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Squash washing 1997 season

A report prepared for the
New Zealand Buttercup Squash Council Inc.

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CONTENTS

1 EXECUTIVE SUMMARY

1. Y14258 increased rot incidence.

2. Veggie Wash had no impact on rot levels but facilitated soil removal.

3. Fishoil 1 and 3, and FRI oil appeared to reduce rot incidence in small scale tests.

4. The treatments altered the microbial flora of the fruit surface.

5. None of the materials tested effectively arrested Fusarium disease once it was established.

6. For the 1998 season we recommend:

   a. Testing Fishoil 1 and 3 and FRI oil on a larger scale in the early part of the season with the aim of running a full scale packhouse trial when the Manawatu harvest starts.

   b. Evaluating Veggie Wash in two full scale packhouse trials.

   c. Determining the relationship between microbial populations on the fruit surface, washing methods and at subsequent rot incidence.

   d. Developing a laboratory assay to see if we can find a natural material that will arrest Fusarium development once it is well established.

2 INTRODUCTION

In this project we have investigated the use of organic materials to improve squash washing. The aim has been to find materials that will minimise the force needed to remove soil so that there is as little damage to the cuticle as possible, and that at the same time reduce the incidence of rots.

The desired outcome is to have less fruit rejected for hard to dislodge soil than at present, and fewer losses to storage rots, less weight loss and better out-turns.

We first became aware of the possibility of using these materials through a project with an asparagus packhouse to better remove grit and pests from export spears. As the project has developed we have been able to access a wider range of materials.

The results are presented in four parts:

1 weight loss
2 disease incidence and fruit failure
3 effects on established fusarium infection
4 effects on surface microflora.

3 WEIGHT LOSS

Weight loss in transit represents a real cost to the squash industry and we were concerned to ensure that there were no adverse effects on weight loss from the materials tested.

3.1 Methods

Fruit from the Kairanga were left in the various solutions for 10 minutes, and then the soil patches scrubbed with three passes of a nylon brush to simulate a commercial brushing unit. The comparisons were: no washing; water only; Veggie Wash and Y14258. Weights were recorded 2, 7 and 9 weeks after treatment, and treatments were applied within 3 days of harvest. The check periods were loosely designed to mimic commercial milestones: prior to shipment; after marketing in Japan; and the 9 weeks was represent a worst case scenario when there had been significant delays at all points in the chain.

3.2 Results and discussion

Weight losses were greatest from unwashed fruit, and from larger fruit (Figure 3.1). As the experiment progressed the range in weight loss increased; that is the variation between individual fruit was greater. Most weight loss occurred in the first two weeks and after seven weeks.

Weight loss for all the washed fruit followed the same general patterns (Figures 3.2, 3.3, 3.4, 3.5 and 3.6). With fruit only washed in water (Figure 3.2) weight loss followed a similar pattern to the unwashed fruit, although initial weight loss was slightly greater, this was offset by lower weight loss at the end of the experiment. With veggie wash (Figure 3.3) initial weight loss was greater again and generally similar over the subsequent periods. Y14258 gave similar patterns of weight loss to Veggie Wash (Figure 3.4) initially, with somewhat lower levels later in the experiment; there was less variability in the data for Y14258 on large fruit at the end of the trial. FRI oil (+1- asi) (Figures 3.5 and 3.6) reduced weight loss with respect to the other additives. Addition of asi may have increased weight loss a little, especially towards the end of the trial.

Overall, weight loss was greatest at the beginning of the experiment and at the end.

