FINAL PROJECT REPORT # PH01-138

Title: **Hexanal vapor to control storage decay**

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OBJECTIVES:

- Identify optimal hexanal concentration, temperature, and duration required to control *Penicillium expansum* (blue mold), and *Botrytis cinerea* (grey mold).
- Determine optimum concentration and length of exposure required to fumigate apples in commercial storage rooms.
- Determine the effect of hexanal fumigation on stored apple aroma.
- Evaluate the potential for combining hexanal with MCP to control post harvest decay.

SIGNIFICANT FINDINGS (2001/02) (Year One)

! Hexanal used as a surface fumigant eliminates blue and grey mold on inoculated fruit.

! Hexanal reduces the amount of mold that develops in wounds. It is most effective on grey mold.

! The best rates and duration to control post harvest pathogens are 2 mg/l for 24 hours or 4 mg/l for 18 hours at 20^{o}C (68^{o}F).

! Of the apple cultivars tested, Gala is the most sensitive to hexanal, Red Delicious the most tolerant.

! Hexanal at concentrations above 12 mg/L for 48 hours at 1^{o}C (34^{o}F) or 2mg/l for 48 hrs at 20^{o}C (68^{o}F) is phytotoxic to apples and causes scald like discoloration.

SIGNIFICANT FINDINGS (2002/03) (Year Two)

- Hexanal used as a surface fumigant eliminates grey mold on inoculated fruit at a range of temperature from 5^{0}C (40^{o}F) to 20^{0}C (68^{o}F), while blue mold is eliminated only at 15^{0}C (59^{0}F) and 20^{0}C (68^{o}F).
- Vangard 75WG (Cyprodinil) applied two weeks before harvest, reduced the amount of *Botrytis* and *Penicillium* that develops in wounds.
- Gala apples fumigated with 2 mg/l of hexanal for 24 hours had significantly improved aroma.
- Freshly cut Fuji apples may release up to 0.5 mg/l of natural hexanal.

SIGNIFICANT FINDINGS (2003/04) (Year Three)

➢ The use of hexanal had no deleterious effect on apple quality.

➢ There was no increase in the amount of hexanal in the tissue of fumigated apples.

➢ A second fumigation with 3.0 mg/l of hexanal after storing apples for five months removed surface postharvest pathogens such as Blue and grey molds.

METHODS

Large Scale Efficacy Trial.

1. Pre-harvest/Fumigation Experiment

Gala apples were treated two weeks prior to harvest with an application of Vangard 75WG (Cyprodinil), a systemic fungicide, using a rate of 6.2g/10 litres (10 oz/acre). Gala apples (3 boxes of each treatment) were harvested on the day of the fumigation. The harvested the fruit were immediately placed in the cold room and air cooled to 15^0C (59^0F). The treated apples were then fumigated at 3.0 mg/l for 24 hours. The level of hexanal during the fumigation was monitored by using a gas chromatograph (GC Model 910, Questron Technologies Corp. Mississauga, Ontario, Canada) 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). Following fumigation, the chamber was vented and the apples were boxed and placed in the cold room at 1^0C (34^oF). The trial was replicated four times. The fourth replicate was used for quality analysis (fruit firmness, titratable acidity (TA) and soluble solids). After 6 months of storage at 1^0C (34^oF), the apples were removed from storage and placed in the ripening room at 20^0C (68^oF) for one week then rated for decay.

2. Bin Quantity Experiment.

Bins of apples, McIntosh (Rep 1), Jonagold (Reps 2 & 3) were harvested on the day they were treated with hexanal. The control bin was place in a cold room at 1^0C (34^oF) immediately after harvest. Each fumigated bin replicate was fumigated with 3.0 mg/l of Hexanal for 24 hours at 15^0C (59^oF), then placed into cold storage. After five months of storage the apples were rated for decay. Prior to rating for decay, sub-samples from each bin/treatment were removed for a post storage experiment. This was replicated three times.

3. Post Storage Experiment

After five months of storage and before the apples in the bin quantity experiment were rated for decay, 10 apples per treatment were removed and placed into a crisper. Half were fumigated with 3mg/l hexanal for 24 hours. Control apples were not fumigated but remained at 20^0C (68^oF) for the 24 hours. After fumigation, each apple was wounded four times with a sterile wounding device. Apples remained at 20^0C (68^oF) for one week, and then rated for decay.

Fruit Quality Analysis

One of the concerns expressed to us was whether or not fumigating at 15^0C (59^oF) would affect the quality of the apples in storage. Though this was not one of our original objectives for this study, it was a valid concern. A number of tests were utilized to address this concern.

