and after 6 weeks of storage, were negative for detection from internal core tissues as determined by an enrichment broth recovery method. A second detection method, polymerase chain reaction (PCR), also was employed; processing of core samples by this method is ongoing.

Objective 4.
We monitored postharvest survival *E. amylovora* pear and apple fruit. Cells of the pathogen were applied to the fruit skin, and then a small puncture wound was made at the site of cell placement. Fruit were processed through an SOPP dump tank and stored at 0-4ºC. Prior to dump tank submersion, populations of *E. amylovora* were between 10^1 and 10^3 viable cells per fruit. After 2 weeks of storage, an average of 10 viable cells were recovered from 8% of fruit; after 4 and 7 weeks of storage, E. *amylovora* had declined to undetectable levels (Fig. 1).

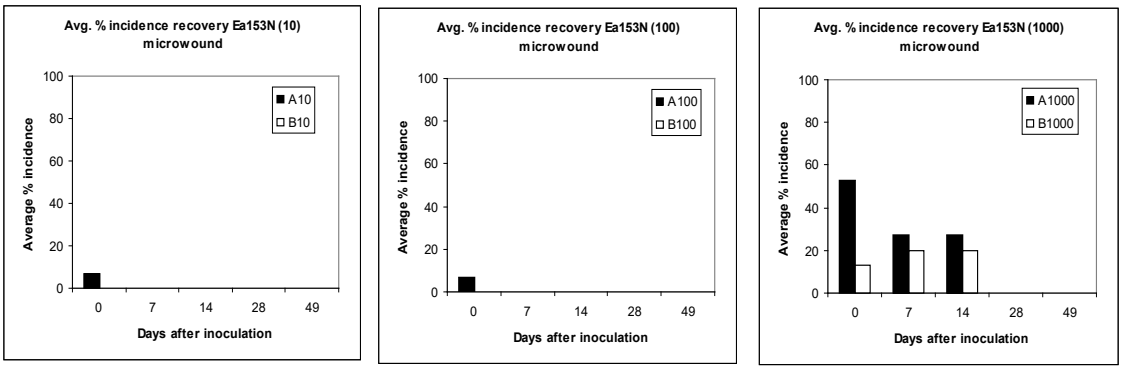


Figure 1. Average percent recovery of Ea153N from micro wounded fruit. A represents d'Anjou pear, B represents Braeburn apple, and 10, 100, and 1000 represents primary inoculum concentrations.

Proposal for 2005:

Objectives 1, 2, 3 and 4 from 2004 will be repeated.

Literature Cited:

1. Llop, P., Caruso, C., Cubero, J., Morente, C., and Lopez, M.M. 1999. A simple extraction procedure for efficient routine detection of pathogenic bacteria in plant material by polymerase chain reaction. J. Microbiol. Methods 37:23-31.
2. McManus, P.S., and Jones, A.L. 1995. Detection of *Erwinia amylovora* by nested PCR and PCR-dot-blot and reverse blot hybridizations. Phytopathology 85:618-623.
3. Miller, T. D., and Schroth, M. N. 1972. Monitoring the epiphytic population of Erwinia amylovora on pear with a selective media. Phytopathology 62:11750.01182.
4. Roberts, R. G., Hale, C. N., van der Zwet, T. Miller, C. E., and Redlin, S. C. 1998. The potential for spread of *Erwinia amylovora* and fire blight via commercial apple fruit; a critical review and risk assessment. Crop Protection 17: 190.028.

Budget:
Proposed duration of objectives: 3 years
Current year: 2005 (3 rd)
Current year request: $0

Budget in 2004: $0
Budget in 2003: $36,426

Principal support for 2005:
USDA FAS via Northwest Horticultural Council $63,000 ($56,000 OSU; $7,000 ARS Wenatchee)

Other funding sources:
Oregon Agricultural Experiment Station
USDA ARS collaborators (Wenatchee and Corvallis)

CONTINUING PROJECT **YEAR 1/2**

Project Title:	New approaches to decay control of pear
PI:	Robert A. Spotts
Organization:	OSU Mid-Columbia Ag Research and Extension Center
Project staff:	Maryna Serdani (Research Assistant)
	Gordon McCarty (Bioscience Research Technician)
Cooperators:	Ag Canada (Peter Sholberg, Dan O'Gorman, Paul Randall)
	New Zealand HortResearch (Trish Virgin, Monika Walter)
	Lincoln University (Alison Stewart)
Funding History:	Year initiated: 1979
	Funding in 2004-2005: $43,526
	Funding requested for 2005-2006: $48,862

Objectives:

1. Effect of MCP on control of decay of pear.
2. DNA techniques for rapid, accurate detection of decay spores (real time PCR).
3. Studies on decay pathogens with an emphasis on gray mold and bull's-eye rot.
4. Evaluation of pyrimethanil (Penbotec) and *Cryptococcus infirmominiatum* (CIM) for control of postharvest decay of pear.
5. Evaluation of *Muscodor* for decay control.
6. Evaluation of chlorine dioxide-emitting paper for decay control.
7. Preharvest fungicides for control of postharvest decay.

Methods:

1. Effect of MCP on control of decay of pear and wound healing.

A. MCP treatment and effect on stem end decay.

Year #2 – The effect of MCP and fungicides that are applied as fumigants is being studied in cooperation with Dr. Peter Sholberg and Paul Randall, Pacific Agri-Food Research Centre, Summerland, BC. Initial hexanal fumigation was done at Summerland but expanded later to include MCP/hexanal trials at Hood River with both Anjou and Bosc pears.

B. MCP and wound healing.

There is the possibility that MCP treatment delays wound healing, which in turn, would result in increased infection and decay. To determine this, d'Anjou pears will be wounded, then exposed to 0, 20, 50, and 100 ppb MCP. Wounds will be inoculated with spores of *P. expansum* at 0, 1, 3, and 5 days after MCP exposure, stored at 30º F, and evaluated after 3 months.

2. DNA techniques (real time PCR) for rapid, accurate detection of decay spores.

We have developed new, rapid, accurate methods to determine the concentration of decay spores in dump tank and flume water so decay control decisions can be made based on previously established spore threshold values. The method is based on DNA analysis with real time PCR.

Research is being done to adapt these techniques for use in postharvest water handling systems. We have determined that the method is highly sensitive, quantitative, and specific. Two key questions still need to be resolved before the PCR technique can be used. First, does the DNA of dead spores in the water break down rapidly enough so that they will not be counted along with live spores? Second, do chemicals such as chlorine, SOPP, flotation salts, and ethoxyquin interfere with the analysis? Preliminary data look encouraging, and additional research is in progress.

Studies initially are being done under controlled lab conditions with spore suspensions of *B. cinerea* (gray mold), *P. expansum* (blue mold), *M. piriformis* (mucor rot), and *N. species* (bull's-eye rot) in water. Next, spores will be suspended in dirty dump tank water and the experiments repeated. Some packinghouse dump tank sampling was done in 2004 and will be expanded in 2005.

3. Studies on decay pathogens with an emphasis on gray mold and bull's-eye rot.

A. Fruit surface Penicillium and Botrytis spore levels and fruit decay.

This study has been expanded to several orchards in Oregon, Washington, and New Zealand and focuses on the relationship between spores on the fruit surface and the amount of decay developing in stored fruit. The goal is to accurately predict the storage decay risk level for each orchard at harvest. We have completed the first full year of research and will begin the second year in February 2005 in New Zealand.

