FINAL PROJECT REPORT
WTFRC Project Number: PR-07-705 (WSU Project #13L-4164-1210)

Project title: New technologies to control storage scald of Anjou pear

PI: Eugene Kupferman
Organization: WSU Tree Fruit Research and Extension Center
Telephone/email: 509-663-8181 x239; kupfer@wsu.edu
Address: 1100 N. Western Avenue
City: Wenatchee
State/Province/Zip: WA 98801

Co-PI: Peter Sanderson
Organization: Pace International
Telephone/email: 509-679-6680 PeterS@PaceInt.com
Address: 25 N. Wenatchee Ave.
City: Wenatchee
State/Province/Zip: WA 98801

Cooperators: Dr. Chang-Lin Xiao, WSU-TFREC; Michael Young, Stemilt Fruit; Bob Gix and Eric Strutzel, Blue Star Growers; Larry Blakeley, Blue Bird Growers.

Total project funding: **Year 1:** $38,633 **Year 2:** 38,060 **Year 3:** Terminated after 2 years

Other funding Sources

Agency Name: Pace International
Amount awarded: Residue analysis, funds for room cleaning and fruit disposal, chemicals and use of thermofogging equipment.

WTFRC Collaborative expenses:

Item	2008-2009	2009-2010
Stemilt RCA room rental	6,368	6,368
Crew labor	0	0
Shipping	0	0
Supplies	0	0
Travel	0	0
Miscellaneous	0	0
Total	6,368	6,368

Budget History

Item	Year 1: 2007	Year 2: 2008
Salaries	13,125	10,920
Benefits (47.3%)	6,208	5,165
Wages	9,000	7,600
Benefits (11.5%)	1,035	875
Supplies	21,643	12,000
Miscellaneous	0	1,000
Travel	500	500
Total	38,633	38,060

RECAP OF OBJECTIVES:

1. **Refine the methodology of thermofogging of the antioxidant ethoxyquin into pear storage rooms by varying the timing, concentration and fan cycling.**
   a. Determine the effect of time between harvest and ethoxyquin application.
   b. Determine the effect of multiple, lower concentration, applications on residue levels.
   c. Evaluate and improve ethoxyquin dispersion in storage room.
2. **Determine whether the phytotoxicity of ethoxyquin can be reduced by rinsing the fruit after it has been drenched ("split-drenching").**
3. **Correlate residue levels with incidence of storage scald.**

SIGNIFICANT FINDINGS—THERMOFOGGING:

a. <u>Determine the effect of time between harvest and ethoxyquin application.</u> Commercial quality fruit was treated 2, 11 and 15 days after harvest.
   - Residue levels were too low to determine if scald was related to application timing.
   - Scald incidence was inversely proportional to ethoxyquin residue level and was controlled at 1.0 ppm.
   - Delay in the initial application of ethoxyquin led to higher phytotoxicity.
   - Fruit on the top of the bins had more severe phytotoxicity than fruit lower in the bin.

b. <u>Determine the effect of multiple, lower concentration applications on residue levels.</u> Commercial quality fruit was treated twice at low concentrations, once 2 days after harvest and again 60 days after harvest.
   - The first application produced low residue levels (< 0.5 ppm). The residue levels after the second application were much higher (3.2 ppm to 5.6 ppm).
   - Despite the higher residue levels there was no phytotoxicity on fruit following long-term CA storage.
   - Residue levels after the initial application were too low to determine if scald was related to multiple applications.

c. <u>Evaluate and improve ethoxyquin dispersion in storage room</u>. Strategies to improve dispersal of ethoxyquin throughout the room included utilization of a free-flowing air manifold fitted with a fan to provide active and passive ventilation of the room to move ethoxyquin through the storage room and different types of bin covers.
   - Manifold: Both active and passive ventilation of the room via the manifold improved dispersion of ethoxyquin throughout the room, within the bins, and the stacks; however, uniformity of residue was not improved.
   - Bin position: Fruit in bins on the top of each stack had higher concentrations than those in bins within the stack.
   - Within bin: The top layer of fruit in each bin had a higher concentration of ethoxyquin than fruit in the middle or bottom of bin.
   - Bin covers: Covers were tested to reduce the concentration on fruit in the top bin. All types of bin covers tested reduced deposition of ethoxyquin on the fruit in the top bin of each stack.
     - In the uncovered bins, many residue levels exceeded the legal limit.
     - In bins covered with wooden palettes, residues exceeded goal levels and sometimes exceeded the legal limit.
     - Plastic sheeting used as bin covers reduced the chemical application to below target levels.

