2007 Northwest Pear Research Review
February 12-13, 2007
Hood River Inn, Oregon

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9:45	50	Shekarriz	Near real-time ethylene sensor for pear post-harvest applications	06
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FINAL PROJECT REPORT

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

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Budget History:

Item	**Year 1:** 2003	**Year 2&3: *****	**Year 4:** 2006
Salaries	19200		16200
Benefits	10176		11010
Wages	1000		
Benefits	50		
Equipment			
Supplies	1500		1290
Travel	2500		250
Miscellaneous	2000		250
Total	36426		29000

***In 2004 and 2005, the project was funded by USDA FAS via Northwest Horticultural Council: $63K per year divided between OSU and USDA ARS Tree Fruit Res. Lab. Wenatchee.

Objectives:

Overall:

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

In 2006:

1-06. Evaluate capacity of *Erwinia amylovora* to colonize and persist on the calyx of pear fruit.
2-06. Evaluate survival of *Erwinia amylovora* on in fruit wounds during cold storage.
3-06. Develop concise summary of 2003-06 data for peer and regulatory review.

Significant findings (overall):

- Over three years, we detected a few cells of *Erwinia amylovora* from only one fruit of ~5000 d'Anjou pear fruit sampled from commercial orchards in the Rogue, Hood River, Yakima, Wenatchee and Okanogan Valleys of the Pacific Northwest.
- *Erwinia amylovora* has a limited survival time on surfaces of healthy pear fruit, and that the survival rates are not different from those observed on mature, symptomless apple fruit.
- Over two years, we were unable to detect *Erwinia amylovora* inside mature symptomless pear fruit harvested from diseased pear trees located at Medford, OR and Wenatchee, WA.

Significant findings (2006):

- Calyx end survival of *Erwinia amylovora* on pear fruit is similar to that observed on apple with high populations detected near petal fall; these populations decline through the summer to nearly undetectable levels at harvest. The few cells of the pathogen that remain detectable at harvest become undetectable by the end of a 6-week cold storage period. In contrast to *Erwinia amylovora*, the non-pathogenic bacterial epiphyte, *Pantoea agglomerans* persists on calyx ends of most fruit through the summer and the period of cold storage.
- For a second year, we found that mature symptomless pear fruit contaminated with *Erwinia amylovora* and subsequently wounded requires an initial dose of >10,000 cells at the wound site to allow for persistence of the pathogen on the fruit through a 7 week cold storage period. By comparing the magnitude of this dose to its likelihood (as determined in our other studies), we conclude the risk of *Erwinia amylovora* establishing itself in small wounds on mature symptomless pear is very small.

Background. Export of winter pears grown in the Pacific Northwest into countries where fire blight does not occur is restricted by phytosanitary concerns over the possible contamination of fruit with the fire blight pathogen, *Erwinia amylovora*. Similar concerns have been applied to apples, but research, risk assessment analyses and trade resolution proceedings have concluded that introduction and successful establishment of *E. amylovora* into a new geographic region via commercial shipments of apple fruit is very unlikely. Reasons for this low likelihood include: 1) viable cells of *E. amylovora* are detected on mature apple fruit only rarely, 2) *E. amylovora* has a low pathogenic capacity on mature apple fruit, and 3) a pathway that demonstrates successful infection of susceptible host material via fruit borne inoculum has never been documented. The purpose of this study is to investigate if the reasons for the low likelihood of movement of *Erwinia amylovora* with apple fruit also hold true for mature, symptomless pear fruit.

Methods:

Objective 1-06. *Calyx end survival.* Field trials were conducted in d'Anjou and Bosc pear and Gala apple orchards to evaluate survival of *E. amylovora* on calyx-end of fruit. Freeze-dried cells of *E. amylovora* strain 153N, a non-pathogenic mutant of Ea153 (Ea153 HrpL-), and *Pantoea agglomerans* C9-1 were resuspended in water were sprayed onto flowers at full bloom. Fluorescent microspheres (1μm in diameter) were co-inoculated with the pathogen to track flowers that received the inoculum spray. Flowers and immature fruit were sampled over the summer and processed for recovery of the inoculated strains. At each sample time, 8 flowers or 5 fruit from each of 3 replicate trees were placed individually into a plastic bag, and transported to the lab chilled on ice. Sterile washing buffer (50 ml of 10 mM phosphate buffer, pH 7) was added to each bagged fruit followed by sonication for 2 minutes to dislodge bacteria. The wash buffer was passed through a 0.2 μm filter membrane to capture the bacteria; the filter was placed onto Miller-Schroth medium and incubated for 7 days at room temperature. At harvest, 300 fruit per treatment per cultivar (2700 fruit total) were processed through a SOPP (sodium ortho-phenylphenate) dump tank followed by 6 weeks of cold storage. Periodically, fruit were sampled to the measure residual bacterial population in association with calyx tissues as described above.

Objective 2-06. *Postharvest survival in wounds on fruit.* At harvest, mature symptomless fruit of d'Anjou pear and Braeburn apple were harvested and transported to the lab. Fruit were surface disinfested in 10% bleach, rinsed in sterile water, and air dried. A 10 μl drop of re-suspended, freeze dried cells of *E. amylovora* was placed onto a marked location on the surface of each fruit. The number of pathogen cells in a drop was zero, 1000 or 10,000 cells. Once the inoculum was air dry (~ 1 hour), a wound was introduced at the inoculation site with a small finishing nail secured to a wooden block. After wounding, fruit were incubated at room temperature for 24 hours, followed by a dump tank treatment in 1.5% SOPP for pear or 100 ppm bleach for apple, and then placed in 0-2ºC cold room for up to 49 days. Surviving pathogen populations were enumerated on day 0 (pre and post dump tank), 7, 14, 28, and 49 days. For each sample, the tissue surrounding a wound site was removed from 15 fruit per cultivar per inoculum treatment. This tissue was macerated in 4 ml of sterile phosphate buffer (pH 7.0). The maceration buffer was passed through a 0.2 μm micropore filter. The filter was incubated on the surface of Miller-Schroth medium.

Results:

Objective1-06. *Calyx end survival:*
For the calyx end survival experiment, by mid summer, immature fruit sprayed with Ea153N (pathogenic strain) had a recovery incidence of 6% of fruit with populations that averaged 100 cells per fruit; for Ea153 HrpL$^-$ a (non-pathogenic strain applied a higher dose than the pathogenic strain), the recovery incidence was 23% of fruit with populations that averaged 100 cells per fruit; for *P. agglomerans* C9-1, the recovery incidence was 97% of fruit populations that also averaged 10,000 cells per fruit. No *E. amylovora* (pathogenic or non-pathogenic) was detected on calyx tissue after immersion treatment or during storage. *P. agglomerans*, however, persisted on 97% of fruit assayed at harvest or during the storage period. Fluorescent microspheres were observed on 100% of blossom and midsummer samples, but declined somewhat for fruit sampled at maturity (80, 74, and 80% for d'Anjou, Bosc and Gala, respectively).

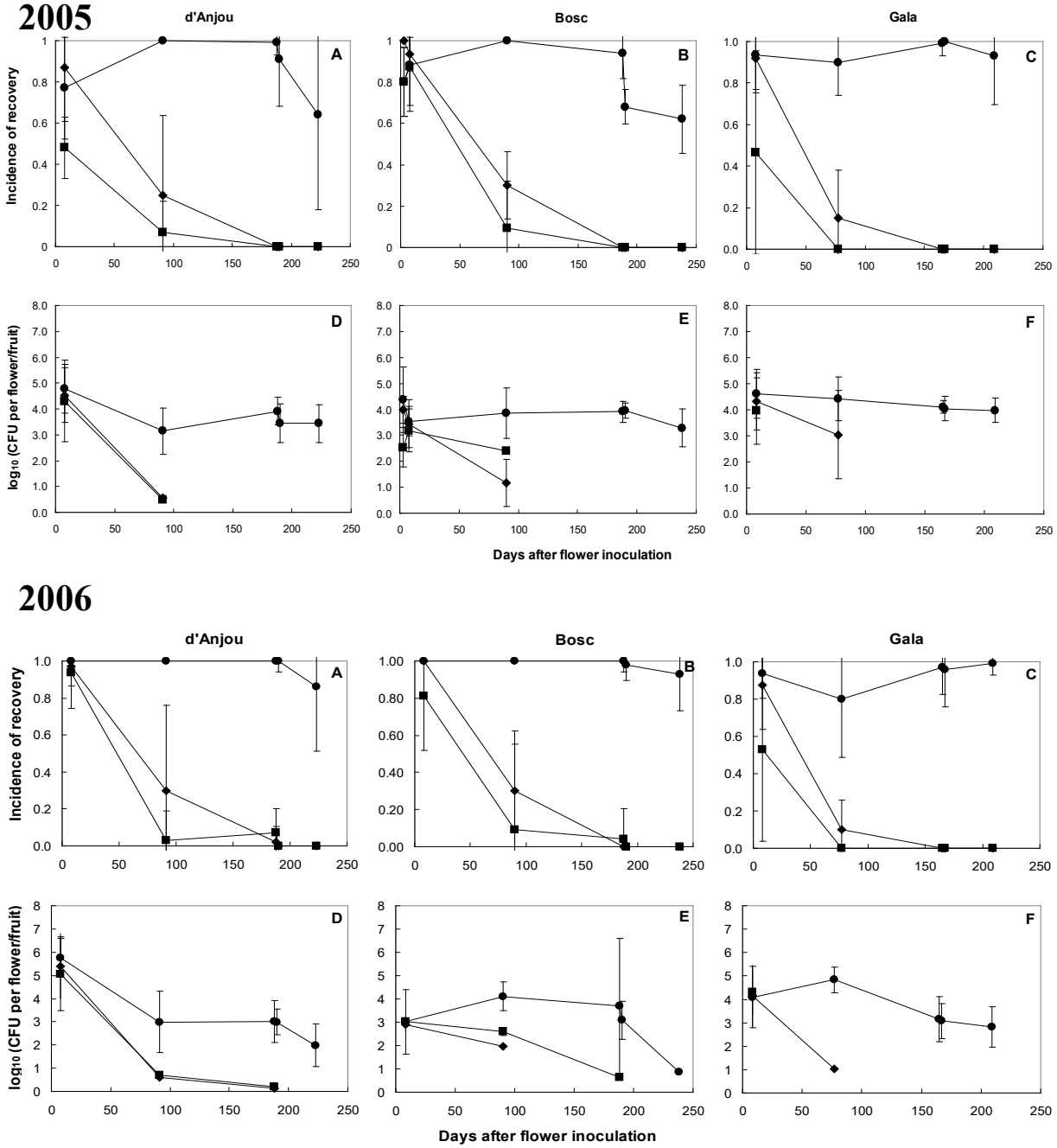

Fig 1. A-C, Incidence of recovery and, **D-F**, mean size of detectable populations (expressed as $\log_{10}$(cfu) per fruit) of *Erwinia amylovora* strains Ea153N (■) and Ea153HrpL$^-$(♦), and Pantoea agglomerans C9-1 (●) on floral and calyx tissue after inoculation in orchards located near Medford ('d'Anjou' pear) and Corvallis, OR ('Bosc' pear and 'Gala' apple) during summers of 2005 and 2006. Inoculum (1 x 10^7 cfu/ml for Ea153HrpL$^-$ and C9-1; 1 x 10^5 cfu/ml for Ea153N) was misted onto flowers at full bloom. For each sample time during the growing season, 8 flowers or 5 fruit were sampled from each of three replicate trees per experiment, washed individually, and dilution plated and/or filter assayed onto Miller-Schroth medium. For sample dates at harvest and at pre-and post-storage, 90 fruit per bacterial strain (bulk harvested for treated trees) were individually assayed in each season. Vertical bars drawn through each point represent $\pm$ one standard deviation of the mean.
Objective 2-06 *Postharvest survival in wounds on fruit.*

Zero, 1000 or 10000 cells of the pathogen were applied to the fruit skin, and then a small puncture wound was made at the site of cell placement. Fruit were processed through an SOPP or bleach dump tanks and stored at 0-2ºC.

The recovery of Ea153N introduced to mature fruit near small skin punctures prior to storage was dependent on the size of inoculum dose. Of the doses of inoculum evaluated in 2004 (water control, and 1 x 10^1, 1 x 10^2 and 1 x 10^3 CFU per wound site), only the highest dose resulted in pathogen recovery from fruit after the dump tank treatment (Table 1). At this dose, Ea153N was detected on 27% of d'Anjou pear after 7 and 14 days of cold storage (0 to 2°C, regular atmosphere) and on 20% of Braeburn apple after 7 days storage; the pathogen was not detected on any fruit (n = 480) stored longer than 14 days. When detected, the mean recovered population size of Ea153N was <1 x 10^2 CFU per fruit (Table 1). A dose of 1 x 10^3 CFU per wound site yielded similar results in the 2005 and 2006 experiments with no detection of the pathogen beyond 7 or 14 days from apple and pear, respectively.

The dose of 1 x 10^4 CFU per wound site, evaluated in 2005 and 2006 experiments, increased persistence of the pathogen on fruit relative to a dose of 1 x 10^3 CFU per wound site, which was evaluated in all years of the study (Table 1). For pear, at the highest dose, populations of the pathogen between 1 x 10^2 and 1 x 10^3 CFU per wound site were detectable on 13 to 37% of fruit at 49 days after inoculation; on apple, smaller populations (between 1 x 10^1 and 1 x 10^2 CFU per wound site) were detectable on 13 to 20% of apple fruit up to 28 days after inoculation. For both fruit types, the incidence of detection of Ea153N was highest at the pre-dump tank assessment (except for pear 2006 with NaOCl as the disinfestant) followed by consistently smaller rates of recovery as storage time progressed. In contrast, estimated population sizes for Ea 153N in the vicinity of wound sites were consistently an order of magnitude higher after 7 days of cold storage compared to samples taken at shortly after inoculation ('pre-dump tank' in Table 4). In pear, incubation of fruit at room temperature for 30 days after storage resulted in detection of Ea153N on 2 of 15 fruit in 2005 (average of 5 CFU per wound site), but no detection of the pathogen on similarly treated fruit in 2006.

NEXT PAGE: Table 1. Effect cold storage on percent of fruit with detectable populations of *Erwinia amylovora* strain Ea153N, and the mean recovered population (CFU) after small wounds[u] were created near inoculation sites on mature symptomless pear and apple fruit near harvest

Table 1. Effect cold storage on percent of fruit with detectable populations of *Erwinia amylovora* strain Ea153N, and the mean recovered population (CFU) after small wounds[u] were created near inoculation sites on mature symptomless pear and apple fruit near harvest.

Host/ Year[u]	Inoculum dose/Dump tank[v] disinfestant	Pre-dump tank	Post-dump tank	Weeks in cold storage[w] 7	14	28	49	Post-cold storage[x]
Pear								
2004	1 x 10^3 SOPP	100%[y] 96 cfu	53% 43 cfu	27 % 50 cfu	7% 80 cfu	0%	0%	n.d.[z]
2005	1 x 10^3 SOPP	20% 3 cfu	0%	0%	0%	0%	0%	0%
	1 x 10^4 SOPP	67% 43 cfu	23% 10 cfu	43% 220 cfu	47% 204 cfu	57% 261 cfu	13% 90 cfu	13% 5 cfu
2006	1 x 10^3 SOPP	34% 5 cfu	10% 29 cfu	10% 3 cfu	7% 239 cfu	0%	0%	0%
	1 x 10^4 SOPP	90% 137 cfu	47% 163 cfu	67% 1376 cfu	47% 793 cfu	54% 263 cfu	37% 222 cfu	0%
	1 x 10^4 NaOCl	64% 40 cfu	30% 101 cfu	67% 272 cfu	47% 317 cfu	54% 428 cfu	27% 230 cfu	0%
Apple								
2004	1 x 10^3 SOPP	100% 88 cfu	13% 1 cfu	20% 11 cfu	0%	0%	0%	n.d.
2005	1 x 10^3 NaOCl	13% 4	0%	0%	0%	0%	0%	n.d.
	1 x 10^4 NaOCl	50% 70 cfu	7% 96 cfu	3% 115 cfu	3% 86 cfu	0%	0%	n.d.
2006	1 x 10^3 NaOCl	47% 6 cfu	27% 7 cfu	20% 29 cfu	0%	0%	0%	0%
	1 x 10^4 SOPP	87% 18 cfu	27% 5 cfu	53% 153 cfu	13% 83 cfu	13% 13 cfu	0%	0%
	1 x 10^4 NaOCl	73% 18 cfu	20% 2 cfu	53% 232 cfu	27% 68 cfu	20% 31 cfu	0%	0%

Key to table:

[t]Fruits were inoculated by placing a 10 µl drop containing the indicated number (dose) of colony forming units on the fruit surface. After the drops were dry, a 1 x 3 mm deep skin puncture was introduced at the site of inoculation with a finishing nail.

[u]Pear experiments were conducted with cv. 'd'Anjou'. Apple cv. 'Braeburn' was used in Corvallis experiments and in Wenatchee in 2006; cv. 'Gala' was used in Wenatchee in 2005.

[v]SOPP = 1.5% sodium ortho-phenylphenate; NaOCL = 100 ppm sodium hypochloride, which are the standard disinfectants used in commercial flotation systems for pear and apple, respectively. After inoculation, fruit were incubated at room temperature (20-22ºC) for 24 hours prior dump tank immersion.

[w]Storage temperatures averaged 2 and 0ºC in Corvallis and Wenatchee, respectively.

[x]After cold storage, fruit were incubated at room temperature (20ºC) for an additional 30 days before assay.

[y]Percent of 15 (2004 and post-storage samples in 2005 and 2006) or 30 fruit (2005 and 2006; 15 from each location) with detectable populations of Ea153N. Mean population size (CFU per fruit) and standard deviation are computed only for fruit on which Ea153N was detected.

[z]Not determined.

Discussion

It was our goal to have three to four location years to support the conclusions for each of the objectives outlined above in the 'significant findings' section of this report. Prior to the 2006 season, the two objectives of this the study where we considered the data incomplete concerned survival of the pathogen on the fruit calyxes, and understanding the potential for pathogen survival in wounds.

In the calyx end survival study, the data indicate that *E. amylovora* can survive on the calyxes for a short period after bloom, but that survival is characterized by small numbers of cells that are declining over time. Surviving populations of *E. amylovora* were detected only rarely at harvest, and in both 2005 and 2006, the pathogen could not be detected on any fruit by the end of a 6- to 7-week cold storage period. In contrast, populations of the non-pathogenic bacterial epiphyte, *Pantoea agglomerans* persisted on calyxes of most fruit through the summer and the period of cold storage, indicating that this organism is better adapted to epiphytic survival than is the fire blight pathogen. Moreover, fluorescent microspheres were recovered from nearly all sampled fruit from which we attempted to isolate *E. amylovora*; this indicates that a similar proportion flowers received an initial dose of the pathogen, and that lack of detection of the pathogen at harvest and during storage was not due to the flowers escaping the initial bacterial spray. With the inoculations of wild type Ea153N in pear in 2006 (and the Bosc pear and Gala apple inoculations in 2005), the incidences of blossom blight were very high (70 to 85% of blossom clusters), demonstrating virulence in the pathogen isolate. Similar patterns of survival resulted from the virulent and avirulent strains of *E. amylovora*, suggesting that virulence (the ability to cause disease) is not strongly associated with the ability to survive epiphytically. Based on data from both seasons, there were no apparent differences in the patterns of calyx end survival on pear compared to those observed on apple.

The microwound inoculation study, although realistic in its emulation of industrial fruit handling practices, represented an improbable scenario in that a small wound (mimicking a stem puncture or other small abrasion induced during handling) was placed on fruit at a position that coincided with a concentration of pathogen cells confined within an area that represented approximately one thousandths of the total fruit surface. With this experiment, our overall goal was to understand, in a dose response framework, how the reportedly greater susceptibility of pear fruit relative to apple would influence pathogen persistence in storage when intimately associated with wounded host cells. The results confirmed our expectation that wounded pear fruit provided a somewhat more conducive environment for persistence of *E. amylovora* compared to wounded apple fruit, but this result was dose-dependent, with the difference between the hosts only apparent at the highest concentration of pathogen inoculum (i.e., 1 x 10^4 CFU in close proximity to the wound site). For both pear and apple, the data also showed small but consistent increases in pathogen population size over the first 7 to 14 days of cold storage, followed by declining incidence of detection and population size in the latter portion of the storage period. These increases in recovered population size during the early part of the storage period, however, were considerably smaller than reported for growth of *E. amylovora* in a nutrient broth incubated at 2°C for 20 days (Taylor and Hale 2003). Macroscopically, both pear and apple fruit remained symptomless at the wound sites, although a very small, necrotic discoloration was apparent at the base of the wound of some fruit that were split with a knife. Pear fruit that received the highest dose of Ea153N also were used to monitor the ability of the pathogen to grow on fruit after the 49 day cold storage period. Pears incubated at room temperature for 30 days post-cold storage showed a low incidence of detection with an estimated population size that was two orders of magnitude smaller (i.e., 5 CFU per fruit) than observed on fruit sampled at the end of the cold storage period (Table 1). Consequently, winter pears are apparently a poor substrate for growth of *E. amylovora* when allowed to ripen at room temperature after cold storage.

Collectively, the results of all experiments in this study can be compared to a peer-reviewed risk model developed to assess the probability that successful establishment of *E. amylovora* in a disease-free area could occur by importation of commercial apple fruit (Roberts et al. 1998). In that model, independent probabilities (P) were assigned to steps of the introduction pathway: P_1, the fruit is infested with *E. amylovora*; P_2, *E. amylovora* survives storage; P_3, contaminated fruit is discarded near host; P_4, host is receptive; and P_5, *E. amylovora* is transferred to the new host and infection occurs. Using these probabilities and an estimate of the potential number of fruit exported annually to the new area, Robert's et al. found the likelihood of successful introduction of fire blight to the new area ranged from once every 11,000 to 38,000 years, depending on the level of phytosanitary precaution taken prior to export. In this likelihood estimate, their derived values for P_1, depending on phytosanitary scenario, ranged from 0.001 (one in a thousand fruit) to 0.035 (one in 30 fruit), which are considerably higher than the 1 in 5600 fruit that we obtained in our surveys of commercial pear orchards. Similarly, Roberts et al. estimated P_2 to be 0.1 (one in ten fruit), which also was greater than we observed in the calyx survival and postharvest epiphytic survival experiments (L. Pusey, reported previously), where fruit were in cold storage for periods of 7 and 8 weeks, respectively. Published values of P_3 and P_4 estimated for the apple hold similar values for pear, although in absolute terms, the potential number of pear fruit exported would be considerably less than apple (fresh pear production in the Pacific Northwest is one eighth that of apple). P_5, the probability that '*E. amylovora* is transferred to new host and infection occurs' is largely dependent on how well this pathogen, starting from a relatively small number of cells on a mature symptomless fruit, could increase its population size on the discarded fruit to enable its transfer to the receptive host, most likely by a visiting insect. Although, the results of the microwound study indicate that mature pear, compared to apple, is somewhat more suitable for growth and persistence of *E. amylovora* in wounds during a storage event, this difference was not large, especially at levels of inoculum reasonably expected to occur under natural conditions. By the end of the storage (and the required chilling)

period, our data also indicated that mature pear fruit were an unsuitable substrate for continued reproduction of this pathogen.

In summary, we found that *E. amylovora* has a limited survival time on surfaces of healthy pear fruit, and that the survival rates are not different from those observed on mature, symptomless apple fruit. Calyx end survival of *E. amylovora* on pear fruit is similar to that observed on apple with significant populations detected near petal fall; these populations declined through the summer to low numbers at harvest, and become undetectable after a 7-week cold storage period. We were unable to detect *Erwinia amylovora* as an endophyte in mature symptomless pear fruit harvested from diseased pear trees. In three years of survey, we detected a few cells of *Erwinia amylovora* from only one fruit of 5600 d'Anjou pear fruit sampled at harvest from commercial orchards in Pacific Northwest region of the United Sates. Pear fruit contaminated with *E. amylovora* and subsequently wounded required an initial dose of >10,000 cells at the wound site to allow for persistence of the pathogen on the fruit through a 7 week cold storage period. By comparing the magnitude of this dose to its likelihood, we conclude that epiphytic survival of *E. amylovora* through a summer survival phase and a postharvest chilling period is unlikely given the unrealistically high population size required for persistence.

Literature Cited:

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 17:19-27.

Taylor, R. K., and Hale, C. N. 2003. Cold storage affects survival and growth of *Erwinia amylovora* on the calyx of apple. Letters in Applied Microbiology. 37:340-343.

FINAL PROJECT REPORT
WTFRC Project Number:

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

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	9,000	4,000	4,200
Benefits	4,680	2,080	2,730
Wages			
Benefits			
Equipment			
Supplies	1000	300	300
Travel	450	300	300
Miscellaneous	1000	1000	1000
Total	16130	7680	8530

Objective:
Field-test an optimized biopesticide strategy in combination with oxytetracycline.

Significant findings:

- Temperature conditions in western Oregon during spring 2006 were favorable for fire blight development. Consequently, as measured against what is typical in commercial pear and apple orchards, the amount of disease that developed in the experimental orchards was extreme. Thus, even antibiotic standards performed below their longer term averages.
- Nonetheless, our experiments concerned with fire blight control continued to show excellent results with what we term an 'integrated strategy', which is one biopesticide treatment followed by one oxytetracycline treatment.
- In Bartlett pear, all treatments that involved a biopesticide applied once near full bloom followed by a single application of Mycoshield provided a significant level of disease control. In Golden Delicious apple, disease intensity was extremely high with two of the integrated treatments providing significant control of fire blight compared to water treated controls; antibiotic standards did not. In Rome Beauty apple, disease intensity was heavy; the mixture of A506 AprX- and BlightBan C9-1 followed by Mycoshield also provided a substantial level of disease control.
- *Pantoea agglomerans* strain C9-1 was registered by US EPA on April 10, 2006 as BlightBan C9-1. NuFarm Americas intends to market BlightBan C9-1 in combination with *Pseudomonas fluorescens* strain A506 AprX- , which is a mutant selection of the active bacterium in BlightBan A506 (also a registered product). Use of A506 AprX- still requires EPA approval.

Background

Fire blight, caused by the bacterium, *Erwinia amylovora,* is a serious disease of pear and apple. The pathogen overwinters in cankers and moves to flowers as temperatures warm in spring. On flowers, the pathogen grows rapidly to attain an infective population size. Diseased flowers become necrotic; the pathogen then invades shoots and can progressively kill larger branches. Once infected, pruning is the only management option to reduce disease. Consequently, control focuses on spraying antibiotics and/or biopesticides onto flowers to prevent initial infections.

Antibiotics were first registered for fire blight suppression in the 1950s. Streptomycin kills cells of the pathogen and provides a ~80% reduction in disease if the pathogen population is sensitive to this antibiotic. In contrast, oxytetracycline is bacteriostatic and is less effective (~40% control). Streptomycin-resistant populations of the pathogen are widespread in the western U.S., and thus, oxytetracycline is used widely for fire blight control.

Two biopesticides are currently registered for fire blight suppression. Serenade (AgraQuest) is an air-dried fermentation culture of *Bacillus subtilis*. BlightBan A506 (NuFarm Americas) is a freeze-dried culture of *Pseudomonas fluorescens* strain A506. These products have provided a ~25% reduction of disease in small-scale, pathogen-inoculated trials, but have been somewhat more effective in orchards when combined within a conventional antibiotic program.

Numerous experiments have shown that strains of *Pantoea agglomerans* are the most effective biopesticides for fire blight suppression. NuFarm Americas has recently completed registration of *P. agglomerans* strain C9-1 under the trade name BlightBan C9-1. They intend to market C9-1 a combination product with a mutant selection of the active bacterium in BlightBan A506 (strain A506 AprX -).

Recently, our experiments concerned with fire blight control have focused on what we term an 'integrated strategy', which is one biopesticide treatment followed by one oxytetracycline treatment. To date, the integrated strategy has resulted in greater disease control than either biopesticides or oxytetracycline applied alone. Over a longer time period, we also have improved the effectiveness of the biopesticides BlightBan A506 and BlightBan C9-1. One improvement requires co-application of BlightBan A506 with the iron chelate, FeEDDHA. This non-phytotoxic chelate induces A506 to produce a potent antibiotic that inhibits E. amylovora (the antibiotic is not produced when iron is absent). A506 plus FeEDDHA already has some adoption among Oregon pear growers. The other improvement involves a derived knockout mutant of A506 that is deficient in an extracellular protease; this strain is called A506 AprX -. When A506 AprX - and C9-1 are applied as a combination, deletion of A506's ability to make the extracellular protease lengthens the half-life of the antibiotics produced by C9-1. In our earlier trials, the combination of A506 AprX - with C9-1 has been the most effective biopesticide treatment. Moreover, the effectiveness of one application of the combination of A506 AprX - with C9-1 followed by one application of oxytetracycline (i.e., the integrated strategy) has provided consistent control, which in many trials has approached equaled the control obtained from two applications of streptomycin.

MATERIALS AND METHODS

Materials tested. The commercial formulation of the biological agent, *Pantoea agglomerans* C9-1S (BlightBan C9-1, NuFarm) was evaluated for disease control in mixture with *Pseudomonas fluorescens* strain A506 (BlightBan A506, NuFarm). The iron chelate Sequestrene 138 (6% FeEDDHA, Becker Underwood, Ames, IA) was combined some of the bacterial treatments. We included treatments consisting of an extracellular protease-deficient deletion mutant of *P. fluorescens* strain A506 called A506 AprX - in mixture with BlightBan C9-1. We also included several treatments where biological control applications were followed by a single application of Mycoshield. Disease control efficacy by an avirulent *hrpL* mutant of Ea153 alone and in mixture with A506 AprX - and BlightBan C9-1 also was assessed. The *hrpL* mutant and A506 AprX - were cultured and freeze-dried in the Johnson laboratory for use in field trials. Additional treatments included Physpé (plant defense elicitor extracted from brown algae, Goëmar, Saint-Malo, France), and famoxate and Tanos (chemical agents manufactured by DuPont, Wilmington, DE). The chemical agents Agri-mycin 17 (streptomycin sulfate 17% a.i, NuFarm Americas, Burr Ridge, IL) and Mycoshield (oxytetracycline calcium complex, 17% a.i, NuFarm) were included as standard controls.

Experimental protocol. Biological agents, antibiotics and experimental chemical materials were evaluated for control of fire blight in a 46-yr-old 'Bartlett' pear orchard, a 26-yr-old 'Gold Delicious' apple orchard, and a 48-year-old 'Rome Beauty' apple orchard. All orchards were spaced 20' x 20' and located at the OSU BPP Field Laboratory near Corvallis.. The experiments were arranged in a randomized, complete block design with 4 replications and 12 to 17 treatments applied to single tree plots. Blossom cluster density on individual trees was estimated prior to bloom; cluster counts and tree location in the orchard were considered in assignment of trees to blocks in the plot design.

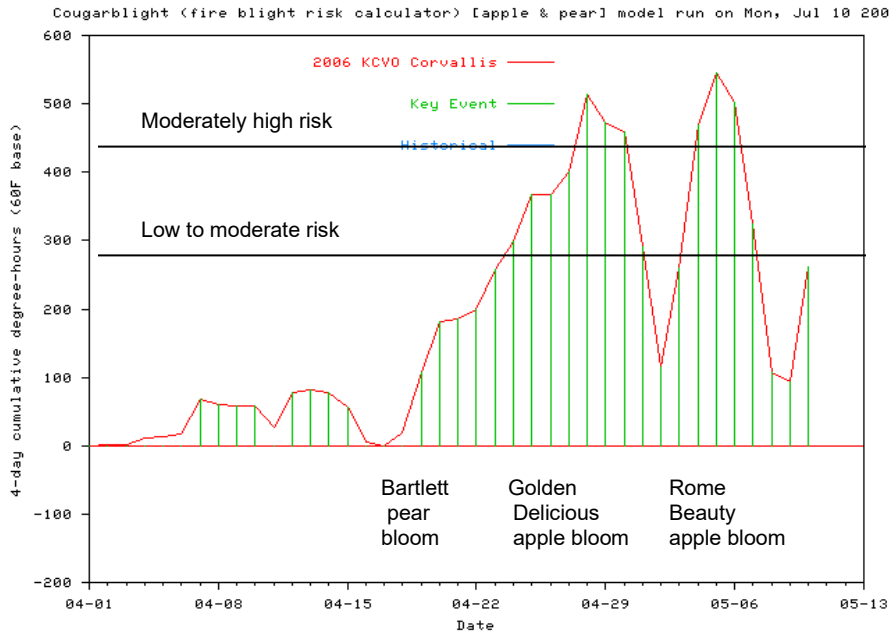
Treatments were applied during early morning on the following phenological stages (dates are in the data tables): green tip (Physpé only), popcorn; Physpé only, 30-40% bloom (Physpé, and some biocontrol bacteria treatments), 80- 90% bloom (all treatments) and full bloom (antibiotics and chemical agents). Treatment suspensions (except second application of Mycoshield were sprayed to near runoff with backpack sprayers equipped with hand wands; because of the large number of trees that received the second application of Mycoshield, this treatment was applied with a motorized, 25 gal tank sprayer equipped with a hand wand (~0.75 gal/tree). The same motorized tank sprayer was used to fog a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin- and oxytetracycline-sensitive pathogen strain), which was prepared at 5×10^6 (pear) and at 1×10^6 (apple) CFU per ml. The pathogen inoculation occurred 2 days after the 80-90% bloom treatments.

Incidence of fire blight was determined by counting blighted blossom clusters (i.e., strikes) on each tree during weekly inspections from 4 to 25 May. Blighted blossom clusters were removed immediately after counting. Total number of blighted blossom clusters per tree (log_{10}-transformed) and disease incidence (total diseased clusters/total number of clusters per tree, arcsine-square root transformed) were subjected to analysis of variance.

RESULTS

Weather conditions during bloom. Temperature conditions in western Oregon during spring 2006 were favorable for fire blight development. The COUGARBLIGHT disease risk model indicated a building risk period during bloom of Bartlett pear, and high risk periods during bloom of Golden Delicious and Rome Beauty apple (Fig. 1).

Fig. 1. Fire blight risk as estimated by COUGARBLIGHT, Corvallis. Oregon, 2006.



Bartlett pear. Trees used in the study averaged 1037 blossom clusters per tree. Symptoms of fire blight were observed first on 30 April. Disease intensity was moderate with symptoms of fire blight developing on 16% of inoculated blossom clusters treated with water only **(Table 1; at end of report).** Because of a cold front and associated rainfall that occurred at full bloom (1.2 inches of precipitation from April 14 to 16), the concentration of pathogen inoculum applied to the plot area on 13 April prior was five times greater than we have typically used for similar field experiments. Agri-mycin 17 provided a high degree of fire blight control. As measured by either the mean number or mean incidence of infected blossom clusters per tree, all treatments that involved a biological applied once near full bloom followed by a single application of Mycoshield provided a significant level of disease control. The mixture of A506 AprX - and BlightBan C9-1 followed by Mycoshield was the only treatment that was similar statistically to the result obtained with Agri-mycin 17.

Golden Delicious apple. Trees used in the study were moderately sized with an average of 1340 blossom clusters per tree. In the evening of 3 May, all trees were fogged with water to compensate for extremely dry conditions during bloom. Symptoms of fire blight were observed first on 3 May.

Disease intensity was extremely high **(Table 2)**; symptoms of fire blight developed on ~ 100% of inoculated blossom clusters treated with water-only. Intense disease pressure was likely due to usually warm weather (up to 26°C) after inoculation of flowers with the pathogen. Under these conditions, the standard chemical treatments AgriMycin-17 and Mycoshield failed to significantly reduce the incidence of fire blight compared to water treated controls. Of all of treatments, only two integrated disease control methods provided significant control of fire blight compared to water-treated controls. The treatments consisting of 1) BlightBan C9-1 combined with A506 AprX - and FeEDDHA applied once near full bloom followed by a single application of Mycoshield or 2) BlightBan C9-1 combined with BlightBan A506 applied once near full bloom followed by a single application of Mycoshield provided a significant level of disease control by analysis of mean number of infected blossom clusters per tree and transformed disease incidence data.

Rome Beauty apple. Trees used in the study averaged 625 blossom clusters per tree. Symptoms of fire blight were observed first on 22 May. Disease intensity was heavy with symptoms of fire blight developing on 39% of inoculated blossom clusters treated with water only **(Table 3)**. Average daily temperatures were 68°F ranging from 58 to 91°F and two major rain events. Agri-mycin 17 provided a high degree of fire blight control. The mixture of A506 AprX - and BlightBan C9-1 followed by Mycoshield also provided a substantial level of disease control.

Fig. 2

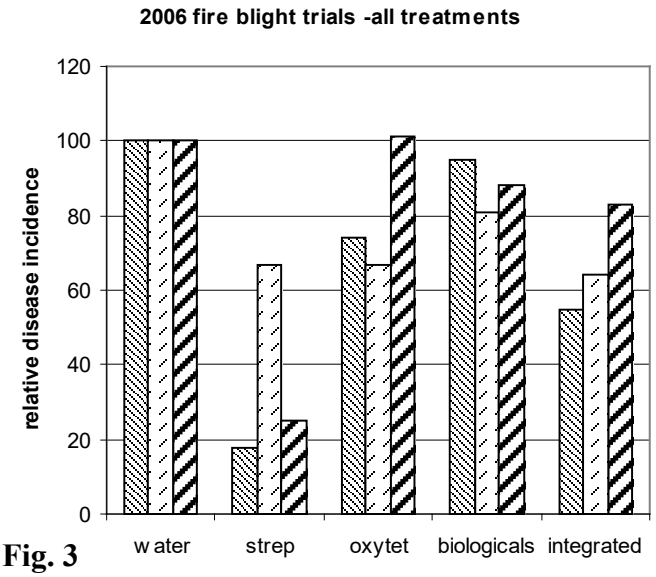
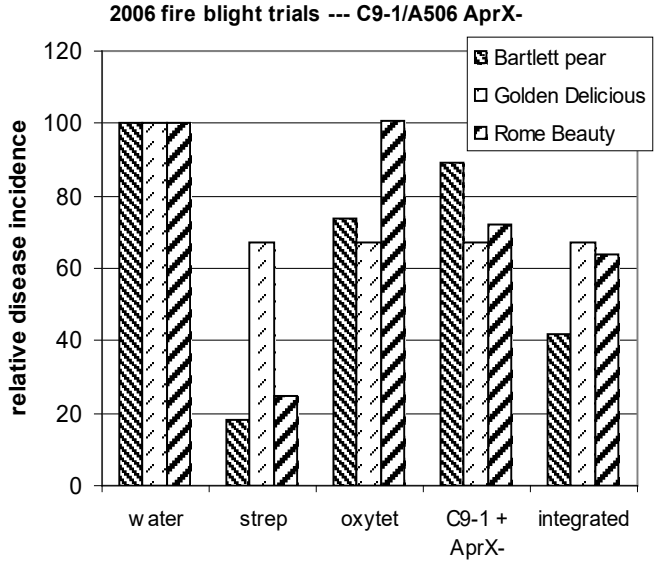


Fig. 3



Overall performance of treatment groups. With treatments scaled relative to the amount of disease observed on the water treated control, Agri-mycin 17 (streptomycin) provided an average of 60% contro1 of fire blight caused by streptomycin-sensitive strain Ea153N (Fig. 2). Mycoshield averaged 20% control, and the average control of all treatments that involved **only** a biopesticide was 12%. The average control obtained from all treatments that followed an 'integrated' treatment regimen (a biopesticide follow by Mycoshield) was 37% (Fig. 2). The most efficacious biopesticide treatment, *P. agglomerans* C9-1 combined with *P. fluorescens* A506 AprX -, provided and average of 34% control (Fig. 3). One application of C9-1 plus A506 AprX - followed by Mycoshield reduced the relative incidence of disease flower cluster by 44% (Fig. 3).

DISCUSSION

As measured against what is typical in commercial pear and apple orchards, the amount of disease that developed in the experimental orchards was extreme. Fire blight infection was promoted by favorable weather conditions in combination with inoculation of the pathogen onto the trees at full bloom. The high disease pressure is the principal reason the Mycoshield (oxytetracycline) performed below its longer term average (40 to 50% disease reduction) in all three trials. Nonetheless, as we have observed in previous seasons, the 'integrated strategy' resulted in greater disease control than either biopesticides or oxytetracycline applied alone. This strategy is has evolved from years of research trials (Fig. 4 next page) involving biopesticides, antibiotics and other chemical agents. A draw back of experimental trials is that the inoculation event introduces the pathogen to flowers all at once, whereas in a commercial orchard, pathogen populations build slowly over a period of several days. Consequently, we predict that integrated treatments will perform better in commercial orchards than we observe in our inoculated plots.

A fire blight forecasting model has been adapted to employ the integrated biopesticide and antibiotic strategy (illustrated in Fig. 5 next page; publication: Johnson, K. B., Stockwell, V. O. and Sawyer, T. L. 2004. Adaptation of fire blight forecasting to optimize the use of biological controls. Plant Disease 88:41-48)

In April 2006, BlightBan C9-1 was granted a registration and tolerance exemption by EPA (Fig. 6 next page). NuFarm has indicated to us that they will now pursue registration of A506 AprX- (a strain of Blight A506 that enhances effectiveness C9-1). A506 AprX- is a protease-deficient mutant of previously registered strain A506. Their intention is to market BlightBan C9-1 in combination with A506 AprX-.

Fig. 4. Summary of fire blight trials conducted at Oregon State University from 1991 to 2006. All treatments scaled relative to the amount of disease observed on the water treated control.

