2004 Northwest Pear Research Review 19 February 2004
Wenatchee Convention Center

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CONTINUING PROJECT REPORT **YEAR 3/3**
WTFRC Project No: PH-02-227 **ARS Project No.** 5350-43000-003-011T

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

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.

Methods:
1. Bin storage of pears
   Cultivar: 'd'Anjou'
   A. Effect of re-application of antioxidants for control of scald:
   Treatments: Check, Ethoxyquin @ 1700 ppm (drench), DPA @ 1700 ppm (drench & thermofog) + fungicide (Pembotec), (4 replications x 8 treatments = 32 bins). Fruit to be held in CA at Stemilt (1.5% O_2 & 1.0% CO_2) until February, commercially packed and evaluated after 30, 60 and 90 days additional RA storage.

   B. Commercial bin storage:
   Treatments: Check, Ethoxyquin, Mertect and Captex (at labeled rates).
   Storage: CA with two atmospheres (1.5% O_2 & 1.0% CO_2 and 1.5% O_2 & 3.0% CO_2) 16 bins in each room. Pears are to be removed from storage and evaluated at monthly intervals starting in January.

2. MAP of 'd'Anjou' and 'Bartlett' pears. (Pallet Covers)

RA storage (only) 45 and 90 days 'Bartlett' pears; 120 and 180 days 'd'Anjou' pears
8 pallets of each cultivar.
Pallet covers to be provided by Life Span.
Fruit will be evaluated and compared to fruit stored with pallet covers in RA and CA.

3. Pear tolerance to high carbon dioxide

Requirements: 27 Small CA chambers
Oxygen concentrations: 1.5, 2.0, and 4.0%
Carbon dioxide concentration: 1, 3, and 5%
Storage time: 'd'Anjou' - 60, 120 and 180 days
'Bartlett' - 60, 90 and 120 days (harvested: 22 Aug using 3 grower lots)
'Bosc' - 60, 120 and 180 days
Pears required: 1440 pears per cultivar, or 500 pears from each of three growers for each cultivar under consideration.

4. EURO Box, Use of Ethoxyquin (will repeat for 2nd year)

Cultivar: 'd'Anjou'
Treatments: (trays impregnated with Ethoxyquin)
1. Check
2. 1000 ppm
3. 2000 ppm
4. 3000 ppm

Storage:
1. RA at 45 and 90 days
2. CA at 120 and 180 days

Cooperators: Wrap Pack and Blue Star

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 consideredl for scald control. When packing pears 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 or 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 or 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 or pears in poly bags after an additional 90 days 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: 2002-2005

Current year: 2004-2005

Project total (3 years): **$108,838**

Current year request: **$19,810**

Year	Year 1 (2002)	Year 2 (2003)	**Year 3 (2004)**
Total	$46,638	$46,500	**$19,810**
Current Year breakdown			
Salaries[1]	29,178	31,500	**13,700**
Benefits	12,460	13,500	**4,110**
Supplies	5,000	1,500	**2,000**
Miscellaneous	100		
Equipment (repair)			
Total	$46,638	$46,500	**$19,810**

[1]Salary for temporary technician (2 year term).

PUBLICATIONS:

Drake, S.R., L.G. Neven and P.G. Sanderson. 2003. Carbohydrate concentrations of apples and pears as influenced by irradiation as a quarantine treatment. J. Food Proc and Pres. 27:165-172.

Drake, S.R. and D.C. Elfving. Quality of packed and bin stored 'Anjou' pears as influenced by storage atmosphere and temperature. Accepted: J. Food Qual. April, 2003.

Drake, S.R., D.C. Elfving and P.G. Sanderson. Influence of float materials on the quality of 'd'Anjou' pears after regular and controlled atmosphere storage. Accepted: J. Food Proc and Pres. December, 2003.

Mielke, E.A. and S.R. Drake. Control of storage-related physiological disorders of 'd'Anjou' pears by integrated reduced dosage of Ethoxyquin and low oxygen treatments. Accepted: J. Food Qual. May, 2003.

Drake, S.R., E.A. Mielke and D.C. Elfving. Maturity and storage quality of 'Concorde' pears. Accepted: HortTechnology December, 2003.

Drake, S.R., D.C. Elfving, S.L. Drake and D.B. Visser. Quality of modified atmosphere packaged 'Bartlett' pears as influenced by time and type of storage. Submitted: J. Food Proc. and Pres. Sept., 2003.

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

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

Project Title: 1-MCP Interaction with Fumigants for the Control of Post Harvest Disease on Pears.

PI: Peter Sholberg, Paul Randall, 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. That was no negative interaction between hexanal and 1-MCP.

2. The quality of the d'Anjou pears were not significantly changed by being treated with hexanal and or 1-MCP at 15^0C (59 ^{o}F) for 28 hours.

Methods:
2003-2004
Half the d'Anjou pears were treated two weeks prior to harvest with a preharvest application of Vangard 75WG (Cyprodinil, a systemic fungicide, registered on grapes for the control of *Botrytis* at 6.2 g/10 litres (10oz/ac)) and no preharvest spray on the remaining pears (Check). d'Anjou pears were harvested on the day of the fumigation. The harvested fruit, was immediately placed in the fumigation chamber and air cooled to 15^0C (59^oF). The pears were then fumigated in a 1 m^3 chamber with 3 mg/l of hexanal for 24 hours or with 30 ppb (0.027g) of 1-Methylcyclopropene (1-MCP) for 4 hours or 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 boxes with top pad and lid, and placed in the cold room at 1^0C (34 ^{o}F). A fourth rep was done and used for quality analysis (fruit firmness, pH, titratable acidity (TA) and soluble solids). All the replicates will be evaluated for post harvest decay and quality at the end of January 2004.

Table 1. Description of the various treatments.

Treatment	Description
Check Pick & Cool	No pre harvest spray, pears were picked, stored in cold room at 1^0C (34^oF) immediately after harvest.
Check No Hexanal	No pre harvest spray, pears were picked, left at 15^0C (59 ^{o}F) for duration of treatments (28 hours), then placed in cold room at 1^0C (34 ^{o}F).
Check Hexanal (3 mg/l for 24 hrs).	No pre harvest spray, pears were picked, placed at 15^0C (59 ^{o}F) and treated with 3 mg/l hexanal for 24 hours, and then

	stored in cold room at 1^0C (34 ^{o}F).
Check 1-MCP (30 ppb for 4 hrs)	No preharvest spray, pears were picked, placed at 15^0C (59 ^{o}F) and treated with 1-MCP, and then stored in the cold room at 1^0C (34 ^{o}F).
Check Hexanal (3 mg/l for 24 hrs) + 1-MCP (30 ppb for 4 hrs)	No preharvest spray, pears were picked, placed at 15^0C (59 ^{o}F) and treated with 3 mg/l hexanal for 24 hours, then treated with 1-MCP for four hours, and then stored in cold room at 1^0C (34 ^{o}F).
Check 1-MCP (30 ppb for 4 hrs) + Hexanal (3 mg/l for 24 hrs)	No preharvest spray, pears were picked, placed at 15^0C (59 ^{o}F) and, treated with 1-MCP for four hours, then treated with 3 mg/l hexanal for 24 hours, and then stored in cold room at 1^0C (34 ^{o}F).
Vangard(10 oz/acre), Pick and Cool	Preharvest spray of Vangard, two weeks before harvest. Picked, boxed and placed in cold room at 1^0C (34 ^{o}F) immediately after harvest.
Vangard (10 oz/acre), No Hexanal	Preharvest spray of Vangard, two weeks before harvest. Picked, left at 15^0C (59 ^{o}F) for duration of treatments (28 hours), then placed in cold room at 1^0C (34 ^{o}F).
Vangard (10 oz/acre), Hexanal (3 mg/l for 24 hrs)	Preharvest spray of Vangard, two weeks before harvest. P picked, placed at 15^0C (59 ^{o}F) and treated with 3 mg/l hexanal for 24 hours, and then stored in cold room at 1^0C (34 ^{o}F).
Vangard (10 oz/acre), 1-MCP (30 ppb for 4 hrs)	Preharvest spray of Vangard, two weeks before harvest. Picked, placed at 15^0C (59 ^{o}F) and treated with 1-MCP, and then placed in cold room at 1^0C (34 ^{o}F).
Vangard (10 oz/acre), Hexanal (3 mg/l for 24 hrs)+ 1-MCP (30 ppb for 4 hrs)	Preharvest spray of Vangard, two weeks before harvest. Picked, placed at 15^0C (59 ^{o}F) and treated with 3 mg/l hexanal for 24 hours, then treated with 1-MCP for four hours, and then stored in cold room at 1^0C (34 ^{o}F).
Vangard (10 oz/acre), 1-MCP (30 ppb for 4 hrs) + Hexanal (3 mg/l for 24 hrs).	Preharvest spray of Vangard, two weeks before harvest. Picked, placed at 15^0C (59 ^{o}F) and, treated with 1-MCP for four hours, then treated with 3 mg/l hexanal for 24 hours, and then stored in cold room at 1^0C (34 ^{o}F).

2003/2004

Quality Analysis

From previous work (Sholberg; Randall, 2003) the optimum temperature to apply hexanal is 15^0C (59 ^{o}F) and 1-MCP is at 20^0C (68 ^{o}F) (Chen et al 2002a, 2002b). For this initial trial, a temperature of 15^0C(59 ^{o}F) was used. Does leaving the fruit at this temperature affect the overall quality of the pears during storage? A number of tests were designed to measure the quality of the pears placed in air storage at 1^0C (34 ^{o}F) after treatment.

Fruit Firmness: Using a pressure tester Model EPT-1 with a 11.7 mm tip, the various treatments were checked for fruit firmness; Brinkmann 719S Titrino was used to determine pH, and titratable acidity (TA) by titrating 15 mls of fresh juice to pH 8.2. An AO Scientific Instruments (Buffalo New York) , digital refractometer ABBE MARK II was used to determine soluble solids The various treatments were checked for these values immediately after fumigation, then 51 days later, and at 82 days. Pears flesh firmness will be tested in mid-late January 2004, before and after they have been allowed to ripen at 20^0C (68 ^{o}F) for seven days.

Headspace Analysis: Nine d' Anjou pears from each treatment, were sliced into 8 pieces using a fruit sectionizer and randomly placed into clear standard gauge cryovac bags. The bags were immediately sealed with a Swiss vac bag sealer, Type Minor 2. Each bag was previously fitted with a homemade septum consisting of a 2 cm^2 piece of yellow highway tape with a blot of Permatex blue sensor-safe gasket maker (Permatex Canada Inc, Mississauga, Ontario, Canada). The headspace was sampled one hour later using a one ml syringe and injecting the sample into the gas chromatograph. The bagged pears were repeatedly sampled at various times over the next 150 hours.

Tissue Analysis: A minimum of two pears per replicate were used. Each pear was cut into eight slices using a fruit sectionizer. A core from four slices per fruit was taken using a #4 coring tube. Five grams of tissue was added to 10 mls of 0.1M HCl. The sample was homogenized for 60 seconds using the Brinkmann Homogenizer. Five mls of the fruit slurry was placed in a 25 ml vial, and sealed. The samples were then incubated for one hour in a water bath at 60^0C (140^oF). A 1 cc headspace sample was taken using a BD 1 ml sub-Q syringe, and injected into a gas chromatograph. A standard was made using 5 µl hexanal in 10 mls 0.1 M HCl and then shaken to mix. A 0.5 ml sample was added to 4.5 ml 0.1 M HCl in a 25 ml vial. This sample was incubated for one hour at 60^0C (140 ^{o}F). A one cc headspace sample was injected into the gas chromatograph.

Results and Discussion:

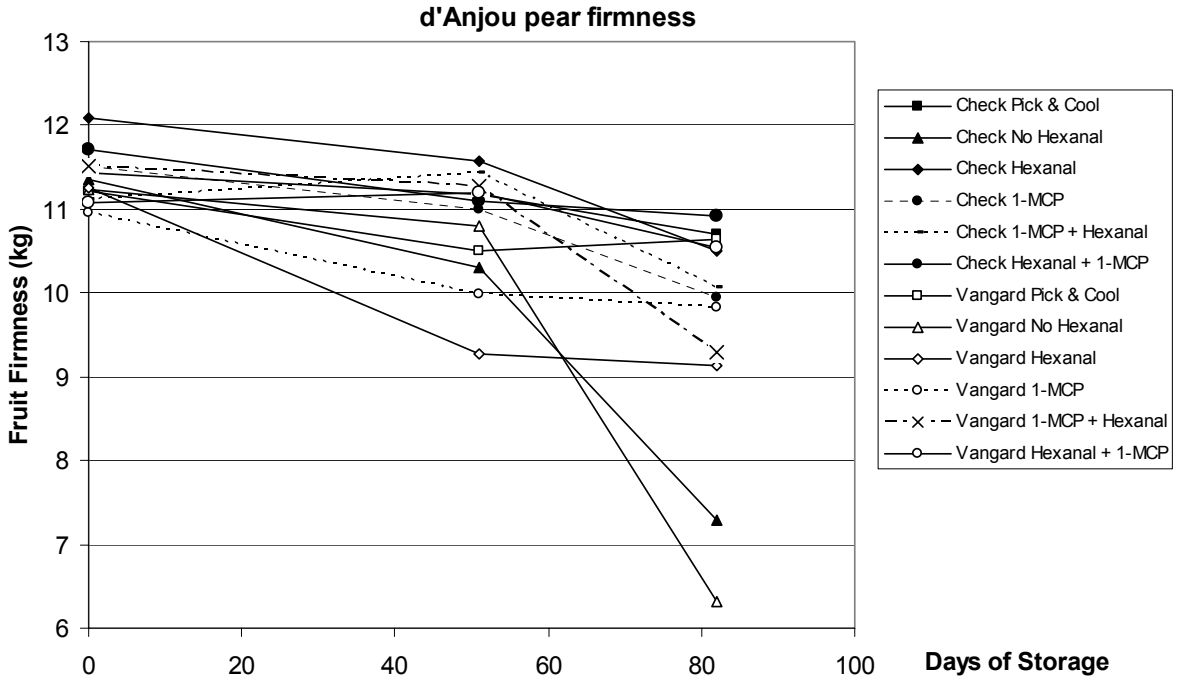


Figure 1. d'Anjou pears fruit firmness over time in air storage of 1^0C (34 ^{o}F).

The pears that remained at 15^0C (59 ^{o}F) but not treated with hexanal, 1-MCP or combination (see Table 1 for description of the various treatments) showed a decline in the fruit firmness and hence quality of the fruit. Treated d'Anjou pears had the similar firmness (see Figure 1 and Table 2) as those which were harvested and immediately placed into the cold room at 1^0C (34 ^{o}F). At 82 days the Check Hexanal and 1-MCP treated pear have the highest firmness. It is important to note that this is the result of a single replicate (Rep 4). At the end of January 2004, all four replicates will have firmness tested before and after they have been allowed to ripen at 20^0C (68 ^{o}F) for seven days.

Table 2. Anjou quality (Firmness) in kg

Treatment	Day 0	Day 51	Day 82
Check Pick & Cool	11.44	11.17	10.70
Check No Hexanal	11.35	10.30	**7.29**
Check Hexanal	12.08	11.58	10.50
Check 1-MCP	11.52	10.99	9.94
Check 1-MCP + Hexanal	11.12	11.43	10.06
Check Hexanal + 1-MCP	11.71	11.10	10.91
Vangard Pick & Cool	11.21	10.51	10.65
Vangard No Hexanal	11.23	10.80	**6.32**
Vangard Hexanal	11.25	9.28	9.14
Vangard 1-MCP	10.95	9.98	9.83
Vangard 1-MCP + Hexanal	11.52	11.28	9.30
Vangard Hexanal + 1-MCP	11.07	11.20	10.54

Headspace Analysis

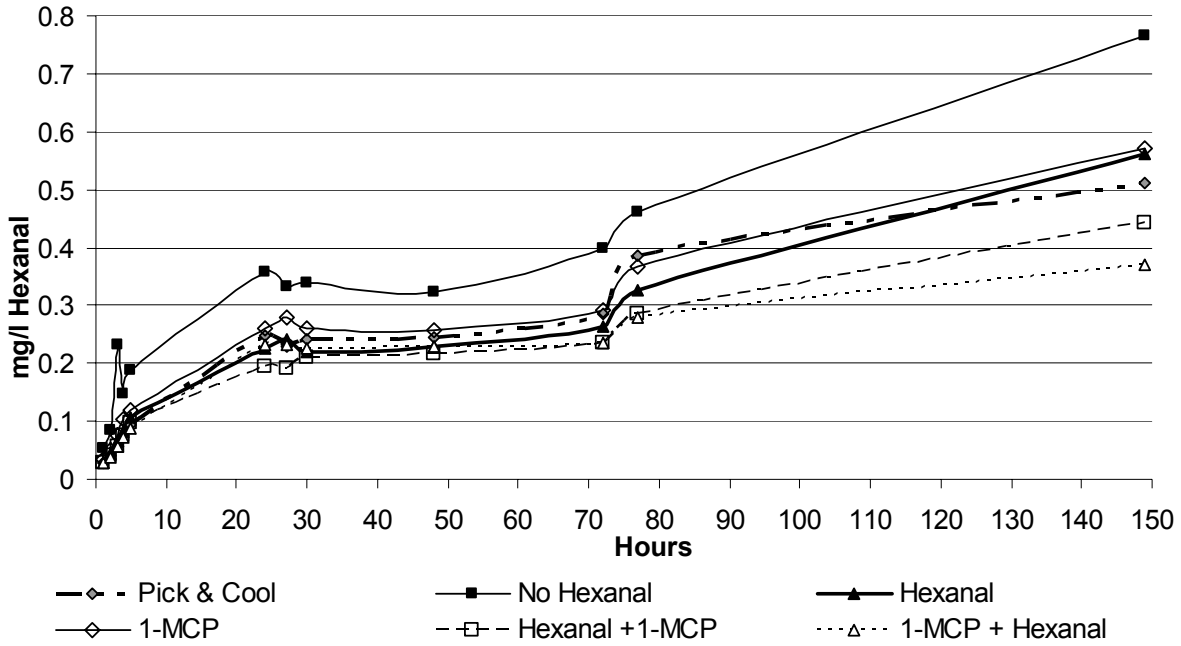


Figure 2. Quantity of hexanal (trans 2 Hexanal) released into the headspace of bagged d'Anjou pears

Figure 2 shows the amount of hexanal released from the bagged d'Anjou pears over 150 hours. The non-fumigated d'Anjou pears which had remained at 15^0C (59 ^{o}F) for 28 hours, have a higher level of hexanal than either the treated pears or those harvested and placed immediately into the cold room at 1^0C (34 ^{o}F). This maybe an indication of fruit maturity as apples which are more mature generate more volatiles (Fellman et al. 2003). The non treated d'Anjou pears were yellow in color compared to the treated pears, and were softer and juicer when sliced with the sectionizer. Note that there is not an increase of in the amount of hexanal in those samples which had been fumigated with hexanal.

Tissue Analysis

The d'Anjou pears which remained at 15^0C (59 ^{o}F) but were not treated, had a higher level of hexanal than both the pears which were immediately placed at 1^0C (34 ^{o}F) and those pears treated with hexanal, 1-MCP or combination of Hexanal and 1-MCP at 15^0C (59 ^{o}F). Furthermore, there is no indication that there are higher levels of hexanal in pears treated with hexanal at the rate used in this trial.

Table 3. The amount of hexanal in pear tissue, both those treated with and without hexanal and or 1-MCP.

Treatment	Check	Vangard
Pick & Cool	0.0361 mg/l	0.0327 mg/l
No Hexanal	**0.0608 mg/l**	**0.0505 mg/l**
Hexanal 3mg/l 24 hrs	0.0339 mg/l	0.0219 mg/l
1-MCP (30 ppb)	0.0312 mg/l	0.0335 mg/l
Hexanal & 1-MCP	0.0270 mg/l	0.0337 mg/l
1-MCP & Hexanal	0.0277 mg/l	0.0310 mg/l

The three methods used to analyze pear quality showed that the quality was not significantly changed by being treated with hexanal and or 1-MCP at 15^0C (59 ^{o}F) for 28 hours when compared to the pears which were harvested and placed immediately into cold storage (Pick & Cool).

Proposed Research for next year

Small Scale Efficacy Trials. The trials will be done at Hood River, Oregon and PARC, Summerland. Hexanal and MCP will be monitored with a gas chromatograph during fumigation of the fruit. The rates for hexanal will be based on the research we are currently conducting. The rate of MCP will be based on the recommended rates of Dr. P. Chen/ Dr Bob Spotts. Hexanal, and other suitable fumigants, will be applied before 1-MCP, with 1-MCP and after 1-MCP treatment on whole pears inoculated with various post harvest pathogens.

The pears will be evaluated as follows:

1. At one month intervals pears will be wounded and placed at 20^0C and evaluated on the control of post harvest decay.

3. After 3 months storage 1-MCP treated pears will be placed at 20^0C (68 ^{o}F), fumigated with hexanal, and evaluated one week later for ripeness.

4. Remaining pears will be evaluated for postharvest decay, quality and aroma at the end of storage period.

Budget:
Project Title: 1-MCP Interaction with Fumigants for the Control of Post Harvest Disease on Pears
PI: Paul Randall, Peter Sholberg
Project Duration: 2003-2005/6
Project Total (3 years) $40,000

Item	**Year 1** 2003-2004	**Year 2 2004-2005**	**Year 3** 2005-2006
Salary	7,000	**11,000**	11,000
Benefits	1,800	**2,700**	2,700
Materials and supplies	600	**600**	600
Travel	600	**700**	700
Total	**10,000**	**15,000**	**15,000**

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

Literature cited:

Chen, P.M., Spotts, R., Mattheis, J.P., Drake, S.R. 2002a Postharvest Physiology of Winter Pears. Presentation at Northwest Pear Research Review 4-5 Feb 2002, Yakima, WA. Pp98-103.

Chen, P., Roberts, R., Rudell, D. 2002b Management of Pear Fruit Ripening with 1-Methylcyclopropene (MCP). Presentation at Northwest Pear Research Review 4-5 Feb 2002, Yakima, WA., Pp124-128

Fellman, JK; Rudell, DR; Mattinson, DS; Mattheis JP; 2003 Relationship of harvest maturity to flavour regeneration after CA storage of 'Delicious' apples. Postharvest Biology and Technology 27: p39-51.

Sholberg, P.L., Randall, P.M. 2003. Use of Hexanal Vapor for Aroma Production and Decay Control. Progress report to Washington Tree Fruit Research Commission Winter Pear Control Committee.

CONTINUING PROJECT REPORT

Project title: Phacidiopycnis Rot of Pears
PI: Chang-Lin Xiao, Assistant Plant Pathologist
Organization: WSU-TFREC, 1100 N. Western Avenue, Wenatchee WA 98801
Cooperators: Bob Spotts, OSU Hood River; Dana Faubion, WSU Extension, Yakima

Objectives:
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.

Significant findings:
- The fruiting bodies (pycnidia) of *P. piri* containing viable spores were available throughout the pear growing season from March to September 2003.
- Fungicides Captan, Dithane, Flint, Scholar, TBZ, Vangard and Ziram at the label rate and $1/10^{th}$ label rate were effective in inhibiting spore germination of *P. piri*. Procure was effective at the label rate but less effective at lower than $1/10^{th}$ the label rate.
- Ziram applied at two 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, in comparison with the non-treated control. Elevate, Procure and Scala applied at two weeks before harvest did not provide a satisfactory control in comparison with the non-treated treatment.
- 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 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 no Phacidiopycnis stem-end rot after six months of storage, indicating that TBZ drench may be effective in reducing the infection in stems that established near harvest.
- Inoculated fruit developed more Phacidiopycnis stem-end rot as the time of TBZ application was delayed. This indicates that TBZ applied on the packing line is likely not an effective eradicant of established infections in pear stems of non-drenched fruit (fruit not treated with TBZ shortly after harvest) that had been stored for a period of time before packing.

Methods:

Inoculum availability of *P. piri* was monitored in two commercial orchards from early spring to harvest during the pear growing season in 2003. Weather data were recorded in these two orchards. At each sampling time, 10 trees were arbitrarily selected in each orchard. Ten dying or dead bark samples and 10 dying or dead fruit spurs were collected from each tree. Samples were examined for the presence of fruiting bodies (pycnidia or apothecia) of the fungus. If pycnidia or apothecia of the fungus were present, three pycnidia or apothecia were selected and crushed in sterile water. Pycnidiospores or ascospores were streaked on acidified potato-dextrose agar to test viability.

To determine susceptibility of pear fruit to infection by *P. piri* at different times during the pear growing season, pear flowers during bloom and fruit at different growth stages were inoculated with the fungus. Inoculation was performed on April 12, May 28, July 9, August 7 and September 4. At each inoculation time, at least 30 flower clusters or fruit on each of four single-replicate trees were sprayed with spore suspensions, and the same numbers of flower clusters or fruit from another four trees were treated with sterile water as controls. All fruit was harvested and stored in air at 32°F and decay development was monitored monthly, starting three months after harvest.

Eight fungicides were tested *in vitro* for their effectiveness in inhibiting spore germination of the fungus. Spore suspensions were mixed with each fungicide to adjust fungicide concentrations to five different levels in the final spore suspensions. Ten µl of each fungicide-treated spore suspension from each treatment were placed on each of three glass slides. The slides containing spore suspensions were placed on moist filter paper in plastic Petri plates and incubated at 68°F for 16 hours. Percentage of germination was determined by examining 100 spores per replicate under a microscope.

To evaluate preharvest fungicides for control of Phacidiopycnis rot originating from infection of wounds on the fruit surface, fungicide treatments including Elevate, Procure, Scala and Ziram, and a non-treated control were applied two weeks before harvest. Fruit was harvested. Thirty fruits from each replicate of each treatment were wounded and inoculated with spore suspensions of *P. piri*. Fruit was tray-packed and stored in cardboard boxes in air at 32ºF. Decay was recorded after six and eight weeks of storage.

To evaluate the efficacy of postharvest treatments with fungicides and biocontrol agents for control of Phacidiopycnis rot, surface-disinfested pear fruit was 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. Treatments were as follows:

(1) Control (Pha spore suspension only); (2) Pha + Cryptococcus
(3) Pha + Aspire (at the label rate); (4) Pha + Bio-Save 110 (at the label rate)
(5) Pha + TBZ (16 oz/100 gallons of water); (6) Pha + Scholar (12 oz/100 gallons of water)

After inoculation, fruit was 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 was 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 was 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 was kept in moist containers at room temperature overnight. Fruit was then stored in RA. Part of the inoculated fruit was treated with TBZ at 1, 10, 20, and 30 days after inoculation. Fruit was evaluated periodically for decay development for up to six months.

Results and discussion: Inoculum availability of *Phacidiopycnis piri* in the orchard.

At each sampling time during the pear growing season in 2003, all sampled trees had viable pycnidia present on either bark or fruit spurs and 67-100% trees had pycnidia of the fungus containing fresh conidia inside (Table 1). From March to September, 55-76% of the bark samples and 5-30% of the spur samples had viable pycnidia; 7-52% of sampled bark and 8-23% of sampled spurs had pycnidia containing fresh spores. The data indicate that viable inoculum of the fungus appeared to be available during the pear growing season from March 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. A second-year experiment is needed to confirm the results on inoculum availability.

Table 1. Inoculum availability of *Phacidiopycnis piri* in two commercial Anjou pear orchards.

Date	Orchard	Type of samples	% 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
6-Mar	FS	Bark	100	100	70	76	76	17	13
29-Apr	FS	Bark	100	100	100	73	66	40	7
		Spurs	100	100	90	22	19	13	0
	JW	Bark	100	100	70	55	55	20	2
		Spurs	60	60	30	11	10	6	0
10-Jun	FS	Bark	100	100	100	60	58	35	3
		Spurs	90	90	80	19	18	18	0
	JW	Bark	100	100	100	57	55	29	5
		Twig	50	50	30	5	5	3	0
21-Jul	FS	Bark	100	100	80	66	70	24	9
		Spurs	90	90	90	30	30	23	3
	JW	Bark	100	100	70	62	60	15	1
		Spurs	70	50	50	15	11	11	0
18-Sep	FS	Bark	100	100	100	67	65	52	19
		Spurs	100	90	90	32	23	17	1
	JW	Bark	100	100	56	68	68	7	14
		Spurs	67	67	67	10	10	8	0

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

In 2003, inoculation of fruit was conducted four times during the growing season. All inoculated fruit and fruit from the non-inoculated controls was harvested and stored in air at 32°F. 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 are presented in Fig. 1. The data indicate that fungicides Captan, Dithane, Flint, Scholar, TBZ, Vangard and Ziram at the label and $1/10^{th}$ label rates were effective in inhibiting spore germination of *P. piri*. Procure was effective at the label rate but less effective at lower than $1/10^{th}$ the label rate. It appears that isolates from the Wenatchee area were relatively more sensitive to TBZ and Ziram than those from the Hood River and Medford areas.

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.

Ziram significantly reduced both incidence and severity (size of the decay) of Phacidiopycnis rot that originated from infection of wounds on the fruit surface. All other fungicides did not provide a satisfactory control in comparison with the non-treated treatment in this trial (Fig. 2). Phacidiopycnis rot has the ability to spread from decayed fruit to the surrounding sound fruit in storage. Reduction of decay severity (size of the decay) by Ziram would be beneficial in reducing secondary infections during storage.

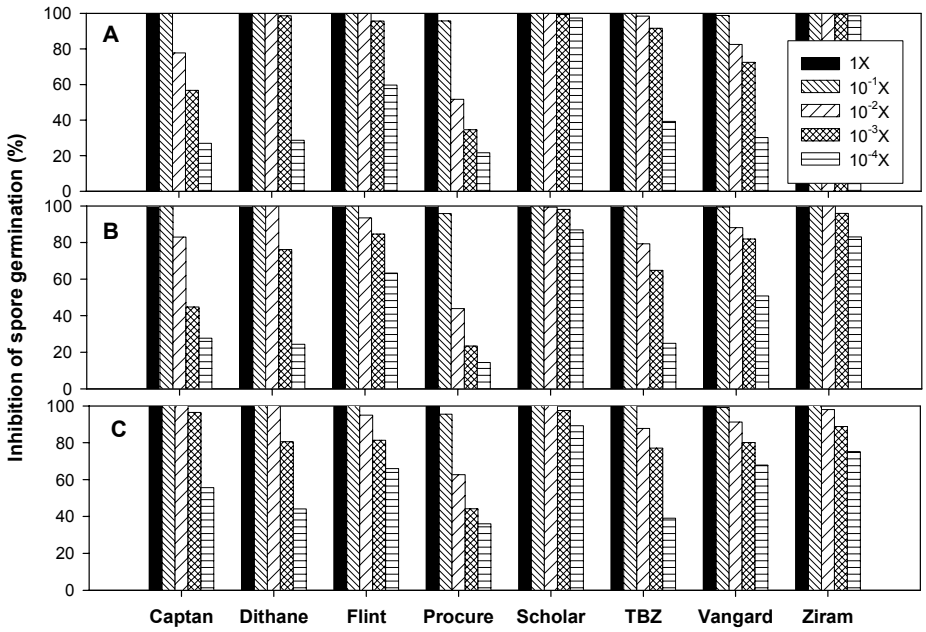


Fig. 1. Effectiveness of selected fungicides in inhibiting spore germination of *P. piri*. Five different rates (X represents the label rates) were tested for each fungicide. Data are the means of two representative isolates from each area. **A:** the Wenatchee area; **B:** the Hood River area; **C:** the Medford area.

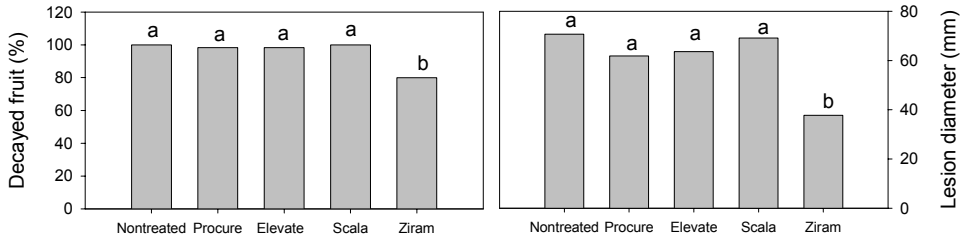


Fig. 2. Percentage of Phacidiopycnis rot and lesion diameter (decay severity) on Anjou pears that were either not treated or sprayed with Elevate, Procure, Scala and Ziram at two weeks before harvest. Fruit was wounded and inoculated at harvest with spores of the fungus *Phacidiopycnis piri* and then stored in air at 32°F for two months.

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

Two experiments were conducted in the 2003-04 storage. The first decay evaluation was done at two months after fruit inoculation. The experiments are still in progress for further decay evaluations. As of December 18, 2002, no decay developed on fruit treated with TBZ and Scholar. The biocontrol agent BioSave was also very effective (Fig. 3); *Cryptococcus laurentii* strain 87-108 reduced Phacidiopycnis rot by 50% compared with the non-treated control. Aspire was not effective in controlling Phacidiopycnis rot in this experiment. The data 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*.

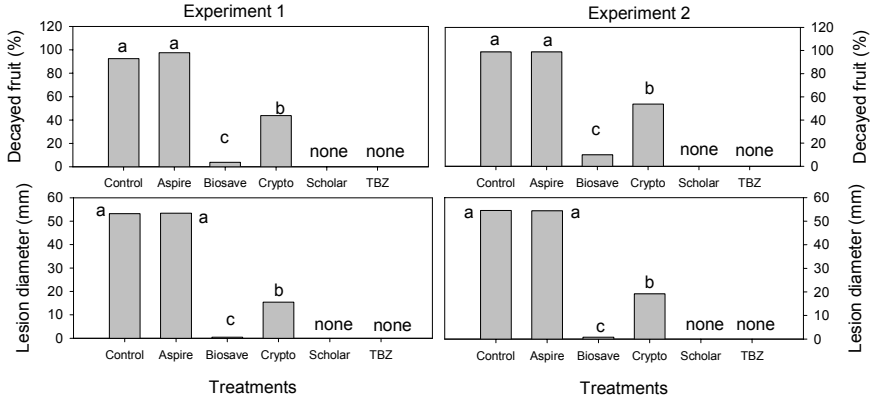


Fig. 3. Efficacy of TBZ and three biocontrol agents, the *Cryptococcus laurentii* strain 87-108 (Crypto), BioSave and Aspire in controlling Phacidiopycnis rot originating from wound infections by *Phacidiopycnis piri*.

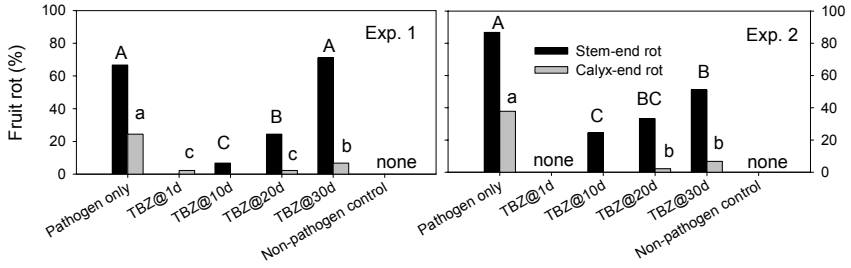


Fig. 4. Effects of timing of a postharvest treatment with thiabendazole on Phacidiopycnis stem-end and calyx-end rot. Inoculated fruit was treated with TBZ at 1, 10, 20, and 30 days after inoculation. Data represent the decay after six months of storage. Bars with different letters (uppercase for stem-end rot and lowercase for calyx-end rot) above them are significantly different.

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

The experiment was conducted in a research block. Fruit was inoculated with the pathogen at three weeks before harvest, and Ziram was applied at two weeks before harvest. All fruit was harvested and stored in RA. The experiment is still in progress for decay evaluation and results will be presented later.

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

Two experiments were conducted in the 2002-03 storage season. Inoculated fruit treated with TBZ one day after inoculation had no Phacidiopycnis rot after six months of storage. The percentage of inoculated fruit that developed stem-end rot increased as the time of TBZ application was delayed (Fig. 4). Significantly lower percentages of fruit treated with TBZ developed Phacidiopycnis calyx-end rot compared with the non-treated control. The decay incidence of calyx-end rot in the pathogen-inoculated control was relatively low in this experiment. Results on control of the calyx-end rot need to be confirmed. The data from this study suggest that a postharvest drench with Mertect (TBZ) may be effective to reduce newly established infection in stems. However, TBZ applied on the packing line is likely not effective to eradicate established infection in pear stems of non-drenched fruit that had been stored for a period of time before packing. Experiments conducted in the 2003-04 storage season are still in progress and results will be reported later.

Proposed research for 2004:

Justification:

Postharvest decay is an ongoing concern for long-term storage of winter pears. Our research has shown that Phacidiopycnis rot is an important component of storage rot in pears. Phacidiopycnis rot causes three types of decay on Anjou pears: stem-end rot, calyx-end rot, and rot originating from wound infection. Infection of pear fruit by the fungus occurs in the orchard prior to storage. Understanding of when the inoculum of the fungus is available and susceptibility of pear fruit to infection by *P. piri* in the orchard is an essential step to developing and implementing control measures. We have found that a few commercially available fungicides are effective to inhibit growth of the fungus in *in-vitro* tests. These fungicides are used to control orchard diseases. The non-target effects of selected fungicides on *P. piri* in the orchard need to be evaluated. Such information would be beneficial to the pear industry in choosing fungicides to control target diseases as well as non-target ones such as *P. piri*. Research is needed on effectiveness and timing of pre- and postharvest fungicides, including new fungicides such as pyrimethanil (Penbotec), in controlling Phacidiopycnis rot.

Objectives:

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.

Methods:

In 2004 we will continue to monitor inoculum availability in two commercial orchards and establish possible correlations between inoculum and weather variables. At each sampling time, 10 trees will be arbitrarily selected in each orchard. Ten dying or dead bark samples and 10 dying or dead fruit spurs will be collected from each tree. Samples will be examined for the presence of fruiting bodies (pycnidia or apothecia) of the fungus. If pycnidia or apothecia of the fungus are present, three pycnidia or apothecia will be selected and crushed in sterile water. Pycnidiospores or ascospores will be streaked on acidified potato-dextrose agar to test viability.

To determine seasonal susceptibility of pear fruit to infection by *P. piri* at different times during the growing season, pear flowers will be inoculated with the fungus during bloom and fruit at different growth stages will be inoculated with the fungus. Weather data will be recorded in the orchard. All fruit will be harvested and stored in air at 32˚F for decay evaluation.

An experiment will be conducted in a research block to evaluate effects of selected fungicides on establishment of *P. piri* on predisposed twigs and development of pycnidia of the fungus on infected twigs in the orchard. Second-year twigs of Anjou trees will be wounded with a coke borer, sprayed with an aerosol

tissue-freezing product at about 4-cm segments, and then inoculated with mycelium plugs of the fungus. Inoculation sites will be covered with moist cheesecloth and wrapped with Parafilm, which will be removed at two weeks after inoculation. Inoculated twigs will be treated with fungicides Dithane, Flint, Procure, Vangard, or Ziram at two and four weeks after inoculation. At six weeks after inoculation, twigs will be removed from trees and examined for presence and viability of fruiting bodies (pycnidia) of *P. piri*. Isolation of *P. piri* will also be made to determine whether the fungus survives in twigs treated with fungicides.

To evaluate effectiveness of preharvest fungicides in controlling Phacidiopycnis rot originating from stem and calyx infections, fruit will be inoculated with the fungus during the pear growing season. Part of inoculated fruit will be sprayed with Ziram (at 14 days before harvest) and the rest left without Ziram. All fruit will be harvested and stored in air at 32˚F for decay evaluation.

To evaluate preharvest fungicides for control of Phacidiopycnis rot originating from infection of wounds, fungicide treatments including Ziram applied at 14 and 5 days before harvest and a non-treated control will be applied two weeks before harvest. Fruit will be harvested. Thirty fruits from each replicate of each treatment will be wounded and inoculated with spore suspensions of *P. piri*. Fruit will be tray-packed and stored in cardboard boxes in air at 32˚F. Decay will be recorded.

To evaluate the efficacy of postharvest treatments with fungicides, including new fungicides Penbotec and Scholar, surface-disinfested pear fruit will be wounded, inoculated and treated with fungicides. After inoculation, fruit will be tray-packed and stored in air 32˚F for decay evaluation.

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.

CONTINUING PROJECT 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:

- Trees with Bartlett or Conference interstems had the smallest TCSA and the smallest canopy volume (CV). Interstemmed trees produced fewer fruit in 2002 than did the controls. Interstems did not affect total per acre yields when plant density was adjusted for tree size.

- Bartlett trees with *P. betulifolia* rootstocks produced 30% larger fruit as compared to trees with seedling Bartlett rootstocks.

- 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 produced almost twice the fruit as compared to the non-interstem control.

- Pyrodwarf, Pyro II, and OHxF 97 rootstocks had no effect on 4-year-old Bartlett, Comice, and Concorde pears.

- 3-year-old d'Anjou trees were larger with Fox 16 rootstocks as compared to 708-12 rootstocks; however, Bartlett trees with Fox 11 rootstocks were significantly larger than trees with 708-36 rootstocks. D'Anjou and Bartlett trees with 708-36 rootstocks produce twice as much fruit as trees with any other rootstock. Fruit size was not affected by either rootstock or crop load.

- 2-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. 1994 *Pyrus communis* interstems – **terminated 2003.**

b. 1994 Bartlett trial – **terminated 2003.**

c. 1996 Interstems – terminate 2005.

