VG99061
Improved control of fungal storage rots of Japanese Squash

F Hay, R Mudford, L Breaden, K Clayton-Greene
Tasmanian Institute of Agricultural Research and Harvest Moon Forth Farm Produce Pty Ltd

Know-how for Horticulture™

VG99061

This report is published by the Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry and Harvest Moon Forth Farm Produce Pty Ltd.

Cover price: $22.00 (GST Inclusive)
ISBN 0 7341 0196 1

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

Final Report

HRDC VG 99061

Improved control of fungal storage rots of Japanese squash

Dr. Frank Hay[1], Mr. Robert Mudford[1], Ms. Lisa Breaden[2], Dr. Kevin Clayton-Greene[2]

[1] Tasmanian Institute of Agricultural Research, University of Tasmania North West Centre, P.O. Box 447, Burnie, TASMANIA

[2] Harvest Moon Forth Farm Produce Pty. Ltd., Leith Road, Forth, TASMANIA

Summary

Approximately 1000 tonnes of Japanese squash (*Cucurbita maxima*) is exported from Tasmania to Japan each year. Periodically, shipments of squash have been affected by fungal storage rots. Rejection of squash is of concern to the industry as there is potential for significant financial losses. In addition the presence of high levels of fungal rots can adversely affect the reputation of the product from the State. Acetic acid vapour at concentrations of approximately 5 μL per litre airspace has been shown experimentally to reduce rots caused by several fungal pathogens on a wide variety of fruits. Acetic acid acts by killing spores on the fruit surface. The purpose of this study was to investigate the use of acetic acid vapour as a fumigant for reducing fungal rots of Japanese squash. Squash (40 per treatment) were placed in wooden half-tonne bins and placed in a shipping container of 28.7m^3 internal capacity. Fruit were treated with acetic acid vapour at rates of 1, 2, 4, 8 or 16 μL/L of total airspace for either 20 or 40 minutes. Fruit were removed following treatment and stored at 11°C along with untreated (control) fruit. Squash were examined at intervals between 7 to 77 days after treatment. Acetic acid treatment had no effect on the number of squash rotted over this period and low concentrations of acetic acid slightly increased the percentage surface area covered by fungal rots. Acetic acid treatment had no effect or only minor effects on some of the quality attributes of squash (flesh colour, skin colour, weight loss, estimated sugar content, flesh firmness) and did not lead to phytotoxicity. One of the main fungal rots encountered in this study (*Fusarium culmorum*) often developed as a ring of cottony mycelium from around the stalk or button end of the squash which indicated that it might be developing from inside the fruit. The presence of fungal pathogens within the squash might in part explain the poor efficacy of acetic acid treatment, which would act as a surface sterilant. Further trial work will be required to determine whether acetic acid will be useful as a commercial fumigant of Japanese squash.

1. Introduction

Acetic acid fumigation has been shown experimentally to have potential as a post-harvest treatment for controlling a wide variety of fungal rots in several different types of fruit. This project examined the effect of concentration and duration of acetic acid vapour on fungal rots of Japanese squash in a semi-commercial situation.

1.1 Japanese squash

Japan imports approximately 130,000 tonnes of Japanese Squash (*Cucurbita maxima* Duchesne) or Kabocha annually. The main suppliers to the Japanese market are New Zealand, Mexico and Tonga. Tasmania is a relatively small supplier, exporting approximately 1000 tonnes of whole Kabocha to Japan per annum. Tasmania aims to supply the Japanese market between March and May, which coincides with the end of the New Zealand season and the beginning of the domestic season in Japan. Tasmania is the only state in Australia able to export whole fruit to Japan, due to quarantine restrictions on export from areas where fruit fly are present.

Exports of Japanese Squash from Tasmania and other countries have in some seasons been severely affected by fungal rots before and during shipment to Japan. In New Zealand, Hawthorne (1988) identified 14 fungal pathogens of *C. maxima* during storage, but only *Fusarium culmorum*, *Fusarium solani* and *Didymella bryoniae* were considered major pathogens. Research into the prevention of fungal storage rots in Kabocha has focused on several areas: (a) best practice procedures for harvesting and postharvest handling to prevent damage to the fruit (King and Wishart 1991), (b) identifying agronomic practices and pre-shipping storage conditions which may influence the development of rots in storage (Hawthorne 1990, Wright and Grant 1999), (c) optimising storage conditions (Beever and Yearsley 1985), (d) breeding varieties with better storage qualities (Grant and Carter 1991) and, (e) understanding

wound repair and other physiological processes (Sharrock and Parkes 1990; Sutherland and Hallett 1993).

1.2 Post-harvest treatments

There has been limited published research into the prevention of fungal rots of Kabocha using chemical treatments. Hawthorne (1989) used pre-harvest treatments of fungicides to prevent storage rots, but results were inconsistent. In addition the increasingly stringent with-holding periods for fungicides prevent this strategy from being adopted commercially.

One of the most commonly used postharvest treatments to reduce rots of Japanese Squash in storage is sodium hypochlorite in the form of a dip prior to grading. Sodium hypochlorite dips have reduced postharvest rots in a wide variety of fruit and vegetables including apples (Motokura *et al.* 1995), mandarins (Agar *et al.* 1995) and tomatoes (Abdel-Mallek *et al.* 1995). However, other researchers have shown that sodium hypochlorite has little effect on the incidence of storage rots and in some cases has increased rotting due to damage caused to the product (Bartz and Kelman 1986; Sangchote *et al.* 1994; D'Aquino *et al.* 1998). The effectiveness of sodium hypochlorite in killing fungi is increased in low pH solutions. However, low pH increases the production of noxious chlorine gas and increases the likelihood of bleaching of fruit (Boyette *et al.* 1993). In addition, the activity of chlorine is decreased considerably in dips that are high in organic matter. Therefore fruit needs to be relatively clean before chlorination to prevent sodium hypochlorite being inactivated by dirty wash water (Boyette *et al.* 1993). Ensuring that wash water is kept sufficiently low in organic matter to retain fungistatic activity of sodium hypochlorite is difficult to achieve on the North West Coast of Tasmania, where crops are often grown in heavy Kraznozem soils.

1.3 Other post-harvest treatments

Acetic acid has been used in liquid form to reduce storage rots of a wide variety of fruit and vegetables (Banwart 1981). Various forms of acetic acid are inhibitory to a range of bacteria, yeasts and fungi and have long been used as anti-microbial agents for food. Acetic acid, acetates and diacetates are classified as food additives in the USA, which come under the category of 'Generally Regarded as Safe' (GRAS) based on toxicological studies and many years of experience with safe use in foods. Acetic acid has food-dependent concentration limits of 0.15-0.8%, up to 3% in sauces and gravies and 9.0% in condiments. Acetic acid has been used in combination with propionic acid in liquid form to preserve grain (Sauer *et al.* 1992). Peracetic acid has been demonstrated to be a promising postharvest treatment for stonefruit (Mari *et al.* 1999).

The treatment of produce by fumigation has some advantages over dipping in that fumigation does not require tank or spray systems, produce is kept dry, and fumigation can be done in airtight storage rooms or shipping containers without additional handling. Natural volatiles have been investigated as fumigants for a number of years as a means of controlling postharvest decay in a many fruits and vegetables (Wilson & Wisniewski 1989). Volatiles investigated include benzaldehyde, methyl salicylate, ethyl benzoate (Wilson *et al.* 1997), acetaldehyde and propanal (Mattheis & Roberts 1993). Although effective, these compounds have not been adopted commercially due to relatively high cost, lack of semi-commercial trials and regulatory requirements (Sholberg & Gaunce 1996).

