

FINAL PROJECT REPORT**WTFRC Project Number:** PH05-506**Project Title:** Acetic Acid Vapors to Decontaminate Bins & Storage Rooms

PI:	Paul Randall	Co-PI(2):	Peter Sholberg
Organization:	Agriculture & Agri-Food	Organization:	Agriculture & Agri-Food
Telephone:	250-404-3331	Telephone:	250-494-6383
Email	randallp@agr.gc.ca	Email:	sholbergp@agr.gc.ca
Address:	PO Box 5000, 4200 Hwy 97	Address:	PO Box 5000, 4200 Hwy 97
City:	Summerland	City:	Summerland
Province/Zip	BC, Canada, V0H 1Z0	Province/Zip:	BC, Canada, V0H 1Z0

Co-operators: Okanagan Similkameen Cooperative Growers Association, Terry Zeller
BC Fruit Packers, Dan Worley

Budget History:

Item	Year 1: 2004-05	Year 2: 2005-06	Year 3: N/A
Salaries	19,500	19,500	
Benefits			
Wages			
Benefits	2,925	2,925	
Equipment	775	775	
Supplies	600	600	
Travel	700	700	
Miscellaneous	500	500	
Total	25,000	25,000	

Objectives: (Year one)

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.

Significant findings: (Year One)

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 when the relative humidity is above 75%. The corrosive effects can be reduced by rinsing the exposed metal with water, immediately after fumigation and maintaining the relative humidity below 70%.
4. Acetic acid is comparable to Storox for sterilizing CA rooms, but acetic acid is more effective in sterilizing bins.

Objectives: (Year Two)

1. Survey of the contamination levels of *Penicillium* spp. on the floors and walls of cold storage rooms in British Columbia.
2. Fumigate small (100 m³) to medium (2700 m³) sized cold storage rooms using specially developed techniques to assess efficacy on several room types.
3. Investigate the possibility of using steam or high pressure hot water to sanitize cold storage rooms.
4. Investigate the sanitation of bins with acetic acid to control *Penicillium* spp. and codling moth.
5. Investigate the relationship between relative humidity and effectiveness of acetic acid on pathogens and corrosion.

Significant findings: (Year Two)

1. The CA room survey showed a variable level of *Penicillium* spp spores depending on the composition of the wall, plywood and fire retardant fibre walls had the highest level of *Penicillium* spp. contamination.
2. Acetic acid treatment of CA rooms was effective in reducing the amount of *Penicillium* spp. present on the walls to zero.
3. Steam/pressure washing of pallets was very effective in reducing the amount of *Penicillium* spp.
4. Acetic acid is an effective bin sanitation material, averaging 72 to 100% reduction in *Penicillium* spp. on bin surfaces.
5. Acetic acid is effective in killing codling moth larvae and pupa but only at a rate of 40 mg/l and if the larvae were in plastic containers.
6. Relative humidity must be above 50% for acetic acid to be effective in reducing the pathogen spore load and below, 65% to minimize the corrosion in CA rooms and bins.

Methods / Results/ Discussion (Year Two)

Objective 1. (Survey of wall contamination in CA storage)

Methods Wall swab samples were taken in various CA rooms from 2 packing houses in the North Okanagan (Table 1) and 5 packing houses in the South Okanagan (Table 2) between March and June 2005. The walls of these CA rooms were composed of different materials ranging from galvanized metal, painted plywood, styrofoam, concrete foam, white or grey fire retardant mineral fibre (white/grey FRF). Samples were taken by dipping a sterile swab into a sterile tube containing 10 ml of sterile distilled water (SDW) and swabbing over an area of 10 cm² by using a template. For a single wall area, 5 swabs were taken across the wall and placed into the same tube. For the walls covered with the white or grey fire retardant fibre, 5 sample areas were collected from across the wall by removing a 2 cm² area each time and placing it into a sterile tube. Each tube containing the swabs was vortexed for 30 seconds before plating. Aliquots of 100 µl were then pipetted on to 100 ml Petri plates containing lactic acid potato dextrose agar (LPDA). This was repeated three times for each sample. The plates were incubated at 20°C for 5 days or until the fungal colonies could be identified and counted.