Figure 3.1; Weight loss of unwashed squash in three size classes

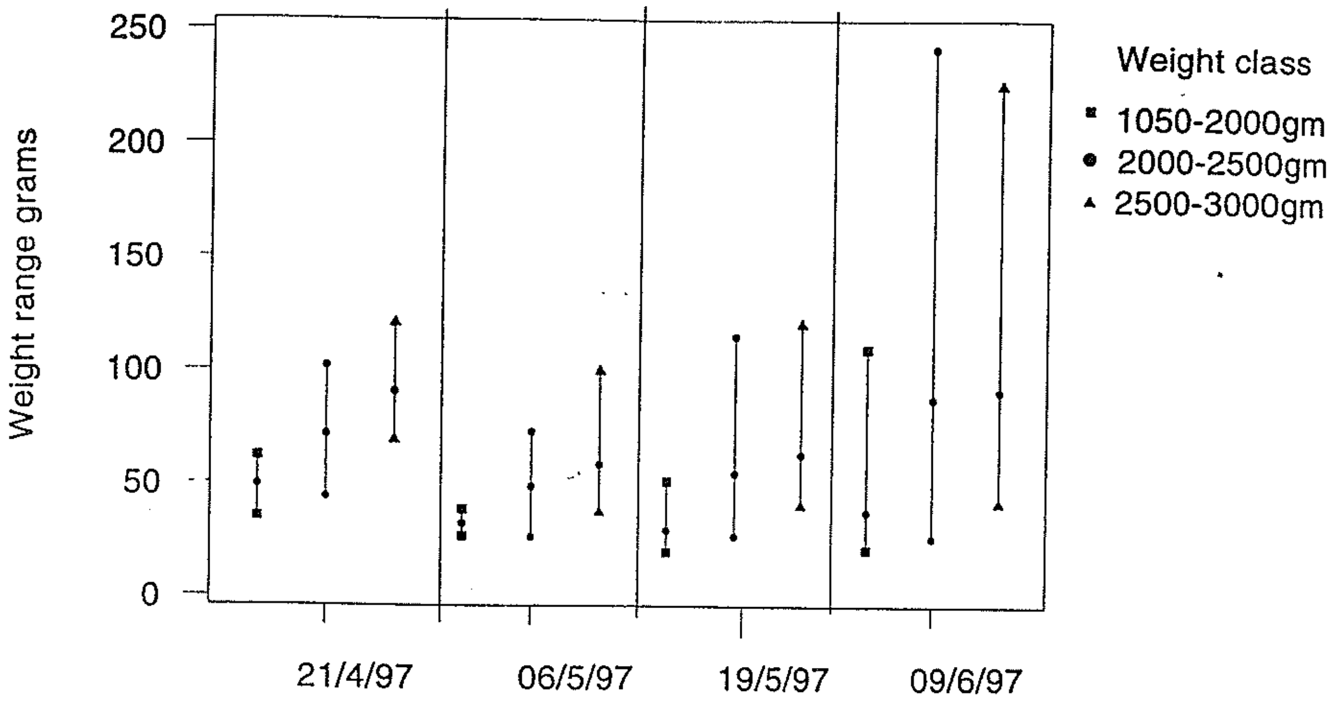


Figure 3.2; Weight loss of squash washed in water in three size classes

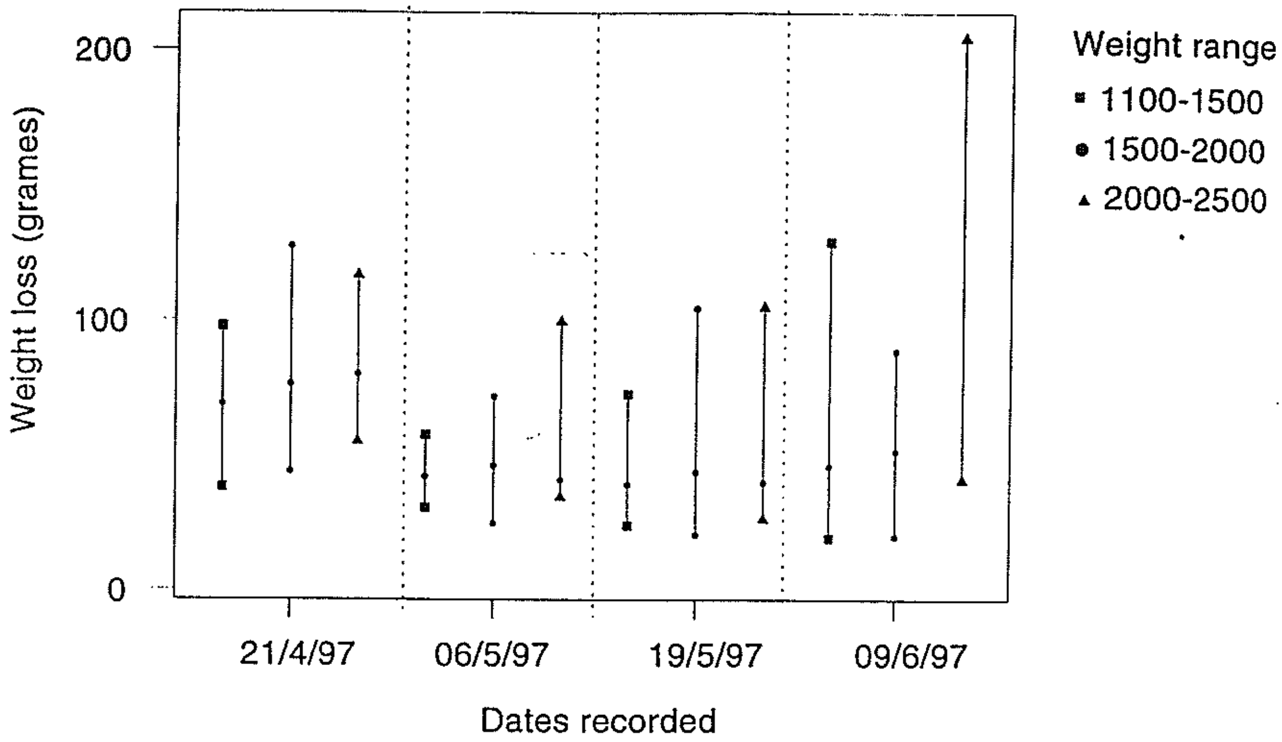