1. Fruit Firmness: Using a pressure tester (Model EPT-1, Lake City Technical Products, Kelowna, BC, Canada) equipped with an 11 mm tip, the various treatments were checked for fruit firmness.

2. Fruit TA. Titratable acidity (TA) was determined by titrating 15 mls of fresh juice in 75 mls distilled water to a malic acid endpoint (pH 8.2) using a Brinkmann 719S Titrino (Brinkmann Instruments, Rexdale, Ontario, Canada)

3. Fruit Soluble Solids. Soluble solids were determined with an AO Scientific Instruments digital refractometer ABBE MARK II (Buffalo, New York). The various treatments were checked for these values immediately after fumigation, 34 days later, 76 days and after 199 days of cold storage.

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

5. Tissue Analysis: A minimum of two apples per replicate were used. Each apple was cut into eight slices using a fruit sectionizer. A core from four slices per fruit was taken using a #4 coring tube. Five grams of tissue was added to 10 mls of 0.1M HCl. The sample was homogenized for 60 seconds (Brinkmann Homogenizer, Rexdale, Ontario, Canada). Five mls of the fruit slurry was placed in a 25 ml vial, and sealed (Each sample was replicated three times). The samples were then incubated for one hour in a water bath at 60^0C (140^oF). An one cc headspace sample was taken using a BD 1 ml sub-Q syringe, and injected into a gas chromatograph. A standard was made by mixing 5.0 µl of hexanal in 10 ml 0.1 M HCl. A 0.5 ml sample was added to 4.5 ml 0.1 M HCl in a 25 ml vial. This sample was incubated for one hour at 60^0C (140^oF). An one cc headspace sample was injected into the gas chromatograph.

Statistical analysis. Analysis of variance procedure (SAS Institute, Cary, NC) was applied to the data and following a significant F test means were separated with Duncan's Multiple Range Test (P=0.05).

RESULTS AND DISCUSSION

Large Scale Efficacy Trial.

1. Pre-harvest/Fumigation Experiment

The results of the large scale efficacy trial is shown in figures 1a, b, c, d. Figure 1a shows the amount of "decay" to mold free Gala apples after 200 days in storage at 1^0C (34^oF), and then at 20^0C (68^oF) for one week. There was very little flesh decay present. Most of the "decay" was grey or blue mold growing on the stem or in the calyx end of the apple.

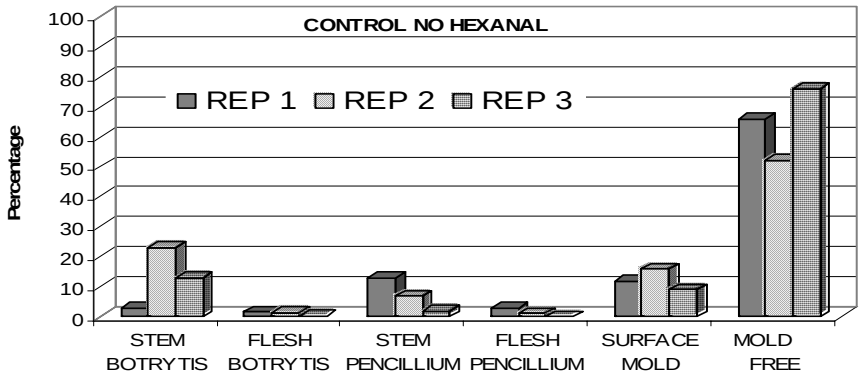

Figure 1a. Results of large scale trial, Gala apples, no fumigation.

Figure 1b shows the result from the three hexanal fumigated replicates. The second replicate had 20% surface mold contamination, and the third rep had 28% stem *Botrytis*. There is very little flesh decay present and great variability between replicates.

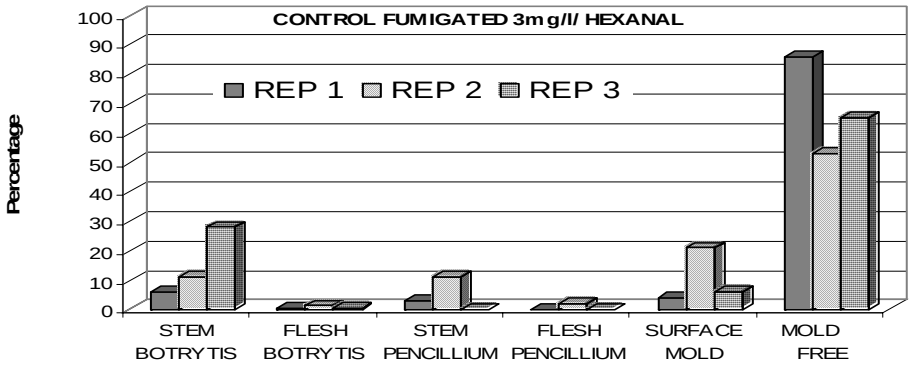

Figure 1b. Results of large scale trial, Gala apples fumigated with 3.0 mg/l hexanal for 24 hours.

