**FINAL REPORT**

**Project Title:** Acetic acid vapors to decontaminate bins & storage rooms.

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**Objectives:**

1. Determine if acetic acid vapors would be effective in eliminating the various post harvest pathogens (*Penicillium expansum*, *Botrytis cinerea* and *Alternaria alternata*) from wooden bins and various surfaces in cold storage rooms.

2. Use acetic acid vapors as a phytosanitary treatment to eliminate *Erwinia amylovora* from the surface of harvested mature apples.

3. Corrosion study of various metals to see the effect of acetic acid vapors on them at various rates, duration of application, and the effect of washing exposed metals following fumigation.

4. Compare the effect of acetic acid vapors to commercial products (i.e. chlorine wash, Storox (Pace International)) to decontaminate bins and storage rooms.

The goals of this project are to determine:

1. whether or not acetic acid can be used as a fumigant to eliminate post harvest pathogens from the surfaces of bins and the walls of cold storage rooms; and

2. if the methods developed can be applied in a commercial setting.

Accomplishment of these goals would be to reduce the amount of decay due to post harvest pathogens during storage.

**Significant findings:**

1. A rate of 6 mg/l or higher of acetic acid is effective in eliminating post harvest pathogens from the surface of bins and storage room surfaces.

2. Acetic acid can reduce *Erwinia amylovora* from the surface of harvested mature apples. Unfortunately, the rates required also produces phytotoxic response in the fruit.

3. Acetic Acid is corrosive on copper. The corrosive effects can be reduced by rinsing the exposed metal with water, immediately after fumigation.

**Methods:**

**General**

The PARC facility was used to test various combinations of time (from 30 mins to 120 minutes), rate of acetic acid application (2 to 15 mg/l), temperature ($25^0$C ($77^o$F)) and pathogens. These trials were done in small chambers 26 l (6.9 gal US) pots, and 1 $m^3$ (35.3 $ft^3$) chambers.

The apples were fumigated with various levels of a acetic acid (AA) (816 to 4896 ppm) (2 to 12 mg/l) for various periods of time (30 to 120 minutes) at $25^o$C. The relative humidity was adjusted to 70% if necessary, by evaporating water in the chamber. Food Grade AA was evaporated by heating with a small electric heater. The AA concentration was monitored by withdrawing a 1 ml sample of air from the chamber with a 1 ml syringe shortly after the start and at regular intervals during fumigation. The gas sample was injected into a Model 910 gas chromatograph (GC) (Questron Technologies Corp. Mississauga, Ontario) and within approx. 1 minute the concentration of the AA was known. At the end of the fumigation, the chamber was vented and the fruit removed.

**Objective One – Sanitation of bins and walls**

All trials were done in 1 $m^3$ (35.3 $ft^3$) chambers. Three panels (10 $cm^2$) on each mini bin and three panels on the chamber walls, were inoculated with each of the following pathogens (*Penicillium* ssp, *Botrytis cinerea*, and *Alternaria spp*). An airbrush (Paache Airbrush Company, Harwood Heights, IL) was used to spray on the inoculum. The bins and walls of the chamber were allowed to air dry before the addition of AA. The AA levels were monitored by the GC. After fumigation the inoculated areas were swabbed with a sterile swab and placed in a test tube that contained 10 mls of sterile distilled water. The tubes containing the swabs were vortexed for 15 seconds before serial dilutions were made. 100µl aliquots for each sample were spread onto 100 ml plates containing lactic acid Potato Dextrose Agar (LPDA). Plates were made in triplicate and incubated at $20^0$C. Plates were counted after two days and were checked for additional colonies for up to seven days.