While acetic acid has traditionally been used in liquid form as an anti-microbial agent, Roberts and Dunegan (1932) showed that vapour of acetic acid could prevent germination of spores of the brown rot fungus of stone-fruit, *Monilinia fructicola*. Research was discontinued because concentrations of acetic acid used in their experiment caused blackening of the fruit. More recent research by Dr. Peter Sholberg and associates at Agriculture and Agri-Food Canada in British Columbia has

shown acetic acid to have considerable potential for controlling post-harvest diseases. Sholberg and Gaunce (1995) reported that fumigation with acetic acid prevented decay in apples, grapes, kiwifruit, pears and tomatoes inoculated with *Botrytis cinerea*, and in apples, oranges and pears inoculated with *Penicillium* spp. Acetic acid fumigation controlled *Botrytis* and *Penicillium* decay in table grapes (Sholberg *et al.* 1996), and in association with modified-atmosphere packaging, controlled *Botrytis* in grapes and strawberries (Moyls *et al.* 1996). Similarly Sholberg (1998) reported good control of *Monilinia fructicola*, *Penicillium expansum* and *Rhizopus stolonifer* on cherry, *Penicillium expansum* on pome fruit and *Penicillium digitatum* on citrus.

Sholberg and Gaunce (1995) reported several qualities of acetic acid which potentially make it a good fumigant:

- It kills spores of a wide range of fungal pathogens at low concentrations (around 5 μL/L).
- It is effective at low temperatures and a range of humidity, although effectiveness is increased at high humidity.
- It is a natural compound that poses little residual hazard at concentrations necessary to kill fungal spores.
- It can be applied *in situ* within cool rooms, thereby negating additional handling of produce.
- It is effective on a range of produce at concentrations that do not cause phytotoxicity to fruit.

Because it is commonly found in food products ranging from 0.25% in baked products to 9.0% in relishes, potential problems of residues are not likely to be of concern for acetic acid (Sholberg *et al.* 1996). The Food and Agriculture Organisation has set no limits on the acceptable daily intake for humans (Anon 1973). However, as for other volatiles, the concentration and duration of treatment must be adjusted for particular types of fruit to ensure phytotoxicity does not occur.

1.4 Purpose of the project

The purpose of this trial was to determine if acetic acid fumigation could be effective at reducing fungal storage rots of Japanese squash on a semi-commercial scale.

2. Materials and methods

Japanese squash were obtained from Queensland and were used for the experiment approximately three weeks after picking. Fruit had received some damage in transit with surface bruises and cuts. For this reason, it was considered unnecessary to inoculate fruit with fungi or to inflict further damage to artificially enhance their propensity to rot.

Glacial acetic acid (AnalaR grade, 99.8%) was obtained from BDH laboratory supplies. Squash were placed in wooden half-tonne bins (Plate 1) and placed in a shipping container of 28.7m^3 internal capacity (Plate 2). Fruit were treated with acetic acid vapour at rates of 1, 2, 4, 8 or 16 μL/L of total airspace for either 20 or 40 minutes with container vents closed. The container was then vented for 15 minutes following treatment. Fruit were removed following treatment and stored at 11°C in a similar container with vents open, along with untreated (control) fruit. For each treatment combination there were 40 fruit. For each treatment, the required volume of acetic acid was poured onto a series of blotting papers separated by plastic mesh and suspended above a tray (Plate 3). Sufficient blotting paper was used to absorb all the acetic acid. Container doors and vents were closed and two personal fan heaters (EWT 2200 W) sited in front of the blotting paper were turned on, to allow rapid evaporation of acetic acid. The fan inside the container was also turned on to recirculate air within the container and ensure rapid distribution of vapour around the fruit. Initial tests of this technique showed that acetic acid was completely evaporated within 5-10 minutes. Treatments were applied by an operator wearing a safety Tyvek Barrier Man coverall (Dupont), full-face mask (Drager) and gloves (Plate 3), as considerable fumes were given off at the higher concentrations.

Fruit were treated on 1/10/1999. Fruit were assessed on 8/10/1999 (7d) (n=40), 14/10/1999 (13d) (n=40), 11/11/1999 (43d) (n=40), 24/11/1999 (56d) (n=40), 1/12/1999 (63d) (n=30) and 15/12/1999 (77d) (n=10). Fruit were assessed for the amount of fruit surface covered by fungal rot and the amount of fruit surface blackened. Fruit were stored at 11°C until 24/11/1999, at which time fruit were maintained at room temperature to simulate shelf-life. Fruit with more than 10% of surface area rotted were removed at each assessment time to reduce contamination of adjacent fruit.

On 1 and 7 December, 1 and 2 weeks after removal from the container, ten fruit per treatment were measured for skin and flesh colour, fruit firmness, moisture content of flesh and Brix %. Skin colour was assessed using an arbitary scale (1=light green, 2= medium green, 3= dark green) (Plate 4). Skin was sliced from the fruit at 3 points around the horizontal circumference and a fruit pressure tester (FT327) fitted with a 5mm diameter head was used to measure the resistance of the flesh to compression to a depth of 1 cm. Squash were then sliced vertically either side of the stalk and the colour was assigned a visual score (dark=7, medium = 7/1, light = 7/2) according to the Wilson Colour Chart (Wilson 1938).

The percentage moisture content was measured gravimetrically by weighing flesh (50 ± 1 g) before and after drying at 100°C overnight. For soluble solid measurement, 50 (± 1 g) grams of flesh was weighed to 3 decimal places, placed in 150 ml of distilled water in a measuring cylinder and the total volume recorded. Flesh was macerated in a Waring blender for 60 seconds. Juice was strained through a paper towel in a filter funnel to remove non-soluble solids and the juice further clarified by straining through a Whatman number 1 filter paper in a funnel. Two to three drops were placed on a Shibuya hand refractometer and Brix measurements recorded. The dry weight of macerated flesh was estimated from the sub sample that had been dried. The measurement of Brix % of juice was used to estimate the grams of sugar per volume of juice and converted to estimated grams of sugar per gram dry weight of flesh.

At each sampling time, 10 selected fruit per treatment were weighed. Fruit weight was expressed as a percentage of the original weight on 8/10/1999.

Data were analysed by Genstat 5 statistical software. The number of squash rotted and percentage surface area rotted from 11/11/1999 to15/12/1999 and skin colour on the 1/12/1999 and 7/12/1999 was statistically analysed by means of a Generalised Log-Linear Mixed Model Analysis assuming a Poisson distribution and using a Log-Link function to linearise data. Log-linear modelling produces the simplest model that adequately describes the data using maximum likelihood criteria (i.e. the deviance statistic) obtained by reducing the parameters in a fully parameterised log-linear model, one at a time until there is no advantage in further reducing the model. Flesh colour was analysed by logistic regression for a binomial population using a logit link function. All other data was analysed by analysis of variance.

Plate 1. Japanese squash in half-tonne bin ready for treatment with acetic acid vapour.

