2006 Northwest Pear Review
February 16, 2006
Clarion Hotel, Yakima, Washington

Time	Page	PI	Project Title	Funding period
8:00		Schmitten/McFerson	Welcome - Federal Marketing Order Update	
			Final Reports	
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9:00	6	Dunley	Biological control in organic and super-soft orchards	03-05
9:15	7	Dunley	Areawide OPM	03-05
9:30	8	Lacey	Spinosad and granulovirus effects of codling moth	04-05
9:45	17	Hilton	Codling moth control using CpGV—with and without mating disruption	05
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10:15	32	Sugar	Calcium chloride sprays for delaying maturity/Strategies for thinning Bartlett pears	03-05
10:30		Powers	NHC Update	
			Break	
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1	55	Xiao	Control of postharvest decay in pear	05-07
1	59	Johnson	Survival of *Erwinia amylovora* on pear fruit	04-06
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Final Report
WTFRC Project: PR-03-339 **Agricultural Research Foundation** #3740

Project Title: Introduction and propagation of pear rootstocks

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

Specifically, over the previous three years, we have: 1) propagated liners of ca. 450 Horner clones for field testing, 2) begun propagation of three rootstocks from Kazakhstan prior to release by APHIS, 3) propagated liners for advanced testing of rootstocks from the United Kingdom, France, Italy and the U.S., and 4) maintained 20 rootstock clones in tissue culture pending determination of the future of these clones.

Significant Findings:

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

1) **Horner series**. About 450 clones are being tested as pear rootstocks:

- Cuttings from 294 clones were propagated at Corvallis in July, 2001. Two or more liners of each were sent to Fowler Nursery in February, 2002 for grafting to be returned to Hood River for testing.
- The remainder of the Horner series, 148 clones, were propagated in July, 2002. Liners of about 130 were sent to Fowler in February, 2003.
- 77 Horner clones were re-propagated in July, 2003. Liners of 64 were sent to Fowler in February, 2004.
- Horner 4 and Horner 51 were initiated in tissue culture. Horner 4 is propagating well, whereas Horner 51 gradually died out.
- Presumed-Horner 10 was initiated in tissue culture during summer, 2005.
- Grafted trees are now being planted at MCAREC for orchard evaluation.

2) **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.

3) **Liners for advanced trial.**
• Liners of 20 clones were propagated for a same age trial at Hood River. The number of clones was subsequently culled to 11. These liners will be shipped to Hood River late winter 2006.

4) **Rootstock collection.**
• 17 pear rootstocks are currently in tissue culture at OSU awaiting requests for liners for research or transfer of cultures to nurseries. We have 26 rootstock clones at the Hort Farm.

Methods:

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

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

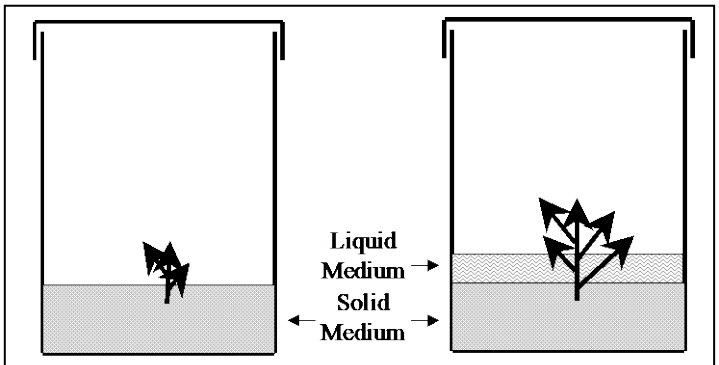

Figure 1. Single-phase vs. double-phase tissue culture.

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

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

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

Results and Discussion:

1) **Horner series.** Small, preliminary studies found some promising rootstocks in this group of about 450 open-pollinated 'Old Home' seedlings. Further testing was warranted. In this situation, tissue culture of 450 clones is inappropriate. Because these are seedlings, however, and have been maintained as small, heavily-pruned trees, rooting potential of softwood cuttings of each clone should be near its maximum. Furthermore, since only 2-5 liners of each clone were required for the rootstock trial, low rooting percentage is not a serious short-term obstacle.

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

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

Two promising rootstocks, Horner 4 and Horner 51 were initiated in tissue culture in July, 2003. Horner 4 is growing very well, Horner 51 gradually died out. Softwood cuttings of Horner 51 also rooted poorly. These experiences suggest that H51 is difficult to propagate. In 2005, we obtained shoots of presumed-Horner 10. The original seedling of H10 was lost. The rootstocks of the four trees on this presumed-H10 are being tested for genetic identity.

2) **Kazakhstan rootstocks.** Several years ago, three clonal pear rootstocks were imported from Russia by Californians Larry Rogers and Jim LaRue. They are purportedly dwarfing. APHIS was willing to make these available to us for preliminary propagation. With the assistance of Gene Milbrath, Oregon Department of Agriculture, to obtain the necessary paperwork, APHIS sent me budwood in February, 2002. We budded it on seedlings in the greenhouse and initiated cultures in late April, 2002. APHIS released these clones in January, 2005.

Table 1. Pear rootstock clones in tissue culture at OSU, December, 2005.

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	

*Included in next field trial

Q29859 multiplies quickly, whereas Q29857 and Q29858 multiply slowly. Rooting of Q29859 is somewhat erratic, but averages about 70%. We will test rooting of the other two clones starting in January 2006 We are currently propagating liners for a trial at Hood River.

3-4) **Micropropagation and testing.** As we have provided rootstock liners for testing, we have maintained a small number of each clone in culture. We presently have 17 clones in culture. If a rootstock merits further testing or commercial propagation, these established cultures will enable us to respond quickly.

Clones 708-2, 12 and 36 from the East Malling, UK, breeding program have been dropped from the program, because of susceptibility to pear decline. 708-13 and 517-9 from that program are still being evaluated.

Eleven rootstocks will be in an even-age field comparison at Hood River. The Q-series and Horner 10 will be included if we can accelerate their growth sufficiently. The Q's are relatively difficult to propagate. Horner 10 was just established in culture summer 2005 and is doing well.

As described in the pre-proposal, we propose a system, whereby promising Horner clones are established in tissue culture at the earliest possible indication that a given rootstock has significant promise. As further evaluation takes place, these cultures can be culled or maintained. When a given clone merits further testing, cultures will be ready for liner production and distribution of cultures to interested commercial nurseries.

Budget:
Project Title: Introduction and propagation of pear rootstocks
PI: Dr. William M. Proebsting
Project Duration: 2003-05
Current Year: 2005
Project Total: $68,048

Year	2003	2004	2005
Total	$23,896	$23,896	$20,256

Details

	2003	2004	2005
Salary, Faculty Research Assistant[1]	$11,373	$11,373	$12,299
OPE	6,028 (50%)	6,028 (50%)	6,764 (55%)
Student Wages[2]	1900	1900	1900
OPE ($3.12/mo.)	95	95	75
Services and Supplies[3]	4,000	4,000	4,000
Travel[4]	500	500	500
Total	23896	23896	25538

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

Other support:
Oregon Hazelnut Commission

FINAL REPORT

Project Title: Field evaluation of new pear rootstocks

Co-PI: Clark Seavert
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No report submitted

Final Report
WTFRC Project #PR-03-342 WSU Project #13C-3643-4431

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

No report submitted

FINAL REPORT
WTFRC Project #PR-03-341 **WSU Project #13C-3643-4385**

Project title: Development of areawide organic insect pest management in pear orchards
PI: John E. Dunley, Associate Entomologist
Organization: WSU Tree Fruit Research and Extension Center

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

Cooperators: Peshastin Creek Growers Association

No report submitted

FINAL PROJECT REPORT **YEAR 2/2**
WTFRC Project # 04-432

Project title: Spinosad and granulovirus effects on codling moth
PI: Lawrence A. Lacey
Co-PI: Steven Arthurs
Cooperators: David Horton and Gene Miliczky

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

Contract Administrator: Chuck Myers, cwmyers@pw.ars.usda.gov, (510) 559-6108

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

SIGNIFICANT FINDINGS:
- Spinosad was effective at suppressing codling moth populations in apple and pear (e.g. < 2% fruit damage in a heavily infested Bartlett pear in an experimental orchard).
- CpGV was less effective than spinosad at preventing fruit damage, but killed the majority of CM larvae that reached the fruit. CpGV appeared to be more effective against the first larval generation of CM.
- The predatory mirid *Deraeocoris* spp. were frequently found in spinosad-treated plots and showed no negative effects of the spinosad treatment.
- Spinosad reduced the abundance of parasitoids, *Anthocoris* spp. and non-target Diptera in small plots, but we did not observe any resultant increase in pest densities.
- No evidence for phytophagous mite resurgence (2 species) resulting from spinosad use.
- Sweep net samples suggested several epigeal taxa were not affected by spinosad treatments.

METHODS
Efficacy of CpGV and spinosad on codling moth
Experimental orchard: In 2004 and 2005 we compared the efficacy of codling moth granulovirus (Cyd-X, Certis, USA) and spinosad (Entrust, Dow Agrosciences) in replicated Bartlett pear blocks at the USDA experimental orchard in Moxee, WA. Treatments were applied in a full-season for program for codling moth using an ATV-mounted 25 gal. airblast sprayer (Hauff Company, Yakima WA). A large tarp was used to minimize contamination between plots and spraying occurred early morning during calm conditions. Applications were made at 7-8 day intervals throughout the season (starting 250 DD post biofix in each generation) in accordance with pheromone trap catches and the WSU phenology model (Beers et al. 1993). The study was a complete randomized block design with 5 (2004) or 4 (2005) replicates for each treatment including an untreated control (12-16 trees per block). Entrust and Cyd-X were applied within recommended label rates (3 fl.oz/A for both) plus sticker (NuFilm17 @ 8 fl.oz/A) at volume application rate of 100 gal./A. Fruit damage was assessed mid season (after the first larval generation) and before harvest from a minimum of 150 fruit/block. Wormy fruit from the virus and untreated control blocks were returned to the laboratory and dissected to assess mortality of larvae inside the fruit.

Commercial orchards: Trials were conducted in commercial orchards where formulations of spinosad (Entrust or Success) and CpGV (Cyd-X or Carpovirusine) were used operationally; mixed pear (Mellow) and Delicious (Knutson). Growers applied spinosad or CpGV in separate blocks (2 replicate blocks per treatment each approximately 1 A). Application rate and frequency were in accordance with label recommendations and localized pest pressure determined on site.

Treatment blocks were sprayed concurrently (within 2 days of each other) at 7-10 day intervals starting at ca. 250 DD. Because spinosad is restricted for resistance management (9 oz/A season for Entrust, 29 oz/A for Success), in the second larval generation it was either alternated with the virus treatment (Mellow) or replaced with an IGR (Intrepid) in the Delicious blocks (Knutson). In a mixed 6 A Golden Delicious/Granny Smith orchard another grower (Ing) replaced part of his OP program (Imidan) with the Carpovirusine formulation of CpGV.

Non-target effects of spinosad and CpGV

In the Moxee trial, beat trays samples were conducted to monitor the abundance of beneficial species including predatory bugs, spiders, lady beetles, lacewings and parasitoids. Pear psylla (Homoptera: Psyllidae), which were the predominant herbivore prey associated with population of beneficial species, were also noted. The central 4 trees in each plot were sampled early in the morning every 1-2 weeks (2004) or 7-10 days (2005) and seasonal trends compared between the different spray treatments. Sweep net samples were also used to census leafhoppers and other non-target taxa on the orchard floor during spraying periods. The central area of each plot was used for collecting samples. Leaf and shoot samples were also monitored for aphids and mites outbreaks within the plots. In addition collections of pear psylla nymphs were made twice to estimate levels of parasitism by *Trechnites insidiosus* or other parasitoids.

Because of anacedotal reports of pest resurgence (aphids and mites) in plots treated with spinosad in 2003 and supporting literature on spinosad's toxicity to certain beneficials found in orchards, we compared late season populations of phytophagous mites in spinosad-treated plots with CpGV and untreated plots at Moxee and a commercial orchard (Mellow).

RESULTS AND DISCUSSION

Efficacy of CpGV and spinosad on codling moth

Experimental orchard: First generation codling moth infestations were not severe in either year (younger fruit were apparently difficult for larvae to penetrate) but significant damage occurred following the second flight, especially in 2005 (Table 1). Entrust worked well at protecting fruit with ≤1.8% injury in both years, compared with up to 37% fruit injury in the untreated blocks. Cyd-X was less effective at protecting fruit in both years, although were fewer deep entries (> ¼″) in virus treated fruit (39 ± 8.7%) compared with untreated fruit (82 ± 4.2%) and some fruit may have still been suitable for processing. The majority of larvae inside virus-treated fruit were dead (also indicated by shallow failed stings). Larval mortality was 71% in 2004 versus 20% on controls and 70% in 2005 versus 23% in controls (at harvest), suggesting the virus is more effective at population suppression in the second generation than prevention of fruit injury. These rates of larval mortality are lower than we have observed with apples treated at equivalent rates of virus (Arthurs et al. 2005).

Commercial orchards: Data from commercial orchards are shown in Tables 2-4.

There was a relatively high initial infestation in the mixed pear orchard (Mellow), with Bartlett the more susceptible variety to codling moth compared with Anjou (Table 2). Both spray programs (i.e. Cyd-X followed by Entrust against the first and second generations respectively and visa versa) were effective at reducing fruit injury and significantly reduced trap catches in the second year. Lowest damage occurred when Entrust was applied against the first larval generation. The grower felt satisfied the programs were effective for CM control and dropped the virus application rate from 3 to 1.5 fl.oz/A in the second year. Larval mortality varied from 63-90% in sprayed fruit in the blocks.

In Delicious, Success was more effective at protecting fruit compared with virus in the 1st generation; although the vast majority (average 92%) of CM were killed in the virus plots indicating the virus was highly effective at population suppression early in the season (Table 3). Virus was less effective compared with an IGR (Intrepid) against second generation larvae (66% mortality), although at harvest fruit damage was similar (3.5 - 4.1%) between the blocks treated with either program. A protracted emergence from a large fruit bin pile was responsible for the increased late season damage in these plots.

Carpovirusine applied at 10-d intervals killed 91-93% CM larvae in the 1st and 2nd generation respectively, but was not as effective as Imidan at protecting fruit (Table 4).

Table 1. Efficacy of spinosad (Entrust) and codling moth virus (Cyd-X) in Bartlett pear over 2 years; % fruit injury in replicated 12-16 tree blocks (Moxee experimental orchard).

Treatment	2004		2005	
	Mid season	Harvest	Mid season	Harvest
Untreated control	0.45	14.8a	3.9a	36.9a
Cyd-X	0.19	11.6a	0.9b	26.0a
Entrust	0.00	1.2b	0.9b	1.8b

Different letters indicate significant differences (P<0.05, Fisher's LSD)

Table 2. Efficacy of spinosad (Entrust) and codling moth virus (Cyd-X) over 2 years; % fruit injury in 1A pear blocks (2 replicates per spray program) in Hood River, OR (Mellow). The Entrust and Cyd-X blocks were switched between the first and second generations to avoid the 9 oz/A cap with Entrust.

Spray program 1st/2nd generation	cv.	2004		2005	
		Mid season	Harvest	Mid season	Harvest
Cyd-X/Entrust	Bartlett	8.3a	na	1.4	1.2
	Anjou	1.9b	1.1	0.4	na
Entrust/Cyd-X	Bartlett	6.2a	na	0.7	0.5
	Anjou	0.8b	2.4	0.0	na

Different letters indicate significant differences (P<0.05, Fisher's LSD)
na = not assessed

Table 3. Efficacy of codling moth virus (Cyd-X or Carpovirusine) versus spinosad (Success) in 1 A blocks of Delicious (2 replicates per spray program). Data shows % CM fruit injury and mortality in sprayed fruit. Success was replaced with Intrepid in the second generation (Knutson, Mattawa).

Spray program 1st/2nd generation	Mid-season (450 fruit/block)		Harvest (1080 fruit/block)	
	% injury	% mortality	% injury	% mortality
CM virus	3.3	92.5	3.5	66.0a
Success/ Intrepid	0.8	80.0	4.1	82.0b

Different letters indicate significant differences (P<0.05, independent T-test)

Table 4. Efficacy of codling moth virus (Carpovirusine) versus Imidan in 6A Golden Delicious and Granny Smith. Data shows % CM fruit injury and mortality in virus sprayed fruit in unreplicated plots (Ing, Hood River).

Spray program (interval)	Mid-season (n = 1056 fruit)		Harvest (n = 3357 fruit)	
	% injury	% mortality	% injury	% mortality
Carpovirusine (10 days)	4.5	91.8	6.3	92.9
Imidan (20 days)	0.25	NA	0.33	NA

Non-target effects of spinosad and CpGV

Data from beating tray samples in the experimental plots are shown in Figure 1. In both years a mid-season peak of pear psylla (1st adult summer generation) was not observed in the spinosad plot, although unaffected by virus treatments. The reasons are unclear, but suggest psylla fecundity or oviposition was reduced by spinosad applications. However, a key psylla predator, the mirid *Deraeocoris brevis*, was apparently unaffected by spinosad treatments, despite the reduced density of its main prey, and undoubtedly contributed to the decline in psylla populations. Although most commonly a pest during bloom, secondary populations of thrips (predominantly *Frankliniella occidentalis*) were suppressed by spinosad treatments. Although less frequent than *D. brevis*, mid-season populations of another predator, *Anthocoris* spp., and to a lesser extent lacewings (*Crysoperla* spp. and *Hemerobius* spp.) were reduced by spinosad treatments. There was no evidence that the large diversity of spiders recovered from the foliage was affected by spinosad treatments. Populations of other beneficials including ladybeetles, *Orius* spp. and hoverflies (Syrphidae) remained fairly low throughout the season. Secondary outbreaks of aphids have been noted by growers following spinosad use, although aphid populations remained below damaging thresholds in our plots. Overall biological control of aphids was effective; a mid-season outbreak of apple aphid (*Aphis pomi*) was noted in one of the Entrust-plots, but quickly bought under control by beneficials. Other secondary pests including western tentiform leaf miner *Phyllonorycter elmaella*, *Campylomma verbasci*, *Geocoris* spp. stink bugs, lygus bugs and leafhoppers (mainly the white apple leafhopper *Typhlocyba pomaria*) were present but remained below damaging thresholds in all plots.

The most significant negative of spinosad on beneficials was noted for parasitoids, of which a wide diversity were noted (Figure 2). Other research indicates spinosad is directly toxic to a wide range parasitoids (Hill and Foster, 2000; Consoli et al., 2001; Mason et al., 2002; Williams et al., 2003). It was not clear if the reduction in parasitoids in our study may have been partly mediated by the reduced prey (psylla), although parasitoids mainly comprised encyrtids (Figure 2), of which 51% were *Trechnites insidiosus* (an important parasitoid of psylla). In conclusion, spinosad treatments negatively affected parasitoids and some predators, but not *D. brevis*, but we did not observe any resultant increase in pest densities. However care should be taken in extrapolating these results to orchards where few or no beneficial species are present to control outbreaks of aphids or other phytophagous pests.

Late season assessments of leaf samples with a leaf brushing machine revealed no difference in the abundance of phytophagous mites between spinosad, virus-treated or untreated plots; species sampled were pear rust mite (PRM), *Epitremerus pyri,* and pearleaf blister mite, *Phytoptus pyri* (Figures 3 and 4).

Sweep net samples also revealed no differences in abundance of several epigeal taxa, notably leafhoppers (comprising 74% *Dikraneura* spp.) (Figure 5). However several non-target dipterans, mainly fungus gnats (family Mycetophilidae) and shore flies (family Ephydridae), were less commonly recovered from spinosad-treated plots. Spider and adults syrphids were also recorded, but may not have been reliably captured. A more complete dataset for 2005 is still being collated.

Figure 1. Psylla, aphids, thrips and non-target taxa monitored with beating trays (Bartlett pear, Moxee 2004). Data show mean for 16 tree plots (4 or 5 replicates). Arrows show timing of spray treatments.

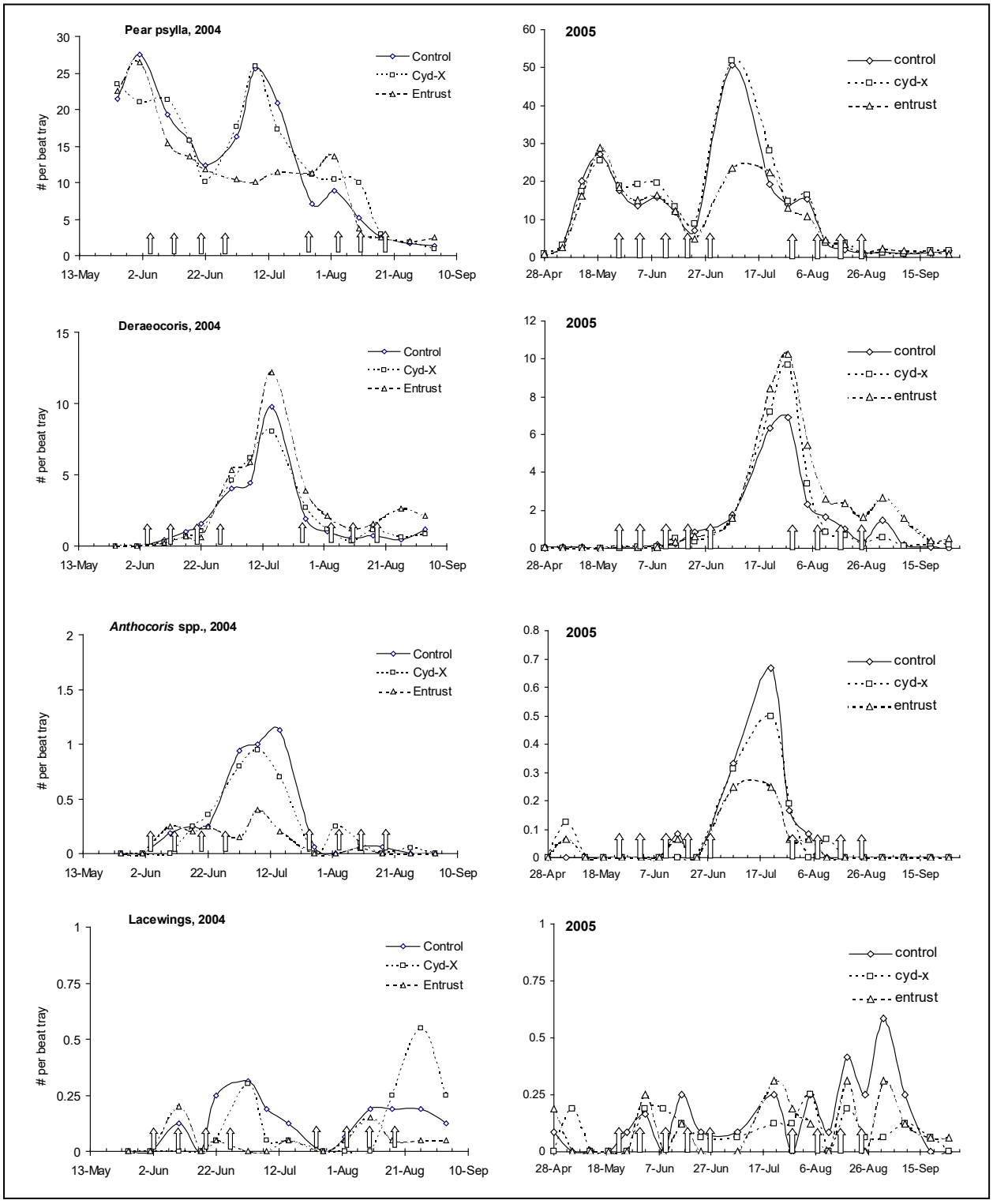
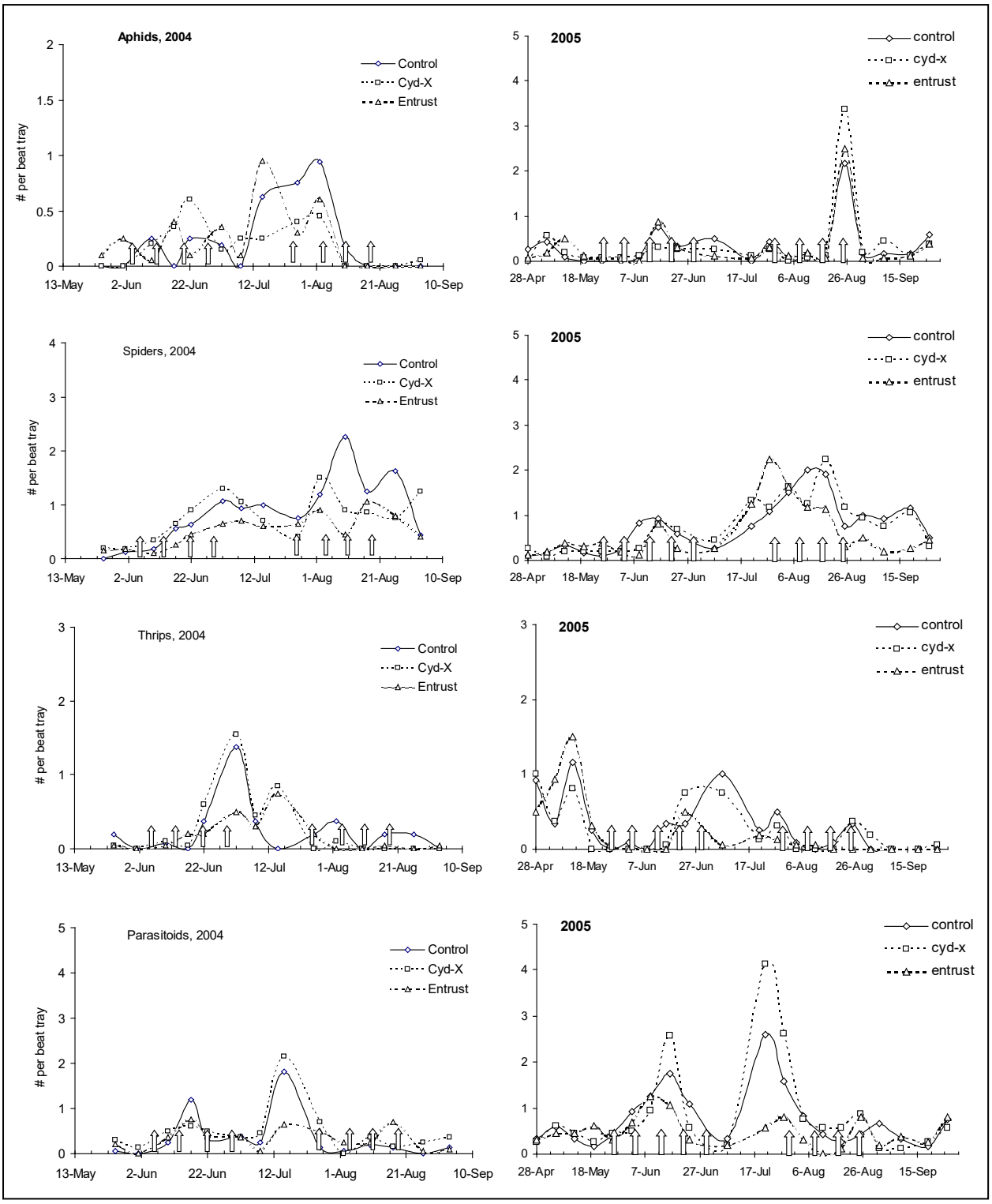


Figure 1. (cont.)



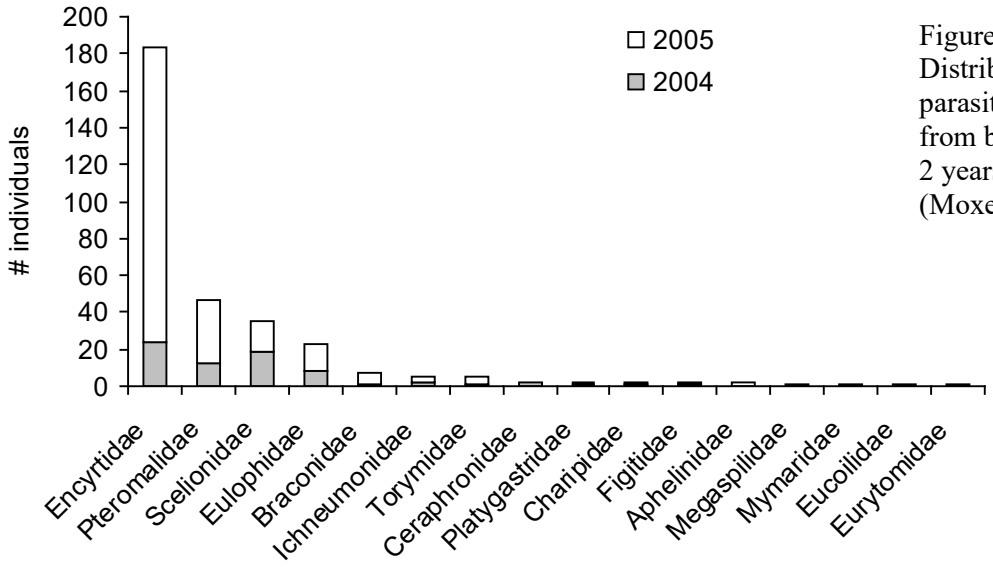


Figure 2. Distribution of parasitoids collected from beat trays over 2 years, Bartlett pear (Moxee)

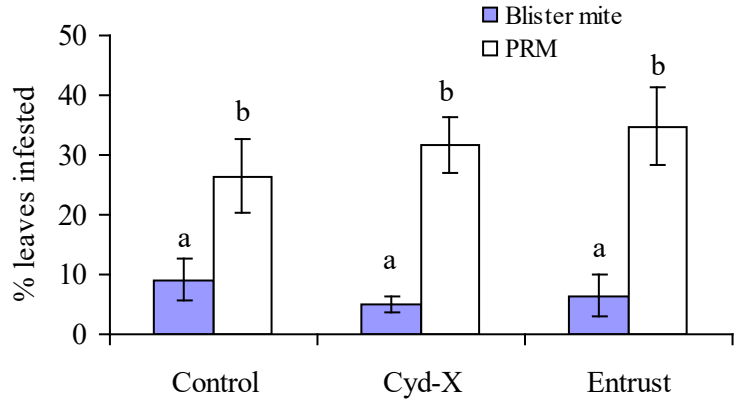


Figure 3. Tests for late season mite resurgence in 2004, Bartlett pear; 12-16 tree blocks replicated 4 or 5 times (Moxee)

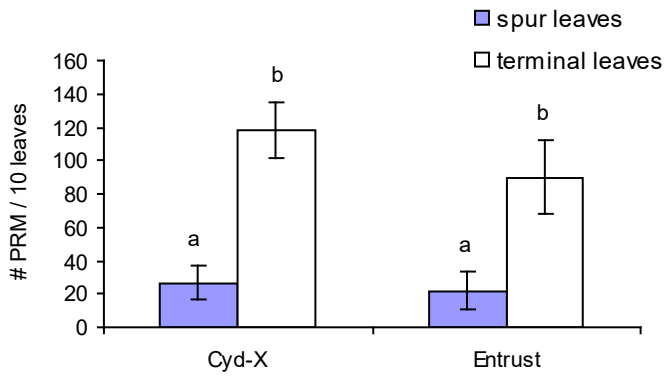


Figure 4. Tests for late season mite resurgence in 2004, Anjou; 1A blocks replicated twice (Mellow)

Conclusion

Codling moth granulovirus was less effective than spinosad at preventing fruit damage, but killed the majority of CM larvae that reached the fruit and was safe for non-targets. Spinosad reduced the abundance of parasitoids, *Anthocoris* spp. and non-target Diptera in small plots. We found no evidence for increased densities of phytophagous pests or mite resurgence resulting from spinosad use.

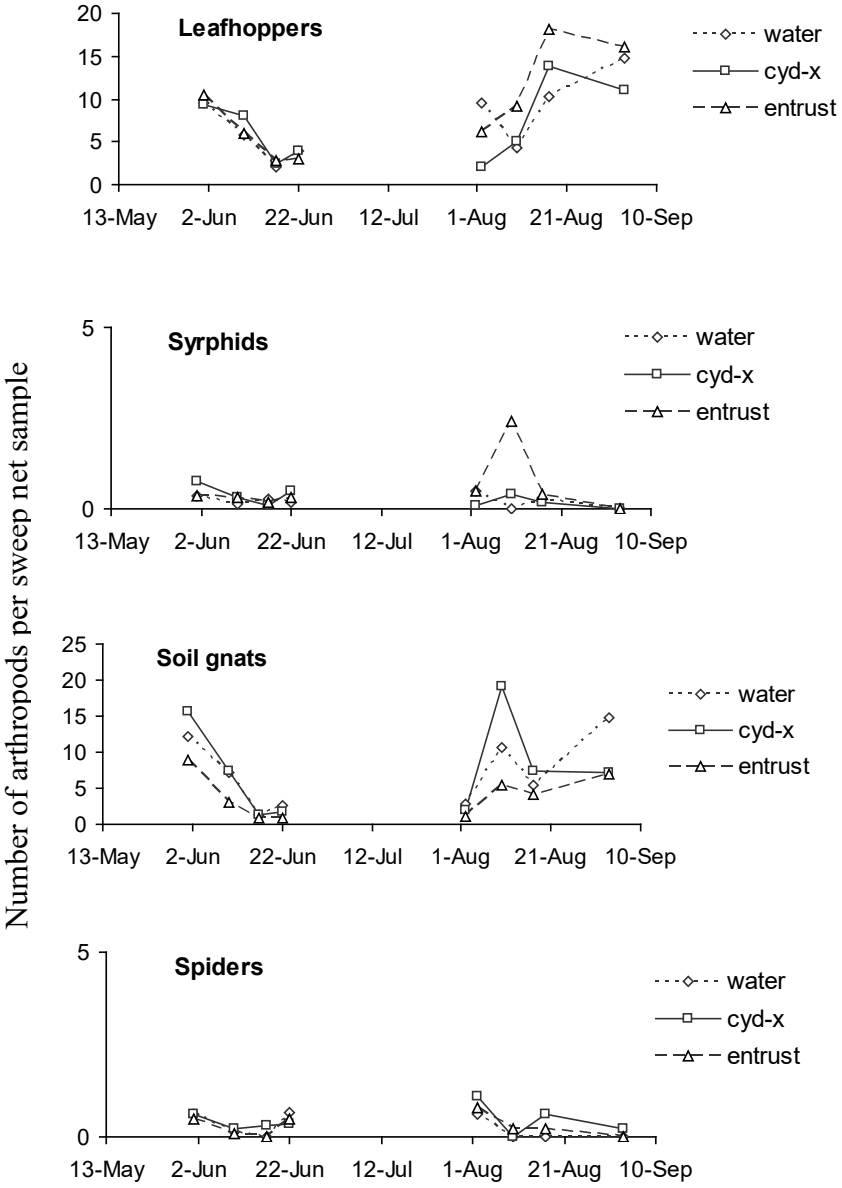


Figure 5. Non-target taxa monitored in sweep net samples. Treatments were applied at approx. 8-day intervals in replicated 12-16 tree plots. (Bartlett pear, Moxee experimental station).

REFERENCES

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*research supported by WTFRC funds

BUDGET SUMMARY

Title: Spinosad and granulovirus effects of codling moth
PI: Lawrence A. Lacey
Project duration: 2004-2005 (2 years)
Project total: $30,000

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

FINAL REPORT

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

PIs: Richard Hilton, Entomologist
Philip VanBuskirk, Professor

Organization: Oregon State University, Southern Oregon Research & Extension Center
569 Hanley Road, Central Point, OR 97502

Contract Administrator: Dorothy Beaton, Agricultural Research Foundation
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Significant findings:

- Under conditions of high codling moth pressure—

1) *Cydia pomonella* granulosis virus (CpGV) applied regularly (10-14 day interval) gave 70% to 85% control of codling moth relative to an untreated check in replicated trials.
2) Mating disruption alone (200 Isomate TT dispensers/ac.) gave 15% to 50% control of codling moth when compared to untreated blocks.
3) Combining CpGV with mating disruption gave 86% to 90% control of codling moth over two years of trials, therefore the bulk of the control in the combination was provided by the applications of CpGV.

- There was no significant difference in successful codling moth entries between CpGV applied on a frequent basis (10-14 day interval) and conventional codling moth treatments (Imidan, Guthion, or Calypso) applied every three weeks, although stings (i.e. unsuccessful entries) were higher when CpGV was applied.

- The addition of encapsulated pear ester to the CpGV did not significantly improve the level of codling moth control.

Results and Discussion:

Tests conducted in 2003 demonstrated that applications of CpGV applied on a 10 day interval gave 80-90% control of codling moth entries in a location with high pest pressure (Table 1). Tests conducted in a grower block where mating disruption was being used and pest pressure was very low indicated that CpGV could be substituted for standard conventional codling moth control materials and still provide a high level of control (Table 2).