- Pulsing of the overhead coil fans did not improve uniformity of residue.
- Plastic bins: The use of plastic bins did not significantly improve the dispersion of ethoxyquin within the bin or between bins.

RESULTS AND DISCUSSION—THERMOFOGGING

The first goal was to fine tune the operating parameters of the thermofogger, so that the residue of ethoxyquin was appropriate and repeatable. Determination of the optimum method to utilize the thermofogger application machine is complicated since the ethoxyquin molecule is larger and heavier than that of DPA. Thus the ethoxyquin molecule falls out of the air more easily than smaller molecules (DPA) or gases (1-MCP). Pace is actively working to increase the efficiency and uniformity of thermofogging applications. Modifications in equipment and the set-up of the experimental chamber required numerous applications using cull pears before repeatability was accomplished. The results of the tests were settings of air temperature and velocity within the thermofogger that optimized particle size.

Dummy bins (bins wrapped in plastic to prevent through-flow) were used in 2007 to better mimic commercial treatments while minimizing the amount of fruit needed to complete these trials. In 2008, bins of cull fruit were used to fill each room to minimize costs while providing a realistic air flow pattern.

a. Determine the effect of time between harvest and ethoxyquin application. Ethoxyquin was applied to commercial quality fruit 2, 11 and 15 days after harvest. Residue levels and phytotoxicity at the top of each bin were higher than those in the middle or bottom (Table 1). Scald incidence was the lowest at the top of each bin. The target residue for this experiment was between 1 and 3 ppm; which was reached only in the top layer of fruit in the 15-day treatment. This fruit had the lowest incidence of scald (5%). Because most residue levels were too low to effectively control scald, the effect of timing cannot be determined.

   Bins that were fogged 15 days after harvest had unacceptably high levels of phytotoxicity, especially on the top layer of fruit (Table 1).

b. Determine the effect of multiple, lower concentration, applications on residue levels. Low concentrations of ethoxyquin were applied twice to commercial quality fruit: once 2 days after harvest, and again 60 days after harvest. The residues analysis after the application 60 days after harvest resulted in very high ethoxyquin residues compared with that obtained after the application 2 days after harvest (Table 2). Whether uneven residue was due to fruit temperature (45 ºF at 2 days, 32 ºF at 60 days) or room temperature (65 ºF at 2 days, 34 ºF at 60 days) at time of application, or other factors, is unknown. However, when the fruit was removed from long-term CA storage, the residues were below the effective limit.

   Scald was effectively controlled in the fruit at the top of the bin; however, the fruit in the middle of the bin had higher scald levels. This is likely due to the low level of ethoxyquin residue after the first application (0.2 ppm) in the middle of the bin.

   Multiple treatments of ethoxyquin fogging show promise as an effective way to control scald while minimizing phytotoxicity, providing sufficient residue remains on the fruit after the first treatment. From this work, it appears that the target ethoxyquin residue level for this first treatment should be ≥ 0.5 ppm. Additional treatments should increase the total residue to ≥ 1.0 ppm. Delaying the first treatment of pears with ethoxyquin until after they have been in storage does little to control scald and can result in high fruit damage.

c. Evaluate and improve ethoxyquin dispersion in storage room. Our experience showed that fruit at the top of the room retained the highest chemical concentration, at times in excess of maximum legal residue. One approach was to determine whether covering the topmost bin in the stack would help reduce the residue on fruit in those bins. Some bins remained uncovered, some were covered with shade cloth stapled over the top of the bins, and some were covered with plastic sheeting stapled on pallets to elevate it above the fruit in the top bin, which would allow normal airflow across the top of the bin so as not to restrict cooling. Fruit in the covered bins had lower residue than fruit without covering (Table 3).

In an attempt to reduce this problem, a manifold was constructed from porous flexible plastic pipe and placed on the floor across the back wall and one side of the room then vented out the door. A fan was fitted to the end of the manifold pipe. When the fan was on, the manifold actively ventilated the room. When the fan was off, over-pressuring by the fogger caused the manifold to passively ventilate the room. Full bins of fruit were stacked three-high under the cloth covered bins to determine chemical dispersion throughout the room. Residue levels were higher when the manifold fan was active (Table 4). There was a greater difference in residues on fruit in the middle of the bin as compared with fruit at the top of the bin when the manifold fan was active (Table 5).