**Objective Two - Phytosanitation of Molds and *E. amylovora* (Fireblight) with AA vapors**

**a. Mold Phytotoxicity trial**

Mature apple fruit consisting of several cultivars were harvested on the day of the trial. A measured concentration of *Penicillium expansum* (Blue Mold) was sprayed on using an airbrush (Paache Airbrush Company, Harwood Heights, IL). Once the inoculum was air dry, the fruit was placed in a 26 l (6.9 gal US) chamber and various rates of AA applied (816 to 4896 ppm) (2 mg/l up to 12 mg/l) for up to 2 hours. After the AA treatment the fruit were removed from the chamber. The apples were then placed in a fumigation chamber and humidity adjusted. After fumigation the apples were wounded, and stored at $20^o$C,for seven days when they were rated for decay.

**b. Phytosanitation trial for Fireblight**

Bisbee red delicious apples were used because they tolerated the highest levels of AA. They were not surface disinfested, but were inoculated with *E. amylovora* on the calyx end. Twenty-five apples were treated on the first day of inoculation (Set 1). The other 25 remained at room temperature and treated three to five days after inoculation (Set 2). This was to simulate fireblight being on the fruit for some time. Once the inoculum was air dry, the fruit was placed in a 26 l (6.9 gal US) chamber and various rates of AA applied (816 to 4896 ppm) (2 mg/l up to 12 mg/l) for up to 2 hours. After the

AA treatment, the fruit were removed from the chamber. Five fruit per treatment replicate were sampled. A 1 cm diameter by 5 mm sample of tissue was removed and placed in 5 mls of sterile MM2 broth (Bereswill *et al* 1998). The test tube was capped and remained at room temperature for 24 hours. The test tube was vortexed for 15 seconds and a 100µl of each test tube was spread over three100 ml plates containing MM2 media. After 24 hours of incubation at room temperature, plates were examined for yellow bacterial colonies and enumerated.

**PCR Methods**

To confirm the identity of the bacterial colonies polymerase chain reaction (PCR) techniques were utilized. Individual colonies were stabbed with sterile pipette tips and transferred to 20 µl solutions of PCR mix containing: 1x buffer (75 mM Tris, 20 mM $(NH_4)_2 SO_4$, 0.1% Tween and 0.1% Triton-X-100), 1.6 mM $MgCl_2$, 1.5 mM dNTP mix, 0.4 µM each, forward and reverse pEA29 plasmid primers, and 1 unit of UltraTherm DNA polymerase. Amplification was performed on a GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA) with the following cycle conditions: 95°C for 2 min followed by 35 cycles of 95°C for 1 min., 58°C for 20 s, and 72°C for 30 sec., and a final extension cycle of 72°C for 7 min.

**Objective Three** **<u>Corrosion Trials</u>**

Twenty plywood blocks (1 7/8" x 1 ½" x ¾") each containing; a copper rivet, aluminium roofing nail, a brass screw, , a galvanized nail and a stainless steel screw were constructed (Figure 1). A numbered block was placed in the fumigation chambers each time a fumigation was conducted. Some of the blocks were subjected to repeated exposure to various rates (816 to 6120 ppm) (2 mg/l to 15 mg/l) of AA for 2 hours. Some of the blocks were rinsed with water after the termination of the fumigation. The effect of the AA vapours on the various metals was visually evaluated.

Figure 1. Corrosion test block

**Objective Four - Sanitation at a commercial storage Facility**

A storage room (91 $m^3$) at the Rutland (Kelowna, BC) packing house will be used for this experiment and a number of empty wooden bins will also be placed in the room and treated with AA. Swabs will be taken before and after fumigation from areas on the walls and on empty wooden bins. Five 10 cm squares will be marked out on the walls, two of which will be inoculated with *Penicillium expansum* at a spore concentration of 1x $10^5$conidia/ml . Swabs will be taken before and after the AA fumigation. This trial is scheduled for the last week of June 2005.

**Results and Discussion:**

**Objective One – Sanitation of bins and walls**

The results of the bin and wall sanitation are shown in figure 2. The only pathogen shown is blue mold, because the recovery of Grey mold and *Alternaria* spores were too low and therefore not comparable between treatments. The blue mold on the wall, bins and foam was reduced as the level of AA increased. The amount of natural inoculum on the foam should be noted. On some section of the foam, the count was as high as 15,000 colony forming units (CFU) per sample. The lower rates of 2 and 4 mg/l reduced the amount of spores on all 3 test surfaces. A rate of 6 mg/l and higher reduced the number of spores to zero.