Figure 1c shows the results from the Vangard (Cyprodinil) treatment. There is considerable variability between replicates, but overall there appeared to be a higher level of surface contamination when compared to the other treatments.

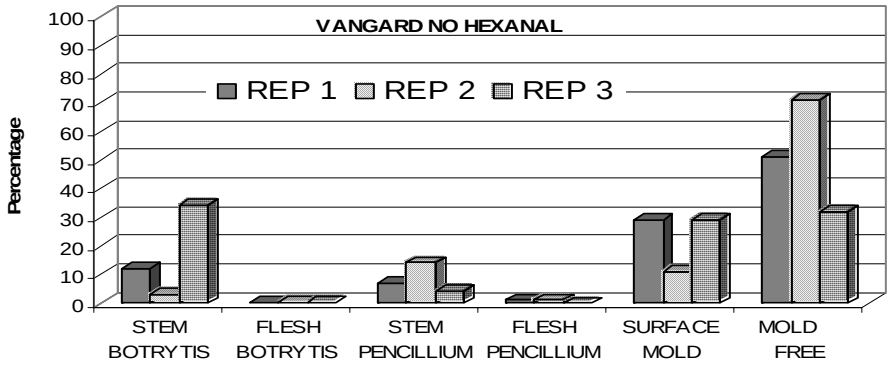

Figure 1c. Results of large scale trial, Vangard (Cyprodinil) treated Gala apples, no Hexanal.

Figure 1 d shows the results from the Vangard-Hexanal treatment. The first and second replicated show similiar amounts of decay and over 80% mold free apples. The third replicate had a slightly higher level of surface contamination and therefore lower amount of mold free apples.

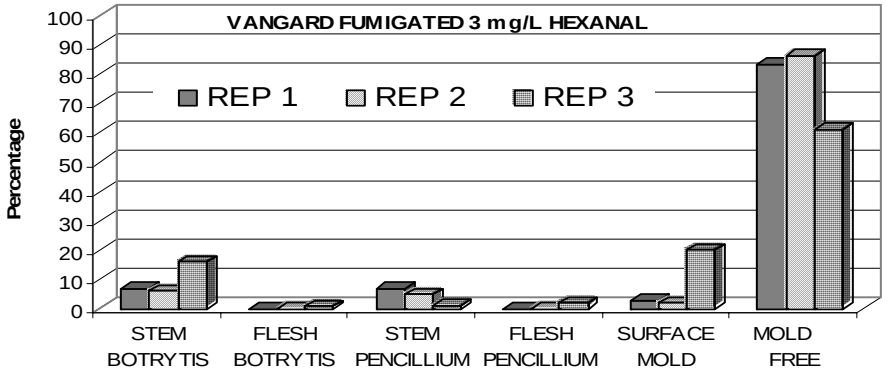

Figure 1d. Results of the Vangard (Cyprodinil) treated Gala apples, fumigated with 3.0 mg/l hexanal for 24 hours.

Figure 2, shows the averaged results for the above four treatments. The Vangard (Cyprodinil) fumigated treatment had the most mold free fruit, and the control fumigated treatment was only slightly higher than the non-fumigated check. The Vangard (Cyprodinil) treatment had the lowest amount of mold free fruit. Statistical analysis of the data did not indicate any significant differences between the treatments at P>0.05.

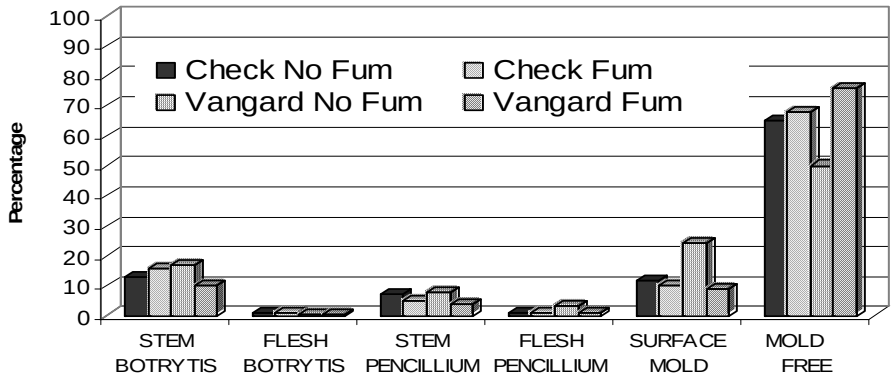

Figure 2. The average of the four treatments.