Figure 3.3; Weight loss of squash washed in Veggie Wash in three size classes

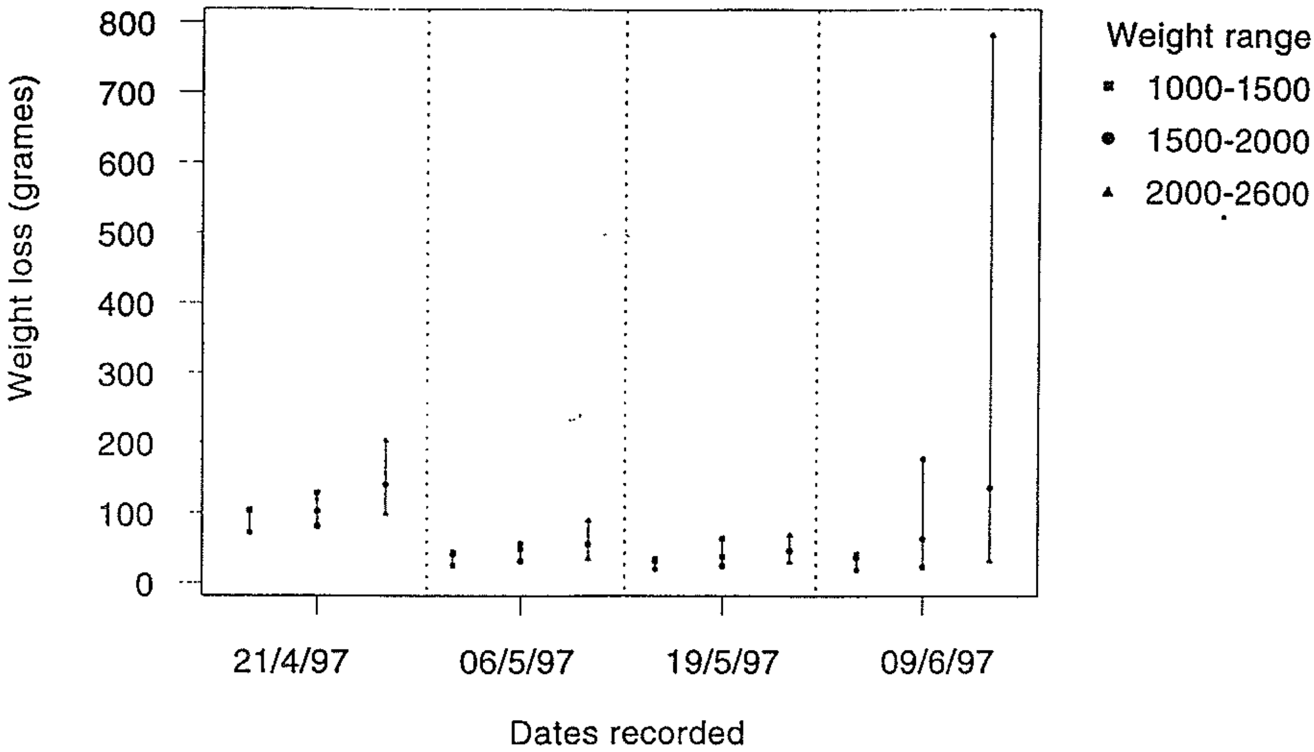


Figure 3.4; Weight loss of squash washed in Y14258 in three size classes.