In orchards with a history of gray mold, spores will be washed from fruit surfaces and counted by standard dilution plating and also analyzed for *Botrytis* with new DNA identification techniques (real time PCR). Stems will be plated to determine presence of decay fungi, and fruit will be stored at 30° F and evaluated for decay after 3, 6, and 8 months. Because the internal resistance of fruit to decay may vary from year to year, a standard test will be developed to measure this factor. Other factors that will be considered that may affect the risk level of each orchard include: tree age, spacing, cultivar, weed control, irrigation method, frequency, and amount, and spray schedule.

B. Bull's-eye rot.

We collected 185 new isolates of fungi from pears with bull's-eye rot. These isolates are mainly from Wenatchee, Yakima, and Hood River and compliment the 400 isolates from Medford and Hood River.

The new isolates will be identified with multiplex PCR. All isolates in the collection will be screened for sensitivity to a large group of fungicides including thiabendazole (Mertect), ziram, thiram, flint, copper, lime sulfur, and the new fungicides Penbotec and Scholar.

The importance of rain and over tree irrigation on bull's-eye rot will be studied for a second year. Fruit from trees irrigated with over tree irrigation and undertree irrigation (control) will be inoculated just prior to harvest with 3 species (*Neofabraea alba, N. malicorticis*, and *N. perennans*) causing bull's-eye rot. Fruit will be stored and decay evaluated after 3, 6, and 8 months.

Effectiveness of postharvest fungicides may vary with time of infection in the orchard and time of application after harvest. These factors will be studied by inoculating fruit at different times during the growing season, then treating at different times after harvest. Fungicides will include Penbotec, Scholar, thiabendazole, and the yeast CIM.

4. Evaluation of pyrimethanil (Penbotec) and *Cryptococcus infirmominiatum* (CIM) for control of postharvest decay of pear

Anjou pear fruit will be harvested and stored at 30° F until use. Fruit will be surface-sterilized with sodium hypochlorite and rinsed with tap water. For the drench experiment, pear fruit will not be wounded. For the line-spray experiment, pear fruit will be wounded (6 mm diameter x 3 mm deep) at two locations midway along the calyx-stem axis. After wounding, the fruit will be stored overnight at 30°F. The next morning, the fruits will be treated with CIM, CIM mixed with pyrimethanil, or pyrimethanil alone (see Table 1 below).

The pyrimethanil formulation used in all experiments is from Janssen Pharmaceutica Inc.

Biocontrol agent. Cryptococcus infirmominiatum (strain YY6), isolated from the surface of a pear fruit, will be cultured on yeast malt dextrose agar and prepared as in previous studies. The cells will be resuspended in SDW and adjusted to the desired concentration with the aid of a spectrophotometer. Actual concentrations will be verified with standard dilution plating.

Pathogen. Penicillium expansum (strain 46, sensitive to thiabendazole) will be grown on potato-dextrose agar and the spore concentration adjusted to obtain 1 x 10^4 conidia per ml.

Drench application. Four boxes (60-70 fruits per box) of pear fruit per treatment will be drenched for 30 seconds per box using a recirculating drencher containing 20 liters of different treatment suspensions (Table 1). Between treatments, the drencher will be surface-sterilized with sodium hypochlorite, then rinsed with tap water. Fruit from each treatment will be stored at 30°F in air. Blue mold incidence (percent of fruits infected) will be recorded after 3 months of storage.

Line spray application . Fruit will be placed on a moving belt on a packingline and passed under a controlled droplet applicator. There will be four replicate boxes of fruits for each treatment. All equipment will be thoroughly washed between application of different treatments and sterilized with ethanol between tests with CIM. Treated fruit will be stored and evaluated as described above.

Table 1

Treatment	Product	Application[a]	Penbotec (ppm)	CIM (cfu/ml)
1	*Control (drench)*	D	na	na
2	CIM	D	na	2 X 10^8
3	PENBOTEC 400 SC	D	500	na
4	PENBOTEC 400 SC + CIM tank mix	D	500	1 X 10^8
5	PENBOTEC 400 SC drench, then CIM line spray	D, then LS	500	2 X 10^8
6	CIM drench, then PENBOTEC 400 SC line spray	D, then LS	1000	2 X 10^8
7	*Control (line spray)*	LS (aqueous)	na	na
8	CIM	LS	na	2 X 10^8
9	PENBOTEC 400 SC	LS (aqueous)	1000	na
10	PENBOTEC 400 SC	LS (wax)	1000	na
11	PENBOTEC 400 SC + CIM tank mix	LS (aqueous)	1000	1 X 10^8
12	CIM, then PENBOTEC 400 SC	LS, then wax	1000	2 X 10^8

[a]D = drench; LS = line spray

5. Evaluation of *Muscodor* for decay control.

Muscodor is a new biocontrol agent that controls a wide range of diseases by releasing a complex of natural volatiles that inhibit growth of fungal pathogens. This study will determine if *Muscodor* controls the 3 main decays of pear.

Anjou pear fruit will be harvested at commercial maturity, surface-sterilized with sodium hypochlorite, and rinsed with tap water. Each fruit will be wounded at one location on the

equator with a metal tool simulating a stem puncture. Fruit will be placed in 8.0 liter plastic boxes. Each wound will be inoculated with 50 µl of a spore suspension containing 2.0 x 10^3 spores per ml of each of the following fungi: *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum*. Rye grain colonized by *Muscodor albus* will be added to the boxes at 0, 0.5, 1, 2, and 4 grams per liter of box space (0, 4, 8, 16, and 32 grams per box). Muscodor will be activated and held 4 hours at room temperature as described above. Boxes will be incubated at 41, 32, and 30° F. There will be three replicate boxes for each pathogen at each temperature and *M. albus* rate. Incubation time will vary from two weeks for *M. piriformis* at 41° F to 3 months for *P. expansum* at 30° F. At the appropriate incubation time for each pathogen x temperature combination, lesion diameter will be measured. Fruit also will be inspected for phytotoxicity.

6. Evaluation of chlorine dioxide-emitting paper for decay control.

   Anjou pear fruit will be harvested at commercial maturity, surface-sterilized with sodium hypochlorite, and rinsed with tap water. Each fruit will be wounded at two locations to simulate stem punctures. Fruit will be inoculated by placing 50 µl of inoculum containing 1000 spores per ml of *Botrytis cinerea* (gray mold), M*ucor piriformis* (Mucor rot), or *Penicillium expansum* (blue mold) into each wound. Inoculated fruit will be placed into standard 40 lb cardboard fruit boxes with perforated polyethylene liners. "Long release" paper will be used, and 3, 5, or 7 pieces placed inside the polyethylene liners of each of three replicate fiberboard trays per box per rate per fungus. Control boxes will have no paper. Paper will be distributed as evenly as possible throughout the box. Fruit will be stored at 30˚ F. Incidence of Mucor rot will be evaluated after 1 month, gray mold after 2 months, and blue mold after 3 months. Fruit also will be visually evaluated for phytotoxicity by comparing fruit from boxes with treated paper with fruit in the control boxes.

7. Preharvest fungicides for control of postharvest decay

   Several fungicides will be applied to Anjou pear trees either 1 or 2 weeks before harvest. Fruit will be harvested and drenched with water containing 500 spores/ml of *P. expansum*. Fruit will be stored at 30˚ F and decay evaluated after 3 months. Fungicides will include Pristine, Topsin M, and Ziram. Decay in treated fruit will be compared with decay in fruit from unsprayed trees and in fruit drenched after harvest with Scholar.