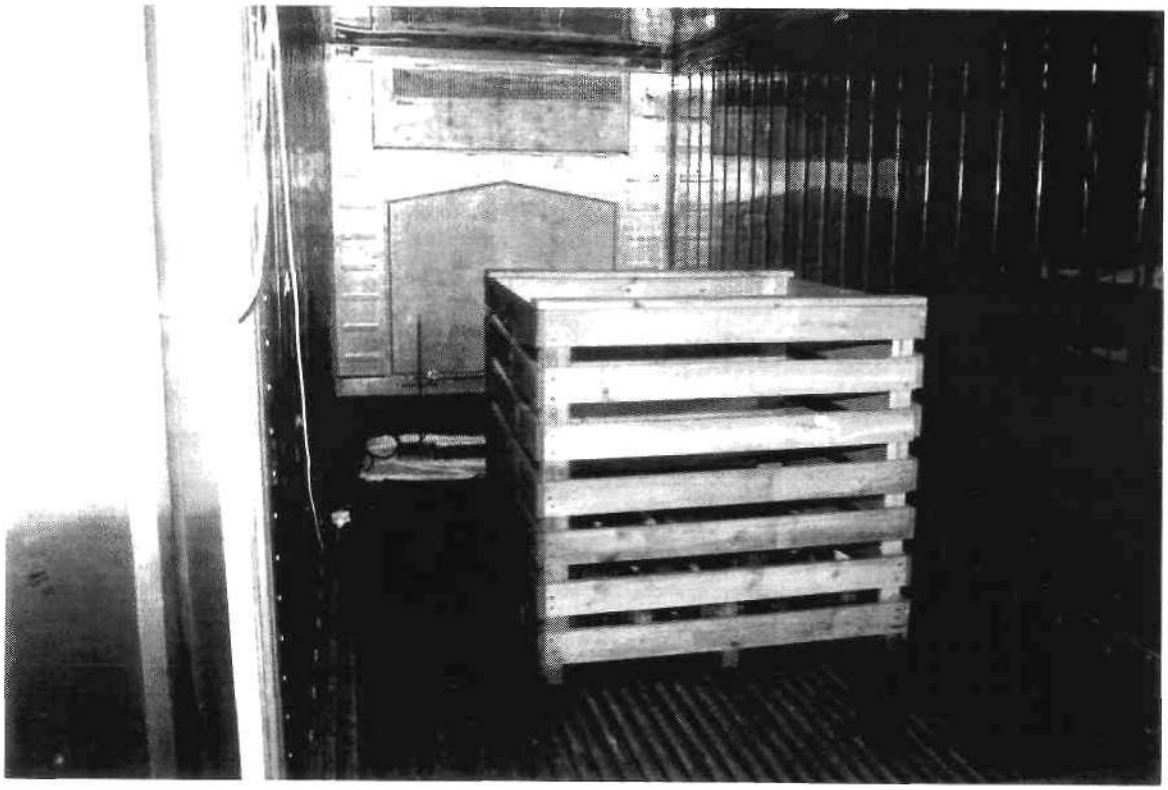


Plate 2. Container used for treating and storing Japanese squash.

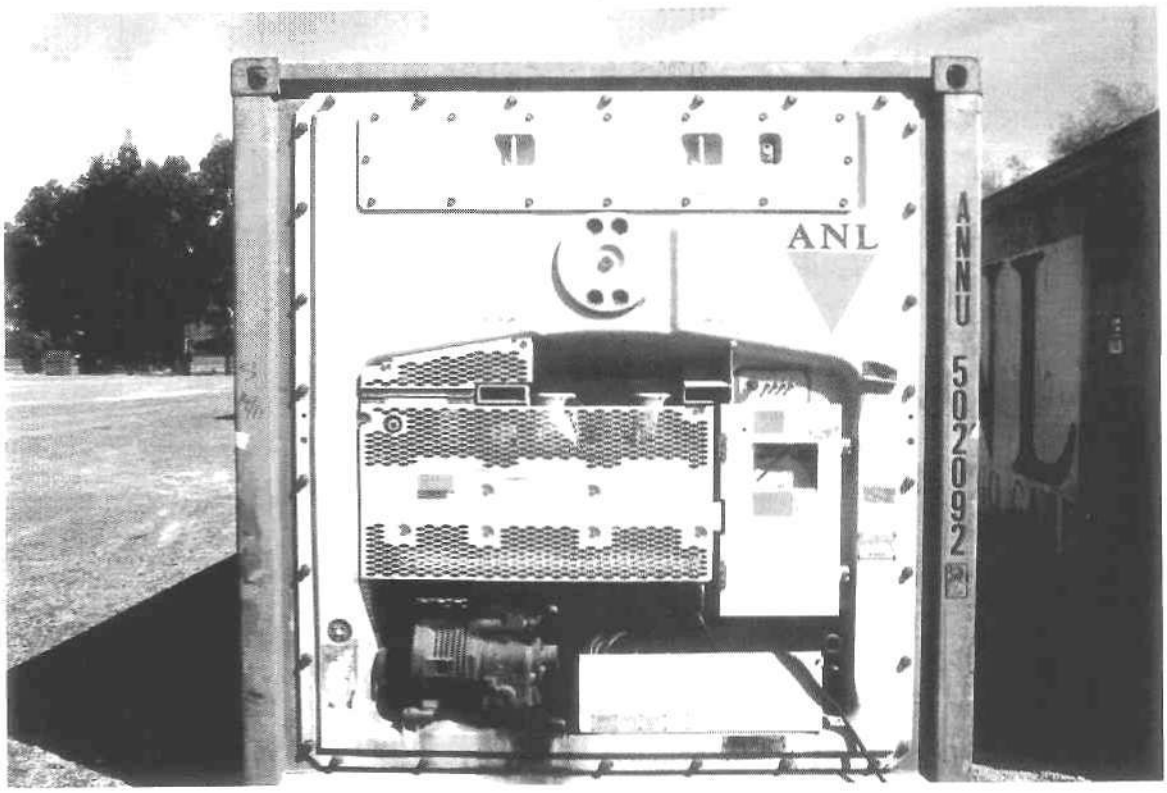


Plate 3. Preparation for acetic acid treatment showing blotting papers suspended between sheets of mesh to absorb acetic acid and fan heaters to promote evaporation.