Additional treatments were applied to compare bin type, the effect of passive vs. active manifold, the use of the room coil fans, and different cover configurations on the top bin. The treatments are listed below.

Treat	Bin type	Manifold	Coil fan	Covers
1	Plastic	Passive	OFF	Plastic (tight) or wooden pallet
2	Wooden	Passive	OFF	Plastic (tight) or wooden pallet
3	Wooden	Active (fan on)	OFF	Plastic (loose) or wooden pallet
4	Wooden	Active (fan on)	Pulsed	Plastic (loose) or wooden pallet
5	Wooden	Passive	OFF	Plastic (loose) or wooden pallet

Based on residue results from earlier trials, the goal for residual ethoxyquin within each treated bin was 1.5 to 2.0 parts per million (ppm). The upper acceptable limit is 3 ppm. The residue samples taken from the top and middle of each treated bin (without bin covers) are reported in Table 6. The residues for the top-of-stack bins using either plastic sheeting stapled to the bin or elevated wooden pallet covers are shown in Table 7.

Statistical analysis was performed using ANOVA-GLM within SAS v. 9.1, to determine if there were significantly different levels of residue among treatment (1 to 5), bin sampling locations (top or middle) or top-bin cover material (plastic or pallet).

Analysis of this data leads to the following findings:

1. The use of plastic bins did not significantly improve the dispersion of chemical within the bin over wooden bins.
2. No treatment dispersed chemical sufficiently to meet goal residue levels within the bins. Although treatment averages show residues within the target range (1.5 to 2.0 ppm), in only 8% of cases did fruit from both the top and middle of the same bin meet the target level, due to uneven distribution within the bins (data not shown).
3. Plastic sheeting bin covers were effective in reducing high concentrations of residue in the top-of-stack bin but also reduced the chemical application to below effective levels in most cases.
4. Plastic sheeting tightly stapled to the tops of the topmost wooden bins restricted penetration of fog so that insufficient residues were achieved within those bins. This effect was not seen with plastic sheeting on the tops of plastic bins (Table 8).

5. Wooden palette covers reduced excessive deposition on fruit from the topmost bins, but residues exceeded target levels and sometimes exceeded permissible levels.

Table 1. Thermofog ethoxyquin residue levels at each application date, followed by percentage of fruit with scald and phytotoxicity (pink staining) after long-term CA storage.

Location in bin	Ethoxyquin residue, scald and phytotoxicity for each application								
	+2 days			+11 days			+15 days		
	Residue (ppm)	Scald (%)	Phyto (%)	Residue (ppm)	Scald (%)	Phyto (%)	Residue (ppm)	Scald (%)	Phyto (%)
Top	0.7	19%	0%	0.6	9%	7%	**1.0**	**5%**	21%
Middle	0.3	41%	0%	0.5	11%	0%	0.6	8%	9%
Bottom	0.3	42%	0%	0.5	14%	0%	0.6	10%	3%

Table 2. Thermofog residues for low concentrations of ethoxyquin applied 2 and 60 days after harvest, including residues after long-term CA storage and incidence of scald and phytotoxicity.

Location in bin	Ethoxyquin residue (ppm)			Scald (%)	Phyto (%)
	2 days	60 days	Following CA		
Top	0.4	5.6	2.6	1%	0%
Middle	0.2	3.2	2.0	10%	0%

Table 3. Effect of covers on top bins on deposition of ethoxyquin applied by thermofogging.

Cover material	Ethoxyquin (ppm)
None	3.8 a
Cloth	2.0 b
Plastic	2.5 b
	(P = 0.006)
Means separated using Tukey's HSD	

Table 4. Effect of moving air with a free-flowing manifold on deposition of ethoxyquin applied by thermofogging within bins of fruit.

	Ethoxyquin Residue (ppm)
Active manifold	2.2 a
Passive manifold	1.3 b
	(P = 0.021)
Means separated using Tukey's HSD	

Table 5. Effect of moving air with a free-flowing manifold on deposition of ethoxyquin applied by thermofogging within bins of fruit.

	Manifold	
Fruit location	**Active**	**Passive**
Top	4.6 a	2.1 b
Middle	2.5 b	1.7 b
	(*P* = 0.044)	
Means separated using Tukey's HSD		

Table 6. Residue levels for the top and middle of the thermofog treated bins (excluding top-of-stack).