Results

Table 1. Results of wall contamination survey in North Okanagan in 2005.

Packing House CA Rooms in North Okanagan (<i>Penicillium</i> spp. only)							
Location	Room	Material	CFUs/cm ²	Location	Room	Material	CFUs/cm ²
Roanoke	61	Plywood	1.1	Winfield	510	Plywood	188
	82	Plywood 04	0		511	Plywood	17,556
	83	Plywood 00	1,113		520	Plywood	1.5
	85	Plywood 00	1,112		508	Plywood	2
	64	Styrofoam	0.5		510	Concrete F	45
	72	Stainless	0.5		511	Concrete F	82
	61	White FRF	3,083		520	Concrete F	261
	63	White FRF	45,083		508	Concrete F	3
	83	White FRF	32,083		525	White FRF	30
	61	White FRF	50		526	White FRF	290
	85	Grey FRF	92				

Table 2. Results of wall contamination survey in South Okanagan in 2005.

Packing House CA Rooms in South Okanagan (<i>Penicillium</i> spp only)							
Location	Room	Material	CFUs/cm ²	Location	Room	Material	CFUs/cm ²
Summerland	232	Plywood	7.6	Oliver	115	Plywood	28
	234	White FRF	503		107	Plywood	1.3
Naramata	253	Plywood	0.2		118	White FRF	9.3
	258	Plywood	0.1	Osoyoos	144	Plywood	0.1
	259	Plywood	3		144	White FRF	2,534
	258	White FRF	77		149	White FRF	257
	259	White FRF	276	Keremeos	129	Plywood	1.5
	253	Concrete F	14.4		129	Concrete F	29.1
					125	Plywood	1.0
					125	Concrete F	2,145

Discussion

Various fungal spores were identified in this study, *Penicillium* spp., *Mucor*, *Alternaria*, and some yeasts. On most of the surfaces checked in this survey, only *Penicillium* spp. was found, which is the most important pathogen of stored apples. The level of *Penicillium* spp. on the various surfaces ranged from 0 to 45,000 colony forming units (CFUs)/cm² (Table 1 & 2).

Objective 2. (Fumigation of storage rooms)

Methods All surfaces were swabbed, as described in Objective 1 above, before and after fumigation. In Keremeos, BC, the CA room air was sampled with a Burkard portable air sampler (Burkard Manufacturing Co Ltd, Rickmansworth, England). A 100 ml Petri plates containing exactly 27 mls of acidified PDA (LPDA) was used to collect the air contaminants and the sampler was run for 1 minute. This device passes 20 litres /min of air across the plate. There were 3 replicated samples per room.

Fumigation Method Once the FRED (Fumigation, Rapid Evaporation Device) was placed in the CA room, relative humidity (RH) was adjusted by evaporating the amount of distilled water (DW) required to bring the room RH up to a minimum of 50% to a maximum of 65%. Temperature was adjusted by the addition of heat provided by small room heaters, ceiling lights and fans. Temperature was raised to a minimum of 19 °C (66.2°F) to 26 °C (78.9°F). The relative humidity, temperature, and the amount of acetic acid used per room is shown in table 3. Once the room RH and temperature was established, the acetic acid was added to the FRED. The room was sealed and locked to prevent entry and the FRED turned on. The acetic acid was evaporated in approximately 45 minutes. The level of acetic acid was monitored with a Gas Chromatograph. The room remained sealed overnight and the room vented in the morning. When it was safe to enter, the corrosion blocks were checked for possible corrosion and the defrost cycle on the cooling system was run to remove any acetic acid residue.

Table 3. Date, location, CA room size used in this study.