Estimated duration:

The first year of two years of MCP studies has been completed in cooperation with Agriculture Canada. The DNA rapid pathogen identification work will include development of methods the first year, then application during the second and third years. Use of spore numbers on fruit surfaces to predict decay in storage requires addition of new orchards and 2-3 years of data to develop a reliable prediction model. Work done in New Zealand will provide an additional growing season per year. Bull's-eye rot studies will require one additional year. Penbotec/CIM studies will be completed in one year. Muscodor, and chlorine dioxide paper will require two years because of the new technology involved. Preharvest fungicides will be evaluated in one year.

2005-2006 Budget requested:

Item	Amount
Salaries and wages	$47,862
Service and supplies	$1,000
TOTAL	$48,862

BUDGET DETAIL

Proposed Title: New Approaches to Decay Control of Pear

Submitted to: WTFRC/Winter Pear Control Committee

Project Leader: Robert A. Spotts

Item	**Amount**
Salaries and wages	
Academic salaries	$34,008
OPE-Academic	$13,854
Services and supplies	$1,000
GRAND TOTAL	$48,862

CONTINUNG PROJECT REPORT **YEAR 2/3**

Project title: Role of systemic resistance in defense against the gray mold pathogen *Botrytis cinerea*

PI: Henrik Stotz
Organization: Oregon State University

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Phone: 541-737-5468

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Cooperator: Robert A. Spotts (Mid-Columbia Ag. Res. & Ext. Ctr.; Hood River, OR)
Ken Johnson (Department of Botany & Plant Pathology, OSU)

Contact Administrator: Dorothy Beaton
Ag Research Foundation
Dorothy.Beaton@oregonstate.edu
(541) 737-3228

1. RESEARCH OBJECTIVES

Our main ***objective*** is to determine whether treatments that induce systemic resistance can be used to manage Botrytis decay. We analyzed the role of ethylene in Botrytis resistance during the previous year. Treatments with propylene or 1-methylcyclopropene (1-MCP) were supposed to influence ethylene biosynthesis and fruit ripening. As a result, we expected differences in Botrytis susceptibility among treatments. Our ***rationale*** was that, once the effects of these treatments on gray mold decay were known, they could be implemented to control postharvest decay.

We proposed to use different pear (*Pyrus communis*) cultivars with and emphasis on d'Anjou, Bosc, and Bartlett. During the course of the last year we compared d'Anjou, a winter pear, with Bartlett, a pear that does not require a chilling period for fruit ripening. In addition, we re-evaluated *Pyrus pyrifolia* cv. Niitaka, a Japanese pear with higher resistance to Botrytis than d'Anjou, Bosc, or Comice. We suggested inoculating fruits with mycelia grown on agar to avoid the effects of wounding. Because this method was highly variable and not effective, we used wound inoculation instead. Fruits that were wounded and mock inoculated served as controls for all experiments. Assessment of the impact of jasmonic acid and salicylic acid, which are inducers of systemic resistance, on gray mold decay was stated as a goal for the activities of the coming year. Robert Spotts provided pear fruits for all of our last year's studies.

During the next year, we will test whether inducers of systemic acquired resistance (SAR) inhibit Botrytis infection. 1,2,3-Benzothiadiazole-7-carbothioic acid S-methyl ester (BTH) is the active ingredient of Actigard. This compound has successfully been used to control various diseases, including fire blight. Because SAR may only be induced in fruits that are attached to plants, pear trees will be treated one and two weeks before harvest. Fruits will be wound inoculated with Botrytis one week after the last treatment with Actigard. In addition to these short-term assessments, fruits will be tested for their long-term performance during postharvest storage.

Pears respond to salicylic acid treatments by inducing defense-related genes (4). We therefore expect that treatment of pear trees with Actigard will activate SAR throughout the plant. We will analyze the expression of pathogenesis-related (PR) proteins (3) or genes (5) to determine whether Actigard treatments lead to SAR in leaves and fruits. The fire blight pathogen *Erwinia amylovora* can be used as a positive control to determine whether SAR has been induced. We expect that Actigard will enhance foliar resistance because salicylic acid treatments have been shown to protect leaves against Botrytis (6). We will test different stages of fruit development for Actigard protection against Botrytis because induced resistance may operate in immature, but not in mature fruits.

2. SIGNIFICANT FINDINGS

- Treatments with propylene and 1-MCP accelerated and retarded disease-related ethylene biosynthesis, respectively, but had no influence on disease progression.
- Co-treatment of pears with 1-MCP and aminoethoxyvinyl glycine (AVG) blocked ethylene and 1-aminocycolopropane-1-carboxylic acid (ACC) biosynthesis for 4 d, but lesions started expanding on day 2 post inoculation.
- 1-MCP inhibited fruit softening in response to Botrytis infection but had little influence on the rate of lesion expansion.
- A mutant of *Botrytis cinerea* with a defect in the polygalacturonase gene *Bcpg1* was less virulent on pear fruits, suggesting that pectin catabolism is important.
- Fruits became more susceptible to Botrytis as they matured during cold storage.
- Niitaka and d'Anjou were the least and most susceptible cultivars, respectively, whereas susceptibility of Bartlett was intermediate.

3. METHODS

3.1. Biological Material and Treatments

Robert Spotts will provide access to pear trees and fruits cv. d'Anjou and Bartlett. We will select three to four trees per spray treatment with Actigard. The rates of application will be 150 to 300 mg a.i./liter (5). We will harvest one to three boxes of fruits. One box of fruits will be used for wound inoculations with *B. cinerea*. The other boxes will be kept in cold storage to compare the postharvest performance of treatment and control fruits. Because Actigard will be supplied by Syngenta (Greensboro, NC) and the supply of this chemical is limited, we will carry out a feasibility study using tomato plants that either express *nahG*, encoding a salicylate hydroxylase, or not.

B. cinerea (B05.10), maintained as a glycerol stock at –80°C, will be cultured on malt extract or potato dextrose agars. Conidia will be harvested according to published procedures (7).

3.2. Plant Inoculations

Fruits will be surface-sterilized with 0.01% sodium hypochlorite for 2 min; then rinsed with deionized water. Fruits will be punctured with a syringe to create a wound that is 4 mm deep and 4 mm in diameter. An aqueous suspension of *B. cinerea* (250 conidia per inoculum) will be used to inoculate pear fruits (8). Mock inoculations in the absence of conidia will be similar.

Pear leaves will be inoculated (1,000 conidia per inoculum) with or without wounding using Gamborg's B5 medium containing 10 mM sucrose and 10 mM potassium phosphate, pH 6 (7).

3.3. Measurement of Decay Parameters and PR Protein/Gene Expression

Lesion expansion will be determined on a daily basis by measuring the diameter of spreading lesions using a caliper.

RNA will be extracted and analyzed by northern hybridization or reverse transcriptase PCR as previously reported (9, 10). Primers for amplification of PR genes have been published and will be used for our studies (5). Alternatively, chitinase and/or β-1,3-glucanase activities will be tested (3).

3.4. Statistical Analysis

Standard statistical tests will be used to compare differences between genotypes, treatments, and their interactions, including analysis of variance (ANOVA) using the SAS program package.

4. RESULTS AND DISCUSSION

Treatment of pear fruits with propylene and 1-MCP accelerated and delayed ethylene biosynthesis, respectively (Fig. 1A). Despite these changes, there was no difference in gray mold susceptibility between treated and control fruits (Fig. 1B), suggesting that ethylene had no influence on disease progression.