Replicated trials were carried out with CpGV in 2004 in two blocks, one without mating disruption (Table 3) and one with mating disruption (Table 4). Again, pest pressure was high and the addition of CpGV provided significant control of codling moth. As the CpGV particles require ingestion, the level of stings was higher where CpGV was used but the level of successful entries by codling moth was not significantly different than standard materials applied on a three week schedule. As these studies were conducted in separate blocks, direct statistical comparisons between blocks could not be made but a general comparison is shown in Table 5 which indicates that the most of the control was the result of the CpGV applications. One difficulty in this comparison is that different cultivars were being compared, Packham's and Bartletts. Both Packham's and Bartlett have an open calyx and are subject to considerable early attack by codling moth but there are also major differences between the two cultivars that may make a direct comparison somewhat questionable.

Trials conducted in 2005 to repeat the 2004 studies were performed in blocks where Bartlett was present so that more direct comparisons could be made. The block with mating disruption was the

same as in 2004 and contained both Bartlett and Anjou. The comparison block also had Bartletts and Anjou in every replicate. Plot sizes were also increased in 2005. An additional replicated study was carried out on single Bartlett trees to examine the effect of combining microencapsulated pear ester with CpGV. In all the 2005 studies, CpGV was applied on a two week interval. As in 2004, the use of CpGV resulted in a significant decrease in the percent successful entries along with a rise in stings. A comparison between the two blocks (Table 6 and Figure 1) gave results similar to those seen in 2004 with Bartletts. Mating disruption by itself reduced entries by about 50% while control with CpGV alone was better than 80%. The combination of CpGV and mating disruption resulted in an 89% reduction in entries. In Anjous, the effect of either tactic by itself was much less than observed for Bartletts but the combination of CpGV and mating disruption gave a 90% reduction in entries. Thus, it seems that with Bartletts the effect of combining the two methods was only additive, whereas with Anjous there was some synergistic effect from combining the two methods. The additional study showed no significant effect from adding the microencapsulated pear ester to applications of CpGV despite the fact that applying the pear ester by itself did reduce early codling moth entries.

It is not unexpected that mating disruption has limited effectiveness under conditions of high codling moth pressure, however, the effectiveness of CpGV, even when stretched to a two week spray interval, is notable. The fact that the bulk of the control when the two tactics were combined could be attributed to the CpGV implies that under conditions of initial high pressure, a program consisting of frequent CpGV applications would most likely be more cost effective than a combined program, particularly if the addition of mating disruption meant a reduction in the number of CpGV applications. An example of this can be seen in a pear orchard transitioning to organic production from 2002-2005 (Table 7). Various control programs were employed over the last four years. In 2002, mating disruption was used in most of the orchard with one block left untreated as a comparison. Codling moth damage was about twice as high in the block without mating disruption. In 2003 the control program was minimal with just two or three oil sprays being applied and, as a result, the Bartlett crop was a total loss due to codling moth injury. With the registration and use of CpGV materials in 2004, codling moth damage was reduced by 75% from the 2003 levels. In 2005 CpGV was again used throughout the orchard with half the orchard being treated with mating disruption. While stings and total damage were lower in the mating disruption treated area, successful entries were not reduced. In this case, the addition of mating disruption did not appear to provide any improvement over the CpGV used by itself.

The results of the replicated tests and the organic orchard demonstration plots show that the use of CpGV can provide a high level of codling moth control. When codling moth pressure is high, the use of CpGV does result in an increased level of stings relative to standard control measures, but many of these stings are superficial and are generally located in the calyx and do not represent economic injury. The use of mating disruption is probably not warranted when codling moth levels are extreme, however when codling moth levels are low then the use of mating disruption may be called for as a way to maintain codling moth at low levels, using additional treatments as necessary.

Table 1. Field trial comparing CpGV products in small blocks with high codling moth pressure (cv. Bartlett).

Treatment	Rate (form./ac)	CM injury	
		% stings	% entries
Check	-----	20.75	51.5
Carpovirusine	400 ml	65.75	9.25
Cyd-X **Nu-Film 17**	3.0 fl oz 16 fl oz	69.25	6.5

Table 2. Comparison of CpGV products in an on-farm trial using mating disruption under conditions of low codling moth pressure (cv. Comice and Bosc)

Treatment	Rate (form./ac)	% CM injury	Mean seasonal trap catch	
			Pheromone trap	DA trap
Standard program **Intrepid** **Assail**	16 fl oz 3 oz	0.03	8.25	9.0
Carpovirusine	400 ml	0	4	4
Cyd-X **Nu-Film 17**	3.0 fl oz 16 fl oz	0	0	2

Table 3. Replicated comparison of CpGV with standard control measures without mating disruption (cv. Packham's).

Material	Rate and Frequency	CM injury		
		% Stings	% Larvae	% Exits
Calypso	4 oz—21 days	18.50 a	7.25 a	3.75 a
Calypso	6 oz—21 days	14.50 a	6.00 a	2.00 a
Calypso	8 oz—21 days	15.50 a	4.25 a	1.75 a
Cyd-X	3 oz—10/11 days	68.00 b	6.75 a	3.00 a
Imidan	5 lb—21 days	12.00 a	5.50 a	1.50 a
Check	-----	21.25 a	32.50 b	31.00 b

Table 4. Replicated trial evaluating CpGV applied at two time intervals (21 days and 10.5 days) in combination with mating disruption.

cv. Bartlett

Material	Rate and Frequency	CM injury		
		% Stings	% Larvae	% Exits
MD alone	-----	40.67 a	31.00 b	12.67 b
Cyd-X	3 oz—10/11 days	67.75 b	7.50 a	1.00 a
Cyd-X	3 oz—21 days	69.25 b	10.50 a	1.00 a

cv. Anjou

Material	Rate and Frequency	CM injury		
		% Stings	% Larvae	% Exits
MD alone	-----	18.00 a	35.00 b	9.67 b
Cyd-X	3 oz—10/11 days	30.25 b	7.69 a	0.25 a
Cyd-X	3 oz—21 days	41.13 c	12.5 a	1.25 a

Table 5. Combined results of trials conducted in 2004 showing the effect of CpGV applications on the level of codling moth injury in blocks with and without mating disruption (cv. Bartlett and Packham's).

Type of CM injury	% CM injury			
	Check plots		Cyd-X every 10/11 days	
	w/o MD	with MD	w/o MD	with MD
Stings	21.25	40.67	68.00	67.75
Larvae	32.50	31.00	6.75	7.50
Exits	31.00	12.67	3.00	1.00

Table 6. Combined results of trials conducted in 2005 showing the effect of CpGV applications on the level of codling moth injury in blocks with and without mating disruption.

cv. Bartlett

Type of CM injury	% CM injury			
	Check plots		Cyd-X every 14 days	
	w/o MD	with MD	w/o MD	with MD
Stings	27.75	40.00	41.75	56.00
Larvae	33.00	13.25	7.25	4.75
Exits	11.75	9.5	0.25	0.0

cv. Anjou

Type of CM injury	% CM injury			
	Check plots		Cyd-X every 14 days	
	w/o MD	with MD	w/o MD	with MD
Stings	15.5	12.0	41.5	35.5
Larvae	37.25	30.5	11.5	4.25
Exits	6.25	6.0	1.25	0.25

Table 7. Injury due to codling moth in a transitional organic pear orchard under various control programs (cv. Bartlett).

Year and CM control program	% CM injury		
	Shallow stings	Entries (exits +larvae)	Total damage (entries + deep stings)
2002 2-3 oil sprays	1.6	19.0	21.8
2002 2-3 oil sprays + MD	1.1	10.6	11.6
2003 2-3 oil sprays	-----	—Total Crop Failure— -----	61.8
2004 4 Cyd-X sprays	47.3	6.5	15.7
2005 4 Cyd-X sprays	40.0	5.0	27.0
2005 4 Cyd-X sprays + MD	24.0	7.0	17.0

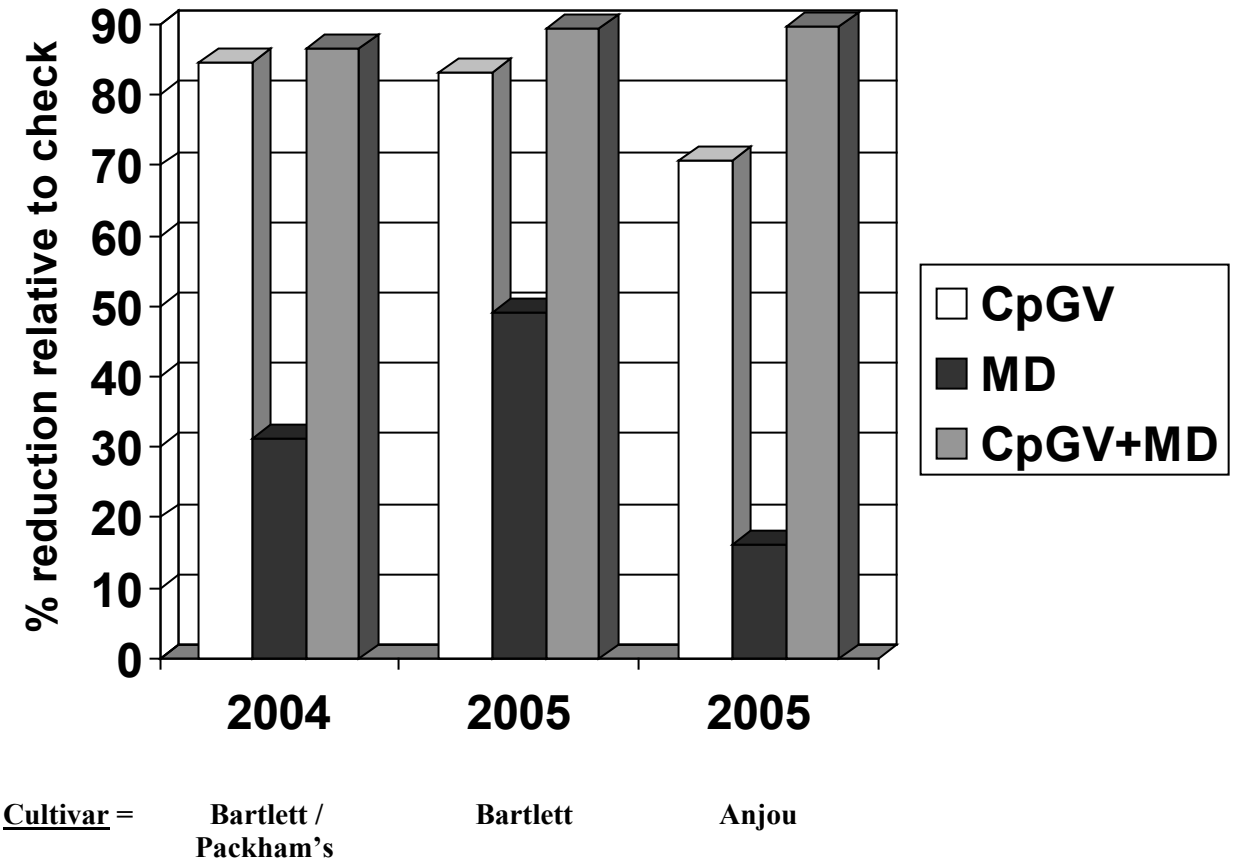

Figure 1. Level of codling moth control (% reduction in entries relative to the untreated check) with CpGV applications, mating disruption (MD), and a combination of CpGV and MD.

Budget:

Project Title: Codling moth control using CpGV—with and without mating disruption.
PI: Richard Hilton
Project duration: 2004-2005
Project total (2 years): $15,000
Current year request: n/a

Item	Year 1 (2004)	Year 2 (2005)
Wages	$7,500	$7,500
Total	$7,500	$7,500

FINAL REPORT

Project title: Ethylene induced resistance to *Botrytis*
PI: Henrik Stotz
Organization: Oregon State University Department of Horticulture
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Corvallis, OR 97331-7304; 541-737-5468
stotzhe@bcc.orst.edu

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

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1. RESEARCH OBJECTIVES

Our main ***objective*** was to determine whether Actigard, an inducer of systemic acquired resistance (SAR), or ethylene can be used to manage postharvest *Botrytis cinerea* decay. Specifically, pears were treated with the ethylene agonist propylene, the ethylene action inhibitor 1-methylcyclopropene (1-MCP), and/or the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase inhibitor aminoethoxyvinyl glycine (AVG) to alter ethylene biosynthesis and fruit ripening. Alternatively, Actigard containing 1,2,3-benzothiadiazole-7-carbothioic acid S-methyl ester (BTH) as the active ingredient was used to trigger SAR. As a result of these treatments, we expected differences in *Botrytis* susceptibility. Our ***rationale*** was that, once the effects of these treatments on gray mold decay were known, they could be implemented to control postharvest decay.

We used different pear (*Pyrus communis*) cultivars with and emphasis on 'd'Anjou', a winter pear, and 'Bartlett', a pear that does not require a chilling period for fruit ripening. We used wound inoculation because mycelial inoculation using agar plugs without wounding was highly variable and not effective. Fruits that were wounded and mock inoculated served as controls for all experiments. Robert Spotts and Joseph Postman (USDA/ARS National Clonal Germplasm Repository, Corvallis, OR) provided pear fruits for all of our studies.

2. SIGNIFICANT FINDINGS

- The *P. communis* varieties 'd'Anjou', 'Bartlett', 'Bosc', 'Comice', and *Pyrus pyrifolia* cv. 'Niitaka' differed in gray mold susceptibility and associated changes in ethylene production and fruit ripening.
- Treatments with propylene and 1-MCP accelerated and retarded disease-related ethylene biosynthesis, respectively, but had little influence on disease progression.
- Co-treatment of pears with 1-MCP and aminoethoxyvinyl glycine (AVG) blocked ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC) biosynthesis for 4 d, but lesions started expanding on day 2 post inoculation.
- 1-MCP inhibited fruit softening in response to *Botrytis* infection but had little influence on the rate of lesion expansion.
- A mutant of *B. cinerea* with a defect in the polygalacturonase gene *Bcpg1* was less virulent on pear fruits, suggesting that pectin catabolism is important for invasion.
- Fruits became more susceptible to *Botrytis* as they matured during cold storage.
- 'Niitaka' and 'd'Anjou' were the least and most susceptible cultivars, respectively, whereas susceptibility of 'Bartlett' was intermediate.
- Actigard accelerated gray mold decay after cold storage of 'Bartlett' pear fruits and did not alter *Botrytis* susceptibility of 'd'Anjou' pears.

3. Methods

3.1. Biological Material

Fruits of the two pear (*P. communis*) cultivars, 'Bartlett' and 'd'Anjou', were collected on the commercial harvesting dates of August 9 (122 DAFB) in 2004 and August 12 in 2005 (124 DAFB), August 27 (142 DAFB) in 2004 and August 30 in 2005 (146 DAFB), respectively, at the Mid-Columbia Experiment Station, Hood River, OR. Fruits were divided in two batches for each cultivar, placed in the polyethylene bags, packed into cardboard boxes, and transferred into cold storage (-1°C). 'Bartlett' was stored for 10 days and 'd'Anjou' was stored for 108 days. These disparate storage conditions have previously been reported to be adequate to elicit similar ripening behaviors in early and late ripening cultivars (1, 2). Pear fruits cv. d'Anjou' that were co-treated with AVG and 1-MCP after 60 d of cold storage and 'Niitaka' (*Pyrus pylifolia*) were harvested at USDA/ARS National Clonal Germplasm Repository, Corvallis, OR, on September 20, 2004. 'Bosc' and 'Comice' fruits were harvested at USDA/ARS National Clonal Germplasm Repository in 2003.

B. cinerea strain B05.10, the mutants *ΔBcpg1* and *ΔBcpme1,* and the double mutant of *ΔBcpg1* and *ΔBcpme1* were obtained from Jan van Kan (Wageningen University, Netherlands) and cultured on potato dextrose agar.

3.2. Chemical Treatments

Freshly harvested or stored pears were transferred to room temperature 1 d prior to chemical treatments that altered ethylene biosynthesis. Pear fruits were exposed in jars to humidified propylene (500 ppm) supplied at a flow rate of 45-60 ml/min for 24 h prior to fungal infection (3). 1-MCP was supplied as SmartFresh™ powder (0.14% active ingredient). According to the direction provided by AgroFresh, 16 mg of 1-MCP was mixed with 1 ml of water to generate 300 ppb of volatilized 1-MCP around pears in a sealed plastic container. Fruits were exposed for 24 h. Control fruits were kept in a sealed container for 24 h without any chemical additions. Pears were soaked in Retain (Valent Biosciences) solution containing 500 ppm AVG with 0.01% Tween 20 for 3 min and then dried. Retain was administrated first and 1-MCP last when pears were treated with both 1-MCP and Retain.

Robert Spotts sprayed pear trees bearing 'd'Anjou' and 'Bartlett' fruits with Actigard one and two weeks prior to harvest. Four trees per treatment were used and the rates of application were 150 or 1,500 mg a.i./liter (4).

3.3. Plant Inoculations

For ethylene-related experiments, 10 and 5 fruits were inoculated per treatment in 2004 and 2005, respectively. For Actigard experiments, 10 fruits per treatment were used. Fruits were surface sterilized by dipping in 0.01 % sodium hypochlorite for 2 min and rinsing with sterilized water. Fruits were treated with chemicals as mentioned above and subsequently inoculated. Fruits were punctured along the equator with the tip of a syringe to generate a 4 mm deep and 4 mm wide hole and then inoculated with a conidial suspension of *B. cinerea* (2.5×10^5 conidia/2 µl in 2004 and 1×10^3 conidia/2 µl in 2005) in water (5). The high concentration was used whenever fungal concentration was not mentioned in the text. The same amount of sterilized water was used for wounded controls. Unwounded control fruits were left intact and were not inoculated. Unwounded control, wounded control, and infected fruits were placed in same moist chambers containing wet a cheesecloth and kept at room temperature.

3.4. Measurement of Ethylene and Lesion Diameters

Ethylene was measured in 2004. Each pear was sealed in a 1-l container for 1 h before each measurement. A sample of 1 ml was withdrawn from the headspace of the container with a syringe. The ethylene concentration of this sample was analyzed using a gas chromatograph (GC) equipped with a 4 ft × 1/8 inch activated alumina column packed with 80/100 mesh and a flame-ionization detector (Gow-Max Instrument Co., Series 580). Flow rates were air = 300 cc/min, hydrogen = 30 cc/min, nitrogen = 30 cc/min, and N_2 was used as a carrier gas. Temperatures were injector 101°C, column 86°C, and detector 104°C. The first measurement was recorded 6 h post inoculation, followed by daily measurements for one week.

Once lesions were visible on the pear surface, lesion diameters were measured daily using a caliper.

3.5. Firmness Measurement

Fruit skin was removed and firmness of fruit flesh was measured as penetration force using a U.S. firmness tester with 0.78 cm penetrometer tip (Western Industrial Supply, San Francisco, California). Measurements were recorded twice per fruit on the non-diseased equatorial area of the fruit 7 d post inoculation.

3.6. Quantification of ACC

ACC was measured as described by Lizada and Yang (6) using pear fruits 7 d post inoculation. Infected and non-infected areas of three pear fruits were cut, pooled, and skin and flesh were frozen in liquid nitrogen. ACC was extracted from 5 g of frozen pear tissue with 10 ml of 5% sulfosalicylic acid. The extraction solution was stabilized by adding 1 μmol $HgCl_2$, and extracts were placed in a test tube sealed with a rubber stopper. A volume of 100 μl of a mixture of 5% NaOCl and saturated NaOH (2:1, v/v), generated on ice, was injected into the test tube. After adding this alkaline hypochlorite solution, the test tube was shaken using a vortex mixer for 5 sec and incubated on ice for 2.5 min. The test tube was agitated for another 5 sec and a volume of 0.5 ml of gas was withdrawn through the rubber stopper using a syringe. Ethylene was measured using a gas chromatograph.

3.7. Statistical Analysis

Data are presented as means ±SE. F-tests were used to compare variances and two-sample t-tests of two-tailed distributions were used to determine significant differences in softening (Microsoft Excel). Differences in lesion expansion were analyzed using the repeated measures procedure of a general linear model (GLM; $\alpha = 0.05$). Contrasts were used to determine significant differences between treatment and control means (SAS Institute, Cary, NC). A significance threshold of $P = 0.05$ was used.

4. RESULTS AND DISCUSSION

Ethylene was always produced when ‘d’Anjou’ and ‘Bartlett’ pears were infected with *B. cinerea*. In the case of ‘Bartlett’ pear fruits, propylene accelerated and 1-MCP suppressed ethylene production (Fig. 1A) and softening (Fig. 3A), but these changes had a relatively small influence on susceptibility to *B. cinerea* (Fig. 1B and C). 1-MCP increased the susceptibility of ‘Bartlett’ pears at harvest maturity. This effect was observed in both 2004 and 2005 trials when low (Fig. 1B; repeated measured option of GLM, $P < 0.0001$) or high concentrations of *B. cinerea* (Fig 1C; $P = 0.0192$) were used, respectively. Propylene only increased susceptibility to *B. cinerea* at harvest maturity in 2004 (Fig. 1B; $P = 0.0011$). This effect of propylene was no longer observed when a lower concentration of *B. cinerea* conidia was used in 2005 (Fig. 1C). Neither propylene nor 1-MCP had any effect on lesion expansion when ‘Bartlett’ fruits were challenged with a low or high concentration of *B. cinerea* after cold storage (Fig. 1E and F). Propylene no longer accelerated ethylene biosynthesis in response to *B. cinerea* once ‘Bartlett’ pear fruits were subjected to cold storage (Fig. 1D). 1-MCP still inhibited ethylene biosynthesis when ‘Bartlett’ fruits were subjected to cold storage (Fig. 1D), but the level of inhibition was less than in mature fruits (Fig. 1A).

Propylene had little effect on infected ‘d’Anjou’ pear fruits. Ethylene production was similar whether or not these fruits were treated with propylene after harvest or cold storage (Fig. 2A and D). Similarly to the situation in ‘Bartlett’ fruits (Fig. 1B), 1-MCP caused a statistically significant increase (Fig. 2B; $P < 0.0001$ and Fig. 2C; $P = 0.0017$) in susceptibility of ‘d’Anjou’ pears at harvest maturity, but not after cold storage (Fig. 2E). Conversely, propylene caused a statistically significant increase in susceptibility of ‘d’Anjou’ fruits after cold storage (Fig. 2E; $P = 0.0117$), but not immediately after harvest (Fig. 2B).

Propylene accelerated and 1-MCP inhibited softening of ‘Bartlett’ (Fig. 3A) and ‘d’Anjou’ pear fruits (Fig. 3B). Softening occurred at a faster rate when pear fruits were infected with *B. cinerea* relative to wounded control fruits. Botrytis infection caused an unexpected

increase in firmness of untreated 'Bartlett' pears relative to wounded control fruits, but this difference was statistically not significant. Despite decreased softening of 1-MCP-treated pear fruits, there was no significant change in susceptibility of stored pears to *B. cinerea* (Fig. 1E).

Co-treatments with 1-MCP and Retain (AVG) were used to suppress ethylene biosynthesis even further in an attempt alter susceptibility to *B. cinerea*. For this experiment, 'd'Anjou' pear fruits were moved to room temperature after 60 d of cold storage. None of the stored fruits produced any ethylene (data not shown), suggesting that the physiology of these pears was similar to fruits at harvest maturity.

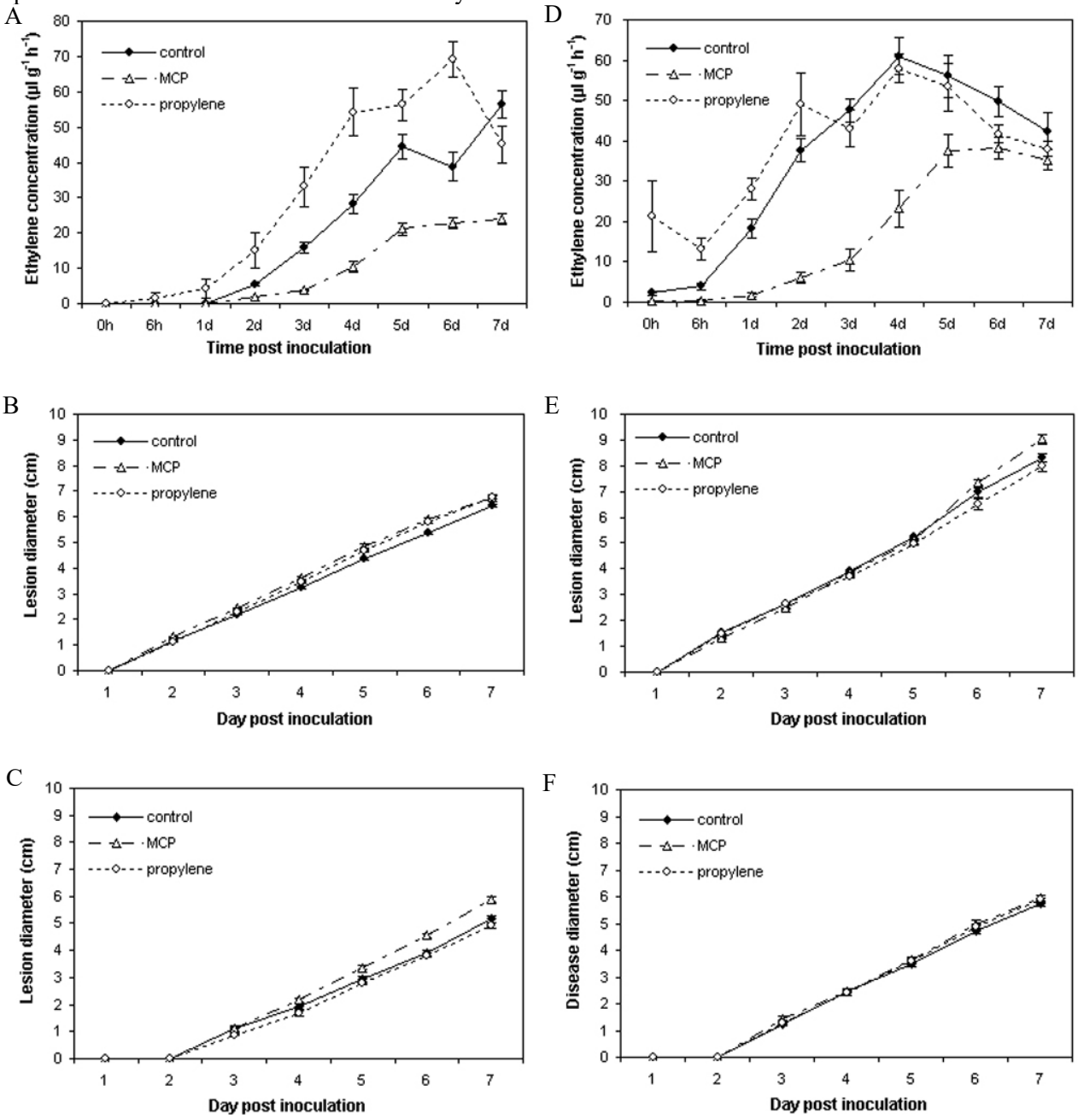

Fig. 1. Effects of 1-MCP and propylene on ethylene production and lesion expansion during infection of 'Bartlett' pear fruits by *B. cinerea*. Fruits were analyzed after harvest (A, B, and C) or 10 d after cold storage (D, E, and F). Results from 2004 (A, B, D, E) and 2005 (C, F) trials are shown. Ethylene production (A and D) and lesion expansion (B, C, E, and F) were measured in 'Bartlett' fruits that were untreated (control) or treated with 1-MCP or propylene prior to inoculation with *B. cinerea*. Fruits were inoculated with 2.5×10^5 (B and E) or 1×10^3 (C and F) conidia per inoculum of *B. cinerea*. The repeated measures procedure of a GLM was used for statistical analysis. Contrasts were used to compare treated with control fruits.

Fruits of ‘d’Anjou’ pears started to produce ethylene 1 d post inoculation with *B. cinerea* (Fig. 4A). Despite pretreatment with Retain (AVG) and/or 1-MCP, ethylene production of infected ‘d’Anjou’ fruits increased significantly 5 d post inoculation (Fig. 4B and C). In addition to ethylene, ACC was detected 7 d post inoculation in ‘d’Anjou’ fruits that were pretreated with both 1- MCP and AVG (Fig. 4D). The presence of ACC suggests that ethylene was produced by the host via conversion of SAM. Despite large

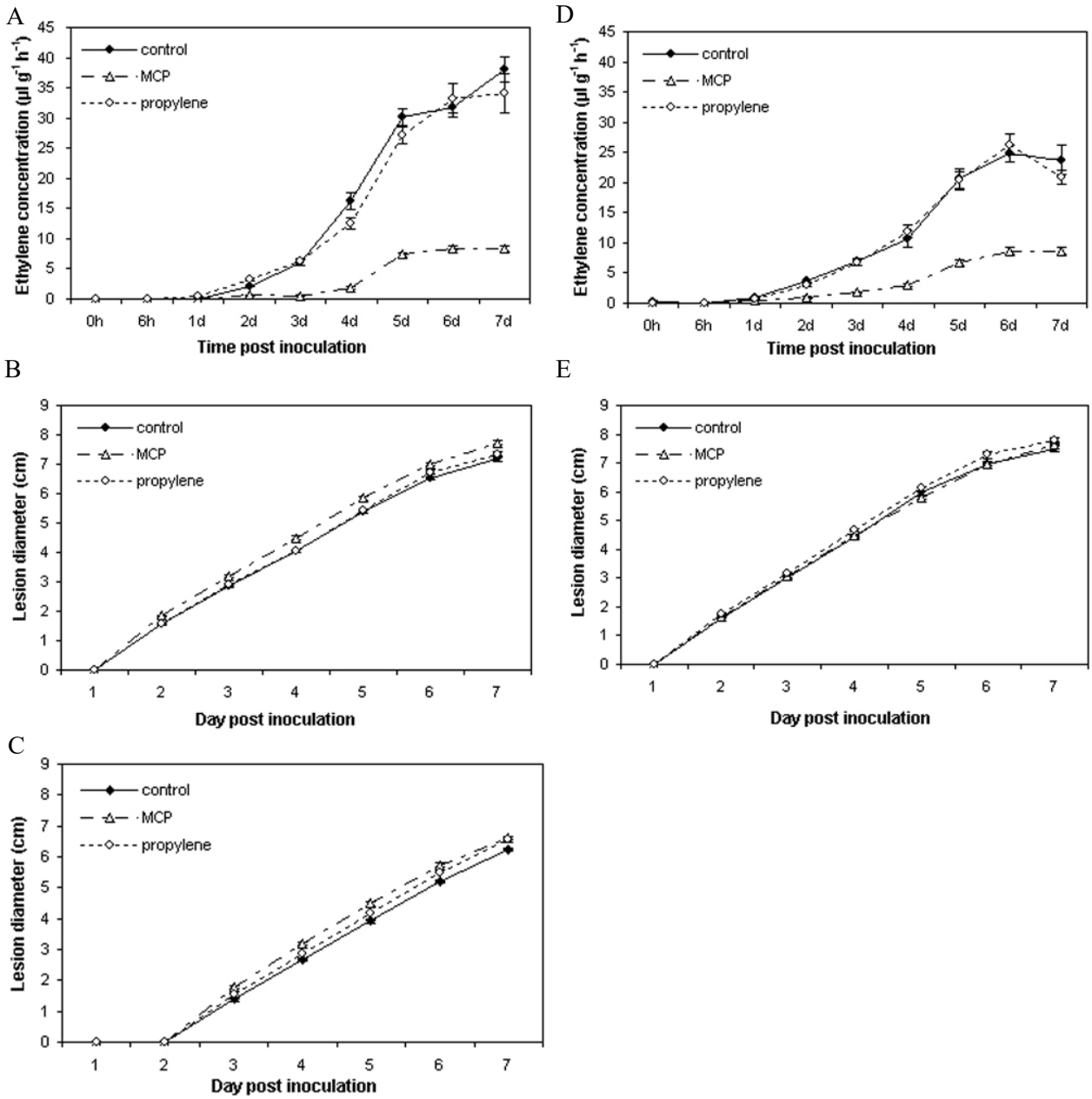

Fig. 2. Effects of 1-MCP and propylene on ethylene production and lesion expansion during infection of 'd'Anjou' pear fruits by *B. cinerea*. Fruits were analyzed after harvest (A, B, and C) or 108 d of cold storage (D and E). Results from 2004 (A, B, D, E) and 2005 (C) trials are shown. Ethylene production (A and D) and lesion expansion (B, C, and E) were measured in 'd'Anjou' fruits that were untreated (control) or treated with 1-MCP or propylene prior to inoculation with *B. cinerea*. Fruits were inoculated with 2.5×10^5 (B and E) or 1×10^3 (C) conidia per inoculum of *B. cinerea*. The repeated measures procedure of a GLM was used for statistical analysis. Contrasts were used to compare treated with control fruits.

changes in ethylene production in response to ethylene inhibitors (Fig. 4A to C), differences in lesion expansion were relatively minor (Fig. 4E). 1-MCP caused a statistically significant increase in susceptibility of pears to *B. cinerea* ($P = 0.0238$). Conversely, AVG caused a statistically significant enhancement of resistance of ‘d’Anjou’ pears to this fungal pathogen (Fig.

4E; P =0.0003). Gray mold susceptibility of pears that were co-treated with 1-MCP and AVG was not significantly different from

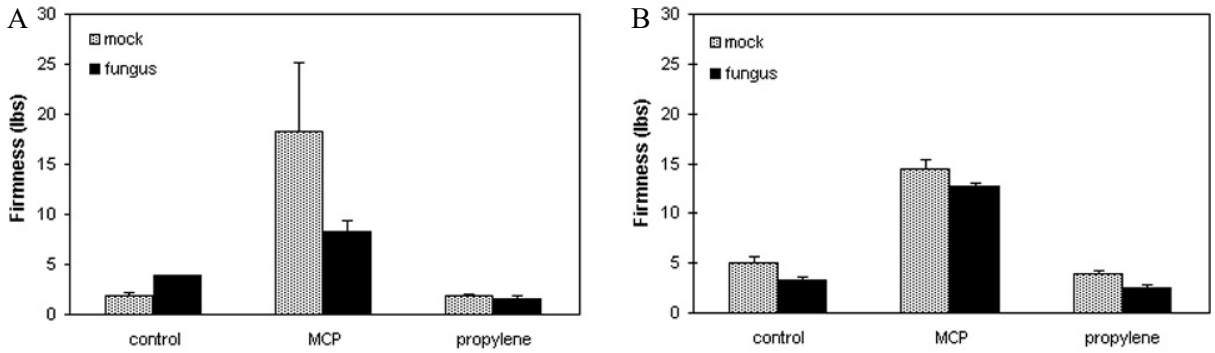

Fig. 3. Effects of gray mold infection and treatments with 1-MCP or propylene on softening of pear fruits. 'Bartlett' (A) and 'd'Anjou' fruits (B) were used after 10 d and 108 d of cold storage, respectively. Firmness of the healthy half of fruits exposed to *B. cinerea* for 7 d was compared to wounded control fruits. Results from the 2004 trial are shown.

untreated fruits, suggesting that the effects of these treatments canceled each other. After cotreatment with 1-MCP and AVG, lesions started to expand 2 d before the onset of ethylene production (Fig. 4C and E), suggesting that the onset of lesion expansion is independently of ethylene biosynthesis.

A mutant of *B. cinerea* with a deletion in the polygalacturonase gene *Bcpg1* exhibited reduced virulence when 'd'Anjou' pear fruits were wound-inoculated compared to wild type *B. cinerea* (Fig. 5A). In contrast, a *B. cinerea* mutant with a deletion in the pectin methylesterase gene *Bcpme1* did not alter lesion expansion in 'd'Anjou' pears (Fig. 5B). The reduced virulence of the *ΔBcpg1 ΔBcpme1* double mutant was likely caused by the deletion in the polygalacturonase gene because the deletion in the pectin methylesterase gene did not alter lesion expansion by itself (Fig. 5B). Thus, the endo-polygalacturonase gene *Bcpg1* increases the rate of gray mold infection in pear fruits, but the pectin methylesterase gene *Bcpme1* appears to be dispensable.

Fruits of 'd'Anjou' pears infected with the *ΔBcpg1* mutant strain were slightly firmer than those infected with wild type *B. cinerea* (Fig. 5C; t-test, P = 0.013). Infection by the *ΔBcpg1* mutant did not change the firmness of fruits treated with AVG and 1-MCP (Fig. 5C; t-test, P = 0.309). Treatment with 1-MCP and AVG caused a 3 to 4 fold increase in firmness of pear fruits that were infected with the fungal mutant *ΔBcpg1*. Despite this major change in fruit softening, there was no change in the susceptibility of pear fruits to the *ΔBcpg1* mutant (Fig. 5A). Conversely, the fungal polygalacturonase gene *Bcpg1* caused small changes in firmness, but large differences in susceptibility of pear fruits to *B. cinerea*, suggesting that pectin degradation is a major factor in gray mold susceptibility. Thus, fruit firmness affected the virulence of neither the *ΔBcpg1* mutant nor wild type *B. cinerea* (Fig. 3 and 5).