Date fumigated	Location- room	Room Size m ³ / ft ³	Bin Capacity	Temp °C/°F	RH	Acetic Acid added	
						litres	mg/l
17 Aug 05	Kelowna - 61	2267 / 80,040	1522	25.5 / 77.9	64%	20	8.8
15 Sep 05	Keremeos - 129	1768 / 62,500	1260	19.9 / 67.8	59%	20	11.3
06 Jun 06	Kelowna - 85	2720 / 96,050	1876	19.7 / 67.5	53%	21	7.7
15 Aug 06	Kelowna - 63	2720 / 96,050	1876	23.0 / 73.4	64%	20	7.4
22 Aug 06	Keremeos - 125	1768 / 62,500	1260	22.0 / 71.6	54%	20	11.3

Results

Table 4. BC Fruit Packers (Roanoke CA Rooms, Kelowna, BC)

Room- Composition	Penicillium spp CFUs/cm ²		% Reduction
	Before fumigation	After fumigation	
61 Plywood	0.9 ± 0.1	0.4 ± 0.2	55.6
61 Plywood	0.6 ± 0.2	0.3 ± 0.1	50
61 White Fire Retardant Fibre (WFRF)	13,783 ± 3,271	3.3 ± 5.8	99.9
61 Grey Fire Retardant Fibre (GFRF)	16.7 ± 10.4	10 ± 0.0	40
61 Floor	1.7 ± 1.0	0.1 ± 0.1	94
85 Plywood	1093 ± 136	18.4 ± 1.9	98
85 Plywood	63 ± 30	12 ± 0.9	80
85 Gray Fire Retardant Fibre (GFRF)	10 ± 0.0	0.0 ± 0.0	100
85 Gray Fire Retardant Fibre (GFRF)	11.7 ± 3.0	0.0 ± 0.0	100
85 Floor	941 ± 86	7.0 ± 2.3	99
63 Galvanized metal	0.9 ± 0.6	0.2 ± 0.0	78
63 Galvanized metal	1.1 ± 0.3	0.1 ± 0.1	91
63 White Fire Retardant Fibre (WFRF)	3,912 ± 1,330	0.0 ± 0.0	100
63 White Fire Retardant Fibre (WFRF)	4,692 ± 1,976	0.0 ± 0.0	100
63 Floor	5 ± 0.8	0.0 ± 0.0	100
63 Over Head Pipes	509,000 ± 74,800	0.0 ± 0.0	100

Table 5. Okanagan Similkameen Cooperative Growers Association (Keremeos)

Room/wall composition	Penicillium spp., CFUs/cm ²		% Reduction
	Before fumigation	After fumigation	
129 Plywood	0.7 ± 0.3	0.0 ± 0.0	100
129 Plywood	2.3 ± 0.5	0.0 ± 0.0	100
129 White Fire Retardant Fibre (WFRF)	14.1 ± 1.7	0.0 ± 0.0	100
129 White Fire Retardant Fibre (WFRF)	33.5 ± 2.7	0.0 ± 0.0	100
129 Air Sample (per 20 l of air)	1.3 ± 0.6	0.7 ± 1.2	50
125 Plywood	0.9 ± 0.5	0.0 ± 0.0	100
125 Plywood	1.1 ± 0.1	0.0 ± 0.0	100
125 White Fire Retardant Fibre (WFRF)	4,260 ± 191	0.0 ± 0.0	100
125 White Fire Retardant Fibre (WFRF)	30 ± 5.0	0.0 ± 0.0	100
125 Floor	2.0 ± 0.3	0.0 ± 0.0	100
125 Air Sample (per 20 l of air)	24 ± 1.5	0.3 ± 0.6	98.8

Discussion

For the CA room treatment in Kelowna, fumigation was most effective on the white/grey fire retardant fibre (W/GFRF). Acetic acid reduced the contamination from 14,500 *Penicillium* spp. CFUs/cm² to zero, and the overhead pipes from over 500,000 spores/cm² to zero. (Table 4).

For the rooms at Keremeos, BC, the *Penicillium* spp. was reduced by 100% on all surfaces (Table 5). The spore load in the air was reduced to less than 1 spore per 20 litres of air. For the five CA rooms treated, the overall effect was a reduction of *Penicillium* spp. on all surfaces especially those areas that would be hard to clean. The corrosion blocks showed no sign of corrosion.