These results need to be tempered by the fact that there was very little decay in all treatments and the variability between the replicates. Most of the "decay" was either *Botrytis* or *Penicillium* contamination (hypae) on the stems or in the calyx end of the apples or *Alternaria* or other molds growing on the fruit surface.

2. Bin Quantity Experiment.

The results of this experiment are displayed in Figure 3. Only in the first replicate (McIntosh apples) was there more mold free fruit in the fumigated bin. Although there is almost 10% Botrytis decay in Rep 1 control, it is difficult to determine if the treatments were effective. In the 2nd and 3rd replicates, (Jonagolds) there were more mold free fruit in the non fumigated bins when compared to the fumigated ones. The fumigated bins of Jonagolds also had a high percentage of soft scald, further complicating our evaluation of decay control. Statistical analysis of the data did not indicate any significant difference between the fumigated and non fumigated fruit.

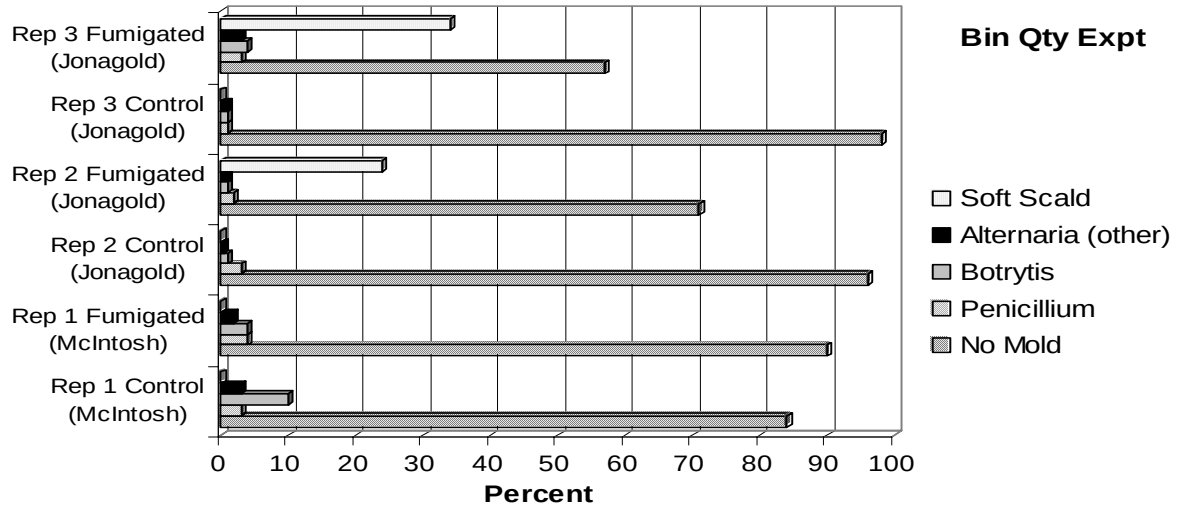

Figure 3. The results of the McIntosh/Jonagold apples Bin Quantity Experiment.

The soft scald was not the result of the hexanal treatment but possibly due to the delay between harvest and placing in the cold room, and the degree of ripeness. There was more soft scald present on the third rep, harvested two days after the 2nd replicate, which supports this hypothesis. The control bins were placed immediately into the cold room after harvest and were on the bottom of the stack, as opposed to the fumigated bins that were on the top of the stack, closest to the cooling fans subjecting the fruit to freezing temperatures. Soft scald, is induced by the reaction of mature or late-picked fruits to temperatures bordering on the freezing point of apples. Severity of the disease is increased by a delay between harvest and cooling (Pierson & Ceponis, 1971). The

3. Post Storage Experiment

The natural level of contamination are clearly shown in the controls (Fig 4a), which were sampled from both the non fumigated and previously fumigated apples from the bin quantity expt. Previously fumigated Gala apples had less decay than the non fumigated Gala apples. McIntosh apples had the most decay and the highest incidence of decay. Both replicates of Jonagold apples had low level of contamination. Fumigating the apples with hexanal (Fig 4b) reduced the amount of decay.