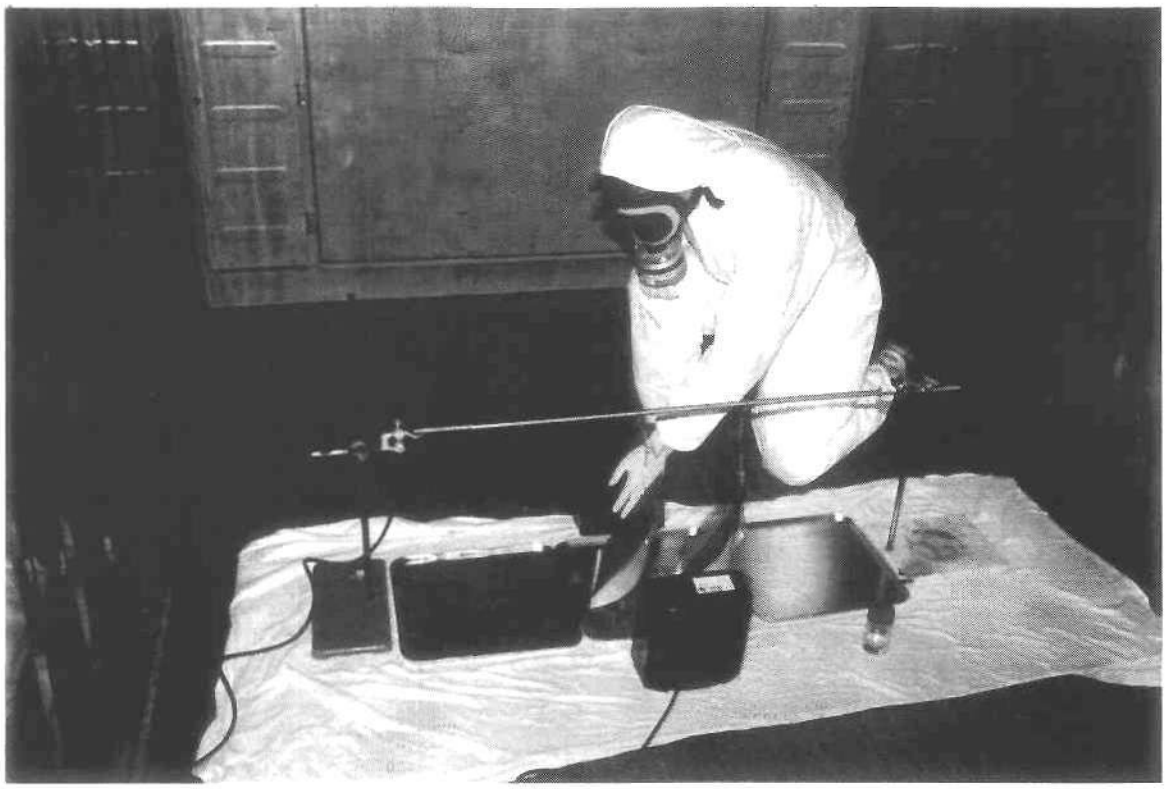


Plate 4. Skin colour categories: 1=light green (left), 2=medium green (centre), 3= dark green (right).

3. Results

Fungal rots were not observed on fruit assessed on the 8/10/1999 or 15/10/1999. On 11/11/1999, one fruit with 50% of surface covered by *Rhizopus* sp. was removed. Fruit remaining at this time had less than 4% of surface area rotted. Of the fruit removed on the 24/11/1999, 54% of rots were due to *Fusarium* sp. (Plate 5) and 33% of rots due to *Fusarium* sp. at the button end of the fruit (Table 1). By 1 December, 45% of rots were due to *Penicillium* spp. and 35% due to *Fusarium* sp. (Table 1). The majority of *Fusarium* occurred at the button end, while the majority of *Penicillium* occurred on the bottom of fruit (Table 1 and Plate 6). On 15 December, 32% of rots were due to *Fusarium* sp. mostly occurring at the button end of fruit, and 48% of rots due to *Penicillium* spp., mostly on the side/shoulder of the fruit (Table 1). Several samples of the *Fusarium* sp. were isolated onto water agar and potato dextrose again in petri dishes and identified as *Fusarium culmorum*.

Table 1. The position of fungal rots on the fruit and percentage of rots caused by fungi at different times after treatment.

		Rots in each category as a percentage of the total number of rots (%)		
Fungal rot	Position	24 Nov	1 Dec	15 Dec
Fusarium	Button	33	15	28
	Stalk	6	10	0
	Bottom	6	0	0
	Side/shoulder	6	0	0
	Ground spot	3	0	4
Penicillium	Button	6	10	12
	Stalk	0	0	4
	Bottom	3	25	12
	Side/shoulder	18	10	20
	Ground spot	3	0	8
Rhizopus	Button	0	5	4
	Stalk	0	0	0
	Bottom	0	5	0
	Side/shoulder	6	10	4
	Ground spot	0	10	4
Other	Button	0	0	0
	Stalk	0	0	0
	Bottom	3	0	0
	Side/shoulder	6	0	0
	Ground spot	0	0	0
Total number of rots[1]		33	20	25
Total number of fruit with rot		33	18	21

[1] n.b. some fruit had more than one type of rot

Plate 5. *Fusarium culmorum* developing from around the stalk of a Japanese squash.

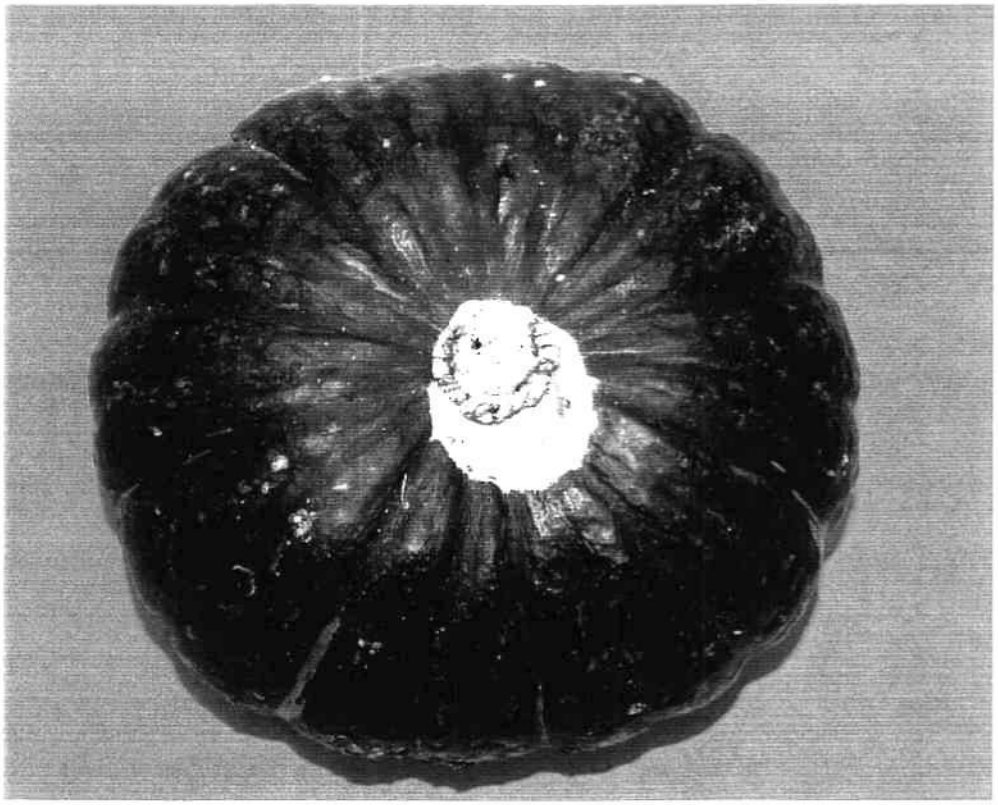
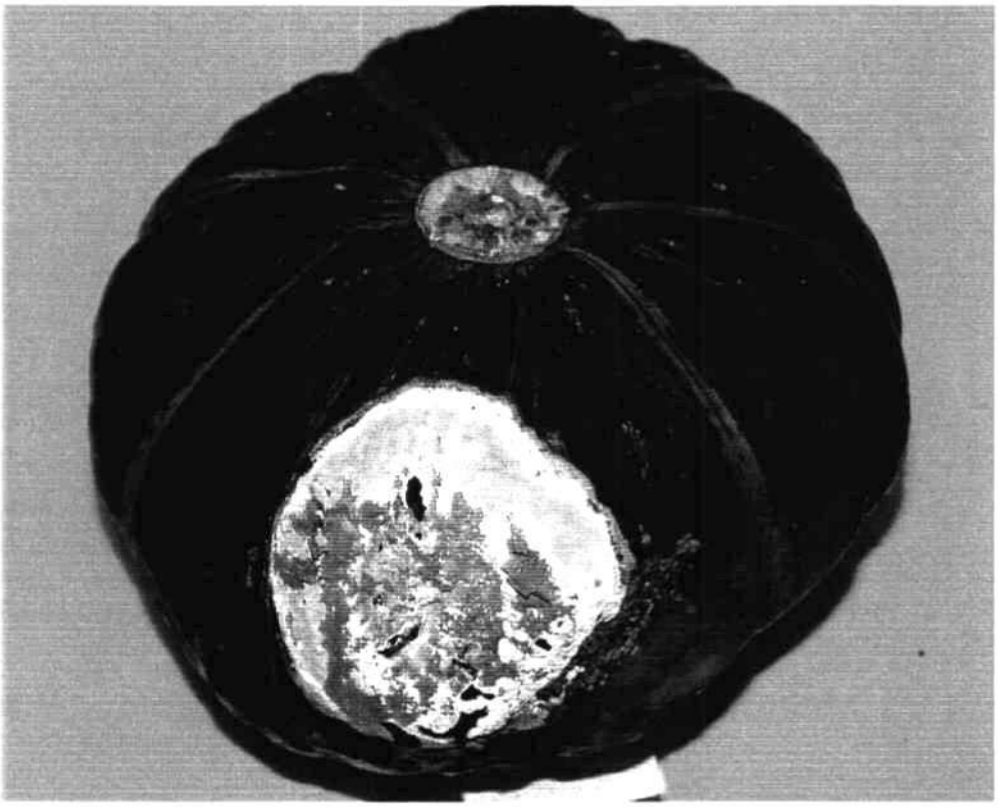