Objective 3. (Steam pressure washer for sanitizing bins and CA rooms)

Methods

A Hotsy model 555SS hot-water pressure washer (Englewood, Colorado, USA) was used to surface sanitize a small (8.4 m³/295 ft³) cold storage room. The unit provides 2.2 gph (8.33lpm) heated water up to 100°C at 1300 psi (89.66 bars).

All surfaces were swabbed as in the method discussed for Objective 1.

Results/Discussion

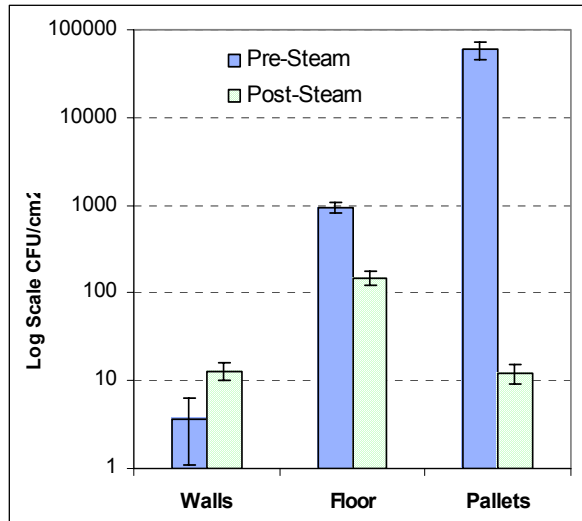


Figure 1. *Penicillium* spp. CFUs per cm² before and after steam treatment

Table 6. CFUs per cm² before and after steam treatment and percent reduction.

	Pre- Steam	Post- Steam	% reduction
Walls	3.7	13	0
Floor	944	150	84
Pallet	59,333	12	99.98

Figure 1 and table 6 show the results of the steam treatment. The steam-pressure washer treatment was effective in reducing the spore load on the floors (84% reduction) and most effective on the pallets (99.98% reduction). The walls showed an increase and this is due to low initial counts and possible splashing of spores from the floor during the steam/heat treatment.

A disadvantage of using this system for cleaning a cold room is the amount of time required to clean the room and the amount of water needed (2.2Gph). For the cleaning of pallets or bins, steam/pressure washing would be ideal for the most contaminated ones.

Objective 4. (Acetic acid fumigation of bins and codling moths)

Methods -Bin Fumigation

Stacks of 3 bins were placed randomly throughout a CA room. The various sides of the bins within the stack were swabbed before and after fumigation as described above. This included the bins inside of the stack to test whether acetic acid vapours would penetrate into the stacked bins at a sufficient concentration to sanitize the bin surface. These fumigations were conducted in conjunction with the room fumigations mentioned in Objective 2.

Methods - Codling Moth Fumigations.

Trays of codling moths were obtained from Sterile Insect Release (SIR) program, Osoyoos, BC. The larvae were raised on the standard diet and were at the 4th or 5th instar. Fifty larvae per treatment were removed from the diet and placed into wooden blocks or 1 ounce plastic solo cups. The larvae were allowed to spin up overnight. The next day the blocks/cup and 8 to 10 pupae were placed into the 23 litre fumigation chambers and treated with different levels of acetic acid vapours. Additional acetic acid was added during the length of the fumigation to maintain a minimal level of acetic acid vapours in the air.

Results/Discussion (Bin fumigations)

Table 7. Results of the bin fumigations in Kelowna BC.

