

## FINAL REPORT

WTFRC Project # PR-01-88

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

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

### OBJECTIVES

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

### SIGNIFICANT FINDINGS

#### Objective 1

Optimal hexanal concentration was 2 mg/l for 24 hrs or 4 mg/l for 18 hours. The most effective temperature was 20°C (68°F) for the control of *Penicillium* and either 15°C (59 °F) or 20°C (68°F) for the control of *Botrytis*.

#### Objective 2

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

#### Objective 3

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

#### Objective 4

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

### METHODS

#### Large Scale Efficacy Tests

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

#### 2002-2003

**WTFRC d’Anjou Pears.** Two bins of d’Anjou pears from Wenatchee, WA were divided into three replicates and hand packed in polylined boxes. The pears were placed in a 1 m<sup>3</sup> chamber at a temperature of 15°C (59 °F) and fumigated at 4 mg/l for 18 hours. After fumigation the polyliner was

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

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

#### 2003-2004

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

Table 1. Description of the various treatments.

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

#### Small Scale Efficacy Tests

##### 2001-2003

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

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

## **2003/2004**

### **Quality Analysis**

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

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

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

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

## **RESULTS AND DISCUSSION**

### **Large Scale Efficacy Test**

#### **2001/2002.**

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

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

**2002/2003**

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

**Results Large Scale Fumigation**

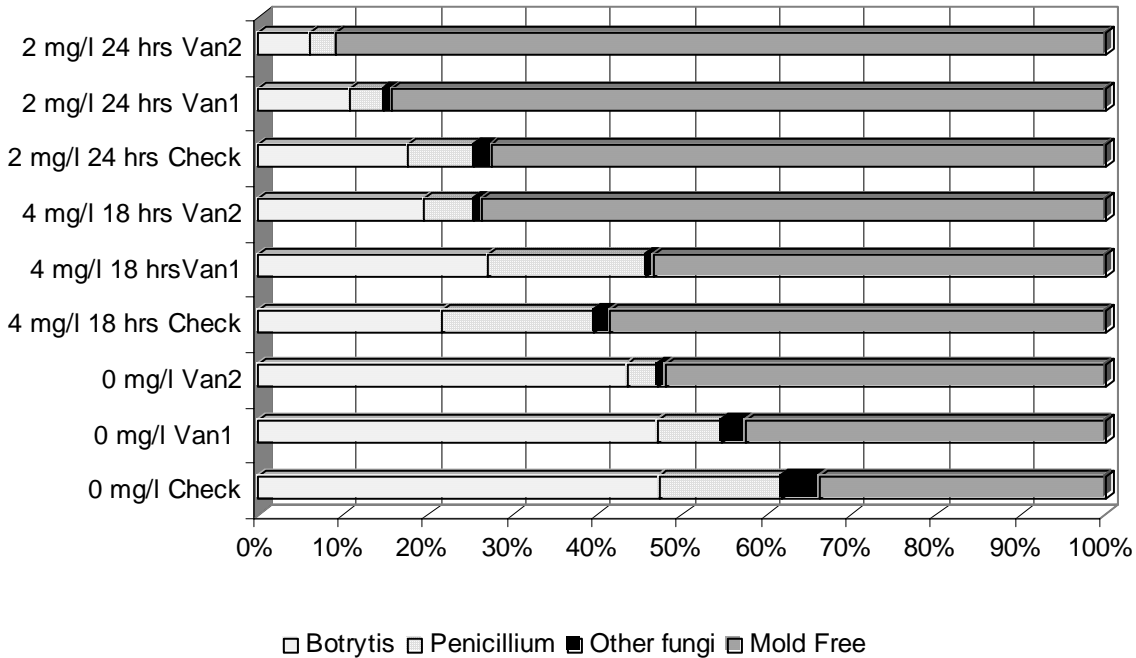


Figure 1. Results of large scale fumigation experiment

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

**2003-2004**

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

**Small scale efficacy tests**

*Botrytis* (Grey Mold)