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

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

f. Pyrodwarf/Pyro II trial – terminate 2009

g. 2001 Fox/708 trial – terminate 2010

h. 2001 Pyronia trial – terminate 2010

i. Continue propagation of 3 Russian rootstocks for a 2005 trial – terminate 2014

j. 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.

k. Begin propagation of *P. betulifolia* (Shaanxi, China), *P. ussuriensis* (Khabarovsk, Russia), and *P. salicifolia* (Arpa Gorge, Armenia) for 2005 trial – terminate 2014.

Objective 2: Determine the fireblight sensitivity of the new rootstocks.

708-36, Fox 11, Fox 16, Pyrodwarf, Pyro 2-33, OHxF 97, OHxF 40, Betulaefolia seedling, and Winter Nellis seedling rootstocks liners were shipped to Kerneysville, WV in the spring 2001, and placed in pots in the greenhouse. D'Anjou, Bartlett, Golden Russet Bosc, and Comice scions on 708-36, Fox 11, Fox 16, Pyrodwarf, Pyro 2-33, OHxF 97, OHxF 40, Betulaefolia seedling, and Winter Nellis seedling rootstocks were shipped to Kerneysville, WV in the spring 2002, and placed in pots in the greenhouse. Rootstock liners for the 2005 and 2006 Northwest pear rootstock trial will be shipped respectively in the spring of 2005 and 2006 and planted in the greenhouse..

When the shoots on the 2001 rootstock liners were actively growing and the main leaders reached at least 20 cm in length in 2002, the plants were inoculated and arranged in a

randomized complete design with 10 blocks. The inoculum was an equal mixture of two virulent isolates, Ea273 and AFRS 554, at 2 x 10^{-8} cfu • ml^{-1} in phosphate buffer. Each tree was inoculated by cutting the unexpanded leaves, plus the top two youngest expanding leaves of a single upright leader shoot with scissors dipped in the inoculum. Following inoculation, the bench was covered with plastic sheeting for 24 hours, and humidity maintained at ~70-85% RH.

Shoot length was measured one week after inoculation, and the length of infection determined at 1, 2, 4, and 6 weeks after inoculation. Infection into 1-year old wood was included in the total lesion length. Length of lesion divided by the total current seasons shoot length equaled the proportional lesion length, which was used as the index of resistance.

The 2002 trees will be evaluated in 2003, the 2005 trees in 2006, and the 2006 trees in 2007.

Results and discussion:

1994 Interstems: In the 10-year trial of d'Anjou, Bartlett, Bosc, and Comice pears with D'Anjou, Bartlett, Bosc, or Conference interstems, cultivar and interstem induced significant cultivar by interstem interactions in tree height, tree diameter, adjusted trees per acre, and fruit weight. Generally trees with d'Anjou and Bosc interstems were largest while trees with Comice interstems were the smallest. Interstemmed trees produced fewer fruit overall than did the controls; however, when planting density was adjusted for trees size there was no significant effect on total yield.

1996 Interstems: In the 8-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.

1994 Bartlett: Trees with Betulaefolia rootstocks had a significantly larger TCSA when compared to trees with seedling Bartlett rootstocks. Trees with Betulaefolia rootstocks were taller, wider, and had a greater CV than trees with seedling Bartlett rootstocks. Bartlett trees with Betulaefolia rootstocks continue to exhibit a 30% increase in fruit size. Fruit size was larger on trees with Betulaefolia rootstocks each year during the nine years of this study. The cumulative fruit number and yield were significantly larger with Betulaefolia rootstocks, as well. Yields were not significantly different when adjustments were made for tree size (2001 data).

1999 English 708 and OH11 Rootstock Trials: In the 5-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. Fruit size was not affected by rootstock or crop load.

2000 Pyrodwarf and Pyro II Rootstock Trial: In a 4-year-old Bartlett, Comice, and Concorde trial with Pyrodwarf, Pyrodwarf II, and OHxF 97 rootstocks, rootstock did not significantly TCSA, yield or tree size.

2001 Hanner Rootstock Trial: In a 3-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 twice as much fruit as trees with any other rootstock. Fruit size was not affected by either rootstock or crop load

.*2001 Pyronia and 708-36 Rootstock Trial:* In a 3-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.

Horner Mother Block Evaluation: Liners from the first 300 of the Horner selections will be shipped from Fowler Nursery spring 2004 and established in Hood River. Additional liners were shipped to Fowler Nursery spring 2003. Several of the selections that had insufficient numbers, or labeling problems that questioned their identity, were repropagated in 2003 and will be shipped to Fowler Nursery spring 2004. Two trees of each selection will be planted beginning in 2004 in a 5' x 5' x 16' double-row planting. The remaining selections will be planted in 2005 and 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.

Fireblight: There were significant differences among clones in shoot length, lesion length, and mean proportional lesion length. Lesion length was greatest for the 1-year old Fox 11 liners and OHxF 40. Proportional lesion length, relating lesion length to shoot growth, was greatest in 708-36 and both sets of the Fox 11 liners, with most infections spreading into 1-year old wood. OHxF87 was the most resistant clone. OHxF 40 had the greatest shoot length and 708-36 had the shortest.

Budget:
Project title: Evaluation of Pear Rootstocks
PI: Eugene A. Mielke
Project duration: Long term
Current year: 2004-2005
Current request: $18,834

Item	2004-2005
Salaries and Wages	$ 8,354
OPE	4,980
Equipment	0
Supplies	5,000
Travel	500
Miscellaneous	0
Total	$ 18,834

CONTINUING PROJECT REPORT

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:

- Rootstock played no significant continuing role in tree survival in any of the plantings.
- Rootstock significantly affected root quality (number and size of roots) in the Hood River d'Anjou planting, but had no effect on growth through the second year.
- Differences in TCSA response to rootstock occurred between the two d'Anjou plantings. TCSAs were more uniform in Hood River.
- D'Anjou trees with Pyrodwarf II roots, Bosc trees with Fox 16 roots, and Bartlett trees with 708-36 roots had the smallest TCSA.
- D'Anjou trees with Pyrodwarf II roots, Bosc trees with Fox 11 and OHxF 87, and Bartlett trees with Pyrodwarf and Pyrodwarf II roots had the greatest increase in trunk growth in 2003.
- Bartlett trees with OHxF 40 roots produced more total shoot growth than trees with 708-36 or Fox 11 roots.
- Rootstock did not significantly affect D'Anjou canopy volume.

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 continued to play no significant role in tree survival in any of the plantings (data not shown). D'Anjou trees with Pyrodwarf II rootstocks had the smallest trunk cross sectional area (TCSA) (Table 1). More differences in TCSA occurred in Cashmere than in Hood River. In Cashmere trees with the OHxF rootstocks had the largest TCSA, while in Hood River trees with all rootstocks except Pyrodwarf II had the same TCSAs. The significant difference in root quality at planting in the Hood River plot did not affect the TCSA at the end of the second growing season. Bosc trees with Fox 16 rootstocks had the smallest TCSA, while trees with OHxF 87 rootstocks had the largest TCSA. It should be noted that Bosc trees with OHxF 87 rootstocks had significantly

larger TCSA than trees with OHxF 40 rootstocks. In the other three plantings there were no differences between the OHxF rootstocks. In the Yakima Bartlett planting trees with Fox 11 rootstocks had significantly larger TCSAs than did trees with either 708-36 or OHxF 40 rootstocks.

Table 1. Effect of rootstock on canopy volume and TCSA in 2-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	9.1 bc	11.57 a	11.7 b	3.4 b	-
Fox 11	10.0 b	11.22 a	9.2 c	5.4 a	-
Fox 16	8.2 c	-	5.6 d	4.6 ab	-
Pyro 2-33	7.9 c	9.06 b	10.3 bc	4.5 ab	8.6 b
Pyrodwarf	9.4 bc	12.22 a	9.2 c	4.4 ab	8.6 b
OHxF 40	12.1 a	11.97 a	9.3 c	4.3 b	-
OHxF 87	12.3 a	11.02 a	13.8 a	4.4 ab	10.3 a
OHxF 97	-	11.88 a	-	-	-
Nellis	9.1 bc	11.57 a	11.7 b	3.4 b	-

[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 produced the greatest increase in TCSA between 2002 and 2003 for the two d'Anjou plantings (Table 2). The greatest increase in TCSA in the Bosc trial occurred with either Fox 11 or OHxF 87 rootstocks. There was no consensus as to rootstock producing the least increase in TCSA between 2002 and 2003.

Table 2. Effect of rootstock on the percent growth increase in TCSA of 2-year-old trees.

	Growth Increase in TCSA (%)[z]				
Rootstock	Cashmere D'Anjou	Hood River D'Anjou	Tonasket Bosc	Yakima Bartlett	Cashmere Bartlett
708-36	238 c	141 b	373 b	96 ab	-
Fox 11	331 abc	141 b	463 a	123 ab	-
Fox 16	313 bc	-	239 e	115 ab	-
Pyro 2-33	481 a	248 a	326 bcd	131 a	169 b
Pyrodwarf	225 c	165 ab	277 cde	131 a	247 ab
OHxF 40	294 bc	158 b	361 bc	89 b	-
OHxF 87	399 ab	159 b	471 a	116 ab	289 a
OHxF 97	-	151 b	-	-	-
Nellis	238 c	141 b	373 b	96 ab	-

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

The total length of 2003 shoot growth was determined in the Yakima Bartlett planting (Table 3). Trees with OHxF 40 rootstocks had significantly more total shoot growth as compared to trees with either 708-36 or Fox 16 rootstocks.

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

Rootstock	Shoot growth[z] Yakima Bartlett (in^3)	Canopy volume Hood River d'Anjou (m^3)
708-36	495 b	0.301
Fox 11	571 ab	0.356
Fox 16	540 b	-
Pyro 2-33	580 ab	0.217
Pyrodwarf	573 ab	0.321
OHxF 40	698 a	0.355
OHxF 87	623 ab	0.332
Nellis	-	0.341

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

Canopy height, diameter, and canopy volume were determined in the Hood River d'Anjou planting. Rootstock did not significantly affect either height or diameter (data not shown) or canopy volume (Table 3). Trees with Pyrodwarf II rootstocks did have a canopy volume that was smaller than trees with the other rootstocks, however due to large variability in the young trees it was not significant.

Project Title: Northwest Pear Rootstock Trial
Co-PIs: Eugene A. Mielke, OSU – Mid-Columbia Ag Res. & Ext. Center
Project duration: Long term

Mid-Columbia Ag Res. & Ext. Center			
Salary & Wages			
Research Technician, 0.085 FTE (12 mos.)	$1,008		
Temporary labor	1,000		
OPE	851		
Sub-Total Salary and Wages		$2,859	
Service & Supplies		3,850	
Travel		500	
Sub-Total (Mid-Columbia Ag Res. & Ext. Center)			$7,209
Yakima County Coop Extension Service			
Salary & Wages			
Data (2 people, 2 days = 32 hr @ $12.50)	400		
Sub-Total Salary and Wages		400	
Service and Supplies		200	
Travel		200	
Sub-Total (Yakima County Coop Extension Service)			$ 800
Wenatchee County Coop Extension Service			
Salary & Wages		0	
Sub-Total Salary and Wages		0	
Service and Supplies		0	
Travel			
Sub-Total (Wenatchee County Co-OP Ext Service)			$ 000
Total			$8,009

CONTINUING PROJECT REPORT **YEAR 2/3**

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 production characteristics of OHxF 40, 69, 87, 97 with scion varieties Bartlett, Golden Russett Bosc and Anjou.
- Compare production using three irrigation treatments targeting soil moisture levels of 70, 80 and 90% of field capacity. For 2003, the calculated volumes (hours x application rate) were 14, 19, and 26 inches respectively, not including post harvest refilling of soil profile.

Significant findings: rootstock

For all varieties:

- OHxF 69 can have trees which lean and may need support.
- Yield, fruit size and fruit quality should be efficiently maintained over time at these densities.
- Fruit quality was assessed at harvest on each plot. There is no clear trend, except firmness and fruit size, brix, and acidity tend to fluctuate with yield rather than other factors when comparing rootstocks.

Anjou:

- In 2003, there were no significant differences due to rootstock. Production was about 40 bins per acre and peaked on 70's
- 2003 crop expressed the largest amount of cork in the trial's history. The plots ranged from 0 to 30% cork in random samples at harvest. No rootstock or irrigation treatment was found to be significantly different. All fruit was sliced across the core five times looking for cork. Samples were placed in RA storage for 90 days and evaluated again for cork. No significant differences were seen.
- Fruit size of OHxF 87 has been successfully maintained equal to fruit size of other rootstocks. It appears that to maximize the potential of OHxF 87, tree densities of 500 to 600 trees per acre (14 x 6) should be considered.
- Attention should be paid to the central leaders of OHxF 87 trees. They can crop heavily, inducing the central leader to break.

Table 1: Anjou production mean values by rootstock

	Pounds	PearNo	BoxSize	Lbs/Tree	pear / tree	Bin / A	Cumulative bins/acre	% cork	Cumulative Kg TCSA
69	1904ns	3046ns	70ns	123ns	196ns	43ns	147b	16ns	365b
87	1690	2745	72	106	172	37	168a	13	435a
97	1951	3190	72	126	206	45	152b	13	353b

Bosc:

- The best performing rootstocks are OHxF 69 and 87. OHxF 69 grew the most pounds per tree, highest number of pears per tree and the best size in 2003
- OHxF 40 continues to be a distinctive last place choice in this trial
- Fruit size was acceptable (peak size 70's) across all rootstocks.

Table 2: Bosc production mean values by rootstock

	Pounds	PearNo	BoxSize	Lbs/Tree	pear / tree	Bin / A	Cumulative bins/acre	Cumulative Kg TCSA
40	1377b	2354ns	75a	86b	147ns	41b	124b	374b
69	1714a	2739	70b	107a	171	50a	163a	486a
87	1548ab	2492	71b	97ab	156	46ab	156a	461a
97	1537ab	2519	72b	96ab	157	45ab	145ab	378b

Bartlett

- OHxF 69 is trending to be the better rootstock choice, yielding more fruit numbers, better fruit size and more pounds per tree than the other rootstocks.
- OHxF 87 consistently has about one box size smaller fruit than the other rootstocks.

Table 3: Bartlett production mean values by rootstock

	Pounds	PearNo	BoxSize	Lbs/Tree	pear / tree	Bin / A	Cumulative bins/acre	Cumulative Kg TCSA
40	1194ns	2260ns	81ns	75ns	141a	35ns	157ns	519ns
69	1472	2329	70	92	146	43	169	518
87	1224	2348	85	77	147	36	139	520
97	1175	1841	69	73	115	35	141	463

Significant findings: irrigation treatment

- In 2003, fruit size declined with volume of irrigation applied.
- Brix varied inversely with water volume applied.
- Firmness in Bosc and Bartlett also varied inversely with volume of irrigation applied.
- With excellent irrigation application efficiency, (no rain shadows or wet spots) pears may be grown successfully with less than two feet of irrigation per acre. Careful monitoring of moisture status of the orchard and visual evaluation for stress of trees would be needed to successfully complete the growing season. This would likely entail significant investment on the part of growers to change irrigation technology and engage soil moisture monitoring practices.
- Fruit size and therefore yield are very closely linked to irrigation applications. There is significant risk during hot seasons such as 2003, for severe crop impacts may occur if water is not immediately available at critical irrigation application times.

Table 4: Anjou production mean values by irrigation treatment

	Pounds	# Pears	BoxSize	Lbs/Tree	pear / tree	Bin / A	% Cork	Firmness	Brix
25	1654ns	3149ns	84a	105ns	199ns	37ns	13ns	14.8ns	14.3a
50	1552	2734	78ab	99	174	35	18	15.2	14.1a
100	1848	2993	71b	118	191	42	14	15.2	13.3b

Table 5: Bosc production mean values by irrigation treatment

	Pounds	PearNo	BoxSize	Lbs/Tree	pear / tree	Bin / A	Firmness	Brix
25	1551ns	2998a	85a	97ns	187a	46ns	16.4a	13.9a
50	1660	2937a	78b	104	184a	49	16a	12.8b
100	1544	2526b	72c	97	158b	45	15.2b	12.2c

Table 6: Bartlett production mean values by irrigation treatment

	Pounds	PearNo	BoxSize	Lbs/Tree	pear / tree	Bin / A	Firmness	Brix
25	1063b	2100b	87a	66b	131b	31b	18.4a	13.1a
50	1305a	2475a	84ab	82a	155a	38a	17.8b	12.4b
100	1266a	2194ab	76b	79a	137ab	37a	17.5b	11.9c

Objectives for 2004:

Repeat the irrigation trials and continue taking rootstock data. The budget reduction reflects no weekly fruit growth measurements in 2004.

Project title: Pear Rootstock and Deficit Irrigation Trial
PI: Tom Auvil
Current year request: $6,500
Current year: 2004
Project total 3 years: $20,460

Original budget request

Total	7560	**6500**	6400

Current year breakdown

Year	2003	**2004**	2005
Wages	6000	**5000**	5000
Benefits (%)	960	**800**	800
Equipment	100	**100**	100
Supplies	200	**200**	200
Soil & leaf analysis	300	**400**	300
Total	7560	**6500**	6400

CONTINUING PROJECT REPORT **YEAR3/3**
WTFRC Project #PR-02-228 **WSU Project #13C-3655-3298**

Project title: Branch induction in pear trees with bioregulators

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

Cooperators: Eric A. Curry, Horticulturist, USDA/ARS, TFRL, Wenatchee, WA
Dwayne Visser, Agricultural Research Technologist II, WSU-TFREC, Wenatchee

Original objectives of the project:
1. Assess potential for cyclanilide-induced shoot formation from buds on 1- and 2-year-old branch sections during the late dormant season prior to budbreak.
2. Assess potential for cyclanilide-induced shoot formation from buds on 1- and 2-year-old branch sections when natural budbreak occurs (early April).
3. Assess cyclanilide concentrations and timings for use after active shoot growth begins for enhancement of lateral branching and rapid canopy expansion in young trees in the orchard.
4. Evaluate the use of proprietary mixtures of 6-benzyladenine and gibberellins A_4 and A_7 (Promalin®) alone or in combination with cyclanilide for effects on branching under orchard conditions.

Modified or additional objectives for 2003:
1. Assess the validity of the "translocated branching effect" theory by treating trunks of trees before budbreak with high concentrations of cyclanilide.

Significant findings in 2003:
- Applications of cyclanilide to trunks during the dormant period strongly increased branching of young 'Bosc' trees the following season.
- Cyclanilide apparently translocates in pear trees from sites of application to shoot tips, where it can inhibit apical dominance.
- It may be possible to improve lateral branching in young, nonbearing pear trees with crown drenches of cyclanilide.
- Growing-season applications of cyclanilide also improve branching.
- Addition of Promalin either with or without cyclanilide does not improve branching in 'Bosc' pear trees treated during the growing season.
- Stimulation of lateral branching with cyclanilide reduces flower-bud formation.

1. Cyclanilide effects applied in fall or spring on budbreak and growth in 'Golden Russet Bosc'/OHxF97 pear (Monitor, WA): Cyclanilide at 5,000, 10,000 or 15,000 mg a.i./liter supplemented with 0.5% v/v spray oil was sprayed on lower scaffold-limb bases and the trunks of 2-year-old 'Bosc' trees in either October 2002 or early March 2003. Some spray solution ran down the trunk onto the crown and nearby soil. No sign of bud growth was present at either spray timing. Branching was rated in fall 2003. Initial budbreak of pre-existing buds was unaffected by treatment. However, when a second flush of budbreak and shoot growth took place in July, cyclanilide treatment greatly increased the number of buds developing into shoots. Most of the buds that grew into shoots were close to terminals on the first flush of shoot growth. The growth response took place up to eight months following treatment and strongly suggests that cyclanilide was translocated from the application sites to the tips of the new shoots that developed in the first flush of shoot growth in 2003.

All cyclanilide treatments produced substantial branching. There appeared to be little difference in amount of branching induced in the second flush due to concentration of applied cyclanilide.

2. Cyclanilide and Promalin effects on lateral branching in fourth-leaf 'Golden Russet Bosc'/OHxF97 pear (Cashmere, WA): On 3 June, 2003, cyclanilide (0, 5, 10 or 20 mg a.i./liter), with or without tank-mixed Promalin (250 mg a.i./liter), was applied by handgun as dilute sprays to fourth-leaf trees when newly developing terminal shoots were approximately 31 cm in length. Lateral branching was rated in fall 2003. Actively growing shoots had slowed elongation considerably at the time treatments were applied. Buds on the spring 2003 flush of shoot growth began to grow within a few weeks of the applications. Second-flush branching was increased in direct proportion to the concentration of cyclanilide applied. The inclusion of Promalin appeared to have little or no effect on the development of new branches from the second flush of shoot growth. A cyclanilide concentration of 20 mg a.i./liter appeared quite sufficient to produce a large increase in budbreak and lateral branching during the second flush. These observations confirm the prediction made in 2002 that cyclanilide concentrations below 25 mg a.i./liter would produce substantial new branch development and also showed that pears are extremely sensitive to cyclanilide.

3. Cyclanilide and Promalin® effects on lateral branching in 'Kalle' (Red Clapps)/OHxF97 pear (Cashmere, WA): On 23 May, 2002, cyclanilide (0, 25 or 50 mg a.i./liter) and/or Promalin (250 mg a.i./liter) were applied by handgun as dilute sprays to third-leaf trees when newly developing terminal shoots were approximately 25 cm in length. Cyclanilide at either concentration was equally effective in increasing the development of lateral branches on previous season's growth (2- to 3-fold increase in branches). In spring 2003, bloom data showed very clearly that flowering was reduced in direct proportion to the amount of cyclanilide applied in 2002. Again, the inclusion of Promalin in 2002 had a minor but non-significant effect on flowering in 2003, partially reflecting its relative ineffectiveness at inducing branching in 2002 at the concentration used.

Methods:
Trials were conducted in suitable commercial pear orchards in Washington. Fall and spring pre-budbreak applications were carried out by spraying cyclanilide on the bases of lower limbs and on trunks in either October 2002 or March 2003. In-season applications were made with a handgun. Single-tree plots in randomized complete-block designs were used in all the orchard trials. All treatments were applied in randomized complete-block designs to permit appropriate statistical analyses of data.

Results and discussion:
season's shoots with cyclanilide induced new lateral branches to form in the following midsummer. These observations prompted a trial that began in October 2002 with the spray treatment of trunks of young 'Golden Russet Bosc' trees to assess whether cyclanilide can be translocated from application sites to other parts of the tree, where its physiological activity can be expressed. The results of this trial in 2003 showed very clearly that cyclanilide can translocate from trunks to shoot tips and can become active up to eight months after application. The results from this trial have already led to the establishment of a new trial for 2004 in which we have duplicated and will duplicate our fall and spring application strategies with known quantities of product per tree to try to quantify this translocated response in terms of grams of cyclanilide needed per treated tree to produce a branching effect. This approach might permit pear growers to easily induce branching in non-bearing pear trees with treatments at off-peak times when other work is limited.

As might be expected, cyclanilide treatments that increase the development of new branches also appear to produce a decrease in flowering the next season. It is not possible to say with assurance that

cyclanilide is antagonistic to flowering in a direct way, since we have not observed a reduction in bloom in the absence of a positive effect on branching. The most likely reason that flowering is reduced is that, as a rule, vegetatively active buds do not produce flowers the next year. There is no evidence to suggest that cyclanilide treatment has any long-term negative effect on bloom.

Acknowledgments:
The assistance and support of the following persons and organizations are gratefully acknowledged: Jeff Cawood, Mike Cawood, Bob Gix, Daryl Harnden, Jeff Henry, Chris Ishida, Chris Olsen, Greg Rains, Dwayne Visser, Bayer Environmental Science, Valent BioSciences, Washington Tree Fruit Research Commission.

Budget:

Project title:	Branch induction in pear trees with bioregulators
PI:	Don C. Elfving
Proposed project duration:	three years (2002-2004)
Current year:	2004
Project total (three years):	$31,512
Current year request:	$ 7,500

Current year breakdown

Item	Year 1 (2002)	Year 2 (2003)	**Year 3 (2004)**
Salaries (technical)[1]	$ 4,500	$4,750	**$4,000**
Benefits (28%)	1,260	1,330	**1,120**
Wages (time-slip)[1]	2,000	2,200	**500**
Benefits (16%)	320	352	**80**
Equipment	0	0	**0**
Supplies[2]	1,500	1,600	**800**
Travel[3]	1,500	1,700	**1,000**
Miscellaneous	500	500	**0**
Total	$11,580	$12,432	**$7,500**

[1] Technical (Dwayne Visser) and time-slip help to set up trials, apply treatments and collect data as needed.

[2] This category includes a variety of miscellaneous supplies, non-capital equipment, consumables, etc. that are needed to carry out the research project. Cell phone charges are allowed.

[3] Treatment applications and frequent data collection in distant sites. Includes vehicle lease-to-purchase, operating and repair costs.

CONTINUING PROJECT REPORT **YEAR 2/3**

1. 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: Dr. Gene Mielke
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 to nurseries. 2) Nurseries are not 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 have been 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 will be sent to Fowler in February, 2004.
- Horner 4 and Horner 51 were initiated in tissue culture in July, 2003.

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.

3) **Rootstock collection.**

- 22 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.

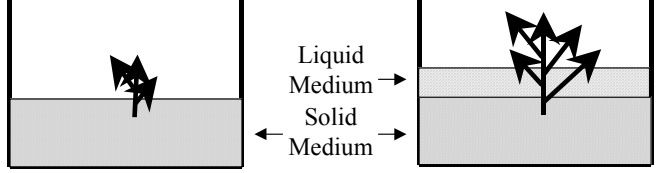


Figure 1. Single-phase vs. double-phase tissue culture.

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.** Gene Mielke is testing production characteristics of this group of over 400 open-pollinated 'Old Home x Farmingdale' seedlings. 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 is growing moderately well. 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, 2003.

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
G28.120	Horner 51

Q29857 and Q29858 are multiplying very slowly. We will re-initiate these in early 2004. Q29859 has multiplied very quickly and we are ready to begin rooting. However, release depends on certification by APHIS. This is anticipated in two to three years.

3) **Micropropagation.** As we have provided rootstock liners for testing, we have maintained a small number of each clone in culture. We now have 22 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. If they grow satisfactorily, we will send them to Hood River in Fall, 2004.

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.

Proposed schedule for 2004

1) **Liner production.** We have 22 pear rootstock clones in tissue culture (Table 1). Except for the Russian group (Q numbers) and perhaps Horner 51, we will produce 20-30 liners of each to provide an even-age set for Gene Mielke to test. The purpose is to enable a direct comparison of these rootstocks. Up to now, these clones have become available over a period of years, which prevents a controlled comparison.

The liners will be rooted in late winter in as short a period as possible. Vigor and difficulty of production varies between clones, but we will attempt to produce as uniform a set as possible.

2) **Russian series**. Q29859 has micropropagated easily, whereas Q29857 and Q29858 are multiplying very slowly. The quality of these cultures is weak. We plan to re-initiate them in culture during late winter on our most advanced medium. We will aggressively treat with double-phase culture as soon as the shoots appear free of internal contaminants. (None of these has been released by APHIS, who expect certification to take two more years.)

3) **Maintenance and research.** While waiting for plot data to provide direction for the next round of propagation and testing, we continue to test improvements of pear propagation. We continue to encounter difficult-to-propagate clones, currently Horner 51, Q29857 and Q29858. Because these are difficult, we have very few shoots for conducting experiments, so progress is slow.

Areas of testing include sets of amino acids, sugars, organic acids, vitamins and hormones that have shown promise in other difficult tissue culture situations.

Budget:

Project Title: Introduction and propagation of pear rootstocks
PI: Dr. William M. Proebsting
Project Duration: 2004-06
Current Year: 2005
Project Total: $75,554
Current year request: $25,538

Year	2004	**2005**	2006
Total	$23,896	**$25,538**	$26,110

Details

	2004	**2005**	2006
Salary, Faculty Research Assistant[1]	$11,373	**$12,299**	$12,668
OPE	6,028 (50%)	**6,764 (55%)**	6,967 (55%)
Student Wages[2]	1900	**1900**	1900
OPE ($3.12/mo.)	95	**75**	75
Services and Supplies[3]	4,000	**4,000**	4,000
Travel[4]	500	**500**	500
Total	23896	**$25,538**	$26,110

[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:
Washington State Department of Agriculture, Nursery Licensing Fee- $14,301
Oregon Hazelnut Commission- $17,000

CONTINUING PROJECT REPORT **YEAR 3/3**

TITLE: Effects of New Insecticides on Natural enemies: Acute toxicity and sublethal effects

CO-PIS Tom Unruh, USDA-ARS Yakima
Dave Horton, USDA-ARS Yakima
Dr. E. Beers, WSU, Wenatchee
Richard Hilton, OSU, Medford
Helmut Reidl, OSU, Hood River
Dr. Nick Mills, U.C., Berkeley

COLLABORATORS Vince Jones, WSU, Wenatchee
John Stark, WSU, Puyallup

OBJECTIVES (2002-3):

1. Test acute toxicity of the next 5 new insecticides to 9 arthropods using standard topical sprays
2. Develop bioassay methods to measure sub-lethal effects on beneficial insects
3. Test sub-lethal effects in those cases where acute effects are modest.
4. Model acute and sub-lethal toxicity data to provide field testable predictions of pesticide effects (New objective since 2002)

Significant Findings:

- Sublethal bioassay procedures have been finalized for 7 test species and data collected on both acute and sublethal effects for each species. These seven are: *Forficula auricularia, Chrysoperla carnea*, *Galenodromus occidentallis, Colpoclypeus florus, Deraeocoris brevis, Mastrus ridibundus* and *Anthocorus nemoralis*.
- Acute toxicity of 6 to 11 insecticides (depending on test species) was measured in replicated bioassays and there were significant differences among species in in their response profile to the insecticides.
- Sublethal bioassays were done for several low to modest toxicity insecticides with each test species and there were significant sublethal effects and obvious differences in response among beneficial species to these insecticides
- *Galendromus occidentalis*: Showed virtually no acute toxicity responses and only modest sublethal effects to Assail, Secure, Envidor and possibly Esteem.
- *Chrysoperla carnea*: Actara, Assail and Provado caused high mortality of larvae or adults but had no effect on egg hatch. Intrepid, Esteem and Success were not acutely toxic to any stage tested but had sub-lethal effects as did Provado and Actara. Intrepid reduced adult fecundity and egg hatch; Esteem prolonged development of the last larval stage. Success reduced adult fecundity.
- *Mastrus ridibundus*. This large parasitic wasp was highly susceptible to Provado Actara and Success in acute toxicity tests. It proved susceptible to the remaining insecticides in sublethal assays (i.e., Actara, Intrepid, Esteem, Success and Assail).
- ***Deraeocoris brevis****:* Assail, Agrimek and Dannitol were acutely toxic to nymphs or adults. Of these Assail had no sublethal effects, and Agrimek extended both nymph and subsequent adult development time and lowered adult fecundity and egg hatch. Success-treated (full field rate) adults laid fewer and less viable eggs. Also, egg hatch in the subsequent generation was lower. Intrepid had no sublethal effects on adults, but treating nymphs at the full rate

increased development time of 4th instars, and lowered fecundity in the subsequent generation.

- *Colpoclypeus florus*: Actara, Assail, Provado, Pyramite and Success proved acutely toxic, and both Assail andSuccess killed more that 50% at a tenth of field rates. Esteem, Azadirect and Pyramite exposure reduced female fertility in the first clutch of eggs laid but this effect disappeared in the second clutch for both Esteem and Pyramite, but not for Azadirect.
- *Anthocoris nemoralis*: Actara, Assail and Provado were acutely toxic at field rate as was Provado and Assail at 10% of field rate. At 10% of field rate, two-week measures of fecundity were decreased by Actara, Success, Intrepid and Esteem.
- *Forficula auricularia:* Provado, Guthion, and Imidan all showed acute toxicity to immatures. Success showed acute toxicity to adults after 15 days. Sublethal bioassays required 7 months from treatment to assessment and still suffer from high control mortality. Earwigs showed a sublethal response to only one insecticide: Intrepid-treated females laid fewer egg masses of smaller size, producing fewer young overall.

Methods:
Bioassay methods are idiosyncratic to the species being tested and are available in detail by request. Acute toxicities are based on topical application of formulated insecticides at field rates or 10% field rates with water controls. All applications employ a potter spray tower. The acute bioassays entail exposing the test insects to topical application and subsequently allowing them to recover, or not, in a clean environment. In the sublethal assays a topical exposure is applied to the test insects but they are then forced to behave in an environment and non-prey food sprayed with the same insecticide. Bioassays deviate by species requirements at this point.

Results and Discussion
Results are summarized in the significant findings section above and in the summary tables (1 and 2) presented below. Some of the surprises are how very different each species response profile is to the insecticides. Also, the largest arthropod and the smalles (earwigs and predatory mites) represent the 2 most tolerant species tested, probably for very different reasons. With the exception of the mite, Provado, Actara, Success, and usually Assail, proved acutely toxic. Esteem was the most likely insecticide to produce sublethal effects.

Table 1. Acute toxicity summaries for 2002 and 2003 combined

Acute Toxicity **Green -- trivial** **Yellow – caution** **Red -- beware**	Earwig *(Forficula)*; Hilton	Lace-wing s *(Chrysoperla)*; **Larvae/Adults** Mills	Predator mite (*Galendromus*); Beers	*Colpoclypeus florus*; Unruh	*Anthocoris nemoralis*; Horton	*Deraeocoris brevis*; Riedl	*Mastrus ridibundus*; Mills
Provado 1.6F	4/1	3 / 3	0	3	4	----	4
Actara 25WDG	--/--	4 / 4	0	3	3	----	4
Intrepid 2F	0/0	0 / --	0	0	0	0/0/0	0
Esteem 0.86EC	0/0	0 / --	0	0	0	0/1/0	0
Success 2SC	--/4	0 / --	0	4	0	0/0/0	4
Assail 70WP	--/1	1 / 3	1	4	4	0/3/4	0
Aza-Direct 0.0987				0			
Acramite			0				
Secure			0				
Pyramite 60W				3			
Agri-Mek 0.15EC	1/0					3/4/4/	
Guthion 50W	4/--						
Imidan 70W	4/--					2/0/0	
Dannitol	--/0					4/4/4	
Dimiln	0/0						
Mitac	--/0						

0=no effect, 1=up to 25% mortalityt, 2 = 25-50% mortality; 3= 50-75% mortality; 4 =75-100% mortality=

Table 2. Sublethal effects of various insecticides on 7 insects tested.

Sublethal effects **Green – none** **Yellow – some** **"—" not tested**	Earwig *(Forficula)*; Hilton	Lace-wing s *(Chrysoperla)*; **Larvae/Adults** Mills	Predator mite (*Galendromus*); Beers	*Colpoclypeus florus*; Unruh	*Anthocoris nemoralis*; Horton	*Deraeocoris brevis*; Riedl	*Mastrus ridibundus*; Mills
Provado 1.6F	0	2/ 0	0	---	--	---	--
Actara 25WDG	---	2 / --	1	---	1	---	2
Intrepid 2F	1	0 / --	0	0	1	1/0	1
Esteem 0.86EC	0	1 / --	1	1	1	---	1
Success 2SC	---	0 / 2	0	0	1	0/2	2
Assail 70WP	0	2 / 0	0	---	---	0/0	2
Aza-Direct 0.0987				1			
Acramite			0				
Secure			1				
Envidor			1				
Pyramite 60W				1			
Agri-Mek 0.15EC	0					0/2	
Guthion 50W							
Imidan 70W							
Dannitol	0						
Dimiln	0						
Mitac	0						

0=no effect, 1=minor effect, 2=large effect (dramatically affects life history) – did not survive well enough to test

Proposed schedule of accomplishments:

Bioassay development is complete. We plan to complete all objective of the proposal in the final year, with year 3 emphasizing the effect of sublethal exposures to key materials, acute and sublethal testing for some materials not examined (specific to each species). Also, several sites will be testing the predicted effect of insecticide sprays on field populations of predators or parasitoids.

BUDGET:

TITLE:	Effects of New Insecticides on Natural enemies: Acute toxicity and sublethal effects
CO-PIS	Tom Unruh
Proposed Project Duration:	**3 years**
Project total:	**$150,000**
Current year request	2003: $50,000

Budget:

Item	2002[1]	2003[1]	**2004[1]**
Salaries	45,000	45,000	**45,000**
benefits	5,000	5,000	**5,000**
IFAFS Matching	86,000	86,000	**0**
Total (WRFRC)	50,000	50,000	**50,000**

[1] $10,000 per location; and funds should be sent to 5 locations as in 2003.

$15,000 charged to Pear commodity

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

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 very good.
- Spider mite and grape mealybug densities were low.
- Codling moth control was very good, despite significant pressure.
- Organic pest management is limited by the control of pear rust mite.
- Natural enemy populations did not increase significantly until late season.
- Costs were variable, and differences in programs were not statistically significant.
- Communication of monitoring data was increased via the web.
  - Sampling frequency, area, and precision were increased.

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 the 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.).

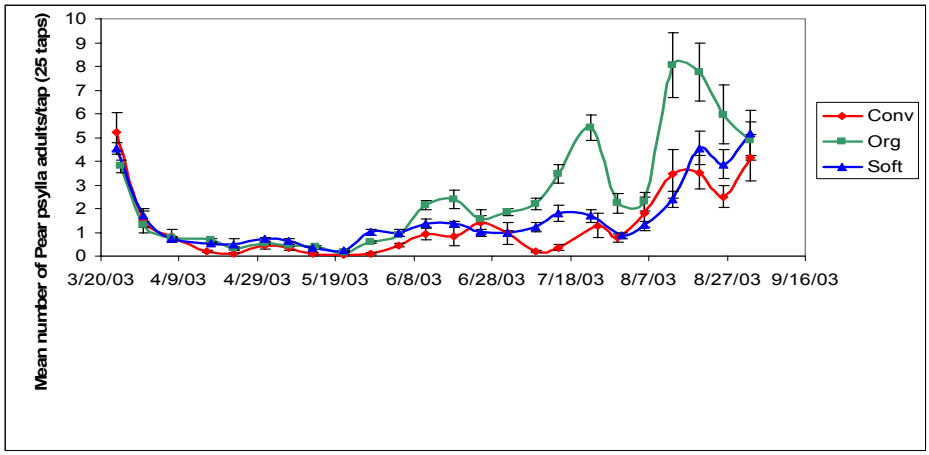
Project growers provided their spray records for the season at harvest. These records were used to verify the assignment of blocks into the three management categories. Spray records will also be used

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, a 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:

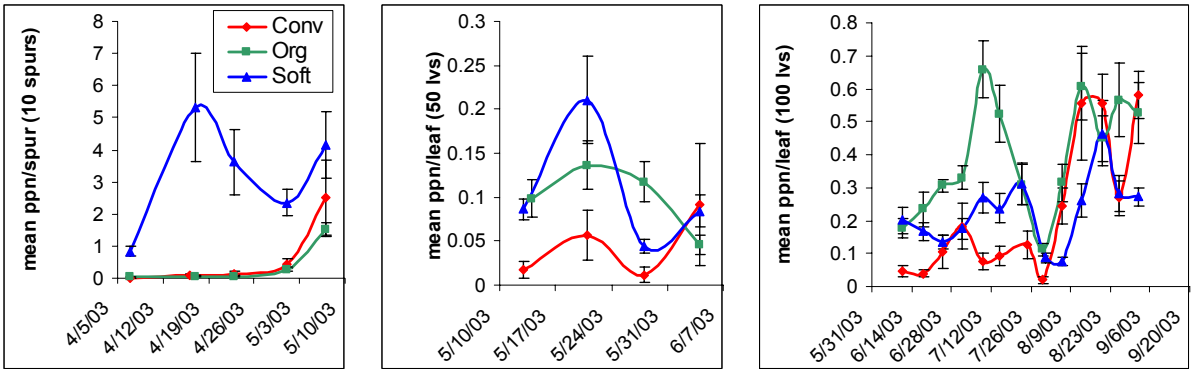
Results from the second year of implementation (first year as a funded project) of areawide organic and soft pest management were positive. Densities of pear psylla, historically the key pest in the Peshastin Creek area, were much lower in all three programs in 2003 relative to 2002. Over the entire 2003 season, there were significantly more pear psylla adults in Organic blocks than Conventional blocks, while Soft blocks were not significantly different from the others (Fig. 1). No differences occurred in the early season, while psylla densities in the Organic were significantly higher than Conventional in the mid- and late seasons.

Fig. 1. Adult pear psylla densities, 2003.