Plate 6. *Penicillium* sp. causing rot on the side of a Japanese squash



Relatively few fruit were rotted at each sampling time (Table 2). Log-linear regression was used to examine whether a fruit was rotted or not in relation to rate of acetic acid, duration of treatment or date of sampling (Appendix 1). The model Constant+rate+duration+date+rot+rate*rot+duration*rot+date*rot was fitted, assuming a Poisson distribution and using a logarithmic transformation. The significance of the model was tested by dropping successive factors from the model (starting with higher order interactions) and recalculating how well the model still fitted the data. The only interaction responsible for a significant ($P<0.05$) change in the model was the date*rot interaction which demonstrated that there were significantly fewer rots present on some sampling dates than on others, as predicted by the null hypothesis. The interactions rate*rot and duration*rot produced no significant change in the model when dropped, indicating that rate and duration of exposure to acetic acid had no significant effect on the occurrence of rots.

Table 2. Mean number of fruit with fungal rot as a percentage of total fruit on different sampling dates

Acetic acid (μL/L)	Duration (min.)	24 November (n=40)	1 December (n=30)	15 December (n=10)
0	-	5.6	13.6	22.2
1	40	5.0	0	25.0
1	20	7.5	14.8	27.3
2	40	7.5	15.4	11.1
2	20	10.0	4.0	11.1
4	40	12.5	8.7	25.0
4	20	5.0	7.4	33.3
8	40	0	3.3	20.0
8	20	5.0	3.6	22.2
16	40	2.5	3.8	22.2
16	20	5.0	3.4	10.0

The mean percentage of fruit surface rotted was generally low at each sampling time (Table 3). The percentage of fruit surface rotted was significantly influenced by the duration of acetic acid treatment ($P<0.001$) and concentration of acetic acid ($P<0.001$) over the sampling times 24/11/1999 to 15/12/1999 (Table 4 & Appendix 4).

Treatment of fruit at 2 and 4 μL/L airspace increased the percentage of surface covered by rot slightly, while 1, 8 and 16 μL/L were not significantly different from the control.

Table 3. Mean percentage of surface rotted or blackened on different sampling dates.

Acetic acid (μL/L)	Duration (min.)	11 November		24 November		1 December		15 December	
		%rot	%black	%rot	%black	%rot	%black	%rot	%black
0	-	0.13	4.78	1.45	8.75	0.37	16.83	1.78	12.78
1	40	0.08	1.68	1.43	3.45	0	11.25	1.25	11.88
1	20	0.08	2.75	0.53	6.48	0.70	8.48	3.27	13.50
2	40	0.1	1.75	0.90	8.25	1.20	15.92	3.44	16.67
2	20	1.4	3.58	0.5	8.83	0.12	10.12	5.56	15.56
4	40	0.25	4.13	1.18	5.55	0.54	17.92	3.75	20.63
4	20	0.15	4.43	0.30	7.98	0.15	20.19	4.44	13.33
8	40	0.05	3.28	0.13	7.33	0.20	18.0	1.0	12.0
8	20	0.03	5.83	0.38	7.80	0.32	12.14	2.60	13.0
16	40	0	3.08	0.13	5.38	0.19	12.77	6.67	10.0
16	20	0.08	4.93	0.20	11.50	0.28	16.38	2.0	15.5

Table 4. Generalised linear mixed model analysis of the effect of concentration of acetic acid on percentage of fruit surface rotted between 24/11/1999 and 15/12/1999.

Concentration	Mean percentage of fruit surface rotted[1]	Predicted mean	
0	0.02	-4.10	c[2]
1	0.03	-3.40	bc
2	0.33	-1.10	a
4	0.09	-2.42	ab
8	0.02	-4.10	c
16	0.02	-4.10	c

[1] Back-transformed means

[2] Means followed by the same letter are not significantly different (P=0.05).

The percentage of fruit surface blackened (Table 3) was influenced by the concentration of acetic acid ($P<0.001$) and duration of treatment ($P<0.001$). Fruit treated with 1 and 2 μL/L acetic acid had significantly less surface blackening compared to the control and other treatments (Table 5). Acetic acid for 40 minutes led to statistically significantly less blackening compared to untreated fruit and fruit treated for 20 minutes (Table 6).

Table 5. Effect of concentration of acetic acid on percentage of fruit surface blackened between 24/12/1999 and 15/12/1999 ($LSD_{0.05}$ = 0.70)

Concentration (μL/L)	Mean percentage of fruit surface blackened
0	3.9 a[1]
1	2.2 b
2	2.7 b
4	4.1 a
8	4.4 a
16	4.0 a

[1] Means followed by the same letter are not significantly different (P=0.05).

Table 6. Effect of duration of acetic acid treatment on percentage of fruit surface blackened between 24/11/1999 and 15/12/1999 ($LSD_{0.05}$ = 0.63).