Collectively, these data permit conclusions about the roles of ethylene, softening, and pectin hydrolysis in gray mold susceptibility. The 1-MCP data was consistent among cultivars and collection dates (Fig. 1, 2, and 4), suggesting that this ethylene action inhibitor increases the susceptibility of mature pear fruits (5 out of 5 datasets) but not stored fruits. The effects of propylene were variable; this ethylene agonist increased the susceptibility of mature 'Bartlett' pears and stored 'd'Anjou' fruits. AVG appeared to enhance resistance, but only one experiment was carried out using this ACC synthase inhibitor. In conclusion, manipulation of ethylene using exogenous treatments has limited influence on gray mold susceptibility of pear fruits. While 1-MCP did not alter gray mold susceptibility of stored fruits, this ethylene action inhibitor retarded softening of *Botrytis*-challenged fruits, thus demonstrating that fruit firmness is not related to gray mold infection. Lastly, deletion of a fungal polygalacturonase gene retards the rate of lesion expansion, implying that pectin catabolism promotes infection (7). It is, therefore, likely that pectin catabolism rather than softening increases gray mold susceptibility of pear fruits during storage.

Actigard sprays in the orchard one and two weeks prior to harvest did not affect gray mold susceptibility of mature pear fruits (Fig. 6A and B). When applied at a concentration of 1.5 g a.i./liter,

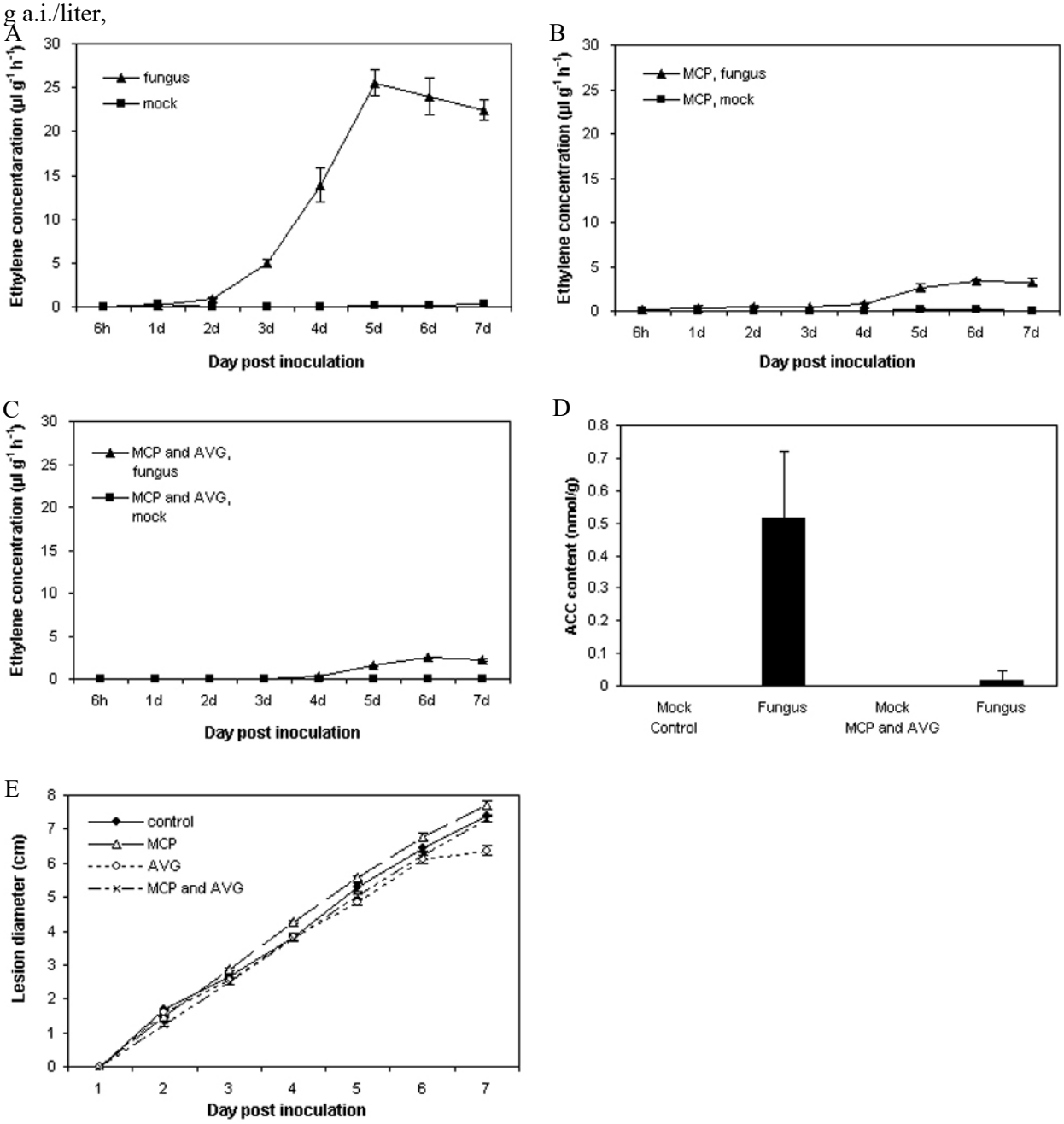

Fig. 4. Effects of 1-MCP and/or AVG on ethylene and ACC production after inoculation with *B. cinerea* of 'd'Anjou' pear fruits 60 d after cold storage. Ethylene production was measured in 'd'Anjou' fruits that were mock-inoculated or challenged with *B. cinerea* (A). Ethylene production in 'd'Anjou' fruits treated with 1-MCP prior to mock inoculation or gray mold infection (B). Ethylene production in 'd'Anjou' fruits treated with 1-MCP and AVG prior to mock inoculation or gray mold infection (C). ACC content was measured in 'd'Anjou' fruits that were not treated (control) or pretreated with 1-MCP and AVG and subsequently exposed to *B. cinerea* for 7 d (D). Lesion expansion in gray mold infected 'd'Anjou' fruits that were not treated (control) or treated with 1-MCP, AVG, 1-MCP and AVG before inoculation.

Actigard increased the rate of lesion expansion when stored 'Bartlett' pears were wound inoculated with *B. cinerea*. Collectively, these data demonstrate that the application of this inducer of SAR could not protect pear fruit after harvest when applied prior to harvest in the field. Analysis of PR gene expression has not yet been conducted because of the lack of a phenotypic effect. Actigard does protect grape fruits

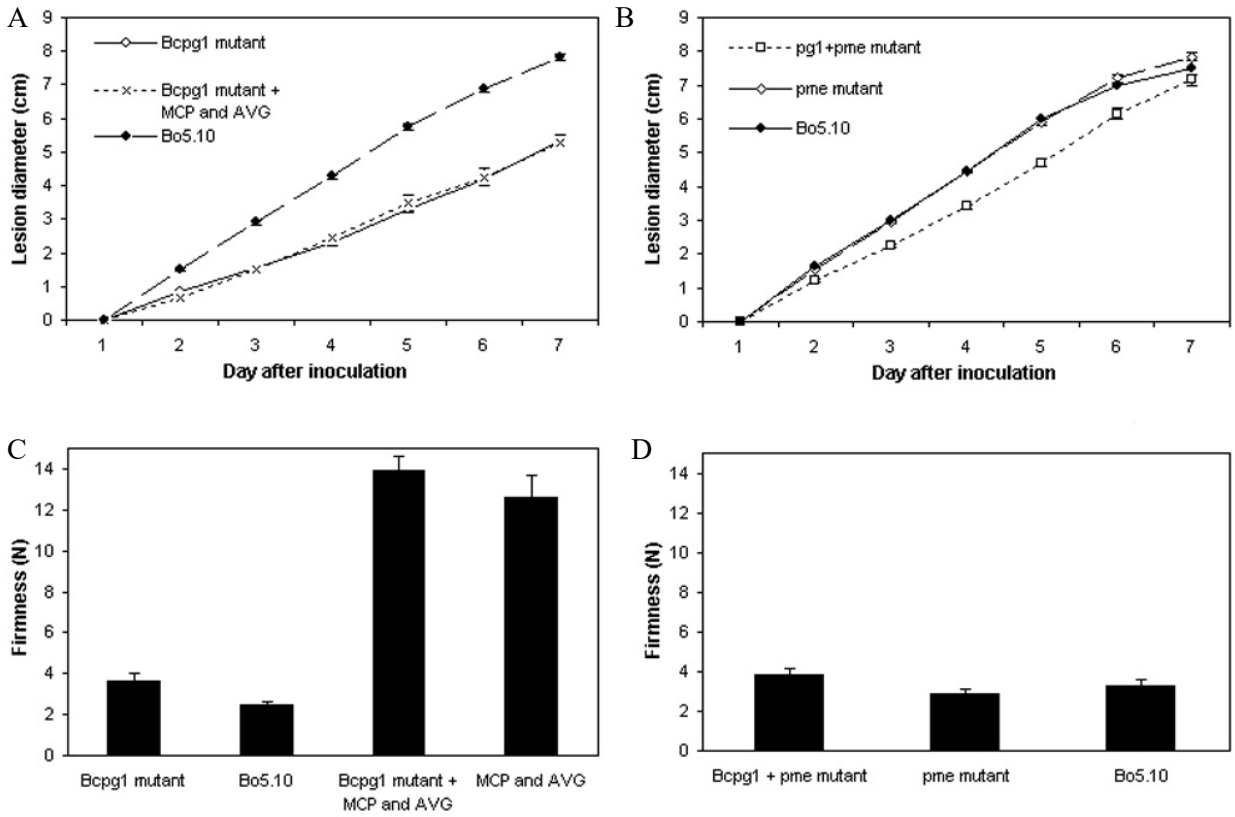

Fig. 5. Unlike pectin methylesterase *Bcpme1*, the polygalacturonase gene *Bcpg1* is required for virulence of *B. cinerea* in pear fruits. Fruits of 'd'Anjou' pears were used after 60 d (A and C) or 108 d of cold storage (B and D). Fruits were pretreated with 1-MCP and AVG and subsequently infected with a deletion strain of *B. cinerea*, *ΔBcpg1*, or the parental wild type B05.10 (A and C). Otherwise, fruits were infected with B05.10, *ΔBcpme1*, or the double mutant *ΔBcpg1 ΔBcpme1* (B and D). Lesion expansion (A and B) or firmness (C and D) was measured.

in the field (8), suggesting that the protective effect of this SAR inducer depends on the attachment of fruits to the plant. Actigard can induce PR gene expression in leaves of apple seedlings (4), but it is unknown whether this SAR inducer affects fruits. However, our preliminary indicate efficacy of Actigard in NahG mutants of tomato (9), which do not accumulate salicylic acid. In these plants Actigard increase resistance to *B. cinerea* in leaves and fruits. In conclusion, Actigard does not alter the susceptibility of pear fruits after harvest either because it is unable to trigger a systemic response in fruits or because SAR does not affect *B. cinerea* (10).

5. REFERENCES

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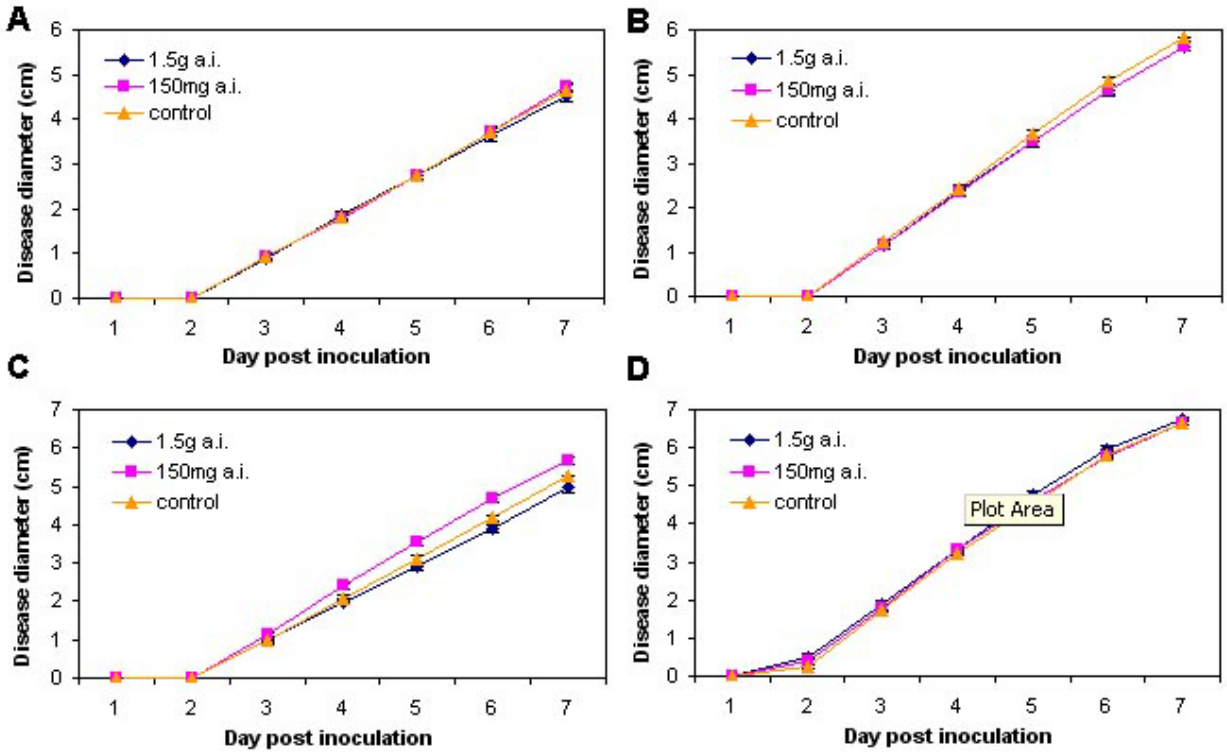


Fig. 6. Effect of Actigard on gray mold susceptibility of pear fruits. 'Bartlett' (A and C) and 'd'Anjou' pear fruits (B and D) were sprayed with 150 mg a.i. or 1,5 g a.i. per liter Actigard one and two weeks prior to harvest. Freshly harvested (A and B) or stored fruits (C and D) were wound inoculated with *B. cinerea*.

6. BUDGET

Project title:	Ethylene induced resistance to *Botrytis*
PI:	Henrik Stotz
Project duration:	2003 to 2005
Final year:	2005
Project total (three years):	$77,020
Current year request:	$29,184

FINAL REPORT

Project Title: Calcium chloride sprays for delaying maturity and improving storage
PI: David Sugar, Professor
Organization: Oregon State University Southern Oregon Research and Extension Center
david.sugar@oregonstate.edu

Contract Administrator: Dorothy Beaton; email: dorothy.beaton@oregonstate.edu
541-737-3228

Objectives:
This project addressed the question: can calcium applications to Bartlett pears during the growing season delay maturity sufficiently to allow delayed harvest, with larger fruit size? Secondarily, the project compared the storage quality of calcium-treated and non-treated Bartlett pears.

Methods:
Replicated, randomized plots were established at the Southern Oregon Research and Extension Center. Treatments were applied by powered handgun sprayer.

Significant Findings:
1. In 2004 plots Bartlett pear trees sprayed three times during mid-late summer with calcium chloride applied at 1, 3, or 5 lbs. actual calcium per 100 gallons of water suffered increasing leaf burn as the dosage increased. Leaf injury at 1 lb. calcium per 100 gallons was minor. In 2005, leaf injury was minor with 1 lb. calcium per 100 gallons, whether applied 3 times or 6 times during the growing season. Calcium-containing products Calcium Metalosate and Nutri-Cal did not cause visible leaf injury.

2. Fruit size and soluble solids, which were diminished in association with leaf injury in 2004, were not affected by any treatment in 2005 (Table 1).

3. Blackened lenticels were observed with dosages of 3 or 5 lbs. calcium at the first harvest in 2004, but were barely noticeable at later harvests. Dark green areas around lenticels were observed in 2005 where 1 lb. calcium per 100 gallons was applied 6 times.

4. Fruit firmness and color were not affected by any treatment in 2005 (Table 1).

5. Calcium treatments in the programs tested did not affect storage quality of the pears (Table 2).

Results and Discussion:
1. Bartlett pear trees appear to be more sensitive than Bosc trees to leaf burn and lenticel blackening by calcium chloride. Excessive leaf burn reduces fruit size and soluble solids. Leaf burn was minor when calcium chloride was applied at 1 lb. calcium per 100 gallons.

2. At dosages that did not causes significant leaf injury, there was no detectable benefit to calcium applications to Bartlett pears. Fruit firmness was not significantly enhanced; there was no indication that if harvest were delayed to increase fruit size, calcium treatments would compensate for the loss of firmness.
3. There was no indication that calcium treatments to Bartlett pears help maintain fruit quality during cold storage.

Table 1. Fruit characteristics in Bartlett pears following various foliar calcium programs.

Harvest 1

Treatment	Average fruit weight (g)	Fruit firmness (lbs)	Soluble solids (°Brix)	Color (hue)
Check	188.3	18.3	13.8	107.8
$CaCl_2$ 1 lb Ca 3 applications	189.5	18.9	13.9	108.3
$CaCl_2$ 1 lb Ca 6 applications	177.4	20.1	13.8	107.8
$CaCl_2$ 5 lb Ca Preharvest	188.9	18.4	14.2	107.7
$CaCl_2$ 1 lb Ca 3 applications + Auxigrow	176.7	19.4	14.0	107.1
Ca Metalosate	178.6	19.1	13.4	105.1
NutriCal	178.3	19.4	13.7	108.3
P value	0.876	0.162	0.735	0.361

Harvest 2

Treatment	Average fruit weight (g)	Fruit firmness (lbs)	Soluble solids (°Brix)	Color (hue)
Check	207.4	15.2	14.1	108.0
$CaCl_2$ 1 lb Ca 3 applications	210.0	16.6	13.7	108.1
$CaCl_2$ 1 lb Ca 6 applications	210.5	17.5	13.9	109.0
$CaCl_2$ 5 lb Ca Preharvest	219.8	16.2	14.2	107.0
$CaCl_2$ 1 lb Ca 3 applications + Auxigrow	205.5	17.1	13.7	107.1
Ca Metalosate	202.3	16.0	14.0	107.1
NutriCal	200.3	17.0	13.2	108.2
P value	0.961	0.164	0.262	0.468

Harvest 3

Treatment	Average fruit weight (g)	Fruit firmness (lbs)	Soluble solids (°Brix)	Color (hue)
Check	233.2	14.2	14.7	105.6
$CaCl_2$ 1 lb Ca 3 applications	242.4	15.5	14.5	105.9
$CaCl_2$ 1 lb Ca 6 applications	213.6	14.5	14.0	105.9
$CaCl_2$ 5 lb Ca Preharvest	227.1	15.1	14.1	105.8
$CaCl_2$ 1 lb Ca 3 applications + Auxigrow	223.6	14.4	14.6	103.8
Ca Metalosate	227.1	15.5	14.5	106.6
NutriCal	234.5	15.0	14.3	106.2
P value	0.904	0.910	0.589	0.849

Table 2. Quality of Bartlett pears grown under various calcium programs, following storage at 31°F.

Evaluation 1 mid-November

	Harvest 1	Harvest 2	Harvest 3
Check	fair	good	fair
$CaCl_2$ 1 lb Ca x 3	fair	fair-good	fair
$CaCl_2$ 1 lb Ca x 6	poor-good	fair-good	fair
$CaCl_2$ 5 lb Ca Preharvest	fair-good	fair-good	fair-good
1 lb x 3 + Auxigrow	poor-good	fair	fair
Ca Metalosate	poor	fair	fair
NutriCal	poor-fair	fair	fair

Evaluation 2 mid-December

	Harvest 1	Harvest 2	Harvest 3
Check	poor	poor	poor
$CaCl_2$ 1 lb Ca x 3	poor	poor	poor
$CaCl_2$ 1 lb Ca x 6	poor	poor	poor
$CaCl_2$ 5 lb Ca Preharvest	poor	poor-fair	poor
1 lb x 3 + Auxigrow	poor	poor	poor
Ca Metalosate	poor	poor	poor
NutriCal	poor	poor	poor

Budget:

Project Title: Calcium chloride sprays for delaying maturity and improving storage
PI: David Sugar

Project Duration: 2004-2005

Item	Amount	
Salaries and Wages	5,505	
Benefits (0.09)	495	
Services and Supplies	800	
Travel	200	
Total	7,000	(Oregon Bartlett Pear Commission)

FINAL REPORT

Project Title: Strategies for thinning Bartlett pears using caustic materials during bloom
PI: David Sugar, Professor
Organization: Oregon State University, Southern Oregon Research and Extension Center
david.sugar@oregonstate.edu

Cooperator: J. McFerson, Ed Vaughn

Contract Administrator: Dorothy Beaton; email: dorothy.beaton@oregonstate.edu
541-737-3228

Objective: This project evaluated caustic materials for thinning Bartlett pears (initiated in 2004).

Significant findings:

1. Applied at 20% bloom, nearly all treatments reduced yield, but fruit size increase was inconsistent (Tables 1 and 2).

2. Applied at 80% bloom, most treatments reduced yield and increased fruit size (Tables 3 and 4).

3. In the two years of study, urea applied at 80% bloom in either 5% or 7.5% solutions resulted in relatively modest reductions in yield with consistent increases in fruit size (Table 5).

4. All data referred to above came from handgun-applied treatments. A replicated plot with 5% urea was also applied by speed sprayer delivering 100 gallons per acre. However, it was observed that the urea was not adequately mixed in the tank, thus the results are considered non-representative (Table 6).

5. In 2004, lime sulfur + Crocker's Fish Oil (CFO) applications, at the rates tested, resulted in misshapen and russetted fruit and sharply reduced production, and CFO alone increased fruit russet. These treatments were not included in the 2005 plot.

6. Calcium chloride at 1.5% affected fruit set and yield in a similar pattern to urea, but did not increase fruit size to the same extent. This suggests that nutrition provided by urea may also be involved in the fruit size response; the benefit of urea sprays at 80% bloom may result from a combination of thinning and enhanced nitrogen nutrition as fruit begin to grow.

Methods: Replicated, randomized orchard plots were established in a uniform commercial orchard. Treatments were applied by powered handgun sprayer, except were indicated.

Results and Discussion:

1. Thinning Bartlett pears at bloom in the Pacific Northwest is risky due to uncertain environmental conditions for fruit set. Nevertheless, methods that result in larger fruit size can be highly valuable to the producer. The main challenge in identifying a suitable material for thinning at bloom is finding an adequate balance between reduction in the crop volume (yield) and increase in fruit size. Since thinning by definition reduces the crop volume, we are seeking a method that minimizes the crop reduction while increasing the proportion of large fruit in the crop.

2. The success of urea treatments at 80% bloom in increasing fruit size raises the interesting possibility that these results reflect the combined effect of fruit thinning and nitrogen enrichment. Comparing the effects of urea at 80% bloom to those of calcium chloride, the similar levels of fruit set and yield, with sharp differences in fruit size, suggest the interaction of crop load and

nitrogen nutrition. Treatments other than urea resulted in either excessive yield reduction or inadequate size increase.

Table 1. Treatments applied at 20% bloom, 2004

	Rate	Fruit set per 100 clusters	Projected tons per acre	Tons per acre relative to check (%)
Water		68.1 a	23.0 a	
Urea	5%	48.9 ab	16.4 ab	- 28.7
Ca chloride	1.5%	44.6 ab	17.0 ab	- 26.1
ATS	3.4 gal/a	29.3 b	8.8 c	- 61.7
Lime sulfur	8%	44.0 ab	15.6 bc	- 32.2
CFO	2%	55.0 a	15.3 bc	- 33.5
LS + CFO	3% + 2%	23.8 b	8.3 c	- 63.9

	Rate	Avg. fruit weight (g)	% size 90 & larger	% 90 & larger relative to check
Water		194.2	32.4 c	
Urea	5%	219.3	57.4 a	+ 77.2
Ca chloride	1.5%	193.3	30.5 c	- 5.9
ATS	3.4 gal/a	207.1	37.4 abc	+ 15.4
Lime sulfur	8%	196.1	33.6 bc	+ 3.7
CFO	2%	201.6	37.5 bc	+ 15.7
LS + CFO	3% + 2%	213.2	51.3 ab	+ 58.3

Table 2. Treatments applied at 20% bloom, 2005.

	Rate	Fruit set per 100 clusters	Projected tons per acre	Tons per acre relative to check (%)
Water		70.3 a	18.3	
Urea	2.5%	43.1 b	16.7	- 8.7
Urea	5.0%	41.1 b	17.6	- 3.8
Urea	7.5%	44.0 b	13.8	- 24.6
ATS	2 gal/a	54.0 ab	14.9	- 18.6
Lime sulfur	4%	48.7 b	16.5	- 9.8
Ca chloride	1.5%	70.8 a	20.6	+ 12.6

	Rate	Avg. fruit weight (g)	% size 90 & larger	% 90 & larger relative to check
Water		189.8 ab	29.2	
Urea	2.5%	190.2 ab	30.3	+ 3.8
Urea	5.0%	182.7 bc	22.3	- 23.6
Urea	7.5%	202.5 a	39.4	+ 34.9
ATS	2 gal/a	189.6 ab	27.5	- 5.8
Lime sulfur	4%	183.8 bc	24.6	- 15.8
Ca chloride	1.5%	171.2 c	16.5	- 43.5

Table 3. Treatments applied at 80% bloom, 2004.

	Rate	Fruit set per 100 clusters	Projected tons per acre	Tons per acre relative to check (%)
Water		76.5 a	23.7 a	
Urea	5%	53.5 b	17.1 ab	- 27.8
Urea	7.5%	54.6 ab	18.7 ab	- 21.1
Ca chloride	1.5%	51.2 b	17.4 ab	- 26.6
Ca chloride	2.25%	45.8 bc	8.9 c	- 62.4
ATS	3.4 gal/a	36.9 b	12.0 b	- 49.4
ATS	5 gal/a	27.7 c	7.7 c	- 67.5
LS	8%	45.1 b	14.5 b	- 38.8
CFO	2%	40.7 b	11.4 bc	- 51.9
LS + CFO	3% + 2%	17.2 c	6.0 c	- 74.7

	Rate	Avg. fruit weight (g)	% size 90 & larger	% 90 & larger relative to check
Water		189.4 d	26.8 d	
Urea	5%	228.5 ab	57.7 ab	+ 115.3
Urea	7.5%	242.7 a	71.3 a	+ 166.0
Ca chloride	1.5%	196.0 cd	34.9 cd	+ 30.2
Ca chloride	2.25%	217.2 a	54.2 a	+ 102.2
ATS	3.4 gal/a	216.8 bc	52.7 bc	+ 96.6
ATS	5 gal/a	229.0 a	60.2 a	+ 124.6
LS	8%	200.9 cd	37.1 cd	+ 38.4
CFO	2%	198.2 cd	38.1 cd	+ 42.2
LS + CFO	3% + 2%	241.6 a	71.1 a	+ 165.3

Table 4. Treatments applied at 80% bloom, 2005.

	Rate	Fruit set per 100 clusters	Projected tons per acre	Tons per acre relative to check (%)
Water		70.3	18.3	
Urea	2.5%	54.0	15.4	- 15.8
Urea	5.0%	56.3	14.5	- 20.8
Urea	7.5%	44.9	11.4	- 37.7
ATS	2 gal/a	47.5	13.7	- 25.1
Lime sulfur	4%	43.4	14.5	- 20.8
Ca chloride	1.5%	56.3	17.5	- 4.3

	Rate	Avg. fruit weight (g)	% size 90 & larger	% 90 & larger relative to check
Water		189.8 bc	29.2 bc	
Urea	2.5%	174.2 d	17.9 c	- 38.7
Urea	5.0%	200.1 ab	39.6 ab	+ 35.6
Urea	7.5%	211.6 a	48.5 a	+ 66.1
ATS	2 gal/a	192.0 bcd	30.7 bc	+ 5.1
Lime sulfur	4%	186.3 bcd	29.6 bc	+ 1.4
Ca chloride	1.5%	182.2 cd	23.7 c	- 18.8

Table 5. Crop reduction and fruit size increase resulting from 5% and 7.5% urea treatments in 2004 and 2005.

Applications at 80% bloom	2004		2005	
	Tons per acre relative to check (%)	% 90 & larger relative to check	Tons per acre relative to check (%)	% 90 & larger relative to check
Urea 5.0%	- 27.8	+ 115.3	- 20.8	+ 35.6
Urea 7.5%	- 21.1	+ 166.0	- 37.7	+ 66.1

Table 6. Crop characteristics in treatments applied by speed sprayer in 2005. Note: due to inadequate mixing of the urea, data may be unrepresentative of 5% solution.

	Fruit set per 100 clusters	Avg. fruit weight (g)	Projected tons per acre	% size 90 & larger
Water	57.9	160.5	21.2	11.9
Urea 5%	52.5	166.6	20.6	15.5

Budget:

Project Title: Strategies for thinning Bartlett pears using caustic materials during bloom

Principal Investigator: David Sugar

Project Duration: 2004-2005

Item	Amount
Salaries and Wages	6,239
Benefits (0.09)	561
Services and Supplies	1,000
Travel	200
Total	8,000 (Oregon Bartlett Pear Commission)

No further funding is requested. Additional work on this topic will be carried out under the proposed project "Pear Storage Decay and Fruit Quality Research".

CONTINUING PROJECT REPORT **YEAR 2/3**

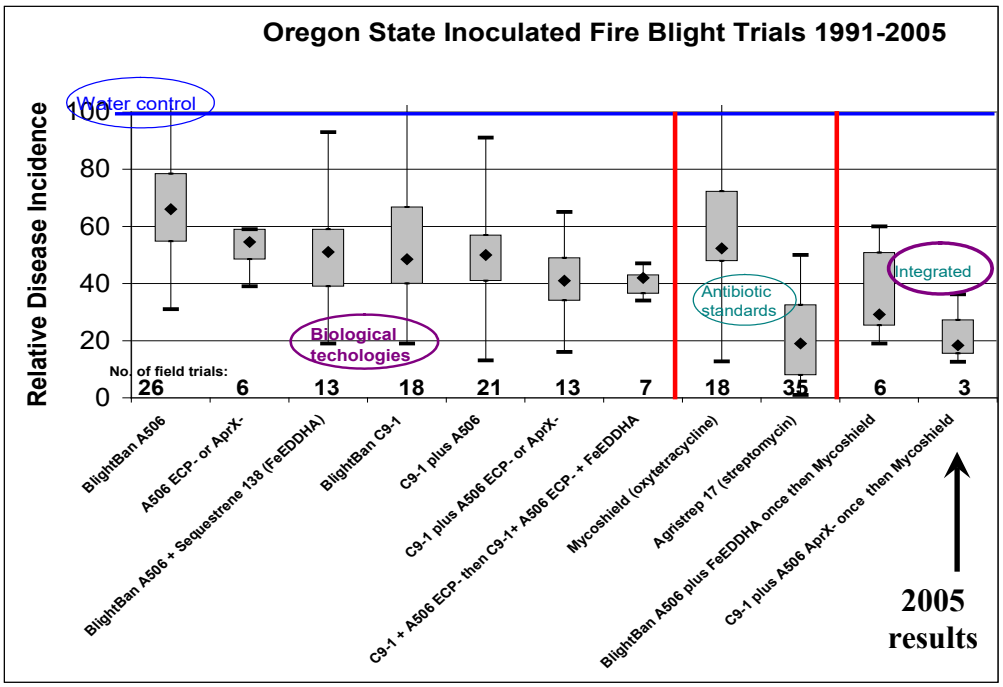
Project title: Integrated management of fire blight of pear and apple
PI: Kenneth B. Johnson
Organization: Dept. Botany & Plant Pathology, Oregon State University Corvallis, OR
Co-PI(s) and affiliation(s): Virginia Stockwell (OSU, Corvallis)
Cooperator(s): David Sugar (OSU, Medford), Joyce Loper (USDA-ARS,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

Objectives:
In 2005: Field-test an optimized biopesticide strategy in combination with oxytetracycline.

Proposed In 2006:
Field-test optimized biopesticide strategies in combination with oxytetracycline.

Significant findings: Our experiments concerned with fire blight control have shown excellent results with 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.

The final data package for registration of *Pantoea agglomerans* strain C9-1 as a biopesticide has been submitted to US EPA by NuFarm Americas; the registration is pending. This strain is one component of our most effective biopesticide treatment (see figure below). The other strain is a mutant of *Pseudomonas fluorescens* strain A506, the active bacterium in BlightBan A506. A registration process for the mutant (named A506 *aprX*-) will be initiated if the C9-1 registration is received.



Methods:
Chemical and biological agents with potential to control fire blight were tested (see results section). This experiment was conducted in a Bartlett pear, Golden Delicious and Rome Beauty apple orchards at the Botany and Plant Pathology Experimental Farm near Corvallis Oregon. Experimental treatments were arranged in randomized block designs with 4 replications of

individual trees. Treatments included alternative products, a water-treated control and standard antibiotic products (streptomycin and oxytetracycline). Treatments timings were varied according to properties of the product, but generally, two application of each product were made. Products were applied to near run-off a hand-directed backpack sprayers. Freeze-dried inoculum of the fire blight pathogen (strain *Ea*153nal, streptomycin sensitive) was applied near full bloom. Beginning in mid-May and ending in July, incidence of fire blight was evaluated weekly by counting and removing the diseased blossom clusters on each tree.

Results:

Bartlett pear. Blossom cluster density on the Bartlett pear trees averaged 588 clusters per tree. Disease intensity was moderate with blight symptoms developing on 10.1% of blossom clusters treated with water-only. Based on analysis of mean strikes per tree, the antibiotic treatment of AgriStrep 17 provided a superior level of disease control. Mycoshield, the biological treatment of A506 AprX- mixed with *Pa*C9-1S followed by treatment with Mycoshield, and Serenade also significantly ($P \leq 0.05$) reduced the incidence of disease compared to that observed on water-treated inoculated control trees. Based on observed disease incidence, Agri-mycin 17, Mycoshield, and treatment with A506 AprX- plus *Pa*C9-1S followed by treatment with Mycoshield provided outstanding control of fire blight (this group averaged an 83% reduction in disease incidence compared to inoculated water-treated trees). Serenade and treatment that included the combination of A506 AprX- plus *Pa*C9-1S also provided significant control of fire blight compared to the water treated control.

BARTLETT PEAR, Corvallis, Oregon, 2005 Fire blight trial

Treatment	Rate per 100 gallons water	Date treatment applied*: 15 March white clusters	24 March 30% bloom	30 March 80% bloom	4 April full bloom	Mean number blighted clusters/ee		Mean % of clusters blighted	
							a*		a*
Famoxate	30 fl.oz.	---§	---	X§	X	65	*	14.9	**
Water	**------**	**---**	**X**	**X**	**---**	**58**	**a**	**10.1**	**ab**
Physpé 4	14 fl. oz.	X	X	---	---	42	a	9.5	ab
Ea153 HrpL$^-$	total 10^8 CFU/ml	---	X	X	---	38	ab	7.9	bc
HrpL$^-$ & C9-1S & A506 AprX$^-$	total 10^8 CFU/ml	---	X	X	---	26	ab	4.8	cd
C9-1S & A506 AprX$^-$	total 10^8 CFU/ml	---	X	X	---	26	ab	4.7	cd
Serenade	8 lb.	---	---	X	X	15	bc	3.6	cd
C9-1S & A506 AprX$^-$ then Mycoshield	total 10^8 CFU/ml 16 oz.	**---** **---**	**---** **---**	**X** **---**	**---** **X**	**8**	**c**	**2.2**	**de**
Mycoshield	16 oz.		---	X	X	8	c	2.1	de
Agri-mycin 17	8 oz		---	X	X	3	d	0.7	e

* Trees inoculated on 1April with 5 x 10^5 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain).
**Means of the sum of strikes per tree followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$. Data were transformed $\log_{10}(x+1)$ 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.

Golden Delicious apple. Trees used in the study were moderately sized with an average of 1240 blossom clusters per tree. Disease intensity was relatively high with symptoms of fire blight developing 23% of inoculated blossom clusters treated with water-only. All treatment scenarios that were initiated with an application of a biological and followed with a later application of Mycoshield provided control that was statistically equivalent to the level of disease suppression provided by Agri-mycin 17 (disease suppression in this group averaged 77%). The lowest

amount of disease occurred on trees that received AprX- plus *Pa*C9-1S followed by treatment with Mycoshield (82% disease suppression). The avirulent, *hrpL* mutant of *E. amylovora* also provided a relatively high level of disease control (75%), whereas a relatively low level of disease suppression (< 60%) was observed on trees that received Serenade, Physpé 4, Bloomtime Biological, Mycoshield, or BlightBan A506 plus Sequestrene 138.