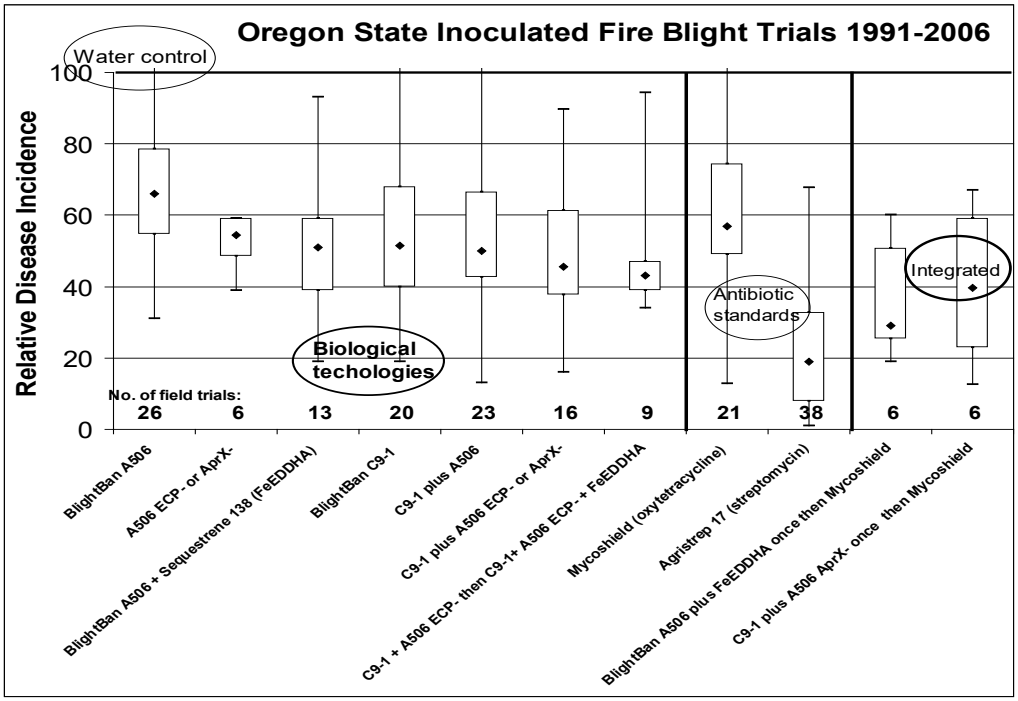


Fig. 5. Working model of the 'integrated' biopesticide/antibiotic strategy. Timing of fire blight treatments is based on bloom stage and a temperature-based disease risk assessment.

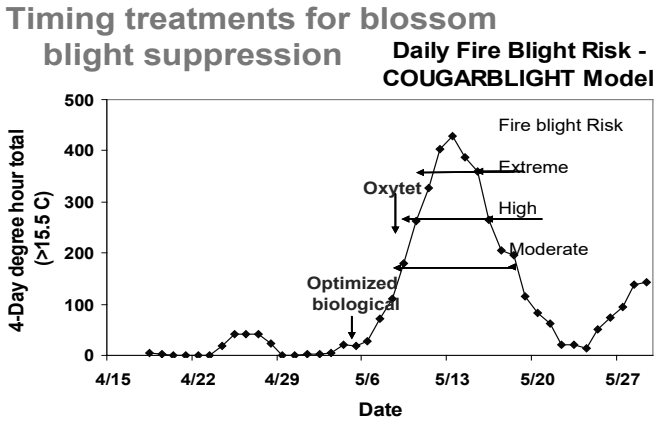
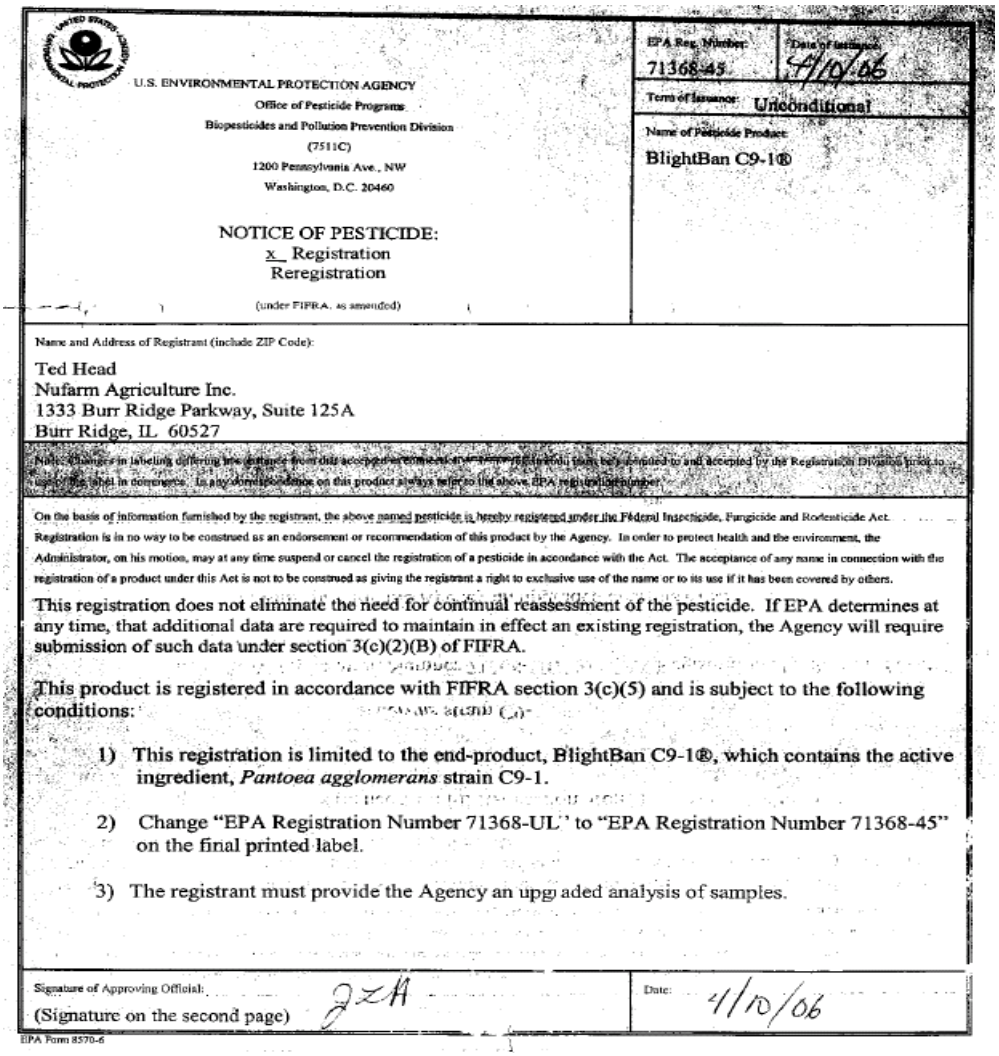


Fig. 6. EPA Registration of Blight Ban C9-1:



U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
(7511C)
1200 Pennsylvania Ave., NW
Washington, D.C. 20460

EPA Reg. Number: 71368-45
Date of Issuance: 4/10/06
Term of Issuance: Unconditional
Name of Pesticide Product: BlightBan C9-1®

NOTICE OF PESTICIDE:
x Registration
Reregistration
(under FIFRA, as amended)

Name and Address of Registrant (include ZIP Code):
Ted Head
Nufarm Agriculture Inc.
1333 Burr Ridge Parkway, Suite 125A
Burr Ridge, IL 60527

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Registration Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered under the Federal Insecticide, Fungicide and Rodenticide Act. Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This registration does not eliminate the need for continual reassessment of the pesticide. If EPA determines at any time, that additional data are required to maintain in effect an existing registration, the Agency will require submission of such data under section 3(c)(2)(B) of FIFRA.

This product is registered in accordance with FIFRA section 3(c)(5) and is subject to the following conditions:

1) This registration is limited to the end-product, BlightBan C9-1®, which contains the active ingredient, *Pantoea agglomerans* strain C9-1.

2) Change "EPA Registration Number 71368-UL" to "EPA Registration Number 71368-45" on the final printed label.

3) The registrant must provide the Agency an upgraded analysis of samples.

Signature of Approving Official: JZH
(Signature on the second page)
Date: 4/10/06

EPA Form 8570-6

TABLE 1. Bartlett pear, Corvallis, Oregon, 2006 Fire blight trial

Treatment	Rate per 100 gallons water	Date treatment applied* 10 April 30% bloom	12 April 90%-bloom	18 April full bloom	Mean number of blighted clusters per tree**		Mean percent of clusters blighted***	
BlightBan C9-1&	2.5 oz.	X	X§	---				
BlightBan A506	2.5 oz.	X	X	---	154	a	17.4	a
BlightBan C9-1 &		X	X	---				
A506 AprX- &	total 10^8	X	X	---				
hrpL mutant	CFU/ml	X	X	---	153	a	15.5	ab
Water control	**------**	**---**	**X**	**X**	**162**	**a**	**15.6**	**abc**
Tanos	12.0 fl oz	---	X	X	153	ab	15.3	abc
Avirulent *hprL* mutant	10^8							
of *E. amylovora*	CFU/ml.	X	X	---	145	abc	14.6	abcd
BlightBan C9-1 &	total 10^8							
A506 AprX-	CFU/ml.	X	X	---	138	abc	14.0	abcde
Physpé	9.7 fl. oz.	X	X	X	114	abc	13.4	abcdef
BlightBan C9-1 &	2.5 oz.	X	X	---				
BlightBan A506 &	2.5 oz.	X	X	---				
Sequestrene 138	16 oz.	X	X	---	108	abc	12.8	abcdef
Mycoshield	16 oz.	---	X	X	116	abc	11.6	abcdefg
Famoxate	11.4 fl oz.	---	X	X	114	abc	11.0	bcdefg
BlightBan C9-1&	2.5 oz.	---	X	---				
A506 AprX- &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	96	bcd	10.7	cdefg
BlightBan C9-1	5 oz.	X	X	---	86	cd	8.9	defg
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506 &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	87	cd	8.8	efg
BlightBan C9-1	5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	90	cd	8.3	efg
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506	2.5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	87	d	8.5	fg
BlightBan C9-1 &	total 10^8	---	X	---				
A506 AprX-	CFU/m.	---	X	---				
then Mycoshield	16 oz.	---	---	X	76	d	6.7	gh
Agri-mycin 17	8 oz	---	X	X	30	e	2.8	h

TABLE 2. Golden Delicious Apple, Corvallis, Oregon, 2006 Fire blight trial

Treatment	Rate per 100 gallons water	Date treatment applied* 22 April 30% bloom	24 April 80%-bloom	27 April full bloom	Mean number of blighted clusters per tree**		Mean percent of clusters blighted***	
Water control	**------**	**---**	**X§**	**X**	**1622**	**a**	**121**	**a**
BlightBan C9-1 &	2.5 oz.	X	X	---				
BlightBan A506 &	2.5 oz.	X	X	---				
Sequestrene 138	16 oz.	X	X	---	1518	ab	114	a
Avirulent *hpr*L-	total 10^8							
E. amylovora	CFU/ml	X	X	---	1445	abc	105	ab
Famoxate	11.4 fl oz.	---	X	X	1408	abc	103	abc
Tanos	12.0 fl oz	---	X	X	1376	abc	102	abc
BlightBan C9-1 &		X	X	---				
A506 AprX- &	total 10^8	X	X	---				
*hrp*L- mutant	CFU/ml	X	X	---	1363	abc	101	abc
BlightBan C9-1	5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	1352	abc	95	abc
Physpé	9.7 fl. oz.	X	X	X	1203	abc	90	abc
BlightBan C9-1 &	total 10^8							
A506 AprX-	CFU/ml	**X**	X	---	1147	abc	81	abc
Mycoshield	16 oz.	---	X	X	1126	abc	82	abc
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506 &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	1124	abc	83	abc
BlightBan C9-1	5 oz.	X	X	---	1103	abc	86	abc
BlightBan C9-1 &	total 10^8	---	X	---				
A506 AprX-	CFU/ml.	---	X	---				
then Mycoshield	16 oz.	---	---	X	1076	abc	81	abc
Agri-mycin 17	8 oz	---	X	X	1015	abc	82	abc
BlightBan C9-1&	2.5 oz.	---	X	---				
A506 AprX- &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	901	bc	67	bc
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506	2.5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	837	c	62	c

TABLE 3. Rome Apple, Corvallis, Oregon, 2006 Fire blight trial

Treatment	Rate per 100 gallons water	Date treatment applied* 26 April 30% bloom	28 April 70%-bloom	1 May full bloom	Mean number of blighted clusters per tree**		Mean percent of clusters blighted***	
Mycoshield	16 oz.	---§	X§	X	259	a	39.6	a
BlightBan C9-1&	2.5 oz.	X	X	---				
BlightBan A506	2.5 oz.	X	X	---	242	a	39.3	a
Water control	------	---	**X**	**X**	**230**	**a**	**39.1**	**ab**
BlightBan C9-1 &		X	X	---				
A506 AprX- &	total 10^8	X	X	---				
*hrp*L- mutant	CFU/ml	X	X	---	242	a	38.9	ab
BlightBan C9-1&	2.5 oz.	---	X	---				
A506 AprX- &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	239	a	38.1	ab
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506	2.5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	231	a	36.9	ab
BlightBan C9-1	2.5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	200	a	32.4	ab
BlightBan C9-1 &	total 10^8							
A506 AprX-	CFU/ml.	X	X	---	176	a	30.2	ab
Avirulent *hpr*L- mutant of *E. amylovora*	10^8 CFU/ml.	X	X	---	193	a	29.9	ab
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506 &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	186	a	29.7	ab
BlightBan C9-1 &	total 10^8	---	X	---				
A506 AprX-	CFU/m.	---	X	---				
then Mycoshield	16 oz.	---	---	X	156	a	25.2	b
Agri-mycin 17	8 oz	---	X	X	60	b	9.7	c

Key to tables:* Trees inoculated on 13 April (Bartlett pear), 25 April (Golden Delicious apple) or 29 April (Rome Beauty apple) with 1 x 10^6 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 $P = 0.05$. Data were transformed log(x) prior to analysis

*** Mean disease incidence values followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$. Data were transformed arcsine (square root(x)) prior to analysis.

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

FINAL PROJECT REPORT
WTFRC Project Number: PR-04-433

Project Title: Harvest and postharvest practices for optimum quality

PI: Jim Mattheis
Organization: USDA, ARS TFRL
Telephone/email: (509)664-2280 x249 mattheis@tfrl.ars.usda.gov
Address: 1104 N. Western Ave
City: Wenatchee
State/Province/Zip WA 98801

Budget History:

Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries*	44,233	49,042	24,857
Benefits	13,270	14,712	12,243
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	2,300	2,300	800
Travel	0	0	0
Miscellaneous	0	0	0
Total	59,803	66,054	37,900

*Salaries: 2004: GS-9 biological science tech., 2005: GS-11 Postdoctoral Research Associate, 2006: GS-11 Postdoctoral salary at 0.5FTE, the other 0.5FTE funding provided by ARS.

Objectives:

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

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

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

Significant Findings 2004-2006:

- Additional measures of fruit firmness/texture may provide additional harvest maturity information.
- Changes in production of several volatile compounds by freshly harvested 'Bartlett' and 'd'Anjou' pears that correlate with optimum harvest date based on firmness are not consistent between lots.
- Ripening capacity of 1-MCP treated 'd'Anjou' pears increases with CA O_2 concentration and CA storage duration.
- Efficacy of 1-MCP can be reduced by sufficient ethylene or CO_2 present during treatment.
- Efficacy of post-storage 1-MCP treatment of 'Bartlett' pears may be dependent on ethylene present during treatment.
- Field application of 1-MCP can slow postharvest ripening of 'Bartlett' pears.
- The low oxygen limit for 'd'Anjou' pears defined by chlorophyll fluorescence is subject to seasonal variation.
- Impacts of 'Bartlett' and 'd'Anjou' storage at the low oxygen limit are cultivar and lot specific and dependent on storage duration.

Results and Discussion:

Indicators of maturity/storability.

Studies over the 3 year project period indicate considerable variability exists between seasons and lots within a season in the progression of fruit quality and physiological parameters analyzed as a function of fruit development. The focus of this objective was to evaluate potentially new means to assess maturity, and the main factors evaluated were non-ethylene, non-respiratory volatile production and additional measures of fruit physical properties. Although changes in volatile production during maturation were observed for both 'Bartlett' and 'd'Anjou, consistent differences across lots and seasons that could be useful as an additional measure of maturity were not apparent. Changes in emission of total or specific volatiles, most often esters, were apparent the week of or the week prior to optimum maturity (based on storage data) but the same patterns were not observed across lots and seasons. The same outcome was observed for analysis of another gas produced by fruit, nitric oxide (NO). While NO production tended to increase during fruit development, consistent changes in NO that may be of utility for maturity assessment were not observed.

Additional measures of fruit texture/firmness may have potential as indicators of storability. An instrument that records information for a number of physical aspects including firmness at multiple points to the core was used for this portion of the studies. A measure of firmness of the inner portion of 'd'Anjou' fruit (M2, 0.32"in to the coreline) in some instances showed changes in values from week to week that were not reflected in the firmness value for the outer portion of the fruit (M1, fruit

surface to 0.32” in). Differences in M2 between lots with similar M1 values were observed as were differences between seasons. As both M1 and M2 values decrease as fruit soften, higher M2 values at harvest may be a factor influencing differences in storability between lots.

	August 11	August 18	August 25	September 1	September 8
Firmness lbs	19.6	19.1	19.0	16.1	15.8
Starch (1-6)	1	1	1	1.5	4.5
Ethylene (ppm)	nd	nd	nd	0.02	nd
Butyl acetate*	13.3	13	848	266	216
Pentyl acetate*	1.6	1.7	87	10.5	1
Hexyl acetate*	3.0	4.4	1879	314	0.14
total esters*	473	2090	4153	1232	1234
NO (nL kg h^{-1})	nd	nd	nd	1.1	2.6

Table 1. Bartlett maturity 2004. NO: nitric oxide. *nM kg^{-1} m^{-3}

Orchard 1	August 3	August 9	August 15	August 22	August 29
Firmness lbs	15.7	14.9	12.4	13.1	12.1
Starch (1-6)	1.2	1.5	2	4.7	4.4
esters*	7	140	42	630	4250
aldehydes*	425	190	210	1080	2465
total detected*	530	335	260	1750	6870

Orchard 2	August 4	August 10	August 19	August 24	Sep 1
Firmness lbs	18.7	18.6	16.8	16.8	14.6
Starch (1-6)	1	1.1	1.1	1.2	1.5
esters*	400	625	555	70	1150
aldehydes*	12,500	755	4310	830	590
total detected*	13,430	1380	4880	920	1770

Orchard 3	August 5	August 15	August 22	August 29	Sep 6
Firmness lbs	19.7	17.4	16.7	18.2	17.4
Starch (1-6)	1	1.1	1	1.4	1.9
esters*	430	105	84	39	270
aldehydes*	5185	680	555	530	775
total detected*	5620	785	640	570	1050

Table 2. Bartlett maturity 2005. *nM kg^{-1} m^{-3}

Orchard 1 '04	August 22	August 30	August 22	August 29	September 6
M1 lbs	15.9	14.4	13.5	12.4	10.8
M2	18.8	18.1	15.8	14.6	14.3

Orchard 2 '05	August 18	August 24	September 1	September 8	September 15
M1 lbs	16.1	14.9	14.6	14.5	13.1
M2	22.1	20.3	20.0	17.5	17.0

Orchard 3 '06	August 30	September 7	September 13	September 20	September 27
M1 lbs	15.6	14.2	14.1	12.7	12.4
M2	22.3	18.6	18.8	17.7	15.8

Table 3. 'd'Anjou' firmness at harvest . Values are lbs. M1: highest pressure in outer portion of fruit, 0-0.32"; M2: highest pressure in fruit region 0.32" to coreline.

Protocols for 1-MCP that ensure predictable ripening

'd'Anjou' 1-MCP/CA: 'd'Anjou' pears treated with 300 ppb 1-MCP at harvest were stored in CA with 0.5% CO_2 and up to 5% O_2. After 6 and 9 months plus 7 days at 68 ^oF , peel color rating (1=green, 5=yellow) increased with O_2 concentration but 1-MCP-treated fruit remained greener than controls. Softening increased with increased O_2 concentration after 6 and 9 months, however, after 6 months, 1-MCP treated fruit did not soften to 6 lbs or less in 7 days. After 9 months, 1-MCP-treated fruit stored at 3 or 5% O_2 softened to 3.8 and 3.4 lbs, respectively. Fruit treated with 1-MCP did not develop scald regardless of storage environment, and decay incidence in some cases was lower in 1-MCP-treated fruit. This trial shows the potential for mitigation of 1-MCP-induced ripening delay over long storage durations by O_2 management during CA storage.

Month	O_2 %	Color d7		Lbs		Scald (%)		Decay (%)	
		C	MCP	C	MCP	C	MCP	C	MCP
3	1	1.7	1	3.1	13	0	0	0	0
	3	1.9	1	2.6	12.9	0	0	0	0
	5	2.2	1.2	2.7	12.7	0	0	0	0
6	1	2.6	1	1.9	12.3	0	0	6	6
	3	3.2	1.6	1.8	10.6	0	0	0	0
	5	3.6	1.7	1.6	9.5	11	0	6	6
9	1	2.4	1.4	2.0	12.4	89	0	6	6
	3	3.6	2.9	2.4	3.8	67	0	33	6
	5	4.0	3.2	2.7	3.4	67	0	44	0

Table 4. Quality of 1-MCP treated 'd'Anjou' pears after storage. Fruit treated with 300 ppb 1-MCP at harvest. Fruit held 7 days at 68 °F after removal from CA. C: untreated control; MCP: 300 ppb 1-MCP at harvest.

Impact of Ethylene and CO_2 on 1-MCP efficacy: 'Bartlett' pears were treated with 0 or 300 ppb 1-MCP with up to 1000 ppm ethylene or up to 4% CO_2 present during treatment. The presence of 1 or more ppm ethylene was sufficient to completely inhibit efficacy of 1-MCP (table 5). CO_2 concentrations of 2 or 4% during 1-MCP treatment reduced the magnitude of 1-MCP responses (table 6). The results indicate ethylene at relatively low amounts during 1-MCP treatment at harvest can prevent treatment effectiveness and that CO_2 present during treatment can also influence fruit response to 1-MCP.

Treatment	Ethylene	Peel color	Titratable acid %	Lbs	Scuffing %
Control	0	5	0.250	2.5	0
1-MCP	0	2.8	0.332	18.2	0
1-MCP	1	4.8	0.244	2.1	0
1-MCP	10	5	0.202	2.1	12
1-MCP	100	5	0.227	2.1	11
1-MCP	1000	5	0.193	2.0	11

Table 5. 'Bartlett' pear quality after 2 months storage in air plus 7 days at 68 °F. Fruit treated with 300 ppb at harvest with 0, 1, 10, 100, or 1000 ppm ethylene. Peel color: 1=green, 6=yellow.

Month	Treatment	CO_2	lbs	color 1-5	IB	decay %
2	Control	ambient	2.4	5.0	89	0
	1-MCP	ambient	17.6	2.6	0	0
	1-MCP	0.5%	17.5	2.7	0	0
	1-MCP	1.0	17.2	2.8	0	0
	1-MCP	2.0	15.2	3.6	0	6
	1-MCP	4.0	2.1	5.0	0	0
4	Control	ambient	--*	--*	--*	100
	1-MCP	ambient	13.8	5.0	0	0
	1-MCP	0.5%	13.5	4.9	0	0
	1-MCP	1.0	12.8	4.8	0	0
	1-MCP	2.0	8.3	5.0	0	11
	1-MCP	4.0	1.2	5.0	100	61

Table 6. 'Bartlett' pear quality after air storage plus 7 days at 68 ºF. Fruit treated with 300 ppb 1-MCP at harvest with ambient, 0.5, 1.0, 2.0, or 4.0% CO_2. Peel color: 1=green, 5=yellow; IB: internal breakdown. --*: all fruit decayed.

Delayed 1-MCP treatment of Bartlett pears: Fruit were treated with 1-MCP at harvest, the day prior to removal from CA, or after removal from CA. After 2 months storage, delayed 1-MCP treatments slowed but did not prevent ripening. Treatment with 1-MCP after 4 months was not effective. Ethylene produced by fruit accumulated to 18 ppm during the 1-MCP treatment after 4 months. The results indicate the benefits in ripening delay from 1-MCP treatment decrease with increased storage duration between harvest and treatment application.

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

Table 7. Bartlett fruit quality after storage. 1-MCP applied at harvest or prior to or after removal from CA. Fruit held 4 days at 68 ºF after removal from storage.

Responses of 'Bartlett' pears to field applied 1-MCP.

An experimental formulation of 1-MCP was applied to 'Bartlett' pear trees in commercial orchards in two seasons. In 2005, two application dates (A:1 week preharvest, 19.0 lbs; B:1 day prior to commercial harvest, 17.3 lbs) and 3 rates were evaluated. Half the fruit from each field application was also treated with SmartFresh® after harvest. Evaluation of fruit after harvest indicated treatment efficacy for slower ripening as well as a possible effect of fruit maturity at the time of application. After 4 months storage in air, treatment effects from field applications on ripening were not apparent, but the efficacy of a post-storage temperature pre-conditioning period was evident for fruit receiving a postharvest application of 1-MCP.

Treatment Date	Control	M0	M1	M2	M3
A	2.3	2.2	2.4	5.4	7.0
B	4.1	3.9	4.6	14.2	14.9

Table 8. 'Bartlett' firmness after harvest. A: Harvest 1 fruit held 5 days at 50 °F plus 7 days at 68 °F, or B:Harvest 2 fruit held 7 days at 68 °F. Control: unsprayed; MCP: 0,1,2,3 relative amounts; M0 is oil only; P: postharvest SmartFresh® application at 300 ppb.

	Control	P	M0	M1	M2	M3
Lbs	6.8	4.7	4.7	3.6	4.1	3.7

Table 9. 'Bartlett' firmness after 4 months storage in air plus a 13 day pre-conditioning period. Control: unsprayed; M: 1-MCP 0,1,2,3 relative amounts, M0 is oil only; P: postharvest SmartFresh application at 300 ppb.

In 2006, 2 field rates were evaluated as was the influence of harvest delay after application. 1-MCP was applied 7 days prior to the date of anticipated harvest. Fruit were harvested weekly for 4 weeks after application. A postharvest 1-MCP treatment (300 ppb) was performed at each harvest date, and fruit were stored at 31 °F: in air for 1 or 2 months; or CA (1.5/0.5 O_2/CO_2) for 4 months. Impacts on ripening at harvest from field treatments were detectable through 3 weeks after treatment. Fruit size increased in the first two weeks after commercial harvest. Field treatment effects on stored fruit were observed through 2 months where field 1-MCP treatments slowed ripening but fruit became acceptably soft (<6 lbs) in four days after removal from storage. Fruit harvested 2 or 3 weeks after commercial harvest did not show treatment effects after 2 months in air storage.

Harvest	Weight	1-MCP A		1-MCP 2A		spreader only		control		SmartFresh	
		day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4
Aug 28	182 g	17.2	16.8	16.8	16.7	16.6	16.3	16.6	15.3	17.1	16.3
Sep 5	217	15.2	14.4	14.8	14.5	13.9	12.0	14.5	13.7	15.0	14.7
11	243	13.6	11.0	13.1	9.6	11.8	6.7	13.8	6.8	12.3	10.3
18	243	10.8	5.0	10.6	4.7	10.0	4.9	10.1	4.0	10.4	8.1

Table 10. 'Bartlett' weight (all treatments) and firmness

Harvest	1-MCP A		1-MCP 2A		spreader only		control		SmartFresh	
	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4
Aug 28	1.1	1.7	1.0	1.7	1.2	2.2	1.5	3.0	1.4	2.0
Sep 5	1.4	2.2	2.0	3.0	2.3	2.5	2.2	3.6	2.2	2.5
11		2.8		3.3		3.3		3.9		3.3
18	3.3	4.2	3.3	4.5	3.5	4.3	3.6	4.9	3.6	4.3

Table 11. Bartlett color 1 and 4 days after 1 month in air. A: 1-MCP 1x; 2A: 1-MCP 2x.

Harvest	1-MCP A		1-MCP 2A		spreader only		control		SmartFresh	
	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4
Aug 28	15.7	13.4	15.6	14.1	15.9	5.6	15.2	3.4	15.3	15.5
Sep 5	14.1	8.0	13.5	6.1	11.8	4.2	13.0	3.1	14.0	13.3
11		10.2		8.5		7.7		6.2		10.2
18	7.1	2.6	8.1	2.9	7.7	2.7	5.7	2.2	7.0	6.8

Table 12. Bartlett firmness 1 and 4 days after 1 month in air. A: 1-MCP 1x; 2A: 1-MCP 2x.

Harvest	1-MCP A		1-MCP 2A		spreader only		control		SmartFresh	
	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4
Aug 28	0	0	0	0	0	0	0	0	0	0
Sep 5	0	0	0	0	0	0	0	0	0	0
11		8		0		17		17		21
18	33	33	21	13	13	19	25	42	29	45

Table 13. Bartlett internal breakdown 1 and 4 days after 1 month in air. A: 1-MCP 1x; 2A: 1-MCP 2x.

Harvest	1-MCP A		1-MCP 2A		spreader only		control		SmartFresh	
	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4
Aug 28	2.3	2.5	2.3	3.2	2.4	3.1	2.3	4.3	2.6	2.8
Sep 5	2.6	3.3	2.8	3.4	3.2	3.8	3.0	4.7	3.1	3.5
11	3.6	4.1	3.8	4.1	3.8	4.9	4.0	5.0	3.5	4.0
18	4.0	5.0	4.0	5.0	4.0	5.0	4.0	5.0	4.0	5.0

Table 14. Bartlett color 1 and 4 days after 2 months in air. A: 1-MCP 1x; 2A: 1-MCP 2x.

Harvest	1-MCP A		1-MCP 2A		spreader only		control		SmartFresh	
	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4
Aug 28	15.4	11.7	14.7	10.3	15.3	3.3	14.7	2.6	15.0	15.1
Sep 5	13.9	4.9	13.1	4.3	10.1	3.5	11.6	3.3	13.7	12.8
11	10.9	3.5	10.4	3.2	8.7	3.2	9.6	3.0	10.8	9.1
18	7.9	3.5	6.7	3.0	8.1	3.8	7.3	3.5	8.6	7.2

Table 15. Bartlett firmness 1 and 4 days after 2 months in air. A: 1-MCP 1x; 2A: 1-MCP 2x. 2 months.

Harvest	1-MCP A		1-MCP 2A		spreader only		control		SmartFresh	
	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4	day 1	day 4
Aug 28	0	0	0	0	0	0	0	0	0	0
Sep 5	0	0	0	0	8	13	0	4	0	0
11	8	4	4	4	17	25	21	25	21	0
18	46	29	50	39	50	14	46	17	42	17

Table 16. Bartlett internal breakdown 1 and 4 days after 2 months in air. A: 1-MCP 1x; 2A: 1-MCP 2x.

Responses of 'Bartlett' and 'd'Anjou' pears stored at the low O_2 limit defined by chlorophyll fluorescence: 2004: The O_2 concentration at which changes in peel chlorophyll fluorescence of 'Bartlett' and 'd'Anjou' pears (3 lots each) occurred were 0.2 and 0.3% O_2, respectively Fruit were stored in CA with 0.5% CO_2 with 1.5 (control) or 0.4 O_2 for 'Bartlett' and 0.5% O_2 for 'd'Anjou'. Responses of both cultivars varied between lots and with storage duration. 'Bartlett' fruit stored at 0.5% O_2 were slightly greener than fruit stored at 1.5% O_2 when fruit was removed from CA. Incidence of core browning, senescent scald, and internal breakdown were reduced by storage at 0.5% O_2. 'd'Anjou' fruit stored at 0.4% O_2 degreened slower, did not develop scald, and softened slower but two lots developed peel speckling compared to fruit stored at 1.5% O_2. Increasing O_2 concentration to 1.5% during storage was not consistently effective to prevent development of speckling.

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

Table 17. 'Bartlett' fruit quality after storage. Fruit were held at 68 ^{o}F for 7 days prior to analysis. Values are means for 3 lots. Trt: treatment; Color: 1=green, 5=yellow; Core B: core browning incidence; sen scld: senescent scald incidence; IB: internal browning and/or breakdown; decay: decay incidence.

Month	Trt	Color d0	Color d7	TA %	Scald %	lbs	Decay %
2	RA	1.5	2.6	0.248	0	1.9	0
	1.5 O_2	1.4	1.8	0.264	0	3.4	0
	0.5 O_2	1.2	1.9	0.274	0	5.8	0
4	RA	2.5	3.6	0.221	0	2.1	4
	1.5 O_2	1.2	2.3	0.247	0	1.9	2
	0.5 O_2	1.0	1.9	0.243	0	2.9	0
6	RA	3.1	3.8	0.185	39	2.6	59
	1.5 O_2	1.4	2.5	0.223	2	1.5	6
	0.5 O_2	1	2.0	0.232	0	2.4	4

8	RA	3.7	4	0.156	91	3.4	72
	1.5 O_2	1.7	2.7	0.206	12	1.8	20
	0.5 O_2	1.4	2.3	0.206	0	2.2	13

Table 18. 'd'Anjou' fruit quality after storage. Fruit were held at 68 ºF for 7 days prior to analysis. Values are means for 3 lots. Trt: treatment; Color: 1=green, 5=yellow; TA: titratable acidity: speckling: peel speckling incidence; scald: superficial scald incidence; decay: decay incidence.

Lot	O_2/CO_2	Speckling %
A	1.5/0.5	0
	0.5/0.5	71
	0.5/0.5 2months	0
	0.5/0.5 4months	0
	0.5/0.5 6 months	61
B	1.5/0.5	0
	0.5/0.5	0
	0.5/0.5 2months	0
	0.5/0.5 4months	0
	0.5/0.5 6 months	0
C	1.5/0.5	0
	0.5/0.5	39
	0.5/0.5 2months	12
	0.5/0.5 4months	44
	0.5/0.5 6 months	61

Table 19. Incidence of 'd'Anjou' peel speckling after CA storage. Fruit were held in CA at 1.5/0.5% or 0.5/0.5% O_2/CO_2 for 8 months, or 0.5/0.5% for 2, 4, or 6 months then 1.5/0.5% to 8 months.

In 2005, 'd'Anjou' pears (3 lots) obtained at commercial harvest were analyzed for changes in the chlorophyll fluorescence signal at O_2 concentrations as low as 0.1%. No change in fluorescence during the analysis of any of the lots was observed through May. The storage O_2 setpoint of 0.4% was accompanied by CO_2 concentrations of 0.5 or less than 0.1% to determine if impacts from CO_2 occur during ultra low O_2 storage. Through 8 months fruit stored at 0.4% O_2 had slower rates of softening, color change and acid loss compared to fruit stored at 1.5% O_2 (Table 20). Superficial scald developed after 6 and 8 months only on fruit stored at 1.5% O_2. No speckling was observed, and no O_2 concentration effects on decay incidence were observed. No evidence of anaerobic metabolism induced by low O_2 treatments was observed via analyses of ethanol, acetaldehyde, and methanol. After 8 months, fruit stored at 0.4% O_2 appeared to have less of these compounds compared to fruit stored at 1.5% O_2.

Months	Treatment	lbs	Color	TA%	scald	decay	EtOH*	Act*	MeOH*
2	1.5 O_2 0.5 CO_2	3.6	1.8	0.230	0 %	0 %	2.1	0	0
	1.5 O_2 0.1 CO_2	3.9	1.7	0.242	0	0	2.2	0	0
	0.4 O_2 0.5 CO_2	11.6	1.5	0.275	0	0	6.3	0	0
	0.4 O_2 0.1 CO_2	11.3	1.5	0.300	0	0	6.9	0	0
4	1.5 O_2 0.5 CO_2	2.2	1.9	0.205	0	0	21	1.2	2.5
	1.5 O_2 0.1 CO_2	1.7	2.2	0.150	0	0	17	1.0	2.3
	0.4 O_2 0.5 CO_2	4.6	1.5	0.241	0	0	33	1.4	1.6
	0.4 O_2 0.1 CO_2	7.7	1.3	0.250	0	0	28	1.3	0
6	1.5 O_2 0.5 CO_2	1.8	3.0	0.193	0	4	58	2.6	17
	1.5 O_2 0.1 CO_2	1.8	3.0	0.191	6	4	65	3.3	20
	0.4 O_2 0.5 CO_2	5.2	2.1	0.207	0	7	55	2.4	0.6
	0.4 O_2 0.1 CO_2	7.2	1.7	0.215	0	2	55	2.6	0
8	1.5 O_2 0.5 CO_2	2.0	2.7	0.202	25	15	127	7.5	53
	1.5 O_2 0.1 CO_2	2.1	2.6	0.196	33	15	161	10	57
	0.4 O_2 0.5 CO_2	5.2	2.2	0.241	0	15	102	4.8	3.7
	0.4 O_2 0.1 CO_2	6.1	2.1	0.287	0	6	90	4.5	1.8

Table 20. 'd'Anjou' fruit quality after storage. Fruit were held at 68 ^oF for 7 days prior to analysis. Values are means for 3 lots. Color: 1=green, 5=yellow; d: days ripening after removal from storage; TA: titratable acidity; EtOH: ethanol, mg/kg; Act: acetaldehyde, mg/kg; MeOH: methanol, mg/kg.

Summary

Characterization of pear maturity at harvest relies primarily on firmness assessment. Other indices evaluated previously and as part of this study including starch hydrolysis, ethylene production, soluble solids content, titratable acidity, and color have not proven to be consistently reliable indicators of physiological development. Evaluations in this study of emission of other volatile compounds indicated detectable changes that were coincident with maturation, however, the changes detected were not consistent across lots or seasons, or occurred after optimum maturity for storage was reached. This lack of consistency relative to firmness measurements appears to limit the applicability of volatile analysis as performed as an assessment of maturity and storability.

A more extensive evaluation of fruit firmness may provide additional information that could be utilized at harvest. The standard method of firmness measurement where only the outer portion of the fruit is assessed provides only partial information regarding fruit physical condition. Softening and changes in texture are not uniform throughout pear fruit. The studies conducted for this project indicated for 'd'Anjou' in particular, detectable changes in fruit firmness and other properties were observed that could be useful as an indication of storability. Considering only firmness values for the outer (0.32", M1) and inner (0.32" in to the coreline, M2) portions of the fruit, several patterns emerged. Instances were observed where firmness changes in the outer portion (M1) were not detected over a several week period were accompanied by significant changes in M2 values over the same period. Another pattern showed consistent differences between lots in M2 values when similar M1 values were measured. Lots with higher M2 values may take longer to ripen as these fruit go into storage with a higher inner as well as overall fruit firmness. The postharvest studies in this project were not sufficient to evaluate possible relationships between these values and storability, therefore, further research is needed to establish the utility of the M1/M2 relationship as an at-harvest tool.

The commercial use of SmartFresh continues to be limited by the uncertainty of ripening by treated fruit after storage. Studies conducted in this project indicated interactions between CA environment

used for 1-MCP treated fruit exist that may provide a means to enhance ripening capacity. While the success of higher O_2 concentrations to promote earlier ripening of treated fruit is consistent with the long history of CA research, a challenge that remains in implementing this type of protocol is an assessment of lot to lot performance under conditions that reflect commercial reality. The CA system used in these studies provides a means to evaluate many combinations of gas composition and temperature. However, the number of fruit and lots testable under our conditions is relatively small and larger scale trials at the commercial or semi-commercial level are needed for validation.

The results of studies evaluating impacts of ethylene and CO_2 present during 1-MCP treatment show these compounds may interfere with treatment efficacy. These studies were conducted using only one rate of 1-MCP, further research is required to determine if other 1-MCP rates can reduce the risk of ethylene and/or CO_2 impacts on treatment efficacy.

Field use of 1-MCP shows potential as another means to impact fruit ripening. For 'Bartlett', a harvest delay of one week in this study resulted in a significant fruit size increase. Results from 2006 indicate the duration of a field 1-MCP application is less than a postharvest treatment, however, fruit firmness continued to decrease following field 1-MCP application. Ideally a delay in firmness loss in the field would assure fruit are packable with minimal scuffing after harvest. Development of a field protocol for 1-MCP that can result in delayed harvest with less of a postharvest response may be a means to increase harvested fruit size with less potential for marketing issues related to ripening.

Long-term storage of pears continues to provide challenges related to quality and disorder control. The potential for storage at O_2 concentrations at less than what is current industry practice was demonstrated for superficial scald control, ripening delay, and lack of accumulation of anaerobic products that could impact consumer acceptance. Storage at <1% O_2 was not without problems, specifically, development of peel speckling occurred only in the lowest O_2 environments. No incidence of pithy brown core was observed, a disorder previously identified by Paul Chen, OSU, retired, as a risk during ultra low O_2 storage of 'd'Anjou'. Speckling was a lot to lot phenomena in these studies, and lot specific factors influencing its development are currently unknown. Future research to provide insight into the physiology of speckling development may ultimately be a means to develop protocols for low O_2 d'Anjou' storage that mitigate the risk of speckling development.

FINAL PROJECT REPORT

Project Title: MCP and edible coatings to extend storage and marketing life of pears

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Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries			21,333
Benefits			12,586
Wages		14,700	
Benefits			
Equipment			
Supplies	15,000	14,700	4,500
Travel		300	500
Miscellaneous			
Total	15,000	29,700	38,919

Significant findings:

- Thermofogging of ethoxyquin substantially controlled superficial scald of Anjou pears. A dosage of 60 g per ton at harvest plus a second fogging at 30 g per ton after two months of storage gave the best control.
- MCP completely controlled superficial scald of Anjou pears. However, it caused a loss of ripening ability. Study of re-initiating the ripening ability is on-going.
- Field applications of MCP decreased scald incidence of Anjou pears.
- MCP (300 ppb) treatment + pre-conditioning after storage extended storage life of Bartlett pears for two months in both RA and CA storage.
- A coating made of soybean oil emulsion reduced the incidence of superficial scald on Anjou pears.
- A candelilla coating increased the shelf-life of Concorde pears for one week.

Results and Discussion

1. **Effect of MCP on scald incidence and ripening ability of Anjou pears**

1) High dose + pre-conditioning (Fig. 1)

Background and objective: Commercially applicable doses of MCP (300 ppb) controlled scald of Anjou pears, but the fruit lost its ripening ability. Therefore, we adopted a pre-conditioning period to re-initiate ripening.