Room/Bin/position swabbed	<i>Penicillium</i> spp. CFUs/cm ²		% Reduction
	Before Fumigation	After Fumigation	
61 Bin 1 Inside	51 ± 2.7	0.6 ± 0.4	98.8
61 Bin 1 Outside	2.5 ± 0.4	1.1 ± 0.9	56

61 Bin 2a Outside Top	0 ± 0.0	0.2 ± 0.2	0
61 Bin 2b Inside Top	115 ± 8.3	0.9 ± 0.2	99
61 Bin 2c Outside Middle	50 ± 10	19.2 ± 16.7	61.6
61 Bin 2d Inside Middle	120 ± 10	0.3 ± 0.3	99.8
61 Bin 2e Inside Bottom	28 ± 3	17.2 ± 3.3	38.6
61 Bin 2f Outside Bottom	28 ± 2.5	0.1 ± 0.2	99.6
61 Bin 3a Inside	3.4 ± 1.7	0.0 ± 0.0	100
61 Bin 4a Inside	21 ± 3	0.0 ± 0.0	100
61 Bin 4b Outside	0.1 ± 0.3	0.0 ± 0.0	100
61 Bin 10 Inside	22 ± 11	0.7 ± 0.0	96.8
61 Bin 10 Outside	1.9 ± 0.4	0.3 ± 0.0	84.2
61 Bin 11 Inside	50 ± 20	0.1 ± 0.2	99.8
61 Bin 11 Outside	0.3 ± 0.3	0.3 ± 0.3	0
61 Bin 12 Outside	1.4 ± 1.4	0.1 ± 0.2	92.9
61 Fibreglass Box 1 Inside	25.3 ± 2.3	0.3 ± 0.3	98.8
61 Fibreglass Box 2 Inside	10.7 ± 4.7	0.2 ± 0.3	98.1
85 Bin 1	19 ± 5	0.2 ± 0.2	98.9
85 Bin 2a	69 ± 2	26 ± 1.7	63
85 Bin 2b	8 ± 5	24 ± 2.2	0
85 Bin 2c	16 ± 1.5	12 ± 1.2	23
85 Bin 3	4 ± 3.4	0.0 ± 0.0	100
85 Bin 4	1.5 ± 0.0	0.0 ± 0.0	100
85 Bin 5	151 ± 11	0.0 ± 0.0	100
85 Bin 5a	23 ± 7	13 ± 2.7	45
85 Bin 5b Inside	16 ± 13	13 ± 0.9	17
85 Bin 5b Outside	4.4 ± 5	5 ± 0.2	0
85 Bin 6	31 ± 5	0.0 ± 0.0	100
63 Bin 1a Outside	1.9 ± 0.8	0.0 ± 0.0	100
63 Bin 1b Inside	8.9 ± 4.1	0.0 ± 0.0	100
63 Bin 2a Outside	1.7 ± 0.9	0.0 ± 0.0	100
63 Bin 2b Inside	4.3 ± 0.0	0.0 ± 0.0	100
63 Bin 3a Outside	2.8 ± 1.3	0.2 ± 0.4	93
63 Bin 3b Inside	28.2 ± 0.8	0.0 ± 0.0	100

Table 8. Results of the Bin fumigations at Keremeos, BC.

Room/Bin/position swabbed	<i>Penicillium</i> spp. CFUs/cm ²		% Reduction
	Before Fumigation	After Fumigation	
129 Bin 1a Outside	0.2 ± 0.2	0.0 ± 0.0	100
129 Bin 1b Outside	0.6 ± 0.2	0.0 ± 0.0	100
129 Bin 2a Outside	16.1 ± 1.6	0.0 ± 0.0	100
129 Bin 3a Inside	8.8 ± 0.8	0.0 ± 0.0	100
129 Bin 3b Outside	1.6 ± 1.6	0.0 ± 0.0	100
129 Bin 4a Outside	1.3 ± 0.7	0.0 ± 0.0	100
129 bin 4b Inside	10.9 ± 0.8	0.0 ± 0.0	100
129 Bin 5 Inside	51.4 ± 10.9	0.0 ± 0.0	100
129 Bin 6 Inside	6.8 ± 0.5	0.2 ± 0.2	97
125 Bin 1a Outside	1.9 ± 0.2	0.0 ± 0.0	100
125 Bin 1b Inside	17.6 ± 1.2	0.0 ± 0.0	100
125 Bin 2a Outside	0.8 ± 0.8	0.0 ± 0.0	100
125 Bin 2b Inside	3.4 ± 0.2	0.0 ± 0.0	100
125 Bin 3a Outside	2.7 ± 0.7	0.0 ± 0.0	100
125 Bin 3b Inside	12.2 ± 0.4	0.0 ± 0.0	100