Golden Delicious Apple, Corvallis, Oregon, 2005 Fire blight trial

		Date treatment applied*							
Treatment	Rate per 100 gallons water	30 March pink	14 April 30% bloom	20 April 80%-bloom	25 April full bloom	Mean number of blighted clusters per tree		Mean percent of clusters blighted	
Water control	**------**	**---§**	**X**	**X§**	**---**	**271**	**a**	**22.8**	**a**
Serenade	8 lb.	---	---	X	X	207	ab	17.1	ab
Physpé 4	14 fl. oz.	X	X	---	---	160	abc	16.8	abc
Bloomtime	22 oz	---	X	X	---	152	abc	14.1	bcd
Mycoshield	16 oz.	---	---	X	X	129	abcd	13.1	bcde
BlightBan A506 &	5 oz	---	X	X	---				
Sequestrene 138	16 oz	---	X	X	---	130	abcd	10.9	bcde
HrpL$^-$ & C9-1S & A506 AprX$^-$	total 10^8 CFU/ml	---	X	X	---	103	bcde	8.5	bcde
Famoxate	30 fl. oz.	---	---	X	X	85	cde	8.4	cde
C9-1S & A506 AprX$^-$	total 10^8 CFU/ml	---	X	X	---	92	cde	8.0	de
BlightBan A506	5 oz.	---	---	X	---				
then Mycoshield	16 oz.	---	---	---	X	69	de	7.6	de
BlightBan A506 &	5 oz.								
Sequestrene 138	16 oz.	---	---	X	---				
then Mycoshield	16 oz.	---	---	---	X	65	e	6.3	de
Agri-mycin 17	8 oz	---	---	X	X	58	e	5.7	de
BlightBan A506 &	5 oz.								
Sequestrene 138	16 oz.	---	X	X	---				
then Mycoshield	16 oz.	---	---	---	X	62	e	5.6	de
Ea153 HrpL$^-$	total 10^8 CFU/ml	---	X	X	---	67	de	5.5	e
C9-1S & A506 AprX$^-$ then	total 10^8 CFU/ml	**---**	**---**	**X**	**---**				
Mycoshield	16 oz.	**---**	**---**	**---**	**X**	**50**	**e**	**5.2**	**e**

Footnotes as above.

Rome Beauty apple. Trees used in the study were moderately sized with an average of 892 blossom clusters per tree. Disease intensity was low with symptoms of fire blight developing ~2% of inoculated blossom clusters treated with water-only. Based on analysis of mean strikes per tree, the antibiotic treatment of AgriStrep 17 provided a superior level of disease control. The biological treatment of A506 AprX- mixed with *Pa*C9-1S at 70% bloom followed by treatment with Mycoshield, or BlightBan A506 plus Sequestrene 138 at 70% bloom followed by treatment with Mycoshield also a high level of disease control (disease suppression with these two treatment combinations averaged 67%). All other treatments provided an intermediate level of fire blight suppression.

Rome Beauty Apple, Corvallis, Oregon, 2005 Fire blight trial

		Date						
Treatment	Rate per 100 gallons water	25 April 30% bloom	27 April 80%-bloom	2 May full bloom	Mean number of blighted clusters per tree		Mean percent of clusters blighted	
Water control	**------**	---	**X**[§]	**X**	**18.8**	**a**	**1.97**	**a**
Ea153 HrpL$^-$	total 10^8 CFU/ml	X	X	---	15.6	ab	1.55	a
BlightBan A506 then Mycoshield	5 oz. 16 oz.	X ---	X ---	--- X	12.0	abc	1.37	ab
C9-1S & A506 AprX$^-$	total 10^8 CFU/ml.	X	X	---	11.3	abc	1.09	ab
BlightBan A506 & Sequestrene 138	5 oz 16 oz	X	X	---	11.3	abc	1.28	ab
BlightBan A506 & Sequestrene 138 then Mycoshield	5 oz. 16 oz. 16 oz.	X ---	X ---	--- X	10.8	abc	1.25	ab
Mycoshield	16 oz.	---	X	X	9.5	bcd	1.16	ab
Erwinia rhapontici strain PINK	total 10^8 CFU/ml	X	X	---	9.0	bcd	1.08	ab
HrpL$^-$ & C9-1S & A506 AprX$^-$	total 10^8 CFU/ml	X	X	---	7.8	cd	.075	bc
C9-1S & A506 AprX$^-$ then Mycoshield	total 10^8 CFU/ml 16 oz.	**--- --- ---**	**X --- X**	**--- X**	**6.8**	**cd**	**0.87**	**b**

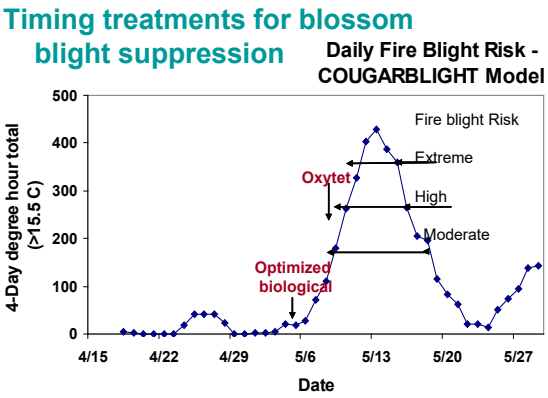
BlightBan A506 & Sequestrene 138	5 oz. 16 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	5.5	de	0.77	bc
Agri-mycin 17	8 oz	---	X	X	3.3	e	0.33	c

Footnotes as above.

Proposal for 2006:

Justification: The goals of this project are to understand to the biology and epidemiology of the fire blight pathogen, to develop and refine control methods for fire blight of pear and apple, and to integrate these technologies into commercial orchard management. In this context, we have evaluated many potential products for blight suppression with an emphasis on biopesticides. Based on recent results, we have begun to investigate combinations of biopesticides with chemical products. In the last two years, we have been encouraged by enhanced blossom blight suppression with a combination of early biopesticide treatment followed oxytetracycline treatment at full bloom. Importantly, fire blight was severe in several of the orchards.

Our objective for 2006 is to continue to evaluate optimized biopesticide strategies in combination with oxytetracycline.



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

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

Budget:

Proposed duration of objective: 1 year + ?

Year	Last Year (2005)	**Current request 2006**	Next year (2007)
Total	7,680	**8,530**	?

Budget specifics

Item	Last Year (2004)	Current Request	Next year (2007)
Salaries	4,000	**4,200**	
Benefits (65%)	2,080	**2,730**	
Supplies	300	**300**	
Travel (Medford)	300	**300**	
Plot Maintenance	1,000	**1,000**	
Total	7,680	**8,530**	

2006 Salary is 1.5 months of a senior faculty research assistant

Support we receive currently from other funding sources:

OSU AES, USDA IR-4 Biopesticide program $14,000 (pending)
USDA-NRICGP: 2004-2006, Research on avirulent *E. amylovora*.
Occasional grants-in-aid of research from chemical companies.

CONTINUING PROJECT REPORT **YEAR 1/3**

Project Title: New approaches to decay control of pear
PI: Robert A. Spotts
Organization: OSU Mid-Columbia Ag Research and Extension Center
Cooperators: Ag Canada (Peter Sholberg, Dan O'Gorman)
New Zealand HortResearch (Trish Virgin, Monika Walter)
Lincoln University (Alison Stewart)

Contract Administrator: Dorothy Beaton (ARF), dorothy.beaton@oregonstate.edu 541-737-4066

Objectives:
1. High tech approach to 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. Evaluation of *Muscodor* for decay control
5. Evaluation of chlorine dioxide-emitting paper for decay control
6. Preharvest fungicides for control of postharvest decay

Significant findings:
- Gray mold in stored pears (Bosc in New Zealand and Anjou in the Mid-Columbia) was related to the amount of DNA *of B. cinerea* on the surface at harvest.
- A real time PCR method to determine the concentration of decay spores (*Penicillium expansum* and *Mucor piriformis*) in dump tank and flume water is almost complete.
- We have identified 569 isolates of *Neofabraea* (bull's-eye rot) from decayed fruit in Washington and Oregon. *Neofabraea perennans* appears to be the most aggressive species
- *M. albus,* a biological control agent, significantly controlled blue mold, gray mold, and mucor rot of d'Anjou pear fruit.
- Preharvest sprays of Pristine, Topsin, and Ziram significantly reduced postharvest decay.

Methods:

1. High tech approach to decay risk prediction

This study in Oregon, Washington, and New Zealand focuses on the relationship between spores on the fruit surface and the amount of decay developing in stored fruit. The goal is to accurately predict the storage decay risk level for each orchard at harvest. Spores were washed from fruit surfaces and counted by dilution plating and also analyzed for pathogen DNA using real time PCR (qPCR). Stems were plated to determine presence of decay fungi. Fruit were stored at 30° F and evaluated for decay after 3 and 6 months. A standard test was developed to measure the yearly change in fruit resistance. Other factors that will be considered that may affect the risk level include preharvest rain and spray schedule.

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. Experiments were done to determine the amount of DNA in single spores, degradation rate of DNA in dead spores, and possible interference of packinghouse chemicals.

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

We have collected and identified, using multiplex PCR, 569 isolates of fungi from fruit with bull's-eye rot. Isolates are from Wenatchee, Yakima, Hood River, Medford, and California. All isolates are being screened for sensitivity to a large group of fungicides including thiabendazole (Mertect), ziram, mancozeb, Flint, copper, Topsin, and the new fungicides Pristine,

Penbotec and
Scholar. Screening is being done with a new ELISA plate protocol.

The importance of rain and over tree irrigation on bull's-eye rot was studied for a second year. Fruit from trees irrigated with over tree irrigation and under tree irrigation (control) were inoculated prior to harvest with *Neofabraea alba, N. malicorticis*, and *N. perennans*. Fruit was stored for 8 months and decay evaluated.

4. Evaluation of *Muscodor* for decay control.

Muscodor is a new biocontrol agent that controls disease by releasing a complex of natural volatiles that inhibit growth of fungi. This study determined if *Muscodor* controls the 3 main decays of pear.

Anjou pear fruit were wounded to simulate a stem puncture. Fruit were inoculated with spores of *Botrytis cinerea, Mucor piriformis,* and *P. expansum*. Rye grain colonized by *Muscodor albus* was added to the boxes at 0, 0.5, 1, 2, and 4 grams per liter of box space. Boxes were incubated at 41, 32, and 30° F. At the appropriate incubation time for each pathogen x temperature combination, lesion diameter was measured. Fruit also were inspected for phytotoxicity.

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

Anjou pear fruit were wounded at two locations to simulate stem punctures. Fruit were inoculated with spores of *B. cinerea* (gray mold), M. *piriformis* (Mucor rot), or *P. expansum* (blue mold). Inoculated fruit were placed into standard 40 lb cardboard fruit boxes with perforated polyethylene liners. "Long release" paper was used, and 3, 5, or 7 pieces placed inside the polyethylene liners. Control boxes had no paper. Fruit were stored at 30˚ F. Mucor rot was evaluated after 1 month, gray mold after 2 months, and blue mold after 3 months. Fruit also were visually evaluated for phytotoxicity.

6. Preharvest fungicides for control of postharvest decay

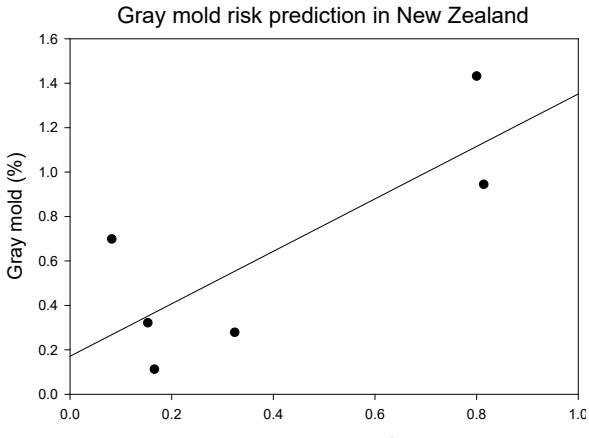
Several fungicides were applied to Anjou pear trees either 1 or 2 weeks before harvest.

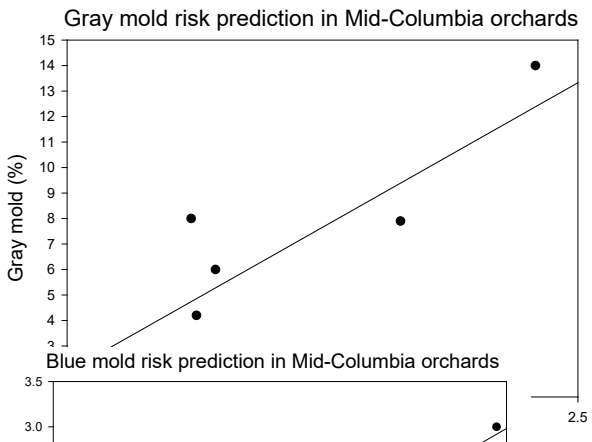
Fruit were harvested and drenched with water containing spores of *P. expansum*. Fruit were stored at 30˚ F and decay evaluated after 3 months. Fungicides included Pristine, Topsin M, and Ziram. Decay in treated fruit was compared to decay in fruit from unsprayed trees and to fruit drenched after harvest with Scholar.

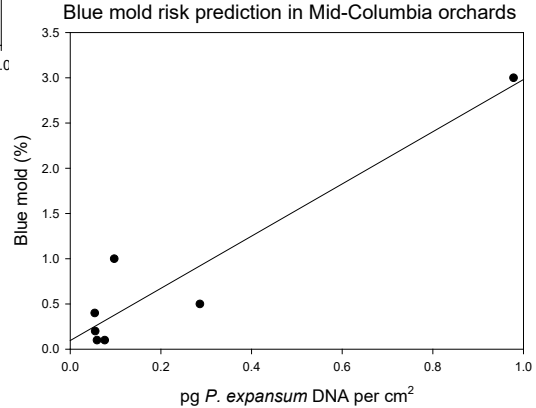
<u>Results and discussion:</u>

1. High tech approach to decay risk prediction

Gray mold in stored pears (Bosc in New Zealand and Anjou in the Mid-Columbia) was related to the amount of DNA *of B. cinerea* on the surface at harvest. Gray mold and *B. cinerea* DNA amounts were considerably higher in Mid-Columbia fruit than New Zealand fruit.







Less than 1% of the New Zealand fruit had blue mold, and decay was not related to *P. expansum* DNA on the fruit surface. However,

blue mold levels were higher in Mid-Columbia fruit, and decay was related to *P. expansum* DNA. No relationship was observed between stem end decay and colonization of stems with either *B. cinerea* or *P. expansum*.

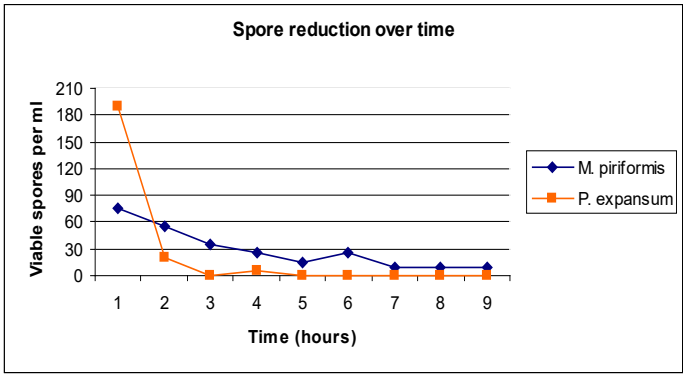
Because data points in the three graphs varied from above to below the regression line, it appears that factors in addition to the amount of surface pathogen DNA are affecting the amount of decay in storage. During the next year, we will be working on a decay risk model that also incorporates preharvest rainfall and preharvest fungicide applications.

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

DNA amount in a single spore: we determined using qPCR that the amount of DNA per spore of *P. expansum* is around 3.5 x 10^{-5} ng. This number is essential when extrapolating amount of DNA, obtained through qPCR, to spore population numbers. This number will also be determined for *M. piriformis*.

Degradation rate of DNA in packinghouse water: chemicals that kill decay spores are added to packinghouse water. We needed to determine whether dead spores will be counted along with live spores when quantifying DNA of spores in the water.

In a commercial packinghouse, spores of *P. expansum* were reduced 77% by SOPP within the first hour after collection and after 3 hours no spores remained viable. Spores of *M. piriformis* were killed at a much slower rate in SOPP. All spores were killed almost instantly in water containing chlorine, with no growth on plates even at time 0.



Sampling was done from packinghouse water containing SOPP at 3 sampling times (T0 + 1hr, T0 + 3hrs and T0 + 6hrs). Within one hour, 82% of *P. expansum* spores were killed in SOPP with Orzan flotation salt. Three hours after sampling, no spores were viable. No DNA was found after 3 hours, indicating that the DNA of dead spores had degraded and did not interfer with DNA measurements. Experiments will be repeated for *M. piriformis*.

Time	Spores/ml	DNA (ng/ml)
T0 + 1	350	1.6 x 10^{-2}
T0 + 3	0	0
T0 +6	0	0

Interference of orchard and packinghouse chemicals with qPCR: Chemicals tested include SOPP, chlorine, Ethoxyquin , and DPA . Results show that there was no significant interference of qPCR by these chemicals. Values are still being determined for flotation salts and pesticides. The experiment will be repeated with *M. piriformis.*

	Water	Ethoxyquin	SOPP	DPA	Chlorine
C(t) value	20.5	20.2	21.0	21.1	21.2

Dump tank sampling was done in 2004/2005 and will be continued in 2006.

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

We have identified 569 isolates of *Neofabraea* from decayed fruit. *N. malicorticis* was found only in samples from California. This fungus is common west of the Cascades. The

new, unnamed species of *Neofabraea* was most common in Medford, but one isolate was found in Hood River. *N. perennans* was the most common species in Wenatchee and *N. alba* the most common in Hood River and Medford. Many additional samples are needed from Yakima to determine which species are most common in that district.

Summary of *Neofabraea* collection - December 2005

Location	*N.alba*	*N.perennans*	*N.malicorticis*	*N sp. nova*	Total
Yakima	0	4	0	0	4
Wenatchee	16	69	0	0	85
Mid-Columbia	111	58	0	1	170
Medford	241	51	0	14	306
California	0	0	4	0	4
TOTAL	368	182	4	15	569

We have just begun to determine the sensitivity of the *Neofabraea* species to various fungicides. The results in the tables are very preliminary, and patterns may change as the number of isolates in the study increases. Thus far, Flint appears to have the least effect on *N. alba* and *N. perennans*.

Effect of fungicides on *Neofabraea alba in vitro*

Fungicide (half dose)	% Isolates inhibited ≥ 75%
Scholar	11
Topsin	78
Penbotec	83
Ziram	100

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

Fungicide (full dose)	% Isolates inhibited ≥75% *N. alba*	*N. perennans*
Flint	12	51
Topsin	61	76
Scholar	64	82
Pristine	79	74
Penbotec	88	71
Mertect	91	79
Copper	94	93
Ziram	94	82
Mancozeb	100	75

N. perennans is the most aggressive species of *Neofabraea*. Preharvest wetness has not had an affect on the incidence of bull's-eye rot. A third year of this study is in progress.

Bull's-eye rot of d'Anjou pear fruit related to *Neofabraea* species and preharvest wetness

	2004		2005	
Species	Wet	Dry	Wet	Dry
N. perennans	74b	100b	98c	90c
N. malicorticis	50b	79b	--	--
N. alba	18a	21a	64b	71b
Control	21a	27a	55a	26a
Average	41	53	73	62

4. Evaluation of *Muscodor* for decay control

M. albus significantly controlled blue mold, gray mold, and mucor rot of d'Anjou pear fruit at 30° F to 41° F. Phytotoxicity needs to be reduced before *M. albus* can be used commercially on pear fruit. As storage temperature was reduced, phytotoxicity decreased. No phytotoxicity was observed at the 4 gram per box rate at 30° F.

Control of pear decays with *Muscodor albus* in 2004-2005

	Percent gray mold[v]			***Percent mucor rot***[v]			***Percent blue mold***[v]		
Rate[w]	41° F	32° F	30° F	41° F	32° F	30° F	41° F	32° F	30° F
0	97b	97b	100c	100c	100c	100c	100c	100c	100d
0.5	6a	50a	72b	72b	39a	50b	61b	89bc	78bc
1	6a	47a	31a	70b	58b	45b	19a	84b	53a
2	3a	56a	67b	51b	61b	22a	14a	55a	69ab
4	3a	56a	53ab	26a	53ab	8a	28a	78b	89cd

[v]Numbers followed by the same letter within columns are not significantly different at P = 0.05 according to least significant difference test.

[w]Rate of *M. albus* per liter.

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

No statistically significant reductions in gray mold, mucor rot, or blue mold were observed with any of the rates of chlorine dioxide emitting paper compared with the "no paper" control. Blue mold was greater in boxes with 7 sheets of paper. The chlorine dioxide emitting paper is a potentially useful tool for decay reduction in fruit crops. However, additional research is necessary to develop paper with the correct amount of chemical to control decay without injuring the fruit.

Control of pear decays with chlorine dioxide emitting paper at 30°F

	Gray mold[x]	***Mucor rot***[x]	***Blue mold***[x]
Sheets[y]	Index[z]	Index[z]	Index[z]
0	37.9a	38.1a	21.8a
3	49.5a	ND	16.8a
5	31.2a	41.3a	16.0a
7	35.1a	33.3a	44.0b

[x]Numbers followed by the same letter within columns are not significantly different at P = 0.05 according to least significant difference test.

[y]Number of sheets of paper per packed box.

[z]Severity x incidence/100.

6. Preharvest fungicides for control of postharvest decay

Scholar, Topsin (1 and 2 week preharvest interval or PHI), Pristine (1 week PHI), and the Ziram standard significantly reduced total decay. Scholar was most effective for control of blue mold and gray mold, and Topsin was most effective for control of gray mold and bull's-eye rot. Pristine applied with a 1-week PHI was more effective than with a 2-week PHI for control of bull's-eye rot and total decay. There was no significant difference between Topsin at 1-week and 2-week PHI. All treatments were significantly better than the ziram standard for control of blue mold, but only Topsin was better than ziram for control of bull's-eye rot.

Table 1. Evaluation of preharvest fungicides for control of postharvest decay of d'Anjou pear fruit at MCAREC, Hood River, OR 2004-2005

		Percent decay			
Treatment and rate/A	PHI (wk)	Blue mold	Gray mold	Bull's-eye rot	Total decay
Control	--	23.2c	8.9c	5.9cd	42.0e
Topsin M 70WP 1.0 lb	2	9.9b	0.7a	0.4a	12.8ab
Topsin M 70WP 1.0 lb	1	7.7b	2.0a	0.3a	11.1a
Ziram 76DF 8.0 lb	2	18.5c	2.2ab	2.3b	26.5cd
Pristine 38WG 0.35 lb + Silgard 309 8.0 oz	1	9.7b	8.2bc	4.1bc	24.1bcd
Pristine 38WG 0.35 lb	1	5.4b	2.6ab	8.4bc	22.8bc
Pristine 38WG 0.35 lb	2	8.3b	7.1bc	16.3de	36.7de
Scholar 50WP 8.0 oz/100 gal	0	0.3a	0.6a	13.2f	15.7abc

BUDGET

Proposed Title: New Approaches to Decay Control of Pear
PI: Robert A. Spotts
Project duration: 2005-2007
Current year: 2006
Project total (3 years): $155,372
Current year request: $51,733

	Year 1(2005)	**Year 2 (2006)**	**Year 3 (2007)**
Salaries	34,008	34,049	36,092
Benefits	13,854	16,684	17,685
Supplies	1,000	1,000	1,000
Total	48,862	51,733	54,777

CONTINUING PROJECT REPORT **YEAR 1/3**
WTFRC Project #PR-05-502 **WSU Project #13C-3661-4366**

Project title: Control of Postharvest Decay in Pear
PI: Chang-Lin Xiao, Assistant Plant Pathologist
Organization: WSU Tree Fruit Research and Extension Center
Address, phone, e-mail: 1100 N. Western Avenue, Wenatchee; 509-663-8181 ext. 229; clxiao@wsu.edu

Cooperators: Dana Faubion, WSU Extension, Yakima
Pear packinghouses

Contract administrators: Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

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 (2005):
- When applied at 7 and 14 days before harvest, Pristine was effective against Phacidiopycnis rot and reduced Phacidiopycnis rot by 54-64% in comparison with the nontreated control. Surprisingly, Pristine was not effective against gray mold on d'Anjou pears in this trial. This is different from what we have observed on apples. We have observed that Pristine applied within 14 days before harvest was effective to reduce gray mold on Red Delicious and Fuji apples. The performance of Pristine on d'Anjou pears needs to be further studied.
- The new compound V-10135 was very effective to reduce incidence of gray mold compared with other treatments, but it was not effective against Phacidiopycnis rot.
- Ziram applied at 2 weeks before harvest reduced gray mold by only 11% and Phacidiopycnis rot by 24% on wound-inoculated fruit as compared with the nontreated control.
- Both Scholar and Penbotec at label rates were very effective to control Phacidiopycnis rot originating from infections of skin wounds. After fruit were inoculated with Phacidiopycnis, a 24-h delay of a treatment with either Scholar or Penbotec did not compromise the efficacy.

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. The registration of the new fungicide Elevate for use on pome fruits is still pending. Elevate will be included in the2006 study. 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 2 months of storage. Data were subjected to analysis of variance, and means of treatments will be separated by the Waller-Duncan K-ratio t test ($P = 0.05$).

To evaluate postharvest fungicides for control of Phacidiopycnis rot originating from wound infections, d'Anjou pear fruit that were not treated with any fungicides within 6 weeks before harvest were used in this trial. All fruit were wounded with a sterile nail head and inoculated by placing 20 µl of conidial suspensions at each wound. Inoculated fruit were dipped in sterile water

(control) or fungicide solutions within 2 h or at 24 h after inoculation. Fruit were tray-packed and stored at 32ºF in RA. Decay development was evaluated at 8 and 10 weeks after inoculation.

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 from the nontreated and fungicide-treated treatments were harvested. 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 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 6 months.

Results and discussion:

Preharvest fungicides for control of postharvest gray mold and Phacidiopycnis rot.

The purpose of this trial was to search for effective preharvest fungicides for control of gray mold and Phacidiopycnis rot originating from infections of wounds on fruit skin. In the trial conducted on d'Anjou pears, when applied at 7 and 14 days before harvest, Pristine was effective against and reduced Phacidiopycnis rot by 54-64% in comparison with the nontreated control (Fig. 1). Surprisingly, Pristine was not effective against gray mold on d'Anjou pears in this trial. This is different from what we have observed on apples. We have observed that Pristine applied within 14 days before harvest was effective to reduce gray mold on Red Delicious and Fuji apples. The performance of Pristine on d'Anjou pears needs to be further studied. The new compound V-10135 was very effective to reduce incidence of gray mold compared with other treatments, but it was not effective against Phacidiopycnis rot. Ziram applied at 2 weeks before harvest reduced gray mold by 11% and Phacidiopycnis rot by 24% on wound-inoculated fruit as compared with the nontreated control. Topsin-M applied at 7 days before harvest reduced gray mold by 15% and was not effective to reduce Phacidiopycnis rot on wound-inoculated fruit.

Postharvest fungicides for control of Phacidiopycnis rot originating from infection of wounds.

An experiment was conducted in both 2004 and 2005. The results of the 2005 experiment will be forthcoming. The results from the 2004 experiment are summarized in Table 1. After 10 weeks in cold storage, 84% of the non-treated fruit had Phacidiopycnis rot. The fruit treated with Scholar at 10 oz/100 gallon did not have decay, whereas 1.3% of the fruit had Phacidiopycnis rot when Scholar was used at both 6 and 8 oz/100 gallon. Only 1.3% of the fruit developed Phacidiopycnis rot when Scholar @ 8 oz/100 gallon was applied 24 h after inoculation, indicating that Scholar may have a post-infection activity against Phacidiopycnis rot. No decay developed on Penbotec-treated fruit. The inoculated fruit treated with Penbotec 24 h after inoculation did not have decay symptoms, indicating that Penbotec may have a post-infection activity against Phacidiopycnis rot.

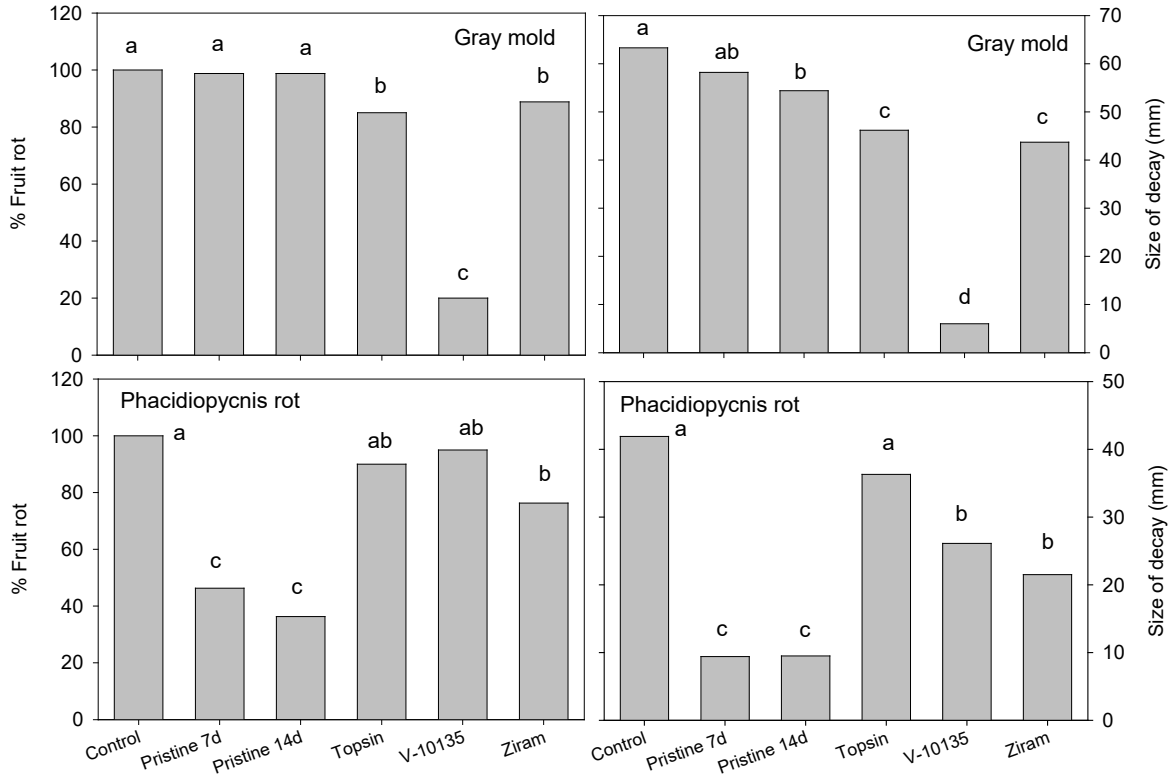


Fig. 1. Effectiveness of preharvest fungicides in controlling postharvest gray mold and Phacidiopycnis rot originating from infections of wounds on d'Anjou pears. Pristine @ 14.5 oz/A were applied at 7 or 14 days, Topsin M @ 1.0 lb/A at 7 days, V-10135 @ 2.5 lbs/A at 7 days and Ziram @ 8 lbs/A at 14 days before harvest.

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

One experiment was conducted in 2005. Pristine, Topsin M, TBZ, Scholar and Penbotec were tested in the experiment. The fruit are still in cold storage for decay development. Results from this experiment will be forthcoming after it is completed.

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. The fruit are still in cold storage for decay development. Results from this experiment will be forthcoming after it is completed.

Table 1. Efficacy of Scholar or Penbotec as a drench treatment for control of Phacidiopycnis rot originating from infection of wounds.

Treatment	% of Fruit Infected	Lesion size (mm)
Nontreated control	83.8	41
TBZ 16 oz applied within 2 hr after inoculation	1.3	0.5
Scholar 6 oz applied within 2 hr after inoculation	1.3	0.4
Scholar 8 oz applied within 2 hr after inoculation	1.3	0.5
Scholar 10 oz applied within 2 hr after inoculation	0	0
Scholar 8 oz applied at 24 hr after inoculation	1.3	0.4
Penbotec 8 oz applied within 2 hr after inoculation	0	0
Penbotec 16 oz applied within 2 hr after inoculation	0	0
Penbotec 32 oz applied within 2 hr after inoculation	0	0
Penbotec 16 oz applied at 24 hr after inoculation	0	0

Budget:

Project title: Control of Postharvest Decay in Pear
PI: Chang-Lin Xiao
Project duration: 3 years
Current year: 2006
Project total (3 years): $84,561
Original budget request: $28,610

Year	2005	**2006**	2007
Total	$26,530	**$28,610**	$29,421

Current year breakdown:

Item	2005	**2006**	2007
Salaries[1]	$13,000	**$14,803**	$15,395
Benefits (37%)	5,200	**5,477**	5,696
Wages[2]	3,000	**3,000**	3,000
Benefits (11%)	330	**330**	330
Supplies [3]	4,000	**4,000**	4,000
Travel [4]	1,000	**1,000**	1,000
Total	$26,530	**$28,610**	$29,421

[1] Salary for a Scientific Assistant (Robin Boal at 0.3 FTE).
[2] Time-slip helpers.
[3] Culture media, chemicals, Petri dish plates, and fungicides. Cost of fruit bought from commercial orchards. Cell phone charges are allowed.
[4] We will be using a leased vehicle.

CONTINUING PROJECT REPORT **YEAR 2/3**

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

Objectives:
In 2005:

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

In 2006:

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

Significant findings:

- Over three seasons, we have been unable to detect *Erwinia amylovora* from ~5000 d'Anjou pear fruit sampled from commercial orchards in the Rogue, Hood River, Yakima, Wenatchee and Okanogan Valleys of the Pacific Northwest.
- *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.
- Over two years, we have been unable to detect *Erwinia amylovora* inside mature symptomless pear fruit harvested from diseased pear trees located at Medford, OR and Wenatchee, WA.
- Pear fruit contaminated with *Erwinia amylovora* and subsequently wounded at harvest require an initial dose of > 10,000 cells in the wound to allow for persistence of the pathogen on the fruit through a 7 week cold storage period.

Justification: Export of winter pears grown in the Pacific Northwest into countries where fire blight does not occur is restricted by phytosanitary concerns over the possible contamination of fruit with the fire blight bacterium, *Erwinia amylovora*. Similar concerns have been applied to apples, but 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. 26 d'Anjou pear orchards in the Rogue, Hood River, Yakima, Wenatchee and Okanogan valleys of the Pacific Northwest were surveyed within one week of commercial harvest. For 17 of the surveyed orchards, no visible fire blight infections were

present whereas 9 orchards had visible fire blight strikes within the orchard or in an adjacent block. 100 healthy fruit were selected from each orchard; 4 from each of 25 randomly selected trees. Each fruit was 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.

Objective 2. Field trials were conducted in d'Anjou and Bosc pear and Gala apple orchards to evaluate survival of *E. amylovora* on fruit surfaces. Treatments were: 1) Freeze-dried cells of the pathogen resuspended in water at 10^7 CFU/ml and sprayed, or 2) ooze from immature pear painted directly onto the calyx end of developing fruits. Inoculation times were June and July. Fifteen fruit per treatment were sampled at 1 hour, and 3, 7, 14, and 35 (and 56 for Anjou and Bosc pear) days after inoculation; these fruit were processed as described under Objective 1.

Calyx-end survival of bacteria was evaluated in the same orchards. Freeze-dried cells of *E. amylovora*, a non-pathogenic mutant of *E. amylovora*, and *Pantoea agglomerans* C9-1 resuspended in water were sprayed onto flowers at full bloom (1μm fluorescent microspheres were co-inoculated with the pathogen to track that flowers that received the inoculum spray.) Flowers and immature fruit were sampled over the summer and processed as described above. At harvest, 300 fruit per treatment per cultivar (2700 fruit total) were processed through a SOPP dump tank followed by 6 weeks of cold storage. Periodically, fruit were sampled to the measure residual bacterial population in association with calyx tissues.

Objective 3. Experimental plots were established in d'Anjou orchards located in Medford, OR and Wenatchee, WA. Flower clusters on trees were inoculated with the fire blight pathogen in early spring, and disease symptoms were allowed to develop and advance through the summer on two or more limbs on each tree. At harvest, 100 fruit from diseased trees at each location were assayed for the external presence of *E. amylovora* following methods described under Objective 1. After a 6-week cold storage period, the same fruit were assayed for the internal presence of the pathogen. Stem and calyx tissue were removed from the fruit and discarded; the core tissue was removed with a flamed no. 9 cork borer. DNA was extracted from a subsample of core tissue; recovered DNA was subjected to polymerase chain reaction (PCR) with primers specific for pEA29, a plasmid that is ubiquitous in and specific to *E. amylovora*. Remaining core tissues were incubated in an enrichment medium prior to plating on Miller-Schroth agar.

Objective 4. 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 (~ I 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% SOPP (sodium ortho-phenylphenate) for pear or 100 ppm bleach for apple, then placed in 3ºC 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 and discussion:

Objective 1. In 2005, a total of 2600 fruit were sampled from 17 apparently disease-free orchards and 9 orchards with disease in or adjacent to the plot. Bacteria (e.g., *Pseudomonas* and *Pantoea* spp.) were recovered occasionally from fruit washings, but *E. amylovora* was not recovered from any commercial sample. A few cells of *E. amylovora* were recovered from one fruit harvested from an experimental orchard in Wenatchee, Washington (Table 1).

Table 1. Incidence of *E. amylovora* on d'Anjou pear fruit with from commercial and experimental orchards in 2005.