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

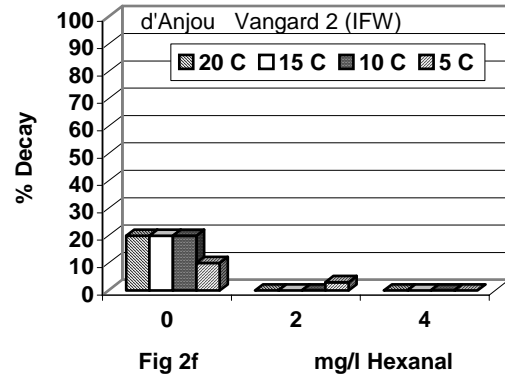
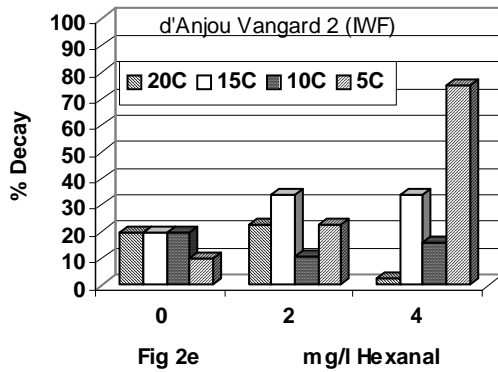
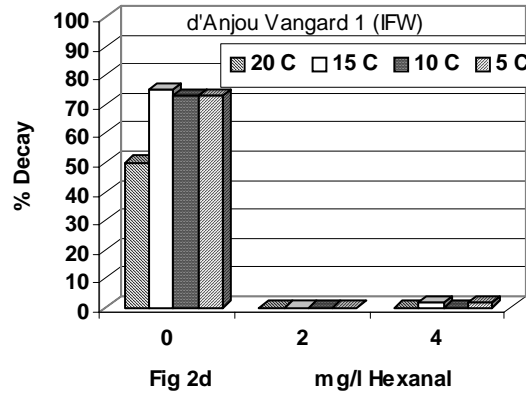
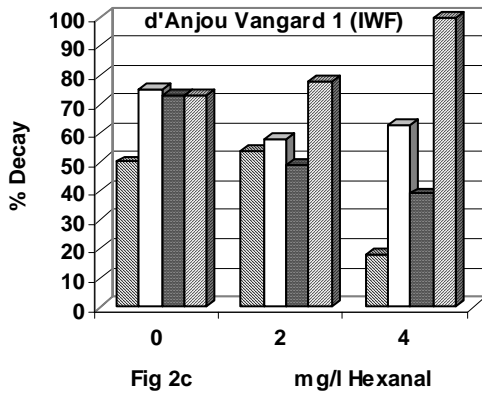
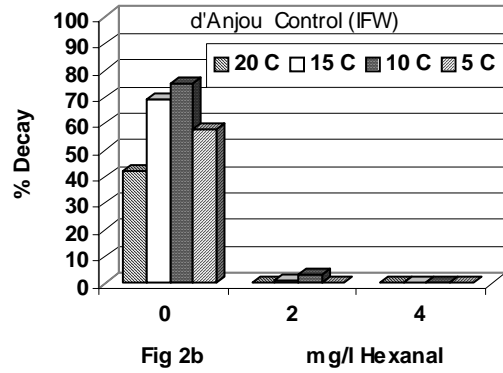
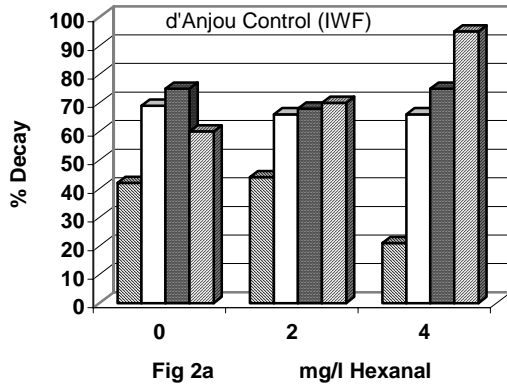


Figure 2. The effect of hexanal on (grey mold) inoculated, wounded, then fumigated (IWF) d' Anjou pears (Fig 2a, 2c, 2e) vs inoculated, fumigated then wounded (IFW) d' Anjou pears (Fig 2b, 2d, 2f). Hexanal used as a surface sterilant on d' Anjou pears, shows an almost total reduction of decay (right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