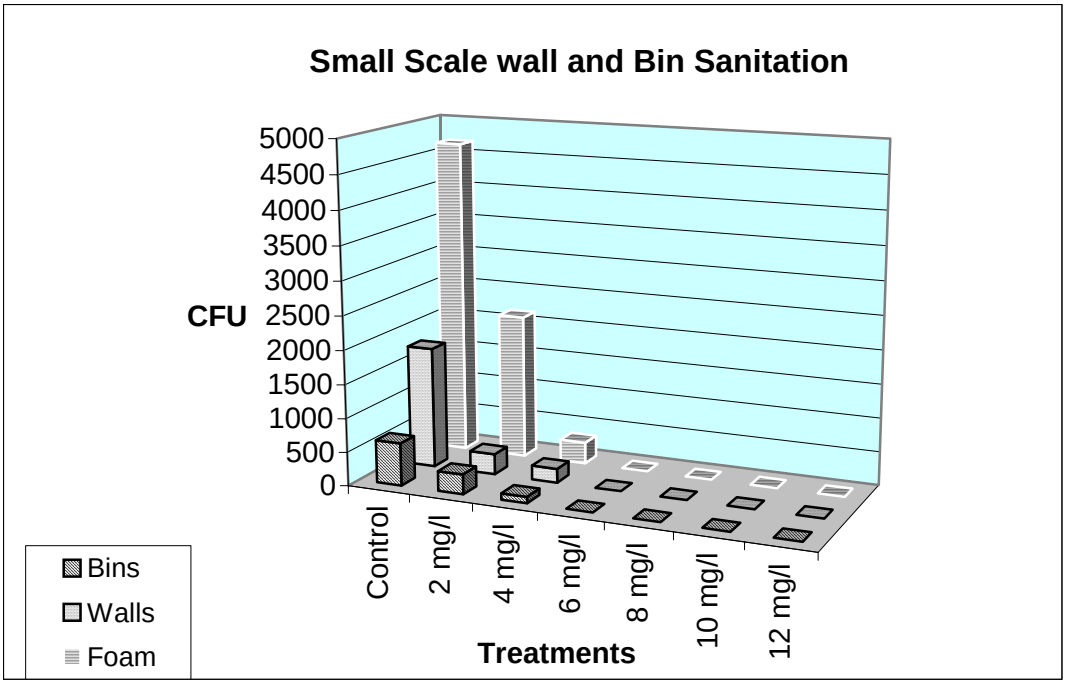


**Figure 2.** Results of the small scale fumigation of storage room walls and bins.

**Objective Two - Phytosanitation of Mold and *E. amylovora* (Fireblight) with AA vapors**

**Phytotoxicity Trial**

A number of trials were conducted at 25$^{o}$C to determine AA effectiveness and its phototoxic effect on different varieties of apples (Tables 1 & 2). The level of acetic acid vapor required to reduce these pathogens on apples and not damage the fruit surface is minimal.

Table 1. AA rate vs time duration for fumigation of 'Jonagold' apples.

| | 120 Minutes | | 60 Minutes | | 30 Minutes | |
|---|---|---|---|---|---|---|
| mg/l | % Decay | Phyto | % Decay | Phyto | % Decay | Phyto |
| 0 | 96 | No | 100 | No | 100 | No |
| 2 | Not tested | Not tested | 4 | Some LB | 0 | No |
| 4 | 0 | Some LB | 0 | Some LB | 0 | Some LB |
| 6 | Not tested | Not tested | 0 | Yes | 0 | Yes |
| 8 | 0 | Yes | 1 | Yes | 3 | Yes |
| 12 | 1 | Yes | Not tested | Not tested | Not tested | Not tested |
| 15 | 0 | Yes | Not tested | Not tested | Not tested | Not tested |