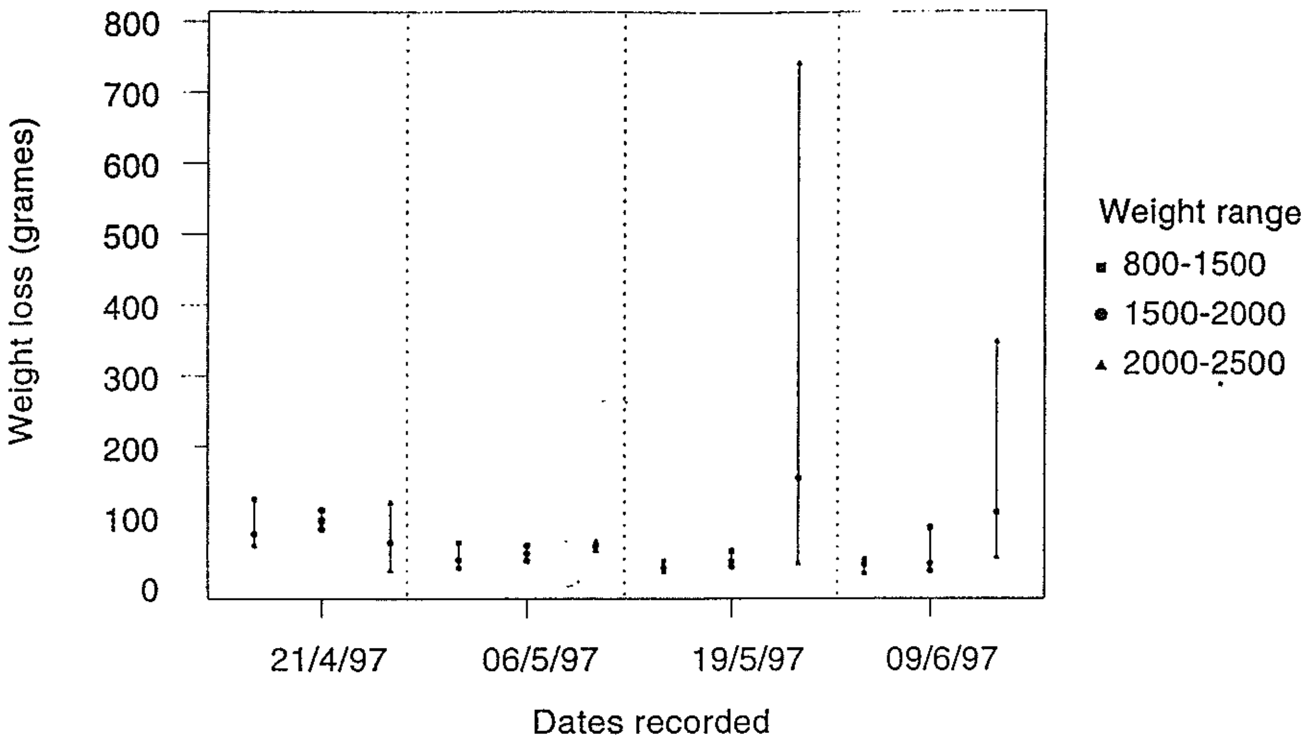


Figure 3.5; Weight loss of squash washed in FRI Oil in three size classes.

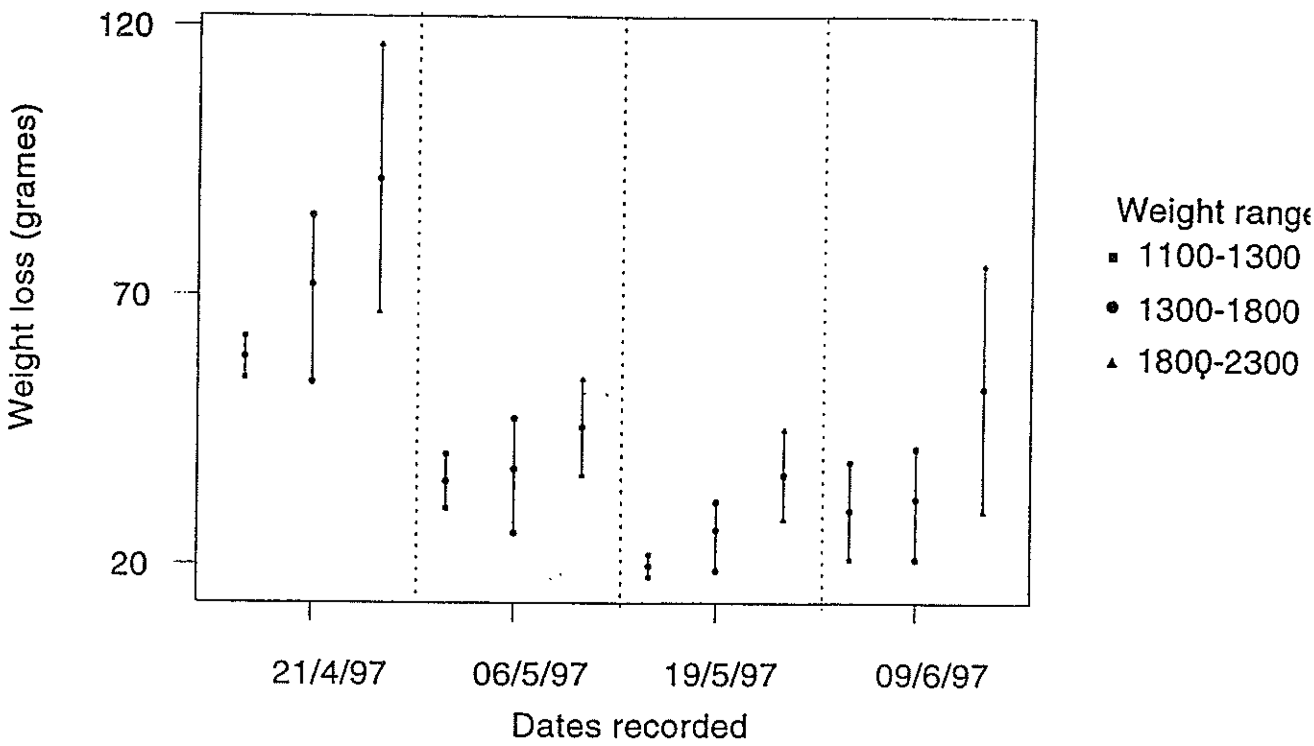
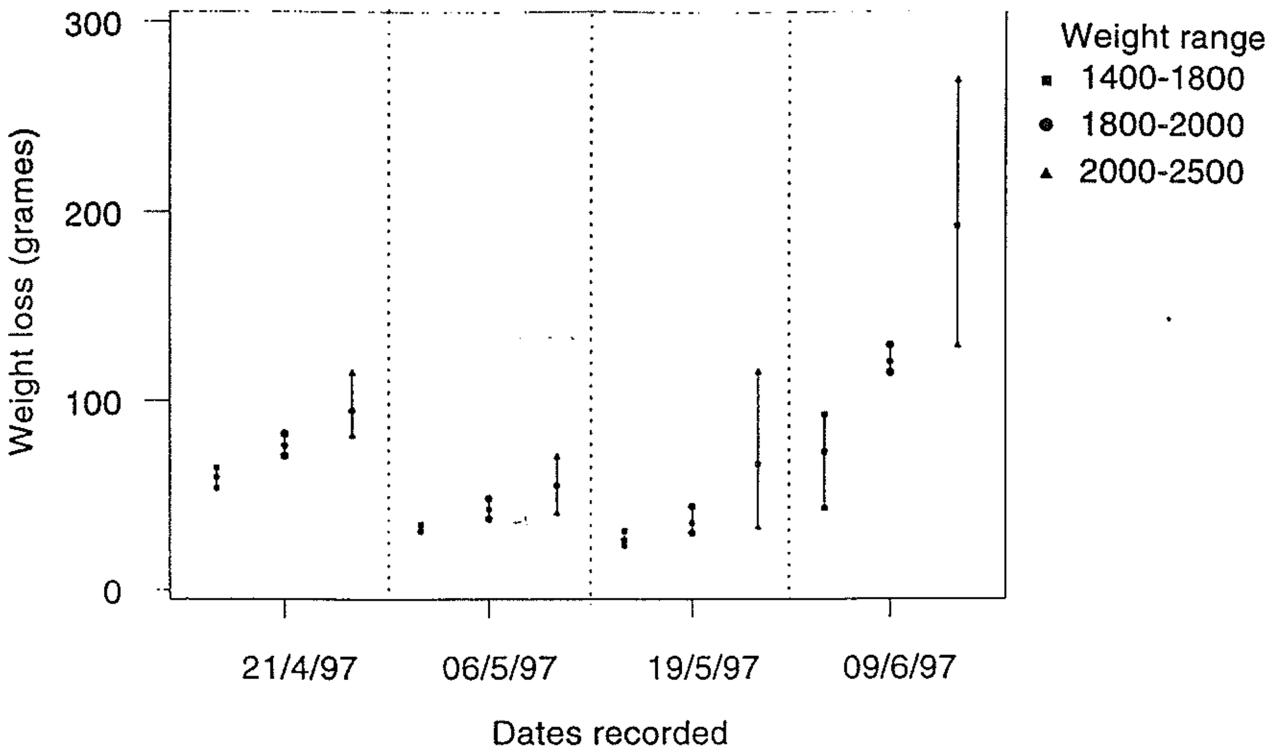