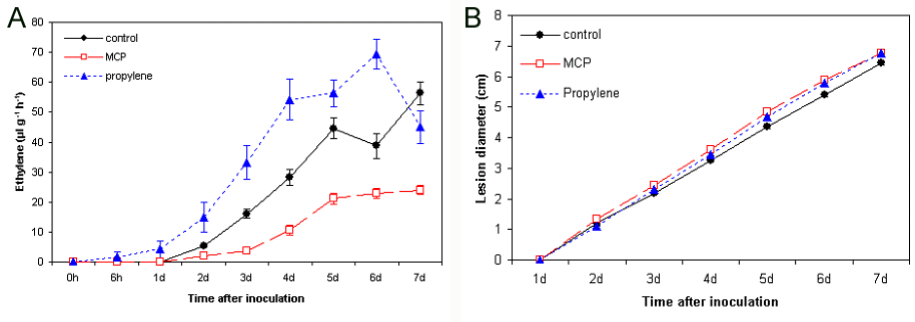

Figure 1. Ethylene agonists and antagonists affect ethylene biosynthesis but not gray mold susceptibility. **(A)** Bartlett fruits at harvest maturity

Botrytis caused disease even when a co-treatment of 1- MCP and AVG was used

treated for 1 d with propylene (500 ppm) or 1-MCP (300 ppb). Ethylene was measured using a GC with FID detector. **(B)** Lesion expansion was measured using a caliper.

to block ethylene biosynthesis (Fig. 2B and D). Because ACC was detectable in infected fruits at the end of the experiment (Fig. 2C), ethylene biosynthesis in the presence of 1-MCP and AVG was likely of plant origin. ACC would not be expected to accumulate if ethylene originated from the fungus because ACC is not an intermediate of ethylene biosynthesis in *B. cinerea* (1).

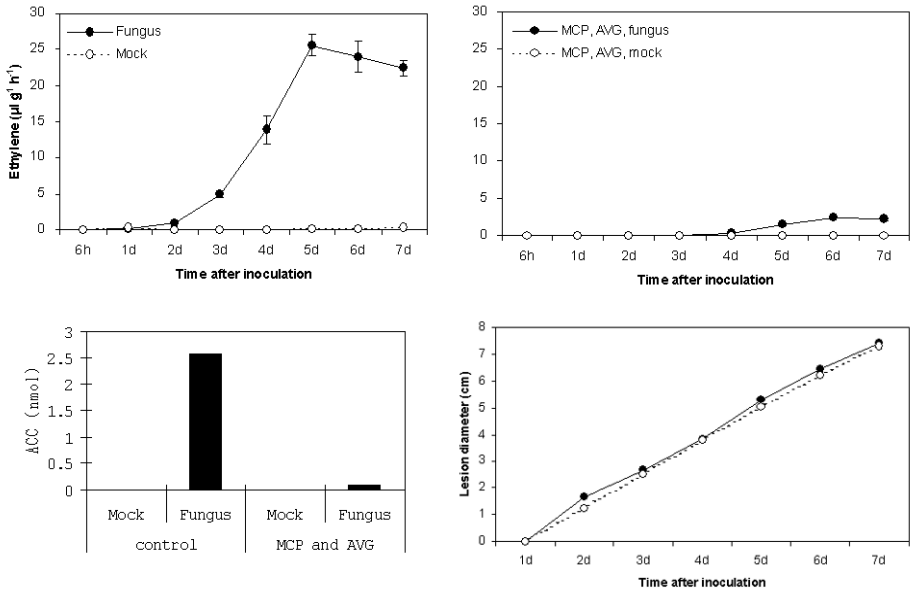

Figure 2. Gray mold infection despite inhibition of ethylene biosynthesis. **(A)** Ethylene production in d'Anjou pears at harvest maturity after wounding or gray mold infection. **(B)** Inhibition of wound- or pathogen-induced ethylene biosynthesis by 1-MCP and AVG. **(C)** ACC content in pear fruits treated or not treated with 1-MCP and AVG. **(D)** Lesion expansion was measured with a caliper.

Fruit firmness had no influence on Botrytis infection (Fig. 3). 1-MCP completely inhibited Botrytis-induced softening of fruits (Fig. 3A). At the end of the experiment, 1-MCP-treated fruits were firmer than control fruits or fruits that were treated with propylene. Botrytis caused softening of control fruits and propylene-treated fruits

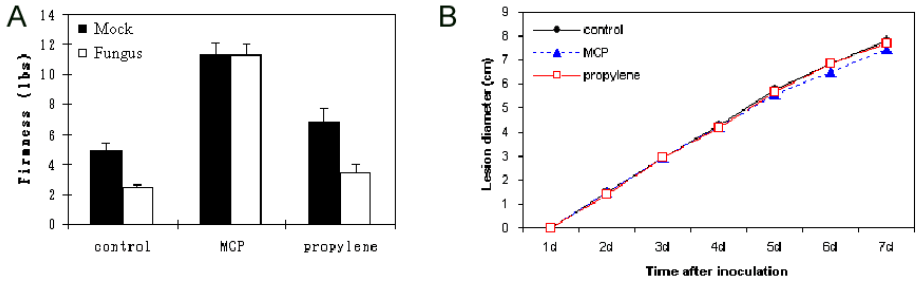

Figure 3. Firmness has no effect on Botrytis decay. **(A)** Decrease in firmness of d'Anjou pear fruits that were stored for 2 months at -1°C. Fruit softening was recorded at the end of the experiment with a UC firmness tester. **(B)** Lesion diameters were measured with a caliper.

Despite this effect on fruit firmness, 1-MCP had little influence on lesion expansion (Fig. 3B). Thus, reductions in firmness are a consequence of Botrytis infection but not causally related to disease development.

Despite the lack of effect of ethylene, we observed differences in Botrytis susceptibility that were related to fruit development, cultivar, and pectin metabolism (Fig. 4). Both d'Anjou (data not shown) and Bartlett fruits were more susceptible after storage at -1ºC compared to fruits that were freshly harvested (Fig. 4A). Consistent with our previous data, the Japanese pear variety Niitaka was less susceptible than d'Anjou (Fig. 4B). Bartlett pears were intermediate in gray mold susceptibility. A *B. cinerea* mutant with a deletion in the polygalacturonase gene *Bcpg1* (2) was significantly less virulent than wild type fungus, suggesting that pectin degradation has a major impact on lesion expansion (Fig. 4C).

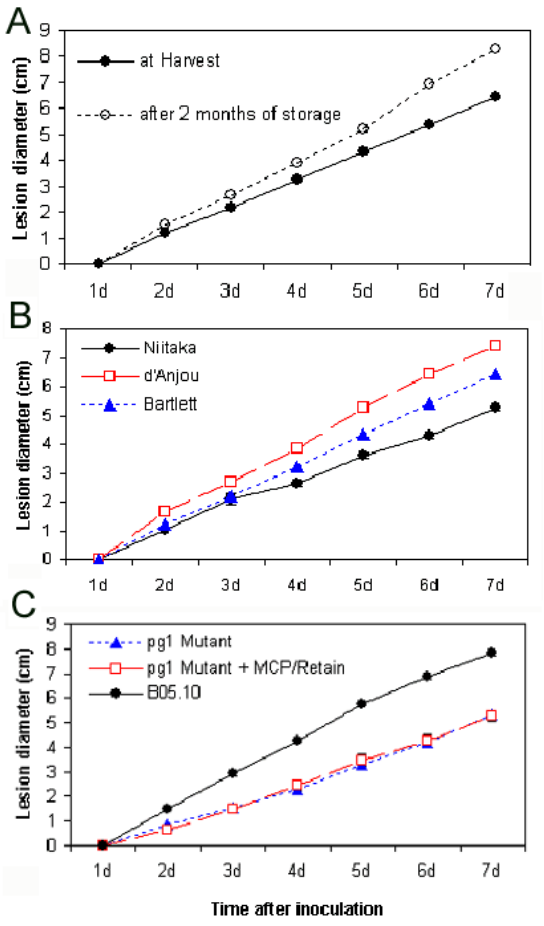

Figure 4. Gray mold susceptibility is influenced by fruit development, cultivar, and pectin metabolism. **(A)** Cold storage increases susceptibility. **(B)** Cultivars differ in Botrytis susceptibility. **(C)** Mutation in the polygalacturonase gene *Bcpg1* reduces virulence.