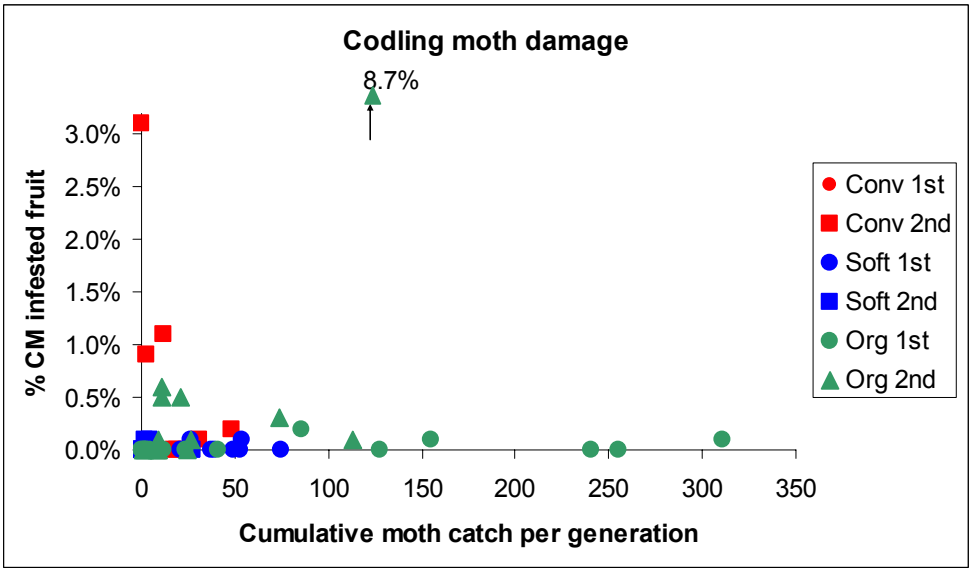
Densities of pear psylla nymphs were higher in the Organic blocks than Soft blocks in the early season, although neither was different from Conventional blocks (Fig. 2a). There were no significant differences for nymphs mid-season (Fig. 2b), while the Organic had significantly more nymphs than the Soft program in late season (Fig. 2c). No differences in egg densities occurred throughout the season.

Fig. 2. Pear psylla nymph densities, 2003. a. early season; b. midseason; c. late season.



While codling moth pressure was higher than expected, control was successful throughout the project (Fig. 3). The codling moth trap catch for the overwintering generation (first flight) was extremely variable within the project, with the cumulative catch for the flight ranging from 0 to 310 moths per block. Damage from codling moth was very low in the first generation, even in blocks with very high trap catch; control was provided primarily by spinosad (Entrust) and granulosis virus (Cyd-X), in combination with mating disruption. Because of the excellent control, those blocks with high first flight trap catch saw greatly reduced second flight. Two blocks, one Organic and one Conventional, did have significant damage at harvest. The Organic block did not use adequate management tactics in either generation, leading to the damage. The Conventional block with 3% fruit injury was involved in a test using sprayable fibers for mating disruption, which may have interfered with the ability of the traps (10X) to adequately monitor the codling moth population. However, no statistical differences in damage occurred between programs.

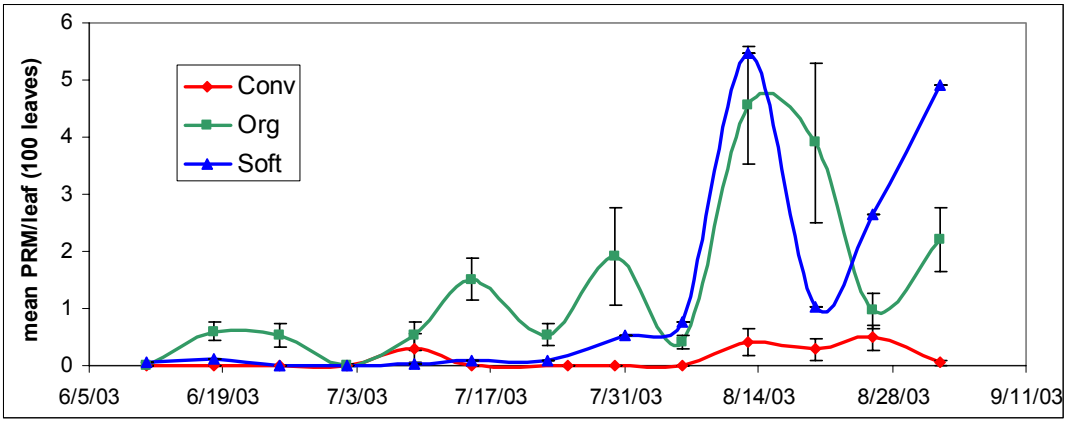
Fig. 3. The relationship between codling moth trap catch (for each program and for each generation) and codling moth fruit infestation.



Twospotted spider mites were very low throughout the Peshastin Creek Valley and did not become a problem. Grape mealybug was also very low in 2003. Pear rust mite was problematic in the Organic and Soft programs; however, there were no differences between management types (Fig. 4).

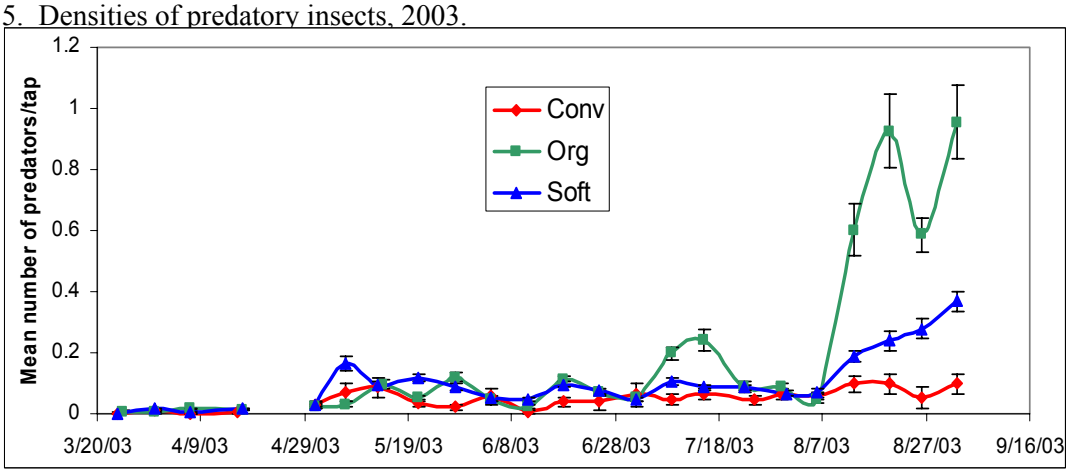
In the late season, pear rust mite increased in both Organic and Soft programs. One Organic orchard (three sample blocks) did sustain economic pear rust mite damage throughout the block; the grower had been unable to apply adequate prebloom control measures (sulfur). This orchard will not be organic in 2004, demonstrating both the potential for long-term economic damage from this pest, and the need for organic growers to be vigilant in managing it.

Fig. 4. Densities of pear rust mite from leaf samples, late season 2003.



Predatory insect densities were low throughout the season in the Conventional program (Fig. 5). Densities were also low in the Soft and Organic blocks; however, there was a significant increase in late season predator populations. This increase followed the late season increase in pear psylla in those programs.

Fig. 5. Densities of predatory insects, 2003.



Program costs for 2003 are still being collected and analyzed; however, the preliminary analysis finds no differences between program costs. For 2002, there were no significant differences between programs ($p=0.74$), due to the variation in pest management expenses within programs. There were again no differences for 2003 costs ($p=0.49$), although in general the organic programs did spend more than in 2002 (likely due to the increased codling moth pressure and the use of spinosad).

The use of the internet for communicating monitoring results and recommendations worked very well. This method proved particularly effective in giving areawide results and was favored over a bulletin board that was placed in the middle of the project. In particular, timely communication of

trap catch and degree-day accumulations allowed for better application of codling moth controls. Nevertheless, the bulletin board will remain at the Peshastin Creek Growers Association, for both ease of access and public information and education.

In summary, the implementation of organic and near-organic pest management on an areawide basis has been successful, relative to conventional programs, for two 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.

Budget:
Project title: Development of areawide organic insect pest management in pear orchards
PI: John Dunley
Project duration: 2003-2005
Current year: 2004
Project total (3 years): $80,624
Current year request: $27,277

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)
Salaries[1]	8,150	**9,155**	9,210
Benefits (27%)	2,200	**2,472**	2,487
Wages[2]	11,000	**11,000**	11,000
Benefits (16%)	1,760	**1,760**	1,760
Supplies[3]	1,350	**1,350**	1,350
Travel[4]	1,540	**1,540**	1,540
Total	26,000	**27,277**	27,347

[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.

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project #PR-03-342 WSU Project #13C-3643-5385

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,

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.
- 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.

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. 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. 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.

Insect (pest and predator) samples were collected weekly by beating tray method from each transect point. Sampling began in late April and continued into September, with the final two samples at two

and three-week intervals. Beginning with the 12 June sample, all insects from the vegetation, and all unknown insects from the orchard, were collected and brought to the lab for identification. Sampling was conducted at night at two sites.

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 techniques were examined in a WSU-TFREC experimental orchard to validate the methods before placing them in the Peshastin Creek Areawide Organic Project; subsequently, trials were established at four of the nine transects in the project area. For sentinel pear psylla, pear shoots infested by pear psylla were collected from the TFREC experimental orchard and were trimmed into sections with one to three leaves. Under 20x magnification in the lab, we removed pear psylla eggs and nymphs to leave a population of only ten nymphs. The remaining nymphs were all between the first and third instars. The proximal ends of the resulting shoots were placed in small floral tubes with water. Small vials affixed to pear tree limbs held the floral tubes in the canopy (1.5 m high on a main scaffold) at each of the sample points in the orchard, and three-foot stakes with wire hoops on them held the cages suspended in the vegetation at points in the surrounding vegetation. Four repetitions of paired shoots—caged (excluding predators) and open (allowing predation)—were arrayed at each sample point. For open cages, the cloth sleeve cage was affixed adjacent to the shoot. After one to four days in the field the shoots and cages were brought back to the lab and psylla nymphs were re-counted.

Sentinel prey were also established using clean pear leaves from greenhouse cuttings, which were artificially infested either by hand-transferring psylla nymphs from orchard shoots or by inoculating with psylla eggs by caging females with the shoots. These shoots were caged and placed in the orchards in the same way as the naturally infested shoots.

Sitotroga eggs, a commonly used food for rearing predatory insects in the lab, were also tested as substitutes for pear psylla in later trials. Frozen Sitotroga eggs were purchased from Rincon-Vitova, Visalia, CA. A dilute 1:10 solution of mucilage was used to glue 20 flash-frozen eggs onto 1.5 x 4.5 cm tags of card stock. The cards were then caged and deployed in the same manner as the shoots.

Results and discussion:

In a preliminary analysis of data from the first year of monitoring along transects some differences were found, as expected. However, the mean densities of predators over the season in each of the management environments were not significantly different between programs (Fig. 1). This is likely due to the within-season variation in predator density; great fluctuations in density occurred through the 18 weeks of sampling, with particular increases in density late in the season (Fig 2).

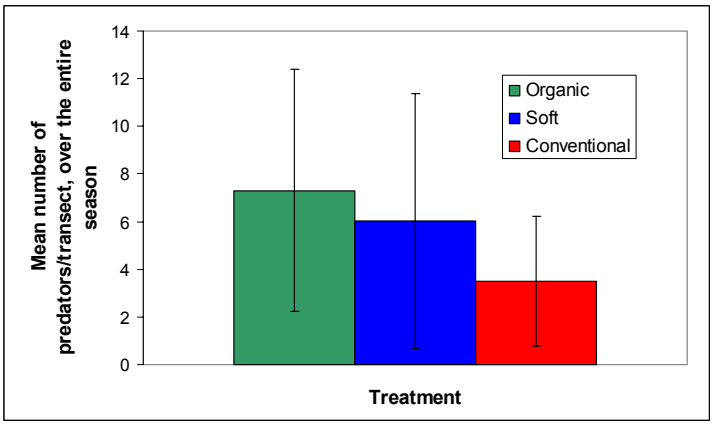
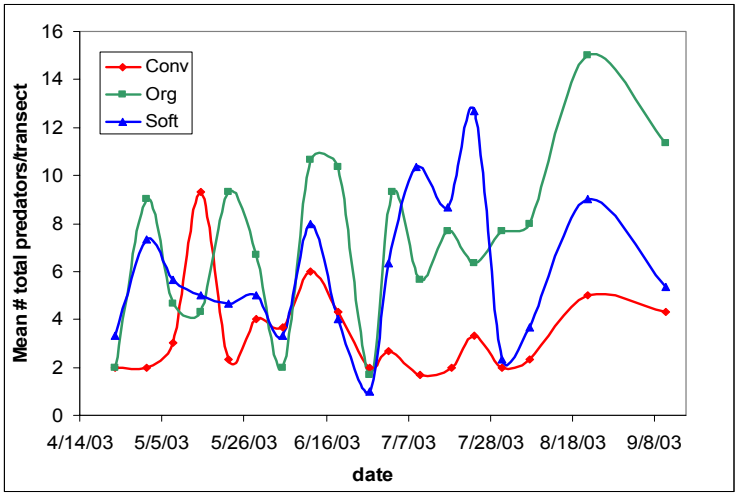


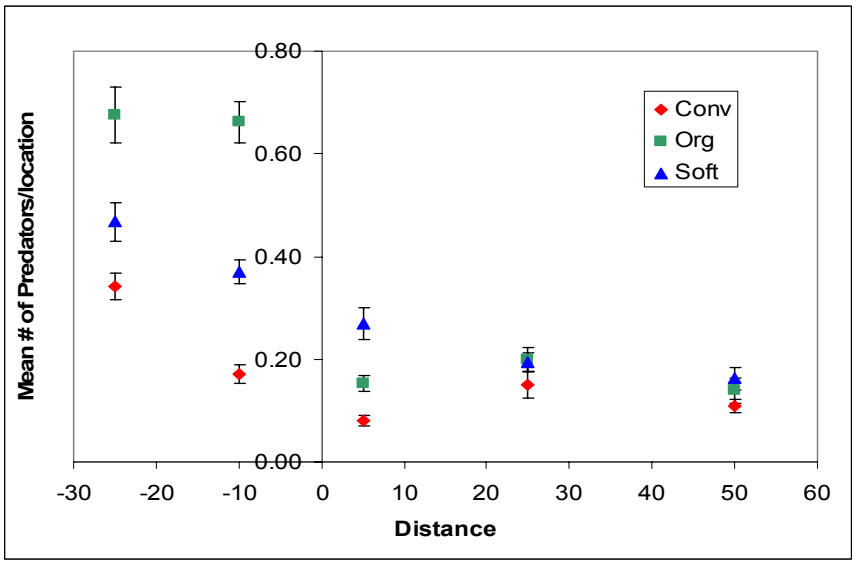
Fig. 1. Mean density of predators along transects in three different pest management programs over the 2003 season.

Fig. 2. Mean density of predators in weekly samples along transects in three different pest management programs.



Distance along the transects also had significant effects on predator density (Fig 3). Predator densities were highest in surrounding vegetation. For Organic orchards, predator populations were higher 25 m into the surrounding habitat than the other programs and did not decrease 10 m from the orchard border. Alternatively, the predator populations in vegetation surrounding Conventional orchards were reduced compared to Organic and declined in the samples closer to the orchards. Soft orchards were found to have a trend intermediate to the Organic and Conventional orchards. There were no differences in predator populations within orchards, however, Soft and Organic orchards had more predators 5 m from the border into the orchards. Overall border effects were strongest in Organic orchards (which had the highest native-vegetation predator densities) and Conventional (which had the lowest in-orchard densities at 5 m), while the decline in populations moving into the orchard was less sharply evident in the Soft program.

Fig. 3. Mean density of predators along transects in three different pest management programs. Transects originate 25 m outside of orchards and terminate 50 m into orchards.



Because this experiment takes place within the Peshastin Creek Areawide Organic Project, comparisons can be made with predator densities, sampled weekly from 41 sites (17 Organic, 19 Soft, and 5 Conventional) within the region. Fluctuations in predator densities were moderated when observed over the entire region, and the increase in predator densities in the late season is quite marked (Fig. 4). The late-season increase was greatest in the Organic blocks, but there were also increases in Soft blocks. Pear psylla densities also rose at this time (Fig. 5).

Fig. 4. Predator densities in the Peshastin Creek Areawide Organic Project, 2003.

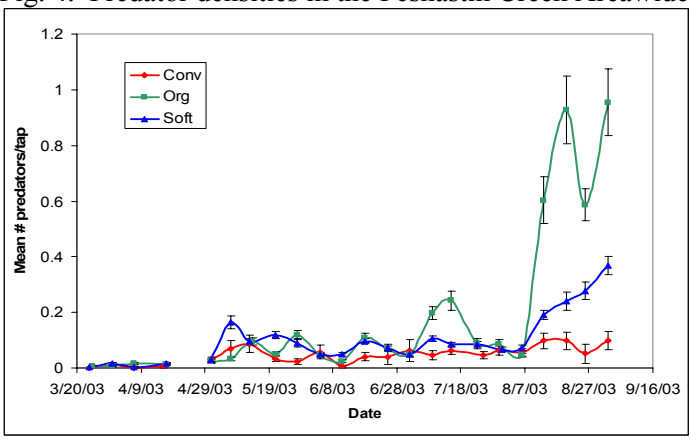
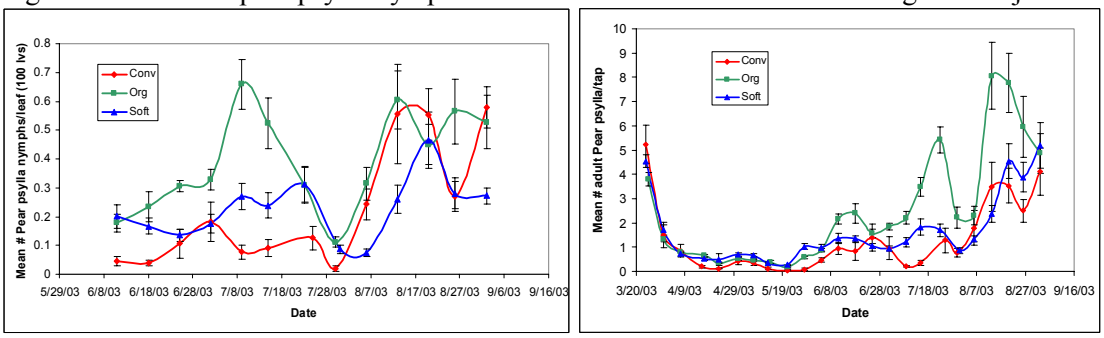


Fig. 5. Densities of pear psylla nymphs and adults in the Peshastin Creek Organic Project.



Results from the sentinel prey trials were preliminary and were used to develop the methodologies that will be used in 2004. Several limitations to the technique were identified, and the methods were modified to overcome them. For example, several time periods of exposure to predation were examined for using pear psylla nymphs on shoots. Even a short time period is difficult during hot weather; the shoots quickly dehydrate, and the nymphs leave the leaves or die. Shoots with nymphs will be used only in the early season in 2004, along with Sitotroga eggs. The relationship between predation on pear psylla nymphs and on Sitotroga eggs will then be examined, and the Sitotroga eggs will possibly be used as surrogate prey. This comparison will also be conducted in the early season using *Deraeocoris brevis* and *Campylomma verbasci* in the laboratory, in both no-choice and choice tests.

The monitoring along transects will be continued at the same sites in 2004. Additionally, the sentinel prey (both pear psylla nymphs and Sitotroga eggs) will be placed at the sampling distances along transects. The presence and impact of biological control will be better quantified following a second year of study.

Budget:
Project title: Biological control in areawide organic and "super-soft" pear orchards
PI: John E. Dunley
Project duration: 2003-2005
Current year: 2004
Project total (3 years): $98,270
Current year request: $32,454

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)
Salaries[1]	16,300	**18,310**	19,775
Benefits (27%)	4,401	**4,944**	5,340
Wages[2]	6,000	**6,000**	6,000
Benefits (16%)	960	**960**	960
Supplies[3]	2,300	**700**	700
Travel[4]	1,540	**1,540**	1,540
Total	31,501	**32,454**	34,315

[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, optivisors. Cell phone charges are allowed under this grant.
[4] Travel: local travel to research plots only.

CONTINUING PROJECT REPORT

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

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

Objectives:
In 2003:

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.

Proposed In 2004 (all ongoing):

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.

Significant findings:

- A survey of commercial d'Anjou pear fruit orchards in four growing regions of the Pacific Northwest was conducted to estimate the incidence of contamination of fruit with indigenous *Erwinia amylovora*. One thousand fruit were sampled from 10 apparently disease-free orchards. Bacteria (e.g., *Pseudomonas* and *Pantoea* spp.) were recovered occasionally from fruit washings, but *E. amylovora* was not recovered from any commercial sample.

- We monitored survival of *Erwinia amylovora* on developing pear and apple fruits in orchards. Method of inoculum production represented treatments: cells fresh from solidified medium, freeze-dried cells, and cells in air-dried ooze from diseased fruits. Immediately after spraying, *E. amylovora* was recovered from 64% of fruit (n = 420) at populations of 10^3 to 10^5 CFU/fruit. Incidence of recovery and population size declined rapidly with time regardless of fruit species or method of inoculum production. At 7 and 14 days after inoculation, recovery of *E. amylovora* declined to 6 and 1 % of sampled fruit, respectively. At 35 days, a total of 8 CFU of *E. amylovora* on two fruits were recovered from the 330 sampled. Fire blight symptoms were never observed on inoculated trees or fruit.

Justification: Export of winter pears grown in the Pacific Northwest into countries where fire blight does not occur can be 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 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 project is to investigate if the first two reasons cited above that contribute to unlikely movement of *Erwinia amylovora* via apple fruit also hold true for pear. The results expected to form the basis of a risk assessment analysis for evaluating the potential for introduction of fire blight into a new geographic region via shipments of commercial pear fruit.

Methods:

Objective 1. Three d'Anjou pear orchards in the areas surrounding Wenatchee and Yakima, Washington and Hood River, Oregon, and one d'Anjou pear orchard in Medford, Oregon, were surveyed. One hundred fruit were sampled from each orchard within one week of commercial harvest. Twenty-five trees were selected randomly within each orchard. No visible fire blight infections were present for each selected tree and its surrounding (adjacent within and between row) trees. Four healthy fruit (without visible symptoms of fire blight or other disorders) and located at a height between four to six feet from the orchard floor were selected from each tree. 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. Individual fruit were washed within each re-sealable bag. 50 ml of sterile buffer (10 mM phosphate buffer, pH 7.1) was added to each bag followed by vigorous massage and sonication for 3 minutes. A 250 µl sample of the sonicated fruit wash was spread onto Miller-Schroth medium and incubated at 25°C. The remaining sonicate was filtered through 0.2 µm membranes; each membrane was then incubated on Miller-Schroth medium for differential recovery of the pathogen. Positive (*E. amylovora* 153N) and negative controls (buffer) were processed simultaneously to ensure quality of the isolation protocol. Colonies were counted after three days of growth on Miller- Schroth medium. Bacterial colonies resembling *E. amylovora* onto Miller-Schroth were subjected to further evaluations: colony morphology on three media, ability to produce ooze on immature pear fruit, lack of ability to fluoresce under ultraviolet light, and analysis by GC-FAME.

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. Method of inoculum production represented treatments: cells fresh from solidified medium, freeze-dried cells, and cells in air-dried ooze from diseased fruits. The isolates of the pathogen was *E. amylovora* strain 153N ("N" denotes resistance to nalidixic acid); wild-type parental strain Ea153 also was inoculated in several trials. Developing fruit were sprayed to near-run off with inocula suspended in water at 10^5 and 10^7 CFU/ml at three separate times on three replicate trees per treatment during the summer (June, July, and August, 2003). Three fruit from each tree (15 total per treatment per inoculation timing) were harvested at 1 hour, and 3, 7, 14, and 35 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.

Results and discussion:

Objective 1. One thousand fruit were sampled from 10 orchards located in the Wenatchee, Yakima, Hood River and Medford production areas of Washington and Oregon. These orchards were apparently disease-free; that is, no fire blight was detected in the 2003 pear production season and no visible symptoms of fire blight were detected on any tree surveyed. 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 contamination of d'Anjou pear fruit with *Erwinia amylovora* from orchards in 2003.

Orchard location[x]	Harvest date	No. fruit	Control Ea153N	*Erwinia amylovora*	*Pseudomonas* spp.[z]	Other spp.
Hood River, OR	9/08	100	2/2 [z]	0%	10%	100%
Hood River, OR	9/08	100	2/2	0	9	100
Hood River, OR	9/08	100	2/2	0	2	100
Yakima, WA	9/10	100	2/2	0	5	100
Yakima, WA	9/10	100	2/2	0	2	100
Yakima, WA	9/10	100	2/2	0	4	100
Medford, OR	9/15	100	2/2	0	6	100
Wenatchee, WA	9/17	100	2/2	0	9	100
Wenatchee, WA	9/17	100	2/2	0	6	100
Wenatchee, WA	9/17	100	2/2	0	7	100

[y]Orchard fruits were randomly sampled at each location; 100 fruit from each commercial orchard.
[y] Number positive for the detection *Erwinia amylovora* 153N in two control samples processed with the 100 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 cells harvested fresh from media, freeze-dried cells, or cells in air-dried ooze

Trees used in the study were large, averaging 50 to 200 fruit per tree. During the experiments, daily maximum temperature averaged 88ºF (max. temp. 105ºF). Symptoms of fire blight on fruit were not detected on any inoculated, experimental tree.

Immediately after spraying, *E. amylovora* was recovered from 64% of fruit (n = 420) at populations of 10^3 to 10^5 CFU/fruit. Incidence of recovery and population size declined rapidly with time regardless of fruit species or method of inoculum production. At 7 and 14 days after inoculation, recovery of *E. amylovora* declined to 6 and 1 % of sampled fruit, respectively. At 35 days, a total of 8 CFU of *E. amylovora* on two fruits were recovered from the 330 sampled (Tables 1-3).

Table 1. Population of recovered *Erwinia amylovora* from inoculated d'Anjou pear fruit in 2003

Average colony forming units[x]							
			Days after inoculation				
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	6/25 to7/30	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^3	6/25 to7/30	9.4×10^3	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^5	6/25 to 7/30	5.8×10^4	1.3×10^2	4.0×10^1	0.0	0.0
Check	-	7/21 to 8/28	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^5	7/21 to 8/28	2.2×10^3	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/21 to 8/28	1.6×10^4	0.0	0.0	0.0	0.0
Ea153N Fresh	10^5	7/21 to 8/28	0.0	0.0	0.0	0.0	0.0
Ea153N Ooze	10^5	7/21 to 8/28	4.3×10^3	2.1×10^1	0.0	0.0	0.0
Ea153 Freeze-dried	10^5	7/21 to 8/28	4.6×10^3	0.0	0.6×10^0	0.0	0.0
Ea153 Fresh	10^5	7/21 to 8/28	5.5×10^3	0.0	0.0	0.0	0.0
Check	-	8/22 to 9/26	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^6	8/22 to 9/26	1.6×10^5	5.6×10^3	0.0	0.0	0.0
Ea153N Fresh	10^6	8/22 to 9/26	1.9×10^5	1.2×10^4	9.3×10^1	2.6×10^0	0.0
Ea153N Ooze	10^6	8/22 to 9/26	1.6×10^5	1.6×10^4	0.0	0.5×10^0	0.6×10^0

[x]Average colony forming units are represented as the mean of 15 fruit per treatment.
[y]Treatments include *Erwinia amylovora* 153N and *Erwinia amylovora* 153 wild-ype inoculated onto fruit as freeze-dried cells, harvested fresh cells, or air-dried ooze cells suspended in water.
[z]Colony forming units (CFU) of inoculum concentration applied to fruit.

Table 2. Population of recovered *Erwinia amylovora* from inoculated Bosc pear fruit in 2003

Average colony forming units[x]							
			Days after inoculation				
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	6/23 to7/28	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^3	6/23 to7/28	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^5	6/23 to 7/28	0.0	0.0	0.0	0.0	0.0
Check	-	7/21 to 8/25	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^5	7/21 to 8/25	1.2×10^2	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/21 to 8/25	1.3×10^3	0.0	0.0	0.0	0.0
Ea153N Fresh	10^5	7/21 to 8/25	0.0	0.0	0.0	0.0	0.0
Ea153N Ooze	10^5	7/21 to 8/25	2.7×10^3	4.9×10^3	1.0×10^3	0.0	0.0
Ea153 Freeze-dried	10^5	7/21 to 8/25	0.0	0.0	0.0	0.0	0.0
Ea153 Fresh	10^5	7/21 to 8/25	2.0×10^2	0.0	0.0	0.0	0.0
Check	-	8/21 to 9/25	0.0	0.0	0.0	0.0	NT[z]
Ea153N Freeze-dried	10^6	8/21 to 9/25	1.6×10^5	4.0×10^3	1.7×10^1	0.0	NT
Ea153N Fresh	10^6	8/21 to 9/25	8.9×10^4	1.6×10^4	6.0×10^1	0.0	NT
Ea153N Ooze	10^6	8/21 to 9/25	4.0×10^4	4.5×10^1	2.0×10^1	0.0	NT

[w]Average colony forming units are represented as the mean of 15 fruit per treatment.
[x]Treatments include *Erwinia amylovora* 153N and *Erwinia amylovora* 153 wild-type inoculated onto fruit as freeze-dried cells, harvested fresh cells, or air-dried ooze suspended in water.
[y]Colony forming units (CFU) of inoculum concentration applied to fruit.

[z]NT = not tested due to harvest.

Table 3. Population of recovered *Erwinia amylovora* from inoculated Braeburn apple fruit in 2003

Average colony forming units[x]							
Days after inoculation							
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	7/21 to 8/28	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^5	7/21 to 8/25	5.4×10^3	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/21 to 8/25	3.5×10^5	0.0	0.0	0.0	0.0
Ea153N Fresh	10^5	7/21 to 8/25	3.0×10^4	0.0	0.0	0.0	0.0
Ea153N Ooze	10^5	7/21 to 8/25	5.0×10^4	2.9×10^2	0.0	0.0	0.0
Ea153 Freeze-dried	10^5	7/21 to 8/25	0.0	4.6×10^1	0.0	0.0	0.0
Ea153 Fresh	10^5	7/21 to 8/25	0.0	3.6×10^3	8.0×10^1	0.0	0.0
Check	-	8/21 to 9/25	0.0	0.0	0.0	0.0	NT[z]
Ea153N Freeze-dried	10^6	8/21 to 9/25	1.6×10^5	5.6×10^3	0.0	0.0	NT
Ea153N Fresh	10^6	8/21 to 9/25	1.9×10^5	1.2×10^4	9.3×10^1	2.6×10^0	NT
Ea153N Ooze	10^6	8/21 to 9/25	1.6×10^5	1.6×10^4	0.0	0.5×10^0	NT

[w]Average colony forming units are represented as the mean of 15 fruit per treatment.
[x]Treatments include *Erwinia amylovora* 153N and *Erwinia amylovora* 153 wild-type inoculated onto fruit as freeze-dried cells, harvested fresh cells, or air-dried ooze suspended in water.
[y]Colony forming units (CFU) of inoculum concentration applied to fruit.
[z]NT = not tested due to harvest.

Proposal for 2004:
Objectives 1and 2 from 2003 will be repeated.

Literature Cited:

1. 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.
2. 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: 2 to 3 years
Current year: 2004
Current year request: $0
Budget in 2003: $36,426

Principal support for 2004:
USDA 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 REPORT

Project title: Integrated management of fire blight of pear and apple

PI: Kenneth B. Johnson
Organization: Dept. Botany & Plant Pathology, Oregon State University, Corvallis

Co-PI(s) and affiliation(s): Virginia Stockwell (OSU, Corvallis)
Cooperator(s): David Sugar (OSU, Medford), Joyce Loper (USDA-ARS, Corvallis)

Objectives: *In 2003:*

3. Evaluate new products for fire blight suppression.
4. Field-test mixtures of beneficial bacteria optimized for compatibility of their biological mechanisms
5. 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 2004 (all ongoing):

3. Evaluate new products for fire blight suppression.
4. Field-test mixtures of beneficial bacteria optimized for compatibility of their biological mechanisms, including refinement of use of iron with BlightBan A506
5. Evaluate potential for epiphytic growth of *Erwinia amylovora* on common flowers frequented by honey bees but which are not hosts of fire blight

Significant findings:

- An inoculated field trial was conducted in apple to evaluate alternative products for suppression of blossom blight. GWN-9250 (gentamicin sulfate), a mix of *Pseudomonas fluorescens* strain A506 Ecp- and *Pantoea agglomerans* strain C9-1 with or without Sequestrene 138 (FeEDDHA), *P. fluorescens* strain A506 (BlightBan A506) plus Sequestrene 138, BlightBan A506 plus Sequestrene 330 (FeDPTA) resulted in significantly ($P \leq 0.05$) fewer diseased blossom clusters compared to the water treated control. A new formulation of oxytetracycline (Fireman, NuFarm Americas) was the most effective product tested reducing the incidence of blight by 66%.
- An additional inoculated field trial was conducted in pear to further evaluate the effect of the iron chelates on performance of *Pseudomonas fluorescens* A506 (BlightBan A506) for fire blight suppression**.** A mix of *Pseudomonas* fluorescens strain A506 Ecp-, *Pantoea agglomerans* strain C9-1 with Sequestrene 138 (FeEDDHA), and BlightBan A506 plus Sequestrene 330 (FeDPTA) resulted in significantly ($P \leq 0.05$) fewer diseased blossom clusters compared to the water treated control. In both the apple and the pear trials, BlightBan A506 by itself did not provide significant disease suppression.
- For a second year, flowers from plants that are not hosts of fire blight disease were evaluated in growth chamber for their ability to support epiphytic growth of *Erwinia amylovora.* Plants of the rose family - peach, cherry, plum, blackberry, service berry – supported high populations of the fire blight pathogen. In addition, high populations of *E. amylovora* developed on flowers of big leaf maple, scotch broom, and snowberry. In general, flowers of mustard, dandelion, and clover were relatively poor hosts for epiphytic *E. amylovora*.

Methods:

Objective 1. New chemical and biological agents with potential to control fire blight were tested (see results section). This experiment was conducted in a Rome Beauty apple trees at the Botany and Plant Pathology Experimental Farm near Corvallis Oregon. (A second 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 (7 May). Beginning 30 May and ending 22 July, incidence of fire blight was evaluated weekly by counting and removing the diseased blossom clusters on each tree.
Objective 2. The iron-chelates FeEDDHA (Sequestrene 138) and FeDPTA (Sequestrene 330) were tested. We consider FeEDDHA a better choice then FeDPTA as it makes iron available to *Pseudomonas fluorescens* A506, but not the fire blight pathogen *Erwinia amylovora*. FeDPTA is a weaker chelate but is less expensive. Field trials were conducted on mature Bartlett pear and Rome Beauty apple trees at the Botany and Plant Pathology Experimental Farm near Corvallis Oregon. The commercial formulation of the biological agent, *Pseudomonas fluorescens* strain A506 (NuFarm Americas, Houston, TX) was evaluated for disease control alone or in combination with the Sequestrene products. Sequestrene 138 is FeEDDHA 6% a.i. and Sequestrene 330 is FeDPTA, 10% a.i.; both products were from Becker Underwood, Ames, IA. We measured the titer of the bacterium in BlightBan A506 at *ca*. 8 X 10^9 CFU/gram. We increased the amount of product applied to a rate of 16 grams per 12 L to yield a bacterial concentration of *ca*.1 X 10^7 CFU/ml, which is the recommended concentrated rate on the product label.. Standard antibiotic products (streptomycin and oxytetracycline) were included as controls.
Objective 3. In a growth chamber, flower-bearing branches were collected from: willow, maple, *Prunus* spp. (cherry, peach, plum), clover, dandelion, mustard, blackberry, service berry, broom, rhododendron, and apple. Bouquets of flowers were inoculated with standardized suspensions of 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).

Results and discussion:
Objective 1.

Trees used in the study averaged ~500 blossom clusters per tree. Disease pressure was moderate and symptoms of fire blight developed on about a fifth of the blossom clusters on water-treated trees. Agrimycin applied near full bloom and following pathogen inoculation provided good control of fire blight (50% fewer strikes than water-treatment). Based on analysis of mean strikes per tree, BlightBan A506 combined with Sequestrene 138 or Sequestrene 330, A506Ecp- combined with *Pa*C9-1S, Fireman, and Agrimycin resulted in significantly ($P \leq 0.05$) fewer diseased blossom clusters compared to water treated controls. Analysis of variance based on arcsine (square root (relative disease incidence)) revealed that the treatments BlightBan A506 combined with Sequestrene 138 or Sequestrene 330, A506Ecp- combined with *Pa*C9-1S, Bloomtime Biological, Gentamicin, Mycoshield, Fireman, and Agri-mycin 17 significantly ($P \leq 0.05$) reduced the disease incidence compared to water-treated controls. The product BlightBan A506 did not provide significant control of fire blight compared to treatment with water. The product Fireman provided significantly better control of fire blight compared to the Mycoshield used in this trial. With the exception of BlightBan A506 suspended in water and Bloomtime combined with Ecp-, all of the biological control agents suppressed fire blight to a level that did not differ significantly from the antibiotic products Fireman and Agri-mycin 17.

Rome Beauty Apple, Corvallis, Oregon 2003 Fire blight trial

		Date treatment applied						
Treatment	Rate per 100 gallons water	1 May	5 May	9 May	Mean number of blighted clusters per tree		Mean relative incidence of fire blight	
BlightBan A506	18 oz.	X	X		129	a*	1.29	a**
Water control	----------	X	X		102	**ab**	1.00	**ab**
Mycoshield (200 ppm)	16 oz.		X	X	79	bc	0.78	bc
Gentamicin (60 ppm)	8 oz		X	X	76	bc	0.72	cde
Bloomtime & A506 Ecp-	140 oz. Bloomtime & fresh cells	X X	X X		74	bc	0.74	cd
Bloomtime	140 oz.	X	X		63	bcd	0.63	cdef
BlightBan A506 & Sequestrene 330	18 oz. BlightBan & 16 oz. Seq. 330	X	X X	X	52	cd	0.54	cdef
Agri-mycin 17 (100 ppm)	8 oz.	X	X	X	52	cd	0.50	cdef
BlightBan A506 & Sequestrene 138	18 oz. BlightBan & 16 oz. Seq. 138	X	X X	X	50	cd	0.51	cdef
A506 Ecp- & *Pa*C9-1S & Sequestrene 138	Fresh cells & 16 oz. Seq. 138	X	X X	X	43	cd	0.43	def
A506 Ecp- & *Pa*C9-1S	Fresh cells	X	X		41	cd	0.39	ef
Fireman (200 ppm)	16 oz.		X	X	34	d	0.35	f

*Means 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 relative disease incidence followed by the same letter are not significantly different according to Fischer's protected least significance difference at P = 0.05.

Objective 2.

Blossom cluster density on the Bartlett pear trees ranged from ca. 500 to 1800 clusters per tree. Disease pressure was very light (likely due to cool wet weather during pear bloom) and symptoms of fire blight developed on only ca. one percent of the blossom clusters on water-treated trees. Agrimycin and Mycoshield applied near full bloom and following pathogen inoculation provided good control of fire blight (69% and 51% reduction, respectively, in the disease incidence on water-treated trees). Based on analysis of mean strikes per tree, treatment with BlightBan A506 combined with Sequestrene 330, A506Ecp- combined with *Pa*C9-1S and Sequestrene 138, ErB, Mycoshield, and Agrimycin resulted in significantly ($P \leq 0.05$) fewer diseased blossom clusters compared to water-treated controls. Analysis of variance based on arcsine-square root transformed disease incidence, only Agrimycin significantly ($P \leq 0.05$) reduced the disease incidence compared to water-treated controls.

It is difficult to make solid conclusions on the efficacy of various treatments for control of fire blight from data in the Bartlett pear plot where the disease incidence was very low. Nonetheless, in both trials, the commercial formulation of BlightBan A506 by itself failed to provide significant disease suppression. In contrast, 3 of 4 treatments of BlightBan A506 mixed with an iron chelate provided significant disease control (averaging 52%). Moreover, the forth (nonsignificant) treatment of BlightBan plus iron provided 45% control.

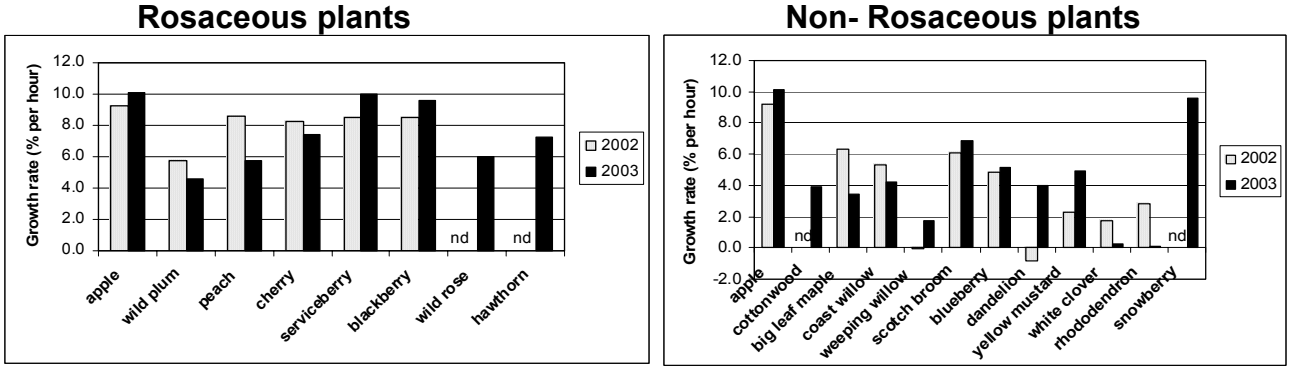
BARTLETT PEAR, Corvallis, Oregon 2003 Fire blight trial

		Date treatment applied*						
Treatment	Rate per 100 gallons water	5 April	8 April	15 April	Number of blighted clusters per tree		Disease incidence	
Water control	----------	**X**	**X**		**15.6 ± 2.9**	**a****	**0.0149**	**a*****
BlightBan A506	18 oz.	X	X		13.6 ± 2.3	ab	0.0147	a
C4-98	Fresh cells	X	X		10.2 ± 5.9	abc	0.0091	ab
A506 Ecp- & *Pa*C9-1S	Fresh cells	X	X		10.2 ± 1.7	abc	0.0102	ab
BlightBan A506 & Sequestrene 138 & Mycoshield	18 oz. BlightBan & 16 oz. Seq. 138 & 16 oz Mycoshield	X - -	X X X	- X X	9.4 ± 0.9	abc	0.0101	ab
BlightBan A506 & Sequestrene 138	18 oz. BlightBan & 16 oz. Seq. 138	X -	X X	- X	8.6 ± 2.6	abc	0.0100	ab
A506 Ecp- & Eh252	Fresh cells	X	X		8.4 ± 1.9	abc	0.0098	ab
BlightBan A506 & Sequestrene 330	18 oz. BlightBan & 16 oz. Seq. 330	X -	X X	- X	7.4 ± 2.1	bc	0.0067	ab
A506 Ecp- & *Pa*C9-1S & Sequestrene 138	Fresh cells & 16 oz. Seq. 138	X -	X X	- X	7.4 ± 1.9	bc	0.0095	ab
ErB	Fresh cells	X	X		7.4 ± 2.0	bc	0.0069	ab
Mycoshield (200 ppm)	16 oz.		X	X	5.6 ± 1.3	c	0.0073	ab
Agri-mycin 17 (100 ppm)	8 oz		X	X	5.0 ± 1.3	c	0.0046	b

* Plot inoculated on 10 April with 2.3 x 10^5 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain).
**Means of the sum of strikes per tree followed by the same letter are not significantly different according to Fischer's protected least significance difference at P = 0.05. Means are presented ± the standard error.
***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.