Figure 3.6; Weight loss of squash washed in FRI Oil with AS1 added to improve emulsification, in thrre size classes.



4 ROT INCIDENCE

Rots are a significant cost to the squash industry and a reduction in rots of 1% of the total export crop would increase profitability of the industry. In this experiment we looked at cleaning and rot incidence.

4.1 Methods

The squash were sourced from the Kairanga, through Morgan Laurenson/Linton Horticulture on 5 April 1997. On 7 April they were treated: not washed, washed in water, washed in 1% Veggie Wash, or washed in 1% Y14258. They were then held in an airy shed and rot incidence and size was recorded on 21 April, 6 May, 19 May and 9 June 1997. Any fruit that had a lesion more than 100 mm diameter was discarded.

4.2 Results and discussion

In Tables 4.1 - 4.4 the data are summarised: number of squash left per treatment; the number of fruit with lesions; the size classes of lesion and the number of fruit discarded as unusable.

Table 4.1 Fruit quality: unwashed squash

		Sample period (weeks after treatment)		
		2 weeks	7 weeks	9 weeks
Number of fruit left		214	200	140
Number of fruit discarded		0	14	60
Number of fruit with lesions		6	57	13
Lesion size	> 10 mm	0	15	1
	10 - 30 mm	2	24	5
	30 - 50 mm	4	11	1
	50 - 70 mm	0	6	1
	70 - 90 mm	0	1	5

Table 4.2 Fruit quality: washed squash

		Sample period (weeks after treatment)		
		2 weeks	7 weeks	9 weeks
Number of fruit left		497	484	433
Number of fruit discarded		0	13	51
Number of fruit with lesions		8	62	68
Lesion size	> 10 mm	1	24	6
	10 - 30 mm	1	22	22
	30 - 50 mm	2	13	20
	50 - 70 mm	0	2	7
	70 - 90 mm	0	1	13

Table 4.3 Fruit quality: Veggie Wash treatment

		Sample period (weeks after treatment)		
		2 weeks	7 weeks	9 weeks
Number of fruit left		434	418	357
Number of fruit discarded		0	16	61
Number of fruit with lesions		7	80	55
Lesion size	> 10 mm	1	40	14
	10 - 30 mm	4	21	9
	30 - 50 mm	0	12	18
	50 - 70 mm	1	6	7
	70 - 90 mm	1	1	7

Table 4.4 Fruit quality: Y14258 treatment

		Sample period (weeks after treatment)		
		2 weeks	7 weeks	9 weeks
Number of fruit left		583	548	444
Number of fruit discarded		6	35	104
Number of fruit with lesions		76	144	69
Lesion size	> 10 mm	10	42	11
	10 - 30 mm	36	43	21
	30 - 50 mm	18	29	15
	50 - 70 mm	7	23	13
	70 - 90 mm	5	7	9

In the unwashed fruit rot incidence peaked after seven weeks. In washed fruit and with Veggie Wash rot incidence increased up to the end of the experiment. Y14258 effectively destroyed the fruit.