**Penicillium (Blue Mold)**

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

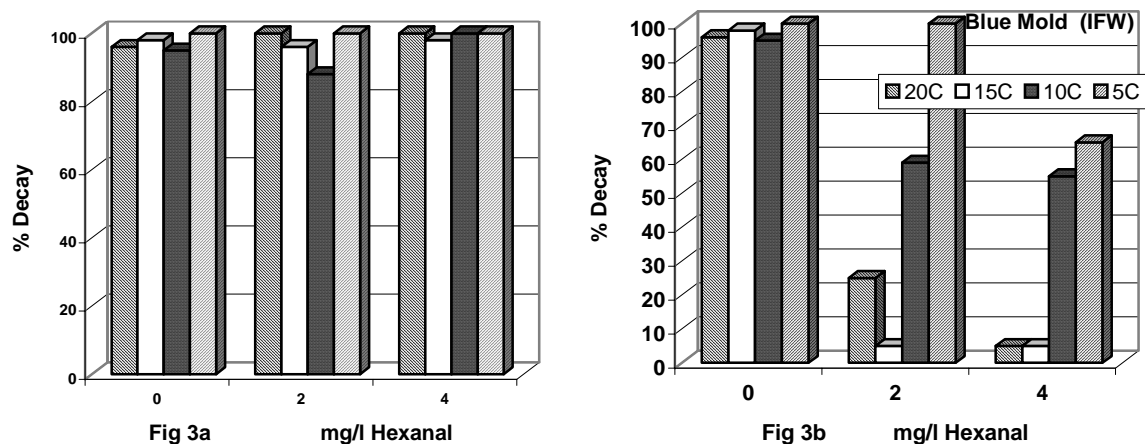


Figure 3. The effect of hexanal on (Blue mold) inoculated, wounded, then fumigated fruit (IWF) (Fig 3a) vs inoculated, fumigated then wounded fruit (IFW) (Fig 3b) at a range of temperatures.

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

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

## Quality Analysis

### Fruit Firmness

Table 4 and Figure 4 show the results for fruit firmness of the various treated d’Anjous.