Methods:

- 1-MCP: 300 ppb at 70°F for 24 hours
- Pre-conditioning: at 50-70°C for 5-20 days

Report:

- Superficial scald: Completely controlled scald after 6 mths in RA or 9 mths in CA
- Ripening ability: did not reach eating quality regardless of temperature and time of pre-conditioning (6 lb, Fig. 1).

2) Short treatment + pre-conditioning (Fig. 2)

Objective: To improve ripening ability by delaying harvest, and decreasing MCP treatment time and temperature.

Methods:

- Harvest maturity: commercial, one- and two-week(s) delayed
- MCP: 300 ppb at 33°F for 6 hours
- Pre-conditioning: at 50°F for 5-15 days

Results:

- Superficial scald: unacceptable incidence after 6 mths in RA or 9 mths CA (Fig. 2A)
- Ripening ability: Most of the treatments did not reach eating quality, except when harvested two-weeks delayed + stored for 6 mths in RA or 9 mths in CA + pre-conditioning at 50°F for 15 + shipping at 33°F for 2-3 weeks (FF $\leq$ 6 lb, Fig. 2B).
- Sensitivity of pears to 1-MCP is higher in one-week delayed fruit, but lower in two-weeks delayed one.

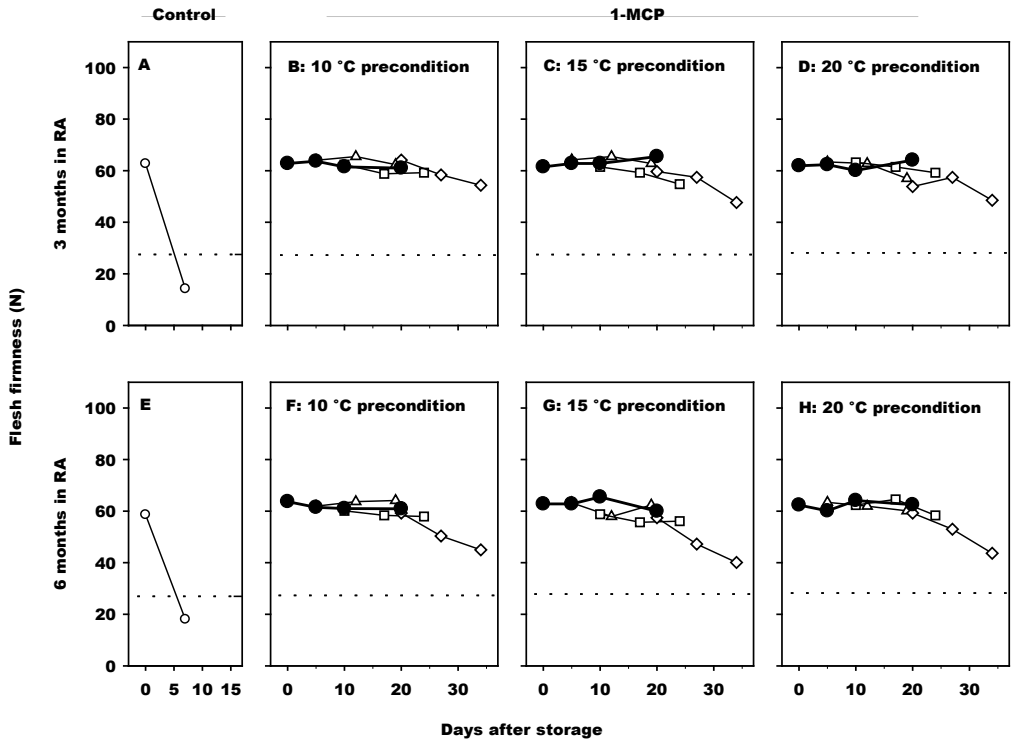

Fig. 1. The effect of MCP treatment on softening of Anjou pears. Fruit were treated with 300 ppb MCP at harvest and stored at 30F for up to 6 months before preconditioning.

3) Low dose + pre-conditioning (Table 1)

Objective: To improve the ripening by decreasing MCP dose to 50 ppb

Methods:

- MCP: 50 ppb at 33°F for 24 hours
- Pre-conditioning: at 50°F for 5-15 days

Results:

- Superficial scald: ~0 after 4 mths in RA or 6 mths in CA (Table 1) and unacceptable incidence after 6 mths in RA or 8 mths in CA.
- Ripening ability: With 5 days of pre-conditioning, fruit softened to eating quality (6 lb, Table 1)

4) Low dose of MCP + delayed ethoxyquin combination (Fig. 3)

Background and objective: 25 ppb of MCP reduced scald without inhibiting ripening. For full control of scald, a delayed ethoxyquin treatment was applied within 60 days of storage. Ethoxyquin is labeled to be used within 7 d after harvest, but for practical purposes it is difficult to perform the application within such a narrow window.

Methods:

- 1-MCP: 25 ppb at 70°C for 24 hours.
- Ethoxyquin: after 1, 7, 30 or 60 days of cold storage, 1000 ppm ethoxyquin

Results:

- Superficial scald: controlled for up to 5 mths in RA.
- Ripening ability: ripened normally.

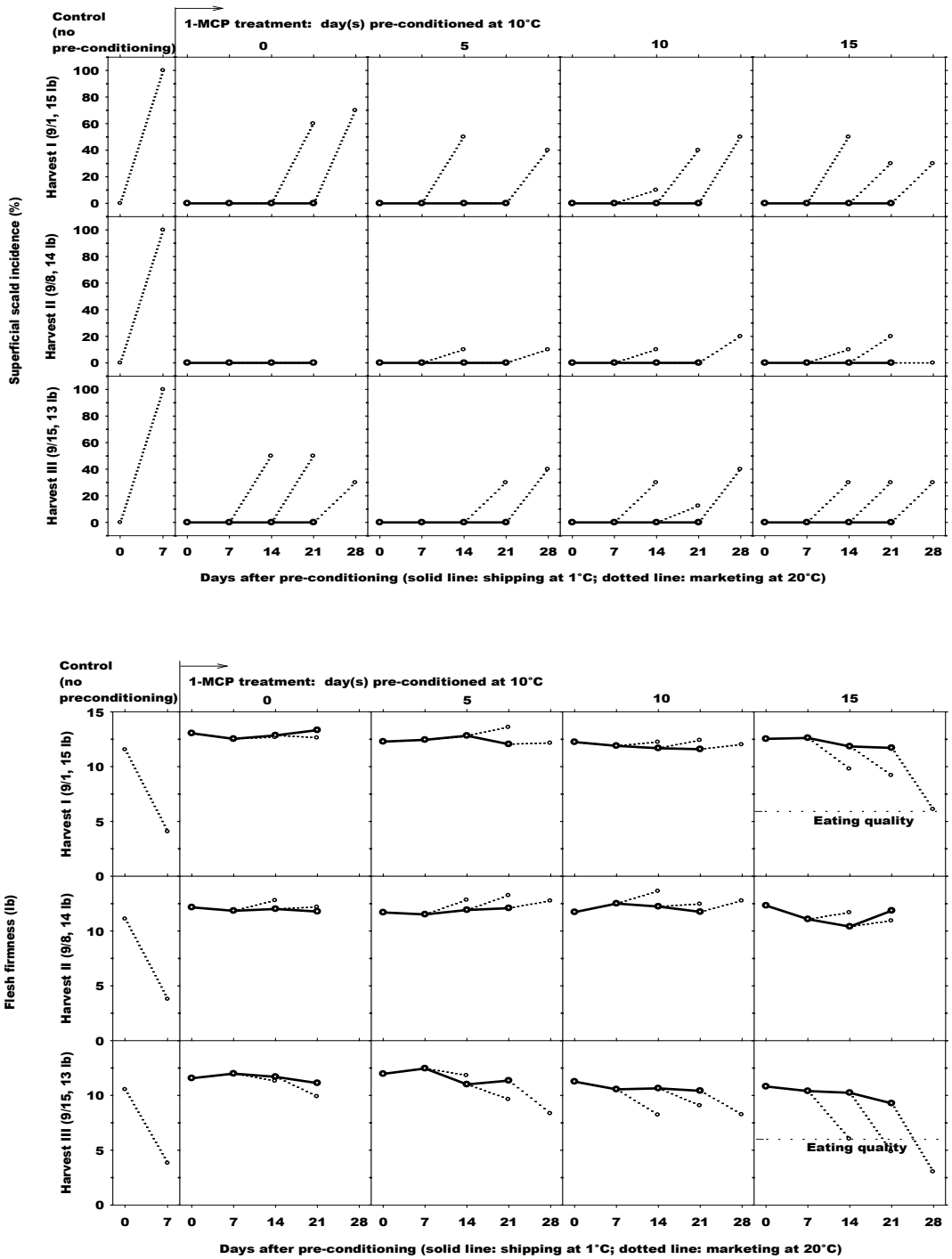

Fig. 2. Effect of MCP treatment on superficial scald incidence (upper, A) and flesh firmness (bottom, B) of Anjou pears. Fruit were harvested at commercial harvest maturity, or one or two week(s) delayed. MCP treatment was applied immediately after each harvest. Fruit were stored at 30F for 6 months before preconditioning.

Table 2. Effects of harvest maturity and postharvest 1-MCP application on ripening behavior and superficial scald of Anjou pears. Fruit were harvested at commercial maturity or 10 days delayed respectively. 1-MCP (55 ppb) was applied at harvest and after 2, 4 and/or 6 months of air or CA storage. Preconditioning was applied for 1-MCP treated fruit for 5 days at 50°F prior to ripening (no preconditioning for the control fruit) for 7 d at 68°F..

Harvest	1-MCP application time (months)	Air				1-MCP application time (months)	CA			
		FF (lb)		Scald			FF (lb)		Scald	
		Before ripening	After ripening	Incidence (%)	Index [z]		Before ripening	After ripening	Incidence (%)	Index
		4 months					***6 months***			
Commercial harvest	Control	14.3	3.1	56.7	1.6	Control	13.8	3.4	26.7	1.0
	0	15.1	2.7	10.0	0.6	0	14.8	4.6	2.5	0.1
	0+2	15.2	2.7	13.3	0.7	0+4	14.6	2.9	0.0	0.1
delayed harvest	Control	13.0	3.6	60.2	1.8	Control	13.3	4.3	30.0	1.0
	0	14.6	4.8	3.3	0.3	0	13.6	6.1	0.0	0.1
	0+2	13.3	4.9	10.0	0.5	0+4	13.6	6.6	0.0	0.2
F-value and significance										
	Harvest (H)	15.52**	8.11*	0.96	0.33	Harvest (H)	6.4	7.83*	0.03	0.07
	MCP (M)	4.21*	0.65	25.92***	15.86***	MCP (M)	1.3	2.04	7.16*	12.39**
	H x M	1.12	1.26	0.19	0.55	H x M	0.3	1.47	0.06	0.04
		6 months					***8 months***			
Commercial harvest	Control	12.6	3.6	68.3	2.3	Control	12.7	3.8	8.3	0.2
	0	13.2	3.3	22.4	0.8	0	14.5	2.8	22.6	0.8
	0+2	13.3	2.7	20.0	1.1	0+4	13.7	2.1	24.9	0.9
	0+4	13.8	2.5	26.7	1.0	0+6	13.6	2.1	28.8	1.0
	0+2+4	13.3	3.0	43.3	1.4	0+4+6	13.9	2.9	10.4	0.6
delayed harvest	Control	12.1	4.3	83.3	2.7	Control	12.2	3.0	15.3	0.5
	0	12.8	3.3	30.0	1.1	0	13.4	4.4	0.0	0.0
	0+2	12.7	3.6	36.7	1.2	0+4	14.1	3.3	3.3	0.3
	0+4	12.7	2.7	23.0	1.0	0+6	14.0	4.3	0.0	0.0
	0+2+4	13.1	4.9	31.8	1.0	0+4+6	14.0	3.4	6.1	0.2
F-value and significance										
	Harvest (H)	1.97	6.91*	0.00	0.07	Harvest (H)	0.27	8.27*	11.42**	11.53**
	MCP (M)	1.32	4.13*	4.14*	3.88*	MCP (M)	4.01*	0.76	0.26	0.51
	H x M	0.22	1.55	0.29	0.22	H x M	1.14	3.04	2.49	2.22

1. Ripening behavior: A 5-day preconditioning was needed for 1-MCP treated fruit. (Fruit did not soften to 6 lb without preconditioning)
2. Scald control: 1-MCP application at harvest significantly controlled scald for at least 6 months in both air and CA storage.
3. Delayed harvest did not decrease scald incidence, nor did multi-applications of 1-MCP.
3. Unexpected results: After CA storage for 8 months, scald incidence decreased except the treatment harvested at commercial maturity and treated with MCP.

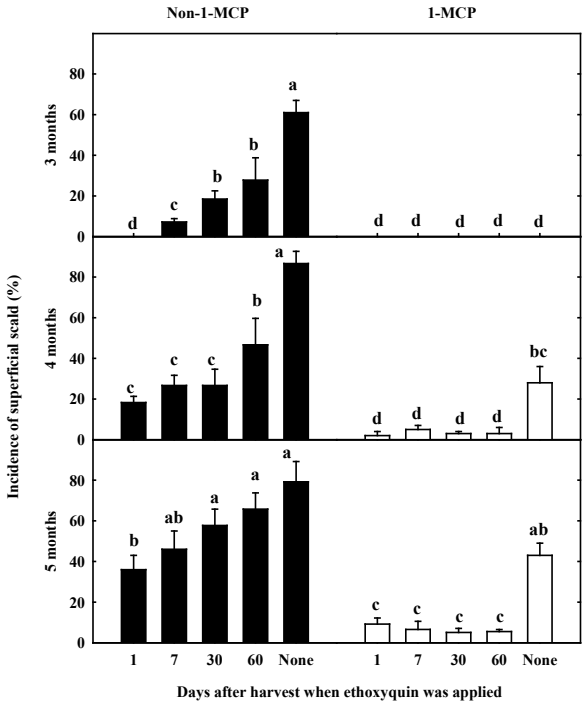

Fig.3. The effect of MCP and ethoxyquin on superficial scald incidence of Anjou pears. Fruit were treated (solid) or untreated (open) with 25 ppb MCP for 24 hours immediately after harvest and then stored at 33°F for up to 5 months. Ethoxyquin drench (1000 ppm) was applied after 1, 7, 30 or 60 days of cold storage. Superficial scald was evaluated after 3, 4, or 5 month storage at 33°F followed by 7 days of shelf life at 70°F. Vertical lines represent SD (n = 3). Within the same storage time (sampling day), vertical bars labeled with the same letter are not significantly different at P = 0.05 using Duncan's multiple range test.

2. Effect of MCP on senescent disorders and ripening ability of Bartlett pears (Table 2)

Background and objective: Storage life of Bartlett pears is relatively short in comparison with winter pears. After 2 months of RA storage or 4 months of CA storage, senescent scald and/or senescent breakdown occur and cause an end of the storage life. The objective of this research was to extend storage and the marketing life of Bartlett pears without a "permanent" loss of the ripening ability.

Methods:

- MCP: 300 ppb at 70°F for 24 hours
- Pre-conditioning: at 50-70°C for 5-20 days

Results:

- Storage life: MCP treated fruit had two months longer storage life in comparison with non-MCP control in both RA and CA storages at 30F.
- Marketing life: MCP treated fruit had one week longer marketing life in comparison with non-MCP control at 70F.
- Ripening ability: The ripening ability of MCP-treated 'Bartlett' fruit recovered in response to many pre-conditioning combinations of 50°-70°C for 10-20 days, as indicated by a decrease in flesh firmness to 6 lb or lower.

3. Thermofogging of ethoxyquin to control Anjou scald (Table 3)

Objectives: To improve efficiency of ethoxyquin application and decrease chemical burn (phytotoxicity) caused by drenching.

Methods:

- Dose: 60 – 90 g/T for the primary fogging at harvest and 30-60 g/T for the second fogging after 2 months of storage.

Results:

- Best treatment based on 2-year results: an initial treatment with 60 g/T dose plus a second fogging of 30 g/T controlled superficial scald as well as drenching at 1000 ppm with less pytotoxicity.

Table 2. Flesh firmness and incidence of internal breakdown of 'Bartlett' pears during shelf life at 20°C

Treatment	Preconditioning		Flesh firmness (N)			Internal breakdown (%)		
	Temperature (°C)	Days	Day-0	Day-7	Day-14	Day-0	Day-7	Day-14
			2 month stored in RA					
Control	0	0	76	7	ND [1]	0	0	57
1-MCP	10	5	83	71	30	0	0	0
		10	81	51	15	0	0	0
		20	77	16	7	0	0	0
	15	5	83	57	18	0	0	0
		10	72	34	14	0	0	0
		20	32	10	ND	0	0	77
	20	5	80	58	13	0	0	0
		10	64	23	11	0	0	0
		20	15	ND	ND	0	73	100
	$LSD_{0.05}$		4	5	2	0	3	2
			4 month stored in RA					
Control	0	0	73	ND	ND	0	97	100
1-MCP	10	5	78	50	21	0	0	0
		10	70	33	ND	0	0	50
		20	32	ND	ND	0	33	67
	15	5	76	38	17	0	0	0
		10	65	24	ND	0	0	63
		20	18	ND	ND	0	77	100
	20	5	76	34	ND	0	0	37
		10	56	19	ND	0	0	63
		20	ND	ND	ND	77	93	100
	$LSD_{0.05}$		6	3	2	2	6	4
			4 month stored in CA					
Control	0	0	79	8	ND	0	0	33
1-MCP	10	5	83	74	31	0	0	0
		10	81	59	24	0	0	0
		20	78	20	9	0	0	0
	15	5	79	72	25	0	0	0
		10	78	47	17	0	0	0
		20	33	10	ND	0	0	53
	20	5	77	68	20	0	0	0
		10	69	25	12	0	0	0
		20	13	ND	ND	0	70	100
	$LSD_{0.05}$		4	6	3	0	3	3
			6 month stored in CA					
Control	0	0	69	ND	ND	0	47	100
1-MCP	10	5	77	54	26	0	0	0
		10	71	35	ND	0	0	63
		20	52	ND	ND	0	57	100
	15	5	74	53	15	0	0	0
		10	69	28	ND	0	0	63
		20	21	ND	ND	0	37	67
	20	5	77	40	ND	0	0	47
		10	58	19	ND	0	0	77
		20	ND	ND	ND	37	67	100
	$LSD_{0.05}$		5	1	1	3	5	6

Table 3. Thermofogging of Xedaquin A (ethoxyquin) and Pyrimethanil control superficial scald and decay of Anjou pears

No.	Method of application	Initial ethoxyquin dose (9/20)	2nd fog ethoxyquin dose (12/1)	Pyrimethanil dose (9/20)	Pyrimethanil residue (11/30)	Ethoxyquin residue (12/1)	Scald				Phytotoxicity				Decay (%)	
							Incidence (%)		Index (4: severe; 3: moderate; 2: slight; 1: very slight; 0: clear)		Incidence (%)		Index (4: severe; 3: moderate; 2: slight; 1: very slight; 0: clear)			
1	Fog (g/T)	0	0	90	1.3		76.8	ab[z]	2.6	b	6.9	d-f	0.2	e-h	0.5	bc
2	Fog (g/T)	0	30	90	4.7	n.d.	64.3	bc	1.9	c	4.4	d-f	0.3	e-h	2.8	bc
3	Fog (g/T)	0	60	90	0.7	2.2	52.3	c	1.7	c	12.3	b-f	0.4	c-f	1.1	bc
4	Fog (g/T)	60	0	60	1.3		16.8	de	0.7	de	2.0	ef	0.1	gh	0.5	bc
5	Fog (g/T)	60	30	60	3.6	2.3	11.4	e	0.5	de	1.6	f	0.1	h	1.3	bc
6	Fog (g/T)	60	60	60	1.3	n.d.	11.8	e	0.5	e	9.6	c-f	0.4	d-g	4.3	b
7	Fog (g/T)	90	0	60	0.7		18.4	de	0.7	de	1.4	f	0.1	gh	0.0	c
8	Fog (g/T)	90	30	60	2.6	2.7	11.6	e	0.5	de	5.9	d-f	0.3	e-h	0.3	bc
9	Fog (g/T)	90	60	60	2.2	4.3	12.3	e	0.6	de	6.6	d-f	0.3	e-h	1.8	bc
10	Drench (ppm)	1000	0	90	7.8		4.3	e	0.3	e	13.0	b-e	0.6	b-d	1.3	bc
11	Drench (ppm)	1000	30	90	3.3	n.d.	6.4	e	0.5	de	14.7	b-d	0.6	b-d	1.9	bc
12	Drench (ppm)	1000	60	60	2.5	2.8	3.3	e	0.4	e	12.7	b-f	0.5	c-e	1.5	bc
13	Drench (ppm)	1500	0	60	2.4		4.0	e	0.4	e	19.3	a-c	0.7	a-c	1.9	bc
14	Drench (ppm)	1500	30	60	4.1	2.5	2.5	e	0.4	e	21.5	ab	0.7	ab	2.2	bc
15	Drench (ppm)	1500	60	60	8.0	2.7	7.4	e	0.4	e	28.6	a	0.9	a	2.1	bc
16	Fog (g/T)	0	0	0	n.d.		84.9	a	3.1	a	2.5	ef	0.1	gh	9.9	a
17	Fog (g/T)	60	0	0	n.d.		29.7	d	1.0	d	1.5	f	0.1	gh	1.9	bc
18	Fog (g/T)	90	0	0	0.3		13.4	e	0.6	de	1.7	f	0.2	f-h	1.5	bc
19	Drench (ppm)	1000	0	0	n.d.		12.4	e	0.7	de	1.7	f	0.2	f-h	3.7	bc
20	Drench (ppm)	1500	0	0	n.d.		7.4	e	0.4	e	4.5	d-f	0.3	e-h	2.0	bc

[z] Mean values (n=6) not followed by the same letter are significantly different (P<0.05) by Duncan's multiple range test.

4. Pear coating development

1) Soybean oil emulsion coating alleviated superficial scald of Anjou pears (Table 4)

Methods: A soybean oil emulsion coating was developed for pears. The major components were soybean oil (The Hain Food Group, Inc., Uniondale, NY), polyoxyethylenesorbitan monostearate and sorbitan monostearate. Soybean oil coatings were diluted to total solids of 5%, and coated onto Anjou pears with gloved hands. Carnauba and carnauba + shellac mixture coatings (both diluted to a total solids of 5%), along with a non-coating control were applied as a comparison. After 4 months of RA storage, coated or non-coated fruit were held at 68°F for up to 2 weeks.

Results: The gas concentration inside the fruit for the various coatings ranged from 6-12 % CO_2 and 14-6 % O_2. Superficial scald was observed in the control fruit with 100% incidence and a scald index of 1.0. Carnauba and carnauba + shellac mixtures decreased the scald index to 0.5-0.7. However, soybean emulsion significantly decreased scald index to 0.28. This coating alleviated the severities of scald but did not exterminate scald. There was no difference between the coating treatments based on scald incidence.

Generally, coating decreases scald by reducing oxygen diffusion from the atmosphere to inside the fruit, slowing oxidations of phenolic compounds, and the aging metabolism of fruit. However, soybean oil adds another function to coating – antioxidant power. Soybean oil contains rich unsaturated acyloxies and other functional molecular structures which capture free radicals and protect fruit from disorders.

Table 4. Effect of soybean oil emulsion and other coatings on internal CO_2 and O_2, weight loss, superficial scald and flesh firmness of Anoju pears. Fruit stored at 30°F for 4 months were transferred to 68°F for 16 hours before applying coatings. Coated and non-coated fruit were then held at 68°F for 14 days.

Coating	Internal gas (%)		Weight	Superficial scald		Flesh firmness
	CO_2	O_2	loss (%)	Incidence (%)	Index	(lb)
			Day 7 at 68°F			
Non-coated	1.4 c [z]	19.3 a	4.1 a	100 a	1.0 a	2.4 c
Carnauba 5%	8.1 b	11.8 bc	1.4 c	96 a	0.52 b	4.1 b
Carnauba + shellac 5%	10.6 a	8.9 c	1.9 b	94 a	0.46 b	4.9 a
Soybean oil	7.1 b	13.4 b	1.6 bc	89 a	0.21 c	4.4 ab
			Day 14 at 68°F			
Non-coated	2.6 c	17.5 a	5.7 a	100 a	1.0 a	1.3 b
Carnauba 5%	7.6 b	12.6 b	2.0 c	100 a	0.63 b	2.7 a
Carnauba + shellac 5%	9.5 a	10.2 c	2.7 b	100 a	0.69 b	2.9 a
Soybean oil	10.1 a	9.8 c	2.6 b	100 a	0.28 c	2.5 a

[z] Means (n = 10) were separated with DMRT (P = 0.05). Means followed by a common letter are not significantly different.

2) Edible coatings for pears (Table 5 and 6)

Background and objectives: The application of coatings to pears prior to marketing is becoming a standard practice. 'Delicious' apple has been a key commodity in the development of fruit coating formulations and technology, and because this cultivar is relatively tolerant to high gas barriers, the coatings developed have tended to emphasize improvement of visual gloss with little need for other effects on the fruit that might result from a high barrier to gas exchange. A shellac coating seems an excellent fit for dark red 'Delicious' apples because it imparts high gloss, hides

bruises and forms a modified atmosphere condition that tends to preserve firmness and prolong shelf-life in this variety.

It is well known that when fruit is separated by a barrier, such as a coating or packaging, from exchange of gases with the atmosphere there is the possibility for the respiration to become anaerobic which is associated with the development of off-flavors. Therefore, coatings and packaging developed for one type of fruit may not be suitable for another.

Pears are sensitive to high levels internal CO_2 levels and have different color in comparison with red apples. They may also differ from apples in the porosity of the peel and the structure of blossom- and stem- ends, and thus the same coating may result in a different modified internal atmosphere, and physiological reactions to a given internal gas composition may also differ. The pear industry usually uses 10 times diluted apple waxes for their pear coating to avoid CO_2 injury. However, there is no research indicating proper pear coating and proper air barrier for pears. These considerations suggest it appropriate to once again determine how to select coatings for pears. There also seems a possibility that the trend in consumer preference for more 'natural' products might lead to less preference for high glossy coatings for pears.

Methods: We selected three coating formulations: shellac, carnauba and candellila, and up to four concentrations of each formulation. One of the intermediate coating formulations was made mostly of candelilla wax, which is considered a GRAS substance, which is allowed by the FDA with no limitations other than good manufacturing practice (CFR, 184.1976). Apples with candelilla wax coatings have a nearly natural, non-coated appearance (preliminary experiments). Other coatings are carnauba wax microemulsion (intermediate gas permeability) and shellac solution (low gas permeability), both materials being commonly used in fruit coatings. These coatings were used with 2-4 months stored pears of 'Anjou', 'Concorde' and 'Bartlett'. The coated or non-coated fruit were held at 68 °F for up to 2 weeks to simulate the marketing conditions.

Results: The gas concentration inside the fruits for the various coatings ranged from 1-18 % CO_2 and 16-2 %O_2 (Table 5). The coatings with intermediate gas permeability (5-10% carnauba and candelilla) gave intermediate values of CO_2 and O_2 in the internal fruit. The coatings with lowest permeability (carnauba 20%) caused high internal CO_2, low O_2, resulting in anaerobic fermentation in pears. Candelilla coated pears showed lowest gloss and provided a more natural appearance (Table 6).

Table 5. Internal CO_2 and O_2 (%) of pears at 68°F for 7 days after application of different coatings.

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

Table 6. Gloss, weight loss, and firmness of 'd'Anjou' pears coated with different formulations after 7 days at 68 °F

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

FINAL PROJECT REPORT
WTFRC Project Number:

Project Title: Ethylene ripening of pears by unconventional means

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Budget History:

Item	Year 1:	Year 2:	Year 3:
R&D Fees	26,240	32,000	30,200
Equipment	400	7,000	4,000
Supplies	500	1,500	8,000
Travel	2,860	7,200	10,000
Accommodation in US		1,600	2,000
Cool store fees		600	600
Taste panel and associated costs at NCSU			5,000
Miscellaneous			
Total	30,000	49,900	59,800

OVERALL PROJECT GOAL

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

OBJECTIVES FOR 2005-06:

- Continue to determine the influence of ethylene concentration and length of conditioning period at 20°C (68°F) on subsequent softening and aroma production by 'Green Anjou' (in USA) and 'Comice' (in New Zealand) after one and 3 weeks of cold storage. (This included work carried out in March 2006, which is the main focus of this report).
- Test the use of ERCs for pre-conditioning 'Green Anjou' in boxes immediately prior to and during transport to the East Coast. Conditioned fruit to be compared in terms of eating quality and cosmetic attributes with fruit given the current industry standard conditioning, after all have been further ripened to a similar extent upon arrival. (This was completed in October 2005 and was the focus of our previous report).

Significant findings during the entire three year project:

In our experience, the following observations and conclusions apply to the conditioning of early storage (i.e. within the first month after harvest) Green Anjou in the US and Comice in New Zealand, provided they have been harvested at normal commercial maturity and are free of disorders.

Effects of ethylene concentration

- Warming alone was never sufficient to condition early season fruit adequately, unless they exhibited a high incidence of cork pit. Externally supplied ethylene was normally vital for success.
- Levels as low as 2 ppm ethylene produced definite stimulation of ripening (based on both firmness and aroma).
- Full softening was triggered by lower levels of ethylene than those required to trigger full aroma production. Effects on softening plateaued at about 10 ppm but to trigger full aroma potential required >100 ppm for Anjou, and >25 ppm for Comice.
- Higher levels of ethylene during conditioning resulted in a greater proportion of the fruit becoming autocatalytic (producing their own ethylene) after subsequent ripening.

Effects of temperature during conditioning

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

Effects of length of conditioning period

- Longer periods of ethylene conditioning (e.g. 5 days) were more reliable than 3 days in triggering the capacity for aroma production. Shorter conditioning periods resulted in slower rates of aroma release during ripening. A single day of conditioning in ethylene often resulted in fruit that were capable of softening acceptably but produced little or no aroma.

Influence of period in cold storage

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

Usefulness of ERCs as a method of conditioning

- ERC prototypes were capable of producing and maintaining levels of ethylene sufficient to simply and effectively condition early season pears in a range of packaging, including clamshells, Euro-boxes and bushel boxes, using conventional perforated apple box liners.
- A half-pallet of cold 'Green Anjou' at two weeks after harvest, conventionally wrapped and packed in standard cartons and Euro-packs and sealed under a disposable pallet cover, was conditioned effectively and reasonably uniformly with ERCs in 5 days at ambient temperature.
- Conditioning of early season 'Green Anjou' using ERCs inside conventional cartons and Euro-packs for one day at ambient temperature, followed by gradual cooling before and during trucking across America with the ERCs still in place, resulted in a greater ripening potential and more aromatic and flavorsome fruit (according to a taste panel) than did standard one day forced air ethylene conditioning of pre-warmed fruit in a trailer or three days of warming without ethylene.

Results and discussion

The following section focuses on results from aspects of the first objective of the last year of this project that could not be included in our 2006 report. We then discuss these in relation to results and conclusions from earlier work in this project and by other research groups.

ERC-conditioning of early season US Anjou in clamshells; effects on fruit quality and aroma production

In 2006 we reported on a conditioning trial conducted in the fall of 2005 that tested the use of ERCs for pre-conditioning 'Green Anjou' in boxes immediately prior to and during transport to the East Coast. Conditioned fruit were compared in terms of eating quality and cosmetic attributes with fruit given the current industry standard conditioning, after ripening upon arrival. Early season Anjou were used in this trial, since such fruit, which have not had their chilling requirement satisfied, present the greatest challenge to condition effectively. It is particularly difficult to initiate full flavor and aroma development. These important aspects of fruit ripening are often neglected since they are more difficult to measure than softening.

In order to be able to easily monitor the effects of conditioning by ERCs on aroma development, one aspect of the 2005 shipping trial involved packing fruit into ripeSense® 4-piece clamshells containing sensor labels that change color in response to the accumulation of ripening-related aromas inside the clamshell. 'Green Anjou' pears used in this work were picked near Peshastin WA on 14 September 2005, graded into cherry bins and placed in storage at -1°C (30^0F). Cold fruit (90 ct) were then packed into ripeSense® 4-pack clamshells on 21, 23 and 25 September and conditioned at ambient temperatures (18-20°C) in a packing house until 26 September, when they were returned to the cold

store and subsequently shipped to Raleigh, NC, where they arrived on 5 October. Controls comprised fruit that were packed in clams lacking ERCs, which were conditioned simply by warming to room temperature alone for three days, and others that were given no conditioning whatsoever. There were 50 clamshells per treatment, packed in commercial display boxes during conditioning and shipping.

Ethylene levels in clamshells containing ERCs were in the 50-100 ppm range during the conditioning period at room temperature in Wenatchee. Upon arrival in Raleigh ten days later, ethylene was still present, at levels around 5 ppm, in the clamshells containing ERCs. A trace (0.4 ppm) was found in the control set that had been warmed for three days without ERCs, while no ethylene was present around the control set of fruit in clamshells that had not been given any form of conditioning.

During ripening at 20°C (68°F), aroma and firmness were monitored for each of the clamshell treatments (Fig. 1). The fruit that had been conditioned with ERCs in their clamshells (Fig. 1 C & D) produced much more aroma and softened more rapidly than those of the control group that had just been warmed (B) or that had received no conditioning whatsoever (A). Fruit quality from a cosmetic perspective was perfectly acceptable in the clams that had been given 1 and 3 days conditioning with ERCs at room temperature (C & D), with less than 2% affected by bruising sufficient to render them unsaleable. This was despite arriving in Raleigh at 8 lb and 4.5 lb firmness respectively. The treatment that received 5 days conditioning with ERCs (not shown) exhibited 12% damaged rejects on arrival, presumably attributable to their soft state in transit (6 lb when shipped, 3 lb upon arrival).

Influence of ethylene concentration and length of conditioning period on subsequent ripening of early season New Zealand Comice

Harvest, storage and conditioning. Comice were picked near Wanganui, New Zealand on 15-16 February 2006 at normal commercial harvest maturity (average firmness of 6.02 kg, starch pattern index average of 0.65, average brix of 11.5%) and kept in cool storage -0.5°C (30°F) for two weeks. Fifteen samples of 50 fruit were then placed in perforated plastic bags and transferred to 20°C (68°F) on 27 February. After equilibrating for 24 h, ERCs in varying numbers (1, 2, 4 or 8) were added to the bags in triplicate. As controls, three bags did not have ERCs. Temperature tracking devices (iButton®) were placed inside fruit in a selection of the treatments. All the bags were then enclosed in cardboard boxes. These were not air tight but served to protect the bags from drafts. Ethylene concentrations inside the liners within the boxes were monitored daily using a Drager ethylene meter (calibrated against a gas chromatograph) throughout the conditioning period. After 24 hours of conditioning at 20°C, on 1 March one box of fruit from each triplicated treatment was returned to cold storage at -0.5°C, followed by the second and the third boxes after 72 and 120 hours (3 and 5 days) of conditioning respectively. All ERCs were removed from the boxes just prior to their return to cold storage.

Ripening after a short intervening period (4-8 days) of cold storage. On 9 March (4-8 days after being returned to cold storage, depending on the conditioning period), four ripeSense® clamshells containing aroma sensing labels were filled with fruit from each of the 15 boxes in the cold store, and a further four with fruit that had received no conditioning treatment. The filled clamshells were transferred to 20°C where the fruit were allowed to ripen. Daily measurements were made of ethylene, carbon dioxide and oxygen concentrations in the headspace of each clamshell, and colors of the aroma sensing labels were recorded relative to the standard ripeSense® eight point color scale. In order to monitor changes in firmness non-destructively, a bench top Sinclair IQ tester was used. The fruit were thus briefly removed from the clamshells each day to permit this measurement,

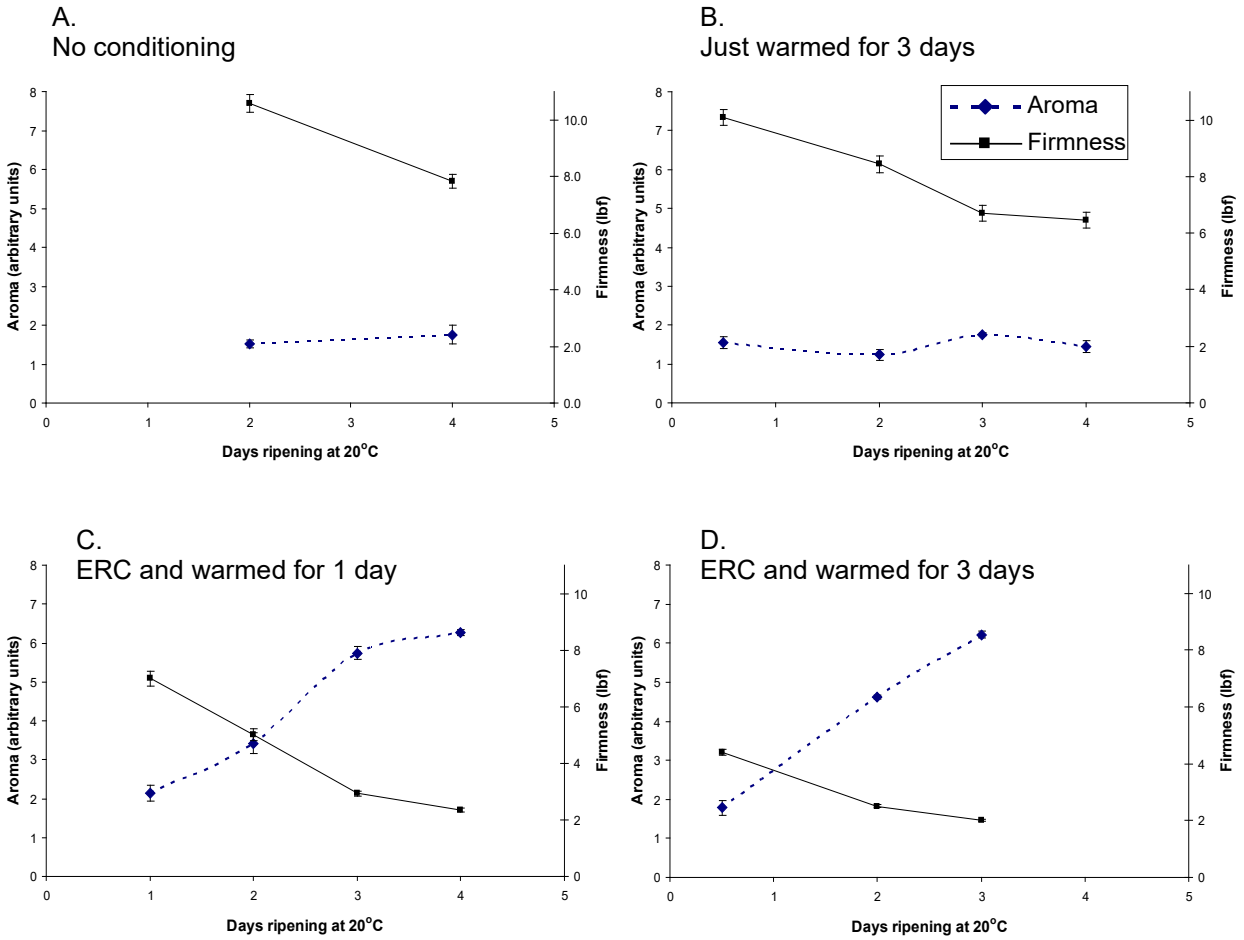

Figure 1 Effects of ethylene from an ERC inside a 4-pack ripeSense® clamshell on the rate of softening and aroma production by early season US Green Anjou pears. One ERC was added to each clamshell when pears, harvested one week earlier, were packed. Resulting ethylene levels inside the clamshells were 50 – 100 ppm. The packed clamshells were left to warm at 20°C for 1 day (C) and 3 days (D) before re-cooling and shipment by truck to North Carolina under normal commercial conditions, followed by ripening at 20°C. Control groups of fruit packed in clamshells without ERCs were either given no conditioning whatsoever (A), or were simply warmed for 3 days without any source of artificial ethylene (B).

after the head space gases had been sampled. Firmness was finally assessed destructively, using a GUSS Fruit Texture Analyser, at staggered times for each conditioning treatment, so that fruit in each treatment had been exposed to 20°C a total of 250 hours (just over 10 days) including both the conditioning and the post storage ripening periods.

The need for prolonged ethylene treatment during conditioning of Comice at two weeks from harvest, if the fruit are to be marketed shortly afterwards, is clearly evident from data summarized in Figures 2, 3 and 4. Fruit exposed to ethylene for 3 days during conditioning produced significantly more aroma (Fig 2B-D), ethylene (Fig 2F-H) and CO_2 (Fig 3B-D) than those given just 1 day of ethylene, even when all were given the same total time at 20°C, including conditioning and subsequent ripening. Prolonged ethylene treatment beyond 3 days had little additional impact on fruit capacity to

eventually produce ethylene (Fig 2F-H) and CO_2 (Fig 3B-D), or to soften (Fig 4), but brought about significant further increases in aroma production capacity when ethylene was supplied at concentrations of 13-50 ppm (Fig 2B-C).

A single day of conditioning produced significant changes in ethylene production (Fig 2 F-H) and firmness (Fig 4), but had little or no significant effects on aroma (Fig 2B-D) or CO_2 (Fig 3B-D) production relative to controls (Figs 2A and 3A) during ripening.

Control fruit (simply warmed without external ethylene for one, three or five days during conditioning), produced only trace amounts of aroma (Fig 2A), ethylene (Fig 2E), maintained flat baseline levels of respiratory CO_2 (Fig. 3A) and remained markedly firmer than any of the conditioned fruit (Fig. 4), even after a total of 10 days at 20°C (which includes the conditioning period). The low level of ethylene (1 ppm) that was detected within the control clamshells during conditioning was evidently insufficient to trigger subsequent autocatalytic ethylene production, which is likely to be involved in activating and accelerating other ripening related changes.