Objective 3.

Non-host plants of the rose family - peach, cherry, plum, blackberry, service berry – supported high populations of the fire blight pathogen. In addition, high populations of *E. amylovora* developed on flowers of big leaf maple, willow, and scotch broom. Conversely, flowers of mustard, dandelion, clover and rhododendron were relatively poor hosts for epiphytic populations of *E. amylovora*.



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* can 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). 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 may be a consideration in determining the risk of blight outbreaks.

Proposal for 2004:

Justification: The goals of our 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 fire blight management. In recent years we have completed studies concerned with understanding how orchard environment affects growth and spread of bacteria on and among pear and apple blossoms. In addition, we have investigated and proposed a decision aid to modify fire blight forecasting models for use with softer control technologies. Our current activities include evaluating new chemical controls, and continuing to investigate, improve and optimize biological control of fire blight with beneficial bacterial. Another objective is concerned with evaluating the potential for epiphytic growth of *Erwinia amylovora* on common, abundant flowers frequented by honeybees but which are not hosts of fire blight disease. Knowledge of the potential for *Erwinia amylovora* to grow on flowers of non-hosts may lead to improved indications as to when regional risks of fire blight epidemics are high.

Objectives (all ongoing):

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

Objective 1 (ongoing): Evaluate new products for fire blight suppression. New chemical products with potential to control fire blight are being developed and tested (e.g. Fireman (new formulation of oxytetracycline, Serenade (*Bacillus subtilis* strain QST 713), Bloom Time Biological (*Panteoa agglomerans* strain 325), Agri-Gent (gentamicin sulfate), VacciPlant (algal derivative), Surround (kaolin clay), Mycosin (stone powder), Apogee (prohexidione-CA), Phyton 27 (copper in organic acid)). The mode of fire blight suppression differs among these products. Our emphasis in testing new materials will be on effectiveness, compatibility with other control products, and potential side effects (e.g. blossom browning, russeting). These experiments will be conducted in blocks of pear and apple located in Corvallis, OR, with treatments repeated in Medford if trees are available. Experimental methods and data collection will be as described above under Objective 1.

Objective 2 (ongoing): Field-test mixtures of beneficial bacteria optimized for compatibility of their biological mechanisms. We have shown that mixtures of bacterial antagonists on pear and apple blossoms are more effective than individual strains and, and less variable in the degree of control obtained. In our recent efforts, we have discovered several ways to enhance the effectiveness of antagonist mixtures. One of these enhancements involved selecting a mutant of *P. fluorescens* A506 deficient in an extracellular protease (strain A506 Ecp-). In a mixture with *Pantoea agglomerans,* the loss protease production by A506 Ecp- prevents degradation of the antibiotic produced by *P. agglomerans*, resulting in a longer effective lifetime for the antibiotic and a greater level of disease suppression.

We have also demonstrated that the addition of an iron chelate, FeEDDHA, to blossoms induces A506 to produce a previously unknown antibiotic, which also enhances disease suppression. In the coming year, we will continue to investigate how to best utilize iron-enhanced biological control of fire blight with A506; to investigate forms of iron best suited for this technology, and to evaluate integration of iron-enhanced biological control with conventional, antibiotic-use recommendations for fire blight control. For example, in some trials, FeEDDHA has not enhanced fire blight control by A506. We suspect that part of the variation in control is due to insufficient coverage of flowers with the iron chelate. Our final applications of A506 with FeEDDHA occurred at 70% bloom, and while A506 can migrate to flowers that open later, the iron chelate cannot; thus, about a third of the flowers were not treated directly with the iron chelate. The purposes of these experiments will be three-fold: 1) determine if variation in efficacy can be diminished if iron is applied after all flowers are open, and 2) determine the persistence of iron chelates on flowers in orchard environments and 3) evaluate promising alternative iron chelates for disease control: FeDPTA (Sequestrene 330, Becker Underwood), FeEDTA, and Fe-amino acid chelate (Metalosate,

Albion, Clearfield, UT). Experimental methods and data collection will be as described above under Objective 2.

Other objectives include investigating if the addition of avirulent (non-disease causing) strains of *Erwinia amylovora* to mixtures of beneficial bacteria enhances disease suppression. We also are testing alternative formulations of gram-negative bacteria that do not require freeze-drying (these formulations have the potential to greatly reduce the cost of biological products).

Objective 3 (3rd of 3rd year): Evaluate potential for epiphytic growth of *Erwinia amylovora* on common flowers frequented by honey bees but which are not hosts of fire blight.

Control of the blossom blight phase of fire blight focuses on the epiphytic growth of *E. amylovora* on floral structures, including stigmas and the hypanthium (nectary or floral cup). Stigmas, which are borne on the ends of the floral style, have been demonstrated to be the primary site epiphytic colonization by *E. amylovora* (1). Bees, and to a lesser extent other insects, are the primary vectors by which bacteria are introduced to stigmatic surfaces. Growth of *E. amylovora* on floral surfaces of important, rosaceous hosts of fire blight has been investigated widely, but the potential for *E. amylovora* to grow on surfaces of flowers that are not hosts of the disease has received little research effort. We believe of this kind of epiphytic growth may be significant in providing inoculum to pear and apple flowers during key periods in the bloom season, and thus, may be an important but little understood risk factor in the initiation of fire blight epidemics.

The book *Plants for Beekeeping in Canada and the Northern United States* (2) provides top ten lists of the most important nectar or pollen sources for bees in the major eco-regions across the continent. In the northwest, this list includes willow, maple, popular/alder, prunus, clover, dandelion, mustard, blackberry, broom, snowberry and rhododendron.

Methods: For this objective, we will collect flower-bearing branches of the above species. In growth chambers, bouquets of flowers will be inoculated with standardized suspensions of freeze-dried cells of *E. amylovora* strain 153Nal. Population size of *Ea*153Nal will be monitored by dilution plating onto selective media. Population sizes among various flower types will be standardized on a per weight basis.

In spring 2004, we also plan to conduct several field inoculations of *E. amylovora* onto non-host flowers (e.g., cherry, pear, blackberry, maple) growing in the Corvallis area. The intention of these field inoculations is to confirm (or refute) data obtained in the growth chamber inoculations.

Literature Cited:

3. Johnson, K.B., and Stockwell, V.O. 1998. Management of fire blight: A case study in microbial ecology. Ann. Rev. Phytopathology 36:227-248.
4. Ramsay, J. 1987. Pages 139-143 in: *Plants for Beekeeping in Canada and the Northern United States.* Intl. Bee Research Assoc., London.

Budget:

Proposed duration of objectives: Objectives 1 & 2: Ongoing; Objective 3: 3rd of 3 year

Item	Last Year (2003)	**Current Request**	Next year (2005)
Salaries	9,500	**9,000**	6,000
Benefits (52%)	4,655	**4,680**	3,120
Supplies	1,500	**1,000**	1000
Travel	450	**450**	500
Plot Maintenance	1,500	**1,000**	1,000
Total	17,605	**16,130**	11,600

2004 Salary is 3.0 months of a senior faculty research assistant

Support from other funding sources:

OSU Agric. Exper. Station, NWHC: Survival of *E. amylovora* on pear fruit 2004 $56,000
USDA: 2003-2005, $297,415 (Stockwell's salary; research on avirulent *E. amylovora*).
Occasional grants-in-aid of research from chemical companies

FINAL REPORT

PROJECT TITLE: Biology and Management of Pear Pests

PI: David Horton
USDA-ARS, Yakima Agric. Research Lab., Wapato, WA

COOPERATOR: Tom Unruh

OBJECTIVES:
Project objectives are to develop new means for controlling arthropod pests in pears, with emphasis on pear psylla. Focus of the project is to improve our understanding and utilization of natural enemies and biological control in pear orchards. The research consists of a mix of basic and applied studies in both the laboratory and field.

SIGNIFICANT FINDINGS:
2001-2002 seasons:

- Finished analysis and publication of mowing project, showing that reduced frequency of mowing in pear orchards led to substantially higher densities of natural enemies in the ground cover and (for some taxa) in the tree canopy.
    - ***Comment****:* This was a project begun originally using WPCC funding. Those data were then used to obtain a 2-year Western Region SARE grant ($110,000). The studies were done at sites in Hood River, Yakima, and Peshastin. Technology transfer included three field days in Hood River and Peshastin, presentations at workshops and scientific meetings, and publication of papers in technical and trade journals (see list at end of this report).
- Compared approximately 20 cover crops for pest and predator populations in small plot trials. Results of these trials were used to select cover crop mix for 2003 studies (see below).
- Showed that *Campylomma verbasci* (the mullein bug) readily feeds and develops on pear psylla and spider mites, and may be a significant source of biological control in orchards.
- Studies of pest and predator overwintering led to:
    - Development of degree-day models for predator emergence from overwintering sites;
    - Demonstration that common mullein is important source of overwintered pests and predators;
    - Improved understanding of environmental and orchard factors that affect densities of predators overwintering in orchards.
- Showed that a common predator of pear psylla (*Anthocoris antevolens*) is actually a complex of reproductively isolated "cryptic" species, which may differ in how readily they colonize pear orchards.
    - ***Comment:*** This was a project begun originally using WPCC funding. Those data were then used to obtain a 3-year NRI grant ($200,000; Co-PI Tom Unruh).

2003 season:

- Compared taxonomic composition of predator communities in ground cover and tree canopy of pear orchards, as indirect means of learning what predator species might move between ground cover and tree canopy. This involved some rearing of immatures for several groups.
- Showed that predator densities in the tree and pear psylla densities were not substantially affected by presence of a cover crop in small plot trials, despite substantially higher densities of predators in the ground cover where the cover crop was planted. Population trends for both pest and predators, however, suggest possible enhancement of biological control in cover crop plots.

- ❖ Collected predators from both ground cover and tree to screen (using ELISA) for evidence of the predators having fed upon pear psylla, again to look for evidence of predator movement between the two habitats. ELISA assays are ongoing. With Tom Unruh.

RESULTS AND DISCUSSION (2003)

Background: My work has shown that it is easy to prompt a build-up of natural enemies in the ground cover of pear orchards (e.g., by reducing mowing frequency or by planting a cover crop). What is not clear is the extent that build-up contributes to biological control in the tree. This lack of understanding is due to several factors:

- ❖ Poor understanding of predator movement between ground and tree;
- ❖ Less than full understanding of taxonomic composition of some groups; specifically, certain groups (e.g., ladybird beetles, green lacewings) found in orchards are species complexes that may be composed of species having fairly strict habitat preferences (i.e., including ground cover species that never move into trees);
- ❖ Plots are often too small to demonstrate biological control, apparently because movement by pests and predators between plots "washes out" any actual treatment effects.

Objectives:

1) Compare predator community composition in tree and cover crop;
2) Test for effects of cover crop on psylla densities;
3) Address effects of inter-plot dispersal (studies delayed until 2004; see new Proposal);
4) Test for evidence of psylla predation in tree-collected and cover crop-collected predators, using ELISA.

Methods: Cover crop plots (2 tree rows wide x 4 trees long) were established in spring 2003 in the pear orchard at the Moxee farm. Plots were composed of a mix of vetch, winter wheat, crimson clover, and winter Austrian pea (a mix shown earlier by me to harbor large numbers of predatory insects). Control plots were composed of resident rye-grass. Five plots per treatment were established in a randomized block design. Trees and ground cover were sampled with beat trays and sweep nets, respectively every 3 weeks. Leaf samples (for immature psylla) were taken every 3 weeks (100 leaves per plot). Lacewings and ladybird beetles were taken to the laboratory for identification, since they often could not be identified in the field; immatures of these groups were reared to adulthood in the laboratory for identification, by feeding them a mix of pear psylla, green peach aphid, and pea aphid. On several other dates, predators were collected from both habitats, placed immediately on ice, taken to the laboratory and put in an ultrafreezer, and eventually analyzed using ELISA for presence of psylla proteins in the predator gut. Presence of psylla proteins in the guts of predators that were collected from the ground cover would be evidence that predators move between ground cover and tree habitats.

Results: Predator densities were considerably higher in the ground cover of the cover crop treatment than the control (grass) treatment (Fig. 1; data exclude spiders and misc. uncommon taxa). There was a suggestion that the effects carried over to the tree canopy, but the differences were not statistically significant (Fig. 2: tree). Thus, as with some of my earlier work, the results are suggestive of enhancement, but not statistically conclusive. Again, we must consider that plot size was too small to allow statistical differences to show, and that dispersal between plots washed out effects.

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

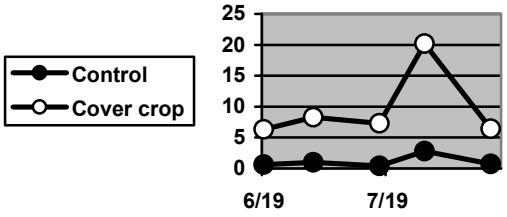
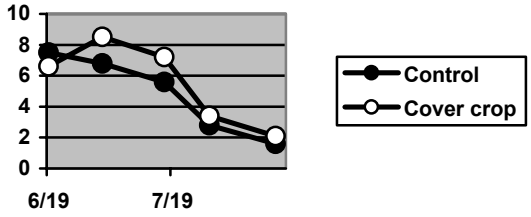


Fig. 2: Tree (number predators per beat tray)



Adult pear psylla were actually more abundant in the cover crop plots than in the grass plots (Fig. 3). This effect was consistent across all 5 blocks, and thus does not appear to be a chance event. It is unclear why adult psylla might prefer trees grown with a cover crop understory, but could include unknown effects of the cover crop on tree nutrition and water, or upon microenvironment surrounding tree. Despite the effects of the cover crop on adult psylla, numbers of psylla immatures per leaf were virtually identical in the cover crop and control plots (Figs. 4-5). When considered in combination with the adult data, results for eggs and nymphs suggest that mortality of immature psylla was higher in the cover crop plots; whether this apparent higher mortality was due to predation is unknown.

Fig. 3: Adult psylla per tray

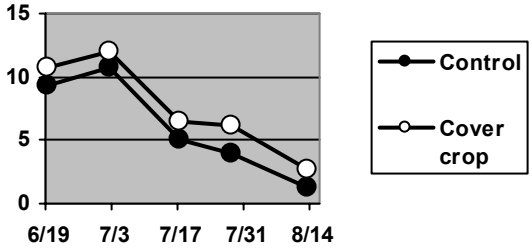


Fig. 4: Psylla eggs per leaf

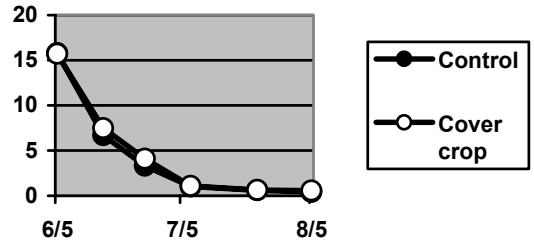
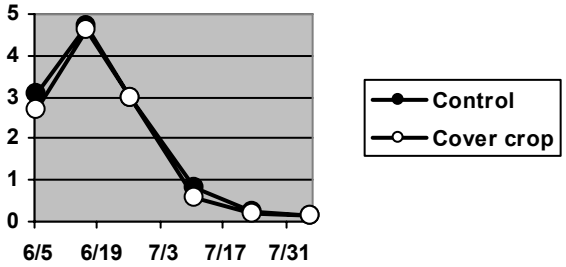


Fig. 5: Psylla nymphs per leaf



Composition of the predator communities is shown in Table 1 (excludes spiders and misc. uncommon taxa). In the ground cover, minute pirate bug and big-eyed bug were most abundant; in the tree, two predators of pear psylla (*Anthocoris*, *Deraeocoris*) were most abundant. Three groups (minute pirate

bug, ladybird beetles, green lacewings) occurred regularly in both the tree canopy and ground cover (Table 1). Closer examination of species' composition for the ladybird beetles and lacewings showed that some species occurred in both habitats very regularly (Figure

Table 1. Percentage composition of major predator groups in tree and in ground cover ranked from highest to lowest.

Ground cover		Tree	
Minute pirate bug	48.5	*Deraeocoris*	57.0
Big-eyed bug	20.2	*Anthocoris*	33.9
Ladybird beetles	10.6	Green lacewings	4.1
Damsel bugs	9.6	Minute pirate bug	2.4
Green lacewings	8.6	*Campylomma*	1.2
Deraeocoris	2.8	Ladybird beetles	0.9
Brown lacewings	0.5	Brown lacewings	0.5

6-7): ladybird beetles - - *Coccinella transversoguttata* and *C. septempunctata*; lacewings - - *Chrysoperla plorabunda*. Thus, for these taxa in particular (including minute pirate bug), we might speculate that manipulation of ground cover to enhance their densities could lead to enhancement in the tree as well. Other species, however, were habitat specialists (Fig. 6-7): ground cover - - *Hippodamia convergens* (beetle) and *Chrysopa oculata* (lacewing); tree - - *Chrysopa nigricornis* (lacewing). Two major psylla predators (*Anthocoris*, *Deraeocoris*) are both habitat specialists, preferring the tree canopy, and their densities are unlikely to be affected directly by cover crop manipulation.

Fig. 6: Percent composition for ladybird beetles

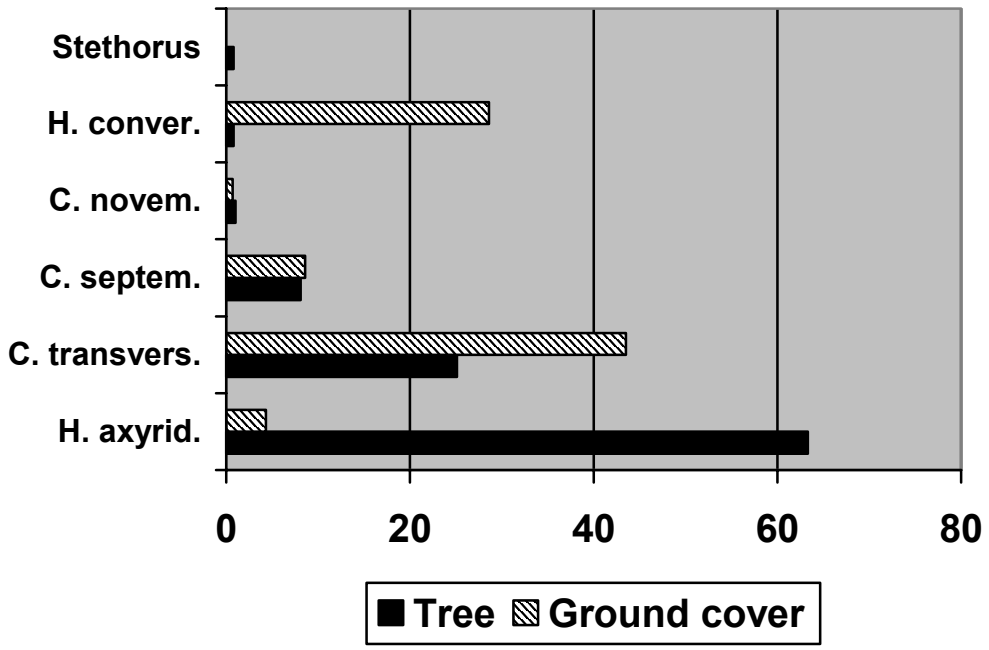
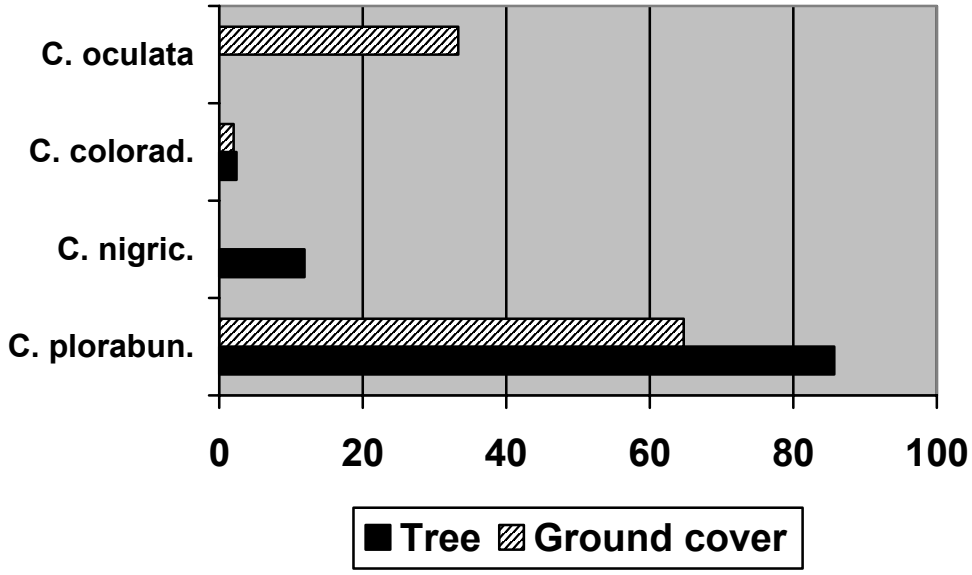


Fig. 7. Percent composition for green lacewings



For the ELISA work, predators were collected from both habitats over the duration of the study. We are currently analyzing the minute pirate bug samples, as we know that this species occurs in both habitats in the orchards. Results will be presented at the research review.

Conclusions: Results indicated that several predator taxa (including minute pirate bug, certain lacewing species, certain ladybird beetle species) utilize both tree and ground cover habitats, thus manipulation of ground cover to enhance numbers of these species potentially could lead to enhancement in the tree. Other taxa, however, were habitat specialists (preferring either the tree or the ground cover), and it seems that cover crop manipulation is unlikely to affect their effectiveness as predators of canopy-dwelling pests such as pear psylla. The ELISA studies are ongoing. A cover crop mix composed of wheat and 3 legumes was shown to provide habitat to high densities of several natural enemy taxa. There was a trend for total predator numbers to be higher in the tree in the cover crop plots as well. Psylla adults were actually more abundant in the cover crop plots than control plots; densities of eggs and nymphs, however, were identical in the two types of plots, suggesting that actual mortality rates of eggs and nymphs were higher in the cover crop plots. Better understanding of predator movements between plots, and between ground cover and tree would be valuable; these questions will be addressed in detail in 2004 (see new Proposal).

TECHNOLOGY AND INFORMATION TRANSFER

Mowing study

- Field days (3)
- Presentations at workshops and scientific meetings (5)
- Journal articles and Proceedings:
  1) **Know when to mow**. *Western Fruit Grower* 120 (#6): 20D-20H
  2) **Evaluating the effects of orchard floor management on biological control in pears**. *Organic Farming Research Foundation Information Bulletin* 10: 22-23.
  3) **Effects of mowing frequency on ground cover insects**. *Proc. Ann. Meeting Wash. State Hortic. Assoc.* 94: 144-147.

4) **Natural enemy communities in pear orchards affected by mowing frequency**. *Good Fruit Grower* 54 (Dec. 2003): p. 50.
5) **Effects of mowing frequency on densities of natural enemies in three Pacific northwest pear orchards**. *Entomologia Exp. Appl*. 106: 135-145.

Predator overwintering studies

- Presentations at scientific meetings (2)
- Journal articles
  1) **Numbers and types of arthropods overwintering on common mullein in a central Washington fruit-growing region**. *J. Entomol. Soc. Brit. Col*. (in press).

Ground cover study (2003)

- Presentation at Wash. State Hortic. Assoc. meeting (Dec. 2003)

BUDGET

PROJECT TITLE: Biology and Management of Pear Pests
PI: David Horton
Project total (3 years): **$80,693**

Year	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Total	34,673	30,050	15,970

Breakdown

Item	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Salaries	25,683	22,260	12,700
Benefits	8990	7790	1270
Materials	0	0	2000
Total	**34,673**	**30,050**	**15,970**

CONTINUING PROJECT REPORT

PROJECT TITLE: Chemical Ecology of Pear Psylla

PI: David Horton
USDA-ARS, Yakima Agric. Research Lab., Wapato, WA

COOPERATOR: Peter Landolt

Comment: This project was initiated as a cooperative effort (with several Israeli scientists) originally submitted to the Binational Agricultural Research and Development Fund (BARD). Proposals were submitted in both 2001 and 2002. Neither was funded. In February 2003, Horton and Landolt initiated several of the behavioral assays that were proposed in the original grant, with the expectation that the additional expertise needed (particularly in chemistry) to fulfill obligations would eventually be obtained elsewhere, should results of the behavioral assays warrant the additional work. Results of these initial behavioral assays are summarized in this report. Funding obtained from the WPCC ($20,230) has not been spent, as the original understanding was to use the funds to supplement the BARD grant. However, we propose here to continue the behavioral assays in 2004, and will use the $20,230 to fund a technician, if the WPCC agrees to this.

OBJECTIVES:
Project objectives are to determine the role of chemical cues associated with the pear tree and with pear psylla in affecting: (1) distribution of pear psylla in orchards; (2) mate-locating behavior of pear psylla; and (3) winterform colonization of orchards. Efforts last year concentrated on improving our understanding of the highly clumped distribution of post-diapause winterform pear psylla in orchards, with emphasis on separating the effects of the host plant from effects of other psylla; and, to develop procedures suitable for assaying the effects of volatile chemicals on psylla behavior.

SIGNIFICANT FINDINGS:

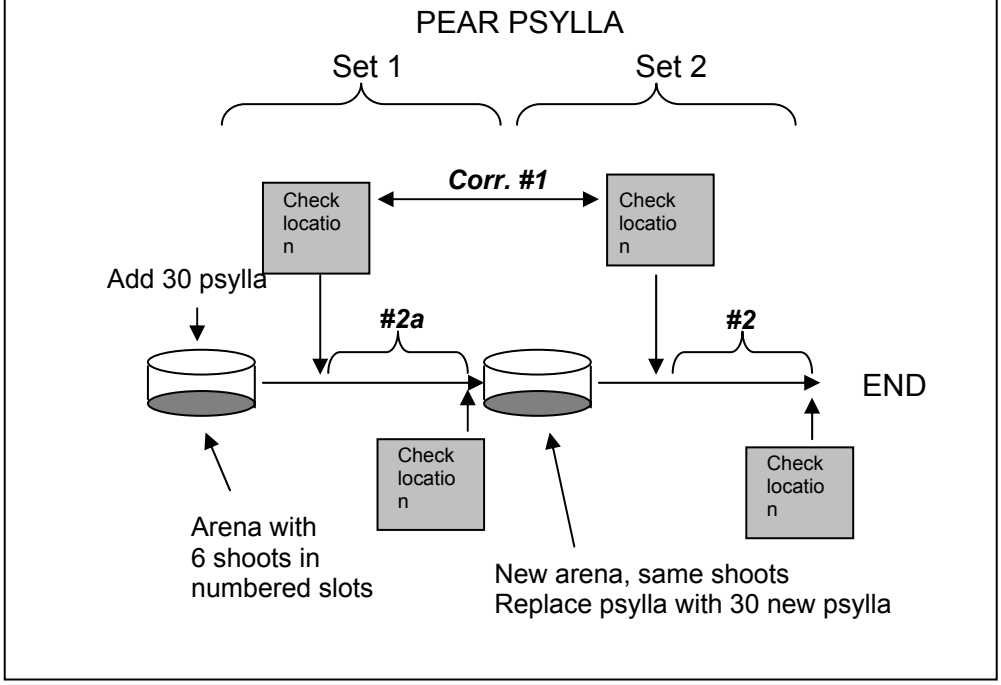
- Post-diapause winterform pear psylla are significantly aggregated on a subset of shoots in the field during the dormant to delayed-dormant period. The effects can be reproduced in the laboratory using field-collected shoots.
- The aggregation behavior appears to be due both to a strong mutual attraction among psylla and to shoot-to-shoot differences in shoot attractiveness; the latter effect was weak in laboratory assays, perhaps due to assay methods; the assays will be adjusted to strengthen tests. It is unknown whether cues affecting the aggregation behavior were chemical.
- There was weak evidence that shoot attractiveness was affected by allowing previous feeding on the shoot. Feeding by sucking insects is known to affect host plant quality in other systems. These assays will also be pursued next year.
- Attempts to develop an assay method that can be used to screen plant or psylla volatiles have so far been unsuccessful. We are currently looking at a modified choice chamber that can be used in a flight tunnel, and hope to have more success with that.

METHODS:
The laboratory assays using post-diapause winterform psylla were done in circular arenas (8 inches diam, 4 inches tall) having a cardboard floor through which holes were punched for insertion of pear shoots. Cut ends of shoots were placed in a container of water. Shoots were collected from a section of the Moxee orchard having few overwintering pear psylla.

Study 1: Demonstrate aggregation of winterform pear psylla under laboratory conditions. Previous work by us (see Results) indicated that psylla are strongly aggregated on a subset of shoots in the orchard during the re-entry period. In March 2003, we collected uninfested shoots from the Moxee orchard, and set the shoots up in our assay chambers (10 shoots per chamber). Sixty winterform psylla (1:1 sex ratio) from the Moxee orchard were added to each chamber, and location of psylla checked 24 hours later.

Study 2: Shoot-to-shoot variation or mutual attraction? To test whether shoot-to-shoot differences in attractiveness explained aggregation, as opposed to mutual attraction among psylla, a large study was done in which field-collected shoots (6 per chamber; March 2003) were provided to psylla, and the shoots were ranked after 3 hrs according to number of psylla settling on them. After 24 hrs, we removed the psylla and moved the shoots to new arenas (knowing, by marking the cardboard floor, what each shoot=s original rank in attractiveness was), and added a new set of psylla (see Fig. 1 for experimental design). After 3 hrs in the new arena, we recorded location of this second set of pear psylla among the shoots. If shoot differences were very important in explaining the aggregation, we expect psylla set #2 to rank shoots from most favored to least favored in the same order as the first set of psylla ranked the shoots; this would lead to a positive correlation coefficient (**Corr. #1** in Fig. 1). We also looked at whether each set of psylla remained aggregated on a specific set of shoots by checking each set of psylla twice: once at the 3 hr interval, and once again at 24 hours (Fig. 1). High correlations at those intervals (correlations **#2a** and **#2b** in Fig. 1) would indicate psylla were showing strong aggregation behavior. If mutual attraction among psylla is most important in explaining aggregation behavior, we expect high correlations in **#2a** and **#2b**, and a comparatively lower correlation for correlation **#1**. A lack of correlation in **#2a** or **#2b** would indicate that psylla distribution was mostly happenstance, and that there was a lot of movement by psylla between shoots in between the first and second samples.

Figure 1. Experimental design



Study 3: Does previous feeding by psylla on shoots affect shoot attractiveness? This was a preliminary study done just before scale spread began in the field. In mid-March 2003, we bagged 12 shoots in the field, and added 10 winterform psylla to each bag; 12 control shoots were also bagged. Psylla were allowed 48 hours to feed. At the end of 48 hours, shoots were cut, taken to the laboratory, and paired (1 control shoot vs 1 conditioned shoot) in chambers. Ten psylla were added to each chamber, and their locations checked 3 hours later.

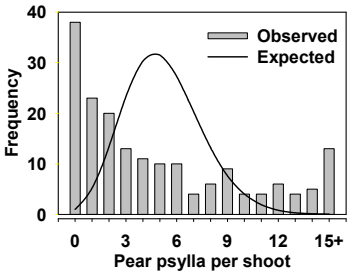
Study 4: Attempt to design assay methods for testing volatile chemicals. Our first attempts included use of flight chamber and use of organdy cages. Neither was successful (see Results). We are now looking at a modified choice chamber that can be used in a flight chamber.

2004: Methods will be similar to those described above, with modification as necessary. Emphasis of the 2004 work will be: (1) tease apart role of mutual attraction vs host plant effects in affecting winterform behavior; (2) address role of sex ratio affecting aggregation behavior of winterforms; (3) test whether previous feeding on shoots affects attractiveness; if so, we would eventually want to compare chemistries of conditioned and control shoots; and we would want to determine whether the change in attractiveness was due to internal chemical changes of shoots caused by feeding vs changes in external chemistry of shoots because feeding psylla themselves leave odors on the shoots; (4) begin work testing whether infestation of psylla nymphs on pear foliage affects preference of summerform adults; (5) continue attempts to develop assay methods for screening volatile chemicals.

RESULTS AND DISCUSSION:

(1) Our previous field sampling showed that winterform psylla are highly aggregated on a subset of shoots (data re-shown from our 2001 sampling study; Figure 2). This aggregation behavior was then replicated in the laboratory in March 2003 using shoots collected from the field (Figure 3; although the trial 3 distribution is approaching randomness rather than aggregation). The aggregated distributions indicate: (a) psylla were mutually attracted to one another; or, (b) shoots differed in attractiveness; or, (c) aggregation was due to a combination of (a) and (b).

Figure 2. Number of shoots having shown number of psylla; 180 shoots sampled (2001 data); expected indicates expectation if distribution was random



(2) Figure 4 shows the correlations obtained from our study designed to look for mutual attraction vs shoot-to-shoot variation. The marginally significant correlation (although weak) for correlation **#1** (Fig. 4A), suggests that the two sets of pear psylla ranked the same set of shoots in a similar manner, which we interpret as evidence that shoots differed in attractiveness. There was an extremely large amount of variation, however, that might be eliminated by changing our assay methods. We will conduct similar trials in 2004, but using paired shoots in an arena, rather than 6 shoots per arena.

Both correlation **#2a** and **#2b** were highly significant and positive (Fig. 4BC), indicating (again) that psylla exhibited aggregative behavior on a subset of available shoots, and that psylla either did not move much between shoots during the 24 hrs or that they did move but returned repeatedly to preferred shoots. The two correlations are much stronger than correlation **#1**, which we interpret (tentatively) as evidence that the aggregation behavior was mostly due to mutual attraction among psylla and less by shoot-to-shoot variation in attractiveness. We will continue to explore this

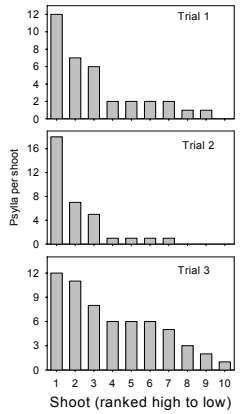


Figure 3. Psylla distribution; laboratory trials

hypothesis in 2004 using paired shoots, which we hope better controls the unexplained variation observed. We will also be manipulating sex ratio of psylla in the chambers, to determine whether both sexes aggregate similarly (our initial impression is that females aggregate apparently somewhat weakly, and that males wander until finding small to large aggregations of females, following which males settle).

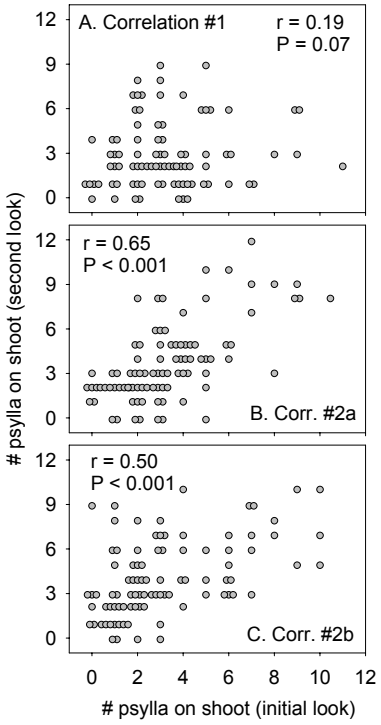
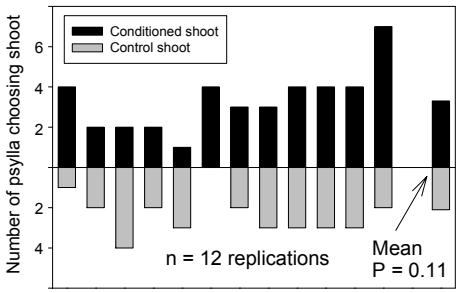


Figure 4. Psylla distributions on shoots

Figure 5. Preference for conditioned vs control shoots



(3) There was only very weak evidence (P = 0.11) that shoots which had been intentionally infested with pear psylla were more attractive to psylla than previously psylla-free shoots (Fig. 5; 61.5% of psylla chose the conditioned shoot). This trial was done just before scale spread began, and allowed only 48 hours of feeding by bagged psylla. We will repeat the experiment earlier in the delayed dormant period, and allow longer intervals of feeding.

(4) Attempts to develop a method for testing response of pear psylla to volatile chemicals have so far been unsuccessful. We first modified a flight tunnel by placing an organdy screen between the chamber that contained the psylla and the chamber containing the volatile source (in this case, a pear seedling; the seedling was paired with an artificial plant, to control color effects). The idea was to see if psylla would accumulate on the half of the organdy screen that was immediately downwind of the pear seedling. This method was abandoned, as we found that psylla mostly wandered about the chamber rather than accumulating on the screen. We then tried enclosing pear seedlings in small organdy cages that just fit over the seedlings. The seedlings and cages were then put into larger cages containing pear psylla. Here, the idea was to determine whether psylla would accumulate on organdy cages placed over pear seedlings vs cages placed over artificial plants. This trial was also unsuccessful, as the psylla mostly wandered around the large cage.

We have returned to the wind tunnel, but now release the psylla in a small organdy screened cage having two arms (similar to Y-tube olfactometer): one arm is downwind of a pear seedling, the second arm is downwind of the artificial plant. These assays are ongoing. If the method looks to be useful, we will compare infested and uninfested plants, and will look for attraction of male psylla to female-infested plants.

Proposal for 2004:

- Continue to address role of host plant vs mutual attraction affecting winterform distribution. Simplify assays to reduce variability among different tests.
- Address role of sex ratio of winterforms affecting aggregation behavior of males vs females, to determine if aggregation is largely due to mating activities. Compare aggregation behavior of males vs females in presence and absence of opposite sex.
- Determine whether previous feeding on shoots by winterform psylla affects attractiveness of shoots.
- Determine whether infestation by nymphs on pear foliage affects preference by summerform adults.
- Continue to modify assay methods for testing volatile chemicals, until a suitable method is developed.

BUDGET

Project title: Chemical ecology of pear psylla
PI: David Horton
Project duration: 2002-2004
Current year: 2004
Project total: $20,230
Current year request: $0

	Year 1 (2002)	Year 2 (2003)	**Year 3 (2004)**
Total	20,230	0	**0**
Current year breakdown			
Salary[a]	7875	0	**0**
Benefits[b]	2375	0	**0**
Wages[c]	9980	0	**0**

[a] The original request was to provide partial support for GS-11 Post-doctoral Research Associate (to supplement BARD funding)
[b] 30%
[c] 130-day GS-3 technician

Comment: The $20,230 has not been spent, and we propose to use the funds in 2004 to partially fund a GS-5 term technician, who has been in Horton's lab for the last 2 years and who assisted in gathering the data presented in this report.

INTERIM FINAL REPORT

Project Title: A biochemical approach to quantifying pear psylla predation in the field

PI: Tom Unruh, USDA-ARS, Wapato WA 98951

CO-PI: Dr. Nina Bárcenas, Colegio Postgraduados, Texcoco, Mex.
Cooperator: Dave Horton

Funding History:
Year initiated: 2001 ($4,000)
2002 ($28,600)
2003 ($28,600)
This project was funded August to August and year 3 is only 30% completed

2004 Objectives: As this project is funded through August of 2004, this final report is premature and is thus entitled Interim final report. We plan on submitting another report in 2005. We have three objectives for 2004 and 2005:

1. Complete digestion profiles for *Orius*, one coccinellid, and an ant predator. We also will add replicates to our existing digestion profiles. These digestion profiles remain important in interpreting results of field studies and for choosing the best primers for the
2. Develop a time budget for feeding by key predator species. We attempted this effort in the field in 2002 and failed due to the small size of predators and the low densities at which they occur. We failed again to execute this in laboratory microcosms in 2003 but will attempt to do that for *Anthocoris, Deraeocoris*, and a lacewing.
3. Use ELISA and PCR to provide estimates of predation frequency by key predators. We now have doubts that a time specific predation rate may be beyond the reach of current technology. However, we are confident that comparative predation rate or intensity data can be collected. We are currently analyzing field specimens from 1999 collected by D. Horton. We will continue to analyze the field data we have collected in 2001-2003 and work with specimens collected by Horton collected in 2003, and that his group will collect in 2004 (see Horton proposal).