Orchard location[x]	Harvest date	No. fruit	Control Ea153N	*Erwinia amylovora*	*Pseudomonas* spp.[z]	Disease in orchard	Other spp.
Selah, WA	9/05	100	2/2 [y]	0%	48%	No	100%
Selah, WA	9/05	100	2/2	0	62	No	100
Selah, WA	9/05	100	2/2	0	89	No	100
Yakima, WA	9/05	100	2/2	0	27	No	100
Yakima, WA	9/05	100	2/2	0	20	No	100
Yakima, WA	9/05	100	2/2	0	22	No	100
Cashmere, WA	9/07	100	2/2	0	43	No	100
Cashmere, WA	9/07	100	2/2	0	56	Yes	100
Cashmere, WA	9/07	100	2/2	0	58	Yes	100
Cashmere, WA	9/07	100	2/2	0	45	Yes	100
Cashmere, WA	9/07	100	2/2	0	67	No	100
Cashmere, WA	9/07	100	2/2	0	21	Yes	100
Wenatchee, WA **exp.**	9/07	100	2/2	1	67	Yes	100
Wenatchee, WA **exp.**	9/07	100	2/2	0	59	No	100
Medford, OR **exp.**	9/09	100	2/2	0	68	Yes	100
Medford, OR	9/09	100	2/2	0	53	No	100
Hood River, OR	9/12	100	2/2	0	53	No	100
Hood River, OR	9/12	100	2/2	0	27	Yes	100
Odell, OR	9/12	100	2/2	0	48	Yes	100
Mallot, WA	9/13	100	2/2	0	71	No	100
Mallot, WA	9/13	100	2/2	0	32	No	100
Mallot, WA	9/13	100	2/2	0	21	No	100
Willow Flats, OR	9/19	100	2/2	0	20	No	100
Parkdale, OR	9/19	100	2/2	0	25	Yes	100
Parkdale, OR	9/19	100	2/2	0	33	No	100

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

Objective 2.

Trees used in the study averaged 50 to 200 fruit per tree. During the experiments, daily maximum temperature averaged 80ºF (max. temp. 102ºF). Recovery of *E. amylovora* immediately after spraying re-suspended cells or painting of ooze was 100% (n = 270). Population size was reduced by 50% after 2 days (Tables 1-3). By 14 and 35 days after inoculation, fruit on which *E. amylovora* could be detected had declined to 44 and 6 %, respectively. A5 35 days, *E.amylovora* was recovered from only 2 fruit of d'Anjou pear (5 cells total) and 2 fruit of Bosc pear (43 cells

total). No cells of the pathogen were recovered at 56 days. Fire blight symptoms were not observed on inoculated trees.

Table 1. Population size of recovered *E. amylovora* from inoculated d'Anjou pear fruit in 2005

			Day after inoculation [x]				
Treatment[y]	CFU[z]	Sample interval	0	3	7	14	35
Check	-	6/09 – 7/14	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	6/09 – 7/14	1.25E+05	7.2E+00	1.1E+00	0.0	0.0
Ea153N Ooze	10^9	6/09 – 7/14	1.28E+07	1.3E+04	5.5E+01	6.0E+00	0.0
Check	-	7/14 – 8/18	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/14 – 8/18	4.2E+05	0.0	9.0E+03	0.0	0.0
Ea153N Ooze	10^9	7/14 – 8/18	8.2E+06	1.8E+05	1.4E+02	2.1E+01	2.0E+00

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

Table 2. Population of recovered *Erwinia amylovora* from inoculated Bosc pear fruit in 2005

			Days after inoculation[x]				
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	6/09 – 7/14	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	6/09 – 7/14	1.2E+04	1.8E+03	0.0	0.0	0.0
Ea153N Ooze	10^9	6/09 – 7/14	1.5E+07	2.0E+05	2.5E+02	1.4E+02	2.1E+01
Check	-	7/14 – 8/18	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/14 – 8/18	5.5E+05	0.0	0.0	0.0	0.0
Ea153N Ooze	10^9	7/14 – 8/18	5.2E+07	8.3E+04	3.6E+01	7.1E+00	0.0

Table 3. Population of recovered *Erwinia amylovora* from inoculated Gala apple fruit in 2005

			Days after inoculation[x]				
Treatment[y]	CFU[z]	Sampling interval	0	3	7	14	35
Check	-	5/25 – 6/29	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	5/25 – 6/29	3.3E+04	1.3E+03	0.0	0.0	0.0
Ea153N Ooze	10^9	5/25 – 6/29	2.3E+07	2.4E+05	1.8E+02	4.4E+01	0.0
Check	-	7/19 – 8/23	0.0	0.0	0.0	0.0	0.0
Ea153N Freeze-dried	10^7	7/19 – 8/23	2.0E+04	0.0	0.0	0.0	0.0
Ea153N Ooze	10^9	7/19 – 8/23	1.9E+05	2.7E+03	7.1E+01	4.7E+00	0.0

Calyx end survival:
For the calyx end survival experiment, by mid summer, immature fruit sprayed with Ea153N (pathogenic strain) had a recovery incidence of 10% of fruit with populations that averaged 1 to 10 cells per fruit; for Ea153 HrpL- a (non-pathogenic strain applied a higher dose than the pathogenic strain), the recovery incidence was 20% of fruit with populations that averaged 1000 cells per fruit; for *P. agglomerans* C9-1, the recovery incidence was 100% of fruit populations that also averaged 1000 cells per fruit. No *E. amylovora* (pathogenic or non-pathogenic) was detected on calyx tissue at harvest or during storage. *P. agglomerans*, however, persisted on 73% 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 (76, 82, and 93% for d'Anjou, Bosc and Gala, respectively).

Objective 3.
For both the Medford and Wenatchee experiments, 100 fruit were sampled from the diseased trees at harvest and analyzed for external and internal contamination by *E. amylovora* 153N. External fruit washings were negative for the detection of the pathogen with the exception of one fruit from Wenatchee (32 cells identified as *E. amylovora* (Table 1)). The pathogen was not detected from internal core tissues of any fruit as determined by an enrichment broth recovery method and by polymerase chain reaction (PCR).

Objective 4.
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 (pear) or bleach (apple) dump tank and stored at 0-4ºC. Pathogen cells were detected on both pear and apple fruit prior to dump tank submersion. After immersion, cells could be detected on pear fruit at the higher inoculum concentration (10,000 cells) but not at the lower concentration (1000 cells). On day 49 (end of storage period), an average of 149 viable cells were recovered on a total of 3 fruit in Corvallis experiment, and 1 viable cell recovered from one fruit in Wenatchee experiment.

After the cold storage period, remaining fruit in the Corvallis experiment were placed at room temperature for 8 weeks. After this warm incubation period, a total nine cells of *E. amylovora* were recovered from two of the fruit.

On Braeburn apple, after 2 weeks of storage, 86 viable cells were recovered from one fruit from the 10,000 cell inoculation, but all other post-dump tank samples were negative for pathogen recovery.

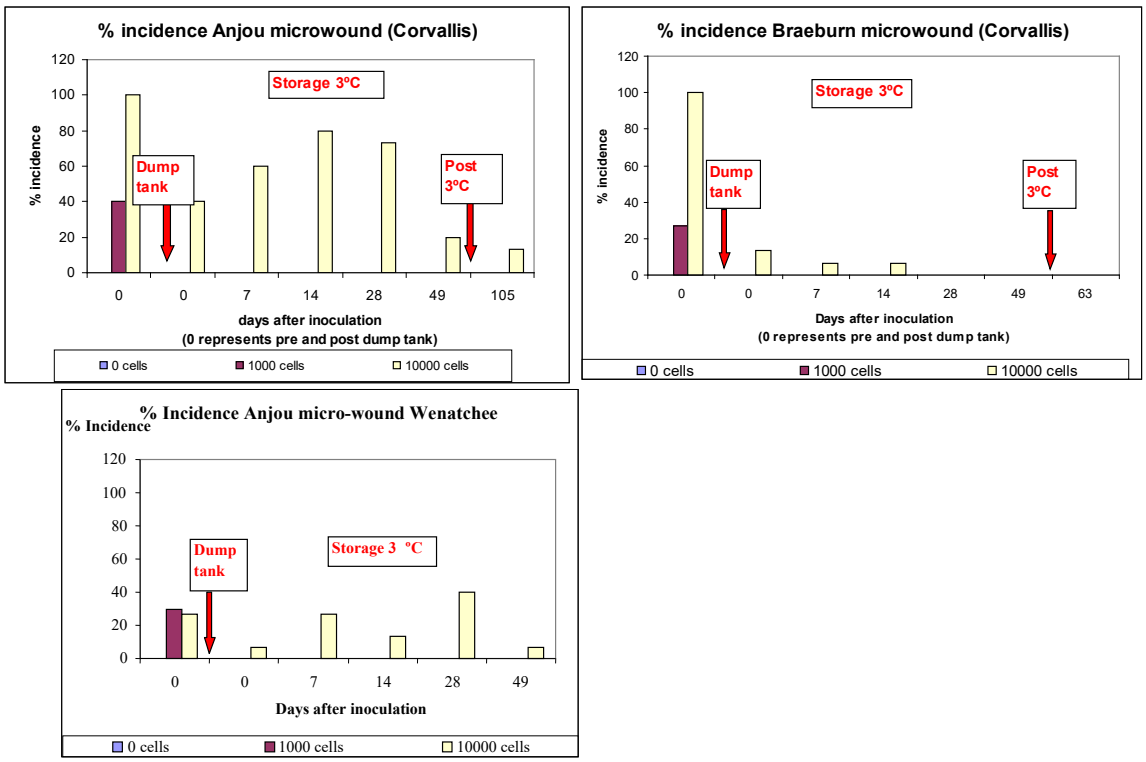


Figure 1. Average percent recovery of Ea153N from micro wounded fruit. Top panels represent Corvallis, OR experiments on d'Anjou pear and Braeburn apple. Lower panels represent Wenatchee, WA experiments on d'Anjou pear and Gala apple.

Proposal for 2006:

It is our goal to have three to four location·years to support the conclusions outlined above in the 'significant findings' section of this report. The two objectives of this the study where we consider the data incomplete concern survival of the pathogen on the fruit calyx, and understanding the potential for pathogen survival in wounds.

With the calyx end survival, the data indicate that *E. amylovora* can survive on the calyx, but survival is characterized by small numbers of cells that are declining over time. We need an additional season to confirm this observation.

In contrast, the microwound inoculation studies are indicating that the tissues of mature pear fruit tissues are more supportive of growth of *E. amylovora* than are mature apple fruit. Importantly**,** the data also show that the likelihood of pathogen persistence in association with pear tissues is dose-dependent. Based on our survival work in experimental orchards, the cell dose at which persistence in a wound may occur (10,000 cells) is at most a transient event. Also, in our three year survey of commercial orchards, no cells of *E. amylovora* cell have been recovered form fruit. We expect that persistence of *E. amylovora* on pear fruit tissue to be closely scrutinized by regulatory authorities, and thus additional data are needed to support the conclusion.

Work we will conduct in 2006

- Repeat orchard studies of pathogen survival in association with calyx end of pear and apple fruits.
- Repeat microwound inoculation studies to fully understand the dose dependent persistence of E. amylovora with pear fruit.
- Analyze and summarize the data from all four objectives. Our goal is to submit the data in autumn 2006 for publication in peer-reviewed journal (*Phytopathology*). Expected title of paper: 'An assessment of risk of movement of *Erwinia amylovora* in association with mature symptomless fruit of winter pear'.

Budget:

Proposed duration of objectives: 1 year

Year	Last Year (2005)	**Current and final request (2006)**	Next year
Total	see below	**$29,000**	project finished

Budget specifics

Salaries **6 mo. FRA**		**16,200**	
Benefits (65%)		**11,010**	
Supplies		**1,290**	
Travel (Medford)		**250**	
Plot Maintenance		**250**	
Total		**29,000**	

Current year: 2006 (4th year of project)

Funding history:

Budget in 2004 and 2005: $0 WPCC/WTFRC, $63,000 each year from USDA FAS via NHC
Budget in 2003**:** $36,426 WPCC

Other funding sources (in-kind):

Oregon Agricultural Experiment Station
USDA ARS collaborators (Wenatchee and Corvallis)

CONTINUING PROJECT REPORT **YEAR 2/3**

Project title: MCP and edible coating to improve storage life and marketing quality of pears
PI: Jinhe Bai
Project Staff: Kristi Barckley (Research Assistant) and Debra Laraway (Technician)
Organization: Oregon State University, Mid-Columbia Agricultural Research and Extension Center
Address, phone, e-mail: 3005 Experiment Station Dr., Hood River, OR 97031,(541) 386-2030
E-mail: jinhe.bai@oregonstate.edu
Cooperator(s): Dr. Robert A. Spotts, Oregon State University, Mid-Columbia Agricultural Research and Extension Center
Dr. Peter Sanderson, Pace International.

Objectives (2005)

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

Objectives (2006)

1. To understand influence of preharvest application, harvest maturity and postharvest application (low-dose multi-application) of MCP on 'd'Anjou' pears, focusing on the ripening capacity and superficial scald incidence of fruit.
2. To develop thermofogging technology of ethoxyquin to prevent superficial scald of 'd'Anjou' pears – multiple application.
3. To understand influence of coating thickness on internal gas concentration and fruit quality of Anjou pears.
4. To improve pear salad quality by delaying harvest.

Significant findings:

- Incidence of superficial scald was decreased by delayed harvest for one week plus postharvest MCP application.
- Regeneration of ripening ability of Anjou pears treated with MCP was improved by decreasing treatment temperature and duration.
- Thermofogging of ethoxyquin substantially controlled superficial scald of Anjou pears. A dosage of 120 g per ton Xedaquin A applied by thermofogging gave similar residue and scald control efficiency as a 1000 ppm drench.
- Thermofogging of DPA substantially controlled Anjou pear scald.
- A soybean oil emulsion was developed and evaluated as a pear coating.

Methods:

1. Preharvest application of MCP (a week before harvest):
   1) Fill spray tank with water, add oil adjuvant and swirl for a few seconds. Add MCP powder (AFXRD-020) in with swirling, and make up the volume. (Do not shake sprayer to mix; swirling only).
   2) Wait at least 5 minutes before applying spray, but no later than one hour after completion of swirling mix.
2. Postharvest MCP application:
   2003-2004: 300 ppb for 24 hour at 68F in a 40 m^3 air-tight room at harvest.
   2004-2005: 300 ppb for 6 hours at 33F in a 40 m^3 air-tight room at harvest (changes were made because Anjou did not ripen).
   2005-2006: 55 ppb for 24 hours at 33F in a 4 m^3 plastic tent at harvest, combined with/without additional treating during storage with same dosage and condition. The

additional treatments are conducted after 2 and/or 4 months of RA storage, and after 4 and/or 6 months of CA storage (low-dose multi-application of MCP, changes were made because of the difficulty in ripening and unsatisfied scald control).

3. Delayed harvest:

We hypothesized that fruit harvested later than commercial maturity will have greater yield, ripen and be less susceptible to scald. Fruit harvested at commercial maturity, one-week and two-weeks after commercial maturity were analyzed for ripening ability and scald incidence.

4. Preconditioning regime:

1) Preconditioning: After 3/4 and 6 months of RA storage and 6 and 8/9 months of CA storage, fruit are transferred from the cold storage to dark preconditioning rooms at 50°-68°F for 5, 10 and 15 days. Then fruit are transferred to 68°F for 7-14 days to simulate marketing life (shelf life).

2) Evaluation of ripening capacity of pears: For Anjou pear the standard is flesh firmness $\leq$ 6 lb.

5. Thermofogging:

Thermofogging technology was used to apply ethoxyquin (trade mark: Xedaquin A) with dosages of 60-120 grams per metric ton (g/T) or DPA (Trade mark: Xedamine A) with dosages of 20-40 g/T. After 4-6 months of storage, evaluate the superficial scald and phytotoxicity.

6. Formulation and evaluation of natural coatings:

Formulate nature coatings with low shininess, high carbon dioxide permeability, and antioxidant (anti-scald) function, and evaluating the proper dilution.

7. Evaluation of superficial:

Incidence: superficial scald was classified according to scald area in which 0.5 cm^2 or less scald area = very slight; 0.6 cm^2 to 1.0 cm^2 = slight; 1.1 cm^2 to 3.0 cm^2 = moderate; and greater than 3.0 cm^2 = severe. Superficial scald which scored from slight to severe was considered commercially unacceptable.

Index: scald index was calculated by

$$\text{Scald index} = \sum SiFi / 4Fn$$

Where I = 0 - 4, Si is scale, Fi is fruit number and Fn is total fruit number.

S0 = 0 (clear); S1 = 1 (very slight); S2 = 2 (slight); S3 = 3 (moderate); S4 = 4 (severe).

Fn = F1 + F2 + F3 + F4 + F0 (clear, no scald).

Results and Discussion

1. Anjou pears were harvested at three maturities, commercial maturity (flesh firmness = 15 lb), one-week delayed harvest (14 lb) and two-week delayed harvest (13 lb). After harvest fruit were immediately treated with 300 ppb MCP at 30°F for 6 h and then were stored at 30°F in either RA or CA. After 3 and 6 months of RA storage, or 6 and 9 months of CA storage fruit were transferred to 50°F for 0-15 days for preconditioning, followed by re-storage at 33°F for 0-21 day(s) to simulate shipping. After various days of preconditioning and shipping the fruit were transferred to 68°F for 7 days to ripen.

After 3 months of RA storage or 6 months of CA storage, superficial scald occurred on the control fruit and the incidence was 100%. However, MCP treatment completely controlled scald. Fruit harvested at commercial maturity and one week delayed did not ripen regardless of preconditioning (50°F) and transportation (33°F) durations. Fruit harvested two weeks delayed ripened after 15 days of preconditioning at 50°F followed by 7 days at 68°F regardless of transportation time at 33°F (data not shown).

After 6 months of RA storage or 9 months of CA storage, superficial scald occurred on not only the control fruit (100%) but also MCP treated fruit (up to 70%). The incidence of scald was higher in fruit harvested at commercial maturity compared to that harvested by one and two-week delay. Furthermore, fruit with one week delayed harvest had lowest incidence (Fig. 1). However, Fruit harvested by two-week delayed was the only group which ripened after preconditioning (Fig. 2). The results indicate that delayed harvest (two weeks) improved ripening ability of fruit treated by MCP, and decreased superficial scald as well.

Last year our MCP treatment condition was 300 ppb for 24 hours at 68F. With this condition, the fruit could not ripen after storage (up to CA 9 months and RA 6 months) and preconditioning (up to 20 days at 50-68°F). This year, the MCP treatment condition was changed to 6 hours at 30°F. However, consistant control of scald could not be obtained. (Fig. 1 and Fig.2)

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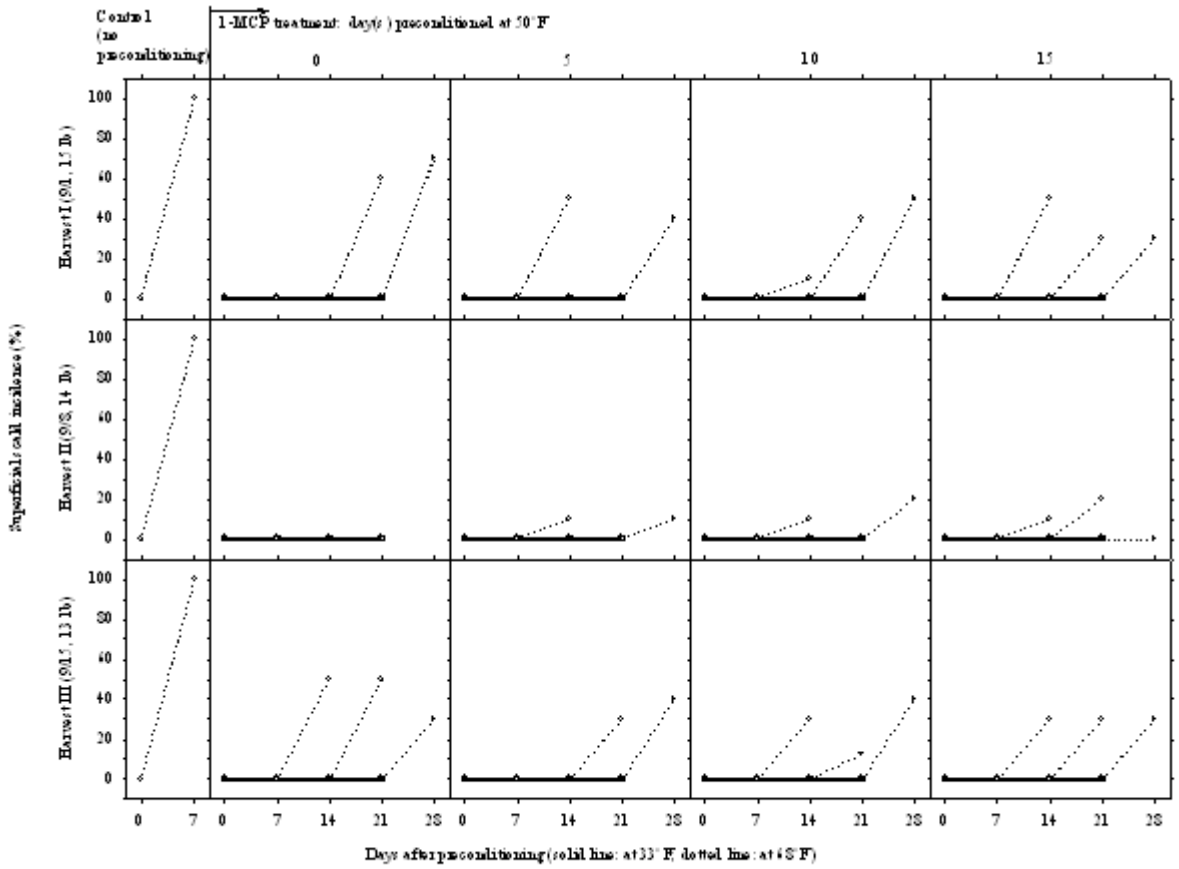

Fig. 1. Superficial scald incidence of 'd'Anjou' pears harvested at three maturities, treated with 300 ppb MCP (6 h at 33°F), and stored at 30°F for 6 months. Stored fruit were transferred to 50°F for 0-15 day(s) for preconditioning. After preconditioning fruit were removed to 33°F for 0-21 day(s) to simulate transportation, and finally by exposing to 68°F for 7 days to simulate marketing.

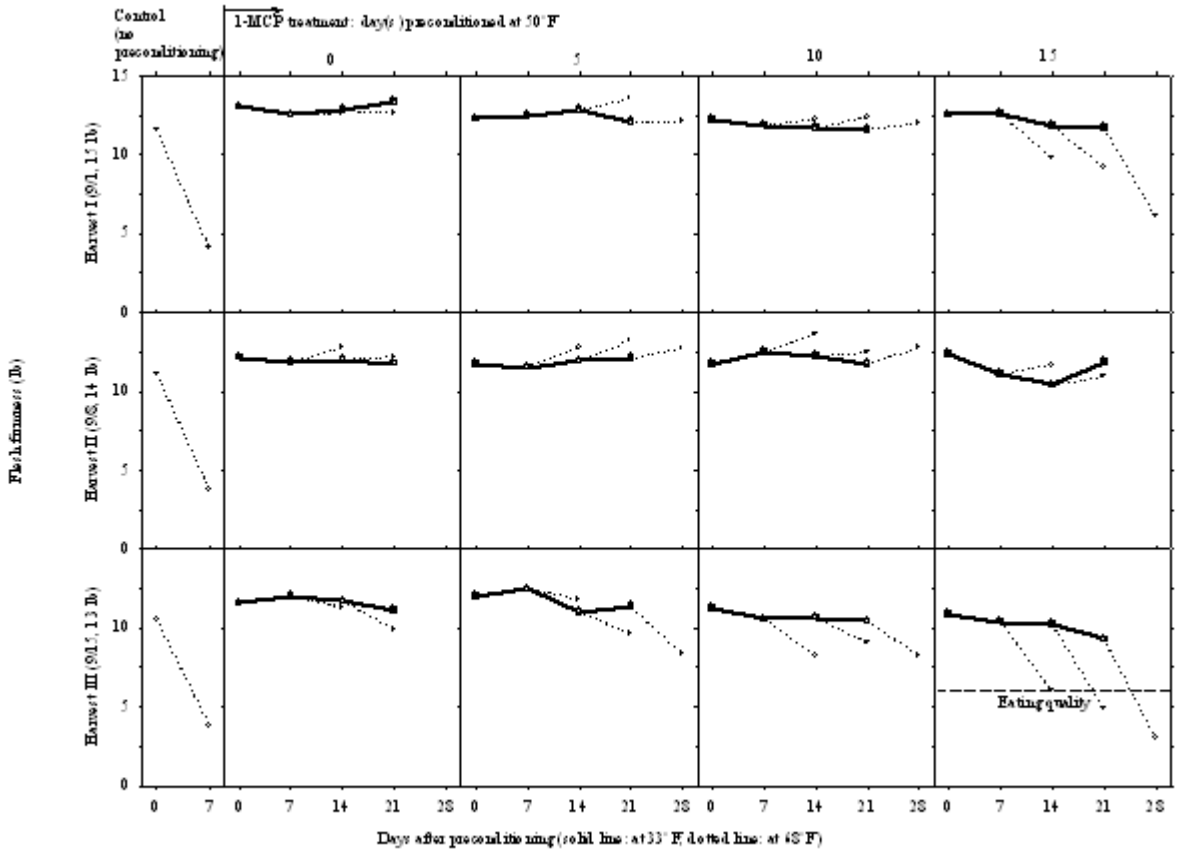

Fig. 2. Flesh firmness of 'd'Anjou' pears harvested at three maturities, treated with 300 ppb MCP (6 h at 33°F) and stored at 30°F for 4 months. Stored fruit were transferred to 50°F for 0-15 day(s) for preconditioning. After preconditioning, fruit were removed to 33°F for 0-21 day(s) to simulate transportation, and finally by exposing to 68°F for 7 days to simulate marketing.

2. Anjou pears were thermofogged with ethoxyquin (Xedaquin A @ 60 g, 90 g, or 120 g/T) or DPA (Xedamine A @ 2 g0, 30 g, or 40 g/T) at harvest and after 70 days of storage. After 5 month storage, fruit were evaluated for incidence of superficial scald and phytotoxicity. As a comparison, fruit treated with conventional ethoxyquin and DPA drench, along with non-chemical control (non-treatment control and water dip control) were also tested.

Best scald control was achieved from dipping fruit in 1500 ppm ethoxyquin (scald incidence of 2.5%), although it was not significantly different from 1000 ppm ethoxyquin drenching and 120 g/T Xedaquin A fogging, as these treatments also controlled scald at levels that were commercially acceptable. Xedaquin A fogging at 60 or 90 g/T and Xedamine A fogging at 40 g/T also significantly decreased incidence of scald. However Xedamine A below 30 g/T was too low to control scald.

Phytotoxicity was observed in fruit treated with 1500 ppm ethoxyquin drench, all rates of Xedaquin A fogging, and 30 g/T Xedamine A foging, although with low incidences (the highest was 1.2% in 1500 ppm ethoxyquin drench).

Ethoxyquin residues on fruit following the initial fog treatment were less than half those from drench applications. Following the second fog application, ethoxyquin residues from the 90 g/T and 120 g/T treatments were comparable to those on fruit following initial dipping at 1000 ppm (Table 1). DPA residue levels were low following the initial fogging but doubled after the second fogging. However, measured residues did not correlate well with dose in either the initial or second applications of either chemical. This was probably due to the treatment chamber dimensions, in which fruit volume was considerably less than total room volume. Under ideal conditions, room volume should be at least ¾ occupied by the target

product.

Xedaquin A showed promise as an effective treatment for control of superficial scald without an increase in the level of phytotoxicity seen in dipped fruit. These results suggest that further trials are warranted (Table 1).

Table 1. Effect of ethoxyquin and DPA applications by drenching or thermofogging on incidence of superficial scald, decay and phytotoxicity of Anjou pears[x].

Treatment			Superficial scald (%)	Phytotoxicity (%)	Ethoxyquin residue (ppm)		DPA residue (ppm)	
					Init.	2nd	Init.	2nd
Non-treated control		-	45.7 a[y]	0	0 d	-	0 c	-
Water		dip	30.7 b	0	0 d	-	0 c	-
Ethoxyquin	1000 ppm	dip	8.0 cd	0	1.4 b	-	-	-
Ethoxyquin	1500 ppm	dip	2.4 d	1.2	2.5 a	-	-	-
Xedaquin A	60 g/T	fog	17.3 c	1	0.6 c	1	-	-
Xedaquin A	90 g/T	fog	16.8 c	0.3	0.7 c	1.6	-	-
Xedaquin A	120 g/T	fog	7.2 cd	0.7	0.8 c	1.7	-	-
Xedamine A	20 g/T	fog	52.5 a	0	-	-	0.3 b	0.8
Xedamine A	30 g/T	fog	46.4 a	0.3	-	-	0.5 a	0.6
Xedamine A	40 g/T	fog	18.0 c	0	-	-	0.3 b	1.1
P value[z]			< 0.001	0.505	< 0.001	-	< 0.001	-

[x] Thermofogging was applied at harvest and again after 70 days of storage with same dosage. Drenching was applied only at harvest.

[y] Means were separated with Duncan's multiple range test at P = 0.05. Means followed by a common letter are not significantly different.

[z] Level of *F*-statistic from one-way ANOVA in which grower lots (2) were treated as block effects.

3. 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 non-coating control were applied as comparison. After 4 months of RA storage, fruit coated or non-coated were held at 68°F for up to 2 weeks. The gas concentration inside the fruits for the various coatings ranged from 7-11% CO2 and 14-8% O2. Superficial scald was observed in control fruit with 100% incidence and scald index 1.0. Carnauba and carnauba + shellac mixture decreased the scald index to 0.5-0.7. However, soybean emulsion significantly decreased scald index to 0.28 (Table 2). These coatings alleviated the severities of scald but did not exterminate scald. There was no difference between coating treatments based on scald incidence (Table 2).

   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 2. 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.

Budget

Project title: MCP and edible coating to improve storage life and marketing quality of pears
PI: Jinhe Bai
Project duration: 2004-2006
Current year: 2006
Project total (3 years): $83,619
Current year request: $38,919

Item	Year 1 (2004)	Year 2 (2005)	**Year 3(2006)**
Salaries [1]			**21,333**
Benefits (59%) [1]			**12,586**
Wages		14,700	
Benefits (%)			
Equipment	15,000	14,700	**4,500**
Supplies [2]			
Travel [3]		300	**500**
Miscellaneous			
Total	15,000	29,700	**38,919**

[1] OSU-MCAREC: Faculty Research Assistant salary (8-month) and the benefits (59%).
[2] Supplies include fruits, chemical, and equipment supplies.
[3] Travel to pear packing houses.

CONTINUING PROJECT REPORT **YEAR 2/3**

Project title:	Managing storage scald in Anjou pears
PI:	Eugene Kupferman
Organization:	WSU Tree Fruit Research and Extension Center, Wenatchee, WA 98801
Cooperators:	Bob Gix, Blue Star Growers Jordan Matson, Matson Fruit Michael Young, Stemilt Fruit Peter Sanderson, Pace International
Contract administrator:	Mary Lou Bricker (mdesros@wsu.edu) 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x 221

Objectives:

The objective of this project is to develop methodology for the control of storage scald in Anjou pears. Activities include the development of a temperature-based risk model for Washington and antioxidant and fungicide treatment strategies to facilitate the long-term storage of fruit in bins.

Most Anjou pears grown in central Washington are hand-wrap packed in the fall prior to long-term storage. The demand by retailers for many new pear packages has resulted in high costs of repackaging otherwise acceptable fruit. Costs would be reduced if pears could be stored in bins in long-term CA and then packed to order. The pear industry has been reluctant to store pears in bins until spring because of potential losses due to scald and decay. Long-term bin storage depends on the ability to treat fruit with effective antioxidants and fungicides without phytotoxicity.

Objectives for the third year of this three-year project include validation of the results of the first two years in four areas:

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.

Expanded objectives for 2006 include:

5. Evaluate the use of thermofogging to control storage scald and decay.

Thermofogging is not new but has only recently been introduced in the USA. European producers have used thermofogging to apply a proprietary formulation of diphenylamine (DPA) to both apples and pears for a number of years with good results; Italian producers have fogged ethoxyquin onto pears, but not Anjou. There have been few, if any, trials on Anjou pears since they are not produced commercially in Europe.

Small-scale experiments undertaken by Dr. Peter Sanderson (Pace International LLC) when working with the WTFRC and over the last two years with Drs. JinHe Bai (OSU) and Steve Drake (USDA-ARS) have shown promise of scald control of Anjou pears by thermofogging with ethoxyquin. A commercial Anjou room in Washington was also treated. This research proposes to thermofog ethoxyquin and the fungicide pyrimethanil on Anjou pears as a method of applying chemicals at harvest without drenching. The WTFRC research rooms at Stemilt will be utilized for this project.

Significant findings:

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

- In 2004, fruit was improperly stored and there are no results applicable to the risk model. However, by coupling the temperature data with scald development in fruit from other objectives, it was apparent that orchards with higher accumulations of cool temperatures developed less scald. Only fruit from only one of three orchards that had virtually no cool temperatures developed scald.
- In 2005, despite a large range in cool orchard temperatures, the number of pears with scald was small. Four orchards accumulated less than 11 hours below 50ºF, but only fruit from one

orchard developed scald (Fig. 1). It appears that the Chen model used in Hood River to predict scald needs to be modified before it will be capable of predicting scald in Washington.

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

- In 2004, the application of the antioxidant wrap within 7 days of harvest significantly reduced scald following long-term CA storage (May 24, 2005). Delaying the application of the antioxidant reduced its effectiveness (Figs. 2 and 3).
- Ethoxyquin-wrap was more effective at controlling scald than DPA paper.

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

- In 2004, drenched fruit stored in CA until February 21 and then held in regular air (RA) for 30, 60 or 90 days developed less scald than undrenched control fruit (Fig. 4).
- The longer the fruit was held in RA following CA storage, the greater the scald in all treatments.
- After 30 days in RA following CA, only fruit from orchards with few cooling hours developed scald; and by 90 days, fruit from all orchards had developed significant scald.
- Pears drenched with ethoxyquin (1350 ppm) developed the least amount of scald.

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

- In 2004, inclusion of TBZ, Scholar or Penbotec did not influence chemical burn.
- Pink-colored chemical burn was found at fruit-to-fruit contact points on a high percentage of fruit treated with ethoxyquin (average of 49% burned fruit) (Fig. 5).
- 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) (Fig. 6).

Methods for 2006 crop year:
The methods used in Objectives 2 through 4 will be refined for the 2006 crop based on the amount of scald encountered when the fruit comes out of storage (spring 2006).

Objective 1: Predict the risk of scald through knowledge of preharvest temperatures.
Five orchards (640-1250 feet in elevation) in the Wenatchee River Valley and three orchards in the Yakima Valley (675-1300 feet) will be utilized for this study. Temperature loggers will be placed within the canopy at least 6 weeks prior to anticipated commercial harvest to record average hourly temperatures. Fruit will be harvested at an average firmness of 14 lbf and stored in RA.

Based on the amount of scald seen in the 2005 crop (only fruit from one orchard developed significant scald), in 2006 the examination period will be extended to allow for additional scald to develop. The first pull-out date, which had been 30 days after harvest, will be moved to 60 days. The evaluations will continue weekly for 14 weeks. Scald will be evaluated after a 7-day ripening period.

Objective 2: Determine the timing of antioxidant application using fruit with different risk levels.
In 2006, fruit from the five Wenatchee orchards will be drenched at harvest with TBZ only or ethoxyquin (1350 ppm) + TBZ. This fruit will be held in RA for 7, 21 or 42 days and then treated with a line spray [ethoxyquin (0, 675 or 1350 ppm) + Penbotec], packed, and stored in long-term CA.

This method has been modified from previous years' work when antioxidants were applied as a paper wrap. The antioxidants will be applied as a line spray to improve coverage and to reflect commercial packing practices. Fruit will be evaluated for phytotoxicity immediately following CA storage and for scald after 7 days of ripening.

Objective 3: Determine the effectiveness of applying antioxidants as a bin drench.
In 2006, fruit from three orchards will be drenched at harvest with a gradient of ethoxyquin (675, 1350, 2000 ppm) in combination with TBZ, Scholar or Penbotec, or with each fungicide alone. Fruit will be held in CA storage at WTFRC research rooms until February. Four samples of each

treatment will be removed from storage. Sample 1 will be evaluated for scald after 7 days of ripening. Sample 2 will be held in RA for 30 days and evaluated after 7 days of ripening. Within 1 week after CA storage, sample 3 will be packed and receive a line spray of Penbotec only, and sample 4 will be packed and receive a line spray of ethoxyquin (not to exceed a total of 2700 ppm for the year) + Penbotec. Samples 3 and 4 will be evaluated for scald after 30 days in RA+7 days of ripening. Additional samples will be removed bimonthly from CA.

This method had been modified from previous years' work to include a second antioxidant application as a line spray. Splitting the application of ethoxyquin may provide a method to effectively control scald using the lowest possible dosage. Additional pull-out dates have been added to extend the storage duration to more closely match commercial conditions.

Objective 4: Determine the potential for chemical burn from antioxidants or fungicides applied as bin drenches.
Fruit used in Objective 3 will be evaluated for phytotoxicity at time of removal from storage. Because of high phytotoxicity from the antioxidants in the 2004 crop, methods have been revised to use lower concentrations applied at different times.

Objective 5: Evaluate the use of thermofogging to control storage scald and decay.
Eight bins of pears from each of 4 growers will be placed in each room. Half the fruit in each room will be thermofogged with pyrimethanil to prevent decay; the remaining fruit will be drenched with Penbotec. The fungicide-treated fruit will be divided between 2 rooms. Ethoxyquin will be thermofogged into one room while the other room will hold the untreated fruit; both rooms will be placed under CA conditions.