Autocatalytic ethylene production was evident as soon as fruit that had been conditioned with ethylene for 5 days were returned to 20°C, and commenced 5 and 8 days later in fruit conditioned for 3 and 1 days respectively (Fig 2F-H).

Increasing the concentration of ethylene from around 13 ppm to around 25 ppm (achieved with two ERCs, data not shown) enhanced aroma production, particularly for the 3 and 5 day exposures, but had no effect on firmness and ethylene production after a total of 10 days at 20°C. Further increases in ethylene concentration to around 50 and 85 ppm (4 and 8 ERCs respectively) produced no greater effects on any of the Comice ripening responses monitored after a total of 10 days at 20°C than those produced by 25 ppm ethylene (data not shown).

Ripening after a longer intervening period (11 weeks) of cold storage On 16 May, after 11 weeks of storage at -0.5°C following conditioning at two weeks after harvest, ethylene levels inside the boxes containing the conditioned fruit in the cold room were measured and rot incidence assessed. Most of the rots (14 out of the total of 19 rotten fruit across all treatments) occurred amongst fruit that had been conditioned for 5 days in ethylene. The remaining sound fruit from each box were put into 15 sets of eight clamshells and permitted to ripen at 20°C. A further set was filled with fruit that had received no conditioning. During this second ripening test, firmness was monitored destructively almost every day during ripening. Aroma was monitored daily in each clamshell but ethylene, CO_2 and O_2 measurements were restricted to early in the ripening period.

Ethylene was detected in all the boxes after 11 weeks while still in cold storage. The lowest levels (5-10 ppm) were found in the boxes of fruit that had simply been warmed for 1-5 days, without ethylene from ERCs. The boxes of ethylene conditioned fruit contained ethylene in the 10-40 ppm range, with the highest levels generally occurring in those boxes that had been conditioned longest (data not shown). Ethylene concentrations in individual boxes did not closely reflect the incidence of rots.

Ethylene was produced in considerable quantities during the first day of ripening by fruit of all treatments, including controls. Levels in the 60-90 ppm range were detected inside all clamshells containing conditioned fruit, in no apparent relationship with period of conditioning nor concentration of ethylene in the range 13-85 ppm during conditioning (data not shown). Those containing control fruit that had received no conditioning, or had been conditioned by warming alone, were significantly slower to produce ethylene (20-40 ppm inside the clamshells after one day of ripening), but all treatments were clearly autocatalytic at this stage.

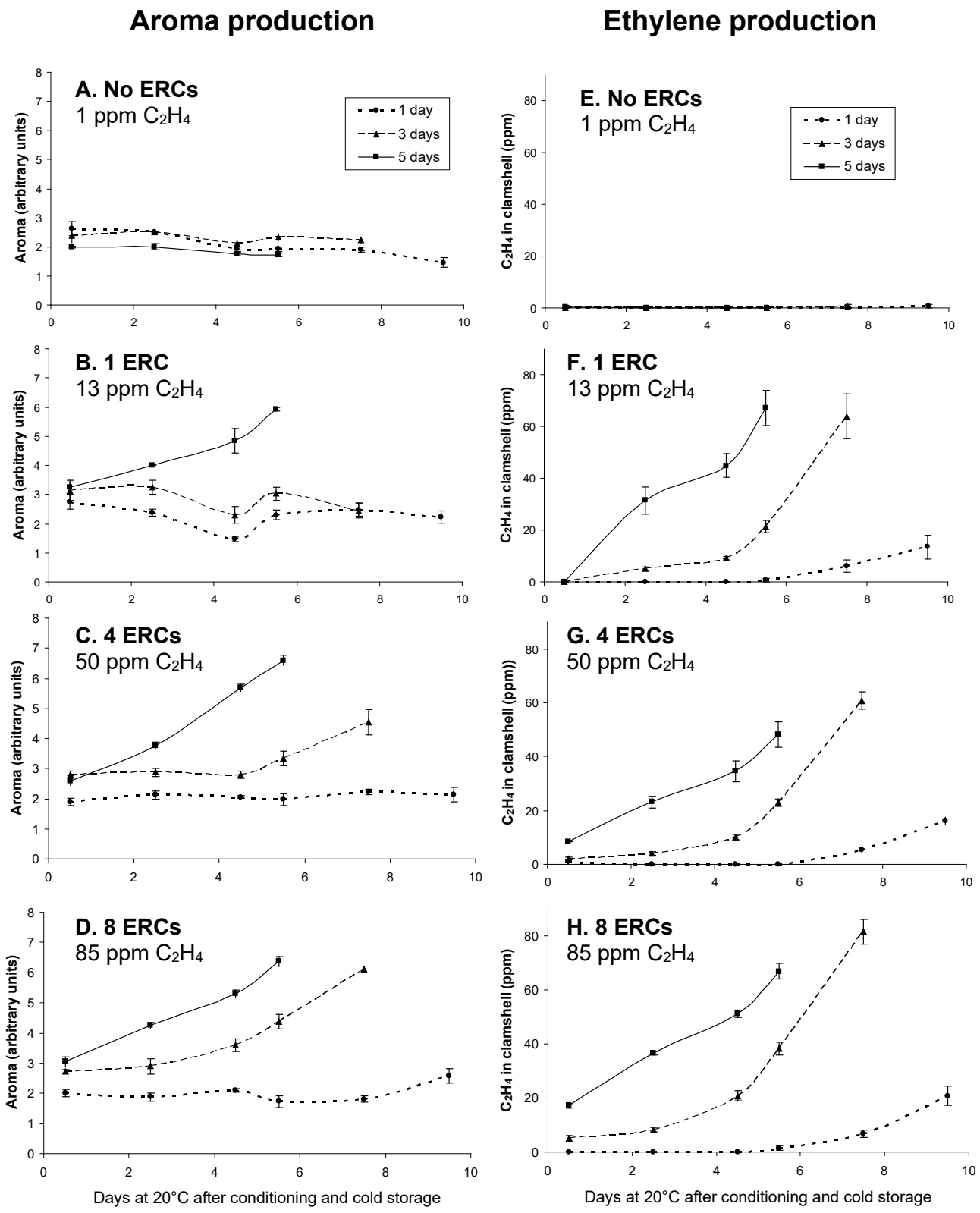

Figure 2. Influence of ethylene concentration and period of exposure during conditioning of NZ Comice pears two weeks after harvest at 20°C on the production of aroma and ethylene during subsequent ripening following a short intervening cold storage period of 4-8 days. To supply ethylene, various numbers of ERCs, as shown, were included with each batch of 50 fruit inside a perforated bag within a cardboard box.

FINAL PROJECT REPORT
WTFRC Project Number: PR-06-609

Project Title: Near real-time ethylene sensor for pear post-harvest applications

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Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	5250		
Benefits	5000		
Wages	3000		
Benefits			
Equipment	1000		
Supplies	500		
Travel	250		
Subcontract	2500		
Miscellaneous	0		
Total	17500		

I. Project Objectives

This was a one year project with a single objective, and that is to qualify sensor accuracy and measurement repeatability under controlled-atmosphere environmental conditions. The response of the sensor to various ethylene concentrations under different temperatures, relative humidity, and atmospheric conditions ($O_2/N_2/CO_2$ ratios) were also to be examined.

II. Significant Findings

Several major accomplishments are highlighted below and further discussions of the results will follow in the upcoming sections.

1. *Impact of Temperature:* Our results indicate that the ethylene sensor generates a stronger signal at higher temperatures and as the temperatures approach the CA room storage temperatures, the sensitivity reduces two almost half of that observed at room temperatures.
2. *Impact of Oxygen to Nitrogen Ratio:* The sensor shows low sensitivity to the ratio of oxygen to nitrogen, and even at a completely oxygen-free environment, the signal is less than 20%.
3. *Impact of Humidity:* The sensor appears to show some sensitivity to humidity, although little measurable differences were observed between normal atmospheric conditions (~50% RH) to the high humidity conditions of CA rooms (>90%RH).
4. *Impact of Interferents:* The sensor appeared to show little to no sensitivity to interferents that might be present in the CA room, such as CO.
5. *CA Chamber Tests for Pear Ethylene Production:* The data for ethylene concentrations measured for Bartlett pear under CA condition show very close agreement to measurements obtained with a GC during the same tests and under the same conditions.

III. Justification and Methods for ETHYLENE Sensing

Ethylene production rate and the amount of ethylene present in the surrounding environment of a single apple and pear (or in general for climacteric fruit) have been shown to affect their quality during various stages of ripening. Further, this information can be used as an indication of the stage of ripeness (or maturity) of the fruit.[1,2] This is especially true in post-harvest where the rate of ripening, scalding, browning, and other issues could prevent high quality fruit from reaching the market.

A number of researchers are currently using various methods supported by a Gas Chromatography system (GC) to research the different aspects of interaction of ethylene and fruit quality at various pre- and post-harvest stages. While significant amount of data has been accumulated and a large of body of literature exists on varieties such as Bartlett pears (and golden delicious apples), little information is available for some of the newer varieties such as Comice pears (and Honey Crisp apples). Research performed on Bartlett pears[3] suggested that very low ethylene concentrations of

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1 Kupferman, E., 1986, "The Role of Ethylene in Determining Apple Harvest and Storage Life," Post Harvest Pomology Newsletter, Vol. 4, No. 1. http://postharvest.tfrec.wsu.edu/pgDisplay.php?article=N4I1C

2 Sansavini, S., F. Donati , F. Costa, and S. Tartarini, 2004, "Advances in Apple Breeding for Enhanced Fruit Quality and Resistance to Biotic Stresses: New Varieties for the European Market," *Journal of Fruit and Ornamental Plant Research,* vol. 12, 2004 Special ed.

3 Bower, J.H., W.V. Biasi, E.J. Mitcham, 2003, "Effect of ethylene in the storage environment on quality of Bartlett pears," *Postharvest Biology and Technology* 28 (2003) 371_/379.

less than 1-ppm have to be maintained to control fruit quality, which is difficult due to high ethylene production of fruit even at -1 °C storage temperatures. For such tight control, it is required to continuously monitor the ethylene levels in the storage facilities. There is currently no cost-effective real-time ethylene sensor in the market that can produce reliable measurements at 0.1 ppm levels required for control in CA and RA rooms.

Fluid Analytics has recently developed a cost-effective electrochemical sensor for monitoring ethylene in air at concentrations of 0.1 ppm and lower. In our electrochemical sensor, selective adsorption of ethylene to a nanoporous gold surface takes place as a prerequisite to sensing. Adsorption onto the surface of gold is restricted to very few compounds with specific molecular structure, namely pi-bond. The nanoporous gold is deposited onto a polymer electrolyte membrane such as Nafion® (registered trade mark of duPont) and acts as the anode for the oxidation of ethylene at a given voltage. A flowing stream of air is passed over the ethylene adsorbing gold electrode. When an electrical potential is applied across the anode and cathode, ethylene that is adsorbed on the gold surface is oxidized to acetaldehyde at the triple-phase boundary, as formulated below:

$$C_2H_4 + H_2O \rightarrow CH_3CHO + 2H^+ + 2e^-$$

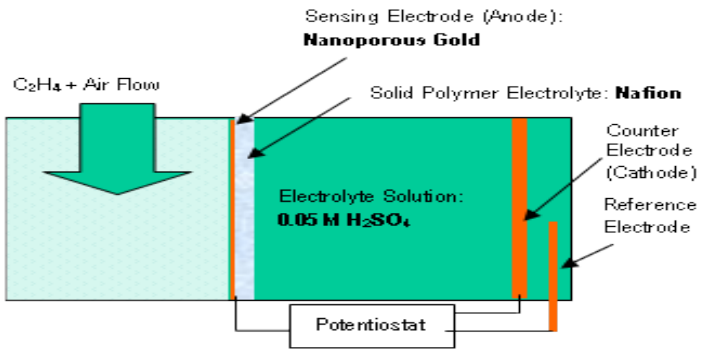


Figure 1. Current ethylene sensing approach.

A sensitive galvanometer is used to measure the current flowing in the cell as a result of the electrons produced by the oxidation of ethylene on the gold anode. The migration of protons through the membrane completes the cell circuit. The current, i, generated by the sensor is a function of a number of parameters, but notably can be linked directly to the partial pressure (or concentration, C) of ethylene in the freestream.

$$i = S \cdot C$$

In the above equation, S is the sensor sensitivity and that is what discriminates our sensor from most other commercially available electrochemical sensors. As S becomes larger, the threshold of detection becomes lower while the resolution of the instrument improves. Our laboratory measurements show that the sensor response is extremely linear for concentrations between 10-ppb and 10-ppm. At the lower end of this range, the sensor provides high resolution down to 10-ppb. More details will be provided in the following sections.

IV. RESULTS AND DISCUSSION

IV.1. Task 1: Prototype Development and Packaging for CA Tests

All engineering, assembly and testing on this system was done at Fluid Analytics. Figure 2 shows a photograph of the completely packaged system. The complete ethylene sensing instrument is packaged in an engineered sheet metal enclosure. The enclosure serves as s a rugged framework for mounting the internal components, while providing a suitable, field-usable protective shell.

Figure 2. Photograph of the packaged system.

Internally, the enclosure is divided into three distinct sections, separated by metal barriers that are integral to the enclosure, and provide additional structural stability. There are provisions on top of the sensor box for electrolyte access ports and access to the fluidics compartment in which the electrochemical cell, the air pump, and all of the associated tubing are located. This section was segregated from the other two as a way of preventing contact between the liquid electrolyte and the electronics. The metal barrier also serves as an electromagnetic shield, to minimize the amount of electrical noise coupled into the sensor cell. The center or the electronics compartment contains the bulk of the electronics: the potentiostat, the main control board, and the front-panel display. The compartment on the right, the power conditioning and management compartment, contains the AC-to-DC power supply. In the future versions, the batteries and corresponding power electronics will be placed in this section. This section is segregated from the main electronics for safety reasons, to avoid the possibility of AC line voltage coming in contact with the electronics. All user-accessible components (air connectors, control buttons, data link, etc.) are located on the exterior, creating a system that does not need to be opened during normal usage.

IV.2. Task 2: Research in Controlled Atmosphere Room at ARS

Prior to any testing in the cold storage facilities, a series of tests were performed to simulate the CA environment in a more controlled laboratory setting. The following sections cover the results of these tests.

2.1. Impact of Temperature Variation on Sensor Response

Knowing that reactions and diffusion are affected significantly by temperature changes, we expected a correlation between sensor sensitivity and temperature. This correlation could then be built into our calibration curves for temperature correction of the data.

In order to test the dependence of sensitivity on temperature, we constructed a controlled temperature box in which to place the sensor; this was to insure that the cell electrolyte was at a controlled

temperature. We also established a stainless steel section of tubing through which all gas would pass prior to entering the sensor; the tubing insured that the gas entering the sensor was at the same temperature as the electrolyte as verified by the inline gas temperature/humidity sensor. Additional temperature probes were placed in the controlled temperature box. A cell was filled with water, placed in the controlled temperature box, and the box temperature was set. The water temperature was monitored over time to establish how long it took for the water to reach the same temperature as the air in the box. Although this time varies depending on the starting temperature of the water and the set temperature of the controlled temperature box, it was found that an equilibrium time of ~ 1 hour was sufficient for all planned temperature tests. Then the cell was filled with the proper electrolyte solution, and temperature testing commenced. Testing included operating at four different temperatures between 10°C and 40°C at 10°C increments. A wide range of ethylene concentrations were tested in order to establish a well defined sensitivity line. A schematic and photo of the temperature test setup is shown in Figure 3.

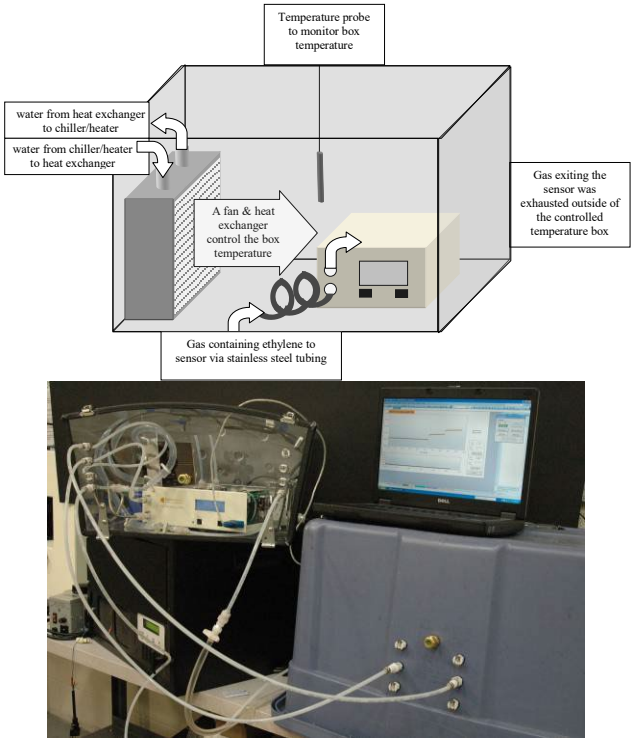


Figure 3. Schematic and photo of test setup for controlled temperature tests.

Using these tests, we were able to find a strong and clear correlation between sensor sensitivity (response) and temperature. The sensitivity of the sensor seems to be linearly correlated to temperature and increased with increasing temperature. These results are shown in Figure 4.

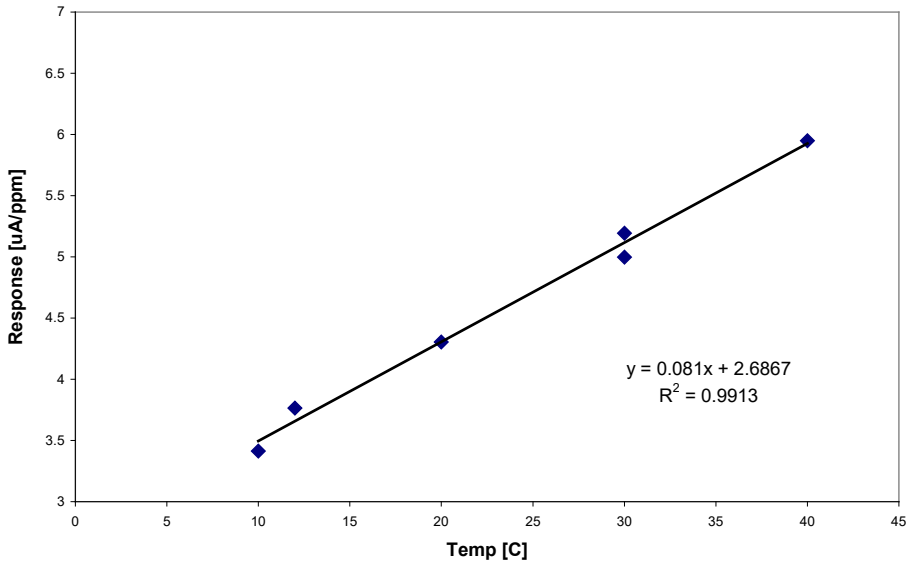
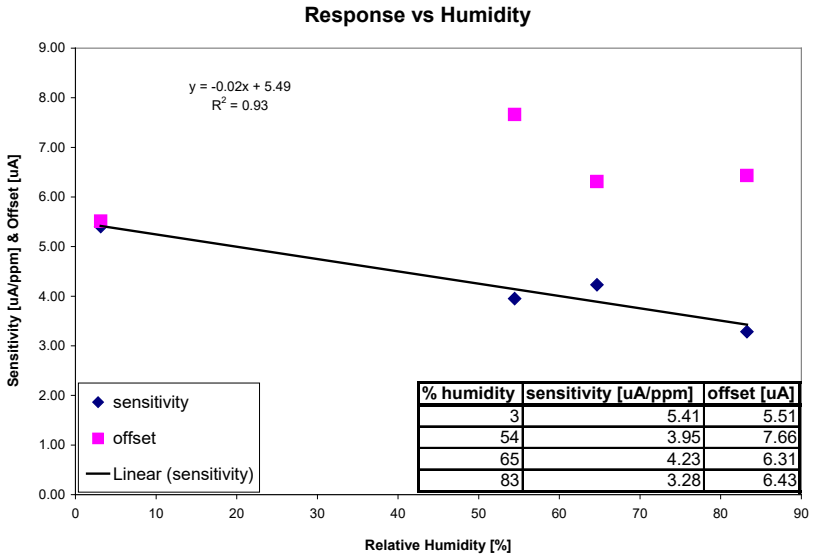


Figure 4. Sensitivity appears to increase with increasing temperature.

2.2. Response of Sensor to Varying Humidity

Varying humidity tests were performed to attempt to assess a correlation between humidity and sensitivity to ethylene for humidity dependent correction. In these tests the flow rate was kept at a constant 500 sccm and was provided from a gas mixing system and cylinder gas. The experiment was setup with gas flowing from the gas mixing system through cells with moistened plain Nafion membranes for humidification. Humidity was varied by varying the number of humidification cells and by changing the water feed rate in those cells. Humidity was recorded using the standard temperature/humidity sensor. Temperature was maintained between 25 °C and 26 °C. Five different concentrations were tested at each humidity level to give an accurate representation of sensitivity. R-squared values of the linear fit of concentration vs cell response at each humidity level exceeded 0.997 in all cases. The plot below shows the results of these humidity tests revealing an inverse relationship between sensitivity and humidity.



% humidity	sensitivity [uA/ppm]	offset [uA]
3	5.41	5.51
54	3.95	7.66
65	4.23	6.31
83	3.28	6.43

Figure 5. Sensitivity appears to decrease with increasing humidity.

2.3. Response in Air

In order to investigate the differences in sensor response at CA condition versus regular atmosphere with high oxygen levels, we constructed an apparatus by which we could mix room air with standard concentrations of ethylene in order to produce a mixture primarily containing room air, but also having a known concentration of ethylene. This was done using a fully functional prototype and relying on the pump to maintain a constant flow rate through the sensor. The outlet of the gas mixing apparatus was placed in a small chamber open to the air and the inlet to the sensor was attached directly to this chamber. When the flow rate of the gas coming from the mixing apparatus was higher than the flow rate of the pump, the chamber would fill with our mixed gas recreating the same conditions as when the sensor is directly connected to the mixing apparatus. When the flow rate of the gas coming from the mixing apparatus was lower than the flow rate of the pump, that gas would mix with room air creating a known concentration of ethylene in what was primarily room air. The flow rate of the pump was measured using an upside down graduated cylinder in a large bath of water. The oxygen-free tests were performed by mixing nitrogen standards with ethylene standards using precision flow controllers in both streams prior to mixing.

The results of these tests are shown in Figure 6. The y-axis is in counts, units proportional to current that are measured by the control board in the sensor prototype. Note that approximately 20% higher sensitivity is realized in the normal atmosphere as opposed to CA condition. However, because our sensor is sufficiently sensitive, the reduction of 20% in the sensitivity does not affect our ability to measure 100-ppb or lower ethylene levels if desired.

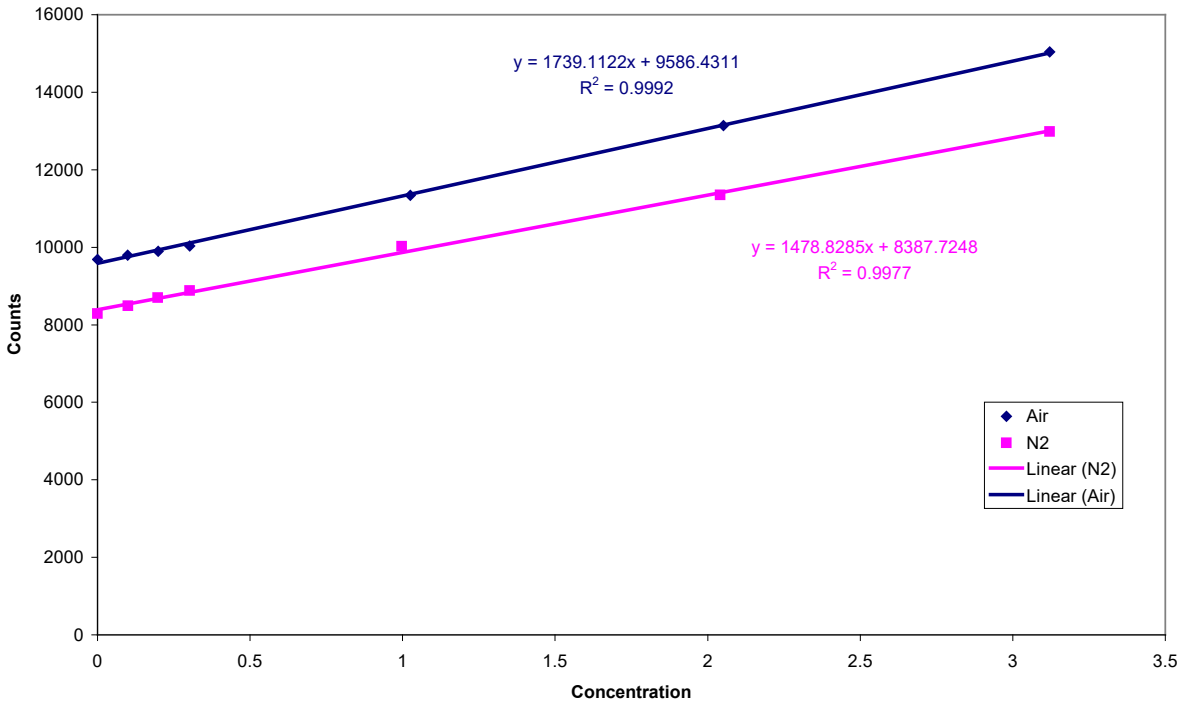


Figure 6. Sensitivity in oxygen-depleted environment (CA) versus normal atmosphere.

2.4. Response of Sensor to CO versus Ethylene

In addition to ethylene we expect that our sensor will respond to other chemicals of similar structure. However, the chemicals that we are interested in testing are those that may be present at the same time while measuring the ethylene levels for postharvest applications. One of the chemicals is CO, knowing that CO has an affinity for adsorption on the surface of gold. A series of tests was performed using the mixing apparatus and an additional cylinder containing 10 ppm carbon monoxide standard mixed with nitrogen to dilute to different known concentrations. A full step test scale of ethylene was performed before and after exposure to carbon monoxide to evaluate possible hysteresis.

The results of these tests are shown in Figure 7. The results of the carbon monoxide testing show that although there is some sensitivity to carbon monoxide, the sensor is more than 40 times more sensitive to ethylene than to CO. This suggests that interference from carbon monoxide is not a significant issue, unless the levels of carbon monoxide are extremely high (higher than the EPA set hazard threshold) or the ethylene measurement requirements are in the 10-ppb levels (which is highly unlikely). Further, no hysteresis effects were observed in our measurements suggesting that the sensor is not "poisoned" by CO as might be the case with platinum-based catalysts.

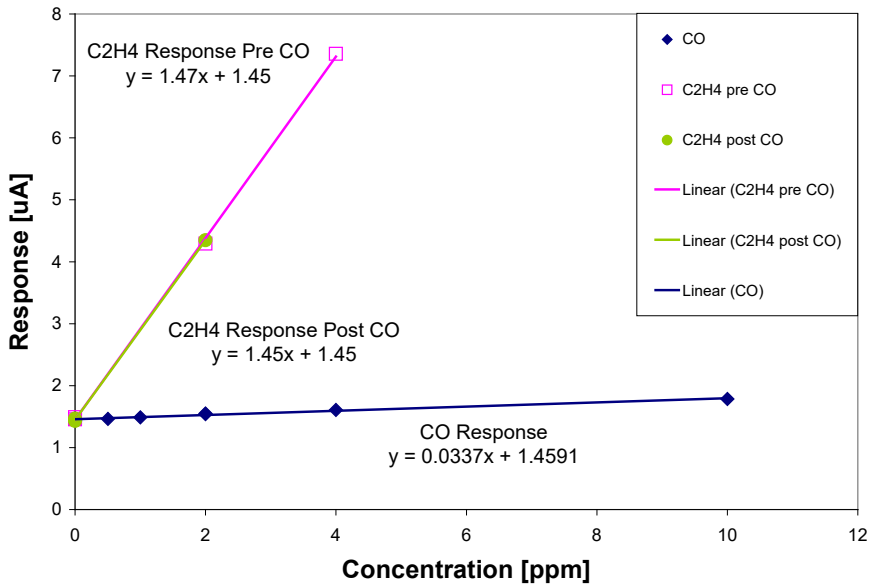


Figure 7. Sensitivity to carbon monoxide in relation to ethylene.

2.5. Summary of CA Room Measurements with Bartlett Pears

A series of tests were performed at USDA-ARS Tree Fruit Research Laboratory in Wenatchee, Washington. Three different CA chambers each with an empty volume of 151 Liters were used. Two of the chambers contained 50 mature green 2006 season Bartlett pears (*Pyrus communis* L.*)* and one chamber was kept empty as control. In order to simulate the humidity condition in the pear-containing chambers, moist paper towels were maintained in the control chamber.

The measurement process included two GC samples that were taken before and after the ETH-1010 measurements with our electrochemical sensor. Careful measured were taken to minimize the reading differences between the two GC samples for each run.

The tests started with the chambers filled with the Bartlett pears and allowed to sit under the recommended CA conditions for more than 24 hours. The gas composition was maintained at 1.5% O_2, 0.5% CO_2, and 98% N_2 and the temperature was kept at 1 °C. Since the background ethylene was low, additional ethylene gas was injected into the chamber in order to make quick comparison between the GC and Fluid Analytics ethylene sensor at the CA conditions. The ethylene injections were performed every 15 minutes at increments of 3 to 5-ppm using a 14,680-ppm ethylene standard, followed by a 0.5 ml sample tested in a HP 5880A GC with flame ionization detector, continuous sampling through the ETH-1010 sensor, and again followed by a second GC sample.

Interestingly, the data from the empty chambers show a good agreement within ±10% of the GC results. Some of the data for chambers filled with Bartlett pear appeared to show a higher level of spread, especially around 10-ppm, where the uncertainty was closer to 15% between the two measurement methods. It is not clear at this point whether or not GC readings were low, ETH-1010 readings were high, or both. The general trend, however, appeared to have excellent agreement.

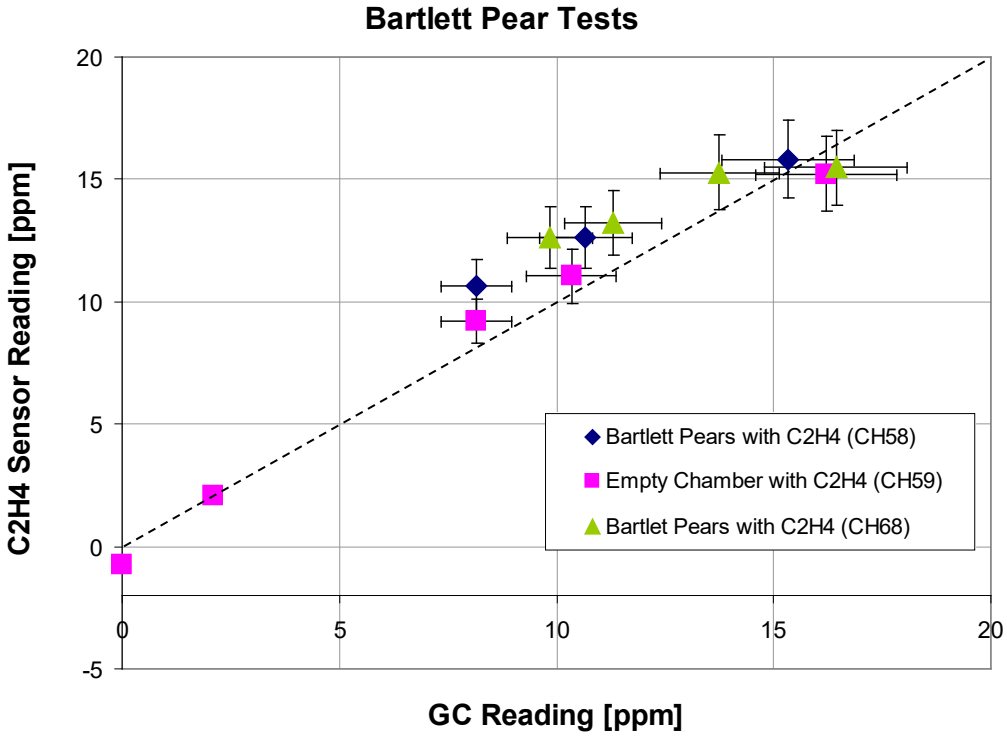


Figure 8. Comparison of HP 5880A GC and ETH-1010 ethylene measurements for empty and Bartlett-filled CA chambers. The error bars are +/- 10% of the reading.

In a separate set of tests with single store-bought organic Bosc pears, we were able to observe a measurable ethylene production rate at room temperature as evidenced in Figure 9. The measured ethylene production rates were approximately 500 ppb/min, which represents the initial part of this curve.

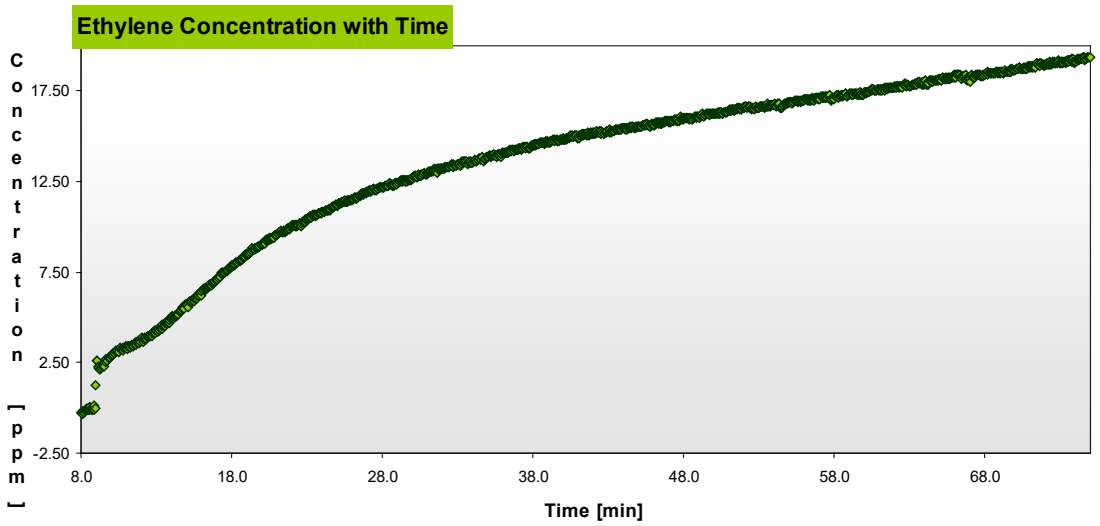


Figure 9. Ethylene production rate for a Bosc pear kept in a 0.5 L container at standard temperature and pressure.

V. Summary

In summary, our technology is differentiated from the competitive technologies in several ways:

1. Accuracy: The current system is able to measure concentrations as low as 10-ppb, which is significantly lower than what other commercially available instruments are capable of, and it is comparable to a GC.
2. Response time: The current sensor is able to make one reading in just a few seconds. This is a marked improvement over a gas chromatography system.
3. Cost: The current price for a scientific grade instrument is <$5,000 and the actual price depends on the added functions and features. Future mass produced instrument is expected to have significantly lower price.
4. Size: Our system is portable and handheld. It measures approximately 10.5”Wx4”Hx10” D and it weighs less than 5 lbs.
5. Ease of use:
    a. It displays the ppm/ppb or concentration of ethylene;
    b. It does not require any additional hardware;
    c. It comes with attachments for different applications;
    d. It is accompanied with appropriate data acquisition and display software that allows the user to store data (internally as well as on a laptop).

VI. Acknowledgements

The PI would like to thank the Washington Tree Fruit Research Commission and the Pear Bureau Northwest for providing funding for this R&D effort.

FINAL PROJECT REPORT

Project Title: Pear storage decay and fruit quality research

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	15,894	15,789	15,736
Benefits	8,106	8,211	9,599
Wages			
Benefits			
Equipment			
Supplies	5,600	5,600	4,265
Travel	400	400	400
Miscellaneous			
Total	30,000	30,000	30,000

Objectives: 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. Fruit quality research (including size enhancement) was included in this project beginning in 2006.

Significant Findings:

1. Postharvest decay control programs.

- Studies on the development of integrated decay control programs established a "backbone" of calcium and ziram treatments during summer, as core treatments for decay reduction.
- Calcium chloride sprays during August and early September were equally as effective as sprays during July and early August for Bosc pears, but a high dose of calcium chloride 1 week before harvest was damaging to fruit and led to greater decay.
- A series of studies to evaluate pre-harvest fungicide treatments identified Flint, Topsin, and Pristine as effective choices.
- The range of pear postharvest pathogens susceptible to each of the available fungicides for pear postharvest decay was unique; that is, each fungicide affects a unique set of pathogens.
- Sequential treatment programs, consisting of calcium and ziram in summer, a pre-harvest fungicide, a postharvest fungicide or biocontrol, and storage in modified atmosphere packaging, were the most effective approaches to decay control.
- Programs suitable for organic production significantly reduced decay, but not as effectively as programs including synthetic fungicides. Calcium in the orchard, BioSave 110 postharvest, and storage in LifeSpan MAP bags was the most effective potentially organic program tested.
- Strains of the blue mold fungus resistant to TBZ (Mertect) were found to be common in decayed pears. All strains collected from packinghouses that were resistant to TBZ were sensitive to Penbotec and Scholar.
- Both Scholar and Penbotec began to lose effectiveness when applied three weeks after spores were introduced into pear wounds prior to cold storage.

2. New technologies affecting pear decay.

- The biofumigant fungus *Muscodor albus* was found to be effective in reducing gray mold and blue mold in pears, but only if inoculated pears were exposed to the biofumigant at room temperature for 24-48 hours prior to cold storage.
- Laser coding of pears to replace the use of stickers, using the technology available through 2006, can lead to a slightly higher risk of decay.

3. Advances in postharvest management of pears.

- Ethylene treatment (100 ppm) of Comice pears for 48 hours at room temperature plus two weeks of cold storage can replace the traditional requirement for 30 days cold storage. Ethylene treatment for 72 hours can eliminate the postharvest chill requirement for Comice, but pears become too soft for long-distance shipping.
- Ethylene treatment (100 ppm) of Bosc pears for 24 hours at room temperature can eliminate the postharvest chill requirement.

- In a variety of tests to find an appropriate protocol for using 1-MCP in Bosc and Comice pears, no treatment was found that extended storage life while allowing consistent, predictable ripening.
- In a study of the chill requirement of Comice pears relative to fruit maturity at harvest, a linear relationship was found between the number of days after the orchard entered the maturity range when fruit were harvested and the number of days of chill required to induce ripening capacity. In other words, with each day later that the fruit are picked, the chill requirement becomes shorter.

4. Pear fruit quality enhancement.

- In 2 of 3 years, 5% and 7.5% urea sprays at full bloom resulted in increased tonnage of Bartlett pears size 90 or larger, while reducing yield of smaller fruit.
- Studies are underway to explore integrating urea treatments with hormone sprays for fruit size enhancement in Bartlett, Bosc, Anjou, and Comice pears.
- Calcium chloride summer sprays were more injurious to Bartlett pear leaves than to Bosc, and calcium treatments did not consistently enhance Bartlett firmness or storage potential.

Methods:

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

Results and Discussion:

I. Postharvest decay control programs.

1. A treatment program consisting of summer calcium chloride sprays, preharvest Pristine fungicide, and postharvest either Scholar or Penbotec fungicide, based on experience in this research project, offers a powerful approach to decay management. In orchards where bull's-eye rot or side rot has been a problem, adding ziram enhances the program. Other preharvest sprays have also been effective: Flint and Topsin M, as will be shown below.

Calcium chloride sprays in summer followed by Pristine one week before harvest increased the resistance of Bosc pears to blue mold (Fig. 1). Resistance to blue mold was determined by wounding the pears and inoculating with the fungus after harvest, then measuring the extent of decay lesion development after 6-8 weeks in cold storage. This study also compared alternative calcium programs; a late summer calcium program (3 lb. actual calcium applied 3 times in August and early September) was equivalent to a mid-summer program (3 lb. actual calcium applied 3 times in July and early August). A single-shot high dose (5 lb. actual calcium) of calcium chloride applied one week before harvest appeared to injure the fruit, and increased the amount of decay, presumably by diminishing natural fruit resistance, facilitating pathogen entry into tissue.

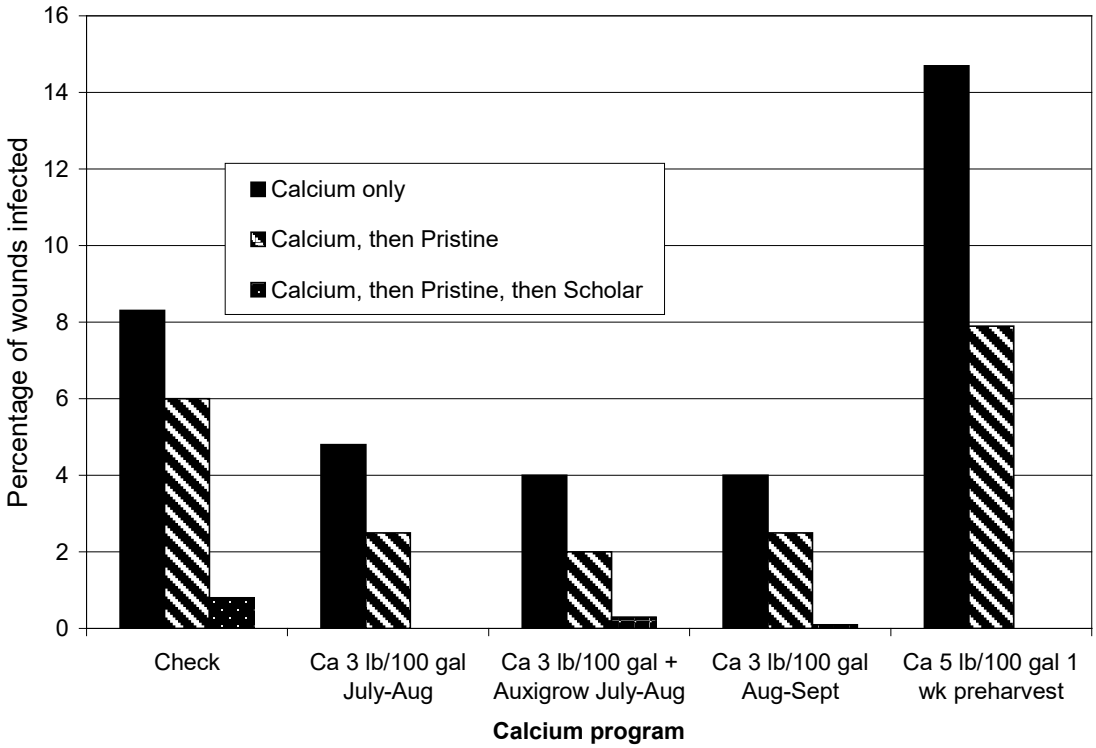

Fig. 1. Decay severity in Bosc pears inoculated after various calcium programs, with and without Pristine treatment one week preharvest, and with and without Scholar treatment postharvest.