OBJECTIVES 2003

1. Complete digestion studies on Lacewings emphasizing short digestion times and evaluate longer amplicons for the bugs (completed)

2. Utilize PCR method to estimate predation rates of pear psylla by of *Anthocoris, Campylomma, Chrysoperla, Deraeocoris* and *Formica* in pear orchards (ongoing).

3. Describe daily rhythm of feeding by predators in laboratory microcosms to support molecular estimates of predation rates in the field (incomplete).

Significant findings in 2002-2003

(These findings are refinements of 2002 and represent 16 months of effort)

1. Compared Monoclonal Antibody and DNA digestion rates in 4 predator species highlighting species differences. Found digestion rates of protein versus DNA highly variable among species
   - Anthocorus and Deraeocoris digest prey much more slowly that lacewings
   - The minute pirute bug, *Orius* sp. showed complete digestion of prey DNA at ingestion
   - The two lacewings differed significantly from each other in digestion patterns of protein

2. Demonstrated importance of size of DNA target for PCR on estimating predation rate and showed method superior to use of monoclonal antibodies
   - A. tomentosus digest prey (halflife) in 1 day with 110 -280 bp amplicons
   - A. tomentosus digest prey (halflife) in 6 hours with 1800 bp amplicon
   - lacewings digest prey remains (110-280 bp and MABS) in less than 2 hrs
   - lacewings have completely digested prey at ingestion for large amplicons (700bp)

3. Existing PCR technology remains too unreliable to accurately estimate predation rates
   - Need more complete DNA extraction and stabilization methods.
   - Need improved primer designs to maximize detection of rare DNA molecules
4. Both PCR and ELISA represent useful tools to estimate comparative predation activity
   - Suitable for comparing insects in high host environments versus low host environments
   - Analysis of field populations ongoing

Results and Discussion

Many past studies have characterized psylla abundance from a combination of leaf counts and beat tray data and predator abundance from beat trays and from these data made inferences on predator importance. This includes some of my own data presented previously to the Winter pear and Tree fruit commissions and earlier data collected by Bob Fye and Brad Higbee at the YARL. Similarly there is a collection of studies of how many psyllas various predators can eat in the laboratory (Fye, Brunner, and others). Unfortunately, only a few studies conducted under very restrictive conditions have experimentally estimated predation of psylla by specific free-roaming predators in the field (Unruh and Higbee, 1994). In sum, we do not yet know which the most important predators of pear psylla are and when they are abundant enough to control psylla below damaging levels in the field.

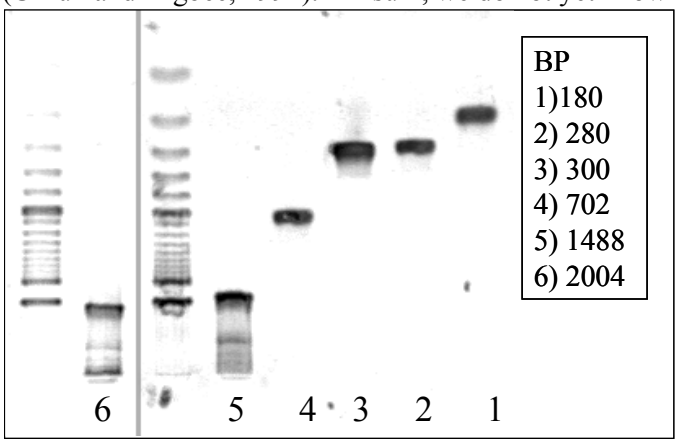


Figure 1. PCR amplification products for primer sets designed to amplify a range in size of segments from the mitochondrial CO1 gene of pear psylla. These primers only amplify psylla and not other insect groups hence they can be used for detection of psylla remains in the guts of predators.

2001: Over the last 6 years we have been studying methods to detect if a predator has eaten a psylla -including specific monoclonal antibodies made against psylla proteins (Horton et al. 1997) and DNA primers that specifically amplify psylla DNA (Agusti, Unruh and Welter, 2003). Dr. Agusti spent 4 months in my laboratory in the winter of 2000-20001. During that period we developed primers that specifically amplify DNA of pear psylla, which can be used to detect "forensic" evidence of psylla in the gut of predators. These primers amplify small pieces of psylla DNA (64- 280 bp). After Dr. Agusti's departure in spring of 2001 we designed primers that amplify larger stretches of DNA (amplicons 300-2,000 bp) as reported in February 2002. (Figure 1from 2002 is reproduced on the left). From this work we have begun to learn details about the digestion biology of key predators.

2002-2003: We completed comparisons between the monoclonal antibody (MAB hereafter) and 3 of the PCR amplicons (280, 702, and 1,800 base pairs, hereafter PCR_{280} etc.) for the predatory bugs, *Anthocoris tomentosus* and *Deraeocoris brevis* and the lacewings, *Chrysopa carnea* and *Chrysoperla rufilabris* (See figures 2-5 below). Surprisingly, the retention time of psylla signal in the gut of Anthocorus and Deraeocoris bug predators is quite long, more than 24 hr. Also, our results with the

bugs show that larger segments of prey DNA are digested more rapidly than smaller segments of DNA. In other words, we can estimate the time since the last meal with greater precision using larger, more rapidly digested, target DNA. The value of short retention times of the prey signal is clear when you consider that some predators may eat more than 1 psylla per hour. A short pulse or signal life is required to have a realistic estimate of predation rate.

The most vivid differences between species is the very rapid digestion of both prey DNA and protein in lacewings compared to the bugs. PCR_{280} and MABs show almost identical digestion rates within *Anthocoris tomentosus and Deraeocoris brevis* with a half life of detection of about 1440 minutes (24 h) but not completely reaching zero (psylla detected) in 2 days when all digestion studies were terminated. In contrast, *Chrysopa carnea* and *C. rufilabris* digested psylla extremely rapidly with a PCR_{110} half life of an hour in *C. rufilabris* and about 2 hours in *C. carnea*. A bizarre result that we are still trying to understand was the extremely rapid digestion of protein in *C. rufilabris*. While protein digestion in *C. carnea* was congruent with DNA digestion, it was much more rapid than small DNA amplicons in *C. rufilabris*. Furthermore, it seemed highly variable, actually rising with time, despite high sample sizes.

The digestion biology of the lacewings differs importantly from the predatory bugs (but see comments below on the mirid, *Orius)*. Both groups inject digestive enzymes into the prey but the lacewings have an incomplete digestive tract as nymphs thus no prey signal is excreted. Furthermore, exo-digestion of the prey, by injected enzymes, is much more thorough and rapid by the lacewings (as evidenced by cadaver examinations) and is especially notable in *C. rufilabris* which digests most of the prey tissues visible the host.

These relationships point out a key strength of the DNA-based method over monoclonal antibodies. Our 2002-3 data indicate that amplicon size must be appropriate the digestion biology of each predator species to be biologically meaningful. We can design DNA primers that best fit a species' digestion biology to more precisely estimate the time since last feeding. In contrasts psylla proteins (as antigens for the antibodies) are likely to be digested at roughly the same rate, independent of protein type.

However, our studies with the minute pirate bug, *Orius tristis* (Miridae) shows that we cannot always adjust PCR amplicon sizes to the digestion biology of the predator. In pilot studies with *Orius* we found that DNA of the prey is apparently completely digested prior to ingestion. That is, *Orius* injects fluids into the prey liquefying it and afterwards sucks in the contents. In our assays, when *Orius* leaves the prey (removes its stylets for 1 minute or more) and is tested immediately, without internal digestion, there is no prey signal in its gut (n=14). In contrast, prey protein can still be detected using our MAB.

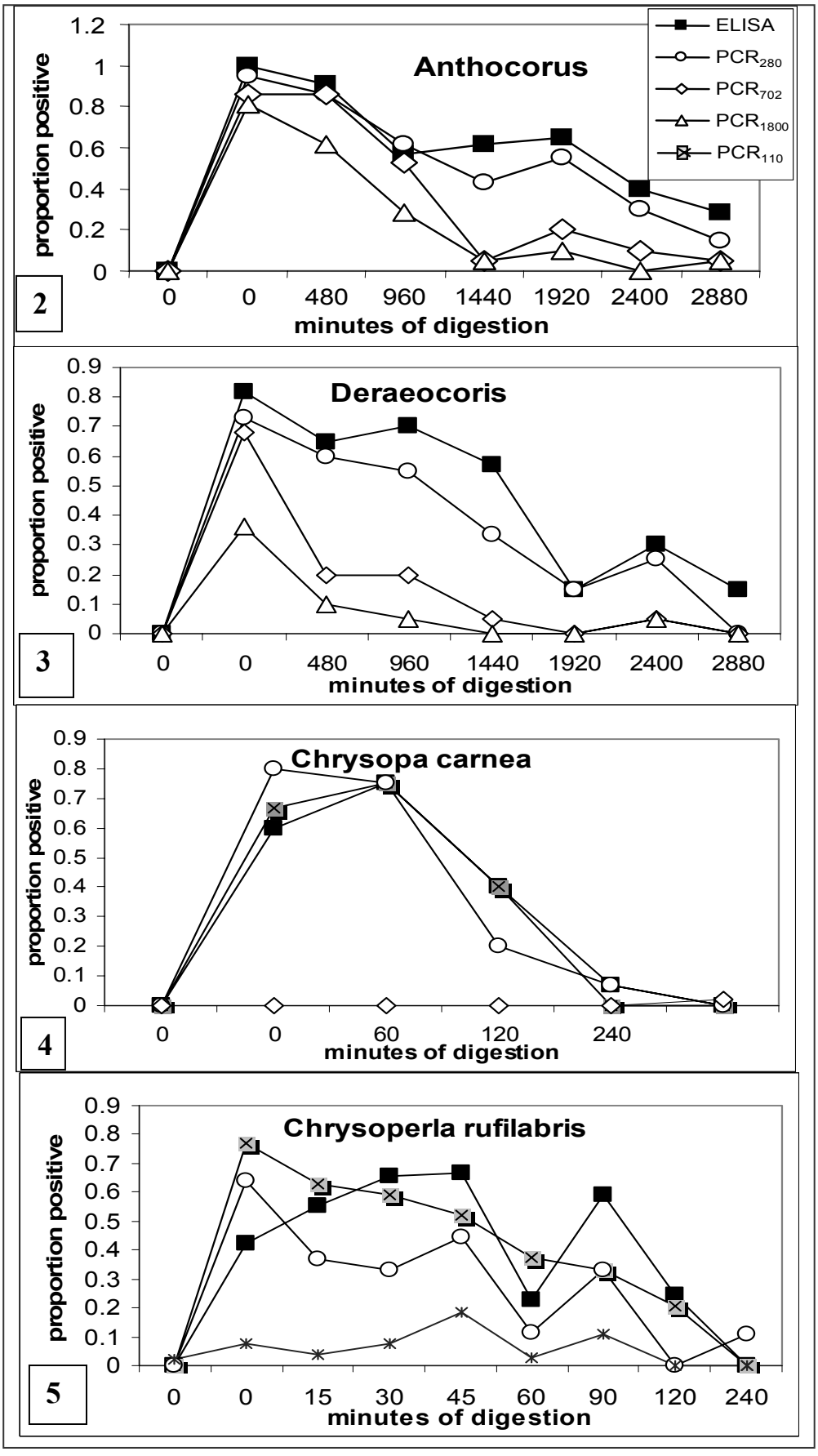
Captions for Figures 2-5. Digestion of psylla protein as measured by ELISA using a psylla MAB and digestion of psylla DNA as measured by PCR of several different sized amplicons

2: For *Anthocoris tomentosus*, ELISA and PCR280 amplicons showed similar digestion profiles that were quite long (Half life of 24 hours) and larger amplicons digested more rapidly.

3. For *Deraeocoris brevis,* patterns are similar to *Anthocoris* with the exceptions that larger amplicons are digested even more rapidly and have lower baselines at zero digestion time.

4. For *Chrysopa carnea* digestion occurred very rapidly, within 4 hours for both protein and DNA (ELISA and PCR methods). Remarkable is that both PCR 110 and 280 amplicons mirror the ELISA digestion curve but larger amplicons (700 and 1800 show no amplification at all)

5. For Chrysoperla rufilabris digestion was even more rapid than in the other lacewing, occurring within 2 hours. There appeared to be high and inexplicable variability in the ELISA pattern with digestion rate estimates sometimes being negative through time. More expected is the digestion curves for the 2 PCR amplicons, 110 and 280. Here the more rapid digestion of the 280 amplicon compared to 110 is what is predicted on d is most consistent with the bug predators.


Anthocorus
ELISA
PCR280
PCR702
PCR1800
PCR110
proportion positive
1.2
1
0.8
0.6
0.4
0.2
0
0
0
480
960
1440
1920
2400
2880
minutes of digestion
2
Deraeocoris
proportion positive
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0
480
960
1440
1920
2400
2880
minutes of digestion
3
Chrysopa carnea
proportion positive
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0
60
120
240
minutes of digestion
4
Chrysoperla rufilabris
proportion positive
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0
15
30
45
60
90
120
240
minutes of digestion
5

FINAL REPORT
WPPC Project # 19906 **Organization Project # 5350-43000-003-008T**

Project title: Manipulation of Pear Fruit Ripening by Control of Ethylene Action
PI: James Mattheis, Plant Physiologist
Organization: USDA, ARS, TFRL, Wenatchee, WA

Cooperators: Paul Chen, Professor Emeritus
Oregon State University Mid-Columbia Experiment Station
Hood River, OR

Rodney Roberts, Plant Pathologist
USDA, ARS TFRL, Wenatchee, WA

Objectives:
A primary objective of postharvest management of pears is to prolong storage life by reducing the rate of fruit ripening. The combination of optimum maturity, refrigeration, postharvest chemical treatments and controlled atmosphere (CA) storage slow ripening and reduce physiological disorders and decay. Controlled atmosphere storage in particular reduces ethylene production as well as the capacity of fruit to respond to ethylene, responses that provide the residual effects of CA after fruit is removed from storage. Because of the importance of ethylene in regulating the processes of fruit ripening, practices that interfere with its production and/or action are useful in the commercial storage of pears.

Researchers at North Carolina State University, Dr. Ed Sisler and Dr. Sylvia Blankenship identified a compound that interferes with fruit ethylene metabolism. This compound, 1-methylcyclopropene (MCP), inhibits ethylene perception and shows great potential as a tool for postharvest management of pears. MCP is a volatile compound that can be applied as a gas treatment without drenching fruit. Results with a number of fruit crops have demonstrated MCP treatment can reduce the rate of climacteric fruit ripening as well as development of a number of physiological disorders.

SIGNIFICANT FINDINGS: 2000-2004

- MCP treatment of 'Bartlett', 'Bosc', 'Comice' or 'd'Anjou' pears with MCP at concentrations between 0.1 to 1 ppm can delay ripening (softening, acid loss, degreening, volatile production) and development of physiological and pathological disorders. Starch and soluble solids metabolism are not significantly impacted by MCP.

- The duration of 'Bosc' responses to MCP have been similar to those of 'd'Anjou', 'Comice' responses have been intermediate between 'Bartlett' and 'd'Anjou'.

- Factors known to influence the duration of pear responses to MCP applied at harvest include cultivar, MCP concentration, fruit maturity when treated, seasonal variation and post-treatment storage conditions (temperature, atmosphere composition).

- Fruit temperature (32 to 68 ^{o}F) during MCP treatment is not a critical determinant of fruit response.

- Peel degreening is slowed but not prevented by MCP treatment. For both 'Bartlett' and 'd'Anjou', degreening can occur prior to softening resulting in a false visual assessment of ripeness.

- Production of volatiles typical of ripe pears is delayed following MCP treatment. Volatile production resumes when ethylene production increases.

- Effectiveness of MCP treatment for 'Bartlett' decreases with firmness loss ($\leq$ 15 lbs) and as the interval between harvest and treatment increases. For 'd'Anjou', treatment delayed 2 weeks or greater may have decreased utility for control of superficial scald.

- Increasing the harvest window for 'Bartlett' fruit down to ~15 lbs has been successful when fruit were treated with MCP soon after harvest.

- MCP treatment of commercially packed boxes of 'Bartlett' or 'd'Anjou' results in typical MCP responses (reduced respiration, ethylene production, slowed loss of quality, delayed onset of decay and development of physiological disorders). However, according to AgroFresh personnel, wet cardboard can absorb MCP and reduce treatment efficiency.

- The duration of MCP responses is dependent on: 1) Storage temperature after treatment. For example, 'Bartlett' fruit treated at 0.5 ppm and stored at 68 ^{o}F ripen within one month of treatment. Response duration increases as storage temperature decreases; 2) Storage atmosphere after treatment. Storage in CA prolongs MCP effects. However, the MCP/CA combination can result in ripening delay for both 'Bartlett' and 'd'Anjou' following removal from CA storage. The delay in ripening after removal from storage decreases with increased storage duration and increased O_2 concentration during storage.

- Effectiveness of MCP treatments applied to partially ripe 'Bartlett' or 'd'Anjou' fruit is determined by fruit ripeness when MCP is applied. 'Bartlett' fruit treated at greater than 13 lbs and 'd'Anjou' greater than 10 lbs have delayed softening compared to controls. Previous storage in CA increases the likelihood of success of ripening delay with a post-storage MCP treatment. The duration of responses to delayed MCP treatment is days to weeks rather than months for fruit treated at harvest and held in cold regular atmosphere (RA) or CA storage.

- MCP treatment applied to 'd'Anjou' pears after > 6 months CA storage does not control development of superficial scald.

- Ethylene treatments up to 10,000 ppm do not result in full recovery of the ripening capacity in 'Bartlett' fruit treated with 0.1 to 0.5 ppm MCP.

- MCP treatment does not increase sensitivity of 'Bartlett' or 'd'Anjou' fruit to CO_2 during CA storage.

- Storage of MCP treated 'd'Anjou' at $\geq$3% O_2 with 0.5% CO_2 may allow fruit to be stored long-term (8-9 months) and ripen after removal from storage.
- Incomplete softening (woody, rubbery texture) that can occur in untreated 'Bartlett' and 'd'Anjou' stored for extended periods in CA can also occur in MCP-treated fruit.

- Low (30-50 ppb) MCP rates slow 'Bartlett' ripening and can prevent 'd'Anjou' superficial scald with minimal delay of ripening. However, technical limitations of delivering this low rate in commercial rooms and lot to lot variability in response to low rates are factors requiring further investigation.

- Pre-shipment conditioning of MCP-treated fruit by holding at warm temperatures accelerates ripening. The duration of warm temperature necessary to initiate ripening decreases with storage duration.

· Decay development in fruit inoculated via fresh wounds is not delayed by MCP treatment.

Procedures:
Pears were obtained from commercial orchards. Fruit were held at 68-70 or 33 ^{o}F in air or CA until MCP treatments were conducted. The treatments were applied to fruit in sealed steel or plastic chambers at 70 ^{o}F for 12 h.

MCP was generated from SmartFresh powder provided by Agrofresh, Inc, a subsidiary of the Rohm and Haas Company. Target MCP concentrations, monitored by gas chromatography, were reached within 10-15 minutes after initiation of gas generation.

Fruit firmness was measured using a Mohr Digi-Test instrument (Mohr and Associates, Richland, WA). Titratable acidity (TA) was determined by titrating fresh juice to pH 8.2, and soluble solids content (SSC) was measured with a refractometer (Atago). Fruit respiration and ethylene production were determined using gas chromatography.

Fruit visual assessments. Peel color was rated 1:green to 5:yellow. Superficial and senescent scald were rated as 1:none, 2: 1 to 33%, 3: 34 to 66%, 4: 67 to 100% fruit surface with light brown discoloration, 5: 1 to 33%, 6: 34 to 66%, 7: 67 to 100% fruit surface with dark brown discoloration. Internal breakdown and core browning (browning within the core line) were rated as 1: none, 2: slight, 3: moderate, 4: severe. Scuffing was rated as 1: absent, 2: present. Decay was rated as 1: absent, 2: present. Objective measures of fruit color were performed using a Minolta colorimeter.

Results and discussion:

Bartlett 2003-04

B2. Pre-conditioning of MCP treated fruit. 'Bartlett' pears harvested at 18 lbs were treated the day of harvest at 300 ppb MCP. Fruit were stored at 33 ^{o}F in air or CA (1.5% O_2, 0.5% CO_2) with up to 20 days at 32, 50, 60 or 70 ^{o}F after 2 or 4 (RA), 4 or 6 (CA) months, returned to 32 ^{o}F for 14 days, then held at 70 ^{o}F for 1 or 2 weeks. Initial 2003 results indicate fruit stored in RA did not require preconditioning for ripening to proceed. Additional results will be presented at the research review.

B3. Post-storage MCP treatment. 'Bartlett' pears harvested at 19.4 lbs were stored at 33 ^{o}F in air or CA (1.5% O_2, 0.5% CO_2). Fruit were treated with 300 ppb MCP after 1, 3 or 5 months, then held at 68 ^{o}F and ethylene production monitored. Fruit were held an additional 5 days after ethylene production exceeded 1 ppm. MCP treatment after 1 or more months RA storage did not delay ethylene production or ripening, however, MCP treatment after 1 month CA delayed ethylene production 1 day. All fruit were soft (less than 3 lbs) and yellow when analyzed.

B4. Response of MCP-treated fruit to storage temperature and O_2. 'Bartlett' pears harvested at 18.7 lbs were treated with 300 ppb MCP, then stored at 33, 37 or 41 ^{o}F in air or CA with 1, 3 or 5% O_2 and 0.5% CO_2 for up to 6 months. After 2 months, MCP treatment and storage in air at 32 or 37 ^{o}F prevented senescent scald and internal breakdown and fruit softened typically in 7 days. Treated fruit stored at 41 ^{o}F in air developed senescent scald. An interaction between temperature and O_2 concentration was evident. Treated fruit stored in CA at 33 ^{o}F did not ripen in 7 days regardless of O_2 concentration. Treated fruit stored at 37 or 41 ^{o}F ripened if O_2 was 3% or higher, all untreated fruit stored under the same conditions were senescent after 7 days.

B5. Lot variability of MCP response. 'Bartlett' pears from 9 grower lots were treated in bins with 300 ppb MCP within 2 days of harvest. Fruit were stored at 33 ^{o}F in air (2 or 4 months) or CA (1.5% O_2, 0.5% CO_2) for 3 or 6 months. Fruit from each lot was preconditioned by holding at 70 ^{o}F for 5 days, returned to 33 ^{o}F for 14 days, then held at 70 ^{o}F for up to 7 days. After 2 months RA or 3 months CA, MCP treatment effects were evident for all lots, however, fruit softening and yellowing were acceptable after 7 days ripening.

'd'Anjou' 2002-2003

A1. MCP rate study. 'd'Anjou' pears from 3 grower lots were treated the day after harvest with 0, 30, 140 or 280 ppb MCP, then stored in air or CA (1.5% O_2, 0.5% CO_2) at 33 ^{o}F. All MCP treatments prevented scald and delayed ripening for fruit from 2 of the 3 lots. For the 3rd lot, 140 ppb was necessary to prevent scald as well as delay ripening.

A2. Pre-conditioning of MCP treated fruit (similar to B2 above). 'd'Anjou' pears harvested at 14.3 lbs were treated the day of harvest at 300 ppb MCP. Fruit were stored at 33 ^{o}F in air with up to 15 days at 70 ^{o}F after 2, 4 or 6 months, then returned to 33 ^{o}F for 2 or 4 weeks. The number of days preconditioning necessary to accelerate ripening decreased with storage duration. After 2, 4 or 6 months, the minimum number of days at 70 ^{o}F to induce ripening after return to cold for 2 or 4 weeks were 10, 5 and 0, respectively.

A3. Post-storage MCP treatment (similar to B3 above). 'd'Anjou' pears previously stored at 32 ^{o}F in air or CA will be treated at 1 ppm MCP, then returned to cold storage for up to 4 weeks followed by up to 14 days at 68 ^{o}F . Results indicated fruit treated with firmness of 9 lbs or higher showed 7 to 14 day delay in softening after MCP treatment. Delayed treatments did not prevent superficial scald but did reduce scuffing and delayed degreening.

'd'Anjou' 2003-2004

A2. Pre-conditioning of MCP-treated fruit. Conducted similarly to B2 above, initial results will be presented at the research review.

A3. MCP and CA O_2 concentration. 'd'Anjou' fruit harvested at 13.4 lbs were treated with 300 ppb MCP then stored at 33 ^{o}F in air or CA with 1, 3 or 5% O_2 with 0.5% CO_2. Fruit will be evaluated after 3, 6 or 9 months. Initial results will be presented at the research review.

A5. Lot variability of MCP response. Conducted similarly to B5 above, initial results will be presented at the research review.

Summary and Conclusions

Experimental use of MCP applied at harvest has consistently resulted in delayed ripening of European pear cultivars including 'Bartlett', 'Bosc', 'Comice' and 'd'Anjou'. Typical responses include delayed softening, degreening, acid loss, volatile production and development of disorders including senescent scald, internal breakdown and decay. When applied in sufficient concentration close to harvest, MCP can be an effective means to prevent development of superficial scald. Fruit in these trials has also remained resistant to scuffing over an extended period compared to non-treated fruit. For all ripening parameters and disorders with the exception of superficial scald, effects of MCP eventually subside and ripening and senescence progress. When ripening resumes, these processes accelerate but not necessarily at the same rates. For example, degreening can occur prior to softening and production of ripening-related aromas. For 'Bartlett', this results in fruit that appears to be ripe

(yellow) but has not fully softened and lacks aroma. For 'd'Anjou', degreening and softening can be coincident resulting in a 'Bartlett' type of ripening where color is indicative of condition.

Table 1. Effects of 1-MCP treatment on quality and ripening of 'Bartlett' pear. Fruit treated with 1-MCP at harvest and stored at 32 ^{o}F in air for 4 months. Color ratings are 1: green, 5: yellow. LSD: least significant difference.

	1 day ripe			7 day ripe		
1-MCP ppm	**Firmness lbs**	**TA %**	**Color 1-5**	**Firmness lbs**	**TA %**	**Color 1-5**
0	14.0	0.30	4.7	3.1	0.25	5.0
0.030	14.6	0.38	4.9	2.9	0.35	5.0
0.300	15.6	0.34	3.2	10.3	0.31	4.8
1.0	16.5	0.34	3.7	7.7	0.31	5.0
$LSD_{0.05}$	1.3	0.04	0.7	1.3	0.03	0.3

Treatment with MCP at harvest provides the maximum duration of response for all cultivars tested. MCP-treated fruit will resume ripening given enough time, but identification of a process to reliably predict how long a period is required requires further research. Resumption of ripening is accompanied by increased ethylene production and respiration rate, however, ripening of MCP-treated fruit is not accelerated by exogenous ethylene. Trials with 'Bartlett' where MCP-treated fruit were exposed to up to 10,000 ppm ethylene did not demonstrate a reversal of MCP effects. Storage temperature, oxygen concentration, MCP concentration, storage duration, harvest to treatment interval, and fruit ripeness when treated all impact the duration of MCP responses. Manipulation of one or more of these factors can impact MCP responses, but all require a high level of management to be successful. Results to date from trials where application rate, harvest maturity, harvest to treatment interval, and storage environment have been examined indicate manipulation of the duration of MCP responses using one or more of these factors is feasible. What remains to be identified are commercial protocols that are consistently effective and provide predictable estimates of the period over which ripening will occur following the preconditioning treatment.

Table 2. 'Bartlett' firmness and incidence of disorders following storage plus 7 days at 68 ^{o}F. Fruit were treated with 300 ppb 1-MCP at harvest then stored in air at 31, 33 or 35 ^{o}F.

					TA		C-D0		C-D7					
			Lbs		% malic		1-5		1-5		CB	Decay	SB	IB
Month	Temp	Treat	Mean	SE	Mean	SE	Mean	SE	Mean	SE	%	%	%	%
0		Initial	17.2	0.3	0.323	0.014	1.0	0.0			0	0	0	0
1	31	check	2.6	0.1	0.263	0.007	1.7	0.1	5.0	0.0	40	0	0	0
		MCP	17.5	0.5	0.354	0.014	1.1	0.1	1.6	0.1	0	0	0	0
	33	check	2.4	0.1	0.260	0.014	1.5	0.1	5.0	0.0	60	0	0	0
		MCP	17.9	0.5	0.342	0.010	1.1	0.1	1.7	0.1	0	0	0	0
	35	check	2.3	0.2	0.233	0.014	1.8	0.2	5.0	0.0	25	0	0	0
		MCP	15.1	0.5	0.309	0.011	1.4	0.1	2.1	0.1	0	0	0	0
3	31	check	3.4	0.5	0.246	0.010	3.5	0.2	4.6	0.1	75	30	0	0
		MCP	16.8	0.3	0.322	0.010	2.2	0.2	2.8	0.1	0	0	0	0
	33	check	4.5	1.3	0.187	0.004	3.7	0.1	4.2	0.1	0	0	0	0
		MCP	12.8	0.6	0.275	0.005	2.3	0.1	3.3	0.1	0	0	0	0
	35	check	.	.	.	.	4.0	0.0	.	.	100	100	90	.
		MCP	8.3	0.7	0.287	0.007	3.4	0.1	4.3	0.1	0	0	0	0
5	31	check	.	.	.	.	.	.	.	.	.	100	90	.
		MCP	10.2	0.8	0.267	0.006	2.8	0.1	4.9	0.1	0	0	0	0
	33	check	.	.	.	.	.	.	.	.	.	10	90	0
		MCP	7.2	0.8	0.244	0.007	4.8	0.1	5.0	0.1	0	20	0	60
	35	check	.	.	.	.	.	.	.	.	.	.	100	.
		MCP	9.7	2.5	0.202	0.010	5.0	0.0	5.0	0.0	0	10	0	90

TA: titratable acidity; C-D0: color when removed from storage (day 0); C-D7: color 7 days after removal from storage; CB: core browning; SB: senescent breakdown and scald; IB: internal breakdown; SE: standard error of the mean.

Preconditioning prior to shipment is currently practiced using ethylene to stimulate ripening. Research conducted over the past two seasons indicates periods at higher than typical storage temperatures can result in accelerated recovery of the capacity to ripen in MCP-treated fruit. While these experimental results indicate the possibility of development of commercially useable protocols, more information is needed to assess the consistency of preconditioning protocols between lots and production seasons.

Table 3. Firmness of 'd'Anjou' pear fruit following preconditioning at various temperatures. Fruit were treated at harvest with 300 ppb 1-MCP, then stored at 32 ^{o}F in air for 4 months. Pears were then stored for 5 days at condition temperatures, then returned to 32 ^{o}F for 2 or 4 weeks. Fruit were then held at 68 ^{o}F for 10 or 20 days.

		Firmness lbs			
Conditioning temperature °F	**after 5 days conditioning**	**+2W 32 °F + 10D 68 °F**	**+ 2W 32 °F + 20D 68 °F**	**+ 4W 32 °F + 10D 68 °F**	**+ 4W32 °F + 20D 68 °F**
32	12.5	11	7.5	10.7	5
41	13.5	10	5	6.2	3.8
50	11	5	2.8	3.3	2.2
59	12	5	1.7	3.2	-*
$LSD_{0.05}$	ns	1.5	1.8	1.4	1.3

*Non-marketable fruit due to senescent breakdown and decay.

Table 4. Firmness of 'Bartlett' pears following 2 months storage plus 7 days at 68 °F. Fruit were treated at harvest with 300 ppb MCP, then stored in air or CA at temperatures and atmospheres indicated. Values are means (n=20), where IB (internal breakdown) or S.Scald (senescent scald) are indicated >75% of

Temp F	**O_2/CO_2**	**check**	**MCP**
32	RA	1.9	5.8
	1/0.5	2.4	15.6
	3/0.5	2.0	16.6
	5/0.5	1.7	13.4
37	RA	IB	2.5
	1/0.5	IB	13.7
	3/0.5	IB	5.8
	5/0.5	IB	8.9
41	RA	S.Scald	S.Scald
	1/0.5	IB	10.5
	3/0.5	IB	3.4
	5/0.5	IB	2.3

Development of decay has been consistently delayed in our trials. While it is evident that MCP applied to field run fruit can delay development of decay, the key word is delay. In all of our trials, marketability of MCP-treated fruit is eventually limited in part by decay. Measures to control decay will continue to be necessary for fruit that is stored after treatment. Results to date indicate similar challenges for use of TBZ, that being the causal organism typically changes from *Botrytis* (grey mold) to *Penicllium* (blue mold) when TBZ has been used. Based on work with the yeast *Cryptoccus laurentii*, MCP appears compatible with this and presumably similar biocontrol agents.

Experiments conducted in the fall of 2003 included application of MCP in research scale CA rooms at the Stemilt RCA facility. Fruit in bins were treated and responses similar to those of small scale laboratory applications have been observed. While these results demonstrate efficacy of MCP for application to fruit in bins, a number of MCP trials in large CA rooms conducted over the past 2 years have failed. There are a number of possible explanations for these failures and identifying what factor or factors are responsible for non-performance when MCP is applied in large commercial rooms requires further research.

MCP is a powerful tool for manipulation of the fruit ripening process. Its availability to the research community allows investigations of fruit development and ripening to be conducted that previously were not possible. However, pear quality at the point of consumption requires a soft, juicy texture, typical aroma, with an attractive appearance. The challenge to use MCP commercially is to 1) have efficacy, and 2) have predictability of ripening. Based on our experience to date, both of these challenges appear to be achievable but may require a level of management beyond what is currently practiced by the fresh pear industry.

Publication:

Argenta, L.C., Fan, X., and Mattheis, J.P. Influence of 1-methylcyclopropene on ripening, storage life and volatile production by 'd'Anjou' cv. pear fruit. J. Agric. Food Chem. 51:3858–3864. 2003.

Project title: Manipulation of Pear Fruit Ripening by Control of Ethylene Action
PI: James P. Mattheis
Project duration:: 2002-2003
Project total (2 years): $54,238

Year	Year 1 (2002)	**Year 2 (2003)**
Total	$26,075	**$28,163**
Current year breakdown		
Salaries[1]	$17,904	**$19,664**
Operations (lab supplies, fruit)	$ 2,800	**$ 2,600**
Employee benefits	$ 5,371	**$ 5,899**
Total	$26,075	**$28,163**

[1]GS-9 biological science technician, 0.5 FTE

This project was started under ARS Project #5350-43000-003-06T in 2000. Due to administrative contract changes it is now under ARS Project #5350-43000-003-08T. Funding for this project has also been received from AgroFresh, Inc., $25,000 per year.

FINAL REPORT
WTFRC Project # PR-01-88

Title: Use of Hexanal Vapor for Aroma Production and Decay Control

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

Co-operator: Peter Sanderson, WTFRC, Wenatchee, WA

OBJECTIVES
1. Identify optimal hexanal concentration, temperature, and duration required to control *Penicillium expansum* (blue mold), *Botrytis cinerea* (grey mold), and *Mucor piriformis* (mucor rot).
2. Determine optimum concentration and length of exposure required to fumigate pears in commercial storage rooms.
3. Determine effect of hexanal fumigation on stored pear aroma
4. Evaluate the potential for combining hexanal with 1-MCP to control post harvest decay and improve pear aroma.

SIGNIFICANT FINDINGS
Objective 1
Optimal hexanal concentration was 2 mg/l for 24 hrs or 4 mg/l for 18 hours. The most effective temperature was 20^0C (68^oF) for the control of *Penicillium* and either 15^0C ($59\ ^o$F) or 20^0C (68^oF) for the control of *Botrytis*.

Objective 2
This was not completed due to logistic factors. We were not sure of the hexanal rates and temperatures to be used and required more results on the use of hexanal prior to using hexanal at the commercial facility. The major concern was how the fumigated pears were to be disposed of.

Objective 3
Sensory panel results showed that hexanal improved the aroma on stored pears.

Objective 4
The rates of 1-MCP for use on pears are not completely known, and that this objective was considered important enough to be a separate project. See progress report on "1-MCP Interaction with Fumigants to Control Decay".

METHODS
Large Scale Efficacy Tests
2001-2002 Two bins of d'Anjou pears, provided by Peter Sanderson (WTFRC) were split into 4 half bins. Two half bins were fumigation in the Fumigation building at 4 mg/l for 48 hours at 2^0C, and two half bins as controls. The level of hexanal during the fumigation was monitored by using a gas chromatograph. After fumigation, the pears were hand packed in polylined boxes with top pad and lid, but no paper wraps was used, and placed into the 1^0C (34^oF) cold storage. A bin of d'Anjou pears from PARC, Summerland, was also split and fumigated as above.

2002-2003
WTFRC d'Anjou Pears. Two bins of d'Anjou pears from Wenatchee, WA were divided into three replicates and hand packed in polylined boxes. The pears were placed in a 1 m^3 chamber at a temperature of 15^0C ($59\ ^o$F) and fumigated at 4 mg/l for 18 hours. After fumigation the polyliner was

closed up, with a top pad and lid. The pears were placed into a 1^0C (34 ^{o}F) cold room. Subsamples were inoculated with both blue and grey mold and fumigated at 2 mg/l for 24 hours, or 4 mg/l for 18 hours.

PARC d'Anjou Pears. All PARC d'Anjou pears were treated two weeks prior to harvest with a preharvest application of Vangard 75WG (Cyprodinil, a systemic fungicide, registered on grapes for the control of *Botrytis*) using two different rates. This allowed for a control (no preharvest spray), Vangard 1 (5oz /ac, approx. 1/2 the grape rate) and Vangard 2 (approx. recommended grape rate (10oz/ac)). d'Anjou pears (3 boxes of each treatment) were harvested on the day of the fumigation. The harvested fruit, were immediately placed in the cold room and air cooled to 15^0C (59 ^{o}F). The treated pears were then fumigated at 2mg/l for 24 hours or 4 mg/l for 18 hours. Following fumigation, the pears were hand packed in polylined boxes with top pad and lid, and placed in the cold room at 1^0C (34 ^{o}F).

2003-2004

All d'Anjou pears were treated two weeks prior to harvest with a preharvest application of Vangard 75WG (Cyprodinil, a systemic fungicide, registered on grapes for the control of *Botrytis* at 6.2 g/10 litres (10oz/ac)). d'Anjou pears were harvested on the day of the fumigation. The harvested fruit, were immediately placed in the cold room and air cooled to 15^0C (59 ^{o}F). The treated pears were then fumigated at 3 mg/l for 24 hours. Following fumigation, the pears were hand packed in polylined boxes with top pad and lid, and placed in the cold room at 1^0C (34 ^{o}F). A fourth replicate was done and used for quality analysis (fruit firmness, pH, titratable acidity (TA) and soluble solids). All the replicates will be evaluated for post harvest decay and quality at the end of January 2004.

Table 1. Description of the various treatments.

Treatment	Description
Check Pick and Cool	No preharvest spray. Pears were picked, placed in polylined boxes, and placed in cold room at 1^0C (34 ^{o}F).
Check No Hexanal	No preharvest spray. Pears were picked, left at 15^0C (59 ^{o}F) for duration of treatments (24 hours), then placed in cold room at 1^0C (34 ^{o}F).
Check Hexanal (3 mg/l for 24 hours)	No preharvest spray. Pears were picked, placed at 15^0C (59 ^{o}F) and treated with hexanal for 24 hours then placed in a cold room at 1^0C (34 ^{o}F).
Vangard (10oz/ac) Pick and Cool	Pre harvest spray of Vangard, two weeks before harvest. Picked, boxed and placed in cold room at 1^0C (34 ^{o}F).
Vangard (10oz/ac) No Hexanal	Pre harvest spray of Vangard, two weeks before harvest. Picked, left at 15^0C (59 ^{o}F) for duration of treatments (24 hours), then placed in cold room at 1^0C (34 ^{o}F).
Vangard (10oz/ac) Hexanal (3 mg/l for 24 hours)	Pre harvest spray of Vangard, two weeks before harvest. Picked, placed at 15^0C (59 ^{o}F) and treated with hexanal for 24 hours then placed in a cold room at 1^0C (34 ^{o}F).

Small Scale Efficacy Tests

2001-2003

Tests to determine hexanal efficacy and phytotoxicity were done by inoculating with a set number of spores (1 x 10^4 CFU/ml) of a decay-causing fungus was misted over the fruit surface, and allowed to dry. The pears were either fumigated then wounded with a sterile wounding device (3mm diameter, 3mm deep) (IWF), or wounded after fumigation (IFW). The inoculated pears were placed in the 1 m^3 chamber. The humidity was adjusted to 80+% if necessary by evaporating water into the chamber.

Laboratory Grade Hexanal liquid was evaporated by heating with a small electric heater. The hexanal concentration was monitored by withdrawing a 250 ml sample of air from the chamber via vacuum pump shortly after the start and at regular intervals during fumigation. The air sample was injected into the gas chromatograph (GC Model 910, Questron Technologies Corp. Mississauga, Ontario) and within approx. 1 minute the concentration in the chamber was known. The GC was outfitted with an 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 chamber used for fumigation was placed at 20^0C (68°F), 15^0C (59 °F), 10^0C (50°F), or 5^0 C (41°F). Fumigated fruit was placed at 20^0C (68°F) for seven days when decay and phytotoxicity were recorded.