Our data suggest that the increased susceptibility of pears in storage is a result of ripening-related pectin catabolism. Fruit softening in general does not have an effect on Botrytis susceptibility. Measures to control Botrytis decay should therefore be directed towards inhibiting pectin metabolism, perhaps by means of calcium sprays. Alternative approaches of improving the resistance of fruits to fungal decay, including activation of systemic resistance (3), need to be considered. The role of ethylene in Botrytis susceptibility can be further evaluated by analyzing fruit trees that express antisense ACC synthase mRNA (Abhaya Dandekar, personal communication).

5. REFERENCES

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BUDGET

Project title: Role of systemic resistance in defense against the gray mold pathogen *Botrytis cinerea*
PI: Henrik Stotz
Proposed project duration: 2003 to 2005
Current year: 2005
Project total (three years): $77,020
Current year request: $29,184

Item	Year 1 (2003)	Year 2 (2004)	**Year 3 (2005)**
Salaries and wages	15,625	18,500	20,714
Benefits (%)	469	492	2,220
Equipment	500	0	0
Supplies	5,500	6,000	6,000
Travel	500	250	250
Total	22,594	25,242	29,184

Budget Justification: Salaries and wages are requested to continue the employment of my graduate student Aya Akagi. She will work together with the PI on the effects of Actigard on gray mold infection of pear fruits. Equipment expenses are not included because we will use existing equipment at Oregon State University. The new estimates for salaries and wages take into account that the pay freezes for students will be discontinued. The salary increase is expected to reach 8%. Expenses for benefits have increased to $500 per term. Another increase of 11% is expected for the coming year. Supplies include the purchase of chemicals, molecular biology reagents, media for plant and microbial growth, and equipment maintenance costs. The supplies budget and travel expenses have stayed constant compared to the previous proposal. Trips between Hood River and Corvallis will be necessary.

Current Support

- California Tomato Commission; Breeding tomatoes for resistance to Botrytis (gray mold); $25,400; 7/1/04 to 6/30/05
- Winter Pear Control Committee; Role of ethylene in resistance to the gray mold pathogen *Botrytis cinerea*; $25,242; 10/1/04-9/30/05
- USDA-ARS Sclerotinia Initiative; Genetic basis of oxalate sensitivity in relationship to Sclerotinia diseases; $54,855; 7/1/04 to 6/30/05

Pending Support

- NSF-IOB; Mechanisms of oxalate sensitivity in plants; $865,129; 4/1/05 to 3/31/10
- USDA-BARD; Genetic analysis of Botrytis virulence and host resistance in tomato; $360,000; 6/1/05-5/31/08
- USDA-ARS Sclerotinia Initiative; Genetic basis of oxalate sensitivity in relationship to Sclerotinia diseases; $61,039; 7/1/05 to 6/30/06
- California Tomato Commission; Breeding tomatoes for resistance to Botrytis (gray mold) II: Segregation of resistance/susceptibility; $28,200; 7/1/05 to 6/30/06

CONTINUING REPORT **YEAR 1/3**
WTFRC Project # **WSU Project #13L-4164-1202**

Project title: Managing storage scald in d'Anjou pears

PI: Eugene Kupferman

Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA 98801

Contract administrator: Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691

Objectives:

The overall objective is to develop a systems approach to the commercial control of storage scald in d'Anjou pears.

Rationale:
Since most fruit is packed prior to storage, the demand by retailers for new pear packages has resulted in high costs of repackaging of otherwise acceptable fruit. It would be helpful if pears were stored in bins. Fear of storage scald is a significant factor in the industry's unwillingness to store pears in bins in CA storage until spring. The other factor is fear of decay, but with new fungicides it may be possible to drench fruit in bins.

Objectives for the second year of this three year project include validation of the results of the first year in four areas:
1. Estimation of the risk of storage scald through knowledge of the effect of preharvest temperatures (summer 2005).
2. Determination of the timing of antioxidant application on fruit with different risk levels (fall 2005).
3. Determination of the effectiveness of applying antioxidants and fungicides as a bin drench (fall 2005).

Expanded goals include:
4. Determine the ability of low oxygen to control scald (fall, winter 2005).

Note: the original description of this project included evaluation of whether thermofogging could apply antioxidant to pear storage rooms. This goal will be deferred to 2006.

Significant findings:

Objective 1: Estimation of the risk of scald through knowledge of orchard temperatures. Data loggers in eight orchards (five in the Wenatchee River Valley and three in the Yakima Valley) developed predictions from 0 days to 88 days in which 10% of the fruit would show scald. Examination of fruit to validate these predictions after 77 and 111 days after harvest is underway.

Objective 2: Determination of the timing of effective antioxidant application on fruit from the five Wenatchee area orchards using scald risk information from the temperature model (above). Fruit from

each orchard were removed from storage at intervals between 7 and 112 days and treated with either diphenylamine (DPA) or ethoxyquin wraps. Examination will take place in the spring.

Objective 3: Determination of the effectiveness of applying antioxidants and fungicides as a bin drench. Ten bins from each Wenatchee area grower were purchased and drenched with a fungicide (TBZ, Penbotec or Scholar) with a half strength antioxidant (DPA or ethoxyquin) following harvest in September. Fruit will be evaluated early in 2005.

Objective 4: Determination of the potential for chemical burn from chemicals (antioxidants and fungicides) used in bin drenches. In October 2004 fruit from the five Wenatchee area growers were drenched in cherry lugs with various combinations of fungicides and ethoxyquin at different concentrations. This preliminary trial showed packers that d'Anjou pears treated with Penbotec (with or without ethoxyquin) developed appreciably more chemical marking than fruit treated with either Scholar or TBZ. When combined with either Scholar or TBZ, fruit treated with ethoxyquin of 1350 ppm or higher developed chemical burn, while fruit treated at 675 ppm did not burn. Fruit from the bin drench (above) will also be evaluated for chemical burn in early 2005.

Methods:
The methods used on Goals 1 through 3 will be refined based on the amount of scald encountered when the fruit comes out of storage. The procedures will be repeated with fruit in 2005 to validate the findings from the first year.

Objective 1: Estimation of the risk of scald through knowledge of orchard temperatures was performed on eight orchards by placing temperature data loggers in the orchards. Temperature data were then analyzed using techniques developed by Ma and Chen and used currently in Hood River to estimate the risk of storage scald. Orchards in the Wenatchee River Valley range in elevation from 640-1250 feet. Fruit from the orchard at 640 feet is not normally placed in long term CA because of a history of scald. It is not the elevation that determines the risk of scald, but rather the accumulation of cold temperatures prior to harvest.