2. A two-year study of alternative decay control programs combining orchard, postharvest, and storage treatments was completed in 2005 (Table 1). Among orchard treatments, Messenger was not shown to be beneficial, while calcium chloride reduced decay. Among postharvest treatments, BioSave 110 and sodium bicarbonate (5%) reduced decay while chitosan and StorOx did not (as used in these experiments). Pears stored in LifeSpan MAP bags had less decay than those stored in standard perforated liners. Pears that had received calcium in the orchard had higher oxygen and lower carbon dioxide atmospheres in the LifeSpan bags, likely indicating a slower rate of respiration. The treatment program consisting of calcium chloride in the orchard, BioSave 110 postharvest, and storage in LifeSpan MAP was the most effective in minimizing decay incidence.

Table 1. Effects of alternative orchard, postharvest, and storage treatments on natural decay in wounded Bosc pears.

Orchard treatment	**Percent of wounds infected**	
	Year 1	Year 2
Check	6.9 a	22.0 a
Messenger	5.7 a	16.8 a
Calcium chloride	3.1 b	7.3 b
Postharvest treatment	Year 1	Year 2
Check	3.4 bc	22.2 a
Chitosan (Elexa 4)	12.6 a	29.6 a
Mertect	3.6 bc	8.3 b
StorOx	4.8 b	22.3 a
BioSave 110	4.0 bc	5.1 c
Sodium bicarbonate	2.8 c	4.7 c
Storage treatment	Year 1	Year 2
Check (Standard liner)	6.4 a	17.8 a
LifeSpan MAP	4.0 b	13.0 a

Combined Effects:

Orchard	**Postharvest**	Storage	**% infected wounds**
Year 1			
Check	Water	Standard liner	5.7 a
Calcium chloride	BioSave 110	LifeSpan MAP	3.3 a
Year 2			
Check	Water	Standard liner	44.2 a
Calcium chloride	BioSave 110	LifeSpan MAP	2.1 b

Orchard treatment	**Average gas content in LifeSpan MAP**	
	Oxygen	**Carbon dioxide**
Check	11.9 a	3.6 a
Messenger	11.9 a	3.7 a
Calcium chloride	13.7 b	2.8 b

3. In laboratory tests, Scholar and Pristine had the broadest range of effectiveness among postharvest pathogens, followed by Penbotec (Table 2). Scholar and Pristine were generally effective at lower concentrations than other fungicides. These results show the excellent potential of newer fungicides to give broad-spectrum decay control. They also stress the value of knowing the target fungi in a pear orchard-packinghouse system for designing the most effective treatment strategy.

Table 2. Minimum concentration (ppm) of fungicides effective against major pathogens in laboratory tests. 10, 100, and 1000 ppm were tested. Dash (-) indicates no effect.

	Mertect	**Penbotec**	**Scholar**	**Pristine**	**Flint**	**Ziram**	**Shield TBZ**
Penicillium-S	1000	1000	10	10	100	-	1000
Penicillium-R	-	1000	10	10	100	-	-
Botrytis	1000	100	10	10	-	100	1000
Cladosporium	1000	-	100	10	10	-	1000
Alternaria	-	100	100	100	-	1000	-
Phialophora	-	1000	10	10	100	100	-

4. A wide array of possible pre-harvest – postharvest fungicide combinations is available for decay control. All of the pre-harvest fungicides tested provided some decay control, even without use of a postharvest fungicide, and all postharvest fungicides provided some decay control, even without use of a pre-harvest fungicide (Table 3). However, combinations of pre- and postharvest fungicides can improve control, and broaden the range of possible pathogens to be controlled.

Table 3. Effect of pre-harvest – postharvest spray programs on natural decay incidence in Bosc pears.

	Total decay (% of wounds infected)							
	Orchard sprays Application timing relative to harvest							
2004 **Postharvest treatment**	**Check**	**Ziram** 1 wk	**Flint** 1 wk	**Topsin** 1 wk	**Pristine** 1 wk	**Ziram** 1 mo. **Flint** 1 wk	**Ziram** 1 mo. **Topsin** 1 wk	**Ziram** 1 mo. **Pristine** 1 wk
None	6.2 a	2.4 a	1.3 ab	1.6 ab	1.1 a	0.5 ab	1.1 a	0.8 a
Scholar	1.2 b	0.0 c	0.0 b	0.0 b	0.0 a	0.0 b	0.0 a	0.0 a
Penbotec	0.6 b	0.5 bc	0.8 ab	0.3 b	0.0 a	0.0 b	0.5 a	0.0 a
Mertect	3.2 ab	2.2 ab	2.7 a	3.5 a	0.3 a	1.1 a	3.2 a	2.1 a

2005 **Postharvest treatment**	**Check**	**Topsin** 1 wk	**Pristine** 1 wk	**Flint** 1 wk	**Ziram** 1 mo. **Topsin** 1 wk	**Ziram** 1 mo. **Pristine** 1 wk	**Ziram** 1 mo. **Flint** 1 wk
Water	47.4 a	18.0 a	8.8 a	33.0 a	13.4 a	13.2 a	16.6 a
Scholar	2.2 c	0.4 b	1.4 b	2.6 b	0.4 c	2.4 b	0.8 b
Penbotec	9.0 b	2.2 b	2.2 b	1.2 b	3.4 b	1.4 b	3.0 b
Mertect	3.2 c	1.6 b	1.8 b	1.4 b	3.0 b	0.4 b	0.8 b
Shield TBZ	1.6 c	1.6 b	1.6 b	0.4 b	0.6 c	1.0 b	0.8 b

5. Penbotec and Scholar fungicides were highly effective in controlling blue mold in pear wounds when applied up to three weeks after spores were introduced into wounds (Fig. 2). This is based on prompt fruit storage at 31 °F following inoculation. At three weeks post-inoculation, decay control was still significantly better than the control, but it was apparent that the ability to inhibit decay was diminishing.

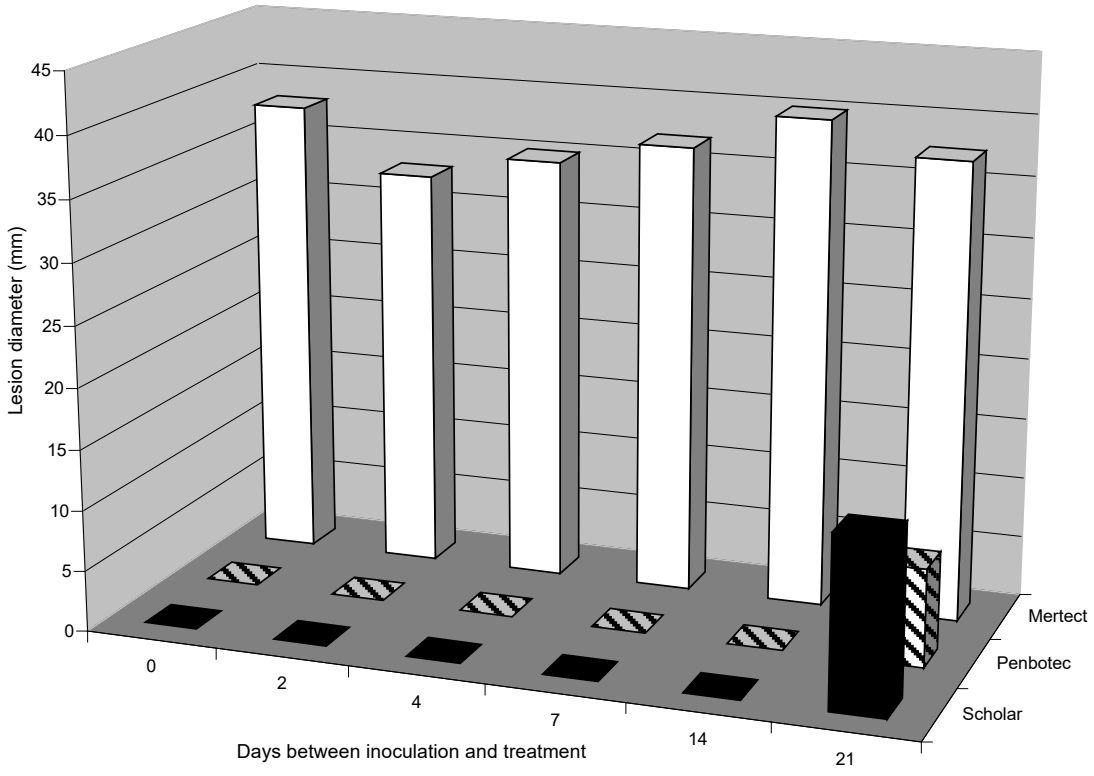

Fig. 2. Effect of length of delay between pathogen inoculation into wounds and fungicide treatment on the severity of blue mold decay in Bosc pears, using a TBZ-resistant strain.

6. Large-scale (10 acre) plots in two commercial orchards were organized in 2005 and 2006 to compare Pristine pre-harvest treatments to standard programs. In a low-decay orchard, no difference was detected, but in a late-harvested high-decay orchard, Pristine applications reduced decay (Table 4).

Table 4. Effect of Pristine pre-harvest sprays on decay in large-scale commercial plots, 2005.

	Percent decay	
	Orchard 1	Orchard 2
Pristine 2 and 1 wk pre-harvest	0.4 a	17.8 b
Standard program (Ziram)	0.9 a	51.0 a

7. In the event that two postharvest fungicides are applied (e.g., drench - line-spray or pre-size line spray – packing line-spray), the various sequences available can provide different results (Table 5). In experimental trials with TBZ-sensitive blue mold, the most effective sequences involved Scholar applied early, followed by either Penbotec or Mertect, or Penbotec followed by Scholar.

Table 5. Effect of different postharvest fungicide sequences when the initial treatment occurs immediately after harvest and the second treatment to the same fruit occurs after three weeks in cold storage.

Treatment applied after harvest (initial)	**Treatment applied 3 weeks after initial**	**Percentage of wounds infected (Blue Mold)**
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 d
Scholar	Penbotec	2.0 d
Scholar	Mertect	1.3 d

II. New technologies affecting pear decay.

1. The biofumigant *Muscodor albus* was highly effective in suppressing blue and gray mold in Bosc pears only when inoculated fruit were held in closed containers with *Muscodor* at room temperature for 24 or 48 hours prior to cold storage (Table 6).

Table 6. Lesion diameters (mm) at wounds inoculated with *Penicillium expansum* or *Botrytis cinerea*, held in closed containers at room temperature for 24 or 48 hours, then stored at 31°F for 2 months.

	Penicillium	*Botrytis*
24 hours exposure:		
Check	13.8 a	14.9 a
Muscodor albus	1.3 b	0.0 b
48 hours exposure:		
Check	18.4 a	20.9 a
Muscodor albus	2.1 b	0.2 b

2. Laser coding may find acceptance as an alternative to stickers in labeling individual pear fruit. Since the coding is accomplished by a certain amount of injury to fruit cells, tests were carried out to determine if laser codes can become entry points for postharvest pathogens. Dip and vacuum infiltration methods with blue mold and gray mold pathogens have thus far shown that laser codes may provide a slightly higher risk of fruit infection (Table 7). In some cases (without fungicide), fungi preferentially grew on laser-coded characters.

Table 7. Incidence of decay in Bosc pears with and without laser coding, following inoculation with decay pathogens by dipping or vacuum infiltration in solutions containing 10,000 spores per milliliter. Following inoculation, pears received either water or Scholar fungicide as a line-spray.

	Total decay incidence					
	Across all inoculation methods		No fungicide		Scholar fungicide	
Blue mold	No fungicide	Scholar fungicide	Dip	Vacuum infiltration	Dip	Vacuum infiltration
Laser coded	27.7 a	8.3 a	21.7 a	33.8 a	10.3 a	6.3 a
No code	16.4 b	2.6 b	16.5 a	16.3 b	4.0 b	1.3 b
Gray mold						
Laser coded	25.0 a	0.6 a	26.3 a	23.8 a	0.0 a	1.3 a
No code	23.9 a	0.6 a	31.6 a	16.3 a	1.3 a	0.0 a

III. Advances in postharvest management of pears.

1. Ethylene treatments were applied to Comice pears for 48, 54, 60, and 66 hours prior to cold storage. Consistent ripening to 5 lbf. or less within 7 days was found with 66 hours of ethylene plus 9 days of cold (Fig. 3). Fruit treated for 66 hours in ethylene plus 9 days cold storage were very close to 9 lbf. firmness prior to ripening, considered a minimum for long-distance shipping (Fig. 3). 54 hours ethylene exposure times did not appreciably reduce the length of time in cold storage needed as compared to the current Comice protocol: 48 hours ethylene plus 2 weeks cold storage.

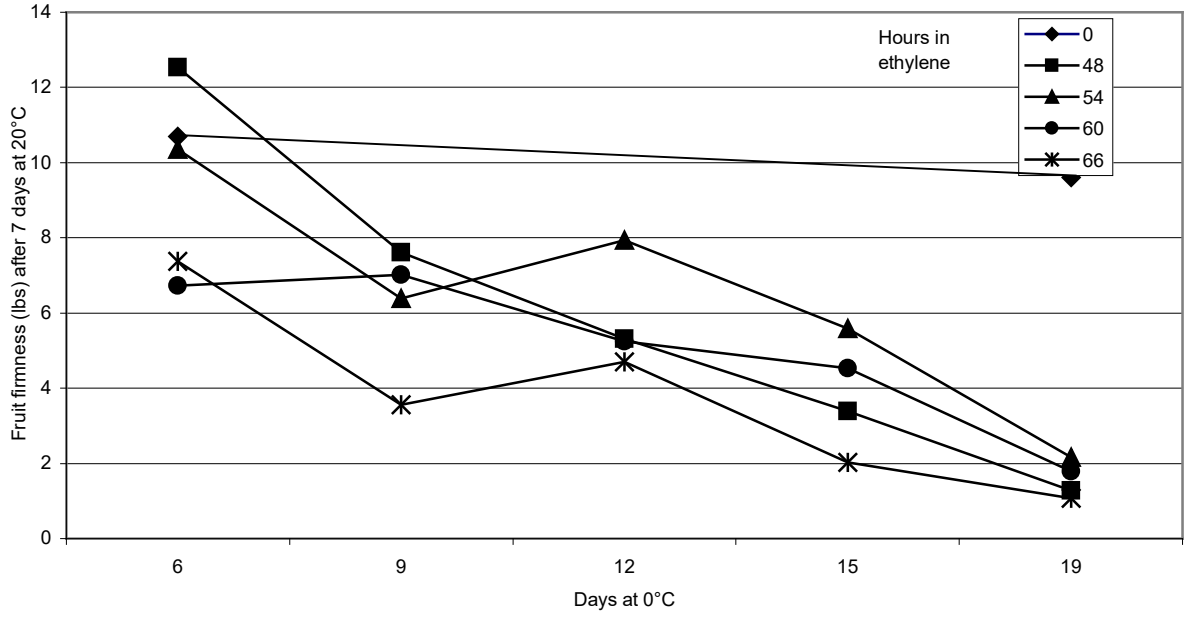

Fig. 3. Effect of ethylene exposure duration and length of cold storage on fruit firmness after 7 days at room temperature (ripe = ~5 lbf.).

2. Attempts to find an appropriate protocol for using 1-MCP in Bosc and Comice pears have been frustrating. No treatment was found that extended storage life while allowing consistent, predictable ripening. Variables tested have included dosage of 1-MCP (from 10 ppb to 1 ppm), fruit maturity at harvest, and exposure to ethylene before treatment with 1-MCP. Although studies with 1-MCP may resume if new information suggests a practical application, thus far I have not seen results that would dependably sustain fruit quality without interfering excessively in the essential ripening process for pears.

3. The relationship between harvest maturity and the length of postharvest chill necessary for inducing ripening capacity was studied in Comice pears. The date that the orchardist identified the orchard as entering the maturity range was the first harvest. Subsequent harvests were conducted weekly for 7 weeks. From each harvest, replicate groups of pears were stored at 31 °F for 5, 10, 15, 20, 25, or 30 days, then brought to room temperature for 7 days, then firmness was measured. A firmness of 5 lbf was considered "ripe". The number of days of chill required decreased in a linear fashion with each later harvest (Fig. 4). This indicates that while the standard 30 days chill requirement for Comice applies to fruit harvested at the top of the maturity range, fruit from later harvest times require shorter chilling duration. From the equation in Fig. 4, the chilling time corresponding to any number of days after entering the maturity range can be calculated.

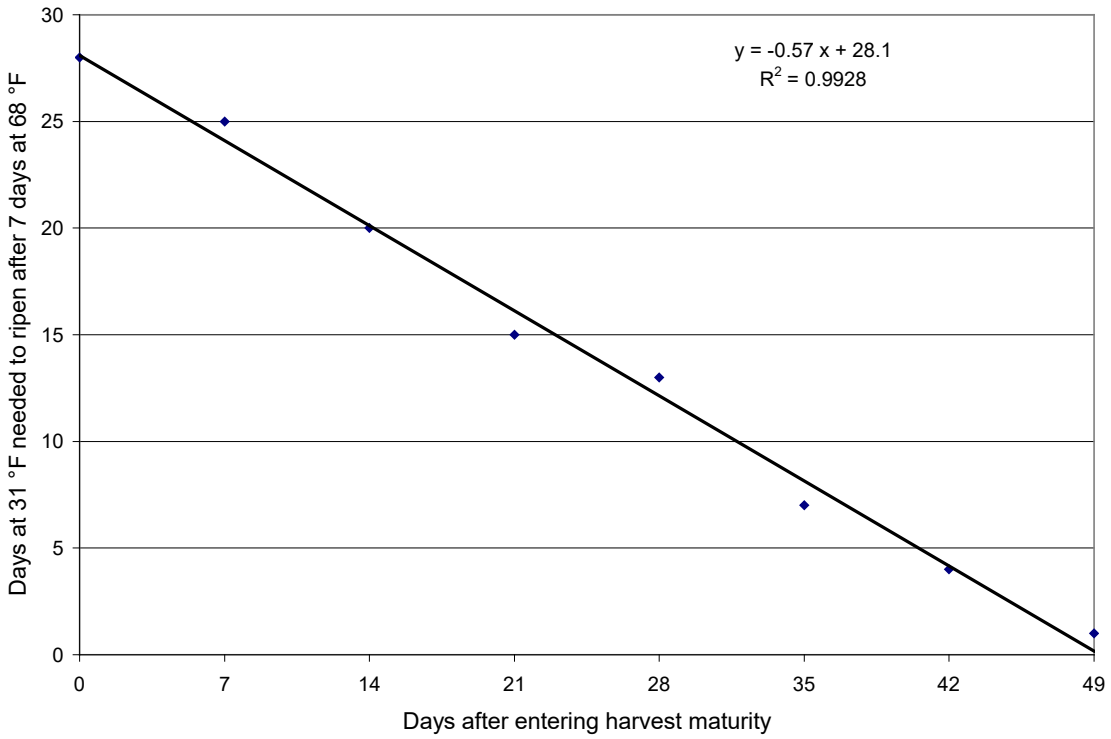

Fig. 4. Relationship of harvest date (relative to the onset of harvest maturity) to the length of postharvest chilling required to induce ripening capacity in Comice pears.

IV. Pear fruit quality enhancement.

1. In 2 of 3 years, 5% and 7.5% urea sprays at full bloom resulted in increased tonnage of Bartlett pears size 90 or larger, while reducing yield of smaller fruit (Table 8). The effectiveness of urea sprays may be dependent on crop load; further testing is needed to understand and predict the outcome of urea sprays. It appears that the effect may be a combination of blossom (fruit) thinning and providing nitrogen to developing fruitlets at a critical time to support fruit expansion. Urea sprays at earlier bloom (20%) have not been effective.
2.

Table 8. Effect of urea sprays at full (80%) bloom on fruit size and yields of Bartlett pear.

2004	Average fruit weight (grams)	Equivalent # fruit per box	Tons per acre	% size 90 or larger	Tons per acre size 90 or larger
Check	189	106	23.7	26.8	6.35
Urea 5%	229	88	17.1	57.7	9.87
Urea 7.5%	243	82	18.7	71.3	13.30
2005					
Check	190	105	18.3	29.2	5.47
Urea 5%	200	100	14.5	39.6	5.81
Urea 7.5%	212	95	11.4	48.5	5.17
2006					
Check	164	122	19.2	8.9	1.76
Urea 5%	186	108	19.4	26.4	5.17
Urea 7.5%	203	99	17.3	38.4	6.02

FINAL PROJECT REPORT
WTFRC Project Number: AE-04-428 (WSU Project No. 13L-3643-6367)

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

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	20,487	21,306	6,112
Benefits	6,146	6,392	2,246
Wages	11,000	11,000	4,142
Benefits	1,760	1,760	455
Equipment	0	0	0
Supplies	3,200	3,200	1,939
Travel	3,200	3,200	1,501
Miscellaneous			
Total[a]	45,793	46,858	16,395*

*Pear portion of expenses only. An additional $33,288 was received from the apple committee.

Significant progress this year:

- We developed a new marking protein (wheat flour) that can be brushed on the tree trunk and used to measure movement of immature insects and spiders moving up and down the trunk.
- We developed a method to correct for the differences in residual marking of our egg and milk marking proteins. This should reduce the likelihood that we bias our estimates of movement between the tree canopy and the ground cover.
- Our studies this year confirm that the ground cover is important to some of the predators and that they move freely between the two habitats.
- Foliage samples this year showed that we rarely detected drift in the canopy originating from the marker applied to the cover crop.
- Foliage samples collected from the cover crop did detect markers that were applied to the canopy of the tree, even when tarps were spread over the cover crop. In one set of experiments (Objective 1B) the drift was minor, but in another (Objective 1D) it was severe enough to invalidate the marker data for insects moving from the canopy to the ground cover.
- Our marker studies demonstrated that we could determine movement patterns that would not be detectable using normal sampling methods; specifically, if the insect has a daily movement pattern that happens at night, normal sampling would not detect that. The markers easily detect that sort of movement and allow us to quantify what proportion of the population moves in that fashion.

Objective 1. Determine the contribution of the orchard ground cover to natural enemy populations and biological control that occur in pear trees.

This year a number of marking techniques were attempted, beyond what we tried in the previous years. These new techniques were aimed at expanding our previous studies and attempting to make sure that there were no problems with drift. There were no rain events during any of the experiments reported below, and under-tree irrigation (low pressure emitters) was applied only on the weekends after the week's samples had already been collected.

1A. Using a wheat flour marker to measure movement up and down a tree trunk.

This technique involved brushing dry wheat flour on the a tree trunk in a band 6 inches wide and then having leafroller larvae and ladybird beetles walk over the residue and determining if they acquired the mark. The wheat flour marker is a newly developed marker. Although the sample size was relatively small, we found that all the insects that walked over the band acquired the mark at extremely high levels. This treatment will be highly effective at measuring the importance of insects moving up and down the tree trunk (*i.e.*, immature insects or spiders) as opposed to flying between the ground cover and tree canopy.

1B. Treating the canopy with milk marker, no ground treatment.

This set of experiments was to determine how efficient the milk marker was at marking insects in the tree canopy and how extensive the drift problem under the tree would be. These experiments were tested over three periods: June 14-19, June 26-30, and July 10-14. Trees were sprayed with 20% whole milk and Sylgard® 309 spreader at 80 ppm. Before applying the milk marker, a tarp was placed below each tree to be treated. After each tree was treated, the tarp was dragged to the new location. Trees were sampled using beating sheets made from sticky cards (to immobilize the insects so that they would not contaminate each other); ground cover samples were shaken over the same sort of sticky cards.

Evaluation of foliage samples from the canopy showed that roughly 63% of the leaves had enough milk present to allow an insect walking across the residue to acquire the marker. Most of the variability came from the last treatment group when only 29% of the leaves had enough milk residue to mark the insects (the first two weekly treatments had 69 and 81% of the foliage marked, respectively). The variability in the leaf residues was reflected in the insect samples taken from the canopy and in the daily samples (taken for three days running in each of the three trials) in terms of the marking found over all insect groups collected. Over all groups and all three experiments, an average of 32% of the insects and spiders collected from the canopy were positively marked. Of the predators, spiders (37.7%), *Anthocoris* (25.5%), *Orius* (22.7%), and *Deraeocoris* (20.7%) showed the greatest levels of marking when more than 20 individuals were collected. If we evaluate the marking on the first two trials only, the marking is considerably higher for spiders (56%) and *Anthocoris* (38.8%); the other predators' values changed little.

Table 1. Percentage of insects positive for the milk marker that was sprayed on the tree. Only insects where > 15 were collected in a location are presented.

Insect/location	% Marked	% Corrected for Marking Efficiency
Tree canopy		
Anthocoris	25.5	Š
Deraeocoris	20.7	Š
Orius	22.7	Š
Spiders	37.7	Š
Psylla	33.5	Š
Overall	31.6	Š
Ground cover		
Orius	8.55	27
Spiders	22.7	72
Geocoris	4.8	15
Lygus	12	38
Overall	10.2	32

The ground collections of foliage were taken from directly underneath the tree canopy and drip-line so that they would likely show the highest amount of drift possible. In fact, the samples did show that there was some drift down to the ground, but over that entire period only 5.6% of the leaves from the ground cover were marked. All but one of the leaves were found to be marked in the first trial, suggesting that there was an error in that particular application, possibly because of moving the tarps before the milk had dried. However, even though the leaves had enough residue to mark an insect, it would still be unlikely. Our previous studies held insects on leaves treated with different amounts of marker for a 24-hour period; but in nature it would be unlikely that the insects would remain on one of the few "hot" leaves for anywhere near that length of time. Our collections of insects within the ground cover did show some with high levels of marking, particularly spiders (22.7%) and ladybird beetles (23.1%), indicating that they probably move between the tree and ground cover frequently, particularly given that the percentage marked in the tree is relatively low. We can use the overall percentage of marked insects collected in the tree to correct for the marker's low marking ability $\left(\frac{100}{\text{Mean \% Marked in tree}} \times \text{\% marked in ground cover}\right)$.
When this is done, it becomes apparent that movement between the two habitats is common (Table 1) and suggests that ground cover management should have a dramatic effect on pest suppression if the predators will switch between psylla and the aphids common in the ground cover.

1C. Marking the ground cover only with the egg marker, no tree treatment.
These experiments were performed from early June to early July. This treatment is similar to work we have done the past few years but examined whether we could decrease the rate of egg used (from 20 to 10%), and we also examined more foliage samples to assess the effects of drift up to the canopy. The canopy samples were all taken from the lowest part of the canopy and thus would have been most likely to have the greatest drift. Our canopy foliage samples showed that 5.5% of the leaves had enough drift to result in marking of insects crawling across those leaves. However, all of the positive samples came on one day, suggesting that the problem was a result of application technique or possibly a short gust of wind during one part of an application.

The percentage of marked insects that were collected from the ground cover was 89.6% over all insects and spiders collected. These results are similar to our data from the past years and allow us to reduce the rates of eggs applied. This should not only reduce costs but should also reduce the importance of drift to the canopy. The high rate of marking in the ground cover also means that the correction factor is only 1.1 for insects that originated in or visited the ground cover but did not acquire the mark there.

Examination of the insects we collected in the tree showed that 36% of the *Anthocoris* were marked as originating in or visiting the ground cover (Table 2). This is roughly twice the percentage of *Deraeocoris* (17.8%) and spiders (15.2%). Green lacewings were also marked 11.7% of the time. Psylla also showed some marking (12%), suggesting that they were collected from the lower part of the canopy or that they fell to the ground and then crawled back up to the tree.

Table 2. Percentage of insects positive for the egg marker that was sprayed on the ground cover. Only insects where > 15 were collected in a location are presented.

Insect/Location	% Marked	% Corrected for Marking Efficiency
Tree		
Anthocoris	36	40.2
Deraeocoris	17.8	19.9
Green Lacewings	11.7	13.1
ladybeetles	6.7	7.5
Psylla	12.3	13.7
Spiders	15.2	17.0
Overall	15.36	17.1
Ground Cover		
Green Lacewings	95.6	Š
Geocoris	79.4	Š
Ladybeetles	95	Š
Lygus	88.2	Š
Nabids	100	Š
Orius	91.6	Š
Spiders	81.5	Š
Overall	89.6	Š

1D. Marking the ground with egg marker and the tree with milk marker.

These treatments were performed from mid-July to mid-August. The foliage samples from the tree showed no drift from the egg marker applied to the ground, but only 42.5% of the leaves in the tree were positive for the milk marker. In the ground cover, 100% of the leaves were positive for the egg marker, but 22.5% of the leaves in the ground cover also showed positive for the milk marker. All of the milk-positive collections from the ground cover came in August, with none in the mid-July collections. The foliage samples thus suggest that we should have seen valid results with the egg marker (movement from ground to tree) but possibly erroneous results from the milk marker (tree to ground).

The insects collected from the ground cover averaged 97% positive for the egg marker (Table 3), similar to our results in 1C above and in previous years. Predator samples collected from the tree were similar to 1C, with *Deraeocoris* being marked 25% of the time and spiders 22.1% of the time. Surprisingly, psylla were marked at 31.3% of the time, again suggesting that there was a drift problem (unlikely according to the foliage samples) or that they fell to the ground and crawled back up to the tree.

The milk marker applied to the canopy marked an average of 37% of the insects collected in the canopy, similar to what we observed in experiment 1B (32%). Again, with this lower level of marking we need a correction factor to help interpret the data. However, given the high level of drift from the canopy to the ground in this experiments, the results of the movement of insects from the canopy to the ground are unreliable.

Overall.

The data from this year provide us with key insights needed to improve our techniques and to help us understand movement patterns. First, in terms of improving techniques, we need to use tarps to cover the cover crop when canopy sprays are applied. To make this more successful, we need to lay out the

Table 3. Percentage of insects positive for milk or egg markers; milk sprayed in the tree canopy and egg on the ground cover. Only insects where >15 were collected are presented.

Insect/Location	% Marked with Egg	% Corrected for Marking Efficiency	% Marked with Milk	% Corrected for Marking Efficiency
Tree				
Deraeocoris	25.0	25.8	28.6	Š
Psylla	31.3	32.3	45.3	Š
Spiders	22.1	22.8	23.2	Š
Overall	27.1	27.9	37.2	Š
Ground				
Geocoris	90.9	Š	3.6	9.7
Lygus	93.4	Š	7.6	20.4
Nabid	100	Š	0.0	0.0
Orius	100	Š	4.5	12.0
Spiders	99.2	Š	7.4	19.9
Overall	97.0	Š	6.2	16.7

tarps below the entire treated area and allow the spray to dry completely before they are moved. This year, tarps were placed under an area of the tree and around it, and after the tree was sprayed they were moved immediately to the next tree to be treated by simply dragging them to the next location. Secondly, we need to change our experimental design to account for the difference in the ability of our milk and egg markers to mark insects as they walk over the dried residue. A way to deal with that problem is to treat the ground cover with egg and the tree canopy with milk in half our replicates and switch the treatments in the other half of the replicates (*i.e.*, tree with egg, ground cover with milk). This will be further strengthened by using the correction factors (described in 1B) to account for the differential acquisition of a mark by an insect walking over the dried residues of the different markers. This will prevent us from underestimating movement in one direction because of variability in marker efficiency. Third, our use of the wheat marker shows that we can easily determine the movement patterns for predators moving up and down the tree trunk, which in some circumstances (especially with immature stages or spiders that do not fly) may provide detailed movement patterns with a much simpler design. Finally, our studies clearly show that we need to expand our foliage samples to act as a check to determine the importance of drift and its possible impact on our findings.

Examination of the data tables also shows a marked discrepancy between the various trials in terms of species present. For example, Tables 1 and 2 show that *Anthocoris* was present in the tree canopy, but Table 3 does not. Most of this variation is a result of seasonal phenology (*Anthocoris* was rarely collected in the latter part of the season). Differences between abundances in the tree versus the ground cover may be a function of predator habitat preference (*e.g.*, *Deraeocoris* in the canopy and only rarely in the ground cover). However, our studies show that one of the key advantages of the markers (over just sampling) is that they allow us to detect movement between the ground cover and the tree canopy that occurs at times we are not sampling. For example, insects collected from the canopy, which test positive for the ground cover marker, could have picked the mark up at any time. Therefore, using markers we were able to detect a daily activity pattern where they move down into the ground cover at night and up into the tree during the day. On the other hand, traditional samples taken at mid-day could never make that connection. A further benefit is that the markers could provide an estimate of how common that sort of movement pattern would be in the general population of insect and spider predators.

FINAL PROJECT REPORT
WTFRC Project Number: #PR-04-431

Project Title: Biology and management of pear pests

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Vince Jones, WSU, Wenatchee

Contract Admin.: Janet Tsukahira, jtsukahira@pw.ars.usda.gov, (510) 559-6019

Budget History:

Item	**Year 1: 23,400**	**Year 2: 23,790**	**Year 3: 24,180**
Salaries	10,000	10,300	10,600
Benefits	3,000	3,090	3,180
Wages	10,400	10,400	10,400
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Total	**23,400**	**23,790**	**24,180**

OBJECTIVES:

Advance our understanding about how cover crops affect biological control of pear psylla:

- Assess taxonomic composition of generalist predator community in cover crop vs tree, and make inferences about habitat preferences;
- Develop methods for marking predators in cover crop and tree canopy, to allow examination of predator movement;
- Develop methods (gut contents analysis) to assess predator feeding on pear psylla, allowing us to determine what species are likely important sources of biological control.

SIGNIFICANT FINDINGS:

- Developed methods (with V. Jones) for marking generalist predators in large sections of habitat (cover crop or tree canopy), and showed that these methods allow us to make inferences about the role of cover crop as a source of natural enemies moving into the tree;
- Developed methods (with T. Unruh) that allow us to determine whether field-collected generalist predators have been feeding on pear psylla, and showed that diet in field-collected predators tracks psylla densities;
- Showed that a complex of predator species occurs in cover crop and tree habitats; by determining densities of each species in either habitat, we were able to make inferences about whether a species is a cover crop specialist, tree specialist, or habitat generalist;
- Marking studies indicated that a percentage of predators do indeed move between habitats, and the movement occurs even in species we initially categorized as habitat specialists;
- In a comparison of cover crop vs grass understory plots, we showed: (1) higher densities of predators in both the understory and tree canopy of the cover crop plots (compared to grass plots); and (2) lower densities of psylla nymphs in the cover crop plots.

- **CONCLUSION:** Results suggested that generalist predators move between cover crop and tree in both directions, and that several of these species regularly contain psylla remains in their guts. We infer from our results that the cover crop acted as a source of predators moving into the tree, and that these predators contributed to statistically significant drops in densities of psylla nymphs.

- **UNKNOWNS (to be assessed in a new project):** We are not certain that predator specimens which colonized the tree canopy from the cover crop were also responsible for prompting the decrease in psylla numbers, because we did not look for both the marker and psylla remains simultaneously in our assayed specimens. Also, the cover crop was obviously highly attractive to generalist predators (based upon predator densities), whereas our marking results suggest that a comparatively low percentage of the predators collected in the tree canopy had originated in the cover crop. It would be useful to develop methods that allow us to prompt higher rates of movement from cover crop into tree.

RESULTS AND DISCUSSION

Plots. A legume cover crop composed of winter pea, hairy vetch, and crimson red clover was used in all studies. Control plots had a ryegrass/fescue understory. Two sets of plots were used (Figure 1). The three large plots were used to develop marker technology. Five smaller plots, each paired with a grass control plot, are the source of our sampling results (taxonomic questions, pest and predator densities). Specimens for gut contents analysis were collected from throughout the orchard.

Sampling studies. Table 1 shows abundance data for 3 taxa of generalist predators (ladybird beetles - Coccinellidae, green lacewings - Chrysopidae, true bugs - Heteroptera), with the 5

numerically most common species in each taxon. The data suggest that the predator community includes habitat specialists (e.g., *Chrysopa oculata* in the cover crop and *Chrysopa nigricornis* in the tree) and habitat generalists (e.g., *Chrysoperla plorabunda* and *Coccinella septempunctata*). From these data, one would hypothesize that a cover crop is unlikely to be a direct source of biological control of pear psylla by species such as *C. oculata* (cover crop specialist) or *C. nigricornis* (tree specialist), but that it could be useful with respect to control of psylla by a generalist such as *C. plorabunda*. However, our marking data (see below) suggest that the data in Table 1 may be somewhat misleading in providing insight into rates of movement between habitats. (PP+) in the Table indicates that Horton has reared the predator successfully on a diet of pear psylla; (PP-) indicates psylla is not an appropriate diet. Asterisks indicate that both adults and immatures were collected.

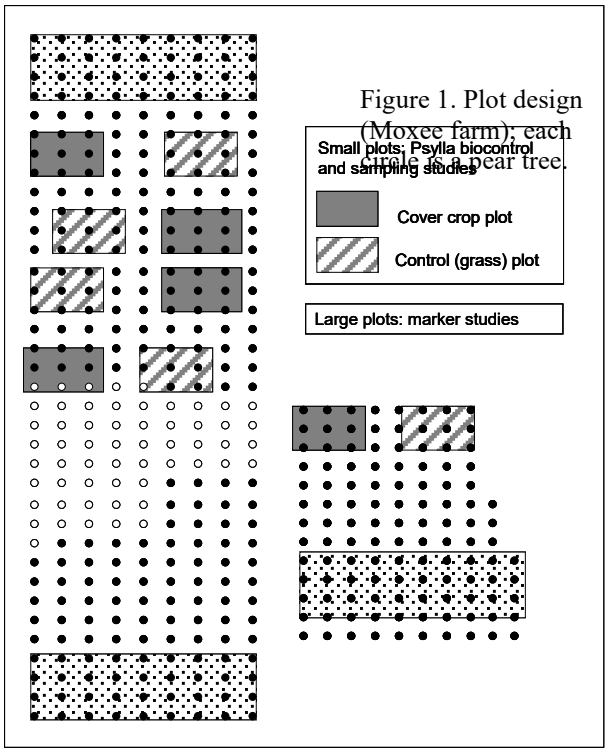


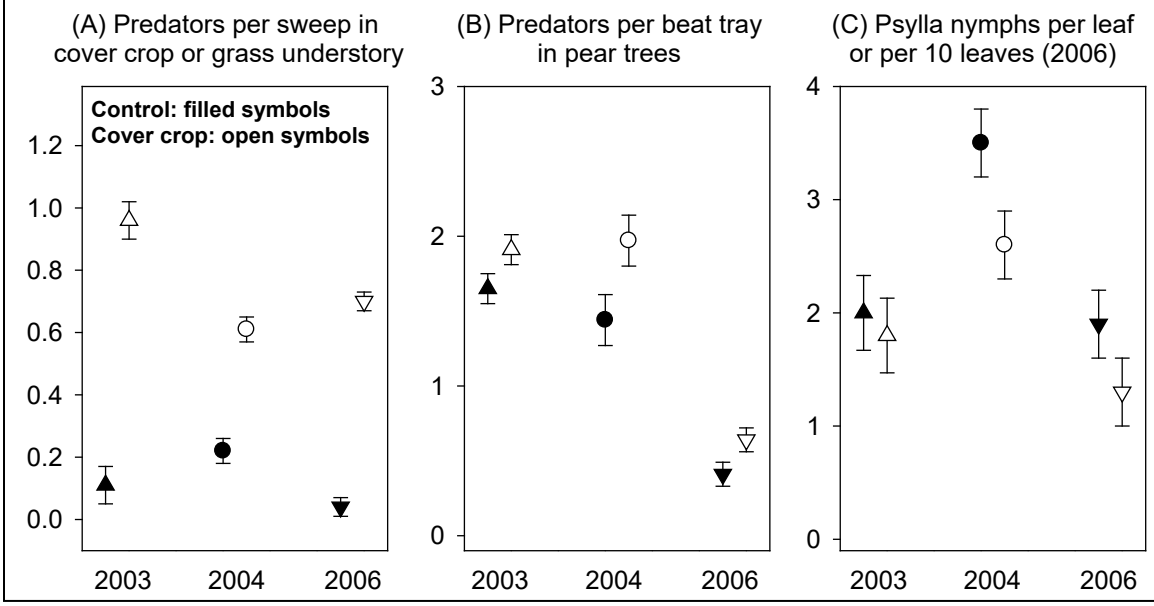
Figure 1. Plot design (Moxee farm); each circle is a pear tree.

Table 1. Numbers of generalist predators in 3 major taxa collected in 2003-06 from a legume cover crop (by sweep net) and pear trees (by beat tray).