**Note:** LB = Lenticel browning. Phyto = Phytotoxicity; a scald like effect on the fruit surface.

Table 2. AA fumigation of 'Gala', 'Red Delicious' and 'Fuji' apples

| | 90 Minutes (Gala) | | 90 Minutes (Red Delicious) | | 60 Minutes (Fuji) | |
|---|---|---|---|---|---|---|
| mg/l | % Decay | Phytotoxicity | % Decay | Phytotoxicity | % Decay | Phytotoxicity |
| 0 | 100 | No | 97 | No | 89 | No |
| 2 | 0 | No | 0 | No | 0 | No |
| 4 | 0 | Yes | 0 | No | 0 | No |
| 6 | 0 | Yes | 0 | No | 0 | Yes |
| 8 | 0 | Yes | 0 | 10 % | 0 | Yes |

The rate of AA required to control Blue mold caused by *P. expansum* is 2 to 4 mg/l for 30 to 60 minutes depending on the apple variety. These fumigation durations are likely too short for fumigation of large volumes of fruit (over 100 bins) but could be used to fumigate smaller numbers of bins such as in a 40 ft container where the vapour could be vented in 15-30 minutes. Furthermore larger volumes could be fumigated where minor fruit damage (lenticel browning) is not a major concern such as in processing apples.

**Results of the *E. amylovora* Phytosanitation experiments**

The results for the phytosanitation experiment are shown in Tables 3. Our goal was to eliminate fireblight bacteria from the calyx end of mature apples. All rates of AA had from slight to 100% phytotoxicity on all the apples. At the lower rates (less than 8 mg/l) the bacteria were allowed to grow. Some of these bacteria had the appearance of *E. amylovora*, and were growing on a selective medium. PCR identification suggested that they were not *E. amylovora*, but positive identification was not made. *E. amylovora* was confirmed as being recovered from the controls and were negative for the fumigated samples (see figure 3). The rate of 10 or 12 mg/l for 60 to 90 minutes appears to have reduced the bacterial counts to near zero, even after enrichment in a 24 hour broth culture. Unfortunately all the apples were severely damaged.

Table 3. Results of fumigation of freshly inoculated 'red delicious' apples.

| **Treatment** | **Replicate** | **% Phytotoxicity** | **Lenticel Browning** | **Calyx end Browning** | ***Erwinia amylovora*** | **Other Bacteria** |
|---|---|---|---|---|---|---|
| Std | 1 | 0 | | | | TMTC |
| | 2 | 0 | | | | TMTC |
| | 3 | 0 | | | | TMTC |
| **Control** | 1 | 0 | | | TMTC | TMTC |
| | 2 | 0 | | | TMTC | TMTC |
| | 3 | 0 | | | TMTC | TMTC |
| | 4 | 0 | | | TMTC | TMTC |
| | 5 | 0 | | | TMTC | TMTC |
| **10 mg/l** 60 mins | 1 | 15 | Yes | Yes | 0 | 0 |
| 1441 ppm hrs | 2 | 10 | Yes | No | 0 | 166 |
| | 3 | 0 | No | No | 0 | 0 |
| | 4 | 15 | Yes | No | 0 | 0 |
| | 5 | 20 | Yes | Yes | 0 | 0 |
| **12 mg**/l 60 mins | 1 | 40 | Yes | Yes | 0 | 0 |
| 1814 ppm hrs | 2 | 90 | Yes | Yes | 0 | 0 |
| | 3 | 10 | No | Yes | 0 | 12 |
| | 4 | 80 | Yes | Yes | 0 | 0 |
| | 5 | 10 | No | Yes | 0 | 0 |
| **10 mg/l** 90 mins | 1 | 10 | Yes | Yes | 0 | 0 |
| 2013 ppm hrs | 2 | 20 | Yes | Yes | 0 | 0 |
| | 3 | 30 | Yes | Yes | 0 | 0 |
| | 4 | 70 | Yes | Yes | 0 | 3 |
| | 5 | 50 | Yes | Yes | 0 | 1 |
| **12 mg/l** 90 mins | 1 | 100 | Yes | Yes | 0 | 0 |
| 2114 ppm hrs | 2 | 100 | Yes | Yes | 0 | 6 |
| | 3 | 100 | Yes | Yes | 0 | 0 |
| | 4 | 100 | Yes | Yes | 0 | 0 |
| | 5 | 100 | Yes | Yes | 0 | 1 |

Note: TMTC = too many to count
Other Bacteria = Not *E. amylovora* but grew on selective medium indicating that they were a closely related species.