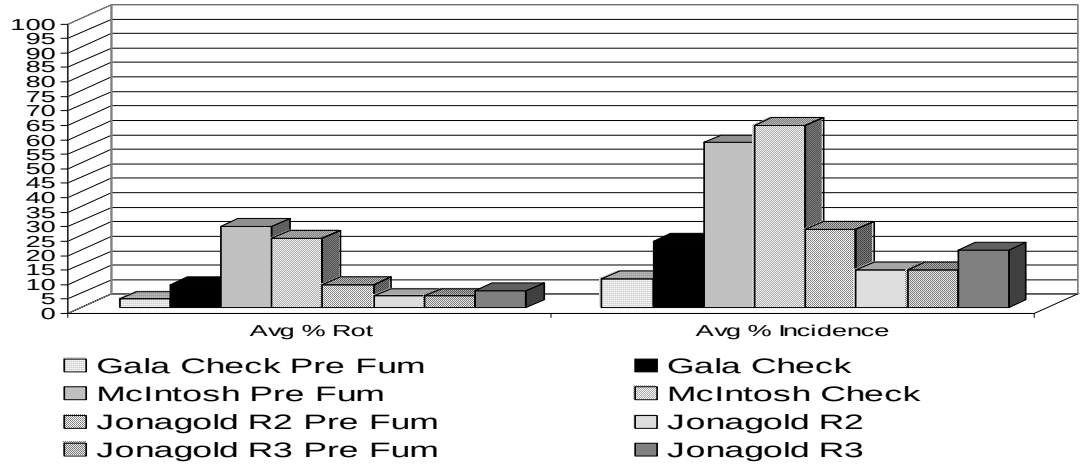

Figure 4a. Non fumigated and previously fumigated apples from the Bin Quantity Experiment.

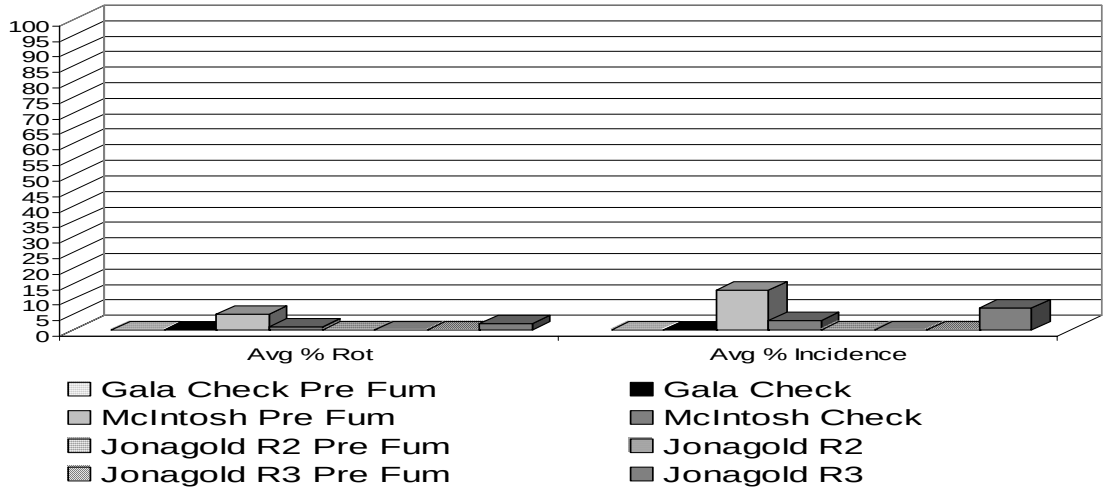

Figure 4b. Results of fumigation of Non fumigated and previously fumigated apples from the bin quantity experiment.

This experiment showed that fumigating the apples immediately after their removable from storage reduces the amount of contamination, and reduces the amount of decay which could develop. Due to variability within the replicates only the Gala apples were significantly different.

Fruit Quality

1. **Fruit Firmness**. Figure 5 shows the Gala apple fruit firmness over the storage period. The overall trend was as expected to happen to fruit in storage. There was a slight initial increase in firmness followed by a gradual decline over time in storage time. From approximately 50 day to 160 days the fumigated apples had a slightly higher firmness when compared to the non fumigated apples. After 200 days in storage both fumigated and non fumigated fruit had similar firmness.