	No. Collected[1] in		Apparent Habitat Preference		
Taxon	**Cover crop**	**Tree**	**Cover crop**	**Tree**	**Habitat generalist**
Heteroptera					
Orius tristicolor (PP+)	662*	24*	X		
Geocoris spp.	329*	0	X		
Nabis sp.	99*	8	X		
Deraeocoris brevis (PP+)	59*	1159*		X	
Anthocoris tomentosus (PP+)	10*	459*		X	
Chrysopidae					
Chrysopa oculata (PP+)	194*	3	X		
Chrysoperla plorabunda (PP+)	132*	155*			X
Eremochrysa sp.	33	111		X?	
Chrysopa coloradensis (PP+)	13	5*			X?
Chrysopa nigricornis (PP+)	4	47*		X	
Coccinellidae					
Hippodamia convergens (PP-)	382*	15*	X		
Coccinella septempunctata	127*	118*			X
Coccinella transversoguttata	116*	36	X?		
Hyperaspis lateralis	112	95			X
Harmonia axyridis (PP+)	11*	159*		X	

Predator densities were substantially higher in the cover crop than the grass understory (Fig. 2A). (Each mean in Fig. 2A-C is a seasonal mean, obtained by averaging over several sample dates per year). Densities of predators in the tree canopy were also higher in the cover crop plots than the control plots (Fig. 2B: by Anova, P = 0.036), suggesting that predators did indeed move from the orchard floor into the tree canopy. Densities of psylla nymphs in the cover crop plots were 90%, 74%, and 68% of the densities noted in the control plots for the three years, respectively, suggesting that the higher densities of predators in the cover crop plots led to biological control of psylla nymphs (Fig. 2C: by Anova, P = 0.028). Note that the data in Figure 2C are expressed as numbers of nymphs per leaf (first two years) or per 10 leaves (third year, in which psylla densities were low all season).

Fig. 2. Small plot (3 trees) trials showing effects of legume cover crop on predator densities in ground cover (A) or tree (B), and densities of psylla in tree (C).



Marking trials (Jones and Horton). Egg white, milk, and wheat flour were used to mark cover crop, tree canopy, and tree trunk, respectively (by Horton). Egg white was diluted to 20% (2004) or 10% (2005-2006) in water, and applied through a boom sprayer attached to an ATV. Milk was diluted to 20% and applied with a hand gun sprayer. Wheat flour was applied in bands to tree trunks by painting the product on. Predators were collected by dislodging them onto sections of cardboard treated with sticky trap, and removing them individually into microcentrifuge tubes for assaying. The specimens were collected by Horton, and shipped to Vince Jones to be processed for presence of markers.

In 2004, we began development of the egg white marker (for cover crop). Over 97% of arthropods collected from the cover crop carried the marker (Table 2), indicating that our application and assay methods were very good. More interestingly, 23% of arthropods from the tree also carried the marker, indicating that these specimens had visited or originated from the cover crop. The study was repeated in 2005 and 2006 to obtain better taxonomic resolution of the lacewings and ladybird beetles (Table 3). Tree specialists, cover crop specialists, and habitat generalists (from Table 1) all carried the marker.

Table 2. Percentage of specimens from tree or cover crop scoring positive for egg white (which was applied to the cover crop only) summer 2004.

	Tree[1]		Cover Crop[1]	
Taxon	**N**	**% Positive**	**N**	**% Positive**
Anthocoris tomentosus	248	21.4	61	93.4
Deraeocoris brevis	98	19.4	70	98.6
Nabis sp.	2	0	24	100
Orius tristicolor	3	33.3	318	97.5
Lygus spp. (pest)*	9	55.6	249	96.4
Chrysopidae*	12	58.3	20	100
Coccinellidae*	15	80.0	238	100
Spiders*	96	14.6	192	96.9
TOTALS	**483**	**23.0**	**1172**	**97.6**

[1]Tree collected insects were all adults; cover crop samples included some immature insects.
*Multiple species

Table 3**.** Presence of a cover crop marker on insects collected from cover crop or tree canopy; June-July 2005-2006. Adult insects only. ND – no data.

	Tree collected		Cover crop collected	
Specialization Group/Species	# Tested	% Positive	# Tested	% Positive
Tree specialists				
Anthocoris tomentosus	138	31.2	13	100
Deraeocoris brevis	267	15.7	46	97.8
Chrysopa nigricornis	3	33.3	ND	ND
Eremochrysa sp.	34	11.8	6	83.3
Harmonia axyridis	24	8.3	ND	ND
Cover crop specialists				
Orius tristicolor	10	20.0	830	97.5
Nabis sp.	2	50.0	52	100
Geocoris spp.	25	24.0	283	86.9
Chrysopa oculata	ND	ND	6	83.3
Hippodamia convergens	1	0	68	98.5
Coccinella transversoguttata	9	0	2	100
Habitat generalists				
Chrysoperla plorabunda	57	12.3	15	93.3
Chrysopa coloradensis	2	0	ND	ND
Coccinella septempunctata	22	4.5	13	100
Hyperaspis lateralis	3	0	5	100
Tree specialists	466	19.7	65	96.9
Cover crop specialists	47	19.1	1241	95.2
Habitat generalists	84	9.5	33	96.7
TOTALS	**597**	**18.3**	**1339**	**95.3**

The milk marker was less effective than egg white at marking predators (Table 4), either because of spray coverage or because insects less readily pick up a milk residue than an egg residue. The results suggest that there was some movement from the tree canopy into the cover crop by predators. Leafroller larvae and ladybird beetle larvae were allowed to walk across tree trunks brushed with wheat flour. All 7 leafrollers and all 12 beetle larvae that were assayed had picked up the marker. We believe that this method can be used to monitor movement between orchard floor and tree canopy by arthropods that use the tree trunk for these movements.

Table 4. Percentage of arthropods collected from tree or cover crop marked with milk; marker applied to tree canopy. 2006 study.

Sample 1		Sample 2	
TREE		TREE	
Anthocoris tomentosus	25.5	*Deraeocoris brevis*	28.6
Deraeocoris brevis	20.7	Spiders	23.2
Orius tristicolor	22.7	Pear psylla	45.3
Spiders	37.7		
Pear psylla	33.5		
COVER CROP		COVER CROP	
Orius tristicolor	8.6	*Orius tristicolor*	3.6
Geocoris spp.	4.8	*Geocoris* spp.	4.5
Spiders	22.7	Spiders	4.7

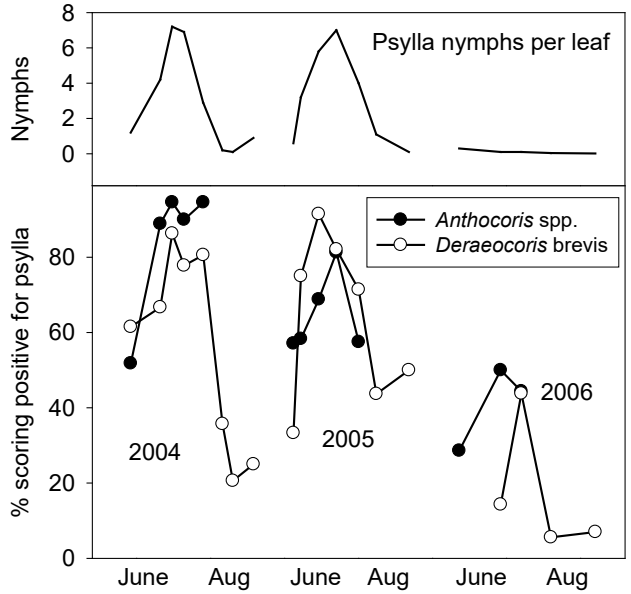
Gut contents analysis (Unruh and Horton). Two methods were developed by Tom Unruh to detect psylla remains in predator guts: (1) a method to detect psylla DNA in predator guts (by use of PCR), and (2) a method to detect psylla proteins (by use of ELISA). In 2004-2006, Horton collected predators from the tree canopy at 2-3 week intervals throughout the summer, and provided them to Unruh for analysis with ELISA; a portion of the 2005 sample was also analyzed using PCR. Also, in 2004-2005, we collected several predator species from the cover crop, to assess whether they might show evidence of having fed on psylla nymphs, which we would interpret as evidence that the predators moved between tree canopy and cover crop. Horton also collected leaf samples on each sample date to determine densities of psylla nymphs in the orchard.

The PCR method successfully identified psylla DNA in the guts of predators collected from the tree canopy or the cover crop (Table 5); the latter result suggests that predators collected from the cover crop had recently visited the tree canopy and fed upon pear psylla. ELISA showed that a small percentage of the cover crop specialist, *Orius tristicolor*, collected from the cover crop, did indeed have psylla remains in the gut (again suggesting some movement to the tree and feeding upon pear psylla). ELISA was used to analyze the seasonal tree collections, and results are shown for two predatory bugs: *Deraeocoris brevis* and *Anthocoris tomentosus* (Figure 3). Both species appear to feed readily on psylla when the pest is abundant, and to be much less likely to have psylla remains in the gut when psylla densities are low. Thus, proportion of specimens scoring positive for psylla were lower in 2006 than 2004-2005, which correlated with yearly differences in psylla densities. Within-season patterns in gut contents results also tracked within-season patterns in psylla densities; this is particularly clear in the 2004 and 2005 data (Figure 3).

Table 5. Incidence of psylla protein (ELISA) or psylla DNA (PCR) in guts of field-collected predators. Specimens collected from cover crop and tree.

	# Assayed	% positive for psylla
COVER CROP		
ELISA (2004)		
Orius tristicolor (June)	87	2.3
Orius tristicolor (July)	137	0.7
PCR (2005)		
Deraeocoris brevis	15	66.7
Hippodamia convergens	15	86.7
Coccinella septempunctata	14	53.3
Coccinella transversoguttata	10	10.0
PEAR TREE (PCR 2005)		
Deraeocoris brevis	15	66.7
Coccinella septempunctata	15	73.3
Harmonia axyridis	15	100.0

Fig. 3. Percentage of field-collected *Anthocoris* spp. or *Deraeocoris brevis* scoring positive for presence of pear psylla proteins (bottom panel); top panel shows psylla densities in orchard on dates predators were collected.



FINAL PROJECT REPORT

WTFRC Project Number: PR-03-339

Project Title: Introduction and propagation of pear rootstocks

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Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	14,126	NA	NA
Benefits	9,182		
Wages	5,000		
Benefits	37		
Equipment	0		
Supplies	500		
Travel	0		
Miscellaneous	0		
Total	28,845		

Objectives: The overall objectives of this project were: 1) help the flow of clonal rootstocks, from research programs towards commercial propagation, and 2) improve propagation of these clones.

Significant Findings:
1) Propagated 2000-2500 liners each of Horner 4, Horner 10 and OHF87 for rootstock trials. These will be shipped to Van Well Nursery in May, 2007.
2) Demonstrated the feasibility of auxin-treated tie-off layering (ATTOL) for propagating pear rootstocks.
3) Three rootstocks from Kazakhstan are difficult to propagate.

Methods:

Layering. Stock plants of rootstock clones OHF 40, 87, 90 and 708-2, 12 and 36, were pruned near ground level in spring, 2006. The resulting shoots were layered during the second week of July. Treatments are described in the Results and Discussion. All layers were covered with clean sawdust contained by roofing felt.

Layers were harvested the second week of December, 2006. Rooting response was scored using the following system: 3 = well-rooted; 2 = acceptable, sufficient rooting to support growth and development; 1 = rooted, but unacceptable; 0 = unrooted.

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.

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).

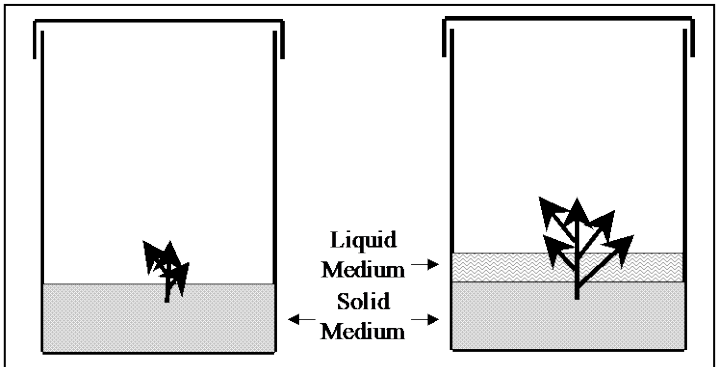

Figure 1. Single-phase vs. double-phase tissue culture.

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.

Table 1. Pear rootstock clones in tissue culture at OSU, December 2006.	
517-9	OHxF 87
708-13	OHxF 97
96FI11	Pyronia
96FI12	Q29857
Fox 11	Q29858
Fox 16	Q29859
96FI15	Horner 4
OH11	Horner 10
OHxF 40	

Results and Discussion.

1) **Horner rootstocks.** A small trial in the 1990's of the so-called Horner collection of pear seedlings found at least two rootstocks, Horner 4 and 10, worthy of more study. This is a remarkable result from such a small sample, suggesting that the entire collection should be screened as quickly and as systematically as possible. Delaying more complete studies of the population's potential could cost a decade or more.

Starting in 2001, we began to clonally propagate all of the Horner seedlings to produce liners for small trials of the entire population. These trees are now at Hood River and will be evaluated in coming years.

• We initiated shoots of Horner clones 4 and 10 into tissue culture and in 2006 produced about 2000-2500 liners of each of these clones plus OHF87 for orchard trials. These liners are growing through the winter and will be transported to Van Well Nursery in May, 2007 for budding and tree development.

• At the 2006 Pear Research Review, we also proposed to initiate tissue cultures of additional Horner clones based on early evaluations of the field trial and grower consultation. The rationale was that as the trials progress and more information becomes available, cultures will either be culled or continued. When interest warrants larger trials of a given clone, the cultures will already be established, enabling more rapid liner production for the next round of trials and a source of cultures for interested nurseries, thus keeping the process moving.

This early propagation strategy has proven very useful for the Oregon hazelnut industry. As Eastern Filbert Blight-resistant cultivars are developed at OSU, promising clones are initiated in culture and are ready for release to nurseries in advance of the variety release.

In 2006, several clones appeared to have promise, although the trees are still young. Discussion among the pear research committee felt it was too early to initiate 10 or 20 clones. Luigi and I would respond that this is the point of our program. Initiating and maintaining a few shoots of several clones is relatively simple compared to initiating clones at a later date when demand may soar. The complication now, however is that the OSU propagation program will end by January 1, 2008. Other tissue culture labs may differ with us on when to initiate prospective clones.

2) **Propagating rootstocks by layering.** Clonal propagation of pear rootstocks is not a trivial problem. Pear does not respond to standard mound layering used for apple. Hardwood cuttings are difficult to propagate, yielding a low percentage of rooting and poor root quality. Softwood cuttings root well, but require significant skill and attention. Most clones respond to tissue culture, but availability is probably doubtful early in a rootstock's development period.

Another facet of the problem is that sales of pear trees in recent years have not been particularly attractive to the nursery industry. As a result, one nursery is the main source of pear liners and these are propagated by **hardwood cuttings**.

Our view is that hardwood cuttings of pear are an inherently limited option for which breakthroughs are unlikely to occur. Tissue culture alone or in combination with softwood cuttings or modified forms of layering offer better options.

Tissue culture of pear is well-established and most genotypes respond, with some notable exceptions. [OHxF 51is difficult, as is the Brossier series.] However, the OHxF series are generally easy to culture, as are Horner 4 and 10. Thus, besides rootstock potential, a criterion for rootstock selection, especially in the Horner series, should be propagation potential.

Until there is a "killer" rootstock that creates a significant market for trees, nurseries will be reluctant to commit major resources to propagating pear liners. In the meantime, tissue culture will probably be the method of choice, if a lab can be persuaded to cooperate. In 2006, pear growers have reportedly teamed-up to ensure larger-scale micropropagation of pear rootstocks.

Softwood cuttings. A useful property of tissue culture is that liners have higher propagation potential for a few seasons afterwards. We have documented that such plants used as a source of softwood cuttings root at higher percentages with high quality root systems. Unfortunately, softwood cuttings are a specialized technique that many tree fruit nurseries are uncomfortable with. Even with the higher propagation potential of cuttings from tissue cultured stock plants, pears demand a highly-regulated mist environment. This presents a challenge that nurseries so far have been unwilling to deal with- given the state of the market.

Mound layering, mechanized to produce millions of liners, revolutionized the apple industry. Pears don't respond well to this technique. Other species share this problem. These species do respond, however, to layering when shoots are girdled and treated with auxin before being layered. This technique, designated auxin-treated, tie-off layering (**ATTOL**), is commonly used by the Oregon hazelnut industry. My hope is that ATTOL can fill the gap between the level of OSU production and the point at which large-scale propagation begins. Perhaps it may even compete with tissue culture at higher levels of production.

As noted above, this effort is based on the fact that until recently, the nursery industry showed little interest in producing pear rootstocks. Adding ATTOL as an option along with tissue culture and softwood cuttings may help more nurseries, or even orchardists, see themselves as pear propagators thereby increasing competition, as well as total liner output.

• In 2006, we tested ATTOL on pear. Own-rooted stock plants established for cutting production were cut off near ground level to stimulate shoot production. We tested: 1) the effects of girdling and IBA application on rooting and 2) the effect of IBA concentration.

Table 2. Effects of indolebutyric acid (IBA) and girdling on root formation on layered pear shoots. All shoots on a stock plant received the same treatment. Treatments were randomized across stock plants. IBA concentration was 20,000 ppm.

		Clone		
Treatment	Response	OHF 40	OHF 87	OHF 97
Untreated	Total Rooting (%)	31.2	0	1.2
	Acceptable Rooting (%)	1.6	0	0
IBA	Total Rooting (%)	37.0	0	4.2
	Acceptable Rooting (%)	9.0	0	0
Girdle	Total Rooting (%)	74.5	41.2	39.2
	Acceptable Rooting (%)	43.4	17.6	17.2
IBA + Girdle	Total Rooting (%)	91.3	61.7	73.7
	Acceptable Rooting (%)	63.3	33.3	44.6

This experiment confirmed that pear responds poorly to simple mound layering. The key factor for stimulating rooting is girdling, as IBA alone had no effect. Based on our observations and the experience of David Smith of the OSU hazelnut breeding program, the quality and location of the girdle are important. Metal rings are most effective, as non-metal materials, such as nylon-ties, expand as shoots grow and fail to girdle the stem.. Care must be taken to ensure a tight fit when the ring is applied. Placement of the ring as close to the base of the shoot as possible may improve rooting and enables shoots to be easily snapped off at harvest.

Girdling and IBA combined were the most effective treatment. Even with this combination of treatments, 30-50% of the rooted shoots were graded unacceptable. An important question is whether

these culled liners can be used? Are we grading too stringently? Are layered pears equivalent to pear hardwood cuttings, which often have poor growth and high mortality, or are they like some layered apple rootstocks which develop few roots during layering, but grow and develop well subsequently?

• Response to IBA concentration varied somewhat (Tables 3-5). Overall, we would recommend use of 20,000 ppm IBA, however 708-36 consistently produced better rooting in response to 5,000 ppm. Clone 708-36 will not be a commercial rootstock, but clones under development should be screened for IBA response.

• The stock plants we used for this study were mature. As a result, our trials provide a conservative assessment of ATTOL. We compared the response of similar-aged stock plants originally propagated by either softwood cuttings or micropropagation. Even after ten years, the micropropagated stock plants were more productive (Tables 3, 4). Young, micropropagated stock plants will respond at least as well, possibly much better. We will conduct a small trial on two year-old micropropagated stock plants in 2007.

Regulating shoot growth will be an important management question for ATTOL. In 2006, we fertilized the stock plants, which proved unnecessary, as many of the shoots were too large for use as liners. In the future, however, years of girdling may reduce vigor. Experience will teach appropriate management.

Table 3. Effect of IBA concentration on rooting of shoots of pear clone 708-12. Stock plants were originally propagated in 1997 from cuttings or tissue culture. All shoots were girdled.

		IBA (ppm)			
Stock Plant	Response	0	5,000	10,000	20,000
Cutting	Total Rooting (%)	33.4	48.9	58.8	53.1
	Acceptable Rooting (%)	11.3	28.8	29.3	36.9
Micropropagated	Total Rooting (%)	44.4	26.2	74.5	85.0
	Acceptable Rooting (%)	12.5	19.0	40.7	68.7

Table 4. Effect of IBA concentration on rooting of shoots of pear clone 708-36. Stock plants were originally propagated in 1997 from cuttings or tissue culture. All shoots were girdled.

		IBA (ppm)			
Stock Plant	Response	0	5,000	10,000	20,000
Cutting	Total Rooting (%)	23.3	60.0	54.3	52.3
	Acceptable Rooting (%)	13.3	43.3	41.0	29.5
Micropropagated	Total Rooting (%)	80.0	100	89.5	82.9
	Acceptable Rooting (%)	50.0	77.8	65.5	75.2

Table 5. Effect of IBA concentration on rooting of shoots of pear clone 708-2. All shoots were girdled.

		IBA (ppm)			
Stock Plant	Response	0	5,000	10,000	20,000
Cutting	Total Rooting (%)	26.4	17.7	50.0	81.7
	Acceptable Rooting (%)	3.8	8.3	30.8	53.3

3)**Kazakhstan clones**.

• In February, 2002, we received budwood from three clonal rootstocks, Q29857, Q29858, Q29859, from Kazakhstan. These were initiated into tissue culture. APHIS released these clones spring, 2005.

Our experience to date is that these clones are generally difficult to micropropagate.

Clone	Approx. Shoot Multiplication Rate	Rooting (%)
Q29857	1x (poor)	NA
Q29858	2x (slow)	ca. 50%
Q29859	4x (good)	ca. 50%

Propagation of Q29859 is feasible, but at this point, the other two have doubtful propagation potential. Repeated application of liquid medium has only limited effect on shoot growth. Rooting of both 858 and 859 has improved from 17% in initial trials to 50% in recent work with the addition of 1% Activated Charcoal in the rooting medium.

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

Project Title: New approaches to decay control of pear

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

Budget 1:
Organization Name: OSU Mid-Columbia Agricultural Research and Extension Center
Contract Administrator: Dorothy Beaton (ARF)
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Item	**Year 1**: 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries	34,008	34,049	**36,092**
Benefits	13,854	16,684	**17,685**
Wages			
Benefits			
Equipment			
Supplies	1,000	1,000	**1,000**
Travel			
Miscellaneous			
Total	48,862	51,733	**54,777**

Footnotes:

Objectives:

1. New model for decay risk prediction
2. DNA techniques for rapid detection of decay spores in packinghouses
3. Bull's-eye rot species in Washington and Oregon and fungicide sensitivity
4. Use of qPCR to determine "residues of CIM biocontrol agent on pear fruit
5. Preharvest and postharvest fungicides for decay control

Significant findings:

- The first version of a 4-factor gray mold risk prediction model has been developed and is being cooperatively validated in major Oregon and Washington pear districts.
- DNA provides rapid, accurate detection of decay spores in packinghouse water.
- 633 new bull's-eye rot isolates have been identified. *N. perennans* is the most common species in Yakima and Wenatchee and *N. alba* the most common in Hood River and Medford.
- The benzimidazoles thiophanate methyl (Topsin) and thiabendazole (Mertect) appear to have the most effect on *N. alba* and *N. perennans*
- A qPCR method was developed to determine threshold "residues" of the biocontrol agent CIM required on pear fruit for optimum decay control.
- The most effective preharvest spray for overall decay control in 2005-6 was a tank mix of Topsin M and Nutraphos 24.
- The new formulation of Scholar (230SC) gave excellent control of blue mold, gray mold, and bull's-eye rot.

Methods:

1. New model for decay risk prediction

The goal is to accurately predict at harvest the gray mold risk level of pear fruit in long term storage. Factors that are being included in model development are pathogen DNA on pear fruit surfaces at harvest, preharvest rain, preharvest fungicide application, and orchard condition. A standard test also was developed to measure the yearly change in fruit resistance.

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

We are developing a qPCR method to determine the concentration of decay spores (*P. expansum* and *M. piriformis*) in dump tank and flume water so decay control decisions can be based on spore threshold values. Dump tank water was collected from three packinghouses, "spiked" with three levels of *P. expansum* spores, and qPCR used to compare spore levels with the traditional plate count method. The relationships between packinghouse water spore loads and decay were determined for blue mold, gray mold, and Mucor rot of Bosc pear and Golden delicious apple and compared with Anjou data from several years ago.

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

In the last year, we have collected and identified, using multiplex PCR, 633 new isolates of fungi from fruit with bull's-eye rot. New isolates are mainly from Wenatchee, Yakima, and Hood River. Selected isolates are being screened for sensitivity to a large group of fungicides with a new ELISA plate lab laboratory protocol as well as with wounded pear fruit.

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

The biocontrol yeast CIM is being developed under an agreement with a private company and is expected to be registered and available by 2009. A real time PCR method was developed to measure the amount of CIM on pear fruit surfaces to assure that coverage is adequate for acceptable decay control. A range of CIM concentrations were prepared, and Anjou and Bosc pears were dipped in the suspensions. Fruit were washed to remove the CIM cells, and cells were quantified by measuring CIM DNA with qPCR.

5. Preharvest and postharvest fungicides for decay control.

Several fungicides were applied to Anjou pear trees either 1 or 2 weeks before harvest. Fruit were harvested and drenched with water containing spores of *P. expansum*. Fruit were stored at 30˚ F and decay evaluated after 3 months.

Postharvest treatments were applied with a recirculating drencher. Drench treatments contained 1000 spores per ml of Penicillium expansum strain 46 to simulate commercial drencher contamination. Treated fruit were stored at -1° C and evaluated for decay after 3 and 6 months. Decayed fruit were removed after the first evaluation to prevent secondary spread. Decay data from both evaluations were combined before statistical analysis.

Results and discussion:

1. New model for decay risk prediction

The first version of a 4-factor gray mold risk prediction model is shown below in Table 1. The model was developed using data from 8 pear orchards in Oregon and Washington in 2004-6 (Table 2). Fruit from moderate risk orchards had more gray mold in 2005-6 than 2004-5, possibly because fruit were more susceptible in the 2005-6 crop (Table 3).

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

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

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

Table 2. Pear gray mold predicted risk vs. actual decay in cold stored fruit from 8 Oregon and Washington orchards

	2004-2005			2005-2006	
Orchard	Predicted risk[1]	Gray mold (%)[2]	Orchard	Predicted risk[1]	Gray mold (%)[2]
1	E	14.0	1	H	8.7
2	H	8.0	2	H	7.3
3	H	7.0	3	H	6.6
4	H	5.9	4	M	5.9
5	H	4.2	5	M	5.1
6	M	2.6	6	M	3.8
7	M	2.2	7	M	3.4
8	M	2.2	8	L	2.1

[1]Risk levels: L = low, M = moderate, H = high, E = extreme.
[2]Decay after 6 months storage at 30°F.

Table 3. Susceptibility of Anjou pear fruit to decay in standardized laboratory conditions

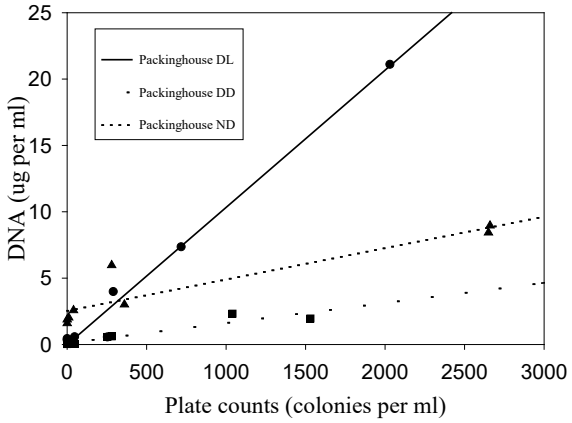
	Infection index[1]		
Year	Gray mold	Blue mold	Mucor rot
2004	22.7b	28.2a	48.4a
2005	28.0c	28.0a	58.2a
2006	13.0a	31.4b	49.2a

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

During the 2006-7 season, the model is being validated in orchards in Wenatchee (Dr. Chang–Lin Xiao), Medford (Dr. David Sugar), and the Mid-Columbia (Dr. Bob Spotts) and will use packinghouse cull analyses when available.

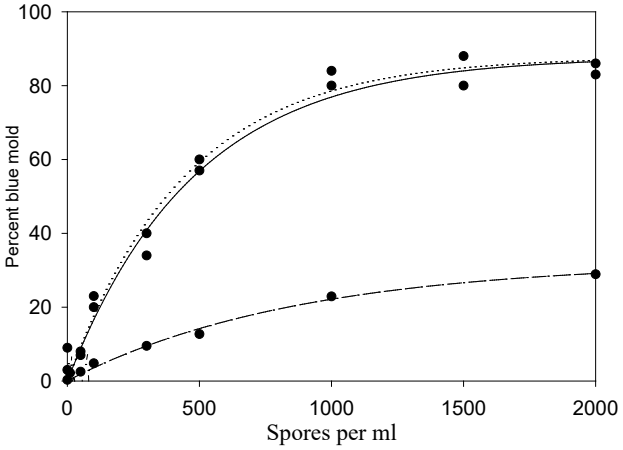
2. DNA techniques for rapid, accurate detection of decay spores in packinghouses

Excellent agreement was found between the amount of *P. expansum* DNA in "spiked" dump tank water from three packinghouses and the spore counts from traditional dilution plates (Fig. 1). However, curves were different for each packinghouse, and reasons for this need to be determined. The experiment will be repeated with *P. expansum* and *M. piriformis*, and additional samples from Oregon and Washington packinghouse water will be analyzed.



Relationship between decay and spore load in water

Blue mold decay is closely related to spore load in packinghouse water systems. We found that the relationship is similar for Bosc pear (solid line) and Golden Delicious apple (dotted line). Both are much more susceptible than Anjou fruit (dashed line). Similar relationships have been determined for gray mold and Mucor rot. Spore loads in packinghouse water should be reduced as much as possible to reduce decay in storage.



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

We have identified 1202 isolates of *Neofabraea* from decayed fruit (633 additional isolates since the last report)(Table 4). *N. perennans* is the most common species in Yakima and Wenatchee and *N. alba* the most common in Hood River and Medford. *N. malicorticis* was not found in any Oregon or Washington orchards but is known to occur on the west side of the Cascade Mountains. The new, unnamed species of *Neofabraea* is most common in Medford. Dr. Chang-Lin Xiao's bull's-eye collection is being processed for identification of isolates.

Table 4. Summary of Neofabraea (bull's-eye rot) July 2006

	Percent occurence				
Location	N.alba	N.perennans	N.malicorticis	N sp. nova	Total
Yakima	0	100	0	0	40
Wenatchee	19	81	0	0	168
Mid-Columbia	64	35	0	1	350
Medford	78	16	0	6	644
TOTAL					1202

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

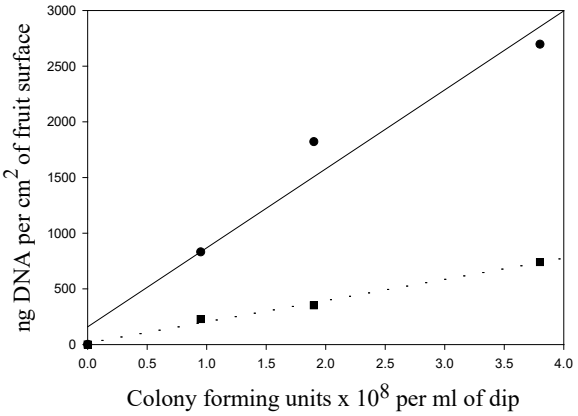
We are determining the sensitivity of the *Neofabraea* species to various fungicides using a lab technique with small ELISA plates. Technical grade active ingredient is placed in micro wells with spores, and increased turbidity indicates germination and growth. Thus far, the benzimidazoles thiophanate methyl (Topsin) and thiabendazole (Mertect) appear to have the most effect on *N. alba* and *N. perennans*, the two most common species in commercial pear orchards (Table 5).

Table 5. Effect of fungicides on *N. alba* and *N. perennans in vitro*

		Relative inhibition	
Fungicide	Tech name	*N. alba*	*N. perennans*
Flint	Trifloxystrobin	++	0
Topsin	Thiophanate	++	+
Scholar	Fludioxonil	+	0
Penbotec	Pyrimethanil	0	+
Mertect	Thiabendazole	+++	++

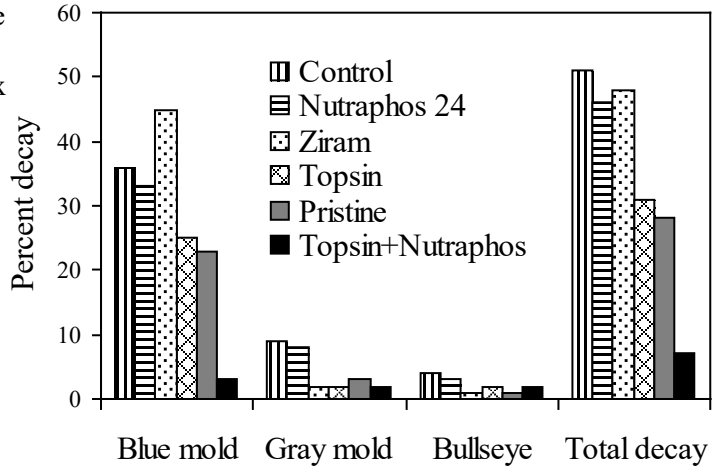
4. Use of qPCR to determine "residues" of a biocontrol agent on pear fruit

The recommended concentration of CIM for decay control is 2 x 10^8 cfu/ml. When Anjou (solid line) and Bosc (dashed line) fruit were treated with this concentration, the amounts of DNA on the fruit surfaces were about 1600 and 400 ng per cm^2, respectively (Fig.3). This method can be used to assure that CIM is being properly applied to pear fruit on the packing line or in the drench and will result in optimum decay control.



5. Preharvest and postharvest fungicides for decay control

Preharvest treatments. The most effective preharvest spray for overall decay control was a tank mix of Topsin M and Nutraphos 24. Topsin alone and Pristine were more effective than Ziram for blue mold control. All fungicides controlled gray mold. Pristine was applied twice, all other treatments once at 2 weeks PHI.



Postharvest treatments. Pristine at 1000 ppm gave significant control of blue mold but not of bull's-eye rot or gray mold. Pristine at 2000 ppm gave excellent control of blue mold and gray mold and was equivalent to Penbotec. Gray mold and bull's-eye rot resulted from natural infections.

Table 6. Control of decay of pear with postharvest drench of Pristine

		Percent fruit infected[z]		
Treatment	Rate product per 100 gal	Blue mold	Bull's-eye rot	Gray mold
Pristine 1000 ppm	35.0 oz	0.3a	2.1ab	2.8b
Pristine 2000 ppm	70.0 oz	0.0a	1.5ab	0.0a
Ethoxyquin 2700 ppm	2.0 quarts	26.2b	4.2b	2.0b
Penbotec 500 ppm	1.0 pint	0.4a	0.0a	0.0a
Water control	---	22.6b	5.4b	5.0b

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

Both formulations of Scholar gave excellent control of blue mold and gray mold. Only Scholar SC controlled bull's-eye rot. Gray mold and bull's-eye rot resulted from natural infections.

Table 7. Control of decay of pear with postharvest drench of Scholar WP and Scholar SC

		Percent fruit infected[z]		
Treatment	Rate product per 100 gal	Blue mold	Bull's-eye rot	Gray mold
Scholar 50WP	8.0 oz	0.7a	7.8b	0.3a
Scholar 230SC	16.6 fl. oz	0.8a	1.4a	0.0a
Water control	---	22.6b	5.4b	5.0b

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

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-05-502 (WSU Project No. 13C-3661-7366)

Project Title: Control of Postharvest Decay in Pear

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Cooperators: Packinghouses

Budget 1:
Organization Name: WSU-TFREC **Contract Administrator:** Mary Lou Bricker; Sally Ray
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Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries[1]	13,000	14,803	**15,243**
Benefits[2]	5,200	5,477	**5,335**
Wages	3,000	3,000	**4,000**
Benefits[3]	330	330	**460**
Supplies[4]	4,000	4,000	**4,000**
Travel[5]	1,000	1,000	**2,500**
Total	26,530	28,610	**31,538**

Footnotes:
[1]***Salary for a Scientific Assistant (Robin Boal at 0.3 FTE).***
[2] Benefits for Robin Boal in 2007 is 35%.
[3] Benefits for a time-slip helper in 2007 is 11.5%.
[4] Culture media, chemicals, Petri dish plates, and fungicides. Cost of fruit bought from commercial orchards. Cell phone charges are allowed.
[5] We will be using a leased vehicle.

Objectives:

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

Significant findings:

- In trials conducted in commercial orchards, Pristine by a ground application reduced the amount of decay in the bins by 45-61% in comparison with Pristine by an aerial application, but the aerial application of Pristine was not effective compared with the nontreated control. The results support our recommendations that a ground application to achieve good coverage is essential to the success of a preharvest fungicide program for control of postharvest rots.
- Gray mold and Phacidiopycnis rot were the two major postharvest rots in field bins (the fruit were not drenched prior to storage) in our trials conducted in commercial orchards. Pristine by a ground application was effective to control both gray mold and Phacidiopycnis rot originating from natural infections.
- When applied within one week before harvest, the residues of Pristine and Ziram on the fruit at harvest significantly reduced infections of wounds (punctures) by Phacidiopycnis rot, but the magnitude of reduction in decay incidence was low to moderate, ranging from 29% to 41%, compared with the nontreated control. The residues of Pristine and Ziram on the fruit at harvest did not protect wounds from infection by gray mold. In comparison with the results on Fuji and Red Delicious apples, it appears that residue levels on the fruit at harvest and susceptibility of the fruit both may affect the effectiveness of Pristine in protecting wounds from infection by gray mold. In Fuji and Red Delicious apples, Pristine applied within two weeks before harvest was very effective to protect wounds from infection by gray mold. D'Anjou pears may be more susceptible to gray mold than apples. A higher level of fungicide residues on d'Anjou pear fruit at harvest may be needed in order to protect wounds from infections by decay-causing pathogens. However, in addition to protecting wounds from infections by decay-causing pathogens, preharvest fungicides applied near harvest also are beneficial to reducing spore load on the surface of the fruit and eradicating some latent infections. Thus, considering all potential benefits, use of preharvest fungicides such as Pristine and Ziram is recommended for control of postharvest rots.
- Topsin and Pristine reduced stem-end and calyx-end Phacidiopycnis rot by 86% and 41%, respectively, in comparison with the nontreated control. It appeared that Topsin was more effective than Pristine for control of stem- and calyx-end Phacidiopycnis rot, but this observation needs to be confirmed in the following-year experiment.
- When applied as a pre-storage drench treatment, all three postharvest fungicides were effective to control stem-end and calyx-end Phacidiopycnis rot. Mertect and Scholar reduced stem- and calyx-end Phacidiopycnis rot by 95% and 88%, respectively, in comparison with the nontreated control. Penbotec was highly effective and no stem- and calyx-end Phacidiopycnis rot developed in the fruit treated with Penbotec.
- The residues of Pristine and Topsin applied at seven days before harvest on pear fruit were able to suppress the fruit-to-fruit spread of gray mold during storage. Topsin was more effective than Pristine in suppressing the fruit-to-fruit spread of gray mold. Among the three postharvest fungicides when applied as a pre-storage drench treatment, Penbotec was not effective in suppressing fruit-to-fruit spread of gray mold, whereas Mertect and Scholar reduced gray mold resulting from fruit-to-fruit spread by 69% and 73%, respectively, in comparison with the nontreated control. These observations need to be further evaluated.

Methods:

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

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

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

Results and discussion:

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

The trials were conducted on the 2005 crop in four commercial orchards. Decay assessment was done in the spring of 2006. Incidence of rots in storage bins varied from orchard to orchard. Orchard 1 and Orchard 2 had 7.6% and 5.2% rots in the nontreated fruit, respectively. The other two orchards had approximately 3% rots. Significant differences in decay control between air and ground applications were observed in Orchard 1 and Orchard 2 (Fig. 1). No significant difference in decay control between the two application methods was seen in Orchard 3 and Orchard 4, likely due to relatively lower levels of rots in these two orchard lots.

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

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

bull's eye rot were low in these trials.

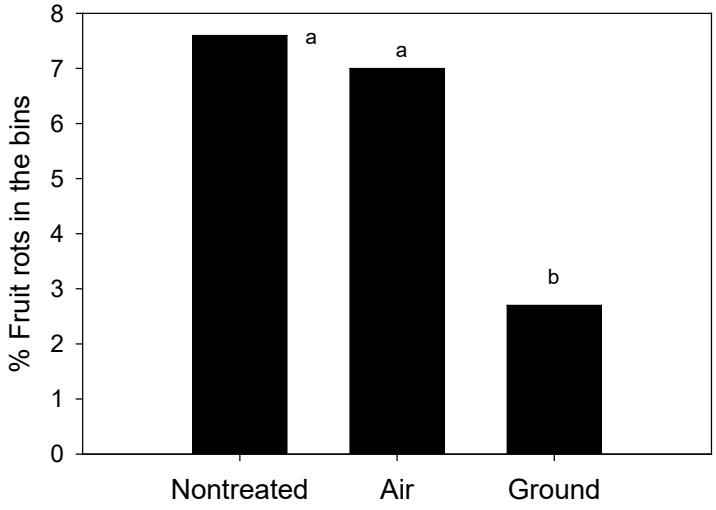

Fig. 1. Comparison of actual losses of d'Anjou pear fruit in field bins between the fruit treated with Pristine applied by a ground application (200 gallons/A) and the fruit treated with Pristine applied by an aerial application. The fruit were not drenched prior to storage. The fruit were stored in CA for five months, at which time decay was assessed. Percentage of fruit rots in field bins was expressed as weight of decayed fruit in the total weight of the fruit in a bin.