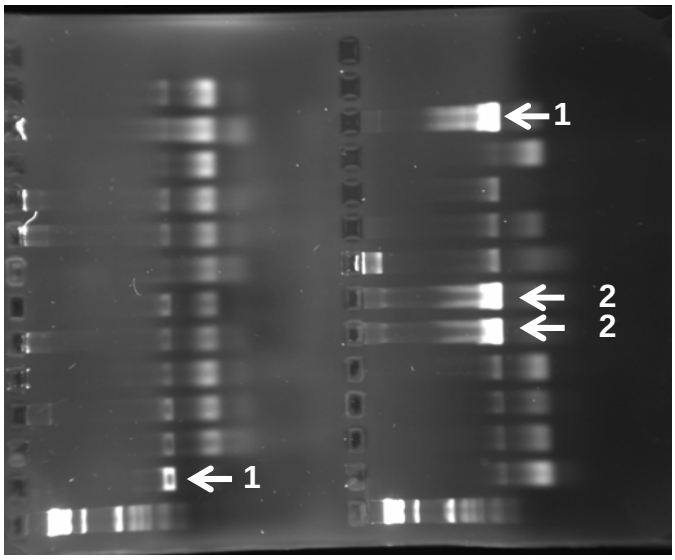


Figure 3. PCR identification of selected bacterial colonies. The number 1 refers to the positive control. The number 2 are from apples inoculated with *E. amylovora*. All of the other colonies were negative.

**Objective Three – Corrosion Trials**

The copper rivet and the brass screw showed the effects of AA vapours. The lowest rate of 2 mg/l caused minor corrosion on the copper rivet. As the rate increase more surface corrosion appeared. The brass screw turned a shade of green due to the exposure of acetic acid. The corrosion also increased as the exposure rate increased. Both effect of corrosion (rust) or discolouration were removed by placing the block under running water. Some minor pitting was noticed under a dissecting microscope at the higher rates of acetic acid.

**Objective Four - Sanitation at a commercial storage Facility**

This objective was originally planned for the second year of the project. However, an opportunity to fumigate storage room (91 $m^3$) in Kelowna is available and will be conducted during the last week of June 2005. The results of this trial will be reported on at the "Review" in July.

**Future Research**

1. Survey of the level of contamination on the walls and floors of cold storage rooms in both Washington and in British Columbia.

2. Fumigation of small (100 m3) to medium (1000 m3) sized cold storage rooms.

3. Investigate the possibility of using steam to sanitize cold storage rooms.

4. Compare the effect of acetic acid vapors to commercial products (i.e. chlorine wash, Storox (Pace International)) to decontaminate bins and storage rooms.

**Literature cited:**

Bereswill, S; Jock, S; Belleman, P.; Geider, K. 1998. Identification of *Erwinia amylovora* by Growth Morphology on Agar Containing Copper Sulfate and by Capsule Staining with Lectin. Plant Disease Vol 82. p158-164.

**Budget**

**Project title: Acetic Acid Vapors to Decontaminate Bins & Storage Rooms.**

**PI: Paul Randall (Peter Sholberg)**

**Project duration:** 2004

**Current year:** 2004

**Project total:** $25,000

**Current year request:** **$**25,000

| Item | **Year 1 (2004/05)** |
|---|---|
| Salaries | **19,500** |
| Benefits (%) | **2,925** |
| Equipment | **775** |
| Supplies | **600** |
| Travel | **700** |
| Miscellaneous | **500** |
| **Total** | **25,000** |