2003/2004
Quality Analysis
One of the concerns expressed to us was whether or not fumigating at 15^0C (59 °F) would affect the quality of the pears in storage. Though this was not one of our original objectives for this study, it was a valid concern. A number of tests were designed to address this concern.

Fruit Firmness: Using a pressure tester Model EPT-1 with a 11.7 mm tip, the various treatments were checked for fruit firmness; Brinkmann 719S Titrino was used to determine pH, and titratable acidity (TA) by titrating 15 mls of fresh juice to pH 8.2. An AO Scientific Instruments (Buffalo New York) , digital refractometer ABBE MARK II was used to determine soluble solids The various treatments were checked for these values immediately after fumigation, then 51 days later, and at 82 days. Pears flesh firmness will be tested in mid-late January 2004, before and after they have been allowed to ripen at 20^0C (68 °F) for seven days.

Headspace Analysis: Nine d' Anjous from each treatment, were sliced into 8 slices using a fruit sectionizer and randomly placed into clear standard gauge cryovac bags. The bags were immediately sealed with a Swiss vac bag sealer, Type Minor 2. Each bag was previously fitted with a homemade septum consisting of a 2 cm^2 piece of yellow highway tape with a blot of Permatex blue sensor-safe gasket maker (Permatex Canada Inc, Mississauga, Ontario, Canada). The headspace was sampled one hour later using a one ml syringe and injecting the sample into the gas chromatograph. The bagged pears were repeatedly sampled at various times over the next 150 hours.

Tissue Analysis: A minimum of 2 pears per replicate were used. Each pear was cut into 8 slices using a fruit sectionizer. A core from four slices per fruit was taken using a #4 coring tube. Five grams of tissue was added to 10 mls of 0.1M HCl. The sample was homogenized for 60 seconds using the Brinkmann Homogenizer. Five mls of the fruit slurry was placed in a 25 ml vial, and sealed. The samples were then incubated for one hour in a water bath at 60^0C (140 °F). A 1 cc headspace sample was taken using a BD 1 ml sub-Q syringe, and inject into a gas chromatograph. A standard was made using 5.0 µl hexanal in 10 ml 0.1 M HCl and then shaken to mix. A 0.5 ml sample was added to 4.5 ml 0.1 M HCl in a 25 ml vial. This sample was incubated for one hour at 60^0C (140 °F). A cc headspace sample was injected into the gas chromatograph.

RESULTS AND DISCUSSION
Large Scale Efficacy Test
2001/2002.
Half bins of d'Anjou pears (2 bins from Washington, and 1 bin from PARC, Summerland, BC were fumigated (Sept 2001) with hexanal at 4 mg/l for 48 hours at 2^0C (36 °F). These pears were placed in polylined boxes and stored at 1^0C (34 °F) until May 2002. The results indicated that hexanal reduced grey mold rot on WTFRC fruit (Pr>f = 0.0216). In this trial hexanal was not effective against blue mold, or stem contamination. This was the first preliminary experiment that was done and treatment conditions were estimated based on previous work with acetic acid fumigation and information found

in the publication by Song et al. (1996). This preliminary experiment provides evidence that hexanal could reduce post harvest decay in stored fruit.

2002/2003
The bin quantity experiments were repeated in 2002-2003 but at a temperature of 15^0C (59 ^{o}F) using a rate of 4 mg/l for 18 hours and 2 mg/l for 24 hours. The pears were cooled to 15^0C (59 ^{o}F) then fumigated. The results from this experiment were evaluated in late Jan 2003. The results are presented in figure 1.

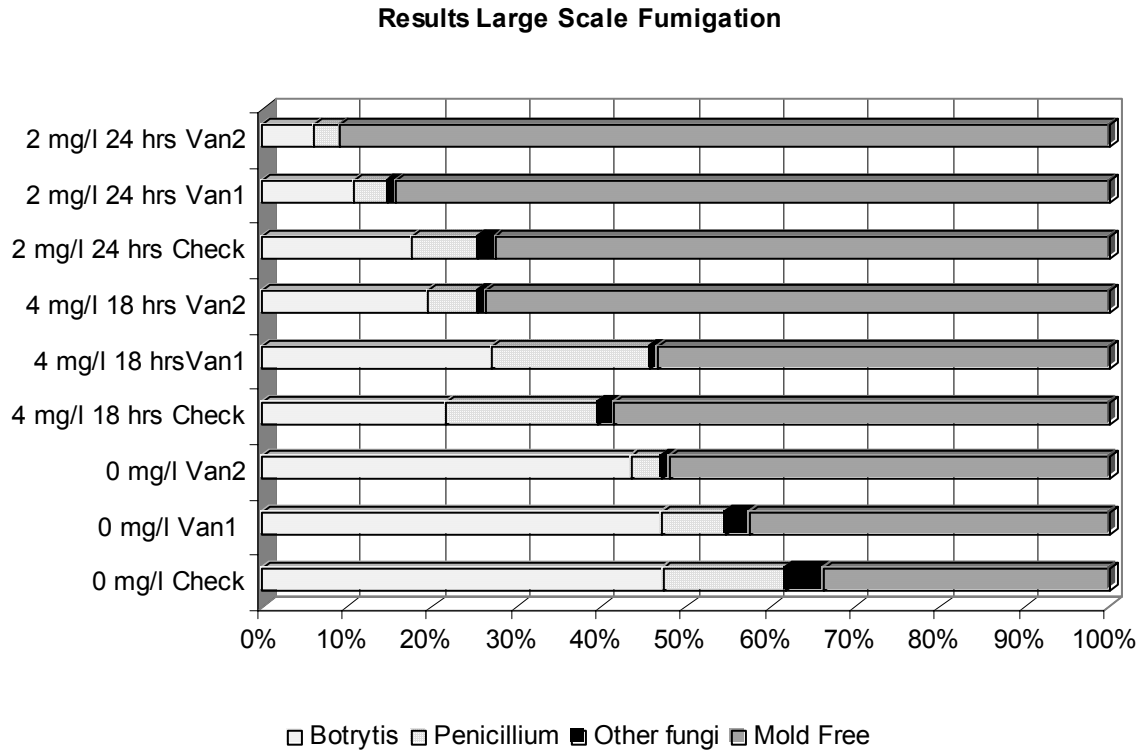


Figure 1. Results of large scale fumigation experiment

2 mg/l of hexanal for 24 hours at 15^0C (59 ^{o}F) was the best rate and duration to control post harvest pathogens on naturally contaminated pears. A single application of Vangard at the rate of 10oz/acre (Van2), two weeks prior to harvest significantly reduced the incidence of decay.

2003-2004
The results of this years experiment will be evaluated in Jan 2004 and the results presented at the Northwest Pear Research Review in Feb 2004.

Small scale efficacy tests
Botrytis (Grey Mold)
The results of d'Anjou pears which were inoculated, wounded then fumigated (IWF) at various temperatures and rates is shown in figure 2 (left half of page Fig 2 a, c, e). Also shown is the effect of Vangard on wounds. Compared to the control (untreated) and Vangard 5oz/acre rate (VAN1), the Vangard 10oz/acre (VAN2) treatment showed a significant reduction in the amount of rot that developed in the wounds. The fumigation rate of 4 mg/l at 20^0C (68^oF) also reduced the amount of decay when compared to the controls. Wounds were fresh when inoculated and hexanal would work better on dry wounds.

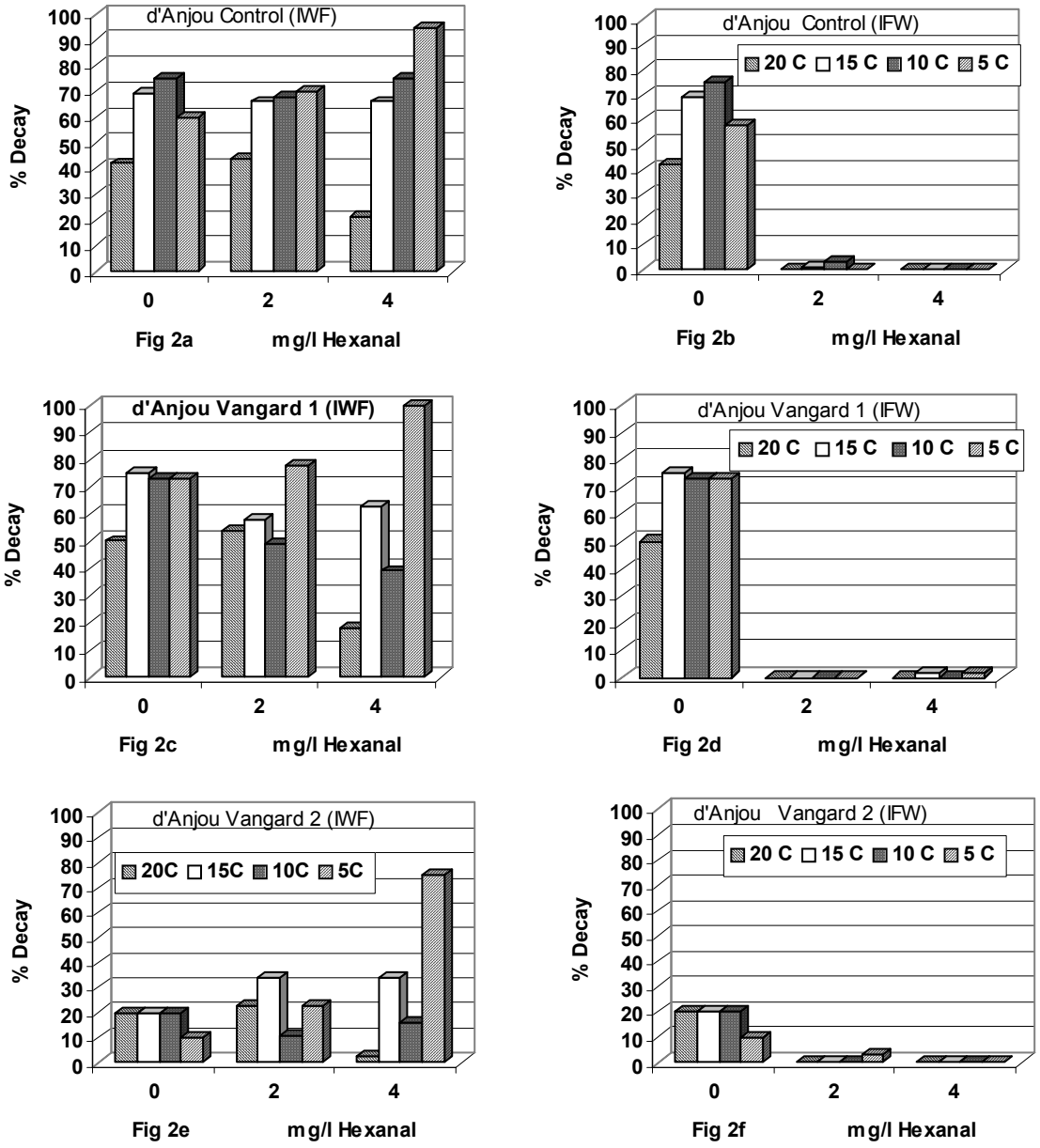


Figure 2. The effect of hexanal on (grey mold) inoculated, wounded, then fumigated (IWF) d'Anjou pears (Fig 2a, 2c, 2e) vs inoculated, fumigated then wounded (IFW) d'Anjou pears (Fig 2b, 2d, 2f). Hexanal used as a surface sterilent on d'Anjou pears, shows an almost total reduction of decay (right half of page Fig 2b, d, f). Hexanal controlled grey mold at low (5^0C, 41 ^{o}F) and high (20^0C (68^{o}F)) temperatures with a low rate of 2 mg/l for 24 hours. Note the effect of the 10oz/acre rate of Vangard in the absence of hexanal (fig 2e & 2f).

Penicillium **(Blue Mold)**

Hexanal research on blue mold was limited to apples but early indication are that the results would be the same on pears. Hexanal does not reduce blue mold in wounds. Hexanal will significantly reduce blue mold on the fruit surface but only at 20^0C (68^{o}F) and 15^0C (59 ^{o}F). (Fig 3a, 3b).

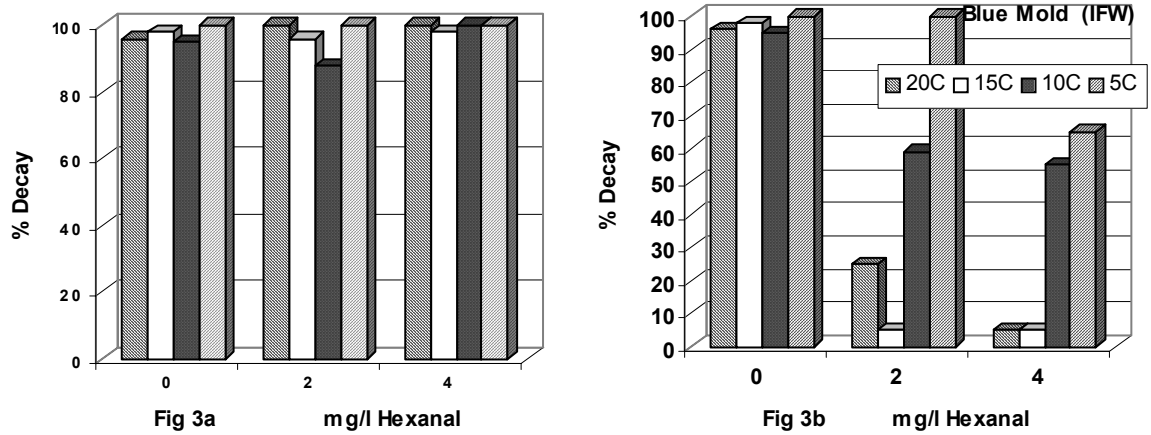


Figure 3. The effect of hexanal on (Blue mold) inoculated, wounded, then fumigated fruit (IWF) (Fig 3a) vs inoculated, fumigated then wounded fruit (IFW) (Fig 3b) at a range of temperatures.

Sensory Panel. A sensory panel of 21 judges rated d'Anjou pears which had been fumigated and then stored for two months. The hexanal fumigated d'Anjou pears had a significantly fruitier aroma, then the nonfumigated pears (GLM procedure Pr>F 0.0002).

MCP and Hexanal. Rates of 1-MCP (1-methylcyclopropene) for use on pears are presently unknown. Pears treated with 1-MCP (apple rate of 100 ppm) do not ripen with removed from storage. 1-MCP apple rates have been defined and an experiment is being conducted on apples only. The results from this experiment were that there were no significant differences in the amount of decay that occurred on apples. No decay occurred on the check apples. The hexanal fumigation proved phytotoxic in this experiment. Approximately 30% of the treated fruit displayed scald-like symptoms. No scald was found on apples not treated with hexanal. This may be due to the apples having been cooled, then warmed, fumigated and then re-cooled as no scald like symptoms were seen in any of the large or small scale trials.

Quality Analysis
Fruit Firmness
Table 4 and Figure 4 show the results for fruit firmness of the various treated d'Anjous.

The pears that remained at 15^0C (59 ^{o}F) for 24 hours, but were not treated with hexanal show lower fruit firmness and hence quality of the fruit by day 82 (see Table 4 and Figure 4). Fumigated pears had the same fruit firmness as those which were harvested and immediately placed into the cold room at 1^0C (34 ^{o}F).

Table 4. d'Anjou quality (fruit firmness in kg)

Treatment	Day 0	Day 51	Day 82
Check Pick & Cool	11.44	11.17	10.70
Check No Hexanal	11.35	10.30	**7.29**
Check Hexanal	12.08	11.58	10.50
Vangard Pick & Cool	11.21	10.51	10.65
Vangard No Hexanal	11.23	10.80	**6.32**
Vangard Hexanal	11.25	9.28	9.14

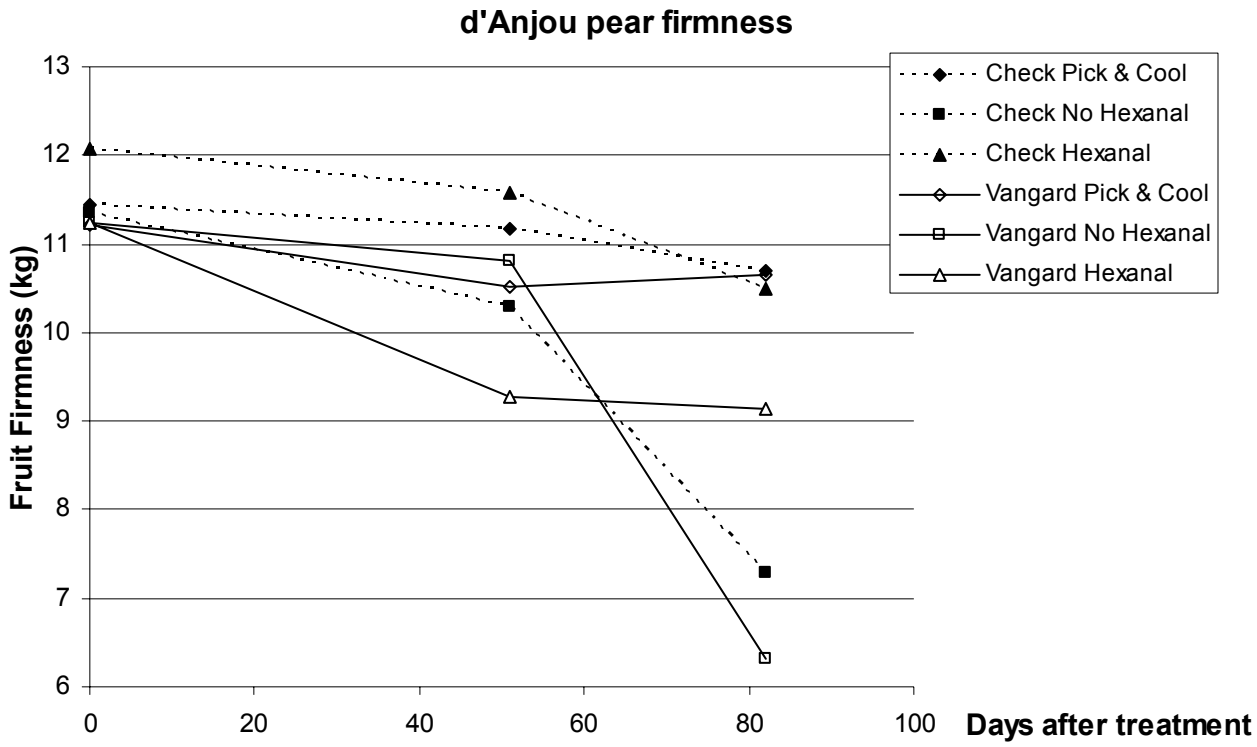


Figure 4. d'Anjou pear fruit firmness over time in air storage at 1^0C (34 ^{o}F).

Tissue Analysis

Table 3. The amount of hexanal in pear tissue, both those treated with and without hexanal.

	Pick and Cool	**No Hexanal**	**Hexanal treated**
Check	0.0361 mg/l	0.0608 mg/l	0.0339 mg/l
Vangard (10 oz/acre)	0.0327 mg/l	0.0505 mg/l	0.0219 mg/l

The d'Anjou pears which remained at 15^0C (59 ^{o}F) but were not treated, had a higher level of hexanal than both the pears which were immediately placed at 1^0C (34 ^{o}F) and those pears treated with hexanal. Furthermore, there is no indication that there are higher levels of hexanal in pears treated with hexanal at the rate used in this trial.

Headspace Analysis

Figure 5 shows the amount of hexanal released from the bagged d'Anjou pears over 150 hours. The non-fumigated d'Anjou pears which had remained at 15^0C (59 ^{o}F) for 28 hours, have a higher level of hexanal than either the treated pears or those harvested and placed immediately into the cold room at 1^0C (34 ^{o}F). This maybe an indication of fruit maturity as apples which are more mature generate more volatiles (Fellman et al. 2003). The non treated d'Anjou pears were yellow in color compared to the treated pears, and were softer and juicer when sliced with the sectionizer. Note that there is not an increase in the amount of hexanal in those samples which had been fumigated with hexanal.

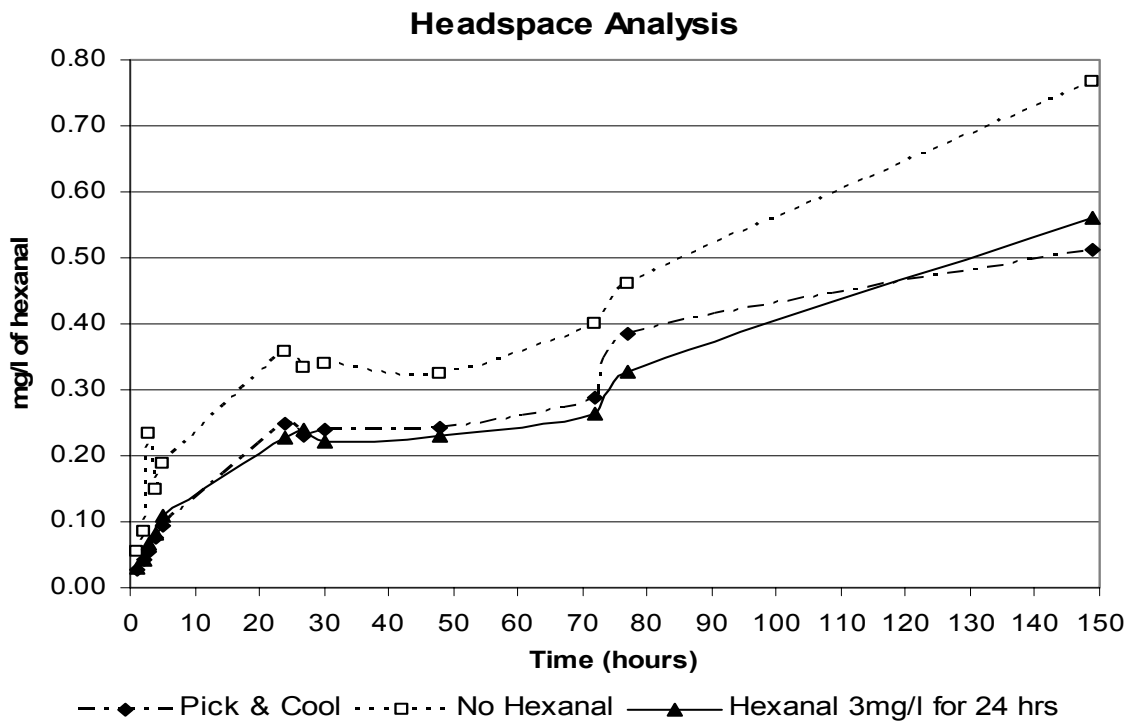


Figure 5. Quantity of hexanal released into the headspace of sliced bagged d'Anjou pears.

The three methods used to analyze pear quality showed that the quality was not significantly changed by being treated with hexanal at 15^0C (59 ^{o}F) for 24 hours when compared to the pears which were harvested and placed immediately into cold storage (Pick & Cool).

Conclusion

This study supports the use of hexanal to control post harvest decay if the fumigation is done at 15^0C (59 ^{o}F) or 20^0C (68^{o}F).

The aroma that develops after the pears have been at room temperature for several days is greater for the fumigated pears then non-fumigated pears.

Using hexanal at 15^0C (59 ^{o}F) for up to 24 hours does not have an effect on the overall firmness of the fruit.

Budget:
Project Title: Use of Hexanal Vapor for Aroma Production and Decay Control
PI: Paul Randall, Peter Sholberg, PARC, Summerland, BC
Project Duration: 2001-2004
Project total (3 years): $22,500

	Year 1	Year 2	Year 3
Year	**2001-2002**	**2002-2003**	**2003-2004**
Salary	6,500	6,500	6,500
Materials and supplies[1]	500	500	500
Travel[2]	500	500	500
Total	**7,500**	**7,500**[3]	**7,500**[3]

1. Supplies include such items as Petri dishes, GC supplies, pears, boxes, packs and hexanal
2. Possible travel to Washington to treat pears at a packinghouse.
3. Funds matched by the Matching Investment Initiative Program of Agriculture and Agri-Food Canada,

References:

Fellman, JK; Rudell, DR; Mattinson, DS; Mattheis JP; 2003 Relationship of harvest maturity to flavour regeneration after CA storage of 'Delicious' apples. Postharvest Biology and Technology 27: p39-51.

Sholberg, P.; Randall P. 2004. MCP Interaction With Fumigants to Control Decay. 2004 Northwest Pear Research Review, 19-20 Feb 04.

Song, J., Leepipattanawit, R., Deng, W., Beaudry, R.M. 1996. Hexanal vapor is a natural, metabolizable fungicide: inhibition of fungal activity and enhancement of aroma biosynthesis in apple slices. J. Amer. Soc. Hort Sci. 12: 937-942.

Project title: **Ethylene ripening of pears by unconventional means**

PI: Dr Keith Sharrock

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CO-PI: Dr Ron Henzell (HortResearch)

Cooperators: Dr Roger Harker (HortResearch), Dr Anna Marin (OSU Portland)

OBJECTIVES FOR 2003:

- Determine the threshold levels of ethylene required at 7ºC and 20ºC to trigger induction of normal ripening of Green Anjou (in US) and Comice (in NZ) after various periods of storage.
- Identify an appropriate level of clamshell ventilation that is sufficient to prevent detrimental modification of respiratory gas levels while permitting an enclosed ERC (ethylene release capsule, HortResearch, patent pending) to promote ripening of Green Anjou and Comice.

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 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.
- Establish whether eating qualities of Green Anjou and Comice are influenced by continuous exposure to ethylene throughout ripening, relative to a conventional restricted 2-3 day exposure to initiate ripening, followed by a return to normal air. Taste panelists will also supply feedback on the likelihood of consumer acceptance of an ERC within pear packaging.

The above objectives differ from those originally proposed in the following respects:

1. Formal taste panel assessments of the effects of continuous exposure to ethylene during ripening have been deferred until 2005, as a cost-saving measure and also to ensure that the best possible use is eventually made of the taste panels and facilities. We will continue to carry out informal small scale taste comparisons in 2004 in preparation for this.
2. Investigation of the influence of ethylene concentration and temperature during conditioning has been extended to include monitoring of aroma production as well as firmness, using our ripeSense labels in a manner proven useful during 2003, but after only two, rather than three, periods of cold storage. The latter change is to avoid the complications caused by fruit producing its own ethylene, which was beginning to occur after 5 weeks of storage in 2003.
3. Application testing of the ERCs has been extended to include their use in standard 20 kg fruit boxes as well as in clamshells.
4. Comice has been substituted for Taylor's Gold since it has more relevance to the US industry.

Significant findings: Although still very early in the project, with the New Zealand season of the first year's research not yet begun, the following interim US results and conclusions seem significant:

- Dose-response curves were evident in the effects of ethylene at increasing concentrations on fruit firmness and aroma production. Ethylene concentration effects were most pronounced if conditioning was attempted within three weeks of harvest.
- Definite stimulation of ripening (assessed on the basis of both firmness and aroma production) resulted from prolonged exposure to ethylene at levels as low as 2 ppm ethylene for 7 days at 20°C. Shorter 3 day exposure required higher levels of ethylene to trigger the same effect.
- Ethylene conditioning at the lower temperature (7°C) did not impact greatly on subsequent fruit softening, but clearly increased aroma production capacity, particularly at one week after harvest.
- Effects of increasing levels of ethylene on firmness plateaued at about 10 ppm, whereas effects on aroma production were still continuing to increase at 100 ppm. More than 100 ppm at 20°C is optimal for stimulation of full aroma potential of fruit during the first three weeks after harvest.
- A single ERC in the central well of a HortResearch 4-pack pear clamshell was capable of maintaining an ethylene concentration of at least 40 ppm for up to seven days, despite ventilation in the base of the clamshell which prevented CO_2 from accumulating to detrimental levels. The ERC induced ripening equivalent, in terms of softening and aroma production, to that induced in pears in the same pack by 3 days in a conditioning room containing 200 ppm ethylene at 20°C.
- Four or five ERCs per 20 kg box were sufficient to significantly accelerate ripening of Anjou tested at 36 days after harvest, even using conventional perforated liners. Larger ERCs will be developed to better suit this application.

Methods

Ethylene threshold and influence of temperature during conditioning Threshold levels of ethylene required to trigger pear ripening were investigated using commercially mature Green Anjou pears stored at -1ºC for 1, 3 and 4.5 weeks after harvest on 28 September 03. Immediately following removal from the cool store, 100 ct fruit were sealed individually in quart-sized glass "canning" jars containing air to which varying amounts of ethylene were added aiming to create atmospheres of 0, 0.5, 2, 10, 30 and 100 ppm, and incubated at 7ºC and 20ºC. The fruit in jars were pre-equilibrated to these temperatures before ethylene was added. Each treatment was replicated four times. To avoid detrimental accumulation of respiratory products and significant depletion of oxygen, each jar was opened and flushed briefly with fresh outside air every 24 hours, before being resealed, re-equilibrated to the incubation temperature and then reloaded with the original quantity of ethylene. CO_2 accumulation was monitored using HortResearch CO_2 sensor labels attached to the inside of each jar. These indicated that CO_2 levels gradually rose to about 3% during each 24 h period between ventilations. Half of the fruit treated with ethylene were removed from the jars at day 3 and the other half at day 7. Both batches of fruit were then placed into HortResearch 4-pack ventilated clamshells in a 20ºC room with a very low ethylene atmosphere. Fruit that had been conditioned at 20°C were allowed to ripen until 10 days after removal from the cool store, while those conditioned at 7°C for 3 and 7 days were ripened at 20°C until 12 and 14 days, respectively, following removal from cold storage. The progress of ripening was monitored by ripeSense aroma detecting labels attached inside the lid of each clamshell. Each batch of fruit was destructively assessed for firmness using a GUSS Fruit Texture Analyzer immediately following one of the designated periods of ripening at 20ºC shown above, at which times the fruit were expected to best exhibit a range of firmness in response to the various ethylene levels.

A similar protocol will be repeated in New Zealand using Comice pears in February/March 2004, but will include an additional higher level of ethylene (200 ppm). We hope to obtain larger jars or other gas-tight containers that will accommodate Comice of typical commercial size.

In the 2004 (US) and 2005 (NZ) seasons we intend to condition and ripen the fruit in 4-pack clamshells, relying on our ERCs to supply ethylene at various levels in the range 1-300 ppm. This will eliminate the labor-intensive use of a separate jar for each fruit and the requirement to regularly ventilate and replenish the ethylene in each. It will also permit use of larger 80 ct fruit and should more closely emulate an anticipated commercial application.

Application testing of Ethylene Release Capsules The effects of ERCs on fruit in clamshells were tested by placing one or two ERCs in the centers of four HortResearch clamshells each containing four 80 ct fruit, freshly removed from cool storage at three weeks after harvest. These and four negative control clamshells containing fruit but no ERCs were incubated in a normal lab atmosphere at 20°C for 7 days. As positive controls, three further clamshells were loaded with fruit from the same batch and conditioned in an ethylene room containing 200 ppm ethylene for three days at 20°C, followed by further ripening for 4 days at 20°C in normal room atmosphere. Ethylene, aroma and CO_2 levels were monitored in the clamshells during ripening by gas chromatography (GC), ripeSense™ and CO_2 sensor labels respectively.

The possible usefulness of ERCs to ripen fruit in industry-standard cardboard boxes was also tested by enclosing four or five standard ERCs (each small enough to fit in the central well of a clamshell) inside 20 kg boxes lined with either of two types of conventional perforated liners or an unconventional non-perforated liner made from a black plastic trash bag. These were either filled with a combination of sealed jars (to occupy space) and at least ten 90 ct Anjou straight from the cold room at 36 days after harvest. The tops of the liners were closed simply by overlapping them and tucking the edges down between the sides and the fruit. Controls comprised batches of ten fruit from the same harvest that were enclosed without ERCs in liners of the above three types and left to ripen at the same temperature as those in lined boxes containing ERCs (i.e. 20°C). Ethylene levels were monitored by GC in air samples removed via a long syringe from inside each of the liners. This sampling required the box lids to be removed, presumably creating more air flow and loss of ethylene through the perforations of the bags than would otherwise have occurred in a closed box.

Results and Discussion

Ethylene threshold and influence of temperature during conditioning

Dose-response curves were evident in the effects of ethylene at increasing concentrations on fruit firmness when conditioned at 20°C (Fig. 1 A & B) and aroma production following conditioning at both 7°C and 20°C (Fig. 1 E-H). Definite stimulation of ripening (assessed on the basis of both firmness and aroma production) resulted from prolonged exposure to ethylene at levels as low as 2 ppm ethylene for 7 days at 20°C (Fig. 1B & F). Shorter 3 day exposure required higher levels of ethylene (e.g. 30 ppm) to trigger the same effect (Figs 1A &E).

Dose related responses to ethylene were particularly pronounced in fruit that had been in cold storage for just one week after harvest, less pronounced after three weeks, and in fruit stored for 4.5 weeks were only evident in aroma production but not in firmness.

The softening effect tended to plateau at lower levels of ethylene (e.g. 10 ppm for 7 days conditioning at 20°C) than did the aroma stimulating effect (still increasing at 100 ppm). Consequently fruit reduced to acceptable eating firmness by low concentrations of ethylene produced less aroma than did fruit conditioned in higher ethylene concentrations. Since aroma and flavor are closely related, fruit conditioned at the higher concentrations of ethylene for 7 days also had most flavor (in our opinion).

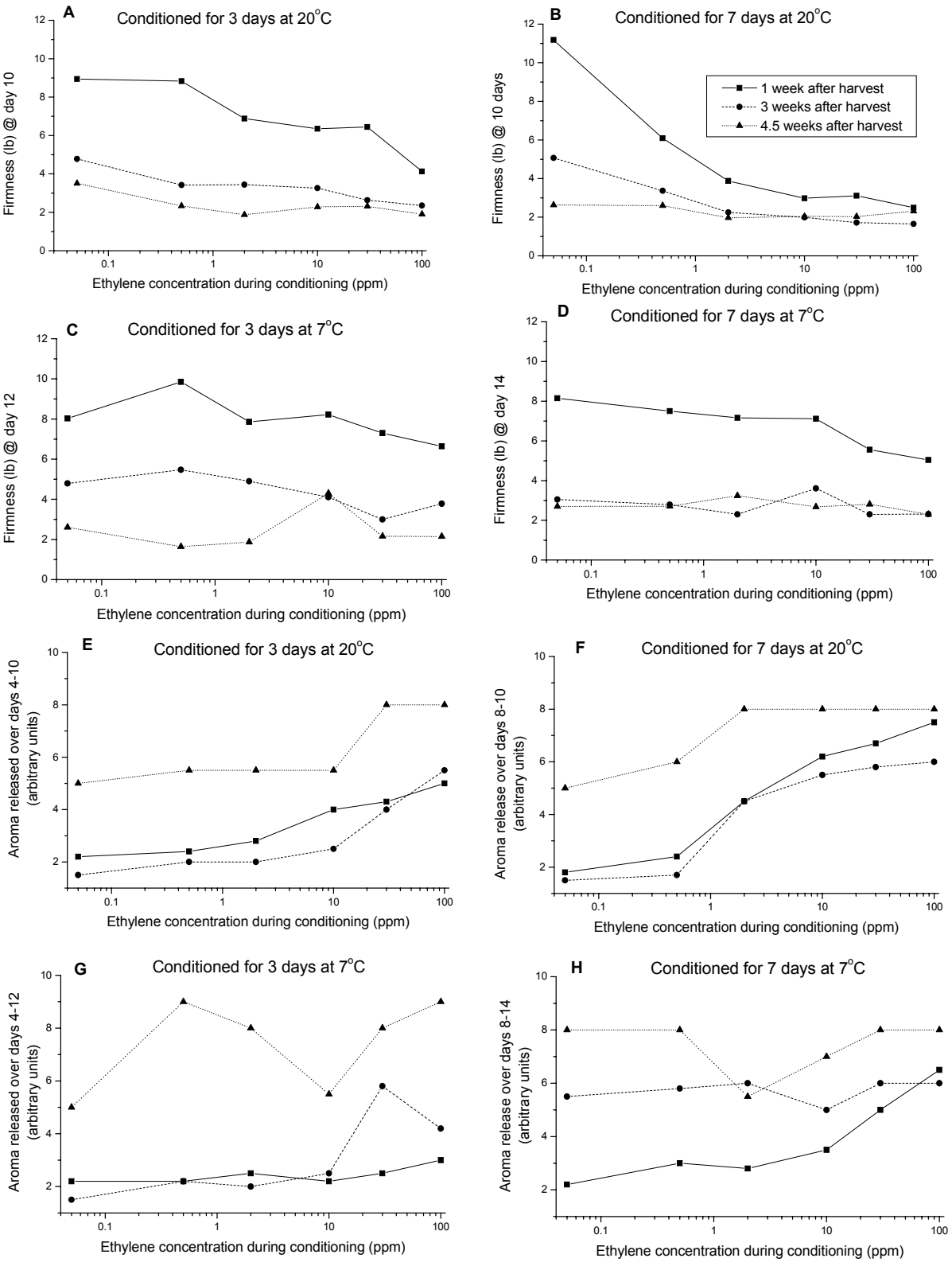


Figure 1. Effects of temperature and period of conditioning on subsequent ripening at 20°C of Anjou pears removed from cold storage at 1 week (■), 3 weeks (●) and 4.5 weeks (▲) after harvest.

A-D show effects on firmness; E-H show effects on aroma production.

Ethylene conditioning at the lower temperature (7°C) did not have a great impact on subsequent fruit softening (Fig 1C&D) but clearly increased the aroma production capacity (Fig. 1 G&H), particularly for fruit at one week after harvest that were conditioned for seven days at 30 and 100 ppm (Fig. 1H).

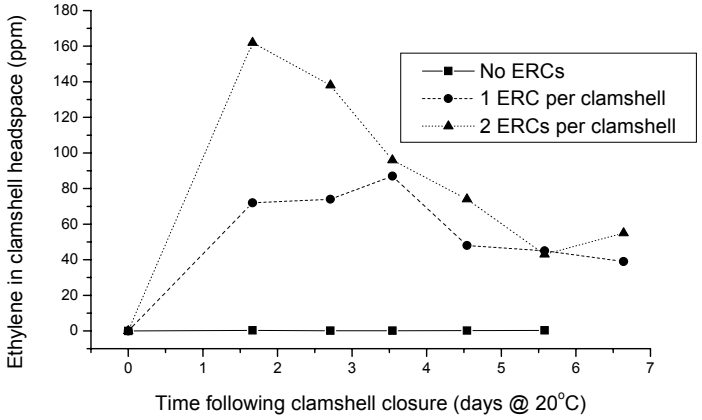
The final assessment begun after 4.5 weeks of postharvest cold storage was compromised by production of significant levels of ethylene by the fruit themselves, which became evident during the post-conditioning ripening period. This included control fruit that had not been conditioned with ethylene. Evidently their chilling requirement had been satisfied earlier than the normal 6-8 weeks. Inadvertent exposure to ethylene at levels up to 2 ppm while fruit were in cold storage at -0.5°C may have been responsible for this reduction in the chilling requirement.

These preliminary conclusions, based on the results of just one season's study, are so far fairly consistent with those of Wang *et al.* (J. Amer. Soc. Hort. Sci. **97**, 9-12, 1972). They found that ripening as measured by softening was triggered by prolonged exposure (20 days) to ethylene at very low levels, even down to 0.05 ppm. They also noted that low ethylene treatment levels could induce softening without inducing the climacteric rise in respiration, consistent with our observation that short exposures to low concentrations could induce softening without stimulating aroma production.

Application testing of Ethylene Release Capsules

A single ERC in the central well of our 4-pack pear clamshell was capable of maintaining an ethylene concentration of at least 40 ppm for up to seven days (Fig. 2). This was despite ventilation in the base of the clamshell which prevented CO_2 from accumulating to detrimental levels above 3%. Higher levels of ethylene were produced and maintained over the first three days by including two rather than one ERC per clamshell (100-160 ppm vs. 70-90 ppm), but no additional impact on fruit ripening was evident after 7 days (Table 1).

Figure 2. Mean ethylene concentrations inside clamshells containing four Anjou pears in the absence or presence of one or two ERCs.



Final firmness and aroma assessments of these same fruit at day 7 showed that ERCs in clamshells induced ripening equivalent to that induced by three days in a conditioning room containing 200 ppm ethylene at 20°C (Table 1).

Table 1. Comparison of the effects of ERCs within clamshells for 7 days at 20°C versus the effects of conventional conditioning room treatment (200 ppm ethylene for 3 days at 20°C) followed by a further four days at 20°C in normal room atmosphere. Fruit had been freshly removed from cold storage at three weeks after harvest. Firmness values are means of 8-16 fruit, aroma of 2-4 clamshells.

Source of ethylene	*No ERC and no external ethylene*	*Single ERC for 7 days*	*Two ERCs for 7 days*	*Conditioning Room (200 ppm ethylene for 3 days)*
Firmness (lb)	5.8	2.3	2.0	2.3
Aroma (by sensor label)	6.0	8.0	7.8	7.8

Four or five ERCs per 20 kg box could sustain for 5 days ethylene levels of >20 or >60 ppm, within perforated or non-perforated liners, respectively (Fig 3A). This was sufficient to clearly accelerate ripening of Anjou, tested at 5 weeks after harvest, even using conventional perforated liners (Fig. 3B).