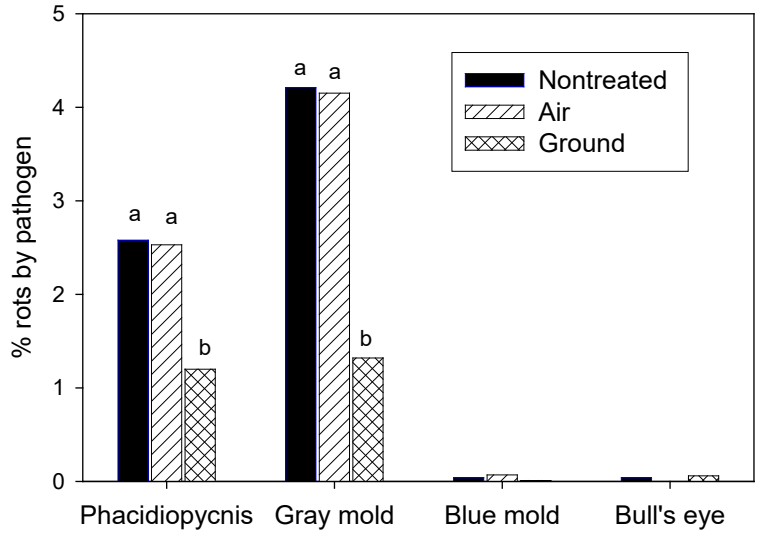

Fig. 2. Comparison of gray mold and Phacidiopycnis rot between the fruit treated with Pristine applied by a ground application (200 gallons/A) and the fruit treated with Pristine applied by an aerial application. The fruit were not drenched prior to storage. The fruit were stored in CA for five months,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

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

One experiment was conducted on the 2006 crop to evaluate preharvest fungicides for control of Phacidiopycnis rot and gray mold originating from infections of wounds on the surface of fruit. The purpose of this experiment was to look at protection of the fruit at harvest by the residues of fungicides applied within two weeks before harvest.

In the worst scenario (fruit were wounded at harvest and inoculated with pathogens), when applied within seven days of harvest the residue of Topsin did not protect wounds on the fruit from infection by Phacidiopycnis rot and gray mold. Pristine and Ziram significantly reduced infections of wounds by Phacidiopycnis rot, but the magnitude of reduction in decay incidence was low to moderate, ranging from 29% to 41%, compared with the nontreated control (Fig. 3). Pristine applied at either one or seven days before harvest and Ziram applied at seven days before harvest did not protect wounds from infection by gray mold (Fig. 3). The results indicated that the residue levels of either Pristine or Ziram on the fruit at harvest were not high enough to protect wounds from infections by gray mold. This is consistent with the results of the experiment we did on the 2005 crop. In comparison with the results we have done on Fuji and Red Delicious apples, it appears that residue levels on the fruit at harvest and susceptibility of the fruit both may affect the effectiveness of Pristine in protecting wounds from infection by gray mold. In Fuji and Red Delicious apples, Pristine applied within two weeks before harvest was very effective to protect wounds from infection by gray mold. D'Anjou pears may be more susceptible to gray mold than apples. A higher level of fungicide residues on d'Anjou pear fruit at harvest may be needed in order to protect wounds from infections by decay-causing pathogens. However, in addition to protecting wounds from infections by decay-causing pathogens, preharvest fungicides applied near harvest also are beneficial to reducing spore load on the surface of the fruit and eradicating some latent infections. Thus, considering all potential benefits, use of preharvest fungicides such as Pristine and Ziram is recommended for control of postharvest rots.

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

Stem-end rot and calyx-end rot are two common types of symptoms of Phacidiopycnis rot in d'Anjou pears. Fruit infected by the fungus at the stem and calyx may not have symptoms at packing, but symptoms develop in the boxes before or after shipping. One experiment was conducted on the 2005 crop, and decay assessment was completed in spring 2006. The results are presented in Fig. 4. The experiment was repeated in 2006 and the fruit are still in storage for decay development.

Two preharvest fungicides were evaluated. Both Pristine and Topsin M applied at seven days before harvest were effective. Topsin and Pristine reduced Phacidiopycnis rot by 86% and 41%, respectively, in comparison with the nontreated control (Fig. 4). It appeared that Topsin was more effective than Pristine for control of stem- and calyx-end Phacidiopycnis rot, but this observation needs to be confirmed in the following-year experiment.

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

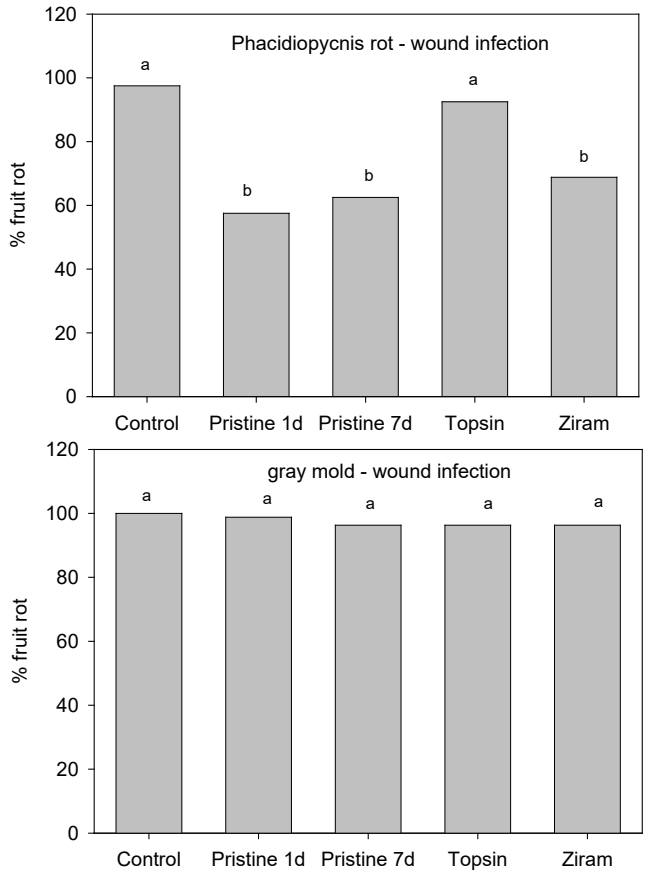

Fig. 3. Effectiveness of preharvest fungicides in controlling postharvest gray mold and Phacidiopycnis rot originating from infections of wounds on d'Anjou pears. Pristine was applied at either 1 or 7 days before harvest. Topsin M and Ziram were applied at 7 days before harvest.

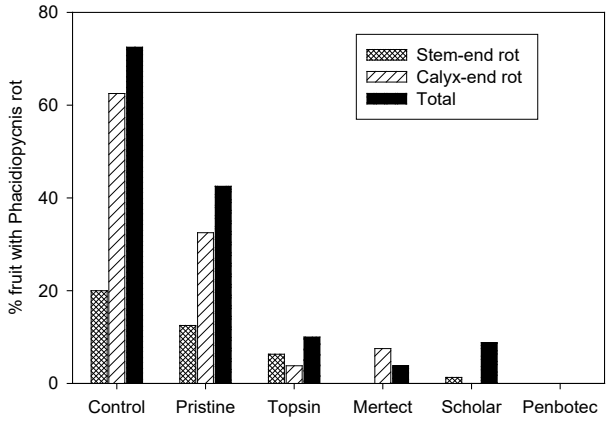

Fig. 4. Control of stem- and calyx-end Phacidiopycnis rot with pre- and postharvest fungicides. The fruit were inoculated with the pathogen in the orchard at three weeks before harvest. Pristine and Topsin were applied at seven days before harvest, and Mertect, Scholar and Penbotec were applied the same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

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

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

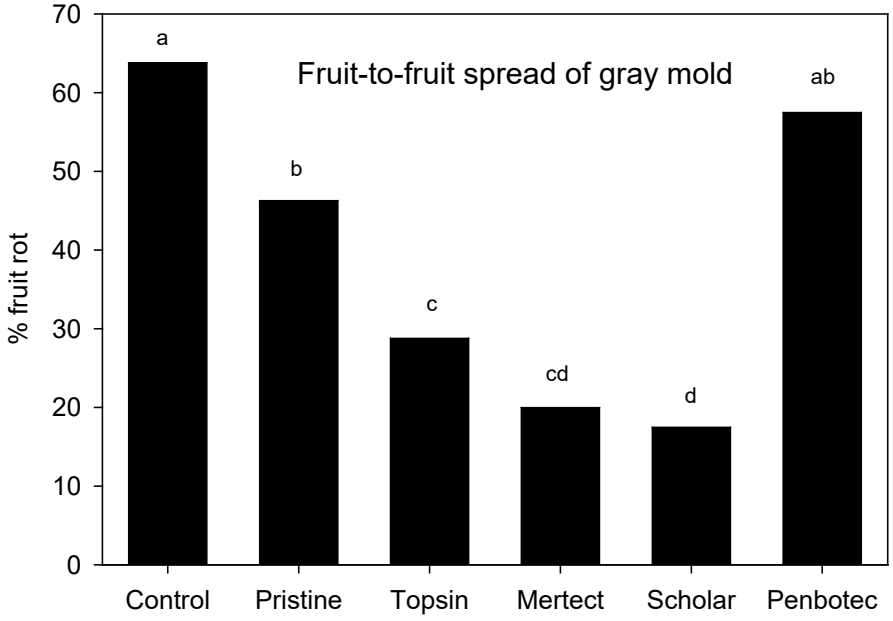

Fig. 5. Effectiveness of preharvest fungicides applied at 7 days before harvest and postharvest fungicides applied as a pre-storage drench treatment in suppressing fruit-to-fruit spread of gray mold in d'Anjou pears during storage.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3
WTFRC Project Number: PR-06-603 (WSU Project #13L-4164-1202)

Project Title: Managing storage scald in Anjou pears

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Budget 1: ***Note: No additional funds are requested for this project in 2007.***

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Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries	16,890	13,301	13,634
Benefits (49%)	5,067	5,985	6,681
Wages	4,500	6,500	7,000
Benefits	720	715	770
Equipment	700	2,000	
Supplies	5,400	15,300	31,000
Travel	1,000	1,500	1,500
Total	34,277	45,301	60,585

Note: This project will not be concluded until June 2007 when the last fruit from the 2006 crop will be evaluated. Under agreement with the Research Commission this work is presented here as a continuing project using the most current data (2005 crop). A final report will be presented in January 2008. No additional funds are requested for this project in 2007.

Objectives:

This project was funded for the crop years 2004-2006 to initiate new research and integrate previous research on the prevention of storage scald on Anjou pears into a systems approach suitable for use by the industry. The specific objectives are:

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

Significant findings:

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

Drs. Ma and Chen (2001) reported good correlations with temperature and scald prediction in Anjou pears in the Hood River region. To date, temperature modeling in the Wenatchee and Yakima Valleys has not resulted in a predictive model for scald risk in Anjous.

- In 2004, fruit from only one of three orchards that had virtually no cool temperatures below 50ºF developed scald.
- In 2005, four orchards accumulated less than 11 hours below 50ºF, but fruit from only one orchard developed scald.
- To date in 2006 (mid-December) the fruit has not developed scald. We are continuing to look at fruit from 8 orchards (5 Wenatchee and 3 Yakima) following 7 and 14 days ripening.

Objective 2: Determine the timing of antioxidant application using fruit with different risk levels.

- In 2004, applying antioxidant as a fruit wrap within 7 days of harvest significantly reduced scald following long-term CA storage. Delaying the application of the antioxidant reduced the effectiveness. Ethoxyquin wrap was more effective at controlling storage scald than diphenylamine (DPA) wrap.
- In 2005, a postharvest drench of ethoxyquin + thiabendazole (TBZ) or TBZ alone was added to the wrap trial. Fruit drenched at harvest with ethoxyquin + TBZ developed less scald than fruit drenched with TBZ and treated with antioxidant wrap 7 days later. These findings from 2004 and 2005 confirmed the importance of applying an antioxidant immediately after harvest.

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

- In 2004, ethoxyquin drenched fruit stored in CA until February 21 and then held in RA for 30, 60 or 90 days developed less scald than undrenched control fruit. Following storage for 30 days in RA-only fruit from orchards with few cooling hours developed scald; by 90 days, fruit from all orchards had developed significant scald. Pears drenched with more than 675 ppm ethoxyquin developed the least scald.
- In 2005, undrenched fruit developed significantly more scald than any of the antioxidant drenches. A 1350 ppm drench of ethoxyquin provided superior control to 675 ppm ethoxyquin or 1000 ppm DPA. Effective concentration was related to specific orchards. The longer the fruit was stored, the more scald it developed (February vs. April vs. May).

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

- In 2004, the inclusion of the fungicides TBZ, Scholar (fludioxonil) or Penbotec (pyrimethanil) in antioxidant drenches did not increase chemical burn.
  - Pink-colored permanent chemical burn was found at fruit-to-fruit contact points on a high percentage of fruit treated with ethoxyquin (average as high as 49% burned fruit).
  - Brown chemical burn was not related to fruit contact and was found on the ethoxyquin-treated fruit (10% burned) and more severely on the DPA-treated fruit (average of 27% burned).
- In 2005, pink burn associated with the ethoxyquin increased with concentration and was unacceptably high (over 90% fruit affected at 1350 ppm) in two of the three orchards. In contrast, pink burn was only a minor problem on fruit from Wenatchee orchard 4 (less than 10% of fruit affected).
  - Brown burn was associated mostly with DPA treatments and was a relatively minor problem on fruit pulled out of storage in February. Brown burn became a serious problem on fruit stored until April and May, and was a more severe problem on fruit from Wenatchee orchard 4, which did not have pink burn.

OBJECTIVE 1: Predict the risk of scald through knowledge of preharvest temperatures.
Methods: The risk of scald was estimated through knowledge of orchard temperatures by analyzing temperature data using techniques developed by Ma and Chen (2001). Using the same orchards in 2004, 2005 and 2006 (5 in the Wenatchee Valley and 3 in the Yakima Valley), data loggers were placed within the canopy 6 weeks prior to anticipated harvest date to record temperatures on an hourly basis.

In 2006, fruit was harvested from each orchard at an average firmness of 14.0 lbf ± 0.5 lbf and stored in RA (32ºF) for 60 days prior to being evaluated for scald. Following RA storage, fruit are evaluated weekly for 14 weeks for storage scald after both 7 and 14 days of ripening. The incidence of scald will be compared to the hourly temperature data for each orchard to correlate cool nighttime temperatures (<50ºF) with scald development.

Results and discussion: In 2005, pears were harvested at the same firmness level (average 14.6 lbf) to reduce the effect of maturity on scald development (Table 1). Fruit were removed from RA storage at weekly intervals from 30 days after harvest to 120 days after harvest, ripened for 7 days and examined for scald. In 2006, fruit maturity was targeted at 14.0 lbf, storage exams started 60 days after harvest and will be continued longer than in previous years to allow additional scald to develop.

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

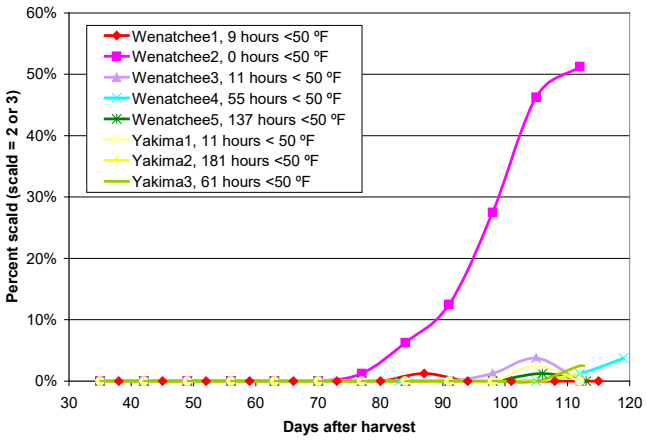

Figure 1. Scald development by orchard, 2005 Crop. Rated weekly starting 30 days after harvest, RA storage, followed by 7 days ripening at 70ºF.

It appears that reliance on accumulated cool temperatures to predict scald may

have to be modified for use in Washington since only one of the three orchards with a low accumulation of cool temperatures (Wenatchee 2) developed any scald in 2005. As of mid-December, no scald has developed in fruit from the 2006 harvest. We will be following fruit from this harvest into 2007 to see when it develops scald. Once we have this data we will test whether other mathematical models can correlate with temperatures with scald.

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

	2005 Crop						2006 Crop				
Orchard	**Harvest**	**Firm (lbf)**	**Hours <50 ºF ***	**Scald prediction****			**Harvest**	**Firm (lbf)**	**Hours <50 ºF***	**Scald prediction** ***	
Wenatchee 1	19-Aug	15.1	9	74d	1-Nov		4-Sep	14.3	28	80d	23-Nov
Wenatchee 2	29-Aug	13.6	0	62d	30-Oct		7-Sep	14.3	9	74d	20-Nov
Wenatchee 3	29-Aug	13.6	11	75d	12-Nov		7-Sep	15.4	32	81d	27-Nov
Wenatchee 4	1-Sept	14.8	55	84d	24-Nov		14-Sep	14.1	76	87d	10-Dec
Wenatchee 5	12-Sept	15.0	137	91d	12-Dec		18-Sep	13.5	145	91d	18-Dec
Yakima 1	23-Aug	15.6	11	75d	6-Nov		31-Aug	14.7	39	82d	21-Nov
Yakima 2	13-Sept	14.7	181	92d	14-Dec		11-Sep	14.9	141	91d	11-Dec
Yakima 3	13-Sept	14.5	61	85d	7-Dec		11-Sep	14.9	57	85d	5-Dec

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

** Predicted days in storage after which 10% of the fruit will show scald symptoms following 7 days ripening at 68ºF, based on the formula: $DIS(10\%)=62.3827 \times ACU^{0.0757}$
DIS = the number of days fruit was held in air storage, ACU = accumulated cold units (hours <50ºF)

OBJECTIVE 2: Determine the <u>timing</u> of effective antioxidant application on fruit.
Methods: In 2006, two bins of commercially harvested fruit from each of the five Wenatchee orchards were drenched with TBZ only or ethoxyquin (1350 ppm) + TBZ within one week of harvest. Bins were stored in RA at 32ºF for 7, 14 and 42 days prior to packing.

After each storage interval, fruit were passed over the wax section of the ARS-1 packingline using one of three line spray treatments: 1) Penbotec only, 2) 675 ppm ethoxyquin + Penbotec or 3) 1350 ppm ethoxyquin + Penbotec. The fruit were tray packed and placed in CA storage. Fruit will be evaluated for phytotoxicity (+0 days) and scald (+7 days) in March 2007.

Results and discussion: In 2004, superior control of scald was obtained when the antioxidant wrap was applied within 7 days of harvest when compared with delayed application (28, 56 or 112 days after harvest). However, even application at 7 days did not provide effective commercial control.

In 2005, to increase scald control, bins were first drenched with either 1350 ppm ethoxyquin or 1350 ppm ethoxyquin +TBZ. Antioxidant wraps were applied 7, 14 or 45 days after harvest. Fruit were stored in CA until May 2006. The most

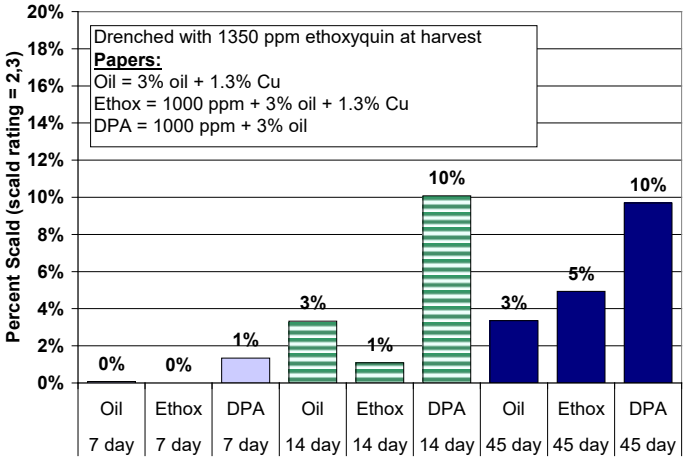

Fig. 2. Scald development by antioxidant wrap type and application date. Average of five orchards. 2005 Crop, CA stored, ripened at 70ºF for 7 days, evaluated May 2006.

effective scald control was an ethoxyquin drench followed by antioxidant wrap applied within 7 days. Ethoxyquin wrap provided superior scald control to DPA wrap (Fig. 2).

In 2006, fruit were drenched and then the antioxidant was applied as a line spray rather than a paper wrap. Fruit will be evaluated in May 2007 following long-term CA storage.

Scald susceptibility in these trials has been very orchard specific. In 2005, Wenatchee 5 developed the most scald, followed by Wenatchee 4. The accumulated cool temperatures in those orchards was higher than that of Wenatchee 1, 2 and 3, which developed only slight amounts of scald (see Objective 1).

With two years worth of data, it appears that antioxidant must be applied within 7 days of harvest to be effective at controlling scald. This third year's data will confirm whether later applications are effective. This could have significant cost savings to the industry if later applications of ethoxyquin are shown to be ineffective at controlling scald.

OBJECTIVE 3: Determine the effectiveness of applying antioxidants as a <u>bin drench</u>.
Methods: In 2006, three bins of commercially harvested pears from three different Wenatchee orchards were purchased and divided into cherry bins for drenching within one week of harvest:

A. Control = TBZ only
B. Ethoxyquin (675 ppm) + TBZ
C. Ethoxyquin (1350 ppm) + TBZ
D. Ethoxyquin (1350 ppm) + Penbotec
E. Ethoxyquin (1350 ppm) + Scholar
F. Ethoxyquin (2000 ppm) + TBZ

Fruit was placed in CA and samples will be removed in January, March and April 2007. At that point, sub-samples will be evaluated for chemical burn. Fruit will be examined for scald after 7 days, returned to air storage for 30 days and then evaluated for scald or passed over the packingline for additional treatment with either Penbotec alone or ethoxyquin + Penbotec. The packed fruit will be held in RA for 30 days and then evaluated for scald and chemical burn. Ethoxyquin concentrations will be 1350 ppm for treatments A through E and 700 ppm for treatment F to stay under the maximum label rate of 2,700 ppm.

Results and discussion: In 2004, fruit from the five Wenatchee orchards were drenched with antioxidants and/or fungicides at harvest, stored in CA until February 21, 2005, and evaluated for phytotoxicity. Additional samples were held for 30, 60 or 90 days in RA and evaluated for scald after 7 days of ripening. Scald appeared on 47% of the undrenched control fruit after 30 days in RA. The amount of scald increased the longer the fruit were held in RA. The ethoxyquin-treated fruit developed the least amount of scald, but even drenching with a fungicide alone reduced scald by approximately half compared to untreated fruit.

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

Undrenched fruit developed significantly more scald than fruit run through any of the drenches that contained an antioxidant. DPA (1000 ppm) drenched fruit developed more scald than fruit drenched with ethoxyquin at a rate of 1350 ppm or higher. Ethoxyquin used at 1350 ppm provided superior control to the 675 ppm rate.

Effective concentration was related to specific orchards. For example, 675 ppm ethoxyquin was an effective scald treatment for Wenatchee 4, which developed less scald than fruit from the other two orchards regardless of treatment.

The longer the fruit was stored the more scald it developed (February vs. April vs. May). Drenched fruit evaluated seven days after CA storage generally had less scald than fruit held for additional time in RA.

There was no significant reduction in scald after the application of ethoxyquin as a line spray following storage, even though the concentration of ethoxyquin was topped off at 2700 ppm. This is another indication of the importance of applying the antioxidant immediately after harvest, rather than following storage.

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

Methods: Fruit used in Objective 3 in 2006 will be evaluated for phytotoxicity at time of removal from storage and after packing. Because of high phytotoxicity from the antioxidants in the 2004 and 2005 crops, methods were revised to use lower concentrations applied multiple times.

Results and discussion: In 2004 fruit-to-fruit contact burn (pink) caused by the ethoxyquin was unacceptably high. The brown non-contact burn on the ethoxyquin- and DPA-treated fruit was also unacceptable.

In 2005, the pink burn from the ethoxyquin treatments increased with increasing concentration and was unacceptably high in all cases (Fig. 3). Again, the orchard factor comes into play because pink burn was only a minor problem on fruit from Wenatchee 4.

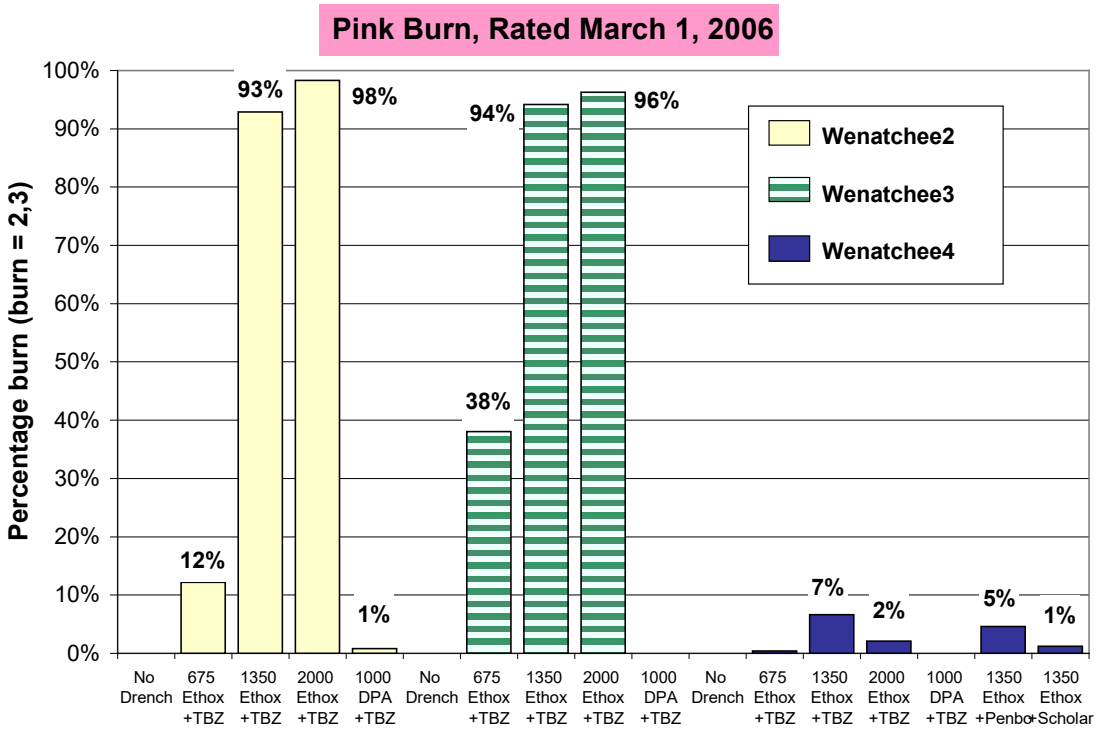

Fig. 3. Pink burn on Anjou pears following ethoxyquin drench and mid-term CA storage. Treatments (l to r): no drench, 675 ppm ethox+TBZ, 1350 ppm ethox+TBZ, 2000 ppm ethox+TBZ, 1000 ppm DPA+TBZ.

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

In drenching experiments conducted over the past two years the presence of pink staining on the ethoxyquin-treated fruit has been a severe problem. Any benefit in scald reduction derived from the application of an ethoxyquin drench has been outweighed by the potential for damage from the treatment. For future projects, the use of a second rinsing drench will be explored to see if the pink residue from the ethoxyquin can be reduced without the chemical losing its effectiveness.

OBJECTIVE 5: Evaluate the use of thermofogging to control storage scald and decay.
Methods: In 2006, 16 bins of pears (4 growers, 4 bins each) were thermofogged with the following treatments:

A. TBZ alone
B. Pyrimethanil alone
C. TBZ + Pyrimethanil
D. Not fogged

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

Results and discussion: In 2006 we began to study the feasibility of thermofogging with ethoxyquin and/or pyrimethanil on Anjou pears in cooperation with Dr. Peter Sanderson of Pace, International. The fruit are now in CA storage until January, when we will pull samples and evaluate for phytotoxicity and scald. Samples for residue analysis were taken at the time of fogging and will be repeated when the room is opened.

Literature cited:

Ma, S., D.M. Varga and P.M. Chen. 2001. Using accumulated cold units to predict the development of superficial scald disorder on Anjou pears during cold storage. J. Hort. Sci. & Biotechnology 76(3):305-310.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: PR-05-500 (WSU Project No. 13C-3655-6299)

Project Title: Branch induction in pear trees with bioregulators

PI: Don C. Elfving Horticulturist
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Cooperator Dwayne Visser, Agricultural Research Technologist III, WSU-TFREC, Wenatchee, WA

Budget 1:
Organization Name: WSU-TFREC **Contract Administrator:** Mary Lou Bricker; Sally Ray
Telephone: 509-335-7667; 509-663-8181 x221 **Email address:** mdesros@wsu.edu; saray@wsu.edu

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries[1]	4,500	4,750	**5,000**
Benefits (34%)	1,530	1,615	**1,700**
Wages[1]	200	220	**240**
Benefits (10% years 1 and 2; 11.5% year 3)	20	22	**28**
Supplies[2]	200	200	**200**
Travel[3]	500	600	**700**
Total	6,950	7,407	**7,868**

Footnotes:
[1] Technical 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 out carry out the research project.
[3] Treatment applications and frequent data collection at distant sites. Includes vehicle lease-to-purchase, operating and repair costs.

Original objectives of the project:

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

New objective of the project:

6. Determine whether cytokinin applications to blind wood in spring can induce renewed spur and/or shoot growth on such wood.

Significant findings:

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

Methods:

Trials were established in both cropping and non-cropping pear trees to determine effects of various bioregulator products on both growth and fruiting behavior. All Washington trials employed single-tree plots in randomized complete-block designs. The trial at MCAREC employed 5-tree plots in a randomized complete-block design. Two trials initiated in 2003, one trial initiated in 2004 and two trials initiated in 2005 were continued in 2006 to observe effects of previous branch induction treatments on carryover branching effects, flowering and tree productivity.

Results and discussion:

A. Effectiveness of cyclanilide as a soil-based branching treatment in the year of planting (Objective 1.)

1. Bronze Beauty Bosc/OHxF87 pear trees planted in April 2005 were drenched with cyclanilide at four concentrations (0, 5, 10 or 20 mg a.i./tree) after their first irrigation in late April.
2. There were no carryover effects of the first-year cyclanilide treatments on shoot growth in 2006 (year 2) of this orchard's life.
3. Similar cyclanilide concentrations applied as trunk drenches in fall 2005 for the 2006 growing season were effective in inducing significant development of weaker lateral

branching. Trees treated in both years 1 and 2 showed the same degree of growth response in 2006 as those treated only in year 2.

4. Five pear cultivars (Anjou, Bartlett, Golden Russet Bosc, Red Clapp's Favorite, selection 014) on OHxF rootstocks at the MCAREC in Hood River, OR were drenched with 0-20 mg cyclanilide per tree in spring 2005, shortly after planting. These trees were not treated again in 2006 and showed no carryover effects from the 2005 treatments.

B. Pruning requirements vs. cyclanilide treatment (Objective 3).

1. Any cyclanilide treatment applied in fall 2003 or spring 2004 to second-leaf Bosc trees led to approximately twice the number of pruning cuts required per tree in spring 2006, after two years of cyclanilide-stimulated increases in lateral branch development.

C. Multi-year treatment strategies with cyclanilide (Objective 4).

1. The trial established with Bronze Beauty Bosc in 2005 clearly showed that growth responses to soil-applied cyclanilide were much stronger for trees treated in year two than for trees treated only in the year of planting. It is clear that no growth-modifying bioregulator treatments should be soil-applied in the planting year, since the root system must re-establish itself in the orchard before the tree becomes capable of responding to such a treatment.
2. Cyclanilide applied as sprays to fourth-leaf Golden Russet Bosc/OHxF97 trees in 2003 increased branching as concentration was increased from 0 to 20 ppm. In 2005, fruit production from these trees was increased in direct proportion to the amount of branching induced by cyclanilide in 2003. In 2006, there was no difference in either bloom or fruit load that could be related to 2003 treatments.

D. Spring vs. fall soil treatment with cyclanilide for branch development in pear trees (Objective 5).

1. Golden Russet Bosc/OHxF87 trees planted in spring 2003 and treated in fall 2003 or spring 2004 with up to 150 mg a.i. cyclanilide as a soil drench showed carryover branching effects in 2005.
2. No carryover branching effects of treatments applied in fall 2003 or spring 2004 were observed in 2006.
3. There was a trend in 2006 to less bloom on trees subjected to higher cyclanilide concentrations applied in fall 2003 or spring 2004, but trees receiving cyclanilide in spring 2004 had fewer fruit than trees treated in fall 2003.
4. Bosc/OHxF87 trees treated with trunk sprays of cyclanilide (0-15,000 ppm) in fall at the end of their second leaf or in spring at the beginning of their third leaf showed a strong increase in branching in response to treatments. In 2006 the trees were in their sixth leaf; there was a larger bloom on cyclanilide-treated trees, but fruit set was less, resulting in no differences in yield per tree in 2006 due to cyclanilide applications in 2003.

Summary:
Cyclanilide is a powerful effector of shoot growth in pear trees. Because pear trees are so sensitive to cyclanilide, it has taken a few years to discover what amounts of product can be used effectively without producing an excessively strong response. Unlike bioregulator effects in most plant systems, including fruit trees, cyclanilide in pear is apparently translocated to shoot tips up to several months or even a year after application. It is not known whether the carryover growth effects we have observed are a result of storage of the bioregulator in the tree followed by later remobilization or whether the product is held in the soil and is taken up later by the root system and moved to the sites of activity, the shoot tips.

When cyclanilide exerts its effect on induction of budbreak and the production of new shoots, we have observed the typical response of reduced flowering as new vegetative growth is produced. However, in one trial we found a significant increase in production two years after treatment. This kind of response would seem to be a logical consequence of the often huge increase in new short shoots and spurs that result from the reduction of apical dominance induced by cyclanilide. We are conducting a series of trials to determine if we can exploit the translocatability of cyclanilide effects in pear to develop novel methods for using this bioregulator effectively in pear orchards to improve the onset of productivity. So far we have concluded that it is not beneficial to treat young pear trees in the year of planting, likely due to the lack of an effective root system. Because pears often do not branch extensively, we believe that a powerful inhibitor of apical dominance such as cyclanilide should be adaptable as a method for reducing the amount of pruning that otherwise would be needed for directing canopy development in pear to produce the short shoots and spurs that result in cropping.

Acknowledgments:
The assistance and support of the following persons and organizations are gratefully acknowledged: Tom Auvil, Jeff Cawood, Mike Cawood, Dick Cox, Randy Cox, Rick Cox, Kevin Forney, Bob Gix, Daryl Harnden, Jeff Henry, Jeff Holmer, Dr. Jim McFerson, Chris Olsen, Major Tory Schmidt, Dr. Clark Seavert, David Smith, Kris Thomas, Janet Turner, Dwayne Visser, Bayer Environmental Science, Cawood Orchards, Cox Orchards, Harnden Orchards, Holmer Orchards, Mid-Columbia Agricultural Research and Extension Center, Washington Tree Fruit Research Commission, and the WSU Agricultural Research Center.

Publications 2006:

Elfving, D.C. and D.B. Visser. 2006. Cyclanilide induces lateral branching in sweet cherry trees. HortScience 41:149-153.

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Drake, S.R., D.C. Elfving, M.A. Drake, T.A. Eisele, S.L. Drake and D.B. Visser. 2006. Effects of ethephon and 1-methylcyclopropene on apple fruit quality at harvest and after storage. HortTechnology 16:16-23.

Drake, S.R., D.C. Elfving, P.L. Pusey and E.M. Kupferman. 2006. Fruit quality of d'Anjou pears after bin storage and late-season packing. J. Food Proc. Preserv. 30:420-432.

Lenahan, O., M.D. Whiting and D.C. Elfving. 2006. Gibberellic acid inhibits floral bud induction and improves 'Bing' sweet cherry fruit quality. HortScience 41:654-659.

Elfving, D.C. 2006. Bioregulator sprays. p. 74-86. In: T.J. Smith (coord.), 2006 Crop Protection Guide for Tree Fruits in Washington. EB 0419.

Elfving, D.C. and D.B. Visser. 2006. Bioregulator effects on growth, flowering and cropping in apple trees. Poster, Wash. State Hort. Assoc. annual meeting, Yakima, WA.

Elfving, D.C. and D.B. Visser. 2006. Branch induction in pear trees with bioregulators.
Poster, Wash. State Hort. Assoc. annual meeting, Yakima, WA.

Visser, D.B. and D.C. Elfving. 2006. Bioregulators for managing growth, cropping and fruit quality in sweet cherry. Poster, Wash. State Hort. Assoc. annual meeting, Yakima, WA.

Smith, D., J. Turner and D. Elfving. 2006. Effects of chemically induced branching on performance of several pear cultivar/rootstock combinations in a high-density planting. Poster, Wash. State Hort. Assoc. annual meeting, Yakima, WA.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: #PR-05-504

Project Title: Chemical ecology of pear psylla

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Cooperators: Dr. Jocelyn Millar (UC Riverside) – funded by NRI
Bob Brown (WSU Master's candidate) – funded by USDA-ARS

Budget 1:
Organization Name: USDA-ARS **Contract Administrator:** Carolyn Yager
Telephone: (510) 556-6019 **Email address:** cyager@yarl.ars.usda.gov

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

Year 3 (2007): Funding requested for GS-11 post-doctoral scientist (C. Guédot; 0.25 FTE) and a GS-5 technician (0.25 GTE). The technician will assist the post-doctoral scientist with behavioral assays to be conducted in tandem with volatiles collection and EAG studies.

OBJECTIVES:

Define how volatile chemicals associated with female pear psylla affect male behavior, with final aims being to isolate, identify, and synthesize chemical attractants. The behavioral studies are being done to define the specific physiological conditions (age, diapause status, mating status) that lead to optimum response by males to female- produced volatiles. Once those conditions are determined, we will collect volatiles from females at those conditions, for isolation and identification of chemical attractants.

Objectives for 2006:

a. Reconfirm seasonal phenology of winterform attractiveness (**completed)**;
b. Assess role of mating status affecting attractiveness of female summerforms (**completed**);
c. Test attractiveness of dead female summerforms to males (this study was done to assess possible role of acoustic communication affecting olfactometer results) (**completed**);
d. Develop field-trapping methods using female psylla as source attractants (**ongoing**);
e. Develop volatiles collection methods (**ongoing**);
f. Develop EAG methods (**ongoing**).
g. Resubmit BARD proposal incorporating data obtained in this WTFRC project (**completed**).

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS FROM 2006:

a. Volatiles from field-collected female winterforms do not attract field-collected males until early to mid-February, coinciding with ovarian maturation and mating in the field.
b. Virgin summerform females and mated summerform females both attract males in olfactometer.
c. Field-collected summerform males are attracted to volatiles from dead females, indicating responses by males in olfactometer are not caused by acoustic signaling.
d. Added a WSU master's candidate (Bob Brown) to this project, whose responsibilities are to develop field-trapping methods; the initial field efforts showed that male summerforms are attracted to female summerforms caged on tree limbs; funding by USDA-ARS.
e. The post-doctoral scientist (Christelle Guédot) on this project visited the fruit fly laboratory in Hawaii to train in EAG methods (ARS-funded trip).
f. Efforts to collect volatiles from summerform females ongoing (C. Guédot).
g. NRI proposal submitted in Dec. 2005 was funded ($230,000). Funds will be split between Wapato and U.C. Riverside. Dr. Jocelyn Millar (UCR), a pheromone chemist, has been added to project to assist in isolation, identification, and synthesis of the attractant.
h. Rewrote and resubmitted the BARD proposal (funding to be split between Wapato and Israel) to conduct pheromone work on the U.S. pear psyllid and the pear psyllid in Israel. Funding will help support a pheromone chemist in Israel, who will collaborate with J. Millar on identifying and synthesizing the attractants.

Objectives for 2007:

a. Assess importance of host plant in affecting attractiveness of winterform and summerform females to males in the olfactometer (**Horton**);
b. Continue to develop volatiles collection methods (**Guédot/Landolt**), and begin to provide collections to **Horton** for olfactometer work and to **J. Millar** (UCR) for chemistry work;
c. Continue to develop EAG methods (**Guédot/Landolt**);
d. Continue to develop field-trap designs (**Brown/Landolt**).

METHODS (for the 2007 Objectives, above)

a. Olfactometer tests will pair females + host plant (shoots for winterforms, seedlings for summerforms) vs females minus plant material (**Horton**). These assays will be done to assess whether we can collect volatile attractants from females in the absence of the host plant, thus allowing us to eliminate host plant odors from the volatile collections.
b. Head space volatiles will be collected from females known from olfactometer tests to be attractive to males (**Guédot/Landolt**). Female psylla will be placed in a volatile collection system composed of a gas collecting jar through which purified air is passed. Volatiles will be collected on SuperQ traps. The trapped volatiles will be extracted with methylene chloride. Extracts will be forwarded to **J. Millar** for chemistry work and to **Horton** for olfactometer work.
c. **Dr. Guédot** will continue efforts to apply standard EAG-EAD technology to male winterform and summerform psylla. Odors from female psylla shown to be attractive to males in olfactometer tests will be used as stimuli.
d. Various types of sticky traps will be assessed for field-implementation, using caged females as attractant (**Brown/Landolt**). Trap location (height in canopy, cardinal direction) will be manipulated. The work will be done in late winter (winterforms) and summer (summerforms)

RESULTS AND DISCUSSION

Seasonal phenology of winterform attractiveness. Field-collected female winterforms were not attractive to males in Y-tube olfactometer trials until early- to mid-February, coinciding with the onset of mating and ovarian maturation in the field (as shown by dissection). **Figure 1** shows percentage of males choosing female-infested shoots in the olfactometer when the infested shoots were paired against psylla-free shoots. These results suggest that production of attractants by female winterforms is tied closely to the diapause syndrome.

Effects of mating status on attractiveness of female summerforms. Data presented last year showed that male summerforms are attracted in olfactometer tests to virgin females. It is difficult to rear large quantities of virgin summerforms from which to extract volatile chemicals, so it was of interest to determine whether mated females (which are easily collected from the field) are also attractive. **Figure 2** shows percentage of males preferring females on seedlings if paired against seedlings alone. The figure indicates that mated females and virgin females (on pear seedlings) both were more attractive to males than pear seedlings alone. A paired comparison of mated vs virgin females showed equal attractiveness to males (**Figure 2**, right-most pie diagram). These results suggest that mated females, like virgin females, produce volatile attractants.