Treat	**Bin type**	**Manifold**	**Coil fan**	**Covers**	**Ethoxyquin residue (ppm)**	
					Top of bin	**Middle of bin**
1	Plastic	Passive	OFF	Plastic (tight) or wooden pallet	2.1	2.1
2	Wooden	Passive	OFF	Plastic (tight) or wooden pallet	3.2	1.7
3	Wooden	Active	OFF	Plastic (loose) or wooden pallet	2.2	1.4
4	Wooden	Active	Pulsed	Plastic (loose) or wooden pallet	1.4	1.5
5	Wooden	Passive	OFF	Plastic (loose) or wooden pallet	2.1	1.7
				Average	***2.2 a***	***1.7 b***

Treatments, *P = 0.1537* (not significant)
Sample position in bin (top or middle), *P = 0.0092*

Table 7. Residue levels for the top and middle of the top-of-stack bins only, by cover type, thermofog trials.

Treat	**Bin type**	**Manifold**	**Coil fan**	**Ethoxyquin residue (ppm)**			
				Plastic cover		**Wooden pallet cover**	
				Top of bin	**Middle of bin**	**Top of bin**	**Middle of bin**
1	Plastic	Passive	OFF	0.9	2.0	3.7	2.3
2	Wooden	Passive	OFF	0.4	0.5	3.6	3.1
3	Wooden	Active	OFF	0.5	1.2	4.1	1.9
4	Wooden	Active	Pulsed	0.6	0.9	3.2	2.1
5	Wooden	Passive	OFF	1.1	1.0	5.4	3.3
			Average	***0.9 a***		***3.3 b***	

Treatments, *P = 0.1537* (not significant)
Sample position in bin (top or middle), *P = 0.0092*
Top bin cover type, *P = <0.001*

Table 8. Interaction of bin and cover types on ethoxyquin residue.

Bin type	**Cover material**	**Ethoxyquin (ppm)**
Plastic	Wooden pallet	3.0 a
Plastic	Plastic (tight)	1.5 b
Wood	Wooden pallet	3.3 a
Wood	Plastic (tight)	0.5 c
		P = 0.007

SIGNIFICANT FINDINGS—SPLIT DRENCHING

Liquid ethoxyquin was applied as a drench together with a fungicide (control), or as two applications in which the ethoxyquin was applied followed by a second drench (4 hours or 7, 21 or 42 days later) with the fungicide. Residue analysis indicated that there was no significant reduction in ethoxyquin following the second drenches applied over a 42-day period. Correlation of scald, burn and residue data for 2007 crop indicate:

1. Split drenching significantly reduced ethoxyquin burn especially when separated by 21 days or more.
2. Scald was reduced in proportion to the level of residual ethoxyquin with zero scald at levels of 1.0 ppm or greater.
3. Ethoxyquin residue levels were greater the longer the interval between initial and second drenches.

Split drenching was performed on the 2008 crop and residue samples were collected. The fruit will be evaluated for burn and scald in spring 2009. An additional split drench interval of 56 days was added to the 2007 protocol. To date, the ethoxyquin residue has not significantly degraded in the 56 days following initial treatment.

RESULTS AND DISCUSSION—SPLIT DRENCHING

Experiments in 2007 have shown that it is possible to obtain consistent and appropriate residue levels of ethoxyquin without phytotoxicity by drenching first with ethoxyquin and then with a fungicide. This led from the observation in previous years that burn developed over time when 'liquid' ethoxyquin residue remained on the fruit. When this 'liquid' residue was removed by washing or brushing the burn did not develop. Correlation of scald, burn and residue data indicate that split drenching significantly reduced ethoxyquin burn especially when separated by 21 days or more. Scald was reduced in proportion to the level of residual ethoxyquin with zero scald at levels of 1.0 ppm or greater and ethoxyquin residue levels were greater the longer the interval between initial and second drenches (Table 9). Residue levels at the time of drenching for the 2008 crop are shown in Table 10.

RESULTS AND DISCUSSION—CORRELATE RESIDUE LEVELS WITH SCALD

For Anjou pears treated with ethoxyquin via a drench solution (1350 ppm ethoxyquin applied within 2 days of harvest), there appears to be an inverse relationship between ethoxyquin residue (measured after final drench) and scald (Table 9). Fruit with the lowest residue level (0.6 ppm) had the highest incidence of scald (10%), and fruit with the highest residue level (1.0 ppm or higher) had the lowest incidence of scald (0%). Ethoxyquin residue levels were not measured after long-term storage for this drenched fruit.

The research in this project has relied upon analysis of ethoxyquin residue performed by the Pace International laboratory using proprietary methodology developed in that laboratory. Without this information and cooperation, the project would not be possible.