Fruit from each bin will be removed starting in mid-January and examined bimonthly. A sample of fruit will be evaluated for phytotoxicity immediately, and a second sample of fruit will be held in RA for 30 days and evaluated for scald after 7 days. Fruit will be analyzed for chemical residue by Pace International.

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

In 2005, pears were sampled at an average of 14.6 lbf to reduce the effect of maturity on scald development. 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.

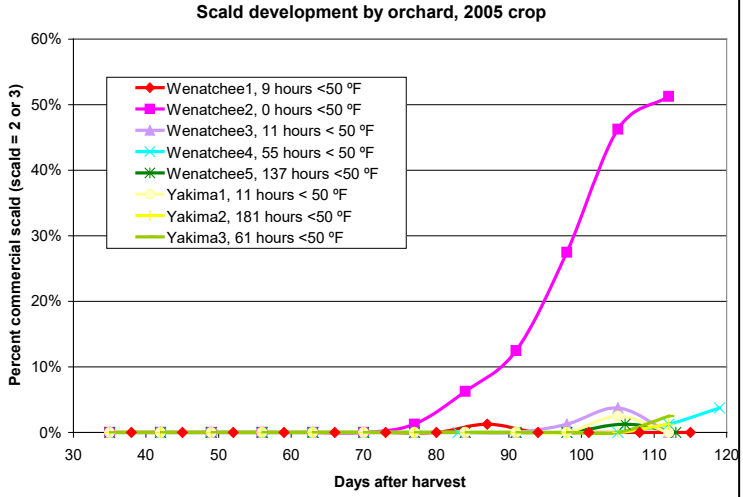

Figure 1. Scald development over time for eight orchards with different temperature profiles. Harvest dates ranged from August 19 (Wenatchee 1) to September 13 (Yakima 2 and 3). Scald evaluation started 30 after RA storage + 7 days ripening at 70 ºF.

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 was 62 days (0 hours below 50ºF) to 92 days (181 hours below 50ºF). Scald only developed on fruit from one orchard within the inspection period (Fig. 1). Attempts at using the protocol developed by Dr. Chen have not been successful; the protocol will need to be modified to reflect conditions in central Washington. Additional temperature information is being analyzed to understand why there is a difference in susceptibility among the orchards.

In 2006, fruit will be harvested at 14.0 lbf firmness from the same 8 orchards, storage time extended to 60 days prior to the beginning of evaluation, and the evaluation period extended by two weeks.

Objective 2: Determine the timing of antioxidant application using fruit with different risk levels. In 2004, pears were obtained at commercial maturity from five orchards in the Wenatchee Valley and held in RA storage for various time intervals (7, 28, 56, or 112 days after harvest). Pears were then drenched with Thiabendazole (TBZ) and wrapped in one of three papers: 1) Control=3% oil + 1.3% Cu, 2) Cu+Ethox=1000 ppm ethoxyquin + 3% oil + 1.3% Cu and 3) DPA = 1000 ppm DPA + 3% oil. The papers were formulated fresh for each time interval by Wrap Pak. Wrapped fruit were held in CA storage until May 24, 2005, and then evaluated for phytotoxicity, decay and scald after 7 days of ripening.

There was better scald control when fruit was wrapped 7 days after harvest although there was no difference between wrap treatments. Even application at 7 days did not provide effective commercial control (Fig. 2).

There was a significant difference in the amount of scald that developed in fruit from two orchards (Orchards 1 and 2) despite having equivalent cool temperatures (Fig. 3).

At harvest, the 2005 crop was drenched with either TBZ only or ethoxyquin (1350 ppm) + TBZ and wrapped with the same papers as in 2004 at 7, 14 or 28 days after harvest. This fruit will be evaluated in the spring of 2006 after long-term CA storage.

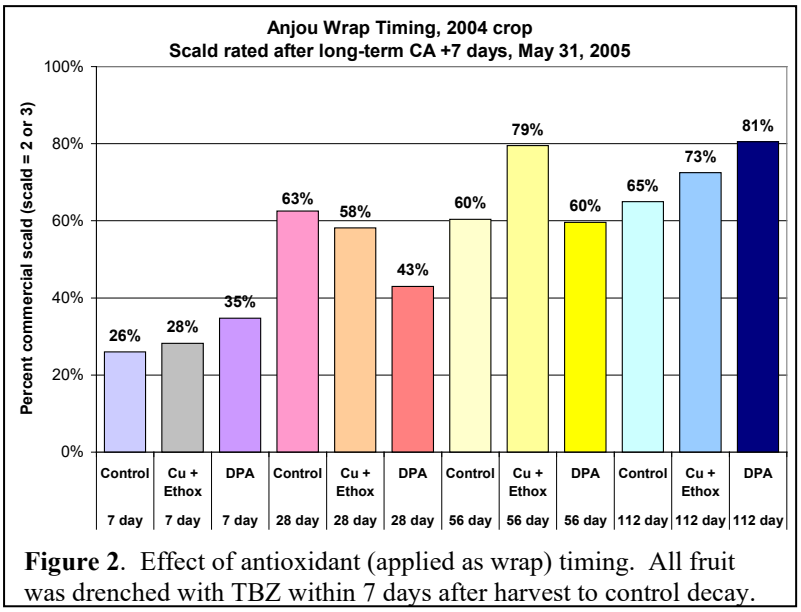

Figure 2. Effect of antioxidant (applied as wrap) timing. All fruit was drenched with TBZ within 7 days after harvest to control decay.

***Objective 3*:** Determine the effectiveness of applying antioxidants as a bin drench. In 2004, fruit from the five Wenatchee orchards were drenched with an antioxidant and/or a fungicide at harvest. The treatments were 1) no drench, 2) TBZ, 3) Penbotec, 4) Scholar, 5) 1350 ppm ethoxyquin + TBZ, 6) 1350 ppm ethoxyquin + Penbotec, 7) 1350 ppm ethoxyquin + Scholar, 8) 1000 ppm DPA + TBZ, 9) 1000 ppm DPA + Penbotec and 10) 1000 ppm DPA + Scholar. Chemicals were applied using a 400-gallon drencher provided by Stemilt. Fruit were stored in CA until February 21, 2005. Chemical residue concentration analysis was performed by Pace International, and all residues were within effective limits. Samples were evaluated for phytotoxicity immediately out of storage. Additional samples were held for 30, 60 or 90 days in RA and evaluated for scald after 7 days of ripening.

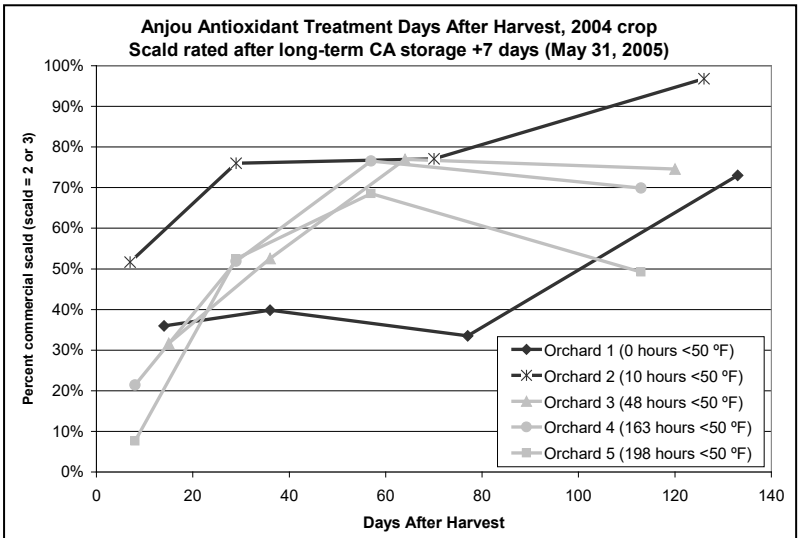

Figure 3. Percentage of fruit with commercial levels of scald damage shown by antioxidant treatment after harvest and orchard. All fruit drenched with TBZ prior to wrapping.

Scald appeared on 47% of the undrenched control fruit stored until February in CA followed by RA for 30 days (Fig. 4). Drenching with any of the fungicides alone reduced scald by approximately half. The amount of scald increased the longer the fruit were held in RA following CA storage.

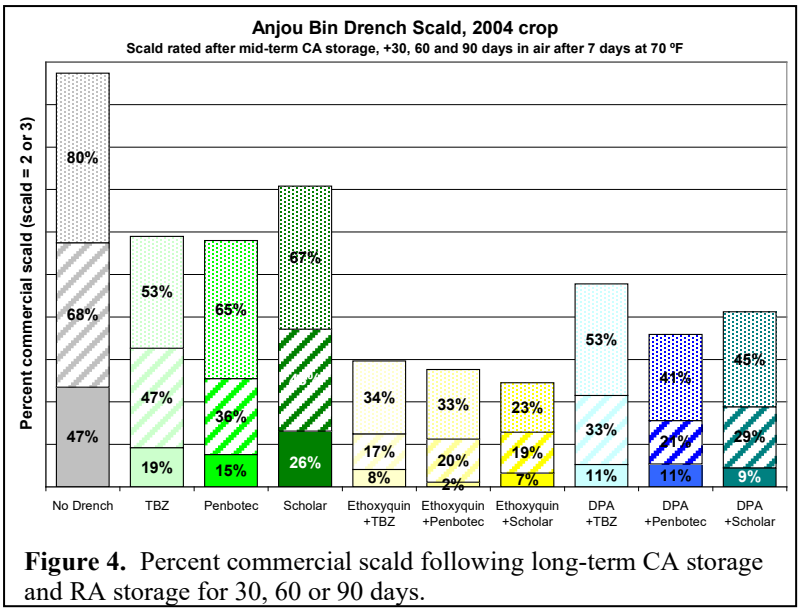

Figure 4. Percent commercial scald following long-term CA storage and RA storage for 30, 60 or 90 days.

In 2005, fruit were drenched using a 200-gallon drencher at WSU. Samples were taken for chemical residue analysis by Pace International, and results have not yet been reported. Fruit are stored in CA at the WTFRC research rooms. In February, four samples of each treatment will be removed from storage and evaluated for scald as follows: 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. Additional samples will be removed bimonthly from CA. The procedure used in 2005 will be repeated in 2006.

***Objective 4*:** Determine the potential for chemical burn from antioxidants or fungicides applied as bin drenches.
The fruit-to-fruit contact burn (pink) caused by the ethoxyquin burn was unacceptably high (Fig. 5). The brown non-contact burn on the ethoxyquin- and DPA-treated fruit was also unacceptable (Fig. 6).

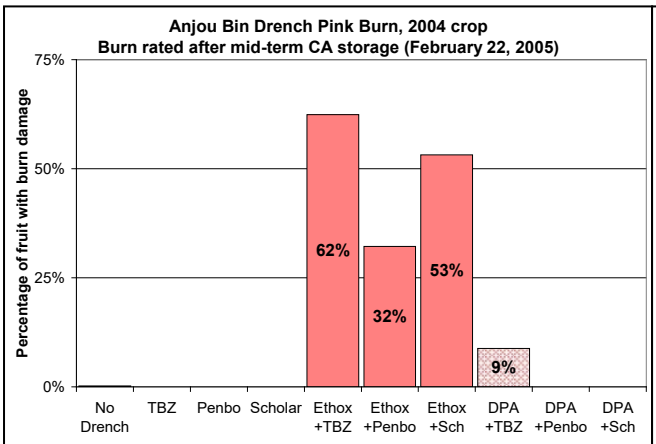

Figure 5. Pink chemical burn by drench treatment. The pink-colored burn was limited to fruit surfaces in contact with another fruit

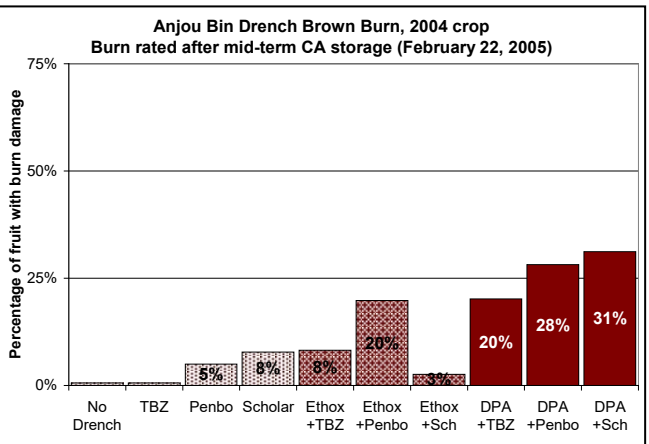

Figure 6. Brown chemical burn by drench treatment. The brown-colored burn was not associated with fruit contact surfaces.

Because of antioxidant phytotoxicity seen in the 2004 crop, the 2005 and 2006 methods have been modified to include a second antioxidant application. Splitting the application may provide a method to effectively control scald using the lowest possible concentration of antioxidant.

Objective 5: Evaluate the use thermofogging to control storage scald and decay.
The application of an antioxidant into a storage room provides several significant advantages to bin drenching, paper wraps or line sprays. With thermofogging there is no drenching of bins at

harvest and no need to dispose of drench solutions. There is no paper to discard at retail and no residue of ethoxyquin on packing equipment.

The thermofogging system has been used in Europe most often with DPA and less often with ethoxyquin and is now in slight commercial use in Chile and under tests in Argentina with good results. It has not been tested on Anjou pears grown in the Pacific Northwest under commercial conditions with the exception of small trials of Drs. Bai at OSU and Drake (USDA) in 2005. Application rate, timing, residue, potential for phytotoxicity and scald control efficacy need to be determined for this method to benefit the Pacific Northwest pear industry.

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.

Budget:

Project title: Managing storage scald in Anjou pears
PI: E. Kupferman
Project duration: 2004-2006 (3 years)
Current year: 2006
Project total (3 years): $131,163
Current year request: $51,585

Note: Because we were not able to lease a Harvest Watch in 2005, there is a carryover of $9,000 to the budget for 2006 from the allocation in 2005 for equipment and additional fruit.

Item	Year 1 (2004)	Year 2 (2005)	**Year 3 (2006)**
Salaries [1]	16,890	13,301	**13,634**
Benefits (49 %)	5,067	5,985	**6,681**
Wages[2]	4,500	6,500	**7,000**
Benefits (11%)	720	715	**770**
Equipment	700	2,000	
Supplies[3]	5,400	15,300	**31,000**
Travel[4]	1,000	1,500	**1,500**
Miscellaneous[5]			
Total	34,277	45,301	**60,585**
Requested funding	34,277	45,301	**51,585**

2006:

[1] Salary: Chris Sater for 6 months (0.75 FTE); benefits are at 49% for 2006.
[2] Time-slip help; benefits are at 11% for 2006.
[3] Supplies: primarily fruit costs and lab supplies. Cell phone charges are allowed. Fruit costs include 44 bins for objectives 1-4 and an additional 64 bins at $250/bin for thermofogging. We have been promised chemicals and residue analysis at no cost from Pace International and other suppliers.
[4] Travel to obtain fruit samples.
[5] Cost of fruit for thermofogging experiments

CONTINUING PROGRESS REPORT **YEAR 3/3**
(Note: this project will conclude in spring 2006)

Project Title:	Ethylene ripening of pears by unconventional means
PI:	Dr Keith Sharrock
Organization:	The Horticulture and Food Research Institute of New Zealand (HortResearch)
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Cooperator:	Dr Eugene Kupferman (WSU Wenatchee)

OVERALL PROJECT GOAL

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

OBJECTIVES FOR 2006:

- Continue to determine the influence of ethylene concentration and length of conditioning period at 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 includes work still to be carried out in March 2006, so reporting on this objective has been deferred until next year).
- 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.

Significant findings

- Ethylene permeated rapidly and uniformly within standard cartons and Euro-packs of 'Green Anjou' pears containing ERCs within the standard polyliner. ERCs maintained minimum ethylene levels of 65 ppm for at least 7 days in these packages.
- 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, resulted in a greater ripening potential and more flavorsome fruit than did standard one day forced air ethylene conditioning of pre-warmed fruit in a trailer or three days of warming without ethylene.
- In-transit conditioning occurred in all the ERC-conditioned packages as they were inadequately chilled after conditioning and retained the ERCs that continued to release ethylene during transport.
- Euro-packs were better than cartons or clamshells for delivering ERC-conditioned fruit to the market place. Less than 1% of the fruit were found to be bruised in Euro-packs, even amongst treatments that had softened to just 3 lb by the end of trucking across the USA.
- Some in-transit bruising damage (<5%) occurred in fruit ERC-conditioned in standard cartons that arrived almost ready-to-eat (2.2-5.2 lb mean firmness), but firmer fruit (7.6 lb mean on arrival) were undamaged and ripened to 2.9 lb mean firmness in five days at ambient temperature.

- Consumers were unable to detect a difference between fruit ERC-conditioned in cartons for 1, 3 or 5 days but preferred them to those that had been simply warmed in cartons for three days.
- 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.

Methods

ERC-conditioning of fruit in cartons and Euro-packs sealed in a standard plastic pallet cover

The prototype ethylene release capsule (ERC) consisted of a small plastic cylinder (20 mm diam. x 35 mm long) that released ethylene at a controlled rate for at least seven days. 'Green Anjou' pears were taken from cold storage after 13 days and conventionally packed into standard cartons and in double-layer vented Euro-packs within standard polyliners. These were used to compile a stack comprising 14 cartons in the two lower tiers and 15 Euro-packs in the three upper tiers, all placed on plastic film covering a slip-sheet on a pallet. Thirty ERCs in two strips three feet apart were then placed on top of the Euro-packs and the load sealed within a standard disposable 80 µm polythene pallet cover. The boxed fruit were then allowed to warm up and condition at about 68°F for five days. Control fruit from the same batch were similarly packaged and sealed but received no artificial ethylene.

A radio-temperature sensor probe was inserted through the plastic cover into a central space between boxes to record internal and external temperatures every 12 hours. The internal air temperature at the start was 50.4ºF. Three narrow Teflon tubes were inserted into a bottom-tier carton, a third-tier Euro pack and a central air gap for daily sampling to measure ethylene, CO_2 and O_2 concentrations.

When the plastic cover was removed, mean fruit firmness was assessed daily on 60 fruit from each of three treated Euro-packs and cartons taken from various locations in the stack. Every second day, 10 fruit were also sampled from each of the four control treatments for firmness testing.

ERC-conditioning of fruit in single clamshells, Euro-packs and standard cartons

'Green Anjou' pears used in this work were picked near Peshastin WA on 14 September 2005, graded into cherry bins and placed in 30^0F cold storage. Cold fruit (90 count) were then packed into 80 count 4-pack clamshells, double-layer vented Euro-packs and cartons on 21, 23 and 25 September. An ERC was placed in the central well of each clamshell. Each Euro-pack containing 47 fruit had 4 ERCs added to the empty pocket in the top layer of fruit. Each carton containing 80 hand-wrapped fruit had 5 ERCs placed in a cavity in the top layer. To minimize ethylene loss, the standard poly-liner in each box type was neatly folded over the top layer of fruit. There were five replicates of each box type and 50 clamshells per treatment.

Fruit from the same batch as above were also conditioned in Euro-packs and cartons using a commercial trailer and typical protocol (warmed for about 12 hours, followed by 1 day in 1000 ppm ethylene at 68ºF). In addition, fruit in all three types of packaging were simply warmed at room temperature for three days without artificial ethylene from ERCs.

For ERC-conditioning, the packages were kept separate on roller racks in a large well-ventilated packhouse in Wenatchee WA that held a stable temperature of 68ºF. To monitor temperatures every thirty minutes during conditioning, storage and shipping, nine i-Button® temperature loggers were placed amongst the fruit inside representative boxes or clamshells. After conditioning for 1, 3 or 5 days, the packages were transferred to a holding cold-storage room at 42-52ºF (47.3ºF mean). Fruit firmness and ethylene levels inside the polyliners were assessed at the end of the conditioning period.

Fruit ripening at Raleigh, North Carolina

The packaged fruit were palletized and transported in a refrigerated truck with a full load of 'Granny Smith' apples from Wenatchee WA to Raleigh NC in about 4.5 days. Upon arrival, the load was transferred to a large well-ventilated basement room in Schaub Hall (Southeast Dairy Foods Research Centre, North Carolina State University). The ethylene concentration in each package was then immediately assessed, the ERCs removed and the fruit within the packages allowed to ripen normally at 68°F for a maximum of five days. This was considered to be a reasonable period in which to expect fruit to reach ready-to-eat condition after delivery. During the ripening period, fruit from various conditioning treatments were sampled regularly to assess firmness, eating quality attributes and transit-related damage (e.g. skin blackening, bruising, scuffing etc.). Sampling involved 24 fruit per clamshell treatment, 25 fruit per Euro-pack treatment (five fruit/replicate from both layers) and 30 fruit per carton treatment (two top, middle and bottom fruit /replicate). As each treatment reached a mean eating firmness of 2.0–2.5 lb it was transferred to a 34°F cold room.

Consumer tests in Raleigh NC

The consumer test involved 122 people of which 68% were female and 62% were aged between 25 and 55 years old. The panelists evaluated fruit from cartons that were warmed from cold for three days or ERC-conditioned for 1, 3 or 5 days, and fruit from Euro-packs that were conditioned for a day at 68°F with ERCs or in a trailer with a forced circulation of air containing 1000 ppm ethylene.

Just prior to the start of the consumer test, all the fruit from these six treatments that were to be tasted were removed from the cold room, pressure-tested at one location on the fruit and then sliced longitudinally into eight uniform pieces. Each person tasted a slice of cold fruit from each treatment and scored it for flavor, texture and overall liking on a 0 (dislike) to 10 (really like) scale.

Results and Discussion

ERC-conditioning of fruit in cartons and Euro-packs sealed in a plastic pallet cover

A half-pallet load of 'Green Anjou' in standard Euro-packs and cartons was effectively conditioned when sealed within a plastic pallet cover containing ERCs and allowed to warm up at ambient temperature for five days. The air temperatures at a central location within the covered pallet ranged between 50 and 66ºF. Oxygen and CO_2 levels were 12% and 8.5% respectively after five days. When the cover was removed, the conditioned fruit were ready to eat after a further 7 days of ripening (Figure 1). In contrast, the untreated fruit sealed within a plastic cover remained hard (12 lb mean firmness) throughout the two week test period at 68-70°C.

The concentration and distribution of ethylene within the load was not a limitation. Ethylene permeated evenly through the packages within about 24 hours from the start and remained between 247 and 126 ppm within a lower tier carton over the following four days. Previous studies (Chen et al. 1996, Facteau and Mielke 1998) indicated that 100 ppm ethylene is sufficient to condition early season 'Green Anjou' in 4 days at 68ºF and have them ready to eat with a further 7 days of warming.

There were no significant differences in the corrected mean firmness and rate of softening between fruit in the bottom four layers of boxes in the pallet. In contrast, fruit in packages in the top layer, with the greatest surface areas exposed to the outside, warmed faster and therefore softened more rapidly than those in the lower layers. These differences might be reduced by adding an extra layer of insulation on top of the pallet. There could also be some advantage in allowing fruit on pallets to warm up for a few days prior to ERC-conditioning under the plastic cover.

ERC-conditioning fruit in single clamshells, Euro-packs and standard cartons, trucked to Raleigh

We had aimed to cool the packages rapidly below 45ºF as soon as the fruit had been conditioned for 1, 3 or 5 days at ambient temperature. Unfortunately this was not achieved. Mean fruit temperatures in the different pack types during pre-transit cooling and trucking were 52-60°F and 42-53°F respectively (Table 1). Our 2004 trials indicated that pears in the presence of ethylene condition to some extent at temperatures of 45ºF or more. The truck atmosphere on arrival contained 2 ppm ethylene. Immediately after removal from the truck, ERC-treated packages, with the ERCs still in place at 10-14 days after their introduction, contained 3-9 ppm ethylene. This compared with 0.2-1.3 ppm ethylene in boxes of fruit conditioned in the trailer or just warmed. Thus fruit in all the packages containing ERCs were exposed to effective conditioning for considerably longer than the intended 1-5 days, including some in-transit conditioning. Not surprisingly, there was a major decrease in fruit firmness between the intended end of conditioning and arrival of fruit in Raleigh (Fig. 2). Some ERC treatments were ready to eat within two days after arrival in Raleigh (Fig. 2).

Table 1. Temperature logging using i-Button® temperature loggers amongst the fruit within representative packs of the various types throughout the various stages of the trial.

	Mean temperatures (°F) within packs						
	During conditioning			**Pre-transit cool storage for 4 days in Wenatchee WA**	**During refrigerated trucking to Raleigh NC for 4.5 days**	**During first 24 h ripening at 71.6ºF in Raleigh NC**	**During first 24 h after transfer to cold room. in Raleigh**
Container	**1 day**	**3 days**	**5 days**				
Std carton	51.3	63.7	62.1	55.6	53.2	57.0	59.6
Euro-2 layer	61.5	64.8	N.D.	59.9	48.8	53.4	53.3
Clamshell	N.D.	66.6	67.5	51.7	41.5	59.5	43.2

N.D. = not done

Heat transfer and thus cooling occurred more rapidly in clamshells and consequently fruit in this packaging was least affected by ethylene released by ERCs after the intended conditioning period. In contrast, fruit in standard cartons cooled more sluggishly and were therefore more receptive to ethylene during pre-cooling and in transit (Figure 2).

A Pear Bureau study (Good Fruit Grower, Sept 2005, p13) found that 79% of consumers expect pears to be ready-to-eat at purchase. Ideally, conditioned fruit must remain sufficiently firm to avoid any risk of bruising during shipping and distribution, but then must ripen rapidly at retail markets to develop juicy, flavorsome and aromatic qualities within a few days. Trial fruit that were conditioned for three or five days with ERCs in cartons all arrived at Raleigh at a mean firmness of less than 3 lb.

Euro-packs were better than cartons or clamshells for delivering ERC-conditioned fruit with minimal in-transit damage. Only 0.6% of conditioned fruit in Euro-packs was bruised on arrival at a mean firmness of 2.8 to 3 lb. In standard cartons, some in-transit bruising damage (3.3%) occurred in ERC-conditioned fruit that arrived almost ready-to-eat (2.2-5.2 lb mean firmness), but firmer fruit (7.6 lb mean on arrival) were undamaged and ripened to 2.9 lb mean firmness in five days at ambient temperature. Up to 16% of the fruit conditioned in clamshells had spin damage and blackening to the neck of the fruit, and damage increased as the mean firmness of fruit on arrival decreased. This was mostly due to the 90-count trial fruit fitting loosely in clamshell cavities designed for 80-count fruit.

As most of the industry packs fruit in cartons, an emphasis was placed on carton-conditioned fruit in the consumer test. Consumers were unable to detect a difference between fruit conditioned in cartons for 1, 3 or 5 days but preferred them to those that had been simply warmed in cartons for three days (Table 2). Fruit ERC-conditioned in Euro-packs for 1 day were preferred to fruit that

had been pre-warmed and ethylene conditioned in a trailer for 1 day. The latter were much firmer (mean 7.1 lb), having not ripened to an ideal eating firmness (1.7-2.3 lb) within the 5 days before tasting (Table 2).

Table 2. Mean scores from a consumer test involving 122 people who each tasted six slices of 'Green Anjou' pear that had been conditioned in different ways. Scores were based on a scale from 0 (dislike) to 10 (really like).

Conditioning treatment (68ºF) and period	Range in firmness of fruit (lb)	Mean flavor score*	Mean texture score*	Mean overall score*
No artificial ethylene, simply warmed in cartons for 3 days	2.8 to 6.6 (mean 3.7)	5.6c	5.6c	5.3d
ERCs in cartons for 1 day	1.7 to 2.3	7.2a	6.7a	6.9a
ERCs in cartons for 3 days	1.7 to 2.3	6.8a	6.5a	6.4b
ERCs in cartons for 5 days	1.7 to 2.3	7.0a	6.8a	6.8ab
Commercial trailer with forced air warming/cooling and1000 ppm ethylene in Euro-packs for 1 day	5 to 9.2 (mean 7.1)	6.2b	5.9bc	5.9c
ERCs in Euro-packs for 1 day	1.7 to 2.3	7.1a	6.3ab	6.6ab

*Means in the same column, followed by the same letter, do not differ significantly at the 5% level.

All ERC-conditioned fruit gave significantly higher mean flavor and mean overall liking scores than the warmed controls that had received no ethylene or the trailer conditioned fruit. Texture scores were also significantly higher for the fruit in cartons with ERCs than in the controls (Table 2).

The trial results are consistent with a recent study on early season 'Comice' pears (Sugar and Basile, 2006) that revealed an increased capacity to ripen as the duration of ethylene exposure is increased. The ERC technology provides a potential alternative method to condition fruit without the need for a controlled conditioning room. This allows (1) longer-term conditioning at no extra cost (2) greater flexibility in coping with bottle-necks in the conditioning chain and (3) the possibility of conditioning individual pallet loads of packaged fruit taken directly from cold-storage.

Further work. We are proposing to further investigate the latter approach through a new project entitled "Conditioning in covered pallets by Ethylene Release Capsules".

References

Chen, P. M., Drake S. R., Varga D. M. and Puig, L. 1996. Precondition of D'Anjou pears for early marketing by ethylene treatment. Journal of Food Quality 19, 375.

Facteau, T. J. and Mielke, E. A. 1998. Proc. 7th International Symposium on Pear Growing. Acta Horticulturae, No 475, 567-574.

Sugar, D. and Basile, S. R. 2006. Ethylene treatment promotes early ripening capacity in mature 'Comice' pears. Horttechnology 16 (1), 89-91.

Acknowledgements

We thank Garry Williams and the staff of Bluebird in Peshastin and Wenatchee WA for their assistance in organizing pears, packaging, facilities and transport to enable these conditioning trials to be carried out. We also thank Gene Kupferman and his staff at WSU for their continued assistance and advice. We are especially grateful to MaryAnne Drake and staff at NCSU for the provision of excellent facilities and for expertly organizing and supervising the consumer test.

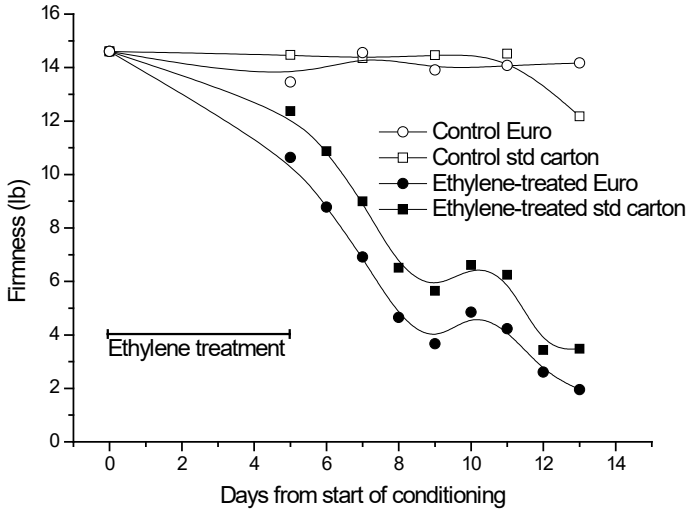

Figure 1. Effects of ERC conditioning in a covered pallet on subsequent fruit softening. Fruit in a mixed pallet of double layer Euro-packs and standard cartons were conditioned using ERCs placed on top of the pallet and sealed inside a plastic pallet cover to trap the ethylene. Control boxes were sealed in 80 μm plastic film (as used in the pallet cover) over the period of conditioning, but with no ERCs included.

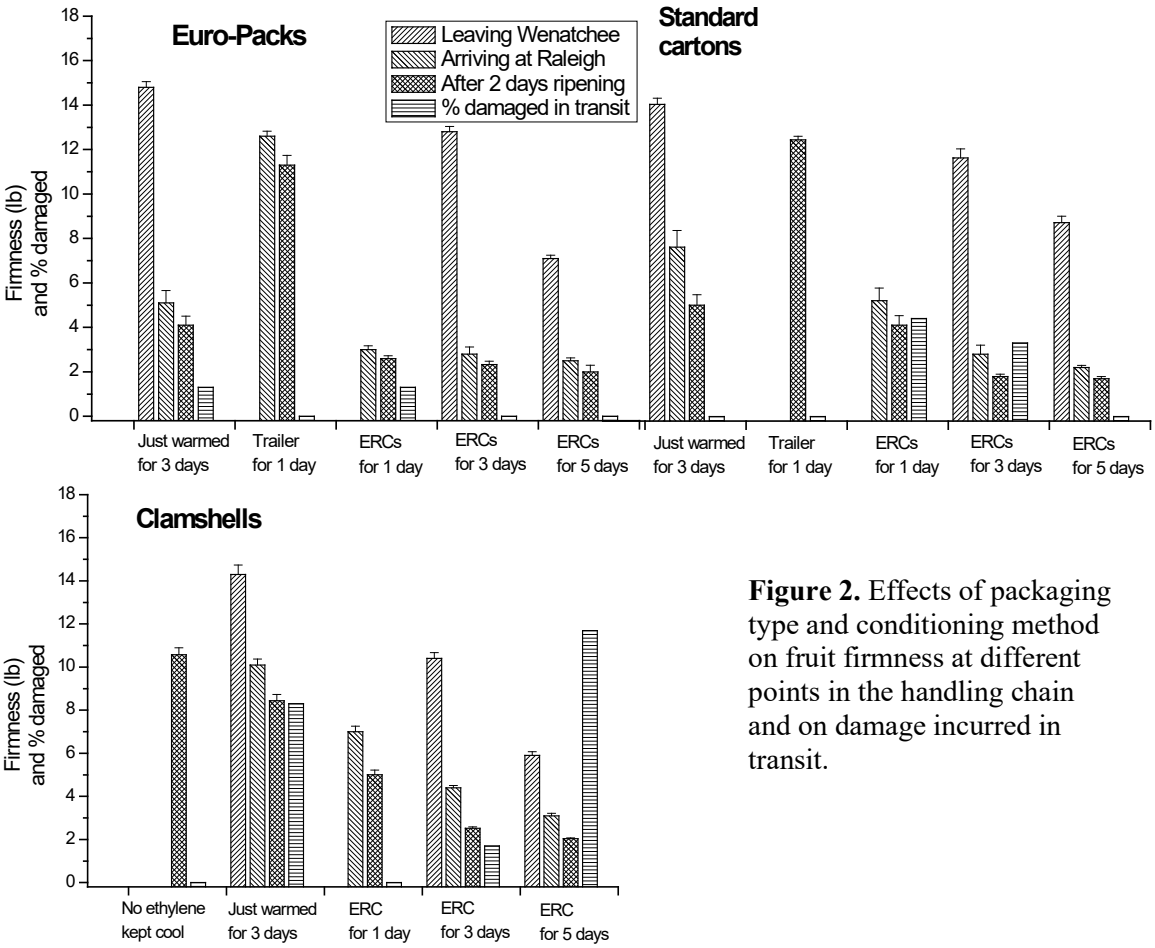

Figure 2. Effects of packaging type and conditioning method on fruit firmness at different points in the handling chain and on damage incurred in transit.

BUDGET

Year 1 (2003)	Year 2 (2004)	Year 3 (2005)	Project total (3 yrs)
$30,000	$49,900	$59,800	$139,700

Request for 2006: $0 for this project.

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project #: PR-04-433 **ARS Project #:** 5350-43000-004-06T

Project title: Management of Harvest and Postharvest Practices for Optimum Quality
PI: Jim Mattheis
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Objectives:

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

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

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

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

Significant Findings:

- Considerable variability exists between orchards in the progression of various indicators of 'Anjou' and 'Bartlett' fruit maturity.

- Field application of 1-MCP can slow postharvest ripening of 'Bartlett' pears.

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

- The low oxygen limit for 'Anjou' pears defined by chlorophyll fluorescence is subject to seasonal variation. Pears used in current year studies did not respond (no spike in the fluorescence trace) to O_2 concentrations down to 0.1%.

- Impacts of 'Anjou' storage at low oxygen are dependent on storage duration and CO_2 concentration.

Methods:

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

1-MCP: Previous research has demonstrated the utility of postharvest applications of 1-MCP for slowing pear fruit ripening. A number of factors including 1-MCP rate, interval between harvest and treatment, post-treatment storage conditions including temperature and CA gas composition impact the magnitude and duration of 1-MCP responses. While consistently effective when applied under experimental conditions, a number of treatment failures have occurred when applied in commercial rooms. It is known from research with apples that ethylene and/or CO_2 present in sufficient concentrations during 1-MCP treatment can reduce or prevent treatment efficacy. One objective is to evaluate whether the same is true for pears, and what amounts of ethylene and CO_2 are critical for influencing 1-MCP efficacy. The range of maturity over which 1-MCP treatments applied at harvest is effective will continue to be evaluated using fruit from sequential harvests as described above.
Reliable ripening after shipment is a key factor currently limiting commercialization of 1-MCP. Treatment of partially ripe fruit reduces the duration of 1-MCP responses to days or weeks, however, fruit treated when too ripe does not respond. Defining what is 'too ripe' for different cultivars in terms of measurable parameters (firmness, color, ethylene production) is one of the goals of this project. Another goal is to identify similar markers to aid in conduct of preconditioning protocols for fruit treated at harvest and held at warmer than typical storage temperatures prior to shipment. These studies will continue to attempt to identify measurable fruit characteristics that are indicative of further ripening in response to defined periods of holding at relatively high temperatures.
Studies to further characterize the relationship between 1-MCP application rate and timing for 'd'Anjou' will focus on the relationship between superficial scald control and effects on ripening. Previous research indicates treatments at 300 ppb or higher delayed 2 to 4 weeks after harvest do not provide complete control of superficial scald. Continued studies examining low rates and timing of 1-MCP application will focus on achieving scald control with less impact on ripening.