In a smaller experiment we looked at the effects of Fishoil 1 at 3 rates, and FRI oil. Fish oil 1 and 3, and FRI oil all appeared to reduce rot incidence.

5 EFFECTS OF WASHING ON FUSARIUM INFECTION

The main experiment looked at prevention of infection; in this small experiment we looked at the possibility that an actively growing *Fusarium* infection could be inhibited by something added to the washing water.

5.1 Method

Organic squash were obtained from Common Property, Te Horo. A nail was used to make 4 wounds in either the distal or proximal surfaces of 15 fruit for each treatment. *Fusarium* was isolated from a squash fruit, bulked up, and a piece of hypha was placed in each hole. When the *Fusarium* had colonised the holes and had started to grow out over the fruit surface, they were immersed in the solution for 10 minutes and then they were left in an airy shed for three weeks, after which they were assessed.

5.2 Results and discussion

The results are shown in Table 5.1. None of the treatments were particularly effective, although this could in part be due to the infection being very well developed at the time of treatment. It may be worth looking at well developed infection.

Table 5.1 Effects of washing additives on well established *Fusarium* infections.

	Number of sound fruit (of 15)
Water only	0
Fish oil 1	
Fish oil 2	
Fish oil 3	
Y14258	
Veggie wash	

6 EFFECTS OF WASHING ADDITIVES ON THE SURFACE MICROBES OF TREATED SQUASH

6.1 Objective

To develop a suitable technique to enumerate microbial populations of bacteria, filamentous fungi and yeasts on the surface of squash fruit treated with different washings in storage.

6.2 Materials and methods

Buttercup squash cv. 'Delica' was harvested from the field was either left untreated, washed or treated with two commercial wash treatments Vegewash and Y14258 on 22 April 1997. The fruits were then stored in large wooden bins and placed in a well ventilated shed. On 4 June 1997, two fruits from each treatment were randomly sampled for a microbial enumeration assay of the fruit surface. Each squash fruit was placed in a sterile autoclavable plastic bag measuring 30.5 x 20.3 cm containing 1.5 L sterile phosphate buffer solution. The sampled fruit was returned to the laboratory where the fruit was held at 1.1 ± 0.5°C until an assay for microbial surface populations was carried out within a 24 hour period.

The bags containing the fruit were shaken on a rotary shaker (Chiltern Scientific Orbital Shaker SS70) at 500 rpm for 10 min and, then placed in an ultrasonic water bath (Ultrasonic Power Corporation Braun-Sonic 1000L) having an output of 200± 10 watts and frequency 20 kHz for a further 10 minutes. The fruit in the bag was occasionally turned to ensure more complete removal of surface microflora was achieved. After washing, 2 mL samples of the buffer were removed and vortexed for 1 min using a Chiltern Vortex Mixer. Tenfold dilutions to 10^{-4} were made in a phosphate buffer.

Yeasts, fungi and bacteria were recovered by spreading aliquots of 0.01 mL of each dilution member onto duplicate plates of agar media. Potato sucrose agar amended with chloramphenicol and rose bengal was used for filamentous fungi and yeasts. Nutrient-broth yeast extract agar supplemented with cycloheximide was used for bacterial counts.

Plates were incubated at 20 ± 1°C for 3-4 days. When the colonies were formed, the number of bacterial, yeasts and filamentous fungi colony forming units were recorded. Colonies were separated into filamentous fungal colonies or mucoid yeast and bacteria on the basis of their macroscopic characteristics.

Surface area of fruit was estimated using the mass area relationship $A=0.02169 \pm 0.0018 + 0.03423 \pm 0.0008\ M$ where A is in M^2 and M is in kg (Nigel Banks pers. comm.) to calculate the number of colony forming units (cfu) per unit area of fruit surface. All sampled fruits were weighed soon after washing.

6.3 Statistical analysis

Analysis of variance was carried out on the estimated number of microorganisms using the statistical package Genstat 5 Release 3.2 for Windows. Most probably numbers (MPN) of microorganisms in the washing were estimated, divided by the surface area of fruit, square root transformed and then analysed to meet the assumptions of the analysis of variance procedure. Results are presented as most probable numbers per cm^2 fruit surface. Back transformed means are included in brackets. For the purpose of discussion untransformed numbers are used.

6.4 Results

The estimated microbial population per cm of untreated squash fruit after six weeks of storage was $4x10^5$, $20x10^4$, $5x10^3$ and cfu/cm^2 for bacteria, yeasts and fungi respectively. The various washing treatments imposed did not affect bacterial and fungi populations significantly ($p>0.05$) (Table 1). However, the yeasts populations were significantly ($p=0.04$) reduced in fruit treated with the commercial washes. Fruit treated with Vegewash reduced yeast populations by 5 folds compared with treatments which did not differ significantly. Moreover, the yeast to fungi count ratio was significantly ($p=0.01$) different between the treatments (Table 1). There is a narrow ratio between yeast and fungi in the Vegewash treatment.

6.5 Discussion

The technique developed was sensitive to detect differences between the microbial populations found on the surface of squash fruit.