Using the same orchards in 2005, data loggers will be placed within the canopy and fruit samples will be harvested at commercial harvest. Fruit will be evaluated weekly for 12 weeks for storage scald beginning after 30 days in RA storage. Scald will be evaluated after 7 days of ripening. Results will be compared with the prediction derived from the orchard temperature data. If we are able to accurately predict the beginning of storage scald, packers will have more flexibility to pack and ship fruit before scald develops or to treat fruit, either by using no antioxidant, a lower concentration of antioxidant, a different antioxidant (DPA), low oxygen or fogging. Each of these alternatives is used in this project on fruit of various risk levels.

Objective 2: Determination of the timing of effective antioxidant application on fruit. Using the same fruit with different levels of risk, fruit will be removed from storage at various times, treated with an antioxidant then placed back into long-term storage. Packers currently treat fruit bound for long-term storage with an antioxidant as soon after harvest as possible. Since it is harvested early, this fruit usually has the highest risk of scald. However, some fruit is stored loose in CA for 60 or 90 days prior to treatment. By treating fruit with a known risk of scald at intervals following harvest, packers will learn how soon fruit must be treated. Fruit samples from each orchard will be removed after storage between 1 and 16 weeks, treated and placed back in to storage until spring when it will be evaluated for scald.

Objective 3: Determination of the effectiveness of applying antioxidants as a bin drench. Packers have been fearful of treating d'Anjou pears in bins due to experiences with both decay and chemical

burn. It is well known that high concentrations of either DPA or ethoxyquin can burn fruit, especially fruit in contact with poly sleeves or each other. However, half-strength antioxidant application may be able to control scald adequately without burn; and with new fungicides, drenching may become a viable alternative.

Drenching will be done on individual bins from each orchard using a chain drencher; and the fruit in bins will be stored in CA for at least 5 months, then packed on a commercial line and evaluated for scald and chemical burn. Fungicides (TBZ, Penbotec or Scholar) with a half-strength antioxidant (DPA or ethoxyquin) will be used. Bins drenched with water only will be used as controls for each lot of fruit. Drench solutions and fruit samples will be submitted for chemical analysis to validate concentrations.

Objective 4: Determine the ability of low oxygen to control scald. Research over a number of years by Dr. Paul Chen has demonstrated that oxygen levels below 1% can control storage scald in d'Anjou pears when the risk of scald is not high. Fruit from the test orchards will be placed into a CA room at the Stemilt facility and oxygen levels set by the HarvestWatch monitor. This work will build on the work of both Drs. Chen and Mattheis, who have worked with the HarvestWatch monitoring system. This system is also under trial in the South Tyrol region of Italy and is working well to control scald on apples. HarvestWatch has been used in commercial facilities in Washington. Bins of fruit from the test orchards will not be drenched or wrapped and will be stored under very low levels of oxygen.

Results and discussion:

Objective 1: Estimation of scald risk based on preharvest orchard temperatures is shown in Table 1.

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

Orchard	Harvest date	Hours <50ºF*	Scald prediction** DIS(10%)=62.3827 x $ACU^{0.0757}$
Wenatchee1	1-Sept	0	0 days
Wenatchee2	8-Sept	10	74 days
Wenatchee3	14-Sept	48	84 days
Wenatchee4	21-Sept	163	92 days
Wenatchee5	21-Sept	198	93 days
Yakima1	3-Sept	9	74 days
Yakima2	14-Sept	101	88 days
Yakima3	14-Sept	46	83 days

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

** Predicted days in storage after which 10% of the fruit will show scald symptoms following 7 days of ripening at 68ºF.

Data as to the amount of fruit that developed scald are not available as this report is being written.

Objective 2: Determination of the timing of effective antioxidant application on fruit. As in Goal 1, this fruit is in storage and has not yet been evaluated. Table 2 shows the dates that fruit were removed and treated with antioxidants and TBZ.

Table 2. Schedule for treatment and removal from storage. Evaluation will occur in the spring with chemical burn evaluated immediately after removal from storage and scald evaluated after ripening.

Harvest date	Orchard No.	Treatment after harvest	Drench and wrap date	Actual days after harvest	Exam date	Chemical burn (%)	Exam date	Scald (%)
1-Sep	Wen1	7 day	15-Sep	14				
8-Sep	Wen2	7 day	15-Sep	7				
14-Sep	Wen3	7 day	29-Sep	15				
21-Sep	Wen4	7 day	29-Sep	8				
21-Sep	Wen5	7 day	29-Sep	8				
1-Sep	Wen1	28 day	7-Oct	36				
8-Sep	Wen2	28 day	7-Oct	29				
14-Sep	Wen3	28 day	20-Oct	36				
21-Sep	Wen4	28 day	20-Oct	29				
21-Sep	Wen5	28 day	20-Oct	29				
1-Sep	Wen1	56 day	17-Nov	77				
8-Sep	Wen2	56 day	17-Nov	70				
14-Sep	Wen3	56 day	17-Nov	64				
21-Sep	Wen4	56 day	17-Nov	57				
21-Sep	Wen5	56 day	17-Nov	57				
1-Sep	Wen1	112 day	12-Jan	133				
8-Sep	Wen2	112 day	12-Jan	126				
14-Sep	Wen3	112 day	12-Jan	120				
21-Sep	Wen4	112 day	12-Jan	113				
21-Sep	Wen5	112 day	12-Jan	113				

Objective 3: Determination of the effectiveness of applying antioxidants as a bin drench.

Fruit were drenched using the 500 gallon drencher at Stemilt, and both drench solutions and fruit were analyzed by Pace International. Drench solution samples were taken after the fruit were drenched instead of prior to drenching, so solution concentration results were significantly lower than the calculated starting concentrations (Table 3). It is not possible to analyze for ethoxyquin levels.

Table 3. Laboratory analysis results of drenching solution concentrations.

Drench solution	Laboratory analysis	
Scholar (8 oz/100 gal)	Scholar = 172 ppm	
Scholar (8 oz/100 gal) + DPA (1000 ppm)	Scholar = 158 ppm	DPA = 848 ppm
Penbotec (500 ppm)	Penbotec = 240 ppm	
Penbotec (500 ppm) + DPA (1000 ppm)	Penbotec = 280 ppm	DPA = 735 ppm
TBZ (500 ppm)	TBZ = 245 ppm	
TBZ (500 ppm) + DPA (1000 ppm)	TBZ = 503 ppm	DPA = 768 ppm

Analysis of fruit indicated consistent residues on the fruit (Table 4).

Table 4. Laboratory analysis results of residue.

Drench	1	2	3	4	5	6	7		8		9	
	Scholar	Scholar (Ethox)	Penbo	Penbo (Ethox)	TBZ	TBZ (Ethox)	Scholar	DPA	Penbo	DPA	TBZ	DPA
Wen1	1.1	1.2	1.9	1.0	2.9	2.0	0.7	1.5	1.6	2.5	1.4	2.0
Wen2	1.5	0.9	1.9	1.1	2.5	1.4	0.8	2.0	0.8	1.3	1.3	1.6
Wen3	1.1	1.2	1.2	1.5	3.4	1.8	0.7	1.8	1.2	1.9	1.5	1.8
Wen4	1.1	1.2	1.5	1.7	2.7	1.7	0.7	1.9	1.5	2.4	1.6	1.9
Wen5	1.2	1.1	2.3	1.7	5.1	2.0	0.7	1.9	1.2	1.9	1.3	2.3
Average	***1.20***	***1.12***	***1.76***	***1.40***	***3.32***	***1.78***	***0.72***	***1.82***	***1.26***	***2.00***	***1.42***	***1.92***

In conclusion, significant progress has been made in developing methodologies for the management of storage scald in d'Anjou pears. Fruit evaluations will determine the exact path of future research.