Table 9. Ethoxyquin drench phytotoxicity (burn), and scald after long-term CA storage, and ethoxyquin residue (analyzed after final drench), 2007 crop. For the split drenches, ethoxyquin (1350 ppm) was applied alone, then followed by a drench of TBZ at the time intervals stated.

Treatment	Burn (%)	Scald (%)	Ethoxyquin Residue (ppm)
Control (single drench)	96	5	0.7
Split drenches			
4 hours	30	10	0.6
7 days	22	1	0.8
21 days	3	0	1.1
42 days	5	0	1.0

Table 10. Ethoxyquin and TBZ residue levels at the time of drenching, drench trial 2008 crop. For the split drenches, ethoxyquin (1350 ppm) was applied alone and then followed by a drench of TBZ at the time intervals stated.

	Residues	
Treatment	**TBZ (ppm)**	**Ethoxyquin (ppm)***
Control (single drench)	2.6	1.0
Split drenches		
4 hours	1.2	0.6
7 days	1.6	NA
21 days	1.0	1.0
42 days	1.0	0.6
56 days	1.2	0.8

* Ethoxyquin residue immediately after TBZ application (2nd drench)
NA – Indicates missing data

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EXECUTIVE SUMMARY

Thermofogging

This has been a joint project with Pace International to determine how best to apply the antioxidant ethoxyquin to Anjou pears in storage using thermofogging. This partnership has been funded for 2 years by the Fresh Pear Committee and Pace International. This initially was planned as a 3 year project, but in the view of the researchers, there is little additional research that can be done on a small scale due to facilities and engineering considerations.

Each thermofogging machine must be calibrated to the size of the storage room in order to determine the correct dosage. Therefore, the first goal was to tune the operating parameters of the thermofogger, so that the residue of ethoxyquin was appropriate and repeatable. This was difficult due the small size of the 40-bin CA rooms at Stemilt. Modifications in equipment and setting up the experimental chamber required numerous experiments using cull pears before the dosing was repeatable.

We determined that location of the bin in the stack, and fruit location in the bin affected residue. Residue on fruit in the topmost bins in the stack often exceeded legal residue limits, which was alleviated when those bins were covered. In initial applications in which relatively low residues were detected, fruit at the top of the bin had minimal scald, while fruit within the bin developed more scald due to insufficient residue. This led to awareness about the lack of information on the appropriate residue level necessary for scald control and the rate of ethoxyquin degradation over time.

In a series of experiments, we determined: 1) ethoxyquin residue levels must be 1.0 ppm or greater to control scald, 2) a delay of more than 2 weeks in the initial application after harvest resulted in serious skin burn, 3) a light initial application close to harvest followed by a second application 60 days later reduced skin burn, and 4) high residues measured after the second application dissipated to acceptable levels after long-term CA storage.

In an effort to reduce the excessive residue on the fruit in the topmost bins, we tried various covers including porous fabric stapled to the bins, sheet plastic stapled to the bins, plastic sheets elevated over the bins, and wooden pallet bottoms covered in plastic. All cover types prevented excessive ethoxyquin residues in top bins. However, plastic sheeting tightly stapled to the tops of wooden bins restricted penetration of fog so that insufficient residues were achieved. This effect was not seen when plastic bins were used. Open structured covers (bin bottoms, pallets covered with plastic and shade cloth) were each acceptable. Bin type (wooden or plastic), room ventilation and room fans did not improve chemical dispersion into the topmost bins.

Distribution of ethoxyquin residues was not affected by bin position in the stack except when the topmost wooden bins were tightly covered with plastic sheeting, which significantly reduced residues. Pressure venting systems, manifolds and pulsed fans did not affect residue distribution among or within bins.

The small room size with high power fans presented challenges that may not be present in large commercial rooms. The thermofogging unit adapted for the research rooms also presented a situation that might not represent a commercial operation. Thus this research cannot proceed beyond what we have accomplished so additional funding is not being requested.

Split Drench

Liquid ethoxyquin was applied as a drench together with a fungicide, or as two applications in which the ethoxyquin was applied followed by a second drench with the fungicide up to 56 days later. This split application may prove to be a useful method of controlling scald while reducing levels of phytotoxicity. Data thus far are positive with regard to fungicide and ethoxyquin residue levels, effective control of scald and reduced phytotoxicity. Fruit from the 2008 crop will be examined in the spring of 2009.