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

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

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

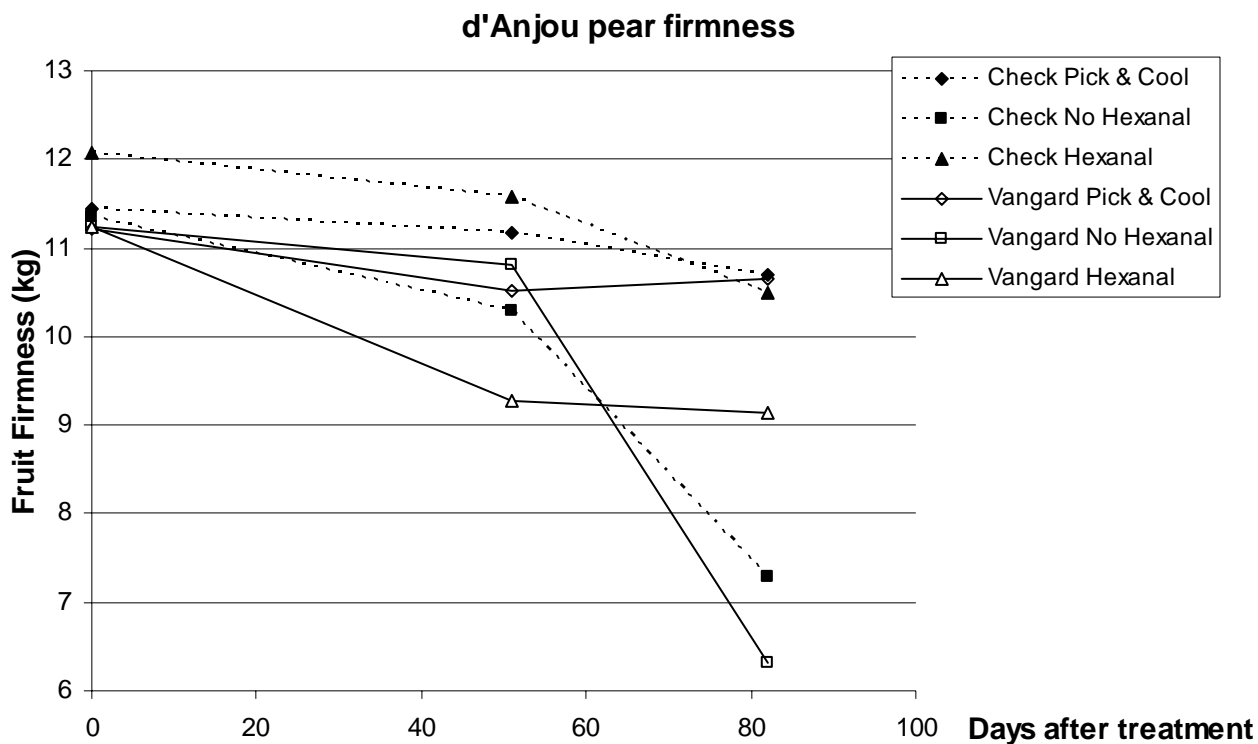


Figure 4. d'Anjou pear fruit firmness over time in air storage at 1<sup>0</sup>C (34 °F).

### Tissue Analysis

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

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

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

### Headspace Analysis

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

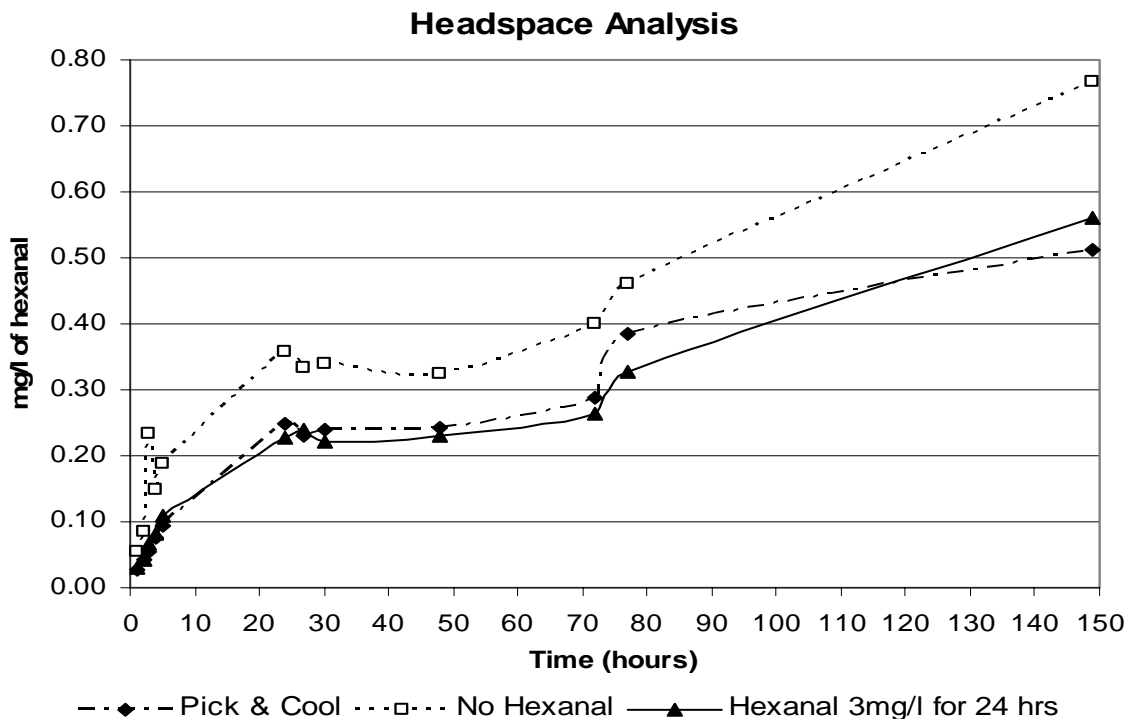


Figure 5. Quantity of hexanal released into the headspace of sliced bagged d'Anjou pears.

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

#### Conclusion

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

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

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



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

	<b>Year 1</b>	<b>Year 2</b>	<b>Year 3</b>
<b>Year</b>	<b>2001-2002</b>	<b>2002-2003</b>	<b>2003-2004</b>
Salary	6,500	6,500	6,500
Materials and supplies <sup>1</sup>	500	500	500
Travel <sup>2</sup>	500	500	500
<b>Total</b>	<b>7,500</b>	<b>7,500<sup>3</sup></b>	<b>7,500<sup>3</sup></b>

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

**References:**

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

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