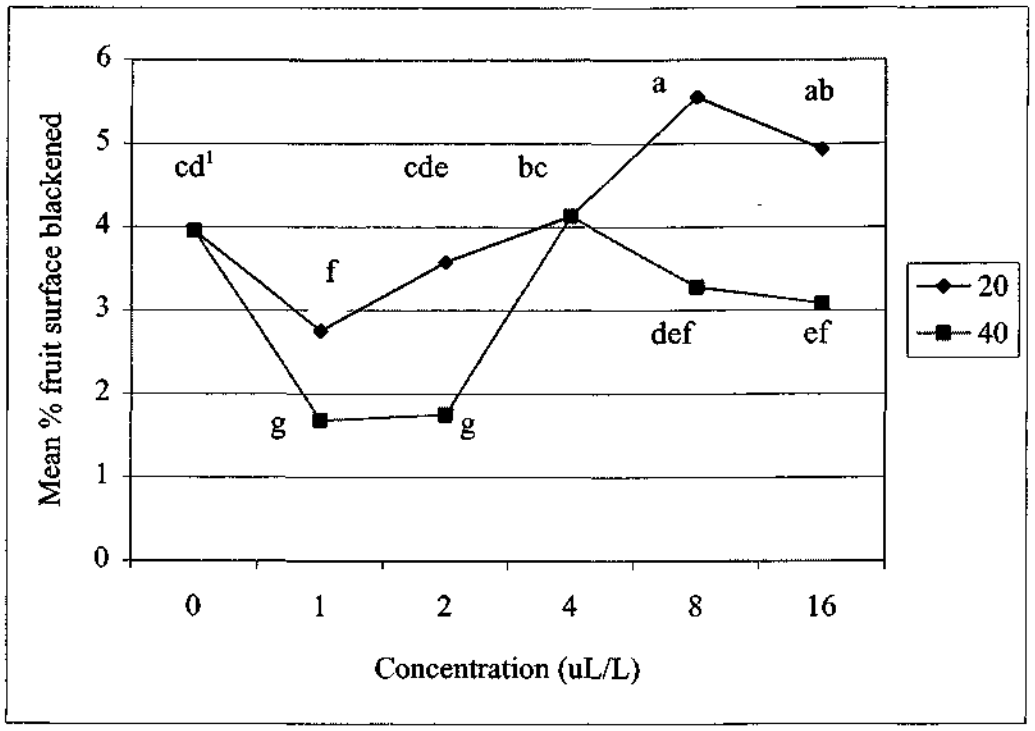
Duration (minutes)	Mean percentage of fruit surface blackened
0	3.5 b
20	4.2 a
40	2.8 c

[1] Means followed by the same letter are not significantly different (P=0.05).

The interaction between concentration and duration had a significant effect on blackening ($P<0.001$). Treatment with acetic acid for 20 minutes caused a reduction in percentage of fruit surface blackened compared to untreated fruit at low

concentrations but an increase at higher concentrations. Treatment with acetic acid for 40 minutes also caused a reduction in fruit surface blackened compared to untreated fruit at low concentrations, an increase at medium concentrations followed by a reduction at higher concentrations (Figure 1).

Figure 1. Interaction between concentration of acetic acid and duration of treatment on the mean percentage of fruit surface blackened between 24/11/1999 and 15/12/1999.



[1] Points followed by the same letters are not significantly different according to LSD (*P*=0.05)

Log linear modelling showed no effect of rate and duration of acetic acid treatment on the skin colour on 1/12/1999 (Appendix 3) or 7/12/1999 (Appendix 4). Similarly, logistic regression analysis showed no effect of rate or duration of acetic acid treatment on flesh colour on 1/12/1999 (Appendix 5) or 7/12/1999 (Appendix 6).

The concentration and duration of acetic acid treatment had no effect on flesh firmness or estimated sugar content on 1/12/1999 (Table 7). Some differences were noted between treatments on 7/12/1999 (Table 8), however no trends were evident.

Table 7. Effect of acetic acid treatment on flesh firmness and sugar content (1/12/1999).

Concentration of acetic acid µL/L	Duration of treatment (min.)	Flesh firmness (kg)	Estimated sugar content (g/gram dry matter)
0	0	9.2	0.64
1	40	9.4	0.66
1	20	10.4	0.64
2	40	9.5	0.64
2	20	8.3	0.65
4	40	8.8	0.68
4	20	9.8	0.61
8	40	9.7	0.64
8	20	9.8	0.63
16	40	8.7	0.59
16	20	9.5	0.65
P=		0.1 (ns)	0.9 (ns)
LSD		-	-

Table 8. Effect of acetic acid treatment on flesh firmness and sugar content (7/12/1999).

Concentration of acetic acid µL/L	Duration of treatment (min.)	Flesh firmness (kg)		Estimated sugar content (g/gram dry matter)	
0	0	9.4	ab	0.75	bc
1	40	9.2	ab	0.78	ab
1	20	9.3	ab	0.76	abc
2	40	9.3	ab	0.69	c
2	20	9.4	ab	0.75	bc
4	40	8.2	c	0.72	bc
4	20	9.0	abc	0.83	a
8	40	8.8	bc	0.73	bc
8	20	9.0	abc	0.75	bc
16	40	9.9	a	0.75	bc
16	20	9.2	ab	0.71	c
P=		0.07		0.03	
LSD		0.90		0.07	

Acetic acid treatment had a significant effect on mean fruit weight as a percentage of fruit weight on 8 October ($P<0.001$). However, there were no clear trends in terms of concentration or duration (Table 9). Fruit weight as a percentage of initial fruit weight declined significantly ($P<0.001$) over the experiment (Table 10).

Table 9. Main effect of acetic acid treatment on fruit weight as a percentage of original fruit weight on 8/10/1999, averaged over all assessment dates.

Concentration of acetic acid µL/L	Duration of treatment (min.)	Mean fruit weight as a % of weight on 8 October
0	0	93.9 ab
1	40	93.8 ab
1	20	93.6 abc
2	40	94.2 a
2	20	93.1 cd
4	40	92.7 d
4	20	92.9 d
8	40	94.2 a
8	20	93.8 ab
16	40	93.6 bc
16	20	92.6 d
P=		<0.001
LSD		0.57

Table 10. Reduction in fruit weight with time as a percentage of fruit weight on 8/11/1999.

	24 Nov	1 Dec	10 Dec	15 Dec
Mean fruit weight as a % of fruit weight on 8 October	95.2 a	93.9 b	92.8 c	92.0 d
P=<0.001, LSD=0.34				

Discussion

Acetic acid treatment was ineffective at reducing the number of Japanese squash rotted or the surface area covered by rots at the concentrations and duration used in this experiment. Squash in these experiments generally had low levels of rot at each sampling time, which made separation of treatments difficult. In addition, many of the fungal rots were caused by *Fusarium culmorum*, which emerged as a developing ring of cottony white mycelium around either the stalk or button. The propensity for this fungus to be associated with these areas on the fruit and the lack of activity of acetic acid as a fumigant at concentrations capable of killing fungal spores suggests that the fungus was located internally. This may be as mycelium within the stalk or button tissue. Alternatively, *Fusarium culmorum* might enter the fruit at an early stage of development and remain as a latent infection until storage. This is known to occur for other fungal pathogens of cucurbits. For example the fungal pathogen *Didymella bryoniae* is known to infect fruit of cucumber not only through wounds on the surface of fruit, but also through the flower (Neergard 1989). The fungus can grow along the stigma into the developing fruit, where it can remain latent for some time before causing an internal rot. The poor performance of acetic acid vapour in this experiment might be partially explained by the inability of the fumigant to penetrate tissue to reach fungi residing internally. This might also explain why the commercially used treatment of squash with sodium hypochlorite at grading is not always successful at preventing rots. However, a proportion of rots in this experiment caused by *Fusarium*, *Penicillium* and *Rhizopus* developed on the ground spot, bottom, side and shoulder of fruit. These types of rots are most likely due to fungi residing on the surface of the fruit and occurred even in treatments receiving the higher concentrations of acetic acid. This suggested that the acetic acid treatment at the concentrations used had been ineffective at sterilising the fruit surface or that infection by these fungi had been sufficiently established prior to treatment that the fumigant was ineffective.