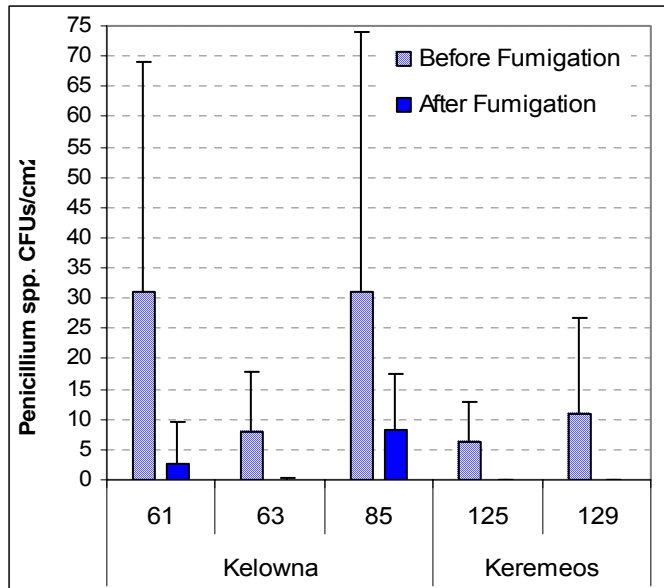


Figure 2. Overall averages of *Penicillium* spp. CFUs/cm² on all bins.

Of the bins treated there was an average reduction of *Penicillium* spp./cm² from 72 to 100% (figure 2). The reduction of 100% was obtained at Keremeos, BC due to the higher mg/l rate of acetic acid used at that location and a lower pre fumigation CFU/cm².

Results/Discussion Codling Moths

The fumigation of the wooden blocks containing the codling moth larvae caused a problem due to the absorption of the acetic acid by the unpainted wooden blocks. There were repeated additions of acetic acid at various times during the fumigation (figure 3). This was done to keep the acetic acid vapours at a high enough concentration to kill the larvae/pupae. Plastic solo cups did not absorb the acetic acid and were therefore at a higher concentration resulting in 100% kill of all the larvae and pupae (figure 4). The results indicate that acetic acid can be used to kill larvae and pupae in bin, but the rate of 40mg/l is 4 times the rate used in the Bin/room fumigations. The use of this higher rate would require a separate room. More research into this aspect is necessary.