The incidence of rots in squash during storage is commonly attributed to various pathogenic fungi such as fusarium and sclerotinia. Although we did not identify the various microorganisms found on the surface of squash in storage higher numbers generally increase the probability of the incidence of rots.

The microbial populations on the surface of squash is considered higher than most of aerial borne fruit probably due to growth habit of the squash vine which bears its fruit close to the ground. The estimated populations of bacterial, yeasts and fungi on the unwashed fruit indicate the populations are generally 10-fold higher than those found in aerial fruit such as grapefruit. Chun & McDonald estimated microbial populations on grapefruit cv. 'Marsh' and estimated populations of bacteria, yeasts and fungi to be between 2-5x10^4, 2-5x10^3, and 1x10^4-6x10^3 cfu/cm^2 depending on the season respectively.

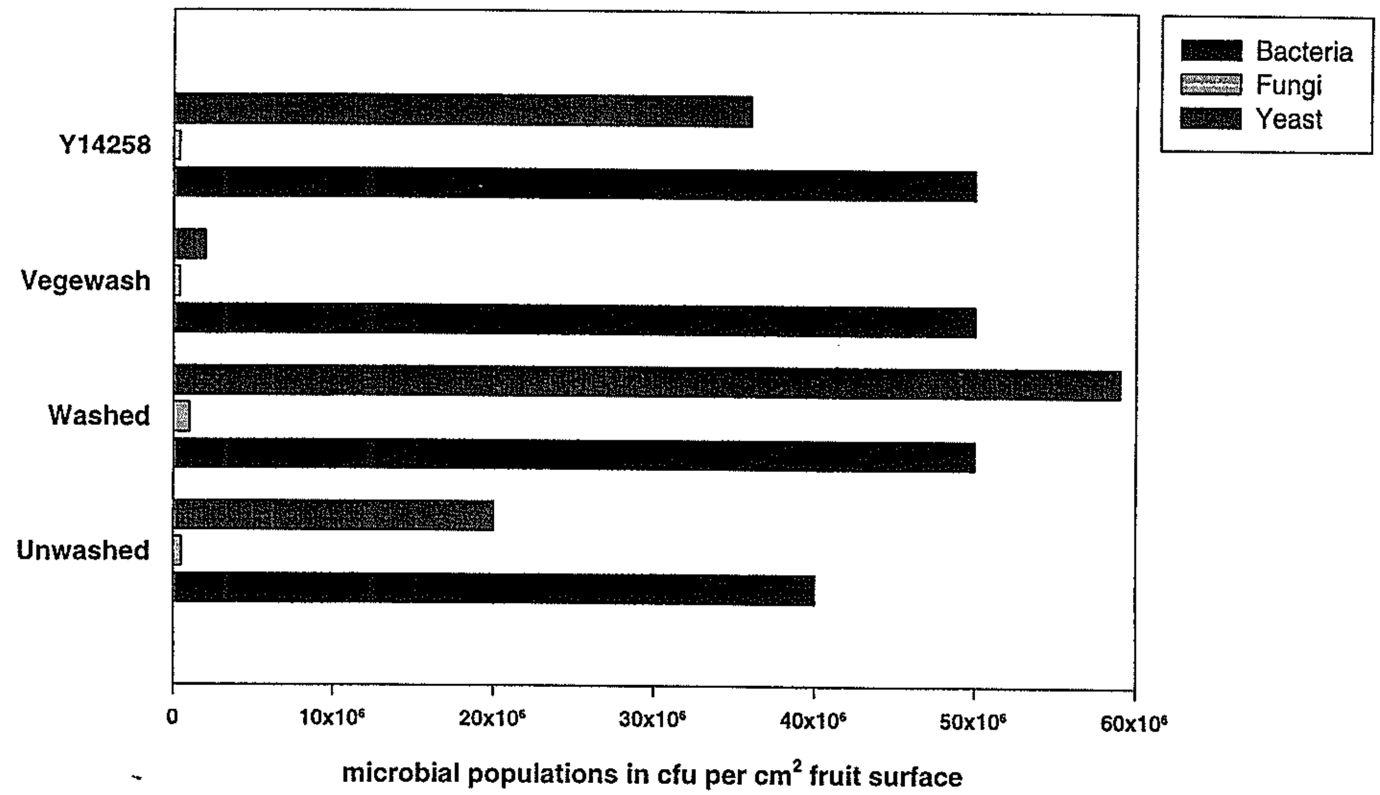
However, fertilizer and pesticide programs both in the field, and in chemical treatment in storage for the control of rots can significantly influence microbial populations (Andrews & Kenerley 1978). The closing in of the ratio between yeast and fungi in the VeggiWash treatment suggest such an occurrence. The tendency of an increased yeast fungi ratio in the Y14258 may suggest something is going on that affected outturn.