Response by males to dead females. Concerns have been raised that our olfactometer tests do not completely eliminate the possibility that male attraction to females (in the olfactometer) is driven by acoustic cues, rather than chemical cues. **Figure 3** shows preference by male summerform psylla for dead summerform females (killed by freezing), when the dead females are paired with an empty jar in the olfactometer. These results indicate that volatile chemicals are driving the male preferences.

Field-trapping assay. Limbs of pear trees were enclosed in white organdy bags (30 cm deep x 15 cm diam) at an unsprayed orchard. The bags were placed one bag per tree in 60 trees, at 2-2.5 m above ground. Either 15 summerform females (N=20 limbs) or 15 males (N=20 limbs) were added to a bag; the remaining 20 bags were left psyllid-free. A 30 x 20 cm section of white plastic hardware cloth (2 mm mesh) was wrapped around each organdy bag; ends were stapled together so that the plastic encircled the organdy bag. The hardware cloth was then coated with tangletrap. After 4 days in the field, numbers of male and female pear psyllids were counted in the tangletrap. **Figure 4** shows that male psyllids accumulated in significantly higher numbers on the female-baited traps than on the male-baited traps or control traps. Females were distributed uniformly among the three types of traps.

Electroantennogram methods. Dr. Christelle Guédot has begun efforts to develop EAG-EAD methods using antennae from male pear psylla. She visited the fruit fly laboratory in Hawaii (funded by ARS) to obtain hands-on familiarity with the technology. She will be visiting Dr. Jocelyn Millar at UC Riverside in spring to obtain additional experience.

Volatiles collection. Volatiles are being collected from summerform females.

NRI and BARD grant proposals. Our olfactometer results are strong enough that we anticipate eventually needing the expertise of a pheromone chemist to assist in isolating, identifying, and synthesizing attractants. Funding was obtained from NRI ($233,000 over 3 years) to fund a pheromone chemist (Dr. Jocelyn Millar, UC Riverside), whose responsibilities include identification and synthesis of the attractants. Approximately half of the NRI funds will remain in the Wapato lab to fund technician help in conducting olfactometer assays. Funds from the BARD project (pending) will be used to support a pheromone chemist in Israel (Anat Zada) and partially support a technician at the Wapato lab. The Israeli chemist will work with the local pear psyllid (*Cacopsylla bidens*), with the assumption that advances made with *C. bidens* will assist us with identifying the attractant(s) from our North American pear psyllid. Jocelyn Millar will work with the North American pear psyllid, and will collaborate with the Israeli chemist in isolating, identifying, and synthesizing the attractants. Allocations of funds from the various sources are summarized in **Table 1**.

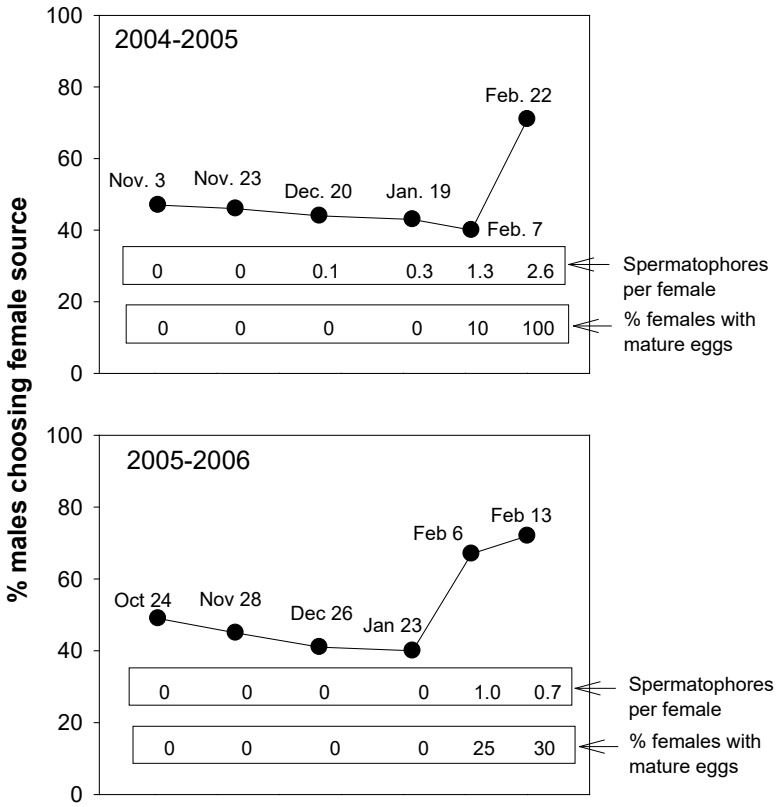

Figure 1. Percentage of winterform males choosing females + pear shoots vs pear shoots alone, as a function of collection date. A subsample of females was dissected on each date to determine mating status and to assess whether the females contained mature eggs.

Figure 2. Percentage of summerform males choosing virgin females + pear seedling, if paired against seedling alone (=controls); mated females + pear seedling if paired against seedling alone; and virgin females paired against mated females.

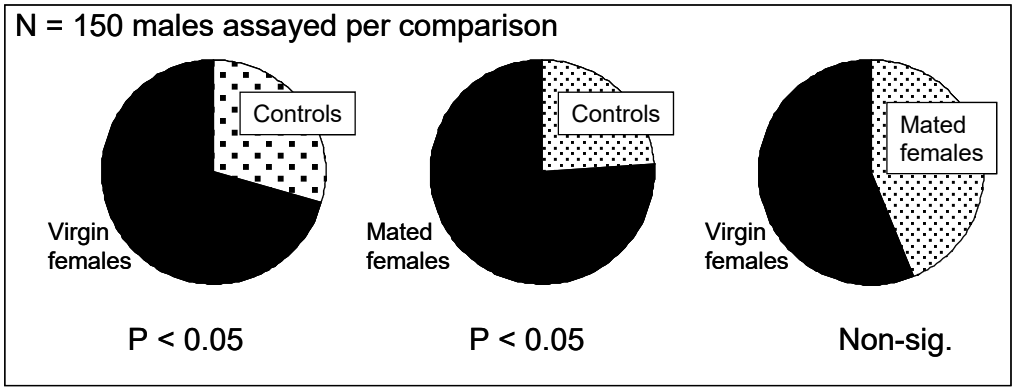

Figure 3. Percentage of summerform males choosing jar of 40 dead summerform females vs empty jar.

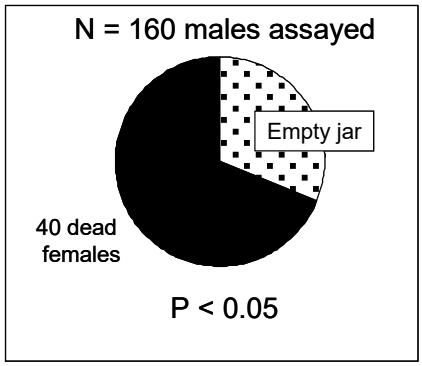

Figure 4. Numbers of pear psylla trapped in tangle-trap covered bags containing 15 female psylla, 15 male psylla, or no psylla. N=20 per trap type.

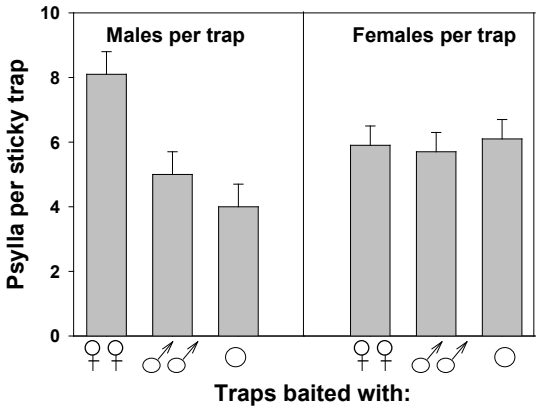

Table 1. Summary of funded, pending, and planned projects as related to current WSTFRC project, and allocation of those funds. BARD: $283,000 for 3 years; NRI: $233,473 for 3 years.

		ARS-WAPATO					Israel and U.C. Riverside
Funding agency	**Status**	**FY 2005**	**FY 2006**	**FY 2007**	**FY 2008**	**FY 2009**	**Pheromone synthesis [1]**
WTFRC	**Funded (this project)**	Technician (0.5)	Post-doc[2] (0.25) Technician (0.5)	Post-doc (0.25) Technician (0.25)			NO
ARS (in-house)	**Funded**		Post-doc (0.75)	Post-doc (0.25)			NO
BARD	Pending			Post-doc (0.5)	Post-doc (0.5)	Post-doc (0.5)	A. Zada (Israel)
NRI	**Funded**			Technician (1.0)	Technician (1.0)	Technician (0.5)	J. Millar (Riverside)
ARS and/or WTFRC	Planned (if necessary)				Post-doc (0.5)	Post-doc (0.5)	
ARS	**Funded**		Master's	Master's			

[1] Summarizes whether funding for identification and synthesis of pheromone is requested in grant.

[2] The post-doc (C. Guédot) will develop and apply the EAD techniques for pear psylla, collect volatiles, and initiate isolation of attractants.

[3] The Master's candidate (B. Brown) is responsible for developing trapping methods for testing attractants.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: PR-06-606

Project Title: Field Evaluation of New Pear Rootstocks

PI:	Clark Seavert	**Co-PI(2):**	Tom Auvil
Organization:	OSU-MCAREC	**Organization:**	WTFRC
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City:	Hood River	**City:**	Wenatchee
State/Province/Zip	OR / 97031	**State/Zip:**	WA 98801

Cooperators: Tim Smith and Dr. William Proebsting

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

Item	**Year 1:** 06-07	**Year 2: 07-08**	**Year 3:** 08-09
Salaries[1]	15,239	17,508	18,342
Benefits (61%)	9,296	10,680	11,188
Wages	1,200	2,000	3,000
Benefits (8.2%)	98	164	246
Equipment			
Supplies[2]	4,600	2,000	25,000[3]
Travel	300	1,000[4]	300
Miscellaneous	200	200	200
Total	30,933	33,552	58,276

Footnotes:
[1]0.50 FTE of a technician.
[2]Nursery tree orders and liners commercially propagated.
[3]Tissue culture services previously provided by OSU, funded by Pear Committee.
[4]One quarter of the cost to travel to International Pear Symposium in May 2007.

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

- Three separate Horner trials will be planted in 2004, 2005, and 2006. These three trials represent 428 different Horner clones.
- Old Home by Farmingdale 87 will be used as a control rootstock.
- Trees will be minimally pruned and trained, and the evaluation period will be five years.

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

- These trials will be conducted on a small scale located at the OSU-MCAREC, and grower sites in Hood River, Yakima and Wenatchee.
  - Finished trees will be available for grower sites in 2009
  - Planting system and scion will be grower's choice
  - Liners will be available for two plantings of plant-in-place, late spring 2007.
- Selections of P2535, Bet # 2291, 517-9, 708-13, 96FI11, 96FI12, 96FI14, 96FI15, Horner 4, OH 11, OHxF 87, Pyronia, and Q29859 will be planted.
- Ten tree replicates per rootstock
- 5 replications per rootstock
- Trees planted in a 12 ft x 4 ft vertical fruiting wall. (907 trees/acre)
- A cultivar that is a good indicator of the characteristics of interest for each growing region, (e.g. Anjou for Hood River, Bartlett for Yakima, Bosc for Wenatchee) will be used.
- Old Home by Farmingdale 87 will be used as a control rootstock
- Trees will be managed to encourage early fruiting and trained to facilitate mechanical assist harvesting.

Objective 3. Identification of new rootstocks for future evaluation

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

Advisory Committee

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

Progress to Date

Initial Horner pre screening

- Using the cumulative data from the Horner 2004 block, 22 of the 249 selections were chosen for review. Upon examination in the data and plots, the tree variance was too great to move any rootstock forward. The consensus was to wait one more season to see if there is a high performing rootstock in this plot.
- The second leaf evaluation was completed in the Horner 2005 planting.
- The Horner 2006 planting was established in the spring of 2006 and the first leaf evaluation completed.

COS High Density planting at MCAREC

- Trees were planted and a trellis system consisting of 8 wires 18 inches apart was established.
- The first leaf evaluation has been completed.
- Two Khazakstan rootstocks, Q 29857 and Q29858 have been propagated by Bill Proebsting and will be planted in place in a high density system at MCAREC in the spring 0f 2007. Depending on their size, they will either be bench grafted or summer budded using Anjou and Bartlett scions.

COS On-Farm Trials

- OHxF 87, Horner 4 and Horner 10 rootstocks have been propagated by Bill Proebsting and will be sent to Van Well's Nursery to be budded and grown out for 2008 planting.

Advisory Committee

- In April 2006 a conference call was made including advisory members from Oregon and Washington to begin the planning for on-farm phases of this project. Topics included the quantity of rootstocks needed, how the initial selections of rootstocks will be made, what tops to graft on, and scheduling a meeting for the fall at Hood River to review the Horner 2004 data for potential winners.
- In November an advisory meeting was held at Hood River to review the data collected for selection of Horner rootstocks for on farm trials. DNA sampling of the Horner rootstocks placed into propagation status was discussed at length. Since a clear target was unable to be identified the topic was left on the table. DNA test of the four Horner 10 plants from MCAREC to verify all four are the same. The mother plant of Horner 10 was lost and a root from one of the four trial trees was used to propagate the trees to date. The last major issue covered during the block tours ranged from pruning and training protocols to discussion of which high density trellis system is most appropriate for on farm trials.

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

Phase	2006	2007	2008
2004 Horner Planting (249 clones)	Complete third leaf evaluation	Complete fourth leaf evaluation and pre-selection of three to four clones for COS 2015 on-farm evaluations	Complete fifth leaf evaluation and continue to select three to four clones for COS 2015 on-farm evaluations
2005 Horner Planting (123 clones)	Complete second leaf evaluation	Complete third leaf evaluation	Complete fourth leaf evaluation and pre-selection of clones for COS 2015 evaluation
2006 Horner Planting (56 clones)	Plant and first leaf evaluation	Complete second leaf evaluation	Complete third leaf evaluation
Other Rootstocks evaluated in a high density orchard system at MCAREC (COS)			
P2535,(13 trees) Bet # 2291(5 trees)	Plant and first leaf evaluation	Complete second leaf evaluation	Complete third leaf evaluation
H-4 (164 trees) OHxF 87 (69 trees)	Plant and first leaf evaluation	Complete second leaf evaluation	Complete third leaf evaluation
517-9 (35 trees) 708-13 (40 trees) 96FI11 (35 trees) 96FI12 (42 trees) 96FI14 (42trees) 96FI15 (25 trees) Horner4 (45 trees) OH 11 (40trees) OHxF 87 (31 trees) Pyronia (11trees) Q 29859 (7trees) Q29857 (50 trees) Q29858 (50 trees)	Planted in place at MCAREC, bench grafted and/or summer budded.	Complete second leaf evaluation Continue training and pruning in a high density fruiting wall system.	Complete third leaf evaluation
Q29857 and Q29858 (50 trees each)	Plants propagated by Bill Proebsting and grown out.	Plant and first leaf evaluation	Complete second leaf evaluation Continue training and pruning in a high density fruiting wall system.

Table 1. The initial twenty two clones selected out of 249 possible in this table are from the cumulative data for the Horner 2004 planting. Tree size and branch angles and branching habits were rated in the winter of 2005. These 22 selections were made by evaluating bloom and fruit set, harvest data for 2006, tree size and branching habits.

			Trunk Circumference											
			(cm)		Total	% incr.	2006 bloom fruit set					Branch		Tree
ROW	TREE	H-ID#	7/04	10/06	Incr.(cm)	04-06	Flwrs	Fruit	Harvest 06	Weight (gms)	#fruit/100	Habit	Angles	Size
5	20	1	1.97	5.31	3.34	169.64	12	1	1	202.3	8.3	1 tier	mod	med
6	19	12	1.88	3.39	1.51	80.21	6	1	1	200.1	16.7	1 tier	mod	sm
14	24	14	1.21	3.12	1.91	157.52	13	0	0		0.0	1 tier	mod	sm
4	3	24	1.80	4.69	2.89	160.39	10	0	1	303.0	0.0	multi/lats	mod	sm
5	7	61	2.02	4.99	2.97	147.03	7	1	1	201.8	14.3	1 tier/lats	mod	sm
20	11	64	1.48	4.97	3.49	235.74	6	2	2	199.4, 179.5	33.3	multi/lats	mod	sm
17	9	78	1.85	4.93	3.08	166.38	11	2	0		18.2	1 tier/lats	mod	sm
4	26	103	1.64	4.40	2.76	168.35	16	0	0		0.0	1 tier/lats	mod	sm
2	5	113	1.99	4.86	2.87	144.07	6	2	0		33.3	tier/lats	mod	sm
16	4	134	1.96	5.29	3.33	170.10	8	0	0		0.0	multi/lats	mod	sm
8	7	161	1.84	4.14	2.30	124.78	6	3	3	161.3, 195.1, 186.8	50.0	1 tier	mod	sm
16	7	178	2.16	4.58	2.42	111.85	10	1	0		10.0	1 tier/lats	mod	sm
3	23	180	1.91	4.21	2.30	120.42	14	2	2	209.0, 168.0	14.3	multi/lats	mod	sm
8	5	194	1.84	3.56	1.72	93.70	14	0	0		0.0	1 tier	mod	sm
16	9	239	2.10	4.88	2.78	132.33	6	1	0		16.7	1 tier/lats	mod	sm
18	30	241	2.23	4.41	2.18	97.62	11	0	0		0.0	1 tier	steep	sm
16	6	265	1.90	4.72	2.82	148.47	22	3	3	202.7, 228.4, 151.3	13.6	multi/lats	mod	sm
12	21	277	1.73	4.35	2.62	151.21	19	2	2	191.4,199.8	10.5	multi	mod	sm
3	5	277	2.01	6.06	4.05	201.29	18	3	3	241.5, 167.3 , (87.3,CodlingMoth)	16.7	multi/lats	mod	med
14	19	279	1.56	4.10	2.54	162.69	15	5	5	221.,241.8,174.9,234.9,212.5	33.3	1 tier	mod	sm
16	14	296	2.18	5.09	2.91	133.39	6	0	0		0.0	multi/lats	mod	sm
1	10	319	2.05	4.55	2.50	121.76	13	2	2	226.8, 215.4	15.4	multi/lats	mod	sm
19	21	319	1.83	2.83	1.00	54.81	6	4	4	170.6,152.8,136.2,160.3	66.7	1 tier	mod	sm
4	31	321	1.42	4.26	2.84	199.72	9	0	0		0.0	multi/lats	mod	med

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

Phase	2006	2007	2008
H-4, H-10	Send cuttings to Bill Proebsting to propagate liners.	Proebsting sends rootstock to nursery to graft and grow out.	Plants grafted and grown at nursery. Nursery sends plants out for distribution to the on-farm trials.
Rootstock selections for on farm trials. (stocks that beat OHxF 87)	Rootstock advisory committee reviews initial harvest and bloom data from Horner 2004.	Selections are made for propagation. Order from North American Plants (NAP).	NAP sends rootstock to nurseries for budding or to cooperator's farms to plant in place for budding or grafting.

Methods

Cultural practices aimed at establishing trees in two to three seasons will be used. The measurements for determining tree size, yield and key yield components, and fruit size are:

Tree size

Trunk cross sectional area (TCSA) provides an index of tree size. Trunk diameter will be measured at 25 cm above the bud union at time of planting and at the end of each growing season. TCSA will be calculated from trunk diameter.

Yield and yield components

Yield will be determined by weighing all fruit per replicate at harvest.

Fruit set (fruitlets/blossom cluster) will be determined by counting flower buds and fruitlets (blossom clusters counted during bloom; fruitlets counted after June drop).

Average fruit weight will be determined by dividing yield by fruit number counted at harvest.

Fruit size distribution will determined with the MCAREC research packing line which provides a frequency distribution based on fruit weight class corresponding to U.S. box sizes or by weighing individual fruit in a random sample of fruit from each replicate.

Parameters such as yield efficiency (yield/unit TCSA) and flower density (flower buds/unit TCSA) will be calculated using the measurements included above.

Discussion

Horner 2004

Yield began in the third year, and initial selections by the advisory committee were based on bloom and fruit set. Branch angles vary, and range from steep to moderate, horizontal, and pendant. The initial list of twenty two clones was sorted using flower clusters and fruit set data. Branching habits were rated in four categories; 1 tier, 1 tier with laterals, multi tier, and multi tier with laterals. Tree sizes range from small to medium to large.

COS 2015 On-Farm Trials

DNA testing of rootstock selections will be done to establish their authenticity before the clones are propagated for on-farm trials. Two clones, Horner 4 and Horner 10, and the standard OHxF 87 have been selected and are currently being grown out as liners by Bill Proebsting. It should be noted that the budget for the third year has been modified due to the retirement of Bill Proebsting and Luigi Meneghelli. Other sources for propagation will need to be found after 2007.
Other rootstocks will be selected when the Rootstock Advisory committee meets in the spring and fall of 2007.

MCAREC High Density COS Block

To achieve maximum performance, continuous growth of 5 inches per week on the central leader is necessary through the growing season. Following planting, the record high temperatures during May kept growth in check, but we were able to get them regulated and growing at the desired rate in July. A daily schedule of irrigation and a weekly application of fertilizer was necessary to keep the block growing at that rate. Weekly measurements were made on the trees starting in July to record the progress and ensure the rate of growth was maintained. Soil samples were taken by our soil scientist and we are awaiting the results.

Identification of new rootstocks for future evaluation

The three Khazakstan rootstocks (Q29857, Q29858, and Q29859) are the latest clones to be propagated and will be planted at MCAREC. Contacts are being made to obtain information on new rootstocks from the INRA program in Angers, France.

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

Project Title: PNW Pear Rootstock Trial

PI: Timothy J. Smith
Organization: Washington State University
Telephone/email: 509-667-6540
Address: 400 Washington St.
City: Wenatchee
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Cooperators: Ed and Darrin Kenoyer (Cashmere Trial)
Geoff and Tyler Thornton (Tonasket Trial)
Ron Wilcox (Yakima Trial)
Esteban Gutierrez, WSU Extension (Tonasket & Cashmere Trials)
Clark Seavert & Janet Turner, OSU Extension (Hood River Trial)
Jennifer Lloyd, WSU Extension (Yakima Trial)

Budget Summary of Total Project:

Projects by Site	Year 1: 2006	**Year 2: 2007**	Year 3: 2008
Yakima, Cashmere	7,618	**7,291**	7,476
and Tonasket			
Hood River	5,788	**6,015**	6,246
Total:	13,406	**13,306**	13,722

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

Item	**Year 1: 2006**	**Year 2: 2007**	**Year 3: 2008**
Salaries	2,667	3,468	3,606
Benefits	907	1,179	1,226
Wages	0	400	400
Benefits	0	44	44
Equipment	0	0	0
Supplies	2000	400	400
Travel	1000	1800	1800
Miscellaneous	0	0	0
Total	6,574	7,291	7,476

Footnotes: Yakima, Cashmere and Tonasket Plot Budgets now unified.
2007-08 0.0996 (five weeks) FTE Extension Coordinator (Tonasket, Cashmere, Yakima)
2007-08 Travel increased to cover five round trips, Wenatchee/Buena
2007-08 Yakima Time slip help, travel and supplies added to this budget, paid out of Yakima in 2006

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

Item	Year 1 2006	Year 2 2007	Year 3 2008
Salaries[1a]	2,688	2,768	2,852
Benefits	1,640	1,688	1,740
Wages[2]	514	605	692
Benefits	46	54	62
Equipment	0	0	0
Supplies[3]	700	700	700
Travel[4]	200	200	200
Miscellaneous	0	0	0
Total	5,788	6,015	6,246

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

In 2002, after several years of preliminary effort, a pear rootstock trial was established in four locations in the Pacific Northwest. Grower cooperators provided sites in Tonasket (Bosc) and Cashmere (D'Anjou), one trial was established on the TFRC property in the mid-Yakima Valley (Bartlett), and one was planted in Hood River at the OSU-MCAREC (D'Anjou). Seven rootstocks were included the first season, and an additional four were planted on these sites in 2005. The trees/rootstocks have been evaluated on the following: 1. survival, 2. suckering, 3. vegetative growth potential, 4. yield, and 5. fruit size. As this was not considered a training systems trial, there was no effort to study the scion/rootstock behavior in an intensive, on-wire, formal training system. That effort would require many more trees than are available on these specific rootstocks. The 2002 trees were planted 10 feet apart in the row and were trained in a free-standing central leader. To date, the "semi-dwarf" plot trees in this system are generally healthy, but have much less vegetative vigor than the standards of the industry. Most of the trees appear as if they would have been quite appropriate if planted at 6 – 7 feet in row and 14 – 15 foot row spacing, with no wire support. Scaffold limbs have been spread early in the training years, except at the Yakima Bartlett plot, where trees were managed as if the fruit was to be processed. In the 2005 D'Anjou trial at Cashmere and the 2005 Bosc trial in Tonasket, the rootstock trial trees were planted at 6 foot row spacings, and are trained on a 4 or 5 wire upright trellis. The 2005 D'Anjou rootstock trial in Hood River was planted at the wider spacing standard of this trial, and may serve as a contrast of rootstock behavior on intensive vs. semi-intensive systems. Pruning and training at the sites other than Yakima has been directed or carried out by local experts, with the intention of bringing the trees into early production, while building a proper framework for the free-standing system.

Objectives:

To continue evaluation of 2002 and 2005 planted pear rootstocks, with emphasis on tree survival, root suckering, vegetative growth potential of the scion, fruit yield, and fruit size.

Significant Findings

Some of the 2002 planted rootstocks are inducing fifth season yields of high quality fruit in excess of those expected in the fifth season of production by most commercial pear producers. The most promising scion / rootstock combinations to date are: D'Anjou or Bosc / OHxF 87, and Bartlett / Pyro II (2-33). These have produced relatively high early yields and a high percentage of large fruit.

Some of the 2002 and 2005 planted rootstocks are exhibiting important negative attributes:

***Tree survival*:** There has been significant tree loss relating to some of the rootstocks at all sites other than Hood River (see table below). The rootstock with the most losses in the first five seasons is 708-36, which appears to have a significant susceptibility to pear decline. Others that have unacceptable problems with decline and/or winter damage include Fox-11 and Fox 16. Of those planted in 2005, it appears that BU-3 is developing health problems.

Suckering: There are numerous, vigorous suckers under every Pyrodwarf rooted tree. The surviving 708-36 has some light root suckering. Trees with Fox 11 rootstock have a few crown suckers (shoots on the rootstock shank above the soil line) on about 30% of the trees. These crown suckers were quite numerous on about 20% of the Fox 11 rootstocks in the second season of growth.

Fruit Size: In the fourth and fifth year, D'Anjou, Bartlett and Bosc trees on Pyrodwarf at the Cashmere and Tonasket sites have produced very significantly smaller fruit relative to the production

on the other rootstocks. The Pyrodwarf-rooted trees were growing well on both sites, but the yields were well below the plot average, and the fruit was smaller than commercially desirable.

Results and Discussion

2002 Planted Trial:
Detailed data is summarized elsewhere, and is available on request from the author. Highlights are below:

Percentage 2006 survival of trees planted in 2002.

	OHxF87	OHxF40	708-36	Fox 11	Fox 16	Winter Nellis	Pyro II	Pyrodwarf
Tonasket Bosc	100	100	80	70	70	-	90	100
Tonasket Bartlett	-	-	-	-	-	-	100	100
Cashmere D'Anjou	100	100	90	100	100	-	100	100
Cashmere Bartlett	100	-	-	-	-	-	100	100
Yakima Bartlett	90	90	30	90	30	-	90	90
Hood River D'Anjou	100	100	100	90	-	100	90	100

Summary of Tonasket Golden Russet Bosc Data:

Bosc-Tonasket. As of Fall 2006 (5th leaf)	Pounds Fruit/ Acre in 5th Year 6x15 ft.	44 lb. Box/ acre, 95% pack, 2006	Total Fruit Weight 04-05-2006	Total Boxes/ acre 04-05 - 06	Box Size (fruit per 44 lb.) 2006	Trunk X-sec area sq. CM	Pounds of fruit per tree in 2006	Pounds Fruit per Sq. CM of Trunk 2006
OHxF 87	39,419	851	55,820	1205	70	63.1	81.4	1.29
OHxF 40	28,895	624	39,740	858	74	54.7	59.7	1.09
708 – 36	18,876	408	31,010	670	82	29.2	39.0	1.34
Pyro II	25,491	550	32,380	699	76	47.9	52.7	1.10
Pyrodwarf	11,072	239	14,730	318	86	47.1	22.9	0.49
Fox 11	14,658	316	19,660	424	74	40.3	30.3	0.75
Fox 16	13,229	286	13,960	301	69	28.9	27.3	0.94

Tonasket Bosc 2006, Percent of fruit in each box size:

	OHxF87	OHxF40	708-36	Fox 11	Fox 16	Pyro II	Pyrodwarf
Size 120	0	0.75	1.5	0.4	0.5	4.2	5.7
110	2.1	1.0	2.3	0.4	0.5	0.8	5.6
100	7.4	2.5	5.5	3.2	0.5	4.2	10.9
90	13.5	6.0	39.5	7.2	1.6	10.0	17.7
80	31.0	13.8	37.1	14.7	7.6	8.3	14.7
70	33.0	34.8	12.1	32.3	21.9	34.2	30.6
60	11.0	30.8	2.0	30.2	39.7	25.8	12.4
50	2.0	10.5	0	11.6	27.7	12.5	2.2

Tonasket Bosc 2006, Extrapolated boxes/acre by box size (95% pack out):

	OHxF87	OHxF40	708-36	Fox 11	Fox 16	Pyro II	Pyrodwarf
Size 120	0	4.7	6.1	1.3	1.4	23.1	13.6
110	17.9	6.2	9.4	1.3	1.4	4.4	13.4
100	63.0	15.6	22.4	10.1	1.4	23.1	26.1
90	114.9	37.4	161.2	22.8	4.6	55.0	42.3
80	263.8	85.8	151.4	46.5	21.7	45.7	35.1
70	280.8	216.8	49.4	102.1	62.6	188.1	73.1
60	93.6	191.9	8.2	95.4	113.5	141.9	29.6
50	17.0	65.5	0	36.7	79.2	68.8	5.3
Boxes/a	851	624	408	316	286	550	239

2005 Planted Trials:

In the Cashmere 2005 rootstock trial, many of the D'Anjou trees on the BU-3 rootstock are weak, and appear ill; exhibiting symptoms of pear decline. Some of the trees on BU-2 are also growing poorly. BM2000, OHxF 87 and Horner 4 rooted trees appear relatively vigorous and healthy. These three rootstocks, two "new" and one standard, show signs of being both healthy and productive, and may provide very interesting data as they come into production on all the sites. The 2005 trial at Cashmere includes D'Anjou on Horner 4, OHxF 87, BM-2000, BU-2, BU-3, and Bartlett on Horner 4. At the Tonasket 2005 rootstock trial, the Golden Russet Bosc trees appear quite healthy on all of the rootstocks, though there are visible differences in relative vigor. The 2005 trial at Tonasket includes Golden Russet Bosc on Horner 4, OHxF 87, BM-2000, BU-3, Pyrodwarf, Pyro II (2-33), and Bartlett on Horner 4. The Horner 4 rootstock appears to be most vigorous, however, it appears to induce the production of relatively horizontal current season shoots, promises to be precocious, and may require less tying or spreading to induce fruiting. The trees on BU-3, while apparently healthy, are producing much less vegetative growth than those on OHxF 87, Pyrodwarf, BM 2000, or a Pyro II (2 – 33).

The 2005 portion of the rootstock trial appears to be growing very well in both the Yakima, and Hood River trial sites. The 2005 Yakima trial has Bartlett on Horner 4, BM-2000, BU-3, Fox 11, Pyrodwarf, 708-36, and OHxF 87.

Plan for the next two seasons:

2007: We will maintain the plots, and take 6th season measurements on remaining 2002 planted rootstocks, 3rd season measurements on 2005 planted rootstocks (fruiting will have started). We will decide again, as a group which of the 2002 rootstocks to drop from evaluation. The Yakima Bartlett trial will receive some TLC in the attempt to restore the health and productivity of the remaining 2002 planted trees. The 2005 planted Yakima Bartletts will be trained to test productivity, rather than the processor style chosen for the 2002 planting.

2008: We will take final fruit measurements on remaining 2002 planted rootstocks, and will take 4th season measurements on 2005 planted rootstocks. This may be the final opportunity to see the more mature fruit production on the 7th leaf trees and the first significant fruit produced on the 4th leaf trees. We would need to plan for the 5th, 6th and 7th seasons of 2005 planting rootstock evaluation, if deemed necessary. As the 2002 plantings will be fully evaluated, the actual time necessary to maintain and evaluate the trial blocks will be significantly reduced as the obviously less interesting roots are eliminated from consideration after the 5th leaf evaluations.

CONTINUING PROJECT REPORT

Project Title: Collaborative WTFRC research projects

PI: Jim McFerson
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Cooperators: Tom Auvil, Felipe Castillo, Tory Schmidt, WTFRC, Wenatchee, WA
Ines Hanrahan, WTFRC, Yakima, WA

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

Item	**Year 1:** 2007		
Salaries*	29,429		
Benefits	9,417		
Wages	10,346		
Benefits	2,224		
Equipment	4,172		
Supplies			
Travel	5,000		
Miscellaneous			
Total	60588		

- **Based on 5 pear projects/year.**
- **Note that an additional $24,000 is paid to Stemilt for room rental on pear-related projects.**

OBJECTIVES:

1. Conduct field trials on crop load management, use of reflective covers, sunburn suppression, russet management, rootstock evaluation, and lenticel-based skin disorders in grower cooperator orchards.

2. Assist WTFRC funded research programs with trial setup, maintenance, and sampling.

3. Manage soil sample collection regarding Penbotec and Scholar registration.

RESULTS AND DISCUSSION

WTFRC field trials

In 2006, the Washington Tree Fruit Research Commission (WTFRC) conducted 65 trials on apple, pear, cherry, peach, and nectarine within its internal research program, covering topics such as crop load management, reflective groundcovers, sunburn suppression, fruit finish, rootstock evaluation, and lenticel-based skin disorders (Table 1). All trials were conducted in grower-cooperator orchards. Funding was used to hire seasonal labor (10 people/year), to repair and maintain equipment, to purchase supplies, and to cover crop loss. Most products evaluated were donated by industry suppliers (Table 2). A number of trails were conducted with financial support from private companies (Table 3). Detailed project reports are included elsewhere in this document.

We have developed strong ties with various organizations specializing in international student exchange (i.e. Experience International, Ohio State University International Agriculture Exchange Program). In recent years, we have hosted interns from Germany, Mexico, and Austria. We encourage students to actively participate in industry events and to educate WA growers about practices in their respective home countries.

Table 1: WTFRC internal program field trials in 2006.

	Apple	Pear	Cherry	Peach	Nectarine
Crop load management	22	3	3	1	1
Reflective fabric*	7	2	5	1	
Sunburn suppression*	1				
Fruit finish*	6				
Lenticel breakdown	4				
Rootstock	9				
				Total:	**65**

* Products donated by industry suppliers

Table 2: Companies and Institutions that contributed materials and services to the WTFRC internal program in 2006.

Contribution	Company
Chemicals	Amvac
	BASF
	Cascade Distributing Co.
	D & M Chemical
	Fine Agrochemicals
	GS Long
	JMS Flower Farms
	Nufarm
	Orcal Inc.
	Pace Intl.
	RainGard
	Rohm and Haas
	Valent
Other supplies	Extenday
	Willow Drive Nursery
	Wilbur-Ellis, Wenatchee
Labor	Crane and Crane
	Fleming's Valley View Orchards
	Stormy Mountain Ranch
	Valley Fruit
	Willow Drive Nursery
Lab space/equipment	USDA-ARS TFRL, Wenatchee
	WSU-TFREC, Wenatchee
Packing line time	Valley Fruit
	McDougall & Sons
Fruit donation	Auvil Fruit Company
	Crane and Crane
	Ron Wilcox

Collaborative Projects

The WTFRC internal program provided technical support with trial set-up, maintenance, and sampling for several WFTRC-funded research programs (Table 3). A growing number of scientists have taken advantage of the opportunity to utilize the internal program's extensive network of industry cooperators when conducting field trials. By using in-state locations in commercial orchards, increasingly relevant data has been generated for Washington growers by research programs around the world.

Betsy Beers: The focus of this project was to evaluate collateral effects of chemical thinners (namely lime sulfur and carbaryl) on populations of phytophagous and predatory mites at WTFRC trial sites. Leaf samples were collected every other week from 5 treatments each at three trials and

delivered to the Beers lab for evaluation. (sample timing: late April to late July)

Curt Rom: WTFRC performed field evaluations of novel organic chemical bloom and postbloom thinners developed by Rom and his graduate student, Jason McAfee. Materials included essential plant oils, organic acids, and organic bases with potential to kill or damage pollen. Trial location, setup, spray applications, harvest, and quality analyses were performed by the WTFRC.

Don Elfving: The internal program continued its ongoing support of data collection for Elfving's PGR work, including whole tree bloom counts in April for three sites and harvest yields in the fall.

Peter Hirst: *a*) Understanding apple flower bud development: *WTFRC* selected 10 trees from both a Gala and a Fuji block, attached tags to 100 buds per tree, and divided buds into 3 categories. Two samples of each type were collected every 10 days until August, when we switched to every 20 days until trial completion in mid October. The samples were fixed in FAA solution and shipped to Purdue for analyses.

b) Mechanisms of apple fruit growth: WTFRC selected 10 trees from both a mature Red Delicious and mature Gala block and hand thinned all trees at full bloom to reduce crop load. Samples were collected weekly starting in May, switching to bi-weekly in July, until trial completion at harvest. Samples consisted of 2 fruit from each tree, which were labeled and measured before being shipped to Purdue.

Steve van Nocker: WTFRC established a simple replicated thinning trial in Gala. Chemical thinners were applied with the Proptec sprayer, followed by 3 intensive sampling events of fruitlet parts. Hundreds of fruitlets were dissected and frozen in the field at each sampling, and eventually shipped to MSU for molecular analyses.

Gennaro Fazio: The main focus of the rootstock plantings is to evaluate new Geneva rootstocks in soils with replant problems (see Fazio report). WTFRC conducts trial layout, planting establishment, data collection, and some horticultural management on an ongoing basis.

Karen Lewis/Tom Auvil: WTFRC moves mobile platforms between locations for industry demonstration and testing, occacionally providing training in platform operation.

FruitGard: WTFRC located rain-vulnerable cherry sites, set up trials, and applied formulations designed to reduce rain cracking. Internal staff also conducted field evaluations, and collected harvest fruit samples.

Ciba-Geigy: Trial location and setup were performed by the WTFRC for 2 trials (Ambrosia and Pink Lady). Apples free of defects were selected and subsequently bagged with two component color enhancement bags about two months before harvest. Roughly a month before harvest the outer bag was removed, ten days later the inner liner was removed and the stencil was applied. At harvest, fruit was transported to the WTFRC lab for stencil removal, followed by treatment with SmartFresh, tray packed, and shipped to the company.

Whiting/Elfving: WTFRC staff helped design the trial, located an appropriate site, laid out the trial, collected field data, harvested sample fruit, and delivered it to the Whiting lab for quality analyses.

Whiting: WTFRC worked jointly with Extenday and Whiting to develop reflective groundcover trials in cherry. Internal staff maintained the trial, collected harvest fruit samples, and delivered them to the Whiting lab for quality analyses.

Fallahi: WTFRC conducted all aspects of trial design, setup, application, data collection, and analysis for peach and nectarine chemical thinning trials. Fallahi advised internal staff regarding treatments and provided Tergitol for use in soft fruit and apple.

Table 3: WTFRC internal program: collaborative support for WTFRC-funded projects.

			WTFRC technical support			
Researcher	**Topic**	**Number of trials**	Site selection	Trial set-up	Data collection	Misc.
Betsy Beers WSU - Wenatchee, WA	Chemical thinner effects on arthropod populations	3	x	x	x	
Curt Rom UA, Fayetteville, AR	Novel chemistries and pollenicides for chemical thinning	2	x	x	x	x
Don Elfving WSU - Wenatchee, WA	Use of gibberellic acid to inhibit flowering in apple	3			x	
Peter Hirst Purdue, West Lafayette, IN	Molecular basis for fruit cell division and expansion	4	x	x	x	x
Steve van Nocker MSU, East Lansing, MI	Molecular control of fruitlet abscission	1	x	x	x	x
Gennaro Fazio Cornell, Geneva, NY	Next generation rootstocks for apple	9	x	x	x	x
Lewis/Auvil WSU - Ephrata, WA / WTFRC	Mechanized assistance of orchard labor	variable	x			x
FruitGard LLC* Wenatchee, WA	Cherry cracking prevention	3	x	x	x	
Ciba-Geigy* Basel, Switzerland	Logo imprinting on apple skin	2	x	x		x
Whiting/Elfving WSU - Prosser/Wenatchee, WA	Use of gibberellic acid to inhibit flowering in cherry	1	x	x	x	
Whiting WSU - Prosser, WA	Reflective fabric to improve cherry quality	4	x	x	x	x
Fallahi UI - Parma, ID	Cropload management in softfuit	1	x	x	x	
	Total:	**33**				

* received financial support from company

Soil sample collection

In collaboration with the Northwest Horticultural Council and EPA, the internal program is managing soil sample collection supporting the registration of two new postharvest fungicides (Penbotec and Scholar). We are currently maintaining 24 sampling sites (Table 3). Soil samples are taken prior to application, directly after application, and post-season. WTFRC is responsible for collecting, shipping, and correct documentation of all soil samples. We anticipate to complete soil sampling in spring of 2007.

Table 3: Wastewater sampling: cost-effective data collection to support registration of postharvest fungicides needed by industry.

	Number of sites in WA	North central Washington	Yakima Valley
Sites established in 2005	11	5	6
New sites added in 2006	13	2	11
Total in 2006	**24**	**7**	**17**