Budget:
Project title: Managing storage scald in d'Anjou pears
PI: E. Kupferman
Project duration: 2004-2006 (3 years)
Current year: 2005
Project total (3 years): $125,751
Current year request: $45,301

Item	Year 1 (2004)	**Year 2 (2005)**	Year 3 (2006)
Salaries [1]	$16,890	**$13,301**	$13,833
Benefits (45%)	5,067	**5,985**	6,225
Wages[2]	4,500	**6,500**	6,500
Benefits (16%-yr 1; 11% yrs 2 and 3)	720	**715**	715
Equipment[3]	700	**2,000**	2,000
Supplies[4]	5,400	**15,300**	15,400
Travel[5]	1,000	**1,500**	1,500
Total	$34,277	**$45,301**	$46,173

Note: In 2004 we obtained antioxidant and fungicides at no cost. We did not incur liquid or fruit analysis costs. It is assumed that these contributions will continue in future years.

2005:
[1] Salary: Chris Sater for 6 months (0.75 FTE)
[2] Time-slip: Nancy Buchanan for 6 months (0.75 FTE).
[3] Equipment: Lease of HarvestWatch.
[4] Supplies: fruit, lab supplies. Cell phone charges are allowed. Original budget did not allocate sufficient funds for fruit purchase. Goal 2 cost over $6,000 for fruit. Goal 4 will cost $8,000.
[5] Travel to obtain fruit samples.

2006:
[1] Salary: Chris Sater for 6 months (0.75 FTE).
[2] Time-slip help.
[3] Equipment: Lease of HarvestWatch.
[4] Supplies: fruit, lab supplies. Cell phone charges are allowed.
[5] Travel to obtain fruit samples.

FINAL REPORT

Title: Storage Decay and Postharvest Quality Research

PI: David Sugar, Professor

Organization: Oregon State University, Southern Oregon Research and Extension Center

Cooperators: R.A. Spotts

Funding in 2004-2005: 30,000

Objective: This research blends activities in the areas of postharvest pathology and physiology. One objective is to further develop a storage decay control program for winter pears in which diverse, independent decay control practices contribute to dependable reduction of postharvest diseases. A second objective is to develop and evaluate methods and materials for the promotion of pear quality during storage.

Significant Findings:

1. It was previously found that Bosc pears treated with 100 ppm ethylene for 24 hr at 68°F could replace the 2 weeks cold storage necessary for Bosc to develop the capacity to ripen uniformly. New results indicate that ripening can occur with a shortened ethylene treatment of 12 hr + 2 days cold, or 6 hr + 7 days cold (Table 1). However, eating quality was unsatisfactory with less than 24 hr ethylene + 7 days cold, or 12 hr. ethylene + 12 days cold.

2. Early harvested Comice pears treated with MCP at 50, 100, or 200 ppb did not ripen adequately after 5 months cold storage (Table 2). Late harvested Comice ripened to good quality following MCP treatment at 50 or 100 ppb after 5 months storage, but did not ripen adequately following treatment at 200 ppb.

3. Early harvested Bosc pears treated with MCP at 50 or 100 ppb failed to ripen after 6 months cold storage (Table 3). Late harvested Bosc showed unacceptable levels of internal breakdown after 6 month cold storage despite MCP treatments.

4. Tests of postharvest and preharvest fungicides against a range of pathogens causing postharvest decay resulted in effectiveness profiles for each fungicide (Table 4). Scholar and Pristine were effective against all pathogens except *Neofabraea alba*, one of the bull's eye rot fungi. Evaluation of decay control treatments using inoculated fruit are in progress.

5. A potential "biofumigant" biological control agent, *Muscodor albus*, was a powerful suppressant of blue mold and gray mold when inoculated fruit were kept in a sealed container for 24 hours at room temperature before cold storage (Tables 5-7). Treatments were much less effective when placed directly into cold storage, except in the case of Cladosporium rot (Table 6). *Muscodor* does not show much activity in reducing blue mold contamination of wooden bin surfaces.

6. Laser labeling of pears does not appear to provide an entry point for decay-causing microorganisms. Decay did not preferentially develop at labels when pathogen spores were pressure or vacuum infiltrated into fruit.

7. Evaluation of other storage decay projects focused on orchard and postharvest integrated management is in progress.

Results and Discussion:

1. Bosc pears typically require approximately 2 weeks of cold storage before developing the capacity to ripen to a buttery texture. Previously it was demonstrated in this project that 100 ppm ethylene for 24 hr at 68°F could replace the chilling requirement in Bosc. An attempt was made to identify shorter periods of exposure to ethylene that would still allow ripening. Using a standard of ripeness of 6 lb. firmness, ripeness was achieved within 7 days at room temperature following ethylene exposure for 12 hr. followed by 2 days cold (31°F), or for 6 hr. followed by 7 days cold. Although ripening was achieved with as little as 12 hr. + 2 days cold, or 6 hr. + 7 days cold, flavor was lacking until fruit received a minimum of 24 hr ethylene + 7 days cold, or 12 hr. ethylene + 12 days cold.

2. Lowering dosage of MCP does not appear to be a sufficient solution to the previously observed problem of excessive inhibition of ripening of Bosc and Comice pears. Late harvest of Comice followed by MCP treatments led to ripening with good quality at 5 months, which may be useful. However, the predictability of this strategy remains to be established. In current tests to be evaluated in spring 2005, Comice and Bosc pears were exposed to ethylene prior to MCP treatments.

3. Effectiveness profiles of fungicides used in pre- or postharvest treatments for pear decay control show a wide range of diversity among fungicides. Scholar and Pristine had the broadest range of effectiveness among postharvest pathogens, followed by Penbotec. These results indicate the value of knowing the target fungi for designing the most effective treatment strategy. They also show the excellent potential of newer fungicides to give broad-spectrum decay control.

4. *Muscodor albus* is a fungus that, growing on grain, emits volatile compounds that can inhibit other microorganisms. This form of biological control, called “biofumigation”, does not involve direct contact between the biocontrol agent and the pathogen or fruit. When placed in sealed containers with pear postharvest pathogens growing on agar in petri dishes, *Muscodor* inhibited growth of postharvest pathogens, as long as the pathogen did not have more than a 24 hour head start in growth. With inoculated fruit, a 24 hour exposure to *Muscodor* at room temperature was necessary prior to cold storage. This treatment was only moderately effective against gray mold, but highly effective against blue mold. Cladosporium rot was controlled by *Muscodor* at cold temperatures, even without pre-treatment in cold. Tests using *Muscodor* to sterilize wooden bin surfaces has thus far not shown much promise.

5. Laser labeling may find acceptance as an alternative to stickers in labeling individual pear fruit. Since the labeling in accomplished by a certain amount of injury to fruit cells, tests were carried out to determine if labels can become entry points for postharvest pathogens. Pressure and vacuum infiltration methods with various pathogens have thus far shown that laser labels do not provide such entry points for decay pathogens.

Table 1. Ripeness of Bosc pears after various combinations of ethylene treatment and post-ethylene cold storage. Pears were harvested at an average firmness of 16 lbs.

	Days at 31°F after ethylene treatment				
Hours in ethylene	**0**	**2**	**7**	**12**	**15**
	Fruit firmness after 7 day ripening period at 68°F				
0	14.3	13.7 a	8.8 a	3.3 a	3.3 a
6	-	13.9 a	3.6 b	3.3 a	3.0 b
12	-	5.9 b	3.5 b	2.7 b	2.6 bc
24	-	2.9 c	2.6 c	2.5 b	2.3 c

Table 2. MCP effect on Comice Pears.