These studies will be conducted using commercial SmartFresh® material obtained from AgroFresh, Inc. Treatments will be applied in small chambers at the USDA, ARS, TFRL or in storage rooms at the Stemilt RCA facility.

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

Results and Discussion:

2005 Results to Date:

Maturity Indicators of 'Bartlett' pears: Loss of firmness, starch, and titratable acidity of 'Bartlett' pears collected from 3 commercial orchards over a 5 week period occurred at a relatively slow rate (Table 1, not all values reported). Low rates of ethylene production were also observed. Changes in fruit crispness had a pattern similar to firmness, and changes in Mohr Digitest measurements did not precede the drop in firmness. Three esters, butyl-, pentyl-, and hexyl acetate, for which maturity related changes were observed in 2004 did not have similar patterns in 2005. Consistent relationships between total ester or volatile productions and fruit development were not apparent.

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

Orchard 1	August 3	August 9	August 15	August 22	August 29
Firmness lbs	18.1	17.3	16.8	17.6	16.1
Starch (1-6)	1	1.1	1	1.2	1.3
Ethylene (ppm)	0.016	0.059	0.012	0.032	nd
Butyl acetate*	3.0	63.6	31.0	10.0	73.7
Pentyl acetate*	nd	nd	nd	nd	8.8
Hexyl acetate*	nd	72.7	10.7	18.5	49.9
Total esters	6.7	140	42	630	4250
Total volatiles*	531	335	250	1750	6870
Orchard 2	August 4	August 10	August 19	August 24	September 1
Firmness lbs	18.7	18.6	16.8	16.8	14.6
Starch (1-6)	1.0	1.1	1.1	1.2	1.5
Ethylene (ppm)	0.075	0.018	0.015	nd	nd
Butyl acetate*	3.6	81.7	28.1	7.5	56.7
Pentyl acetate*	nd	nd	nd	nd	6.2
Hexyl acetate*	21.1	34.2	28.9	11.2	282
Total esters	400	625	555	71	1150
Total volatiles	13430	1380	4880	918	1770

Orchard 3	August 5	August 15	August 22	August 29	September 6
Firmness lbs	19.7	17.6	16.7	18.2	17.4
Starch (1-6)	1	1.1	1	1.4	1.9
Ethylene (ppm)	0.046	nd	0.019	nd	nd
Butyl acetate*	256	22	22	31	198
Pentyl acetate*	nd	nd	nd	nd	1.2
Hexyl acetate*	nd	6.9	2.9	nd	4.4
Total esters	430	105	84	39	270
Total volatiles	5620	785	640	570	1040

Maturity Indicators of 'Anjou' pears: Progression of 'Anjou' fruit maturation contrasts with that of 'Bartlett' pears particularly in starch hydrolysis. No ethylene production was detected during the harvest period. One of the Digitest instrument parameters, quality factor, showed a large decrease over a one week period. The volatile profile showed considerable variation between orchards. Ester production (primarily butyl hexanoate and hexyl butyrate) increased during the later portion of the harvest period. A difference in fruit ripening capacity related to harvest date was evident after one month cold storage (Table 3). Of the maturity related fruit parameters measured, a change in the quality factor appears to be associated with the change in ripening capacity after one month storage.

Table 2. Indicators of 'Anjou' fruit development at harvest. Nd: not detected; *: nmoles/kg/m^3

Orchard 1	August 24	August 29	September 6	September 14	September 21
Firmness lbs	15.1	14.8	14.2	13.6	14
Quality factor	80.4	73.7	59.5	42.6	51
Starch (1-6)	1.6	1.4	1.3	2.4	2.2
Alcohols	nd	nd	nd	nd	nd
Aldehydes	577	371	518	1724	474
Esters	200	17	36	66	42
Total volatiles	777	388	554	1790	516

Orchard 2	August 25	September 2	September 13	September 20	September 27
Firmness lbs	15.7	14.9	12.4	13.1	12.1
Quality factor	83.6	71.2	32.1	39.3	22.8
Starch (1-6)	1.2	1.5	2	4.7	4.4
Alcohols	120	nd	nd	39	100
Aldehydes	570	227	226	1530	980
Esters	14	174	42	5510	3230
Total volatiles	704	401	268	7079	4310

Orchard 3	Aug 30	Sep 6	Sep 13	Sep 20	Sep 26	Oct 3	Oct 11
Firmness lbs	16.4	16.1	14.8	14.1	13.1	13	11.6
Quality factor	76.3	80.7	43.5	41.5	21.6	11.6	7.5
Starch (1-6)	1.2	1.2	1.1	1.8	2.5	5.6	6
Alcohols	8	7	nd	nd	nd	nd	40
Aldehydes	3190	1940	1580	940	1040	1160	1380
Esters	630	250	460	680	950	5570	17970
Total volatiles	3828	2197	2040	1620	1990	6730	19,350

Table 3. 'Anjou' firmness after 1 month air storage, 33 °F plus 7 days at 68 °F.

Harvest	Orchard 1	Orchard 2	Orchard 3
1	12.9	15.7	16.3
2	12.5	7.9	16.1
3	15.3	4.2	8.4
4	5.2	3.2	3
5	3.4	3	3.6
6	--	--	1.9
7	--	--	1.7

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

An experimental formulation of SmartFresh® was applied to 'Bartlett' pear trees in a commercial orchard. 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. After 4 months storage, the efficacy of a post-storage temperature pre-conditioning period was evident for fruit receiving a postharvest application of 1-MCP.

Table 4. '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.

Treatment Date	Control	SF0	SF1	SF2	SF3
A	2.3	2.2	2.4	5.4	7.0
B	4.1	3.9	4.6	14.2	14.9

Control: unsprayed; SF: SmartFresh®; 0,1,2,3: SmartFresh® spray treatment (0 is oil only); P: postharvest SmartFresh® application at 300 ppb.

Table 5. 'Bartlett' firmness after 4 months storage in air plus a 13 day pre-conditioning period.

	Control	ControlP	PSF0	PSF1	PSF2	PSF3
Lbs	6.8	4.7	4.7	3.6	4.1	3.7

Control: unsprayed; SF: SmartFresh; 0,1,2,3: SmartFresh spray treatment (0 is oil only); P: postharvest SmartFresh application at 300 ppb.

Responses of 'Anjou' pears to nitric oxide applied at harvest or during storage.
'Anjou' pears from 3 commercial lots obtained at commercial harvest were exposed to nitric oxide (NO) gas at harvest or during CA (1.5% O_2, 0.5% CO_2). The experiment was conducted to evaluate fruit responses in relation to development of superficial scald. Treatments were applied to fruit in sealed containers with atmospheres containing air or 0.5% O_2. As of January 24, no scald has been observed on any fruit (controls or NO treated) evaluated to date. No impacts of NO treatments on other aspects of fruit quality have been apparent.

Responses of 'Anjou' pear stored at the low O_2 limit as defined by chlorophyll fluorescence:
'Anjou' pears (3 lots) obtained at commercial harvest were subjected at harvest and periodically during CA storage to O_2 concentrations as low as 0.1%. No change in fluorescence during the analysis of any of the lots has been observed to date (January 24). The O_2 sepoint of 0.4% has been accompanied by CO_2 concentrations of 0.5 or less than 0.1% to determine what if any impact

from CO_2 is evident during ultra low O_2 CA storage. Through 4 months fruit stored at 0.4% O_2 had slower rates of softening, color change and acid loss after removal from storage compared to fruit stored at 1.5% O_2 (Table 6). No superficial scald or decay has developed to date. Fruit from one of the orchards has developed some core browning but a clear association with storage conditions is not yet apparent.

Table 6. 'Anjou' fruit quality after storage. Fruit were held at 68 °F for 7 days prior to analysis. Values are means for 3 lots at 2 months, 2 lots at 4 months.

Duration	Treatment	lbs	Color d0	Color d4	Color d7	TA %
2 months	1.5 O_2 0.5 CO_2	3.6	1.0	1.8	1.8	0.230
	1.5 O_2 0.1 CO_2	3.9	1.3	1.4	1.7	0.242
	0.4 O_2 0.5 CO_2	11.6	1	1.3	1.5	0.275
	0.4 O_2 0.1 CO_2	11.3	1.1	1.3	1.5	0.300
4 months	1.5 O_2 0.5 CO_2	2.2	1.7	1.8	1.9	0.205
	1.5 O_2 0.1 CO_2	1.7	1.5	1.2	2.2	0.150
	0.4 O_2 0.5 CO_2	4.6	1.3	1.3	1.5	0.241
	0.4 O_2 0.1 CO_2	7.7	1.0	1.0	1.3	0.250

Color: 1=green, 5=yellow; d: days ripening after removal from storage; TA: titratable acidity:

Budget

Project title: Management of Harvest and Postharvest Practices for Optimum Quality
PI: J. Mattheis
Project duration: 2004-2006
Current year: 2006
Project total (3 years): $163,757
Current year request: $37,900

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

*Salaries: 2004: GS-9 biological science tech., 2005-6: GS-11 Postdoctoral Research Associate.
**2006 GS-11 Postdoctoral salary at 0.5FTE, the other 0.5FTE funding to be provided by ARS.

CONTINUING PROJECT REPORT **YEAR 2/3**

WTFRC Project #PR-05-500 **WSU Project #13-3655-6299**

Project title: Branch induction in pear trees with bioregulators
PI: Don C. Elfving, Horticulturist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Cooperators: Dwayne Visser, Agricultural Research Technologist III, WSU-TFREC, Wenatchee, WA

Contract administrators: Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

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 Bosc pear trees by soil drench resulted in minor growth effects on the central leader but no change in shoot or bud development in 2005.
2. Notching or scoring of bark on one-year-old, vigorous, upright Bartlett pear shoots plus painting those cuts with 5,000 ppm Perlan (cytokinin/GA mixture) doubled branch development compared to untreated trees or trees receiving notching or scoring cuts only. Notching or scoring alone had no effects.
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 (Fig. 1).
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 are extremely sensitive to cyclanilide.
5. In a test of soil drenches of cyclanilide on newly planted trees of five pear cultivars on several rootstocks in Oregon, cyclanilide again showed modest effects on shoot development, likely as a result of the relatively low vigor of these trees as they established their root systems in their first year in the orchard.

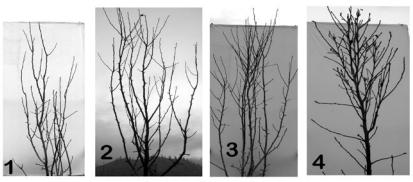

Fig. 1. 'Bosc' trees sprayed in June, 2003 with 0 (1), 5 (2), 10 (3) or 20 (4) ppm cyclanilide to induce branching. Photos taken fall, 2003.

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 trials employed single-

tree plots in randomized complete-block designs. One trial from 2004 was carried over in 2005 to evaluate return bloom responses to ethephon applied in 2004. One trial was carried over from 2003 to evaluate the effects of GA applied in 2003 on flowering and crop load of Fuji apple trees in 2005. New trials were established in 2005 to 1) examine fruiting and return-bloom responses to applications of gibberellic acid, 2) examine the relative merits of leaf removal and cyclanilide for branch induction in sleeping-eye trees and 3) evaluate TDZ for induction of bud activity in latent buds, which are extremely difficult to induce into growth activity.

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 of 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. Growth was modest in this first year. Cyclanilide appeared to reduce the elongation of the central leader to a small extent relative to the quantity in the spring drench, but there were no other effects on branching or bud development.
3. Initial observations suggest that it may be better to delay such a treatment until at least the second year to allow newly planted trees to become well established.
4. Five pear cultivars (Anjou, Bartlett, Golden Russet Bosc, Red Clapp's Favorite, selection 014) on OHxF rootstocks were drenched with 0-20 mg cyclanilide per tree in spring of 2005 shortly after planting. Small but positive effects were observed on lateral branching in the year of planting.

B. Use of proprietary cytokinin/gibberellin mixtures to stimulate lateral branching on one-year-old wood in spring (Objective 2)

1. Vigorously growing Bartlett/OHxF97 trees planted in 2004 and trained to a Tatura trellis were treated with cyclanilide soil drenches (0, 10 or 20 mg a.i./tree) or with notching or scoring of bark with or without application of 5,000 ppm Perlan (Fine Americas) in latex paint.
2. Cyclanilide treatments in 2005 had no visible effect on branching development in treated trees in 2005.
3. Notching or scoring of one-year-old wood at budbreak did not improve branching. Painting either notches or scores with Perlan doubled the amount of branching over that occurring in control trees or those receiving only notching or scoring.

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

1. The trial established with Bronze Beauty Bosc in 2005 will examine the effects of cyclanilide drench treatments applied either in year 1, year 2 or year 3 as single treatments or in years 1 and 2, years 1 and 3, or years 1, 2 and 3 as multi-year treatments. At the end of three years we should have a much clearer idea of when such treatments are effective and whether there are advantages to using such treatments for more than one year.
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.

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. The strong effects of these doses of cyclanilide in fall 2003 and spring 2004 resulted in reduced flowering in treated trees in 2005, although the reduction in yield was insignificant due to the very low production on control trees in 2005.

3. Cyclanilide treatments applied in fall 2003 appeared to have less effect on flowering and shoot growth in 2005 compared to similar treatments in spring 2004, likely due to less bioregulator present in the soil or in the trees in 2005 from those fall treatments.
4. Bosc/OHxF87 trees treated with trunk sprays of cyclanilide (0 to 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 2005 the trees were in their fifth leaf and were just beginning to flower. There were no effects on bloom or fruiting in 2005 related to branch induction in 2003, the year of treatment applications.

E. Stimulation of bud activity on "blind wood" in pear (Objective 6)

1. Thidiazuron (TDZ), a powerful cytokinin, was tested for efficacy in stimulating growth from latent buds on older limb sections (3- to 5-year-old wood) of Kalle (Red Clapp's Favorite) pear trees. TDZ at up to 1000 ppm produced no significant changes in bud development on treated limb sections.

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 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. 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.

Overcoming "blind wood," a problem that can affect pear cultivars, is difficult because the latent buds that populate such wood are extremely difficult to induce to grow. New methods and products are under test to try to overcome this problem.

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Publications 2005:

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Budget:

Project title: Branch induction in pear trees with bioregulators
PI: Don C. Elfving
Project duration: three years
Current year: 2006
Project total (3 years): $22,221
Current year request: $7,407

Year	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)
Total	6,950	**7,407**	**7,864**

Current year breakdown

Item	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)
Salaries (Technical)[1]	4,500	**4,750**	5,000
Benefits (34%)	1,530	**1,615**	1,700
Salaries (Time-slip)[1]	200	**220**	240
Benefits (10%)	20	**22**	24
Equipment	0	**0**	0
Supplies[2]	200	**200**	200
Travel[3]	500	**600**	700
Miscellaneous	0	**0**	0
Total	6,950	**7,407**	7,864

[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 to carry out the research project. Cell phone charges are allowed on this project.
[3] Treatment applications and frequent data collection in distant sites. Includes vehicle lease-to-purchase, operating and repair costs.

CONTINUING PROJECT REPORT **Year 2/3**
WTFRC Project # PR-04-431

Project Title: Biology and management of pear pests
PI: David Horton
Organization: USDA-ARS, Yakima Agric. Research Lab., Wapato, WA
509-454-5639
Horton@yarl.ars.usda.gov

Cooperators: Tom Unruh, USDA-ARS, Wapato
Vince Jones, WSU, Wenatchee

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

OBJECTIVES:
Assess role of orchard floor vegetation as a source of natural enemies attacking pear psylla, using a legume cover crop as a model system. Specific questions to be addressed:

1. How much movement is there between cover crop and tree by predators?
2. Do predators that switch habitats also switch between dominant prey (pear psylla in tree and pea aphid in cover crop)?

Sub-objectives for 2005:

a. Clarify community composition of lacewing and ladybird beetle communities in cover crop and tree (**completed**);
b. Begin laboratory feeding trials assessing suitability of pear psylla and pea aphid for generalist predators (**ongoing; complete in 2006**);
c. Test whether 10% egg solution (rather than 20% used in 2004) can be used to mark predators in cover crop (**completed**);
d. Develop milk marker for marking tree-dwelling predators (**ongoing; complete in 2006**);
e. Develop primers for detecting pea aphid DNA in predators (**ongoing; complete in 2006**);
f. Test pea aphid and psylla primers on field-collected predators (**ongoing; complete in 2006**);
g. Write and submit NRI proposal incorporating data obtained here (**completed**).

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS:

a. Sampling and abundance data suggest that the predator community consists of habitat generalists, tree specialists, and cover crop specialists. However, movement and feeding data (shown below) do not necessarily support these distinctions.
b. A 10% egg solution effectively marked predators in the cover crop. Non-trivial percentages of predators from the tree carried the cover crop marker, which is indicative of movement between habitats. Marked insects included putative habitat specialists, suggesting that my original specialization categories were overly simple. *(with Jones)*
c. A 20% milk marker provided inconsistent marking effectiveness. The studies will be repeated in 2006 using higher volumes of milk solution per tree. *(with Jones)*
d. A primer was developed to detect pea aphid DNA. It is not yet known if the primer is specific to pea aphid, or also detects other aphids; that issue will be resolved in 2006. *(with Unruh)*
e. Pea aphid and pear psylla primers were used to screen field-collected predators for evidence of having fed on these prey species. Some predators scored positive for both prey species, which is evidence of recent habitat and dietary switching. Results again suggest that my preliminary categorizations of habitat specialization are overly simple. *(with Unruh)*
f. Feeding assays showed that pea aphid and pear psylla are not entirely suitable diets for all predator species.
g. Submitted NRI proposal to assess movement and dietary switching by predators in this cover crop – pear system, utilizing technologies developed here for the WSTFRC project. *(with Unruh and Jones)*

Objectives for 2006:

a. Screen pea aphid primer against other aphid species *(with Unruh)*;
b. Correct problems with milk marker *(with Jones)*;
c. Use milk and egg markers to separately mark tree and cover crop, and monitor predator movement in both directions *(with Jones)*;
d. Initiate attempts to manipulate psylla and aphid densities using sugar ester, to develop methods for use in larger scale studies to be done beginning in 2007;
e. Develop methods for marking tree trunks, and assess importance of the tree trunk as an avenue for predator movement between habitats *(with Jones)*;
f. Finish feeding trials testing suitability of pear psylla and pea aphid for common predators.

METHODS (for the 2006 Objectives, above)

a. We will screen pea aphid primer against plum leaf curl aphid, green peach aphid, and green apple aphid using standard PCR protocols;
b. We will double volume of milk marker per tree;
c. Methods used to collect predators and to assay predators for markers were developed earlier; data from 2005 shown below.
d. Psylla and aphid densities will be manipulated using applications of a sugar ester ("Sucrocide"; Dadant & Sons). Laboratory trials by Unruh have shown efficacy of the product in small petri dish assays. Other studies published elsewhere have demonstrated effectiveness of the product against pear psylla in field conditions. The product will be put on at 0.5% concentration, sprayed to drip in both tree and cover crop habitats. Foliage will be sampled in treated and untreated plots to determine effects on aphid and psylla densities.
e. Soy flour will be hand-brushed onto tree trunks. Predators from cover crop and tree habitats will then be screened by Jones for presence of the marker.
f. Standard petri dish assays are being used. Data for 2005 shown below.

RESULTS AND DISCUSSION

Community composition. Sampling data from the last 3 years for the three most important taxa of generalist predators (Heteroptera [true bugs]; Chrysopidae [green lacewings]; Coccinellidae [ladybird beetles]) suggest that the predator community consists of habitat generalists, cover crop specialists, and tree specialists (**Table 1**). The asterisks indicate that both adults and immatures were collected. If these data provide a realistic picture of habitat use (and, our marking and feeding data suggest that the data do not necessarily provide a complete picture; see below), then a cover crop is unlikely to act as an important source of biological control of pear psylla by species such as *Chrysopa oculata* (apparent cover crop specialist) or *Chrysopa nigricornis* (apparent tree specialist), but could act as a source of psylla control for something like *Chrysoperla plorabunda* (habitat generalist).

Feeding trials. I have assayed 6 predator species (including both putative habitat specialists and generalists) for suitability of pea aphid and pear psylla diets (**Table 2**). Development times were slower if fed pear psylla for all predator species; psylla nymphs are substantially smaller than pea aphid, and the slower development on a psylla diet probably was due to the increased number of meals required to complete development on this diet. Larval survival was affected by diet in some species, indicating that these predators are not necessarily equally generalized in diet. Thus, pea aphid was a poor diet for *C. nigricornis* (tree specialist) and *C. coloradensis* (habitat generalist?). On the other hand, pear psylla was a surprisingly poor diet for the very common orchard inhabitant *H. convergens* (convergent ladybird beetle); indeed, no larvae from this species survived to the adult stage on a pear psylla diet. Some putative habitat specialists (*H. axyridis*, *C. oculata*) actually performed very well if fed prey from the less preferred habitat.

Table 1. Numbers of generalist predators in 3 major taxa collected in 2003-05 from a legume cover crop and pear trees.

Taxon	No. Collected[1] in Cover crop	No. Collected[1] in Tree	Apparent Habitat Preference: Cover crop	Apparent Habitat Preference: Tree	Apparent Habitat Preference: Habitat generalist
Heteroptera					
Orius tristicolor	**662***	**24***	**X**		
***Geocoris* spp.**	**329***	**0**	**X**		
***Nabis* sp.**	**99***	**8**	**X**		
Deraeocoris brevis	**59***	**1159***		**X**	
Anthocoris tomentosus	**10***	**459***		**X**	
Chrysopidae					
Chrysopa oculata	**175***	**2**	**X**		
Chrysoperla plorabunda	**72***	**99***			**X**
***Eremochrysa* sp.**	**19**	**95**		**X**	
Chrysopa coloradensis	**12**	**5***			**X?**
Chrysopa nigricornis	**4**	**42***		**X**	
Coccinellidae					
Hippodamia convergens	**162***	**12***	**X**		
Hyperaspis lateralis	**109**	**92**			**X**
Coccinella transversoguttata	**92***	**26**			**X**
Coccinella septempunctata	**42***	**46***			**X**
Harmonia axyridis	**10***	**156***		**X**	

[1]Cover crop insects collected using sweep nets, tree samples using beat trays.
* Both adults and immatures collected

Table 2. Development time for two ladybird beetle species and four lacewing species fed pear psylla or pea aphid (N = 14-20 per predator and diet).

		Larval development time (days)		**Larval survival (% reaching pupal stage)**	
Predator species	**Apparent habitat preference**	**Pear psylla**	**Pea aphid**	**Pear psylla**	**Pea aphid**
Hippodamia convergens	cover crop	29.4	21.9	41.7*	100.0
Harmonia axyridis	tree	41.1	30.3	100.0	100.0
Chrysopa oculata	cover crop	25.8	18.3	100.0	88.9
Chrysopa nigricornis	tree	20.1	16.1	83.3	58.8
Chrysopa coloradensis	generalist(?)	23.8	---	69.2	0.0
Chrysoperla plorabunda	generalist	22.5	21.9	88.9	85.7

* None of the pupae successfully molted to the adult stage.

Marker studies (with V. Jones). Egg white (at 10%) was applied to the cover crop at weekly intervals between mid-June and early-August. Predators were collected from both tree canopy and cover crop, and assayed by V. Jones for presence of the marker (**Table 3**). The egg white effectively marked insects, as shown by rates of marker detection in the cover crop insects (**Table 3**). Non-trivial percentages of specimens collected from the tree carried the cover crop marker, which is indicative of movement between the cover crop and tree. Specimens included

representatives from all of my preliminary specialization categories. Thus, the presence of marked individuals in supposed tree specialists indicates that my preliminary categorizations (in **Table 1**) were overly simple; that is, some supposed tree specialists collected from the tree did carry the cover crop marker. A percentage of immatures collected in the tree also carried the cover crop marker, suggesting that the tree trunk is an avenue for movement between habitats (to be tested in 2006). We had considerably less success in marking tree-dwelling predators with the milk marker, as shown by highly inconsistent readings for tree-collected insects (**Table 4**). The solution was applied as a relatively fine mist from a standard hand-pump sprayer. We will repeat these studies with the milk marker in 2006, using much larger volumes of solution per tree.

Table 3. Results showing the presence of a cover crop marker on insects collected from cover crop or tree canopy. Adult insects unless otherwise specified. ND – no data.

	Tree collected		Cover crop collected	
Specialization **Group/Species**	**# Tested**	**% Positive**	**# Tested**	**% Positive**
Tree specialists				
Anthocoris tomentosus	**100**	**31.0**	**10**	**100.0**
Deraeocoris brevis	**266**	**15.0**	**49**	**95.9**
Chrysopa nigricornis	**1**	**100**	**ND**	**ND**
***Eremochrysa* sp.**	**39**	**7.7**	**5**	**80.0**
Harmonia axyridis	**35**	**8.6**	**ND**	**ND**
Cover crop specialists				
Orius tristicolor	**6**	**33.3**	**411**	**95.6**
***Nabis* sp.**	**2**	**0**	**17**	**100.0**
***Geocoris* spp.**	**3**	**33.3**	**145**	**90.3**
Chrysopa oculata	**ND**	**ND**	**7**	**85.7**
Hippodamia convergens	**5**	**20.0**	**27**	**100.0**
Habitat generalists				
Chrysoperla plorabunda	**17**	**23.5**	**2**	**100.0**
Chrysopa coloradensis	**2**	**0**	**1**	**100.0**
Coccinella septempunctata	**16**	**12.5**	**9**	**100.0**
Coccinella transversogutatta	**6**	**16.7**	**13**	**100.0**
Hyperaspis lateralis	**7**	**0**	**10**	**100.0**
Summary by category				
Tree specialists	**441**	**17.7**	**64**	**95.3**
Cover crop specialists	**16**	**25.0**	**607**	**94.6**
Habitat generalists	**48**	**14.6**	**35**	**100.0**
Other				
Spiders	**259**	**16.2**	**256**	**85.5**
***Lygus* spp. (pests)**	**9**	**66.7**	**264**	**95.5**
Unidentified ladybird beetle	**10**	**10.0**	**9**	**100.0**
Immatures				
Anthocoris tomentosus	**56**	**8.9**	**2**	**50.0**
Deraeocoris brevis	**74**	**5.4**	**5**	**100.0**
Green lacewings	**26**	**11.5**	**5**	**100.0**
Ladybird beetles	**4**	**75.0**	**49**	**85.7**
Totals	**943**	**16.2**	**1296**	**92.8**

Table 4. Presence of milk marker (applied to trees) on tree-collected predators.

Taxon	Number assayed	Number marked	% marked
Anthocoris tomentosus	73	6	8.2
A. tomentosus (nymphs)	24	2	8.3
Deraeocoris brevis	94	4	9.6
D. brevis (nymphs)	104	26	25.0
Orius tristicolor	1	0	0.0
Nabis sp.	1	1	100.0
Lygus spp. (pests)	10	1	10.0
Chrysopa oculata	1	1	100.0
Chrysoperla plorabunda	1	0	0.0
Eremochrysa sp.	28	14	50.0
Coccinella septempunctata	21	3	14.3
Coccinella transversoguttata	4	1	25.0
Harmonia axyridis	20	0	0.0
Hippodamia convergens	7	1	14.3
Hyperaspis lateralis	5	0	0.0
Spiders (mix of species)	92	12	13.0

Gut contents studies (with T. Unruh). Primers developed earlier by Unruh for detecting psylla DNA were used in assays with psylla honeydew, to assess whether predators that feed on honeydew but not psylla might score positive for psylla (leading to overestimation of actual predation on psylla). The pear psylla signal was detected about 20% of the time by PCR if diluted honeydew (50% in water) was used in the PCR instead of psylla tissue. However, the signal disappeared if the honeydew was diluted further with realistic amounts of predator tissue. Primers were next developed to detect pea aphid, and were successfully used in controlled laboratory feeding trials. We then collected ladybird beetles and *Deraeocoris brevis* from the cover crop and pear trees, and assayed them for presence of pea aphid, pear psylla, and aphid + psylla signals (**Table 5**). A substantial percentage of predators from the cover crop contained pear psylla DNA, which is evidence of habitat and dietary switching. A small percentage of predators from both habitats contained both signals (**Table 5**), which is evidence of recent dietary switching. Results for the pea aphid signal may overestimate feeding on this prey species, as we have not yet confirmed that the pea aphid primer does not detect other aphid species; neighboring plum and apple trees were heavily infested with aphids at the time of these pilot studies.

Conclusions from 2005 studies:

Table 5. Presence of pear psylla and pea aphid DNA in guts of field-collected predators as determined by PCR. Adults only were assayed.

		Pear psylla	Pea aphid	Pear psylla + pea aphid
	# assayed	% positive	% positive	% positive
Cover crop collected				
Deraeocoris brevis	15	66.7	46.7	33.3
Hippodamia convergens	15	86.7	33.3	33.3
Coccinella septempunctata	14	26.7	53.3	21.4
Coccinella transversoguttata	10	70.0	10.0	10.0
Pear tree collected				
Deraeocoris brevis	15	66.7	60.0	33.3
Coccinella septempunctata	15	73.3	26.7	20.0
Harmonia axyridis	15	100.0	26.7	26.7

The marker work (with V. Jones) and gut contents work (with T. Unruh) demonstrated that predators exhibit both habitat and dietary switching. We conclude that these new technologies provide powerful tools for assessing movement and diet by generalist predators in this model system, and should be easy to adapt to other systems. We incorporated many of these WSTFRC-funded results into an NRI proposal (Horton, Unruh, Jones; $362,659 for 3 years), submitted in December 2005, to address specific questions about predator movement and dietary switching in response to prey or habitat perturbations in this pear – cover crop system.

BUDGET

Project Title: Biology and management of pear pests

PI: David Horton
Cooperators: Tom Unruh and Vince Jones
Project duration: 2004-2006
Current year: 2006

Project total (3 years): $71,370
Current year request: $24,180

Year	Year 1 (2004)	Year 2 (2005)	**Year 3 (2006)**
Total	23,400	23,790	**24,180**

Current year breakdown

Item	Year 1(2004)	Year 2 (2005)	**Year 3 (2006)**
Salaries[1]	10,000	10,300	**10.600**
Benefits (30%)	3,000	3,090	**3,180**
Time slip[2]	10,400	10,400	**10,400**
Total	23,400	23,790	**24,180**

[1] 33% GS-5 technician. The technician will assist with sampling, collecting predators, sorting predators for shipment to V. Jones, and preparation of predators for gut contents analyses (T. Unruh).
[2] 130 days at $10.00 per hour. The person will assist with collecting and sorting predators.

CONTINUING PROJECT REPORT **YEAR 2/3**

WTFRC Project #AE-04-428 **WSU Project #3643-8366**

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

PI: Vincent P. Jones, Associate Entomologist
Organization: WSU Tree-Fruit Research and Extension Center
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Co-PIs and affiliations: Jay F. Brunner, WSU-TFREC
Tom Unruh, USDA-ARS, Wapato
Dave Horton, USDA-ARS, Wapato
Contract administrators: Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

Objectives:

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

Significant findings:

- The diversity of predators found in the pear tree canopy and the ground cover was similar according to our mathematical diversity indices.

- A significant proportion of several natural enemies species that would be considered "tree species" had visited the ground cover. Even *Anthocoris* and *Deraeocoris* nymphs were found to move between the tree and ground cover, although at ≈1/3 the rate of adult movement patterns.

- We developed a new marking technique using powdered formulations of our markers and traps that allowed us to measure the movement of *Colpoclypeus florus*, a leafroller parasitoid, from the gardens into the orchard.

- Our *C. florus* movement experiment was performed on too small a scale, but even so, a 21□15-foot (315 ft^2) portion of the rose garden affected >2 acres of the adjacent orchard.

- Our movement studies of OBLR between apple and cherry showed that little movement occurred between the two areas.

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

Both last year and this year we applied the egg marker to the orchard ground cover with a weed sprayer mounted on an ATV. Last year we applied the marker as a 20% solution and had 97% marking for insects collected from the ground cover. This year we reduced the rate applied to 10% egg whites, and marking remained nearly the same. Insect samples were collected from both the ground cover and the canopy over the course of the experiment. Ground insects were tested for

presence of the mark to determine marking success, and tree-collected insects were tested for the marker to determine movement between the two areas.

This year we concentrated on expanding our identification of the specimens collected. In particular, we identified the adult ladybird beetles and lacewings to species so that we could determine habitat specificity and the tendency of each species to move between the ground cover and the canopy. We also collected and tested immatures of ladybird beetles, green lacewings, *Anthocoris*, and *Deraeocoris*. For the immature ladybird beetles and lacewings, identification to species was not possible.

Analysis: We wanted to determine the relative difference in species diversity of predators collected from the tree versus the ground cover. We used two different indices of diversity (Simpson's index and the Shannon-Weiner Function); the two have a slightly different basis and theoretical background. Simpson's index varies from 0 (low diversity) to almost 1 (high diversity). The Shannon-Weiner Function starts at 0 (no diversity) to 2.9 (in our samples) with larger numbers showing higher diversity.

We also classified each predator species collected in the tree canopy as to its habitat preference. To do this, for each species we calculated the percentage of the total captures (ground + tree) that

Table 1. Species collected from the ground cover (GC) or canopy collected during summer 2005.

Species	N Ground	N Tree	% Tree Collected	% in Canopy Visiting GC	Preferred Habitat
Harmonia axyridis	0	35	100.0	8.6	Tree
Anthocoris tomentosus	10	100	90.9	31	Tree
Chrysoperla plorabunda	2	17	89.5	23.5	Tree
Deraeocoris brevis	49	266	84.4	15	Tree
Coccinella septempunctata	9	16	64.0	12.5	Generalist
Unknown ladybird beetle	9	10	52.6	10	Generalist
Spiders (various spp.)	256	259	50.3	16.2	Generalist
Hyperaspis lateralis	10	7	41.2	0	Generalist
Coccinella transversoguttata	13	6	31.6	16.7	GC
Hippodamia convergens	27	5	15.6	20	GC
Nabis sp.	17	2	10.5	0	GC
Lygus hesperus	264	9	3.3	66.7	GC
Geocoris sp.	145	3	2.0	33.3	GC
Orius tristicolor	411	6	1.4	33.3	GC
Immatures					
Anthocoris tomentosus	2	56	96.6	8.9	Tree
Deraeocoris brevis	5	74	93.7	5.4	Tree
Green Lacewings	5	26	83.9	11.5	Tree
Ladybird Beetles	49	4	7.5	75	GC
Total	**1283**	**866**			

occurred in the tree. If greater than 70% were collected in the tree, we tentatively considered this a species that preferred the tree; if between 30 and 70% were collected in the tree, the species was considered a "generalist"; and if less than 30% were collected in the tree it would be considered a species that preferred the ground cover. This classification should be viewed as a very rough guide, in part because the sampling methods were different between the tree and ground collections so we may have some differences in efficiencies between the two sampling methods that would distort the percentages.

The proportion of individuals in each species collected in the tree canopies that were positive for the ground cover mark was calculated. This value, along with the habitat preferences classification, was used to determine how important the ground cover was in population dynamics occurring in the canopy.

Results: The values for the two diversity indices were very similar between the predator samples collected from the tree and the ground cover (Simpson's: 0.784 (ground) vs. 0.753 (tree); Shannon-Weiner 0.290 (ground) vs. 0.238 (tree). There was a slight, but not statistically significant, increase in both indices for species collected from the ground, indicating that a slightly greater diversity occurred in the ground cover.

Using the classification system described above, four of the 14 species collected in the tree canopy could be described as "tree species" (Table 1). Of the four tree species, two were commonly collected (*Anthocoris tomentosus* and *Deraeocoris brevis*), with 31% of the *Anthocoris* and 15% of the *Deraeocoris* collected in the tree canopy testing positive for the ground cover marker. Immature *Anthocoris* and *Deraeocoris* were also found to move between the ground cover and the canopy but at roughly 1/3 the rates of the adults. A species of lacewing, *Chrysoperla plorabunda,* and a ladybird beetle, *C. septempunctata,* also moved between the ground and tree, with 23.5 and 12.5% of those collected in the trees testing positive for the ground cover marker, respectively. The ladybird beetle *Harmonia axyridis* was only found in the tree canopy, but 8.6% of that species also tested positive for the ground cover mark.

Spiders (a mix of species) were classified as habitat generalists and were found in nearly equal abundance in the ground and tree collections. In terms of overall abundance, spiders were the second most common predator found in the tree samples (first was *Deraeocoris*). About 16% of the spiders collected in the tree samples tested positive for the ground cover marker.

Conclusions: The ground cover is visited by a number of the different predators, even ones that abundance data would suggest are "tree" species. The *Anthocoris* found in the ground cover likely originated in the tree and only visit the ground cover for short periods (suggested by the high percentage collected in the canopy that tested positive for the ground cover mark and the low numbers found in the ground cover). Surprisingly, even the less mobile immatures move down to the ground cover and back. The importance of the ground cover cannot be further defined with the experiments we have performed to date because we did not collect prey abundance data (pea aphid in the cover crop and pear psylla in the canopy); therefore, the tendency of predators to move between the habitats and switch prey fed upon cannot be determined.