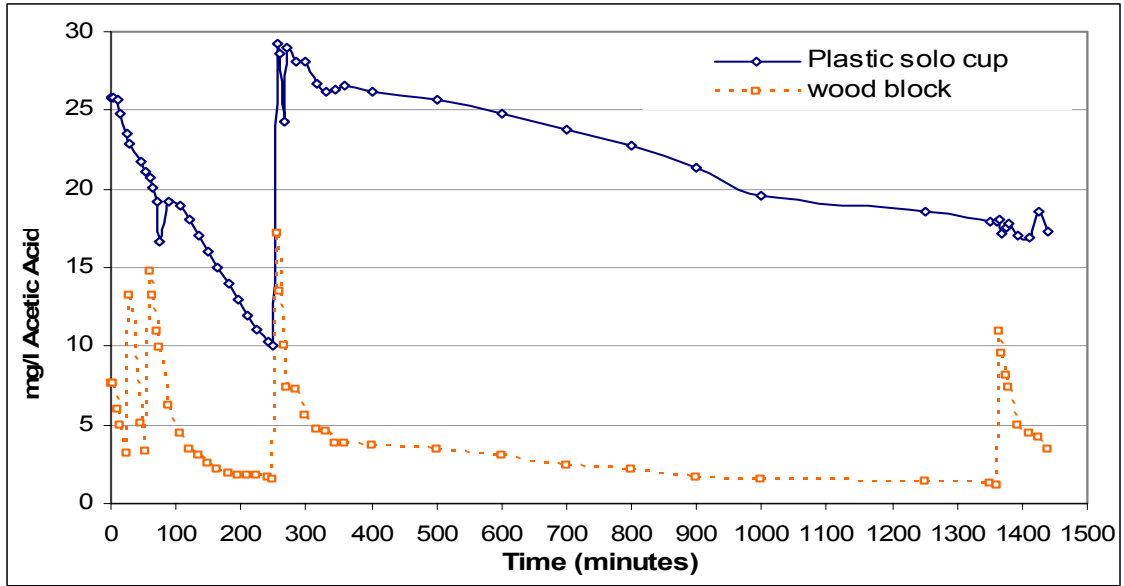


Figure 3. The effect of wood vs plastic on acetic acid concentration during the fumigation of the codling moth larvae/pupae. Note that there were 4 additional injections of acetic acid for the wooden blocks and only one for the plastic solo cups, resulting in a higher mg/l of acetic acid in the chamber.

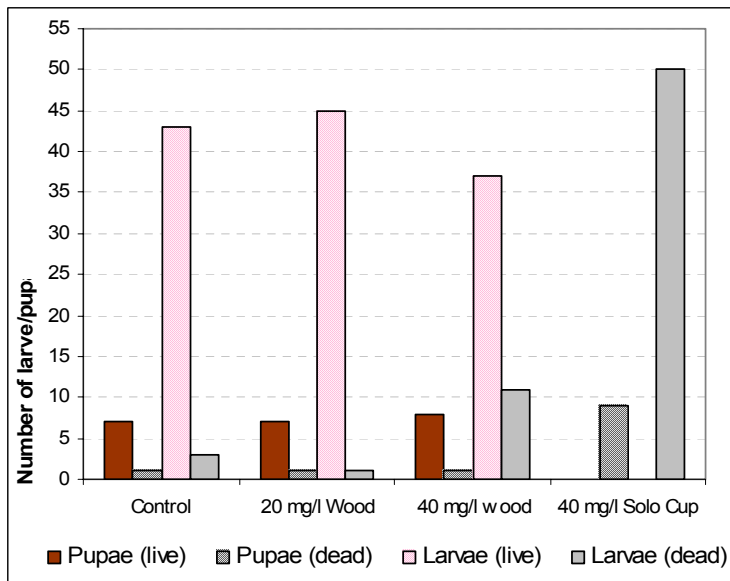


Figure 4. Acetic acid fumigation of codling moth larvae and pupae. Note that there was 100% kill of both larvae and pupae in the plastic solo cups.

Objective 5. (Effect of relative humidity on acetic acid fumigation)

Methods

Inoculum. Fungi from *P. expansum* isolate 1790 and *B. cinerea* isolate B-27 were used. The number of conidia per ml of inoculum was adjusted to $\sim 1.5 \times 10^4$ conidia per ml by diluting with sterile distilled water as needed. A 2 cm by 2 cm sheet of a clear map overlay material were cut, each inscribed with 3 circles of ~ 4 mm in diameter, clipped onto Styrofoam blocks using paper clips, washed with SDW and 95% ethanol and allowed to air dry in a laminar flow hood. Once dried, ~ 10 μ l of the inoculum was placed onto the center of each inoculation circle and allowed to air dry.

Fumigation. The dried Styrofoam blocks were then placed into 23 l fumigation chambers. The relative humidity of the chambers was adjusted to the desired percentage. Measured amounts of 99% Glacial Acetic Acid (Fisher Scientific, Ottawa, Ontario) was added to create treatments of ~ 0.0 (control), 4.0, 6.0 and 8.0 mg / litre of air. Temperature and RH readings in the fumigation chambers were taken every 5 minutes. Acetic Acid concentration was monitored using a Model 910 Gas Chromatograph (GC) (Questron Technologies Corp, Mississauga, Ontario). Fumigations were performed for 120 minutes in duration and the plastic sheets with inoculated sides down, were placed on quarter strength PDA plates and incubated for 18 to 24 hours at $\sim 20^\circ$ C.