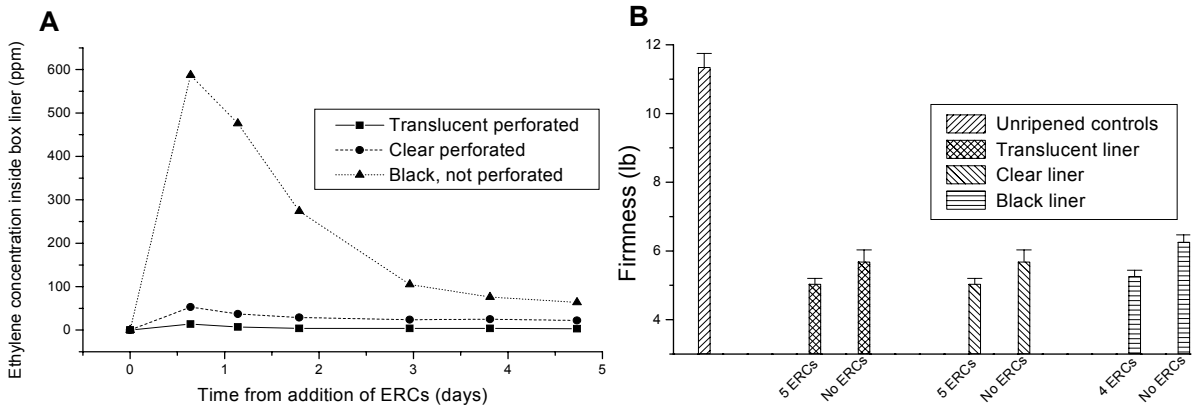


Figure 3. Effects of 4-5 ERCs on ethylene levels (A) and fruit firmness (B) in 20 kg boxes. Firmness of unripened controls was assessed immediately after removal from cold storage at 5 weeks after harvest. Treated fruit were assessed after 5 days at 20°C in various box liners, with or without ERCs.

The most marked effects, both in terms of internal ethylene concentration maintained (Fig. 3A) and on fruit softening (Fig. 3B), resulted from the use of a non-perforated black plastic liner. Such greatly reduced ventilation of the fruit will probably be detrimental to fruit quality in long term storage. Larger ERCs with greater capacity to cope with ethylene losses due to liner perforations will be developed to better suit this application.

The fact that the controls also softened significantly may be partly attributable to the typical background level of 0.3 - 0.6 ppm ethylene in the postharvest laboratory where the boxed fruit were incubated. Also the fruit at this stage were starting to produce their own ethylene, as indicated by ethylene concentrations inside the control liners that exceeded laboratory air levels by up to 1 ppm.

Significance to the pear industry

The first six weeks following harvest is currently a period of sluggish sales for Anjou, when it suffers from the reputation of lacking the capacity to develop full aroma and flavor, although it can be induced to soften by ethylene conditioning. The focus of this project is on the potential benefits of ethylene conditioning by unconventional means when applied early in the storage period, before the chilling requirement has been satisfied and the fruit begin to produce their own ethylene.

The above preliminary results suggest that aroma and flavor qualities of early season Anjou can be greatly improved by continuous exposure to ethylene throughout ripening (rather than using a transient exposure to ethylene to simply trigger ripening). Prolonged ethylene treatment of pears (e.g. for 7 days) is not commercially practical using packhouse conditioning rooms. However, this should soon be possible using ERCs within clamshells, which protect the fruit as they soften.. Larger ERCs to be developed for use within conventional fruit boxes also have the potential to offer a safer, more convenient and cost effective alternative to ethylene conditioning rooms, with the bonus of permitting conditioning to be carried out in transit if required, at no extra cost.

Opinions of packhouse operators and fruit marketers will continue to be sought through discussion and personal demonstrations of the prototypes. Relevant food packaging regulations in USA and New Zealand will be complied with in the design of these prototypes.

Budget

Project title: **Ethylene ripening of pears by unconventional means**
PI: Dr Keith Sharrock
Project duration: 2003-2005
Current year: 2004
Project total (3 yrs): $136,700
Current year request $49,900

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)
R&D Fees[1]	26,240	**32,000**	25,000
Equipment [2]	400	**7,000**	1,000
Supplies [3]	500	**1,500**	1,000
Travel [4]	2,860	**7,200**	7,200
Accommodation in US [5]		**1,600**	2,000
MCAREC cool store fees		**600**	600
Sensory Unit fees [6]			20,000
Total	30,000	**49,900**	56,800

[1] These fees pay for principal investigator's salaries and benefits for five months full time in year 2 and three months 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 purchase of a GUSS Fruit Texture Analyzer (cost $5,500), as used at MCAREC, to permit similarly precise and reliable measurements of fruit firmness during the New Zealand half of the project.

[3] Includes production of prototype ERCs and clamshells, and purchase of fruit.

[4] Includes two return economy class airfares to Auckland-Portland in 2004 and 2005; car rental for six weeks each year; and a food allowance ($25/day) while in Oregon. **N.B.** Travel and accommodation expenses were subsidized by another project which also required us to come to Oregon in 2003, but this will not occur in subsequent years.

[5] Utilizing on site accommodation (trailer home) at MCAREC for 6 weeks in 2004 and 2005. Includes power and gas.

[6] Combined estimate for taste panel tests envisaged at Mt Albert and Portland Sensory Evaluation Units in Year 3.

Title: New approaches to decay control of pear

Project Leader: Robert A. Spotts
Project staff: Maryna Serdani (Research Assistant), Gordon McCarty (Bioscience Research Technician), Briana Thompson (Student Assistant)
Cooperators: Peter Sholberg, Dan O'Gorman, David Sugar, Paul Chen, Paul Randall

Significant Findings:
Stem end gray mold increased in fruit treated with MCP at 30 and 50 ppb but not with 10, 70, and 100 ppb. MCP at 300 ppb did not reduce the amount of wound-related Mucor rot, blue mold, or gray mold. Treatment of fruit with copper/silver ions in dump tank water reduced decay about 15%. A hot water system reduced decay by 23, 33, and 59% for blue mold, gray mold, and mucor rot, respectively. There was no heat injury to the fruit. PCR primers were developed for key decay fungi, and protocols were developed for the new real time PCR, which is more rapid and sensitive than standard PCR. A model is being developed and tested that will accurately rank orchards according to decay risk potential. *Botrytis* is capable of growing on almost any dead or senescing weeds as well as dead pear leaves. In 2003, *Botrytis* spores were present on current as well as 1-year-old blackberry fruit near pear orchards and may contribute to pear gray mold. Decay was closely related to fruit pressure at harvest but not to soluble solids. Gray mold and blue mold increased about 3% for each pound drop in fruit pressure. Mucor rot increased about 5% for each pound drop in pressure. In contrast to last year, fludioxonil (Scholar) did not cause any injury to treated pear fruit.

Objectives:
1. Effect of MCP and CIM, silver ions, and heat on control of decay of pear.
2. DNA techniques for rapid, accurate detection of decay spores
3. Studies on decay pathogens.
4. Fludioxonil phytotoxicity trial.

Results and discussion:
1. Effect of MCP and CIM, silver ions, and heat on control of decay of pear.

A. MCP effect on puncture and stem end gray mold.

Stem end gray mold increased in fruit treated with 30 and 50 ppb but not with 10, 70, and 100 ppb (Figure 1). The MCP rate for Anjou has not been determined but may be about 20 ppb. At this level, MCP is not likely to have any adverse effect on decay.In the second MCP study with 300 ppb MCP, high levels of decay occurred in all treatments (Table 1). MCP did not reduce the amount of wound-related Mucor rot, blue mold, or gray mold.

A. Copper/silver ion generator evaluation.

Treatment of fruit with copper/silver ions in dump tank water reduced decay in both years of the study. Combined results are shown in figure 2. Blue mold was reduced more than gray mold or mucor rot. Inoculum levels in 2003 caused severe decay and possibly masked the benefits of the copper/silver treatment. Overall decay reduction was about 15%.

C. Evaluation of a hot water pressure washer system for decay control.

The hot water system reduced decay by 23, 33, and 59% for blue mold, gray mold, and mucor rot, respectively (figure 3). There was no heat injury to the fruit. This experiment will be repeated in January 2004.

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

PCR primers for *Botrytis, Mucor*, and *Penicillium* were developed at the Pacific Agr-Food Research Center. These were found to be specific for each pathogen and did not amplify other species. The standard PCR was very sensitive and was capable of detecting 10 spores (Figure 4). When orchard samples were tested, chemical inhibitors interfered with the PCR. This problem was solved with a DNA extraction kit designed for soil microbes. Protocols were developed for

the new real time PCR, which is more rapid and sensitive than standard PCR (Figure 5). Breakdown speed of DNA from dead spores remains to be determined. In addition, protocols were developed for a monoclonal antibody test that is specific for *Botrytis*. Future analyses will compare ELISA, real time PCR, and plating. Samples from orchards and packinghouses in Oregon, Washington, and New Zealand will be analyzed in January 2004.

3. Studies on decay pathogens

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

From 1996-1998, data from 14 orchards showed that in two of the three years, the orchards with high numbers of decay spores on the fruit also had the most decay in storage. From 1999-2003, research intensively focused on one orchard that had a high population of *Botrytis*. In 2001, spore numbers in this orchard were low, but gray mold was high (Table 2). Thus, it appeared that spore numbers alone will not always accurately predict storage decay severity, although orchards can be ranked according to decay risk potential in any given year. Other potential predictors include decay fungus in stem tissue and decay in lab-wounded orchard run fruit (Table 3). Also, a measure of the yearly variation in internal fruit resistance to decay is needed. Work done in New Zealand will provide an additional growing season per year.

B. Survival of Botrytis in weeds in orchard plots.

In 2002, survival of *Botrytis* was highest in quack grass and annual bluegrass and lowest on dandelion and clover. In 2003, Botrytis was highest on dead pear leaves but also moderate on dandelion, clover, chickweed, and annual bluegrass (Table 4). *Botrytis* appears capable of growing on almost any dead or senescing weed material as well as dead pear leaves. Thus, orchard weed management may not be an effective means to lower the populations of *Botrytis* in pear orchards.

C. Role of blackberries in the epidemiology of Botrytis on pears.

In 2001 and 2002, Botrytis infected blackberry fruit after pear harvest. These berries dried but remained on the plant and served as a source of spores for infection of the following season's pear crop. Previously, we showed that Botrytis from blackberries causes gray mold on pear fruit. In 2003, rain occurred before pear harvest, and current season blackberry fruit were infected (Table 5). Thus, in 2003, Botrytis spores were present on this years as well as last years berries. Thus, Botrytis-infected blackberries near pear orchards may contribute spores that will infect pears and should be removed where possible.

D. Harvest maturity in relation to decay susceptibility.

Decay was closely related to fruit pressure at harvest but not to soluble solids (Figures 4-9). Gray mold and blue mold increased about 3% for each pound drop in fruit pressure. Mucor rot increased about 5% for each pound drop in pressure. Thus, late harvest of over mature fruit will result in worse decay than when fruit are harvested earlier.

4. Fludioxonil phytotoxicity trial.

No phytotoxicity was observed on any fruit in any of the treatments. This is in contrast to the previous year when Scholar at 4 and 16 oz per 100 gallons plus ethoxyquin at 2700 ppm a.i. caused injury to d'Anjou pear fruit. In that trial, fruit were stored loose in wooden boxes with polyethylene liners. Although the treatment suspensions were allowed to drain off the fruit prior to storage, some fruit were in contact with liquid in the bottom of the box. In the present trial, fruit were place on fiberboard trays and were not in contact with treatment suspension during storage.

Table 1. Effect of 300 ppb MCP on puncture decay of Anjou pear

Decay	MCP	No MCP
Blue mold	98	99
Gray mold	99	100
Mucor rot	100	100

No significant effect of MCP on decay.

Table 2. Botrytis spores on pear surface related to gray mold in a single orchard

Year	Spores per fruit	Gray mold (%)
1999	1987	6.6
2000	140	3.9
2001	0	13.5
2003	8	?

Table 3. Botrytis on fruit, in stems, and decay in wounds at harvest, 2003

	Spores per fruit		Botrytis in stems (%)		Wounds infected (%)	
Orchard	Botrytis	Pen	Botrytis	Pen	Gray mold	Blue mold
A	22	1127	0	7	3.7	1.9
B	6	165	1	2	0.1	0.3
C	8	183	0	1	0.3	0.0

Table 4. Botrytis on orchard weeds, 2003

	Percent colonized by Botrytis		
Weed	30-Jun	30-Jul	29-Aug
Quackgrass	6	4	2
Annual bluegrass	46	41	16
White sweetclover	40	34	36
Chickweed	26	31	33
Pear leaves	40	77	87
Dandelion	40	3	6
Mallow	49	48	13

Table 5. Botrytis colonization of blackberry fruit

	Fruit	Berries infected (%)	
Month	Condition	2002	2003
July	Dry-old	78	88
August	Dry-old	69	89
September	Fresh	0	22

Rain on Sept. 8 and 9, 2003
Berries evaluated Sept. 12, 2003
Anjou harvest Sept. 15, 2003

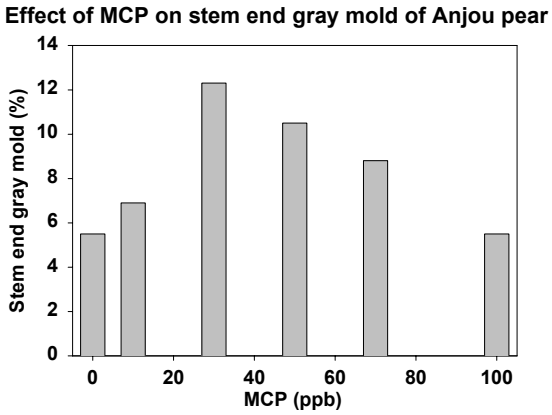


Figure 1. MCP effect on stem end gray mold
LSD = 4.1 at 5% level of significance.

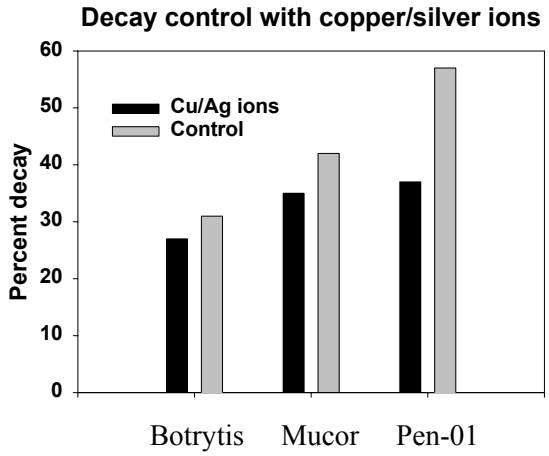


Figure 2. Copper and silver ions for decay control; Decay reduction with ions for each decay is significant; at P = 0.10.

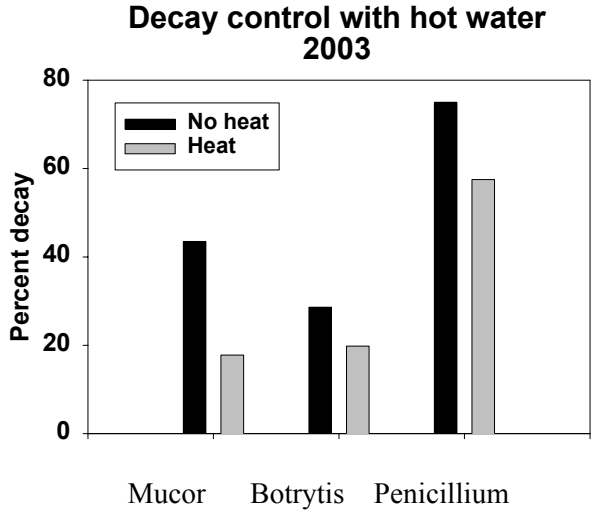


Figure 3. Hot pressurized water for decay control.; Decay reduction with hot water for each decay is significant; at P = 0.05.

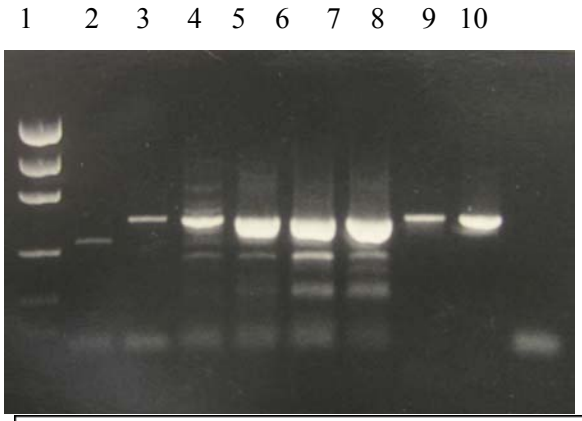


Figure 4. PCR of Botrytis DNA. Lanes are 1) ladder, 2) 1 spore
3) 10 spores, 4) 100 spores, 5) 1000 spores, 6) 10,000 spores
7) 33,0000 spores, 8) mycelium, 9) DNA standard, 10) control

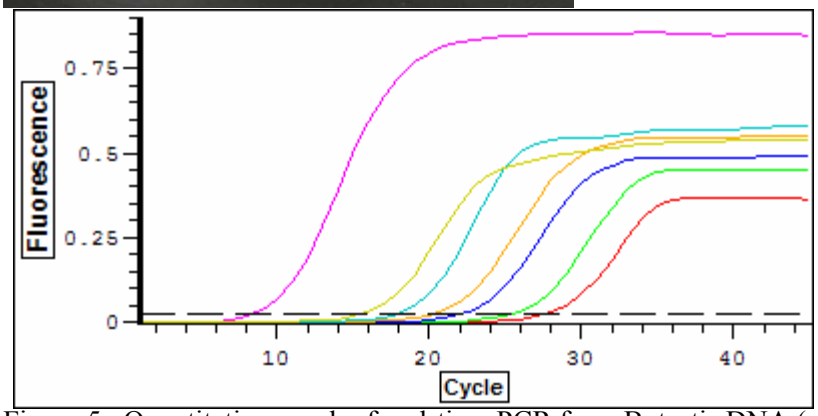


Figure 5. Quantitation graph of real-time PCR from Botrytis DNA (curves 1-3) and a spore gradient (curves 4-7); the higher the cycle number, the less DNA is present

Figure 6. Mucor vs. brix. P = 0.76 not significant.

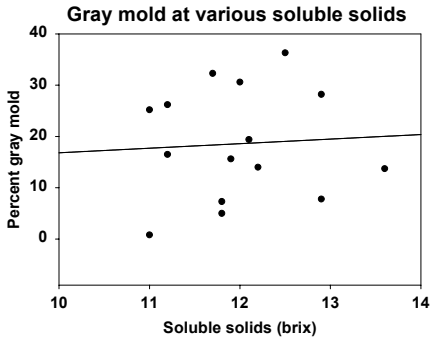


Figure 7. Botrytis vs. brix. P = 0.83 not significant

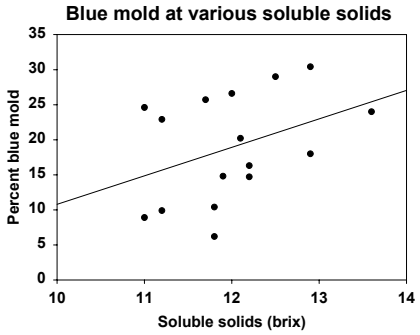


Figure 8. Penicillium vs. brix. P = 0.14 not significant

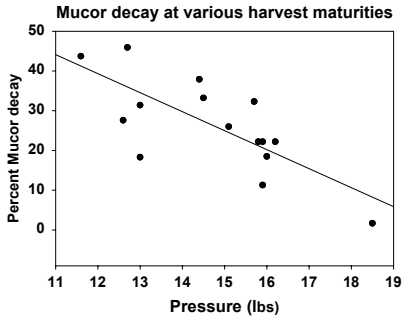


Figure 9. Mucor vs. lbs. P = 0.001 significant

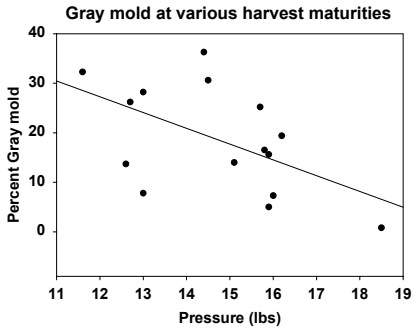


Figure 10. Botrytis vs. lbs. P = 0.04 significant

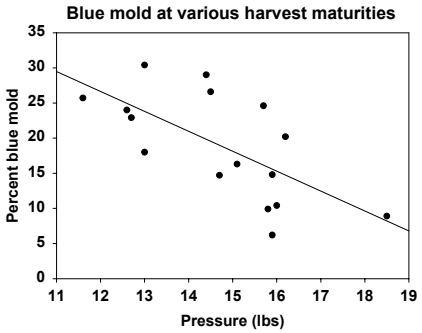


Figure 11. Penicillium vs. lbs. P = 0.005 significant

Budget requested:

Item	Amount
Salaries and wages	$42,526
Service and supplies	1000
TOTAL	$43,526

CONTINUING PROJECT REPORT

Project title: Role of ethylene in resistance to the gray mold pathogen *Botrytis cinerea*

PI: Henrik Stotz

Organization: Oregon State University

Address: Department of Horticulture
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Cooperator(s): Robert A. Spotts (Mid-Columbia Ag. Res. & Ext. Ctr.; Hood River, OR)

1. RESEARCH OBJECTIVES

The stated ***objectives*** of the previous submission were to determine the relationship between ethylene and gray mold decay. Specifically, measurements of (i) ethylene production using gas chromatography coupled with flame ionization detection and (ii) disease progression using a caliper to determine the diameter of expanding lesions were proposed. In addition, treatment of plant material with (i) propylene to accelerate ethylene production and ripening and (ii) 1-methylcyclopropene (1-MCP) to inhibit ethylene production and ripening were suggested. The ***rationale*** of the previous proposal was that, once the effects of these two different treatments on gray mold decay were known, they could be implemented to control postharvest decay.

We have obtained preliminary data on ripening, ethylene production, and decay susceptibility of four pear varieties, namely d'Anjou, Bosc, Comice, and Niitaka. Based on these results, our focus for the next year will be to determine the effects of propylene and 1-MCP on the decay susceptibility of different pear varieties with an emphasis on d'Anjou and Bosc. Beyond the second year, we aim at determining the extent to which jasmonic acid or salicylic acid can be used to protect fruits against postharvest decay through activation of inducible defenses.

Initially, we will test pear fruits that have been stored at -1^{o}C. Bob Spotts will send us fruits of the two varieties d'Anjou and Bosc for this purpose. Each of these two varieties will be treated with either propylene or 1-MCP and subsequently tested for ethylene production and disease progression. Fruits kept in air will serve as controls. We will use agar plugs containing actively growing mycelia for inoculation to avoid problems associated with wound responses. Mock inoculations using plain agar will be used as negative controls.

In addition to the above experiments using stored fruits, we will conduct a trial using freshly harvested pear fruits during the months of August and September. The varieties Comice and Bartlett will be included, as well. Data on Bartlett will be interesting because it is not a "winter" pear. We expect that the result with d'Anjou will be different depending on whether it is freshly picked or comes out of storage because this pear variety requires a cold treatment period of one to two months to acquired the capacity to ripen.

The objectives of the previous proposal were broader and not exclusively focused on pear fruits. For the current project, plant material other than pears is no longer considered. In addition, jasmonic acid and salicylic acid were not previously included as treatment options to control gray mold decay. However, both of these plant hormones are know to contribute to protection against *Botrytis* infections (Liu et al., 1998; Droby et al., 1999; Murphy et al., 2000; Kim and Choi, 2002) and are therefore mentioned in the current application.

The schedule was modified such that we immediately started working on pear fruits and did not consider any other plant material. Our initial experiment was performed on fruits collected in the orchard of the USDA-ARS National Clonal Germplasm Repository, Corvallis, OR.

2. SIGNIFICANT FINDINGS

- The four pear varieties d'Anjou, Bosc, Comice, and Niitaka differed in gray mold susceptibility and associated changes in ethylene production and fruit ripening.
- Wound inoculated Niitaka fruits produced a liquid that inhibited sporulation of *Botrytis cinerea*.

3. METHODS

3.1. Biological Materials

Robert Spotts (Mid-Columbia Ag. Res. & Ext. Ctr., Hood River) will provide pear fruits of cultivars 'd'Anjou', 'Bartlett', 'Bosc', and 'Comice' either at harvest maturity or after storage at –1^{o}C.

B. cinerea (B05.10), maintained as a glycerol stock at –80^{o}C, will be cultured on malt extract or potato dextrose agars. Conidia will be harvested according to published procedures (Guimaraes et al., 2004).

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 inoculated with an agar plug (0.4 mm in diameter) such that the side containing actively growing mycelia touches the skin of a surface sterilized fruit (Urbasch, 1986). Plain agar will be used as a control for mock-inoculations of fruits.

3.3. Propylene and 1-MCP Treatments

Plants and fruits will be treated with 300 ppb 1-MCP for 24 h to block ethylene action prior to pathogen inoculation. A continuous flow system will be used to supply 500 ppm propylene (Starrett and Laties, 1991). Following a pretreatment of 24 h with 500 ppm propylene, plant materials will be removed for pathogen inoculation. Subsequently, inoculated plant material will be reintroduced into the propylene environment or kept in air. A minimum of three fruits will be used per treatment. If necessary, Dr. James Mattheis will be consulted (USDA-ARS, Wantechee, WA).

3.4. Ethylene Measurements and Decay Parameters

Fruits will we weighed. Ethylene production will be measured by manually withdrawing a 1 ml sample from the headspace of a fruit enclosed in a jar containing a volume of 1 l for 1 h. The sample will be injected into a gas chromatograph equipped with a flame-ionization detector. Ethylene will be collected from fruits that have been exposed to the fungus for 6 h, 12 h, and 24 h. Subsequently, measurements will be taken on a daily basis. Ethylene production will be expressed as $\mu l\ g^{-1}\ h^{-1}$.

Lesion expansion will be determined on a daily basis by measuring the diameter of spreading lesions using a caliper. Lesion diameter will be used to compute the area of expanding lesions.

3.5. Statistical Analysis

Triplicate samples will be used for the proposed experiments. Standard statistical tests will be used to compare differences between genotypes, treatments, and their interactions, including analysis of variance (ANOVA) using the SPSS and SAS program packages.

4. RESULTS AND DISCUSSION

Our first experiment was based on individual fruits of the cultivars d'Anjou, Bosc, Comice, and Niitaka. Fruits were either untreated, wounded and mock-inoculated with sterile water, or wound-

inoculated with a conidial suspension of *B. cinerea* (250 spores per inoculum). Disease progression differed among the four different varieties (Fig. 1).

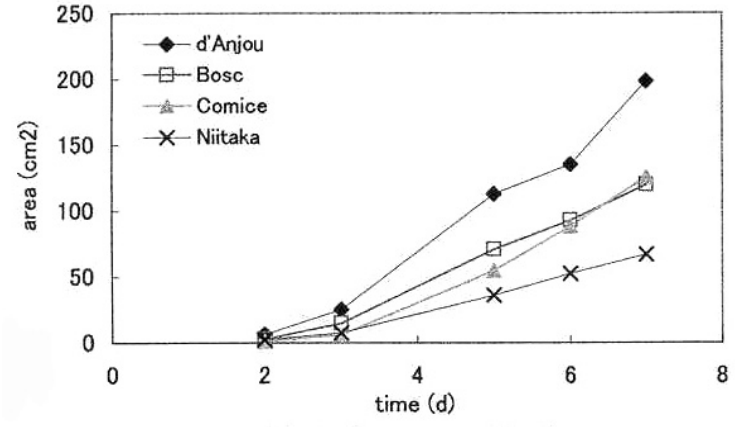

Fig. 1. Susceptibility of four pear varieties to *B. cinerea*. METHODS. Freshly picked fruits were wound-inoculated with fungal conidia. Diameters of expanding lesions were determined on a daily basis and converted into lesion areas ($A = \pi r^2$) by assuming circular lesion expansion.

Interestingly, d'Anjou was most susceptible to *B. cinerea* even though this pear did not ripen when fruits were untreated or wounded and mock-inoculated. The Asian pear Niitaka was the most resistant fruit. This pear released a liquid that inhibited sporulation. Whereas all other fruits promoted sporulation in wounded tissues, asexual fungal reproduction was prevented at the site where Niitaka was inoculated. However, sporulation did occur later in the area surrounding it. Gray mold susceptibility of Bosc and Comice was intermediate. Collectively, these results suggest that pear varieties differ in susceptibility to *B. cinerea*. Moreover, the susceptibility of ripening-incompetent d'Anjou suggests that there is no straightforward relationship between ripening and disease susceptibility.

Ripening and ethylene production varied among the four pear cultivars (Fig. 2). Untreated fruits of Bosc, Comice, and Niitaka exhibited a climacteric rise in ethylene production. The onset of this climacteric occurred between day 3 and day 5 in the case of Bosc (Fig. 2B) and Niitaka (Fig. 2D). Ethylene production of the Asian pear was approximately 25 times lower compared to Bosc. Untreated d'Anjou fruits did not produce any detectable ethylene, in agreement with their inability to ripen. These data show that there is no straightforward relationship between ethylene production and disease susceptibility (Fig. 1).

The pear cultivars we tested differed with respect to ethylene production in mock- and pathogen-inoculated fruits (Fig. 2). The response of the d'Anjou fruit was intriguing because ethylene production could be detected as early as 6 h after inoculation with *B. cinerea* (Fig. 2A). Mock-inoculated fruit supported a minor ethylene peak between day 3 and day 5. The infected d'Anjou fruit changed color, suggesting that *Botrytis*-induced ethylene production triggered the ripening program. Because this pear variety is

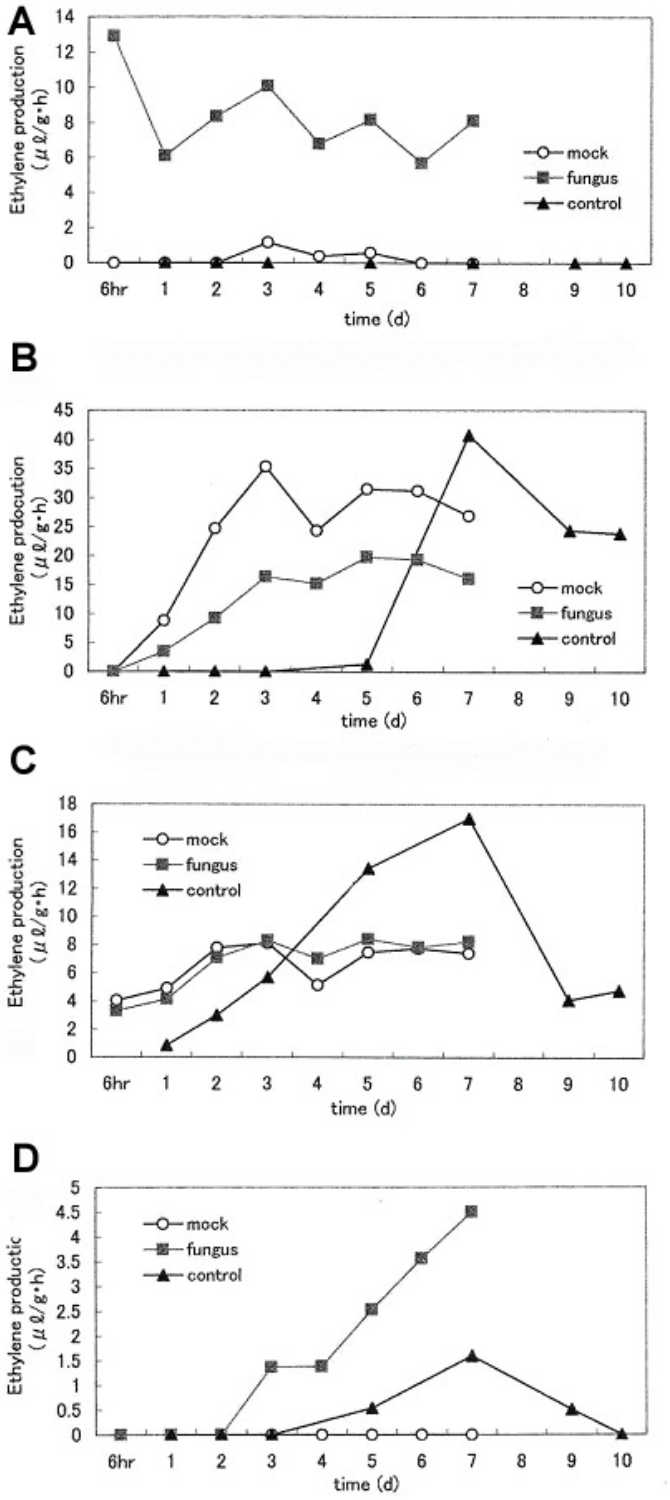

Fig. 2. Ethylene production of **(A)** d'Anjou, **(B)** Bosc, **(C)** Comice, and **(D)** Niitaka fruits that were untreated (control), mock- or pathogen-inoculated. METHODS. Fruits were enclosed in a container for 1 h and the gas collected after that period was analyzed by gas chromatography.

particularly susceptible to *B. cinerea*, it will be interesting to determine whether this is related to ethylene perception of regulation of ethylene synthesis. Bosc (Fig. 2B) and Comice (Fig. 2C) were similar in terms of their response to mock- and pathogen-inoculation. Both of these treatments induced a similar pattern of ethylene production. Niitaka (Fig. 2D), on the other hand, produced ethylene only in responds to fungal infection.

Collectively, these data indicate that the four pear varieties we tested can be classified into three groups. Fruits of cultivar d'Anjou are most susceptible to *B. cinerea*. These fruits produce ethylene and ripen only in response to fungal infection. The second group is comprised of Bosc and Comice. A climacteric rise in ethylene production occurs in both varieties. In addition, Bosc and Comice respond to both mock- and pathogen inoculation. Their sensitivity to *B. cinerea* is intermediate. Lastly, Niitaka is the least susceptible variety. Its ethylene climacteric is relatively small and apparently suppressed by wounding. Based on their varying behavior, we expect these groups to respond differently to 1-MCP or propylene treatments.

The *significance* of these results from an industry perspective is that (i) pear varieties differ in their susceptibility to *B. cinerea* and that (ii) there is no strict correlation between ethylene

production and fruit ripening, on the one hand, and disease susceptibility on the other hand. Future experiments outlined in this application are designed to reduce disease incidence by triggering induced resistance mechanisms after treating fruits with 1-MCP, propylene, jasmonic acid, or salicylic acid. Once this information is available, implementation of these types of chemical treatments are expected to reduce postharvest gray mold decay.

5. REFERENCES

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Liu J, Huang X, Fu Z, He S (1998) Induced resistance effect of salicylic acid on anthracnose of mango fruits (*Colletotrichum gloesporioides*). Journal of Tropical & Subtropical Botany **6:** 245-248

Murphy AM, Holcombe LJ, Carr JP (2000) Characteristics of salicylic acid-induced delay in disease caused by a necrotrophic fungal pathogen in tobacco. Physiological & Molecular Plant Pathology **57:** 47-54

Starrett DA, Laties GG (1991) The effect of ethylene and propylene pulses on respiration, ripening advancement, ethylene-forming enzyme, and 1-aminocyclopropane-1-carboxylic acid synthase activity in avocado fruit. Plant physiology **95:** 921-927

Urbasch I (1986) Resistenz verschiedener Kultur- und Wildtomatenpflanzen (*Lycopersicon* spp.) gegenuber *Botrytis cinerea* Pers. Journal of Phytopathology **116:** 344-351

6. BUDGET

Project title: Role of ethylene in resistance to the gray mold pathogen *Botrytis cinerea*
PI: Henrik Stotz
Proposed project duration: 2003 to 2005
Current year: 2004
Project total (three years): $74,953
Current year request: $25,242

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)
Salaries and wages	15,625	**18,500**	20,350
Benefits (%)	469	**492**	517
Equipment	500	**0**	0
Supplies	5,500	**6,000**	6,000
Travel	500	**250**	250
Total	22,594	**25,242**	27,117

Budget Justification: Salaries and wages are requested to continue the employment of my graduate student. The student will be working together with the PI on the effects of ethylene on gray mold infection of pear fruits. Equipment expenses are no longer included because we use existing equipment at Oregon State University for propylene treatment. The new estimates for salaries and

wages take into account that expenses for students have increased substantially this year. An increase of 10% is projected for in the year 2005. Supplies include the purchase of chemicals necessary for propylene and 1-MCP treatment, media for plant and microbial growth, as well as maintenance costs for gas chromatographic equipment. The supplies budget has been reduced compared to the previous proposal. Travel expenses have been reduced relative to the previous proposal because there will be fewer trips between Hood River and Corvallis than we have previously anticipated.

Current Support

- California Tomato Commission; Breeding tomatoes for resistance to Botrytis (gray mold); $16,000; 7/1/03 to 6/30/04
- Winter Pear Control Committee; Role of ethylene in resistance to the gray mold pathogen *Botrytis cinerea*; $22,594; 10/1/03-9/30/04

Pending Support

- NIH; Guard cells: Mediators of oxalate-initiated diseases; $1,092,575; 7/1/04 to 6/30/09.
- California Tomato Commission; Breeding tomatoes for resistance to Botrytis (gray mold); $25,400; 7/1/04 to 6/30/05
- USDA-ARS; Genetic basis of oxalate sensitivity in relationship to *Sclerotinia* diseases; $114,178; 7/1/04 to 6/30/06

CONTINUING PROJECT REPORT

Title: Epidemiology of Bull's Eye Rot in Pear

Project Leader: David Sugar, Professor
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Objective: To understand the disease cycle of bull's eye rot in pear, so that vulnerable points can be identified and corresponding control measures implemented effectively.

Significant Findings:
1. The species of fungi causing bull's eye rot in the Pacific Northwest are *Neofabraea alba* and *N. perennans*. There is a low incidence of a third, unnamed species.
2. Both *N. alba* and *N. perennans* can cause cankers in pear trees. However, only *N. alba* was found causing cankers naturally. These cankers were small, and occurred along branches or at pruning stubs. Monthly inoculations of wounded pear bark with the bull's eye fungi indicate that pear wood appears to be susceptible from September through April. Inoculations from during late spring through summer did not result in canker development. It is likely that both species can also persist on dead or dying bark of pear trees, without causing distinguishable cankers.
3. Spore production by *N. alba* and *N. perennans* from pear tree cankers follows a seasonal pattern, rising substantially in spring and again in autumn during the latter part of winter pear harvest. These are also periods of relatively high rainfall probability, and are likely to be the most effective times for protective sprays.
4. In fruit covered with bags during the growing season and unbagged for sequential two-week "exposure periods", there was an increase in incidence of bull's eye in the later half of the growing season. In one orchard, peak bull's eye incidence was associated with exposure during late June and also with exposure in late August
5. Fruit infection can take place with as little as 30 minutes of sustained wetness on the fruit surface. Temperature effects were ambiguous; cooler temperatures favored infection in one year and warmer temperatures in the next.
6. Overall incidence of bull's eye rot was five times greater in fruit from orchards using over-tree irrigation. Over-tree irrigation can spread spores from bark or cankers to fruit at any time during the growing season, though more spores are available in spring and fall.
7. In tests on inoculated fruit, Mertect effectively controlled all three species of bull's eye rot fungi. Flint and ziram were moderately effective against *N. alba* but less effective on *N. perennans*. Ziram was the most effective fungicide tested for suppression of spore production on tree cankers and suppression of spore germination. Copper treatments also reduced spore production.
8. During two seasons, the incidence of bull's eye rot developing after 6 weeks at 50° F was not significantly different than after 5 months at 30° F. This is a potentially useful method for predicting the risk of bull's eye rot development in long-term storage lots.

Results and Discussion:
1. *Identity of fungi causing bull's eye rot*. A collection of *Neofabraea* isolates from bull's eye rot on pears grown in Washington, Oregon, and California were screened with species-specific primers in a multiplex PCR reaction (DNA analysis). Isolates were also studied under the microscope to correlate DNA results with observable characteristics of the fungi. *N. alba* was most frequently identified in samples from Oregon and California, while *N. perennans* was most frequently found in samples from Washington. *N. alba* also was identified from tissue of small cankers and pruning stubs on pear trees

using PCR. Bull's eye rot pathogens were isolated from fruit of nine different European pear cultivars, Asian pear, and quince. Overall, *N. alba* was the most prevalent species in 2001 while *N. perennans* was more prevalent in 2002. A third, unnamed species referred to as *N.* sp. nova was identified in samples from Medford, Oregon. Throughout this study the species assumed to be a major pathogen of both apple and pear, *N. malicorticis*, has not been found in pear. It is likely that previous studies mistakenly identified *N. alba* as *N. malicorticis*. Both have similarly shaped spores (macroconidia), but some characteristics differ. The presence of *N. alba* in the Pacific Northwest had not been noted prior to this study.

2. *Tree susceptibility to canker development*. Monthly inoculations of wounded pear bark with bull's eye fungi indicate that pear wood appears to be susceptible from September through April; inoculations from May through August resulted in reduced or no canker development (Figs. 1 and 2). Cankers resulting from inoculations in October through February developed acervuli (spore-producing structures). Later inoculations through the spring produced fewer acervuli. *N. alba* differed from the other two species causing bull's eye in showing a lack of virulence on dormant shoots and in having slightly greater virulence on actively growing shoots of pear than of apple.

3. *Spore production on cankers*. Spore production by *N. alba* and *N. perennans* from pear tree cankers follows a seasonal pattern (Fig. 3). While spore production does not disappear completely during mid-summer, spore production rises substantially in spring and again in autumn during later winter pear harvest. Splash dispersal of spores from tree cankers provides inoculum for new infection of trees and for the fruit infection which appears as bull's eye rot after long-term storage.