Plans for next year: We plan to use at least two markers so that we can mark insects in the canopy as well as those in the ground cover. This should help us to better define movement patterns. We will also attempt to manipulate the pea aphid population in the ground covers using sugar esters to determine if the reduction in pea aphid populations will force more of the ground resident predators into the canopy. On a small scale, we will use the powdered formulations of our markers to determine if we can define the importance of walking versus flying as the normal mode of movement between

habitats for each of the different predator species. We will also collect prey density data in both the pear canopy and the ground cover to help determine the importance of prey density on movement patterns of the predators.

Objective 2. Examine the area of influence ("active space") of a rose/strawberry garden used to bolster parasitism of leafrollers.

Last year, we tried spraying markers on several rose/strawberry gardens but had virtually no luck at recovering marked or unmarked *C. florus* in the adjacent orchards. This year, we developed a new strategy for marking and we designed traps that allowed us to successfully capture adult parasitoids. Increased marking of the parasitoids was accomplished by placing a mesh netting (tulle) over a portion of the rose garden (Fig. 1). The holes in the netting were large enough that *C. florus* could easily pass through but not without coming into direct contact with the netting. We marked the netting by applying soy flour using a hand-held lawn fertilizer broadcaster. The result was that fine particles of soy flour stuck to the netting (see inset, Fig. 1), and the parasitoids contacted the soy flour on the foliage or when walking through the netting.

Fig. 1. Netting over the garden that has been dusted with soy flour. Inset shows a close-up of the soy flour on the netting.

Our research last year showed that *C. florus* was not attracted to yellow or white sticky cards. This year we devised a trap that consisted of an apple shoot infested with a late instar leafroller larva. We punched a hole in the center of a sticky card and inserted the leafroller infested shoot through the card and into a water vial (Fig. 2). These traps were placed at various locations in the orchard adjacent to the rose/strawberry garden. At 3-4 day intervals we would return to the orchard, replace the sticky cards if *C. florus* were on them, and refill the vials with water. The shoots were replaced at weekly intervals. Rolled leaves on the collected shoots were dissected to look for adult *C. florus* (they tend to remain in the feeding roll for 1-2 days after parasitizing a caterpillar).

Fig. 2. Trap used to collect adult C. florus. Shoot is infested with late instar OBLR and the shoot tip is inserted into a floral vial to keep the shoot succulent.

We placed 34 traps in the orchard in late July through the first week of September (Fig 3). We also took a population assessment of the leafrollers in the orchard once during the sampling period.

Results:

The traps allowed us to capture *C. florus* with relatively little effort (at least compared to our previous methods). A total of 182 *C. florus* were captured either on the cards (177) or in the leaf

Fig. 3. Plot layout for C. florus movement experiment. Open circles represent trap locations, filled circles are where parasitoids were collected that were positive for the marker.

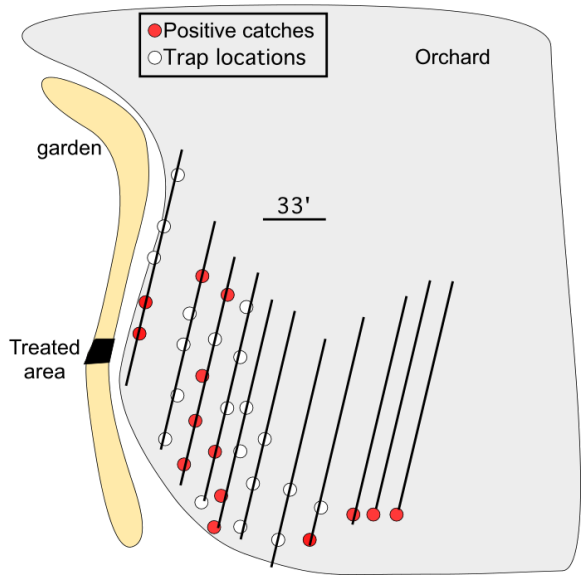
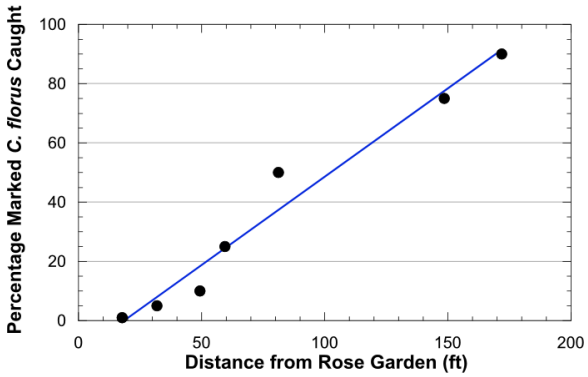


Fig. 4. Percentage of *C. florus* caught at different distances from the edge of the rose/strawberry garden.



rolls (5). Our traps were concentrated near the rose garden, with the maximum distance being roughly 171 feet, through several tree rows of the orchard. The percentage marked was 13.2%, which was slightly higher than the percentage of the rose garden we actually marked. Four individuals were caught at the furthest trap, suggesting that our trap layout needed to be expanded. When we plotted the distance moved versus the percentage of marked individuals captured, 50% of the catch occurred at distances greater than 80 feet from the garden (Fig. 4). However, because we captured multiple individuals at the furthest trap, and because our trapping was concentrated closer to the garden, this figure is too low to be a realistic estimate of the influence of the rose garden. Even so, the 15x21-foot section of the garden influenced >2 acres of the adjacent orchard.

Plan for next year: We plan to increase the distance of our traps from the source gardens and increase the number of gardens we monitor. We will also set up the experiments early in the spring and monitor them throughout the season.

Objective 3. Examine the movement of insect pests from areas of high population density to surrounding managed areas.

We set up an experiment to measure movement of adult leafrollers between apple and cherry crops in late August (after the cherry harvest). The plots were roughly 200 feet deep and 380 feet long (≈1.6 acres each), with the long edges adjacent and parallel to each other. The apple block was sprayed with milk and the cherry block with egg whites. Each plot had 15 traps spaced uniformly throughout the area.

We also evaluated the movement of codling moth between apple orchards under different management schemes. This is reported more fully in our other progress report (AE-04-429), but the emphasis for this report is the movement between the primary (marked) orchard and an adjacent (unmarked) orchard. We marked 3 acres of the primary orchard and trapped throughout this orchard and a portion of an adjacent orchard. The adjacent orchard was between 100-220 feet away from marked area (the two orchards were not parallel to each other) and across an irrigation ditch. We used the combo pheromone/DA lure in all the traps.

Results leafroller movement: We captured 113 moths, 75 in the apples and 38 in the cherries. Overall, 22 (13%) of the captured moths were marked. Of these marked moths, only five of them moved from the area where they acquired the marks. Four of the five marked moths moved from apple to the cherry block, but obviously the number of moths that moved was relatively low compared to the numbers of moths captured. Due to these low numbers of moths trapped out of the area where they were marked, we cannot draw any generalities on movement patterns.

Results codling moth movement: During the first generation, we captured 67 moths in the adjacent orchard, of which 12% originated in the marked plots of the primary orchard. During the second generation, trap catches in general declined, but 28% of the moths caught in the adjacent orchard originated in the marked areas. If we had marked a larger proportion of the primary orchard, it is likely the percentages of moths originating in the primary orchard would at least double for the traps in the adjacent orchard.

Plan for next year: We need to conduct the cherry/apple leafroller movement studies for a longer period and in an area with higher leafroller population levels. The cherry trees we marked were quite large, and we probably had lower spray coverage than in the apple block that consisted of much smaller trees. In addition, the cherry block was treated for leafrollers early in the season, which likely reduced OBLR populations there. We need to find orchards with cherry and apple trees of similar sizes and expand the acreage marked. For the movement of CM, our studies need to be done on a larger scale because our results (as reported in AE-04-429) suggest that movement can be much greater than 800 feet.

Budget:

Project title: The importance of dispersal in biological control and IPM
PI: Vincent P. Jones
Project duration: 2004-2006 (3 years)
Current year: 2006
Project total (3 years): **$**142,334
Current year request: **$**49,683

Year	**Year 1 (2004)**	**Year 2 (2005)**	**Year 3 (2006)**
Total	45,793	46,858	49,683

Item	Year 1 (2004)	Year 2 (2005)	**Year 3 (2006)**
Salaries[1]	20,487	21,306	**24,017**
Benefits (34%)[2]	6,146	6,392	**8,166**
Wages[3]	11,000	11,000	**10,000**
Benefits (11%)[2]	1,760	1,760	**1,100**
Supplies[4]	3,200	3,200	**3,200**
Travel[5]	3,200	3,200	**3,200**
Total	45,793	46,858	**49,683**

[1]Callie Baker, Associate in Research (changed from 0.50 FTE to 0.55 FTE).
[2]Benefits = 30% years 1 and 2, 34% year 3. Time-slip benefits decreased to 11% in year 3.
[3]Time-slip employees.
[4]Lab supplies. Cell phone charges are allowed.
[5]Travel to research plots.

CONTINUING PROJECT REPORT **Year 1/3**
WTFRC Project # PR-05-504

Project Title: Chemical ecology of pear psylla
PI: David Horton, Peter Landolt, and Christelle Guédot
Organization: USDA-ARS, Yakima Agric. Research Lab., Wapato, WA, 509-454-5639
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Contract Admin.: Janet Tsukahira, jtsukahira@pw.ars.usda.gov, (510) 559-6019

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.

Sub-objectives for 2005:

a. Clarify role of diapause affecting male winterform response to female volatiles (**completed)**;
b. Demonstrate that summerform males are attracted to volatiles from summerform females (**completed**);
c. Define role of female age in summerforms affecting male attraction to female-produced volatiles (**completed**);
d. Write and submit NRI proposal incorporating data obtained in this WSTFRC project requesting funding for pheromone chemist (**completed**);
e. Write and submit BARD proposal incorporating data obtained in this WSTFRC project requesting funding for pheromone chemist (**completed**).

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS:

a. Volatiles from field-collected female winterforms do not attract field-collected males until late-February, coinciding with ovarian maturation and mating in the field;
b. Diapause status of female winterforms affected attractiveness to males in olfactometer tests. Females exposed to long-day conditions (to break diapause) were more attractive to males than females kept in diapause by short day conditions. Treatment of diapausing female winterforms with fenoxycarb to prompt diapause termination made females attractive to fenoxycarb-treated males but not to untreated (i.e., diapausing) males in olfactometer tests;
c. Summerform females are highly attractive to summerform males in olfactometer tests;
d. Older summerform females (8-10 days in age) are substantially more attractive to males in olfactometer tests than young (2-4 days in age) females;
e. Hired post-doctoral scientist (Christelle Guédot), to be supervised by Landolt. Dr. Guédot has assisted in olfactometer trials, and is developing EAD methods. She will eventually develop methods for collecting volatiles from females, and methods for isolating attractants.
f. Wrote and submitted NRI proposal incorporating data obtained in this WSTFRC project. The funding request includes support for a pheromone chemist (Jocelyn Millar, U.C. Riverside) to assist with identification and synthesis of the attractant(s).
g. Wrote and submitted BARD proposal incorporating data obtained in this WSTFRC project. The funding request includes support for a chemical ecologist (Vicky Soroker, Volcani Institute) and pheromone chemist (Anat Zada, Volcani Institute) to assist with identification and synthesis of

attractants for the pear psyllid, *Cacopsylla bidens*. The proposal also provides partial support for C. Guédot to work with the North American pear psyllid.

Objectives for 2006:

a. Determine whether the host plant is necessary to make females attractive to males in olfactometer tests.
b. Determine whether virgin summerform females are more attractive than mated females in olfactometer tests.
c. Develop EAD methods to be used eventually in isolating attractants in the headspace mix of volatiles (Guédot).
d. Develop methods for collecting volatiles from known attractive female winterforms and summerforms (Guédot).
e. Begin olfactometer assays with extracts of headspace volatiles.

METHODS (for the 2006 Objectives, above)

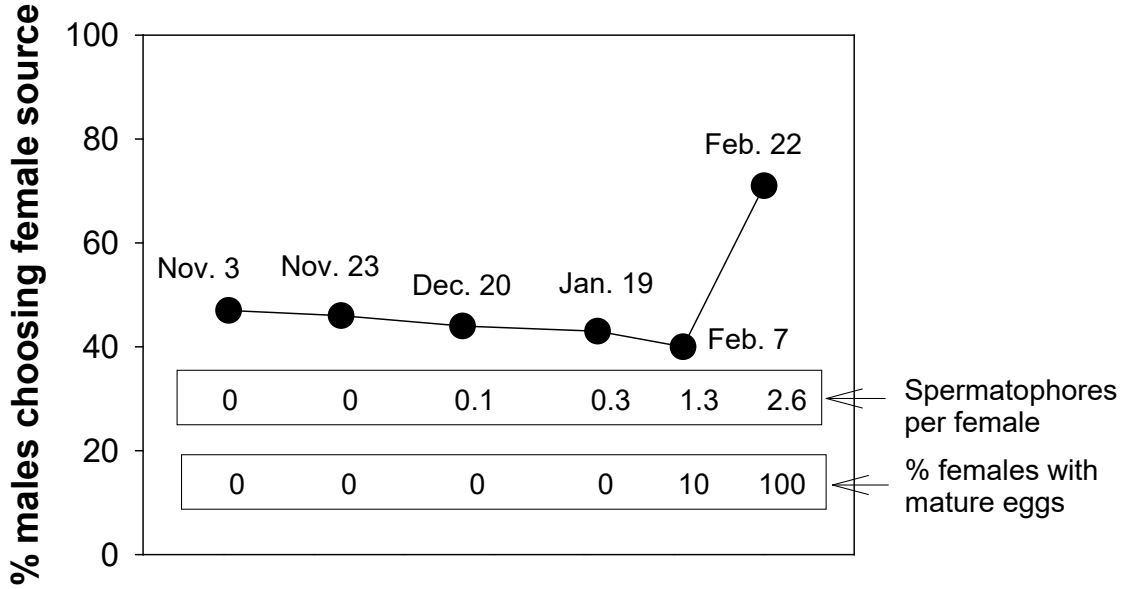
a. Olfactometer tests will pair females + host plant (shoots for winterforms, seedlings for summerforms) vs females minus plant material. Moistened paper towels will be placed in both source containers to provide water to females not having access to host tissues. These assays are done to see whether 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. Virgin and mated summerform females will be paired as odor sources in the olfactometer.
c. Dr. Guédot will continue efforts to apply standard 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. Head space volatiles will be collected from females known from olfactometer tests to be attractive to males. 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. Dr. Guédot will conduct this work.
e. Olfactometer tests will be done with the extracts to confirm that they prompt biological activity in male psylla.

RESULTS AND DISCUSSION

Effects of diapause on attractiveness of female winterforms. Studies begun winter 2004 and finished after last year's pear review showed that field-collected female winterforms were not attractive to males in Y-tube olfactometer trials until late-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. The study ended after the Feb. 22 collection, due to onset of chemical sprays at the orchard. A second set of assays was done using field-collected (early to late December) winterforms which were then exposed in the laboratory for two weeks to long-day conditions (to break diapause) or short-day conditions (to maintain diapause). Both long- and short-day males preferred the arm of the olfactometer connected to the long-day females (**Figure 2**). The numerals at the top and bottom of both figures are the median ovarian scores in the assayed females: < 5 = immature eggs only; 5 = first mature egg seen; 8 = fully laden with mature eggs. The scores confirm that the long-day females were fully egg-laden, while the short-day females for the most part had immature ovaries. A final set of assays was done using fenoxycarb on October-November collected winterforms to prompt diapause termination (assays done by C. Guédot). The studies showed that treated males preferentially chose the arm of the olfactometer connected to treated-females (58.3% of males) if paired against untreated females (41.7% of males); the preference is statistically significant (**Fig. 3, upper panel**). Untreated males failed to show preference (**Fig. 3, lower panel**). Dissection

showed that a percentage (ca. 30%) of the control (untreated) females unexpectedly contained mature eggs. Presence of these post-diapause females in the control groups may explain the relatively weak (albeit significant) results in **Figure 3 upper panel**; i.e., untreated females nonetheless having mature ovaries may have been emitting attractants during the assays, and these odors possibly competed with attractants from the treated females in the opposite arm of the olfactometer.

Figure 1. Percentage of male winterforms choosing female source (vs uninfested shoots) in olfactometer trials as function of collection date. Diapause status of females (ovarian development, spermatophores) determined by dissection.



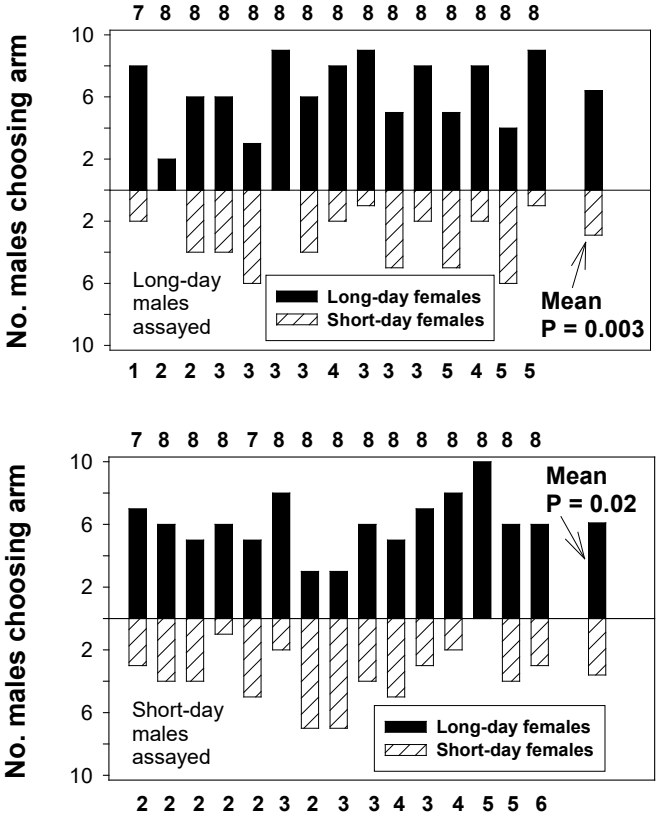


Figure 2. Numbers of male winterforms choosing long-day females (black) vs short-day females (hatched) in olfactometer tests. Upper panel shows assays with long-day males; bottom panel shows assays with short-day males.

Note: Each bar based upon response of 10 males. A given bar may sum to fewer than 10 males, as some males in most assays failed to make a choice.

Numerals at top and bottom of each figure in both panels are median ovarian scores in the

assayed females for a given bar (long day females above each figure, short day females below each figure). For example, in assay #1 with long-day males (upper panel), long-day females had a median score of 7, whereas the short-day females in the opposite arm of the olfactometer had a median score of 1.

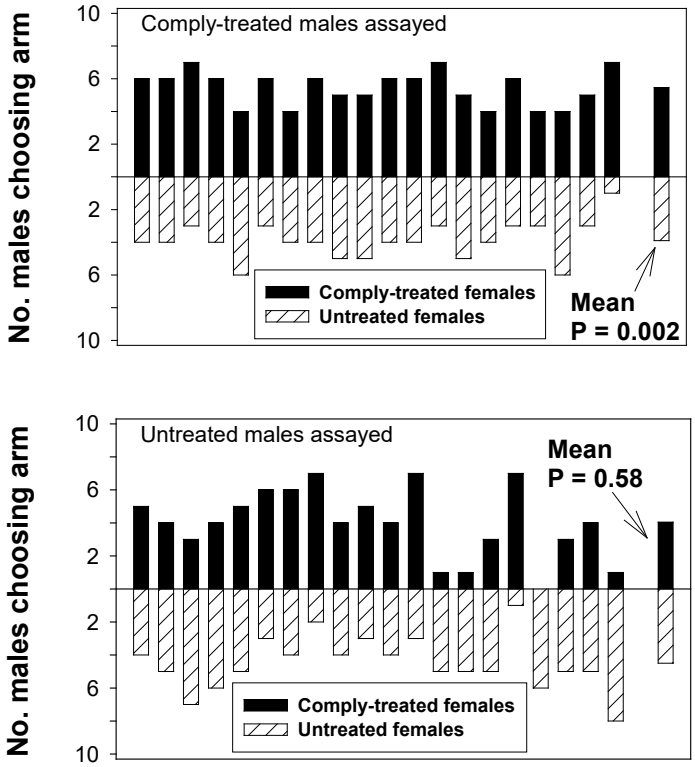


Figure 3. Numbers of male winterforms choosing treatment arm (black) vs control arm (hatched) of olfactometer. Upper panel shows response of fenoxycarb-treated males to treated vs untreated females; lower panel shows response of untreated males to treated vs untreated females. Each bar is based upon 10 males; a given bar may sum to fewer than 10 males, as some males in most assays failed to make a choice.

Summerform assays. Olfactometer trials showed that pear seedlings infested with virgin, 6-8 day old summerform females were significantly more attractive to virgin male summerforms than uninfested seedlings (**Fig. 4**; 64% of males chose the female odor source). Age of females substantially affected their attractiveness, as shown in a second set of assays. Females of 8-10 days in age were significantly more attractive to males in olfactometer trials than females of 2-4 days in age (**Fig. 5**; 75.5% of males chose the older females).

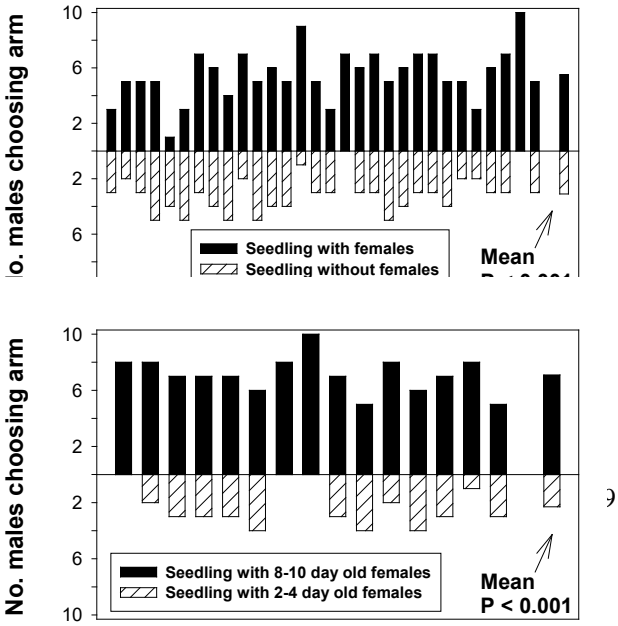


Figure 4. Numbers of male summerforms choosing female source (black) vs control source (hatched) in olfactometer trials. Each bar based upon response of 10 males; a given bar may sum to fewer than 10 if some males failed to make a choice.

Figure 5. Numbers of male summerforms choosing 8-10 day old females (black) vs 2-4 day old females (hatched) in olfactometer trials. Each bar based upon response of 10 males; a given bar may sum to fewer than 10 if some males failed to make a choice.

Electroantennogram methods. Dr. Christelle Guédot has begun efforts to develop EAD methods using antennae from male pear psylla.

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. The chemistry work will be done in conjunction with behavioral (olfactometer) and physiological (EAD) assays, to confirm that isolated or synthesized volatiles prompt biological activity. Funds from BARD and NRI projects will be used to support a pheromone chemist in Israel (Anat Zada) and the U.S. (Jocelyn Millar). 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. Allocations of funds from the various sources are summarized in **Table 1**.

Conclusions from 2005 studies:

Olfactometer trials again strongly support the hypothesis that male pear psylla are attracted to volatiles from female-infested host material. Diapause status in winterforms and age in female summerforms were both shown to affect attractiveness of females to males. Efforts to develop EAD methods are underway. Grant proposals were written and submitted to NRI and BARD, to request funding for support of pheromone chemists; both proposals were written making extensive use of the behavioral data obtained in the WSTFRC-funded project.

		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	Pending			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)	

Table 1. Summary of funded, pending, and planned projects as related to current WSTFRC project, and allocation of those funds. BARD: $287,000 for 3 years; NRI: $233,473 for 3 years.[1] Summarizes whether funding for identification and synthesis of pheromone is requested in grant. BARD: funds will be used by chemist in Israel to identify and synthesize the *Cacopsylla bidens* pheromone. NRI: funds are requested specifically for identification and synthesis of *C. pyricola* pheromone (J. Millar, at U.C. Riverside).

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

BUDGET
Project Title: Chemical ecology of pear psylla

PI: David Horton, Peter Landolt, and Christelle Guédot
Project duration: 2005-2007
Current year: 2006

Project total (3 years): $85,250
Current year request: $39,750

Year	Year 1 (2005)	**Year 2 (2006)**	Year 3 (2007)
Total	19,500	**39,750**	26,000

Current year breakdown

Item	Year 1(2005)	**Year 2 (2006)**	Year 3 (2007)
Salaries[1]	15,000	**27,500**	20,000
Benefits (30%)	4,500	**8,250**	6,000
Supplies[2]		**4,000**	
Total	19,500	**39,750**	26,000

[1] 2006: 50% GS-5 technician and 25% for GS-11 post-doctoral associate (C. Guédot). The technician will conduct behavioral assays and assist with collecting psylla for assays and electroantennogram work. C. Guédot will develop methods for conducting EAD studies and collecting volatiles, and will assist as necessary with behavioral assays.

[2] Glassware and chemicals for EAD work and collection of volatiles.

CONTINUING PROJECT REPORT **YEAR 2/3**

Project Title: Storage Decay and Postharvest Quality Research
PI: David Sugar, Professor

Organization: Oregon State University Southern Oregon Research and Extension Center

Cooperators: R.A. Spotts, C.L. Xiao

Contract Administrator: Dorothy Beaton dorothy.beaton@oregonstate.edu 541-737-3228

Objective: This research blends activities in the areas of postharvest pathology and physiology. One objective is to further develop a storage decay control program for winter pears in which diverse, independent decay control practices contribute to dependable reduction of postharvest diseases. A second objective is to develop and evaluate methods and materials for the promotion of pear quality during storage. It is proposed to include fruit quality research (including size enhancement) into this project beginning in 2006.

Significant Findings:

1. It was previously found that for Comice pears, a program of 48 hours in 100 ppm ethylene at room temperature plus 17 days of cold storage developed early ripening capacity. 72 hours in ethylene reduced the time in cold storage but the fruit were considered too soft for shipping. Current experiments are examining ethylene exposures between 48 and 72 hours.

2. Treating early harvested Comice pears with ethylene prior to MCP treatment at 50, 100, or 200 ppb resulted in very poor quality after 5 months of cold storage. Current experiments are testing MCP on very late harvested Comice.

3. In two years of study, a treatment program consisting of calcium chloride in the orchard, BioSave 110 postharvest, and storage in LifeSpan MAP was the most effective alternative program for minimizing decay incidence.

4. New fungicides Scholar and Pristine were effective against the widest range of postharvest pathogens and at the lowest concentrations, in laboratory tests.

5. A treatment program consisting of calcium chloride sprays in summer followed by Pristine fungicide one week before harvest increased the resistance of Bosc pears to blue mold when they were wounded and inoculated with the fungus after harvest. A late summer calcium program and a single-shot high dose preharvest calcium program were equivalent to a mid-summer program.

6. Laser labeling of pears may provide a slightly increased risk of entry by decay-causing microorganisms. While decay frequency was low, it was higher than expected, at least in the absence of fungicides.

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

Methods

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

Results and Discussion:

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

2. I am very close to giving up on finding a way that MCP can be beneficial for the storage quality of Comice pears. However, in 2005 MCP treatments were applied to Comice pears harvested at weekly intervals in late September and early October. Fruit will be evaluated in February and March.

3. 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.

4. 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.

5. The biofumigant *Muscodor albus* was highly effective in suppressing blue and gray mold in Bosc pears when inoculated fruit were held in closed containers with *Muscodor* at room temperature for 24 or 48 hours prior to cold storage (Table 3).

6. Atreatment program consisting of summer calcium chloride sprays, preharvest Pristine fungicide, and postharvest fungicides is being evaluated. As of late January, one aspect of the evaluation is complete. 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 compares alternative calcium programs; a late summer calcium program (3 lb. actual calcium applied 3 times in August and early September) and a single-shot high dose (5 lb. actual calcium) preharvest calcium program were equivalent to a mid-summer program (3 lb. actual calcium applied 3 times in July and early August).

7. 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. Pressure and vacuum infiltration methods with various pathogens have thus far shown that in the absence of fungicides, laser codes may provide a slightly higher risk of fruit infection. In some cases, fungi preferentially grew on lasered tissue. Current tests included laser coding with and without fungicide application.

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	**Percent of wounds infected**	
	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	**Percent of wounds infected**	
	Year 1	Year 2
Check (Standard liner)	6.4 a	17.8 a
LifeSpan MAP	4.0 b	13.0 a

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

Combined Effects (Year 2):

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

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	-

Table 3. Lesion diameters (mm) around wounds inoculated with *Penicillum expansum* or *Botrytis cinerea*, held in closed containers at room temperature for 24 or 48 hours, then stored at 31°F for two 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

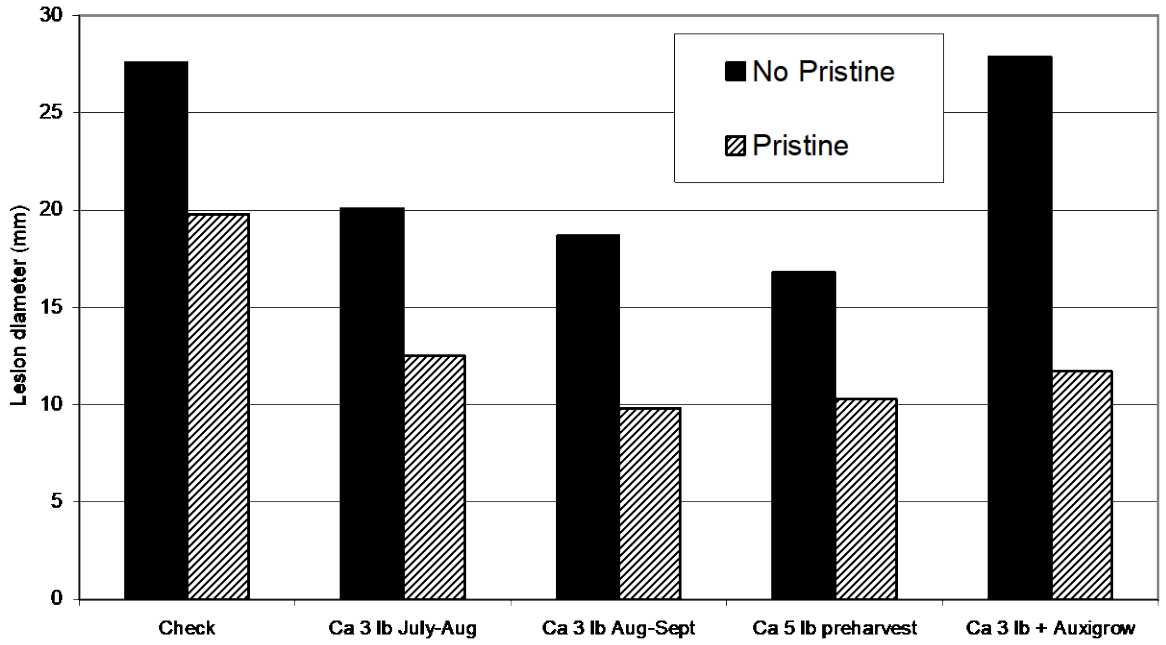


Fig. 1. Decay severity in Bosc pears inoculated after various calcium programs, +/- Pristine treatment one week preharvest.

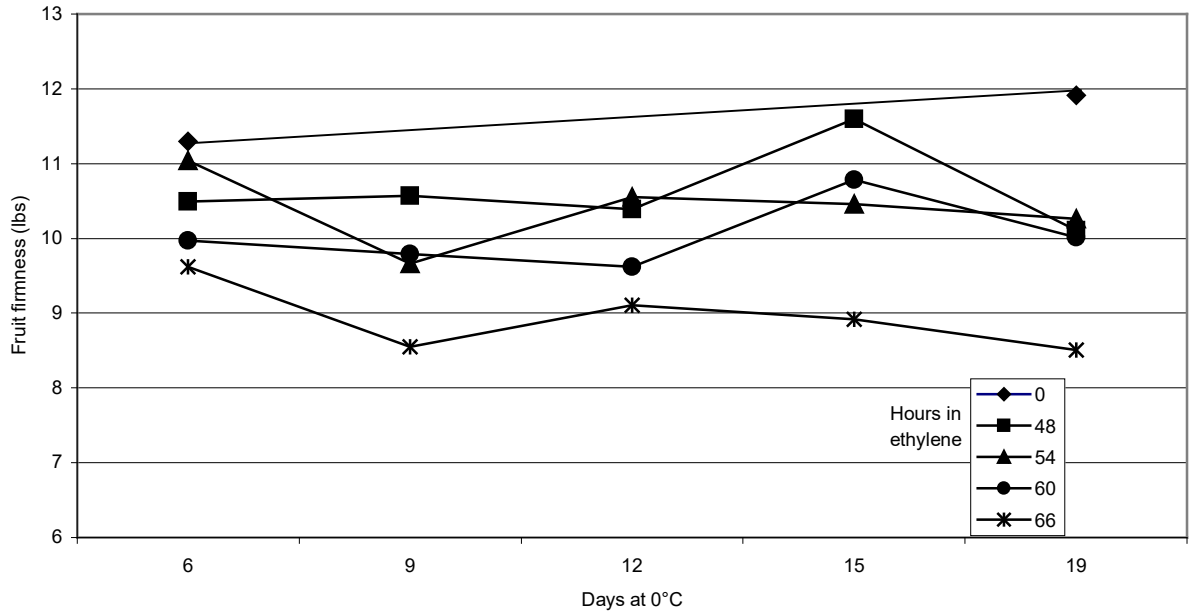


Fig. 2. Effects of ethylene treatment duration on firmness of Comice pears during cold storage.

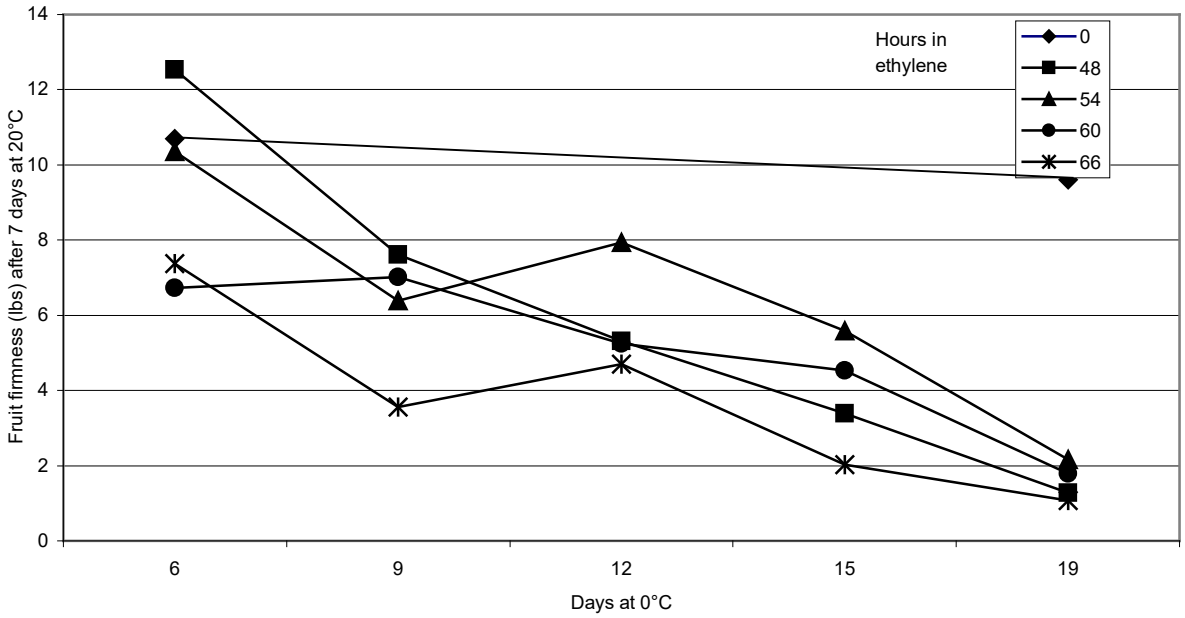


Fig. 3. Effect of ethylene exposure duration and length of cold storage on fruit firmness after 7 days at room temperature (ripe = ~5 lb.).

Budget:

Project Title: Pear Storage Decay and Fruit Quality Research
PI: David Sugar
Project Duration: 2004-2006

Current year: 2006

Project total (3 years): $90,000

Current year request: $30,000

Year	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
Total	30,000	30,000	30,000

Current year breakdown:

	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
Salaries[1]	15,894	15,789	15,736
Benefits	8,106	8,211	9,599
Services and Supplies[2]	5,600	5,600	4,265
Travel[3]	400	400	400
Total	30,000	30,000	30,000

[1] Experimental Biology Technician II, 5.5 months.
[2] Operation of orchard and lab, misc. lab supplies.
[3] Local travel to plots and packinghouses, travel to annual meeting of Fresh Pear Committee.