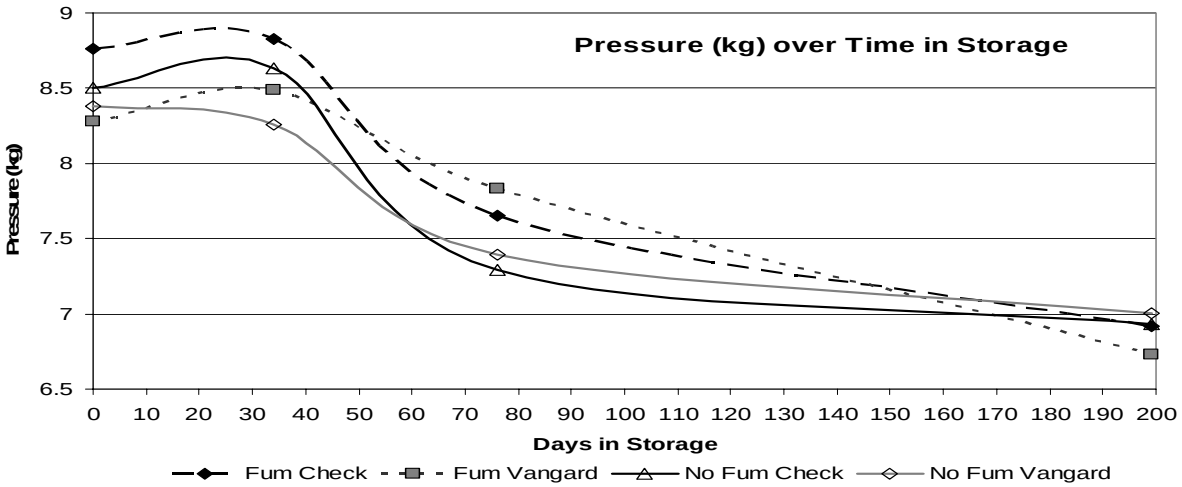

Figure 5. Apple fruit firmness over time in air storage at 1^0C (34^oF).

2. Gala Fruit TA. Figure 6 shows the change in malic acid over time in storage. There was a slight decline in the amount of malic acid over the first 80 days. This trend continued over the 200 days that the fruit was in storage. The fumigated samples had slightly lower levels of malic acid by the end of the 200 days when compared to the non fumigated apples.

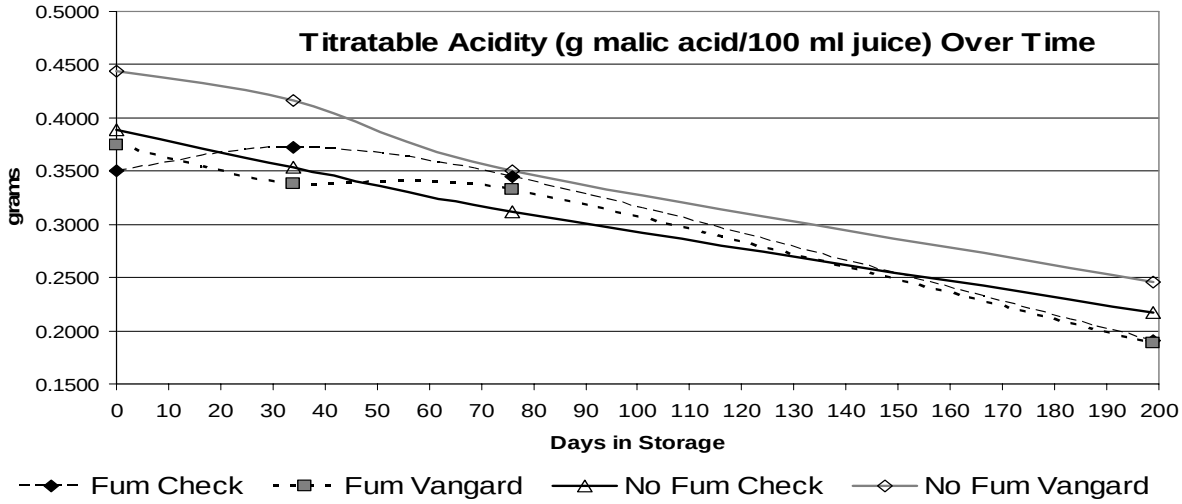

Figure 6. Titratable acidity of Gala apples over time in storage at 1^0C (34^oF).

3. Gala Fruit Soluble Solids. Figure 7 shows the soluble solids over time in storage. The fumigated treatments show a slightly higher level of sugar over the non fumigated apples. The non fumigated Vangard treatment shows an increase of sugar after 200 days in storage. This may be an artifact of the sampling technique as this is not the normal trend.