Evaluation of Spore Death. After the 18 to 24 hour incubation period, the inoculated circles were viewed using a compound light microscope (Carl Zeiss, Germany), and the number of ungerminated spores and germlings within the inoculation circles were counted. The plates were then checked again for growth at 48 hours or more.

Statistical Analysis. For each trial, 12 to 15 replicates per treatment were fumigated and then rated for germination. The average germination rates were calculated using all the replicates of each treatment and the standard error of the means were also calculated.

Results

Table 9. *Botrytis cinerea* mean germination percentages with increasing relative humidity and acetic acid concentration.

Relative Humidity	Control	4mg AA/l	6mg AA/l	8mg AA/l
25%	62.6 \pm 1.5%	26.6 \pm 2.3%	5.9 \pm 1.0%	0.00 \pm 0.00%
30%	70.5 \pm 2.7%	1.8 \pm 0.8%	0.06 \pm 0.06%	0.02 \pm 0.02%
40%	71.1 \pm 2.1%	0.04 \pm 0.04%	0.00 \pm 0.00%	0.00 \pm 0.00%
50%	62.1 \pm 2.6%	0.07 \pm 0.07%	0.00 \pm 0.00%	0.04 \pm 0.04%

Table 10. *Penicillium expansum* mean germination percentages with increasing relative humidity and acetic acid concentration.

Relative Humidity	Control	4mg AA/l	6mg AA/l	8mg AA/l
25%	61.1 \pm 1.6%	33.1 \pm 2.5%	17.8 \pm 3.67%	3.5 \pm 2.1%
30%	60.2 \pm 1.8%	21.2 \pm 2.2%	6.8 \pm 1.96%	0.00 \pm 0.00%
40%	58.5 \pm 1.0%	1.4 \pm 0.3%	0.2 \pm 0.07%	0.05 \pm 0.03%
50%	66.4 \pm 2.1%	0.03 \pm 0.03%	0.0 \pm 0.0%	0.0 \pm 0.0%

Discussion

The control (0 mg AA/l) all resulted in germination percentages that were quite consistent among the species, with the *B. cinerea* germination percentages ranging from 62.1% to 70.5% (Table 1), and the *P. expansum* germination ranging from 58.5% to 66.4% (Table 2). In both cases, germination percentages were not 100% and this could be due to several reasons. Firstly, not all conidia produced by a sporulating culture are viable. Secondly, it is possible that not all the viable spores have germinated by the time the plates were counted which was after an incubation period of 18 – 20 hours for *B. cinerea* and 20 – 24 hours for *P. expansum*. Some of the spores germinated quite early as opposed to some of the other spores, and such germlings were already developing extensive mycelia making counting very difficult if the germlings were allowed to incubate any longer.

From the results of the fumigations at different RH's, it can be seen that there is a relationship between relative humidity and acetic acid concentration. As the relative humidity increases, lower concentration of acetic acid was required to obtain 100% control. It could be possible that in the range of RH% and AA concentrations used in this study, a decrease in one of these two variables could be roughly compensated by increasing the other variable. For example, fumigations performed at a low relative humidity could still cause significant spore death if the acetic concentration was high, while at higher relative humidity fumigations, low acetic acid concentrations still resulted in germination percentages of 0%.

In the first year of this study we had found that when the relative humidity was greater than 75%, copper corrosion increased as the acetic acid concentration increased. Hence, it is desirable to use a low acetic acid concentration in combination with a relative humidity below 65% to minimize corrosion, and still allow effective control of pathogenic spores. From the data obtained through this study, it appears that a relative humidity of ~ 50% in combination with an acetic acid rate of 6 mg AA/l will provide the necessary toxicity to inactivate or kill the conidia of both *B. cinerea* and *P. expansum*.