Low concentrations of acetic acid (2 and 4 μL/L) were shown to statistically significantly increase the percentage of surface covered by rot compared to the control and other treatments, perhaps indicating that low concentrations of acetic acid may stimulate fungal growth. However there is no other supporting evidence for this in the literature. The overall percentage of surface covered at each sampling time was low, ranging from a mean of 0.02 and 0.33% for the control and 2 μL/L treatment respectively over the sampling period 24/11/1999 to 15/12/1999. Therefore, although statistically significant, the increase is of little significance in practical terms. Acetic acid treatment at the concentrations and duration studied did not lead to phytotoxicity on the fruit, as measured by blackening. Higher concentrations (50μL/L) have been shown to be associated with black areas on the fruit, although in these experiments, acetic acid was not the sole cause of blackening (Hay pers. comm.). In the current study, fruit treated with concentrations of 1 and 2 μL/L had less blackening than fruit in other treatments. However, the mean level of blackening ranged from 2.2 to 4.4 percent area of the fruit surface between treatments, so this reduction is considered to be of little practical significance.

Acetic acid treatment had no effect on skin or flesh colour and had no effect on flesh firmness and estimated sugar content after one week at room temperature. Differences between treatments in flesh firmness and estimated sugar content were evident after two weeks at room temperature. However, no clear trends with concentration or duration of acetic acid were evident. The magnitude of the difference was small, with mean flesh firmness ranging between 9.2-9.9 kg and estimated content from 0.69-0.83 g/g between treatments. Acetic acid treatments had a statistically significant effect on weight loss. However this was not related to concentration or duration of acetic acid and the magnitude of the difference was small, with only 1.6% of original weight separating the lowest and highest treatments. Results suggested that acetic acid at concentrations between 1 and 16μL/L for 20 or 40 minutes had little effect on some of the major quality parameters of Japanese squash.

Conclusion

Acetic acid applied as a vapour to Japanese squash at concentrations and duration effective experimentally against a range of fungal pathogens on other fruits was found to be ineffective at reducing the number of rots or the percentage surface area covered by fungal growth. The lack of activity is assumed to be due to the low level of rot in the squash utilised in the study and potentially due to the main fungal pathogen (*Fusarium culmorum*) residing internally within the fruit tissue. Acetic acid treatment appeared to cause little effect on fruit quality, which indicated that higher concentrations or longer duration could be assessed.

Acknowledgements

We gratefully acknowledge funding by Harvest Moon, Forth Farm Produce and the Horticultural Research and Development Corporation. We thank Forth Farm Produce for providing Japanese squash, Bruce McCorkell DPIWE, Newtown laboratories, Hobart for statistical analysis and Jason Scott and Helen Jones for technical assistance.

References

Abdel-Mallek, A. Y.; Hemida, S. K. and Bagy M. M. K. 1995. Studies on fungi associated with tomato fruits and effectiveness of some commercial fungicides against three pathogens. Mycopathologia. 130: 2, 109-116.

Agar, T.; Kaska, N.; Ait-Oubahou, Ad. El. and Otmani, M. 1995. Effect of different harvest dates and postharvest treatments on the storage quality of mandarins. Postharvest physiology, pathology and technologies for horticultural commodities: recent advances Proceedings of an international symposium. Agadir, Morocco. pp 75-82.

Anon 1973. Toxological evaluation of certain food additives with a review of general principals and of specifications. 17th Rep. Joint Food Agricultural Organisation United Nations/World Health Organisation Expert Committee Food Additives. WHO Technical Report Ser. No. 539. FAO Nutrition Mett. Rep. Ser. No. 53.

Baker, K.F. & Heald, F.D. 1932. Some problems concerning blue mold in relation to cleaning and packing of apples. Phytopathology 22: 897-898.

Banwart, G. J. 1981. Basic food microbiology. AVI, Westport, Conn.

Bartz, J. A. & Kelman, A. 1986. Reducing the potential for bacterial soft rot in potato tubers by chemical treatments and drying. American Potato Journal. 63: 481-493.

Beever, D. J. & Yearsley, C. W. 1985. Buttercup squash storage. New Zealand commercial grower, 40: 1, 26. New Zealand Journal of Crop and Horticultural Science. 19: 53-60.

Boyette, M. D., Ritchie, D. F., Carballo, S. J., Blankenship, S. M., & Sanders, D. C. 1993. North Carolina Cooperative Extension Service. North Carolina State University. North Carolina.

D'Aquino, S.; Piga, A., Agabbio, M., & McCollum, T. G. 1998. Film wrapping delays ageing of "Minneola" tangelos under shelf-like conditions. Postharvest Biology and Technology. 14: 1, 107-116.

Grant, D. G. & Carter, B. V. 1991. Breeding of *Cucurbita* spp. in New Zealand. Proceedings of the Agronomy Society of New Zealand. 21: 7-11.

Hawthorne, B. T. 1988. Fungi causing storage rots on fruit of *Cucurbita* spp. New Zealand Journal of Experimental Agriculture. 16: 151-157.

Hawthorne, B. T. 1989. Effects of cultural practices on the incidence of fungal storage rots in *Cucurbita* spp. New Zealand Journal of Experimental Agriculture. 17: 49-54.

Hawthorne, B. T. (1990). Age of fruit at harvest influences incidence of fungal storage rots on fruit of *Cucurbita maxima* D. hybrid "Delica". New Zealand Journal of Experimental Agriculture. 18: 141-145.

King, D. & Wishart, G. 1991. Buttercup Squash: Cultural Guidelines For Export Production. NZ Buttercup Squash Council. pp94.

Mari, M.; Cembali, E.; Baraldi, E. & Casalini, L. 1999. Peracetic acid and chlorine dioxide for postharvest control of Monilinia laxa in stonefruits. Plant Disease 83: 773-776.

Mattheis, J.P. & Roberts, R.G. 1993. Fumigation of sweet cherry (Prunus avium 'Bing') fruit with low molecular weight aldehydes for postharvest decay control. Plant Disease 77: 810-814.

Motokura, Y., Kimishima, E., Sato, S. & Kawai, A. 1995. Fungicidal effect of sodium hypochlorite (NaOCl) on *Monilinia fructigena* (Aderhold et Ruhland) honey on

surface of mature apple fruit. Research Bulletin of the Plant Protection service, Japan No. 31 pp 133-5.

Moyls, A.L.; Sholberg, P.L. & Gaunce, A.P. 1996. Modified atmosphere packaging of grapes and strawberries fumigated with acetic acid. HortScience 31: 414-416.

Neergaard, E de. 1989. Histological examination of flower parts of cucumber infected by *Didymella bryoniae*. Plant Pathology 11: 28-38.

Roberts, J.W. & Dunegan J.C. 1932. Peach brown rot. Tech. Bul. 328. U.S. Dept. of Agriculture, Washington, D.C.

Sangchote, S., Farungsong, N., Farungsong U., Johnson, G.I. & Highley, E. 1994. Factors affecting the incidence of stem-end rot on mango. Development of postharvest handling technology for tropical tree fruits : a workshop held in Bangkok, Thailand, 16-18 July, 1992. ACIAR Proceedings 58 , 80-83.