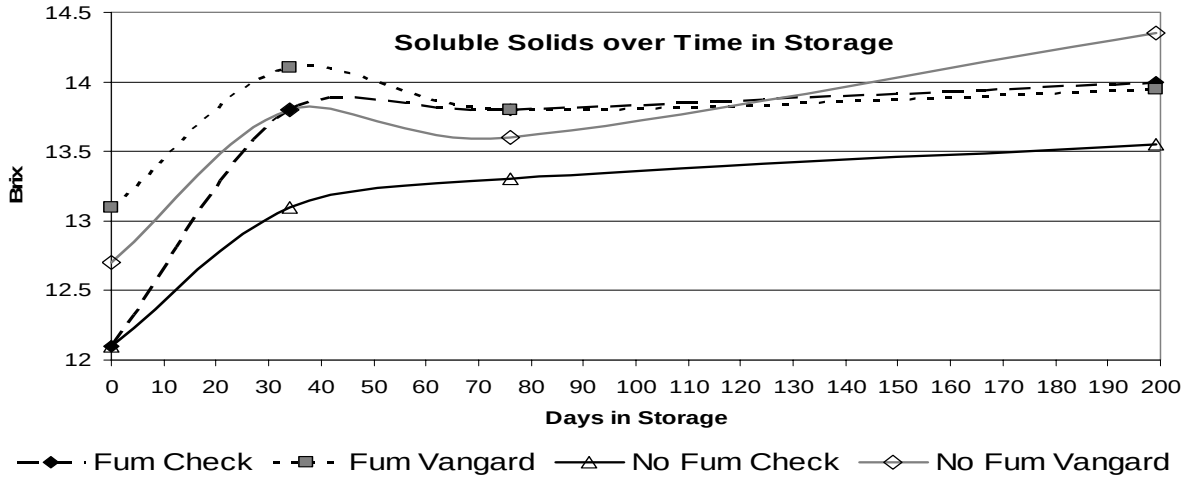

Figure 7. Soluble solids in apple fruit over time in storage 1^0C (34^oF).

Figure 8 show the SS/TA ratio over time in storage. The higher ratio for the fumigated treatments may indicate an effect on the flavour of the fruit.

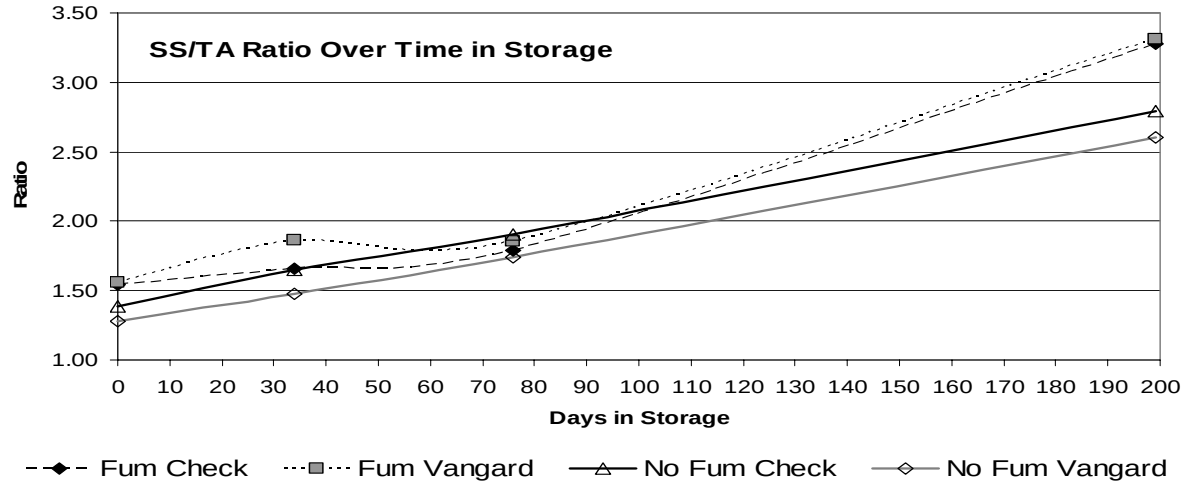

Figure 8. SS/TA ratio over time in storage at 1^0C (34^oF).

The soluble solids and titratable acidity of Gala apples over time is similar to other reports (Mattheis et al 1991). Overall the quality between the non fumigated and fumigated were similar. Whether the slightly higher soluble solids in the fumigated apple translate into a sweeter tasting apple will have to wait until the use of hexanal is approved for apples. Some studies suggest that consumers respond favorably to Gala apples which are firm and of moderate to high sweetness (Marin 2002).