I. Comice early harvest (12 Sept. 2003, 12.2 lb) followed by 5 months storage.

	Fruit firmness (lbs)					**% of fruit ripe (< 5 lb)**					**% of fruit with internal browning**			
	Days at 20 C					**Days at 20 C**					**Days at 20 C**			
MCP (ppb)	**0**	**5**	**7**	**10**		**0**	**5**	**7**	**10**		**0**	**5**	**7**	**10**
0	10.1 a	2.5 a	2.5 a	1.9 a		0	95	95	95		0	0	35	75
50	11.6 b	7.5 b	8.0 b	7.4 b		0	30	5	25		0	0	0	0
100	11.6 b	11.2 c	10.2 c	9.7 c		0	0	0	5		0	0	0	0
200	12.1 b	11.3 c	10.8 c	11.2 c		0	0	0	0		0	0	0	0
Quality: 0 MCP extensive breakdown; all MCP treatments inadequate ripening, coarse and dry.														

II. Comice late harvest (1 Oct., 2003, 10.8 lb) followed by 5 months storage.

	Fruit firmness (lbs)					**% of fruit ripe (< 5 lb)**					**% of fruit with internal browning**			
	Days at 20 C					**Days at 20 C**					**Days at 20 C**			
MCP (ppb)	**0**	**5**	**7**	**10**		**0**	**5**	**7**	**10**		**0**	**5**	**7**	**10**
0	7.3 a	2.0 a	1.7 a	1.0 a		0	100	94	100		0	45	89	90
50	9.8 c	2.2 a	1.6 a	0.9 a		0	95	100	100		0	0	0	35
100	9.0 b	2.0 a	1.3 a	0.7 a		0	100	100	100		0	0	0	30
200	10.2 c	6.3 b	4.6 b	4.0 b		0	20	55	75		0	0	0	0
Quality: 0 MCP extensive breakdown; MCP 50-100 ppb good flavor, text., juiciness at day 5.														

Table 3. MCP effect on Bosc Pears.

I. Bosc early harvest (12 Sept., 2003, 17.0 lb) followed by 6 months storage.

	Fruit firmness (lbs)				**% of fruit ripe (< 5 lb)**				**% of fruit with internal browning**		
	Days at 20 C				**Days at 20 C**				**Days at 20 C**		
	0	**5**	**8**		**0**	**5**	**8**		**0**	**5**	**8**
0	14.1	4.7 a	-		0	90	-		0	10	100
50	15.7	13.2 b	13.0		0	0	0		0	0	0
100	15.9	13.4 b	13.0		0	10	5		0	0	0
Quality: Inadequate ripening in all MCP treatments.											

II. Bosc late harvest (15 Oct., 2003, 13.7 lb) followed by 6 months storage.

	Fruit firmness (lbs)				**% of fruit ripe (< 5 lb)**				**% of fruit with internal browning**		
	Days at 20 C				**Days at 20 C**				**Days at 20 C**		
	0	**5**	**8**		**0**	**5**	**8**		**0**	**5**	**8**
0	11.1	4.7	4.0		0	71	93		19	7	29
50	12.2	4.2	3.4		0	95	90		0	30	30
100	11.1	3.8	3.0		0	100	100		10	10	30
Quality: Inadequate ripening in all MCP treatments. Breakdown in all treatments.											

Table 4. Inhibition of postharvest decay pathogens by various fungicides tested *in vitro*[1].

	Inhibition *in vitro* at 1000 ppm						
	Postharvest fungicides			**Preharvest fungicides**			
Pathogen[2]	**Mertect**	**Penbotec**	**Scholar**	**Pristine**	**Flint**	**Ziram**	**Topsin**
Penicillium expansum	+	+	+	+	+	-	-
Botrytis cinerea	+	+	+	+	-	+	+
Cladosporium herbarum	-	-	+	+	+	-	+
Alternaria sp.	-	+	+	+	-	+	-
Phialophora malorum	-	+	+	+	+	+	-
Neofabraea alba	-	-	-	-	-	+	-
Neofabraea perennans	-	+	+	+	+	+	-

[1] *In vitro* test: filter paper disks soaked in fungicide solutions were place on agar plates freshly seeded with spores of the pathogen. Zones of inhibition (no fungal growth) around disks were observed 3-10 days later. + = growth inhibited, - = growth not inhibited. Note: single isolate of each fungus tested; may not reflect response of other individuals in a genetically diverse population.

[2] *Penicillium expansum* = blue mold *Botrytis cinerea* = gray mold *Phialophora malorum* = side rot
Cladosporium herbarum = Cladosporium rot (symptoms indistinguishable from side rot)
Alternaria sp. = Alternaria rot (symptoms indistinguishable from side rot)
Neofabraea alba = bull's eye rot *Neofabraea perennans* = bull's eye rot

Table 5. Effect of *Muscodor albus* "biofumigant" on decay in Bosc pears exposed at room temperature for 24 or 48 hours prior to 2 months storage at 31°F.

	Botrytis cinerea **(gray mold)**		***Penicillium expansum*** **(blue mold)**	
	Lesion diameter (mm)	**% wounds infected**	**Lesion diameter (mm)**	**% wounds infected**
48 hr. without *Muscodor*	45.9 a	100.0 a	15.7 a	100.0 a
24 hr. without *Muscodor*	32.6 b	97.8 b	9.4 b	88.9 a
48 hr. + *Muscodor*	12.8 c	51.1 c	3.4 c	33.3 b
24 hr. + *Muscodor*	5.6 d	31.1 d	0.0 d	0.0 c
P value	<0.001	<0.001	<0.001	<0.001

Table 6. Effect of *Muscodor albus* "biofumigant" on decay in Bosc pears maintained 2 months at 31°F in LifeSpan modified atmosphere packaging.

	***Botrytis cinerea* (gray mold)**		***Penicillium expansum* (blue mold)**		***Cladosporium herbarum***	
	Lesion diameter (mm)	**% wounds infected**	**Lesion diameter (mm)**	**% wounds infected**	**Lesion diameter (mm)**	**% wounds infected**
Standard liner without *Muscodor*	31.9 a	100 a	14.1 a	100 a	10.3 a	100.0 a
LifeSpan without *Muscodor*	21.1 b	100 a	12.5 b	100 a	2.2 b	30.0 b
LifeSpan + *Muscodor*	14.2 c	90 b	8.8 c	75 b	0.0 c	0.0 c
P value	<0.001	0.002	<0.001	<0.001	<0.001	<0.001

Table 7. Survival of pear postharvest pathogens on agar in petri dishes exposed to *Muscodor albus* at 68 and 31°F.

	Growth of colonies					
	Penicillium expansum		***Botrytis cinerea***		***Cladosporium herbarum***	
	Musc.	**No *Musc.***	***Musc.***	**No *Musc.***	***Musc.***	**No *Musc.***
24 h exposure at 68°F	no	yes	no	yes	no	yes
48 h exposure at 68°F	no	yes	no	yes	no	yes
24 h growth, then 24 h exposure at 68°F	no	yes	no	yes	no	yes
48 h growth, then 24 h exposure at 68°F	yes	yes	yes	yes	no	yes
72 h growth, then 24 h exposure at 68°F	yes	yes	yes	yes	yes	yes
24 h growth, then 1 wk exposure at 31°F	no	yes	no	yes	no	yes
48 h growth, then 1 wk exposure at 31°F	yes	yes	yes	yes	no	yes
72 h growth, then 1 wk exposure at 31°F	yes	yes	yes	yes	yes	yes