Sauer, D.B.; Meronuck, R.A. & Christensen, C.M. 1992. Microflora, pp. 313-339 In: D.B. Sauer (Ed.). Storage of cereal grain and their products.

Sharrock, K. R. & Parkes, S. L. 1990. Physiological changes during development and storage of fruit of buttercup squash in relation to their susceptibility to rot. New Zealand Journal of Experimental Agriculture. 18: 185-196.

Sholberg, P.L. & Gaunce, A.P. 1995. Fumigation of fruit with acetic acid to prevent postharvest decay. HortScience 30: 1271-1275.

Sholberg, P.L.; Reynolds, A.G. & Gaunce, A.P. 1996. Fumigation of table grapes with acetic acid to prevent postharvest decay. Plant Disease 80: 1425-1428.

Sholberg, P.L. 1998. Fumigation of fruit with short-chain organic acids to reduce the potential of postharvest decay. Plant Disease 82: 689-693.

Sutherland, P. W. & Hallett, I. C. 1993. Anatomy of fruit of buttercup squash (*Cucurbita maxima* D.) surface, cuticle and epidermis. New Zealand Journal of Crop and Horticultural Science. 21: 67-72.

Wilson, C.L. & Wisniewski, M.E. 1989. Biological control of postharvest diseases of fruits and vegetables: an emerging technology. Annual Review of Phytopathology 27: 425-441.

Wilson, C.L., Franklin, J.D. & Otto, B.E. 1987. Fruit volatiles inhibitory to Monolinia fructicola and Botrytis cinerea. Plant Disease 71: 425-441.

Wright, P.J. & Grant, D.G. 1999. Effects of pre-shipping storage conditions on buttercup squash quality rots. New Zealand Journal of Crop and Horticultural Science 27: 337-343.

Appendix 1. Log-linear modelling analysis of number of squash rotted from 24/12/1999 to 15/12/1999

Distribution: Poisson
Link function: Log
Fitted terms: Constant + Rate + Duration + Date + Rot + Rate.Rot + Duration.Rot + Date.Rot

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	17	1066.52	62.7365	62.74	<.001
Residual	48	27.48	0.5724		
Total	65	1094.00	16.8307		

* MESSAGE: ratios are based on dispersion parameter with value 1

Drop Date.Rot
Fitted terms: Constant + Rate + Duration + Date + Rot + Rate.Rot + Duration.Rot

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	15	1047.36	69.8238	69.82	<.001
Residual	50	46.64	0.9328		
Total	65	1094.00	16.8307		
Change	2	19.16	9.5811	9.58	<.001

* MESSAGE: ratios are based on dispersion parameter with value 1

* MESSAGE: The following units have large standardized residuals:

Unit	Response	Residual
8	0.00	-2.34
13	0.00	-2.47
56	7.00	-2.41

Drop Duration.Rot
Fitted terms: Constant + Rate + Duration + Date + Rot + Rate.Rot

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	16	1066.30	66.6440	66.64	<.001
Residual	49	27.69	0.5652		
Total	65	1094.00	16.8307		
Change	1	0.22	0.2153	0.22	0.643

* MESSAGE: ratios are based on dispersion parameter with value 1

Drop Rate.Rot
Fitted terms: Constant + Rate + Duration + Date + Rot

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	9	1040.46	115.6067	115.61	<.001
Residual	56	53.54	0.9560		
Total	65	1094.00	16.8307		
Change	5	6.68	1.3365	1.34	0.245

* MESSAGE: ratios are based on dispersion parameter with value 1

Appendix 2. Log-linear modelling analysis of percentage of surface rotted 24/11/1999 to 15/12/1999.

Distribution: POISSON
Link function: LOGARITHM

Random model: Rep
Fixed model: Constant + Duration + Rate

* Dispersion parameter fixed at value 1.000

**** Back-transformed Means (on the original scale) ****

Duration	
0.00	0.10341
20.00	0.04865
40.00	0.01340

Rate	
0.00	0.01665
1.00	0.03330
2.00	0.33297
4.00	0.08879
8.00	0.01665
16.00	0.01665

*** Deviance: -2*Log-Likelihood ***

Deviance	d.f.
2427.42	432

Note: deviance omits constants which depend on fixed model fitted.

*** Wald tests for fixed effects ***

Fixed term	Wald statistic	d.f.	pr.
Duration	34.6	2	<0.001
Rate	84.2	3	<0.001

* All Wald statistics are calculated ignoring terms fitted later in the model

*** Table of predicted means for Duration ***

Duration	0	20	40
	-2.269	-3.023	-4.313

Standard error of differences: Average: 0.5746
Maximum: 0.7468
Minimum: 0.2553

Average variance of differences: 0.3812

Duration	Predicted mean
0	-2.269 a
20	-3.023 ab
40	-4.313 b

*** Table of predicted means for Rate ***

Rate	0	1	2	4	8	16
	-4.095	-3.402	-1.100	-2.421	-4.095	-4.095

Standard error of differences: Average 0.6055
Maximum 0.8044
Minimum 0.2772

Average variance of differences: 0.3857

Rate	predicted mean
2	-1.1 a
4	-2.421 ab
1	-3.402 bc
0	-4.095 c
8	-4.095 c
16	-4.095 c

Appendix 3. Effect of acetic acid treatment on skin colour (1/12/1999)

	Concentration of acetic acid µL/L	Duration of treatment (min.)	Skin colour category Light	Medium	Dark
1	0	0	1	7	1
2	1	40	1	7	1
3	1	20	1	6	2
4	2	40	1	6	1
5	2	20	0	6	3
6	4	40	0	8	1
7	4	20	1	8	0
8	8	40	0	6	3
9	8	20	1	7	1
10	16	40	0	7	2
11	16	20	1	5	3

Appendix 4. Effect of acetic acid treatment on skin colour (7/12/1999)

	Concentration of acetic acid µL/L	Duration of treatment (min.)	Skin colour category Light	Medium	Dark
1	0	0	1	8	1
2	1	40	4	6	0
3	1	20	1	9	0
4	2	40	9	1	0
5	2	20	3	6	1
6	4	40	2	7	1
7	4	20	3	6	1
8	8	40	2	6	2
9	8	20	3	6	1
10	16	40	2	7	1
11	16	20	3	6	1

Appendix 5. Effect of acetic acid treatment on flesh colour (1/12/1999)

	Concentration of acetic acid µL/L	Duration of treatment (min.)	Flesh colour category		
			Dark	Medium	Light
1	0	0	5	3	1
2	1	40	3	6	0
3	1	20	4	5	0
4	2	40	4	5	0
5	2	20	7	2	0
6	4	40	2	6	1
7	4	20	3	6	0
8	8	40	4	4	1
9	8	20	6	3	0
10	16	40	3	6	0
11	16	20	1	8	0

Appendix 6. Effect of acetic acid treatment on flesh colour (7/12/1999)

	Concentration of acetic acid µL/L	Duration of treatment (min.)	Flesh colour category		
			Dark	Medium	Light
1	0	0	4	6	0
2	1	40	7	3	0
3	1	20	5	5	0
4	2	40	5	5	0
5	2	20	2	8	0
6	4	40	4	6	0
7	4	20	2	7	1
8	8	40	4	6	0
9	8	20	3	7	0
10	16	40	5	5	0
11	16	20	4	6	0