4. *Timing of fruit exposure to bull's eye spores*. In two Bosc pear orchards with histories of bull's eye rot, a large number of fruit were covered with paper bags. Bags were then removed from sequential replicate sets of fruit for two-week exposure periods over the course of the growing season. Bull's eye rot appeared in fruit from every exposure period from petal-fall through harvest, probably due in part to the regular use of over-tree irrigation in both orchards (Figs. 4 and 5). However, there was increased incidence of bull's eye in the later half of the growing season. In one orchard, peak bull's eye incidence was associated with exposure during late June and in late August.

5. *Effect of wet period duration and temperature*. Spores don't need long wetness periods to cause infection. They probably carry sufficient amounts of gelatinous material with them when splashed onto fruit to support germination. Results of fruit inoculations inside of controlled-temperature limb cages at MCAREC showed that fruit infection can take place with only 30 minutes of sustained wetness on the fruit surface. Infection appeared to be favored by cooler temperatures in one year, but was favored by warm temperatures in the next, leaving the temperature effect unclear.

6. *Effect of irrigation method*. Splashing water appears to be necessary for spores to be transferred from cankers or bark to fruit. Where bull's eye rot is a problem and over-tree sprinklers are used for irrigation in dry summer climates, alternative irrigation systems should be considered. Several aspects of our study have implicated frequency of rainfall or over-tree irrigation as important factors in bull's eye rot severity. The fungi that cause bull's eye rot produce spores in a gelatinous matrix that readily releases spores into splashing water droplets rather than into dry air. The persistence of bull's eye rot on the trees, as well as the probability of fruit infection are likely to be influenced by the frequency of rainfall or irrigation. In the southern Oregon growing district, the traditionally worst orchards for bull's eye have over-tree irrigation, and in the Mid-Columbia district severe bull's eye rot years have been associated with high rainfall during the growing season. During re-packing at a cooperative packinghouse in Medford in 2002, fruit from 19 orchards were evaluated for decay incidence. Thirteen of the orchards used over-tree irrigation, while six used under-tree irrigation. Overall incidence of bull's eye rot was low, but was five times greater in fruit from orchards using over-tree irrigation. In another experiment, half of the sprinkler risers in an orchard block were brought to ground level, while half were left as over-tree. Fruit were bagged and sequentially exposed in both treatments. In fruit from trees with under-tree irrigation, incidence of bull's eye rot was zero from all exposure periods except for one, during which rainfall occurred (Fig. 6).

7. *Fungicide effects*. Bull's eye infections take place throughout the growing season, but some infections can be controlled by postharvest fungicide applications. Mertect can control all three important *Neofabraea* species (Table 1). However, while some bull's eye rot infection apparently occurs after harvest, postharvest Mertect applications are too late for most infections begun in the orchard, believed to be the principal infection type. Flint and ziram were moderately effective against *N. alba* and *N. malicorticis* but less effective on *N. perennans*. Thiram and Dithane were the least effective fungicides. Ziram, Flint and copper fungicides were tested for their effect on the production of spores on established cankers of *N. alba* and *N. perennans*, and on subsequent germination of spores of these fungi. Spore production by both species was most suppressed by ziram (Fig. 7). Spore production by *N. alba* was also suppressed by Flint (trifloxystrobin) and copper, but spore production recovered more quickly following copper treatment than with ziram or Flint. Germination of spores from treated cankers of both species was suppressed by copper, Flint and ziram (Table 2). Suppression of spore production and germination are important since the probability of successful infection increases with greater numbers of viable spores.

8. *Bull's eye rot prediction*. We first tried to predict the amount of bull's eye rot in pear fruit by freezing sample fruit, then holding them at 50° F to speed up the development of the disease. The method was successful with d'Anjou but not with Bosc. Subsequently, we inoculated Bosc fruit with spores of the pathogen, then held half of the fruit at 50°F without freezing and half in air storage at 30° F, then compared the amount of decay that develops at each temperature. The goal was to obtain equal amounts of decay in both groups, but to see bull's eye rot develop in the fruit at 50° in six weeks rather than in the normal 16 weeks that is commonly needed for visible rot at 30°F. During two seasons, the incidence of bull's eye rot developing after 6 weeks at 50° F was not significantly different than after 5 months at 30° F (Table 3). This is a promising method for predicting bull's eye rot. The incubation time at 50° F may be reduced from 6 to 5 weeks since decay results appeared similar and problems of fruit breakdown were less.

Table 1. Postharvest fungicide dips for control of bull's eye rot of Anjou pear.

		Percent infection			
Fungicide	Rate per 4 liters	*N. alba*	*N. malicorticis*	*N. perennans*	Avg.
Mertect 340F	5.0 ml	5	0	10	5
Flint 50WG	0.445 gm	37	20	73	43
Ziram 76DF	19.2 gm	25	42	65	44
Thiram Granuflo	16.0 gm	87	87	93	89
Dithane DF	7.2 gm	95	85	100	93
Control		100	100	100	100

Table 2. Percentage of germination of conidia of *Neofabraea perennans* isolate MA-0001 from canker washes after fungicide treatment.

	Days after fungicide treatment		
Treatment	12	27	48
Check	91.0 a	95.3a	76.7 a
Copper	65.3 b	71.2a	72.0 a
Trifloxystrobin	76.0 b	93.4a	80.0 a
Ziram	16.0 c	86.7a	77.3 a

Table 3. Incidence of bull's eye rot of Bosc in 50º F short-term storage compared to incidence in 30ºF long-term storage.

	Percent fruit infected	
Season	50ºF / 6 wk.	30ºF / 5mo.
2000-1	64a	84a
2001-2	83a	79a

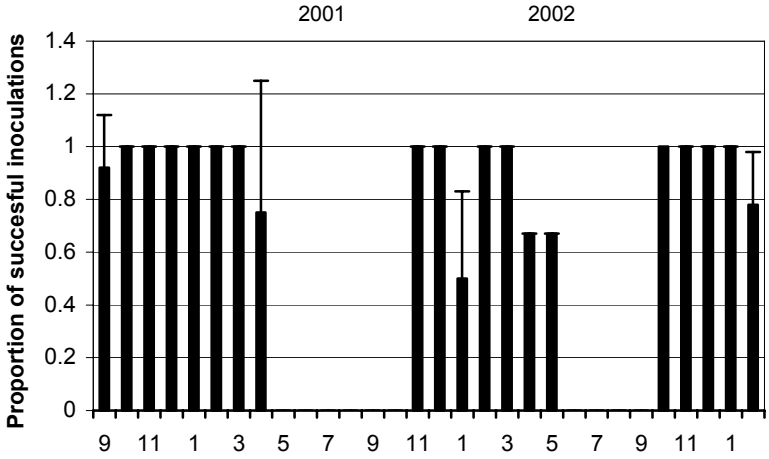


Fig. 1. Proportion (1 = 100%) of wound inoculations of *Neofabraea perennans* that resulted in canker formation in Bosc pear trees.

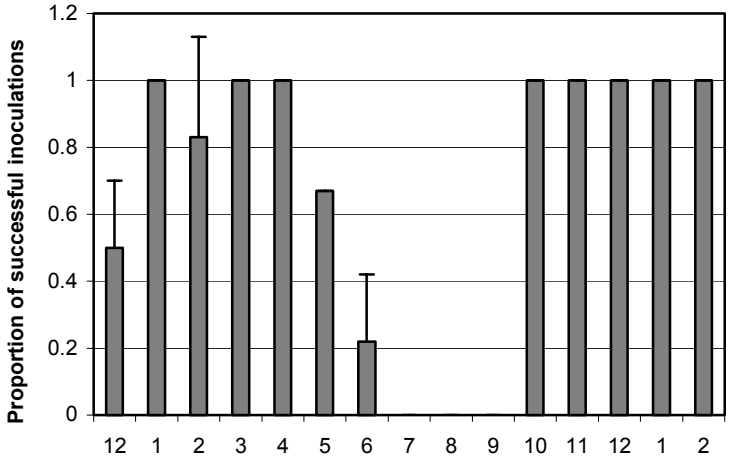
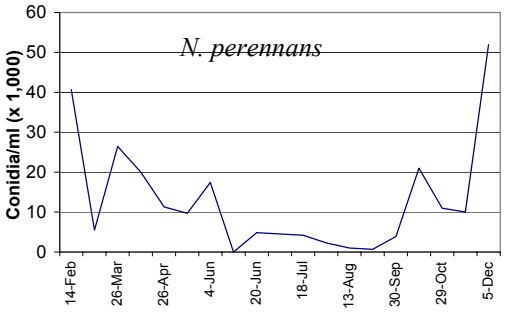


Fig. 2. Proportion (1 = 100%) of wound inoculations of *Neofabraea alba* that resulted in canker formation in Bosc pear trees.

Fig. 3. Seasonal spore production from cankers of *N. perennans* and *N. alba* on Bosc pear trees.



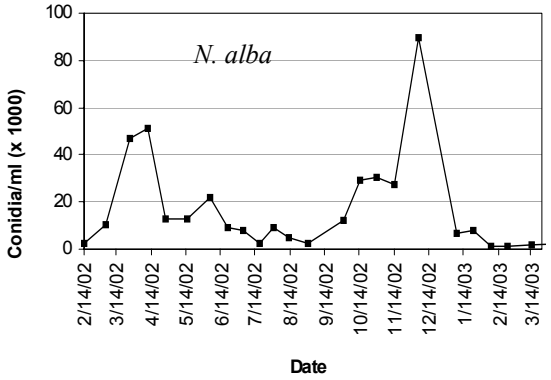


Fig. 4. Incidence of bull's eye rot on Bosc pear fruit corresponding to each of 9 exposure periods in the orchard of SOREC in Medford in 2001. Each period corresponded to a two-week exposure to infestation. Exposure period 0 corresponded to the non-bagged control fruit. Fruit was initially bagged on May 16 and harvested in September 11.

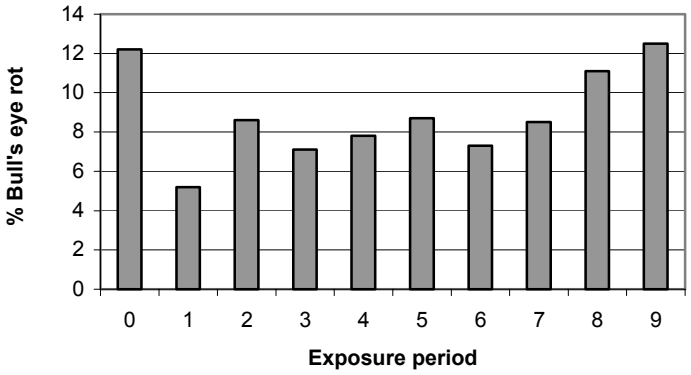


Fig. 5. Incidence of bull's eye rot on Bosc pear fruit corresponding to each of 7 exposure periods in the orchard of SOREC in Medford in 2002. Each period corresponded to a two-week exposure to infestation. Exposure period 0 corresponded to the non-bagged control fruit. Fruit was initially bagged on June 26 and harvested in September 20.

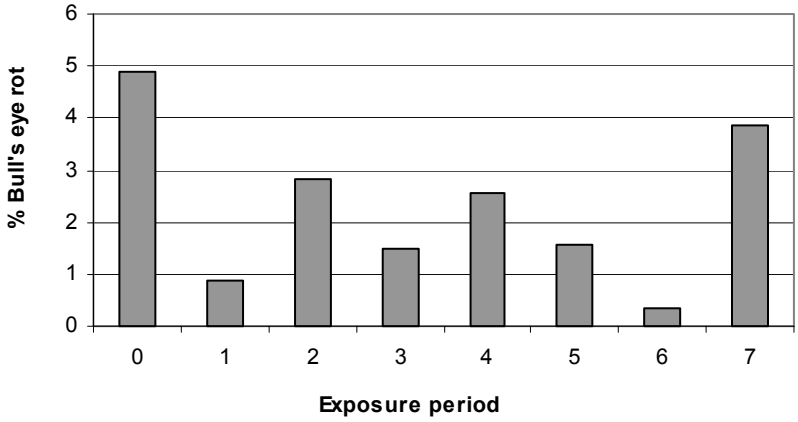


Fig. 6. Bull's eye rot incidence on six exposure periods opened during 2002 on Bosc trees irrigated with over-tree or under-tree sprinklers (top), and rainfall recorded during the study period (bottom).

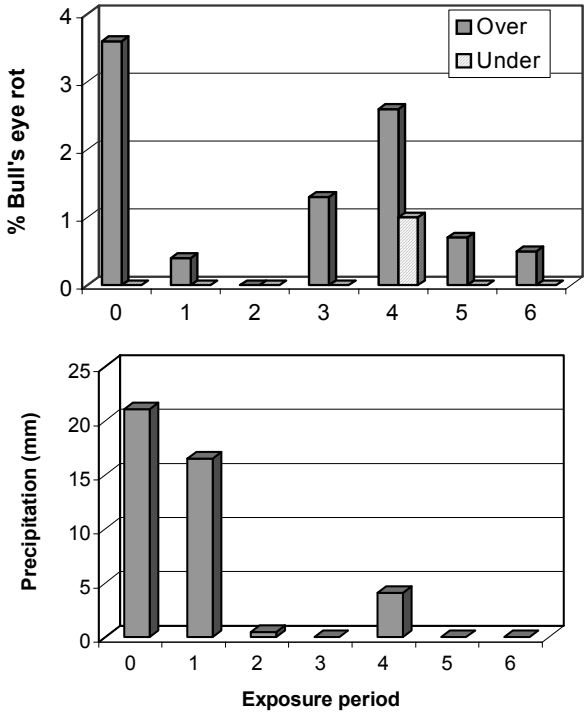
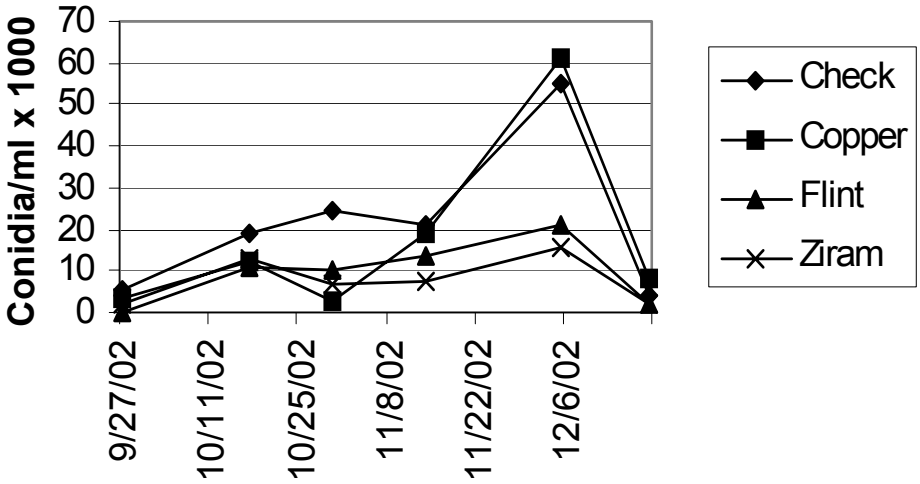


Fig. 7. Production of conidia (spores) by *N. alba* strain MB-0128 from cankers following orchard sprays with various fungicides.



CONTINUING PROJECT REPORT

Title: Storage Decay and Postharvest Quality Research

Principal Investigator: David Sugar, Professor
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Cooperators: R.A. Spotts, P. Sanderson

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. Ethylene treatment at 100 ppm for 24 hours at room temperature was sufficient to eliminate the postharvest chilling requirement for Bosc pears to ripen properly. Comice pears required 48 hours of ethylene plus 2 weeks in cold storage, either before or after ethylene treatment. Ethylene may be applied either before or after the remaining necessary cold period. Ethylene treatment of Comice that had satisfied the chill requirement did not speed up the ripening process at room temperature.
2. In the first year of testing, Bosc treated with 50 ppb ripened to excellent quality at 4 and 6 months after harvest and treatment, superior to untreated check fruit. At 6 months, Bosc treated with 50 ppb were free from core browning which appeared in check fruit. Bosc pears treated with 300 ppb failed to soften adequately throughout the 6-month evaluation period. For Comice pears 50 ppb was insufficient to achieve 6-month storage, while 300 ppb prevented ripening at 6 months.
3. In a sample of 92 isolates of the blue mold fungus collected during commercial repack, approximately 50% were not inhibited by thiabendazole (TBZ), the active ingredient in Mertect, and the remainder were only inhibited by a high dosage. The same isolates were inhibited by Scholar and Penbotec.
4. Mertect, Penbotec, and Scholar controlled a sensitive blue mold strain when applied up to 14 days after the fungus was introduced into pear wounds, but at 14 days Scholar was more effective than Mertect or Penbotec.
5. Probable new fungicide registrations may allow for two postharvest fungicide applications to pears with different materials. Tests of various sequential combinations showed the value of applying the most powerful material early, and that decay may be further reduced by subsequent application of a different fungicide.
6. CIM yeast was more effective in blue mold control when cells were harvested from fresh culture than from 3-year-old cold-stored dry formulation. Iron addition to treatments or to culture plates did not appear to enhance biocontrol.
7. LifeSpan modified atmosphere packaging was suppressive to gray mold development. Adding additional CO2 at bag sealing resulted in higher CO2 levels for about one week, and enhanced gray mold suppression modestly.
8. In a large field trial of various timings for application of Messenger to Comice pears, fruit size and other production characteristics did not appear to be increased, but multiple applications led to increased fruit russet.

Other projects not yet evaluated: alternative orchard and postharvest treatment programs, new preharvest sprays, fungicide timing for bull's eye rot.

Results and Discussion:

1. In this study, Comice and Bosc pears were treated with externally applied ethylene gas to reduce the duration of cold storage necessary before marketing pears capable of ripening. Pears harvested early in the maturity period were exposed to 100 ppm ethylene at 68°F for 24, 48, or 72 hours. For Bosc, an additional treatment of 96 hours was included. Following ethylene treatments, all pears were stored at 31°F and sampled after 3, 10, 17, 24, and 31 days for the ability to ripen, as measured by the extent of softening after 5 days at room temperature. Comice pears without ethylene treatment required approximately 30 days of cold storage to develop the capacity to ripen. 24-hour ethylene exposure reduced the time to approximately 25 days; 48-hour ethylene exposure reduced the time to approximately 17 days; 72-hour ethylene exposure reduced the time to approximately 3 days (since 3 days was the earliest sampling time, the actual requirement may have been reduced to 0) (Fig. 1). Ethylene exposure also stimulated softening during cold storage. Thus 72-hour exposure led to near-immediate ripening capacity, but pears treated for 72 hours may be more vulnerable to injury during transit. The 48-hour treatment reduced the cold storage requirement by nearly half, while retaining greater firmness during storage. In sum, 72 hour treatment may be appropriate for immediate shipment to relatively close markets, while 48 hour treatment may be appropriate for longer distance shipment following a short cold period (plus cold experienced during transit). Bosc pears without ethylene treatment required 17-24 days of cold storage to develop the capacity to ripen. Any exposure to ethylene, from 24-96 hours, resulted in cold storage time being reduced to 3 days (which probably is actually 0) (Fig. 2). Softening during cold storage was notable after 72 or 96 hours of ethylene exposure. Since 24-hour ethylene exposure effectively eliminated the cold storage requirement while retaining firmness during storage, it is apparent that 24-hour ethylene exposure is sufficient for early marketing of Bosc. Ethylene may be applied either before or after the remaining necessary cold period (Fig. 3). Ethylene treatment of Comice that had already satisfied the chill requirement did not speed up the ripening process at room temperature (Table 1).

2. Bosc pears treated with 300 ppb failed to soften adequately throughout a 6-month evaluation period. Bosc treated with 50 ppb ripened to excellent quality at 4 and 6 months, superior to untreated check fruit. At 6 months, Bosc treated with 50 ppb were free from core browning which appeared in check fruit. Preliminarily, 50 ppb appears to be an excellent dosage for treatment of Bosc with MCP, regardless of harvest maturity. Comice pears treated with 300 ppb failed to soften adequately throughout the 6-month evaluation period, without incidence of internal browning at 6 months. Comice treated with 50 ppb failed to ripen at 2 months when harvested early or mid-season, but ripened to good quality when harvested late. At 4 months, Comice treated with 50 ppb from mid-season and late harvests ripened to good quality, superior to untreated check fruit. At 6 months, Comice treated with 50 ppb showed 10% or more internal breakdown after 5 days at room temperature.

3. Resistance to thiabendazole (TBZ) in the blue mold fungus continues to be a problem. In a sample of 92 isolates of the blue mold fungus collected during commercial repack, approximately 50% were not inhibited by TBZ and the remainder were only inhibited by a high dosage (Table 2). The same isolates were inhibited by Scholar and Penbotec. Substantial effort was made to secure Section 18 emergency use of these materials, which thus far has not been successful.

4. Mertect, Penbotec, and Scholar were applied 0, 1, 2, 7, and 14 days after pear wounds were inoculated with a sensitive strain of the blue mold fungus. All fungicides controlled blue mold when applied up to 14 days after the fungus was introduced into pear wounds, but at 14 days Scholar was more effective than Mertect or Penbotec (Table 3).

5. New fungicides Penbotec and Scholar are on the EPA docket for possible registration in the coming year. This may allow for two postharvest fungicide applications to pears with different materials, providing tools for improved control and better resistance management. In tests of various sequential combinations, Scholar appeared to be the most powerful material. Timing differences showed the importance of the earliest application, and that decay may be further reduced by subsequent application of a different fungicide (Tables 4 and 5).

6. CIM yeast was more effective in blue mold control when cells were harvested from fresh culture than from 3-year-old cold-stored dry formulation, even though the applied concentration of stored formulation was based on the viable cell count (Table 6). Literature from China suggested that growing certain yeasts in moderately iron-enriched media enhanced biocontrol of postharvest fruit pathogens. Iron addition to treatments or to culture plates did not appear to enhance biocontrol.
7. LifeSpan modified atmosphere packaging was suppressive to gray mold development (Table 7). Additional CO2 was introduced into some LifeSpan bags from a tank at bag sealing. This resulted in very high CO2 levels for only 12 days, although CO2 levels were higher than in unamended LifeSpan bags for about one week (Fig. 4). Gray mold suppression was enhanced modestly by the initial CO2 "shock".
8. Messenger, a potential stimulant of plant growth systems and natural defense mechanisms, was tested in a large field trial with Comice pears. Various timings of application did not appear to increase fruit size or other production characteristics, but multiple applications led to increased fruit russet (Table 8).

Table 1. Effect of ethylene treatments on the rate of ripening in Bosc and Comice pears that had previous satisfied the postharvest chilling requirement for ripening capacity.

BOSC	Fruit firmness (lbs)		
	No ethylene	24 hr ethylene	48 hr ethylene
Initial	14.2	14.2	14.2
Day 2	12.1	11.6	12.4
Day 4	4.5	4.1	5.0
Day 5	2.7	2.8	3.2
BOSC-High Calcium	Fruit firmness (lbs)		
	No ethylene	24 hr ethylene	48 hr ethylene
Initial	14.6	14.6	14.6
Day 2	10.8	11.9	12.7
Day 4	4.0	4.3	5.0
Day 5	3.0	2.8	3.5
COMICE	Fruit firmness (lbs)		
	No ethylene	24 hr ethylene	48 hr ethylene
Initial	11.7	11.7	11.7
Day 2	7.3	8.1	8.4
Day 4	2.3	3.0	2.3
Day 5	1.4	1.6	1.7

Table 2. Fungicide sensitivity of 92 isolates of *Penicillium* (blue mold) representing 13 orchard sources. The same isolates were tested against each of the three fungicides.

	Percentage of isolates		
Minimum effective fungicide dosage ppm (a.i.)	Scholar	Mertect	Penbotec
10	82.6	0	3.3
100	17.4	0	56.5
1000	0	51.1	40.2
no inhibition	0	48.9	0

Table 3. Effect of fungicide treatment timing on blue mold decay control in Bosc pears. using Penbotec (pyrimethanil 1000 ppm (37 fl oz/100 gal), Mertect (thiabendazole 16 fl oz/100 gal), Scholar (fludioxonil 8 oz/100 gal).

	Percent of wounds infected				
	Days between inoculation and treatment				
	0	1	2	7	14
Water	93.3 a	88.3 a	93.3 a	100.0 a	98.3 a
Penbotec	0.0 b	0.0 b	0.0 b	0.0 b	73.3 b
Mertect	0.0 b	0.0 b	0.0 b	3.3 b	53.3 b
Scholar	1.7 b	0.0 b	1.7 b	0.0 b	20.0 c

Table 4. Control of blue mold decay in Bosc pears with various combinations of fungicide treatments after harvest and three weeks after initial treatment.

Treatment applied after harvest (initial)	Treatment applied 3 weeks after initial	Percent of wounds infected[1]
Water	Water	99.3 a
Water	Mertect	94.7 a
Water	Penbotec	84.7 b
Water	Scholar	82.7 b
Mertect	Water	40.7 b
Mertect	Penbotec	14.7 c
Mertect	Scholar	13.3 c
Penbotec	Water	39.3 b
Penbotec	Mertect	16.7 c
Penbotec	Scholar	8.7 d
Scholar	Water	4.7 b
Scholar	Penbotec	2.0 b
Scholar	Mertect	1.3 b

[1] Different small letters indicate significant differences. Letters apply separately to each three-value vertical grouping, plus the control (water – water).

Table 5. Control of blue mold decay in Bosc pears with various combinations of fungicide treatments after harvest and three weeks after initial treatment. Note: this is the same data as in table 4, grouped by the second treatment.

Treatment applied after harvest (initial)	Treatment applied 3 weeks after initial	Percent of wounds infected[1]
Water	Water	99.3 a
Mertect	Water	40.7 b
Penbotec	Water	39.3 b
Scholar	Water	4.7 c
Water	Mertect	94.7 b
Penbotec	Mertect	16.7 c
Scholar	Mertect	1.3 d
Water	Penbotec	84.7 b
Mertect	Penbotec	14.7 c
Scholar	Penbotec	2.0 d
Water	Scholar	82.7 b
Mertect	Scholar	13.3 c
Penbotec	Scholar	8.7 d

[1] Different small letters indicate significant differences. Letters apply separately to each three-value vertical grouping, plus the control (water – water).

Table 6. Control of blue mold with CIM yeast in combination with iron (Fe) at various concentrations (mM) or grown on agar amended with iron.

	Lesion diameter (mm)
Water	19.8 a
Fe 2.5	18.4 a
Fe 5.0	19.8 a
Fe 10.0	20.8 a
CIM from 3 year old dry formulation	13.8 b
CIM + Fe 2.5	13.4 b
CIM + Fe 5.0	13.1 b

CIM + Fe 10.0	15.4 b
CIM from freshly grown colonies	3.2 c
CIM grown on Fe 2.5	2.2 c
CIM grown on Fe 5.0	3.5 c
CIM grown on Fe 10.0	3.6 c

Table 7. Effect of LifeSpan MAP bags, with and without additional initial carbon dioxide (CO2 shock) on gray mold lesion development in Bosc pears. CO2 concentrations in LifeSpan treatments are shown in Fig. 4.

Treatment	Lesion diameter (mm)
Standard liner	42.8 a
LifeSpan	17.6 b
LifeSpan + CO2 shock	13.8 c

Table 8. Effects of various timings of application of Messenger to Comice pears on production and fruit quality characteristics. Messenger was applied at 6.7 oz/acre in 200 gallons of water per acre.

Treatment timing	Yield (kg/cm^2)	Avg. fruit weight (gm)	% of fruit size 80 or larger	Fruit set (fruit per 100 clusters)	% of fruit with > 25% russet	Sunrash rating
Untreated	0.146	280.3	76.2	12.4	38.2 a	1.23
Postharvest (Oct.)	0.140	283.2	74.2	10.6	47.7 a	1.16
Delayed Dormant	0.116	277.8	77.7	11.7	43.3 a	1.23
Petal Fall	0.161	278.5	75.3	13.8	40.8 a	1.22
Petal Fall + 2 weeks	0.158	264.6	70.3	12.9	45.2 a	1.25
Petal Fall + 4 weeks	0.142	284.1	67.9	12.2	40.8 a	1.19
All Timings	0.112	280.3	74.1	11.7	65.9 b	1.16
P value	0.104	0.702	0.452	0.453	0.007	0.436

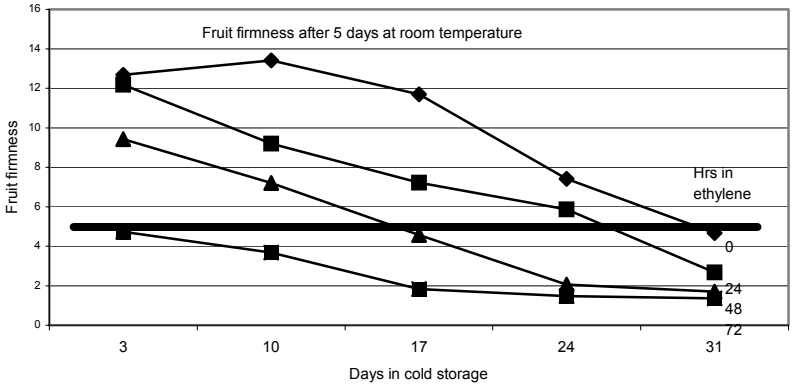


Fig. 1. Comice pears: relationship of hours of ethylene treatment to subsequent postharvest chill requirement for ripening.

Fig. 2. Bosc pears: relationship of hours of ethylene treatment to subsequent postharvest chill requirement for ripening.

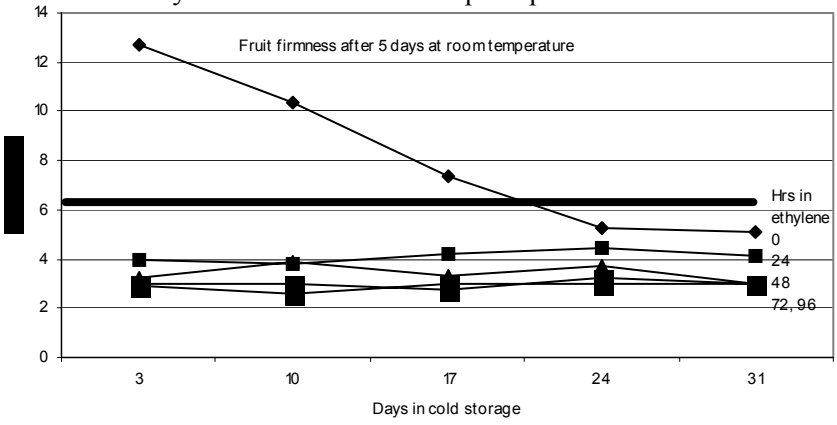


Fig. 3. Comice pears: ethylene requirement for ripening capacity after various lengths of cold storage.

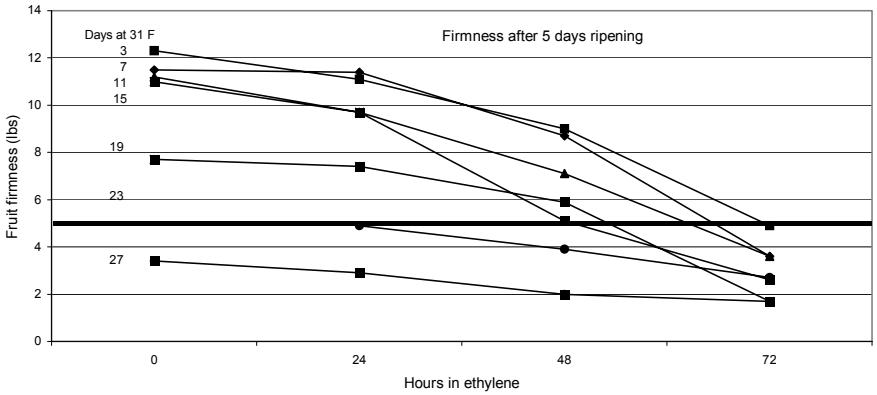
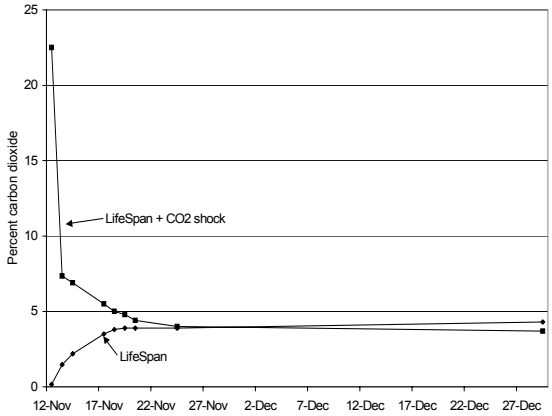


Fig. 4. Carbon dioxide concentrations in sealed LifeSpan bags packed with Bosc pears, with and without initial CO2 shock.



Justification for Proposed Research: Postharvest decay continues to cause significant economic losses in the pear industry. This research program has been focused on the development and evaluation of control tactics for pear decay. However, because of the importance of fruit quality in helping the pear industry meet their objectives, research will expand to include new technologies and opportunities for quality enhancement.

Procedures: The research will be conducted in the orchards and laboratories of the Southern Oregon Research and Extension Center in Medford, and in commercial orchards and packinghouses where appropriate. These areas will be emphasized:

1. Explore strategies to improve postharvest quality of pears. Techniques will include MCP treatments, ethylene management, packaging materials, new products and atmospheres, and combinations of treatments to achieve maximum benefits.
2. Evaluate products, dosages, and timing of chemical applications to maximize benefit for control of postharvest diseases. Test integrated strategies to optimally manage postharvest decay.
3. Continue studies of alternative decay control methods, including biocontrol agents and how they may be used most effectively in different types of pear handling and storage systems.

Estimated Duration: 1 year.

Budget Requested:

Item	Amount
Salaries and Wages	24,000
Services and Supplies	5,600
Travel	400
Total	30,000

CONTINUING REPORT
WINTER PEAR CONTROL COMMITTEE

GEORGE ING, WPCC RESEARCH CO-ORDINATOR

OBJECTIVES:

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

2. Sustaining office activities related to WPCC research.

3. Evaluating varieties with potential.

2003 ACTIVITIES:

1. PROGRAM MAINTENANCE:
Office and local travel, phone, fax, email, mileage, lodging, personal contacts. Attended meetings with pear affiliation. Handling inquiries regarding varieties, cultural practices, research. Interacting with research and extension personnel relative to research projects. Initiating and progressing projects. Communications with other pear entities, particularly Pear Bureau's Kevin Moffitt.

2. INTERNATIONAL:
1. Continual contacts regarding Chinese plant material.
2. Placed grafts of two more varieties from Italian programs.
There are now 4 Italian varieties from two sources, plus 3 from France and 1 from Turkey in the isolation plot. Plot also has one cherry and one apple.
3. Continued dialogue with germ plasm, plant quarantine, breeding and other personnel regarding varieties. Receipt of scionwood is pending for two varieties from Hungary as well as replacement scionwood for an Italian variety that failed. Some dialogue regarding rootstocks.
4. Continue to interact with private breeding program in Italy that has a pear of interest. Established two trees of Rubens, an apple from that breeding program.

3. LOCAL:
1. Two varieties established in previous years had some fruit. The variety from Turkey produced about 40 pears in 2003 and all were much better shape and appearance than the 5 pears from the 2002 crop. Pears from the 2002 crop failed to ripen. From the 2003 crop, harvested at four intervals a week apart, fruit is in cold storage and will be ripened, if possible. A few pears removed in mid December did not ripen. Fruits from the second variety with production are about 12 in number, disappointing as far as russet and will be ripened during the winter.

4. PHYTONUTRIENT RESEARCH:
Set up a project with U of Cal., Davis to do basic phytonutrient tests on red and green Anjou's. Met in Feb. at the site with Dr. Adel Kader, post harvest scientist with a very good reputation. Also met with Dr. Darshan Kelley, a human nutrition scientist with ARS at Davis who deals with foods as related to health and is attached to the Medical School at Davis. Dr. Kelley has done considerable work with California cherries, funded by its cherry commission. Concept was if the phytonutrient work with Dr. Kader showed promise, Dr. Kelley could conduct a human feeding study to ascertain if eating pears changed the level of certain phytonutrients in human blood.

In March, en route to a California cherry symposium I transported 12 boxes of red and green Anjou pears to Dr. Kader at Davis. The pears were from different elevations in the Hood River area,

harvested at different times. Dr. Kader and his staff found that pears have phytonutrients but not in quantities similar to high profile items such as kale, blueberries and red wine. Red Anjou's had anthocyanins in abundance, from peel color, which is good.

Dr. Kader had previously done tests with Bartlett's from California with similar results; beneficial phytonutrients were present but in small quantities.

Based on Dr. Kader's results we did not set up a study with Dr. Kelley. The results of another phytonutrient study, by ARS at its headquarters in Beltsville, Maryland may change the approach. In the meantime, pears are an excellent source of fiber and various nutrients and can be promoted under those parameters.

5. TRAVEL

Attended International Pear Symposium in South Africa, early Feb., 2004. Will write articles, give illustrated talks as appropriate and requested.

6. FUNDS REQUESTED FOR 2004-2005 .... NONE

We have carry-over funds from previous years that will sustain activities for another year.

WPCC PEAR PHYTONUTRIENTS CONTINGENCY

Phenolic Composition and Antioxidant Activity of 'D'anjou' Pears
Adel A. Kader and Betty Hess-Pierce
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Fruits play a significant role in human nutrition and health not only as important sources of vitamins, minerals, and dietary fiber, but also as major sources of phenolic compounds and associated antioxidant activities. Fruits, nuts, and vegetables in the daily diet have been strongly associated with reduced risk of some types of cancer, heart disease, stroke, and other chronic diseases. Although antioxidant activity varies greatly among fruits and vegetables. It is better to consume a variety of products (different colors, textures, and tastes) than limiting consumption to a few with the highest antioxidant activity. we report here on the phenolic composition and antioxidant activity of green and red 'D'Anjou' pears obtained from five locations.

Materials and Methods

Fruit source and handling: Five boxes of green and red D'Anjou pears from various locations (Table1) were delivered to the UC Davis Postharvest Pomology Laboratory (on March 24, 2003). The pears were placed at 20°C (68°F) and 85-90% relative humidity to ripen (to a flesh firmness of 3.5 to 6.0 lb-force) before taking tissue samples. Three replicates of 5 pears each were randomly selected from each box (sampling location). A slice of peel (skin) was removed from the four fruit sides and frozen in liquid nitrogen. All frozen samples were kept at -80°C until used for analysis.

Individual phenolic compounds: Phenolics were extracted using 80% methanol containing 2mMSodium fluoride to prevent oxidation of compounds. Five grams of pears were homogenized in 10ml of extraction buffer using a Polytron homogenizer (Brinkman Instruments, Westbury, N.Y.). The samples were centrifuged for 10 minutes at 10,000rpm and filtered through a 0.45µm filter for analysis by HPLC. Separations was carried out using an HPLC system (Hewlett Packard model 1050 pump) connected to a photodiode array detector (HP Model 1040M Series II) with an autosampler (HP Model 1050), operated by HP ChemStations software (Hewlett Packard, Menlo Park, CA). A reverse phase C_{18} Nucleosil column (150 x 4.6mm; particle size 5µm with a guard column of the same material (MetaChem Technologies Inc., Torrance, CA). The mobile phase consisted of 5% formic acid in a gradient of methanol containing from 5% to 80% final concentration. Compounds were identified by comparison with known standards.

Antioxidant capacity: Using the above extraction for phenolics, antioxidant capacity was measured using a free radical scavenging assay with a commercially available free radical (2,2 diphenyl-1-picrylhydrazyl, $DPPH^+$) measuring the decrease in absorbance at 515nm according to the method of Brand-Williams et al, 1995.

Reference: Brand-Williams, W.; Cuvelier, M.E.; Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm.-Wiss. Technol. 28:25-30.

Results and Discussion

Sampling location: Relatively small differences in phenolic composition and antioxidant activity among sampling locations were noted in phenolic composition of the peel (Table 2) or flesh (Table 3) of green D'Anjou pears and of the peel (Table 4) or flesh (Table 5) of red D'Anjou pears. Green D'Anjou pears from sampling location 62 had a slightly lower antioxidant activity in the peel and

flesh than pears from the other four locations. Red D'Anjou pears from sampling location 56 had a higher antioxidant activity than pears from the other four locations. Such differences may be due to greater preharvest and/or postharvest stress conditions that increase synthesis of some phenolic compounds.

Peel vs flesh: As with other fruits, the peel (skin) or D'Ajou pears is richer in phenolic compounds and antioxidant activity than the flesh. Only chlorogenic acid and catechin were detected in the flesh (Table 6). Anthocyanins were present only in the peel of the red D'Anjou pears, which also contained much higher levels fo flavonols, total phenolics, and antioxidant activity than the green D'Anjou pears (Table 6), The total phenolics and antioxidant activity were similar in the flesh of both the green and red D'Anjou pears (Table 6).

Overall antioxidant activity: Since the peel (skin) is about 15% of the fruit's weight, we calculated the overall antioxidant activity as 85% of that in the flesh + 15% of that in the peel. The overall antioxidant activity (Trolox equivalent) is 52.5 and 87.8 mmoles /100g for green and red D'Anjou pears, respectively. These activities are about 7.8% and 13.0%, respectively, of the antioxidant activity in 100 ml of red wine (675.1 mmoles/100ml), which is often used as a reference point.

Conclusion

Green and red D'Anjou pears are modest sources of antioxidant activity, but should be included along with other fruits as part of a healthy diet. Pears should be eaten with the skin which is a richer source of antioxidant activity than the flesh.