6-1

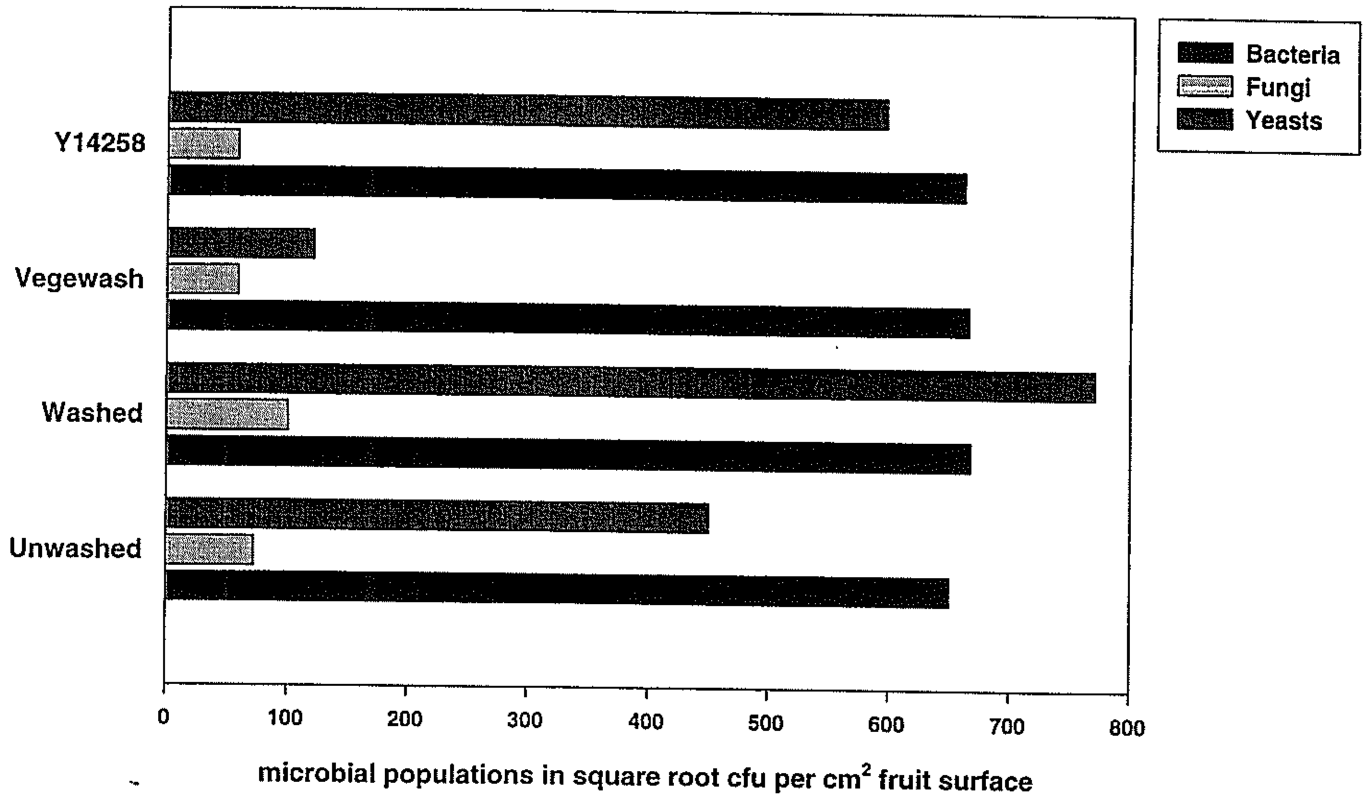
Table 4. The effect of various washing treatments on surface microbial population of squash in storage. Date presented are square root colony forming units ($\sqrt{}$cfu) per cm^2 fruit surface. Back transformed means are included in brackets.

Treatments	Bacteria	Fungi	Yeasts	Yeast/Fungi Ratio
Unwashed	650 (4x10^5)	72 (5x10^3)	450 (20x10^4)	3.4
Washed	667 (5x10^5)	100 (10x10^3)	771 (59x10^4)	3.9
Vegewash	665 (5x10^5)	59 (4x10^3)	121 (2x10^4)	1.1
Y14258	661 (5x10^5)	58 (4x10^3)	596 (36x10^4)	4.7
$LSD_{0.05}$	NS	NS	432	2.1
Overall mean	661 (5x10^5)	73 (5x10^3)	484 (24x10^4)	3.3

The effect of various wash treatments on squash surface microbial populations in storage



The effect of various wash treatments on the surface microbial populations of squash fruit in storage



7 SOIL REMOVAL

We considered soil removal by the materials tested.

7.1 Methods

The soil patch on each fruit was washed and then brushed with a nylon brush. The degree of soiling was determined on 20 fruit from each treatment, and scored 1 - no soil, 2 - slight smearing, 3 - obvious smearing, 4 severe smearing, 5 - soil completely smeared.

7.2 Results and discussion

The average score for the unwashed fruit was 5 as might be expected. For fruit the area washed the average score was 4. For both Veggie Wash and Y14258 the average score was 2, showing that both helped reduce soiling.

8 CONCLUSIONS

1 Y14258 led to too much rot to be further considered.

2 Veggie Wash reduced smearing and had rot incidences similar to washed and unwashed fruit. It should be commercially tested in 1998.

3 Two fish oils and FRI oil gave acceptable rot incidences in small scale trials and should be further investigated.

4 We should look at microbial populations to see if we can predict rot incidences at a later stage in the season.