4. Headspace Analysis

The results of the slices apple fruit sealed in cryovac bags is shown in Figure 9. The McIntosh apples produced more hexanal in the first 24 hours than the Jonagolds. The Jonagold non fumigated apples had a slightly higher level of hexanal than the fumigated ones. The fumigated McIntosh had a slightly higher level of hexanal. Overall the amount of hexanal between fumigation and non-fumigated apples was about the same but McIntosh significantly (P=0.05)

released more hexanal then the Jonagolds. This maybe an indication of fruit maturity because over mature apples generate more volatiles (Panasiuk et al. 1980; Fellman et al. 2003).

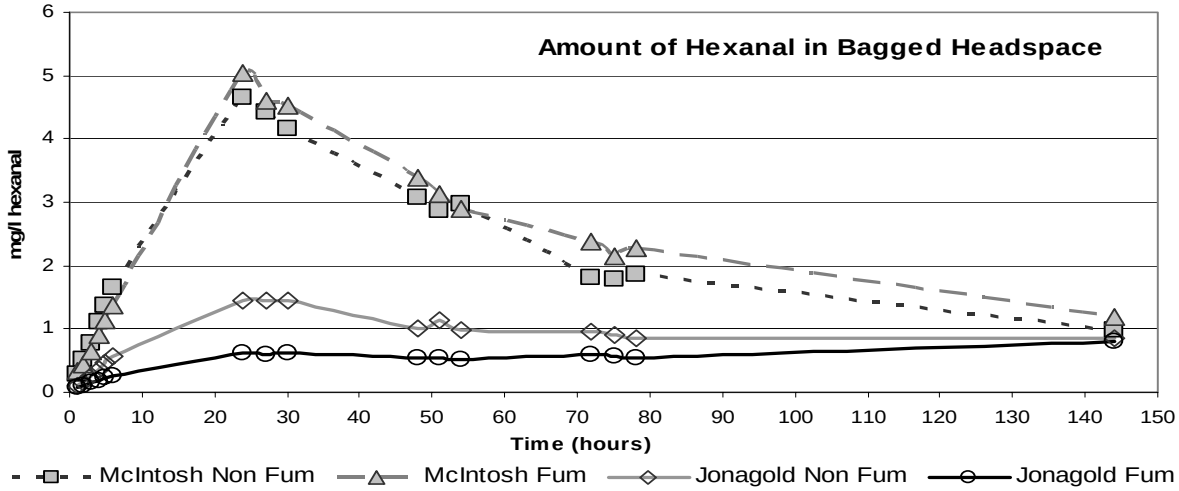

Figure 9. Quantity of hexanal released into the headspace of bagged sliced apples.

Hexanal has been identified as one of several components of apple aroma (Flath et al 1967; Sapers et al 1977).

5. Tissue Analysis: The results from the tissue analysis and their averages are shown in Table 1. The amount of hexanal in the McIntosh apples is similar for both the fumigated and non fumigated fruit. The slightly higher amount of hexanal in the fumigated Jonagold is significantly different when compared to the other three treatments.

Table 1. Results of hexanal tissue analysis of non fumigated and fumigated apples.

Variety/Treatment	19 Nov 03	28 Nov 03	01 Dec 03	Average (mg/l)
McIntosh Non Fumigated	.0697	.0604	.0879	.0727a
McIntosh Fumigated	.0939	.0625	.0873	.0812a
Jonagold Non Fumigated	.0770	.0625	.0884	.0760a
Jonagold Fumigated	.1150	.0919	.1106	.1058b

NB Means with the same letter are not significantly different.

Other researchers have detected hexanal present in apple flesh (Fellman et al. 2003). Apples picked at commercial harvest produced the highest quantities of hexanal and the lowest amounts of acetate esters; this pattern indicates that the commercial harvest time corresponds to unripe aroma quality (Panasiuk et al.,1980; Paillard, 1981; Willaert et al., 1983). The application of aldehydes (which is what hexanal is) and Carboxylic acids resulted in an increase of volatile production (De Pooter et al. 1983). Hexanal is naturally present in apple tissue and this should enable the commission to apply for the use of hexanal on apples as a food additive.

Areas for Further Study

- ✓ A study to investigate the possibility of using a low rate of hexanal just prior to removing the apples from storage to reduce the level of contaminant and improve the aroma of apples treated with MCP and/or stored in CA.

Budget: Hexanal vapor to control storage decay
Peter Sholberg, Paul Randall
Project duration: 2001-2004
Project total: $45,000

Year	Year 1 (2001-2002)	Year 2 (2002-2003)	Year 3 (2003-2004
Salary	14,000	14,000	14,000
Materials & Supplies	500	500	500[1]
Travel	500	500	500[2]
Total	15,000	15,000	15,000[3]

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

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