EFFECT OF WASHING ADDITIVES ON THE INCIDENCE OF ROTS AND AN ENUMERATION OF SURFACE MICROBES IN STORED SQUASH

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ABSTRACT

Postharvest losses through rot in squash fruit exported to Japan result in poor quality or rejected fruit. Mechanical washing aids in the removal of soil, micro-organisms and other debris from fruit surfaces. Washing agents could enhance this removal but their effect on the incidence of rots and on the total number of surface micro-organisms in squash is unknown. This study evaluated the effect of two commercial washing agents on the incidence of rots and used plate-dilution to enumerate microbes on the surfaces of squash fruit in storage. The commercial washing agents Veggi-Wash and the experimental formulation Y14258 reduced the incidence of rots by about 42% and 18% respectively compared to unwashed squash. The estimated populations of micro-organisms on squash fruit without lesions after 7 weeks in storage ranged between 1×10^3 - 1×10^5 colony forming units/cm².

Keywords: MPN, microbial enumeration, squash rot, washing additives.

INTRODUCTION

Squash is New Zealand's fourth largest horticultural export, valued at \$60 million annually (Kerr & Aitken 2000). An estimated 50% of the harvested produce is picked for export, and of that 95% of whole fruit goes to Japan. Postharvest losses of fruit and vegetables in a well-managed shipment are typically between 5 and 10%, but total loss can occur if heavily infected and damaged produce is stored in an unfavourable environment (Eckert 1991). Hawthorne (1988) estimated that the loss of squash fruit in storage increases rapidly after 3-4 months and by six months this loss can exceed 50% of the stored crop. An estimated 80% of rots in squash are caused by soilborne *Fusarium* spp. and 6-16% by *Didymella* spp. (Hawthorne 1988). Chemical control of fruit rots has been available since the early sixties but, the range of postharvest chemicals available are now limited, and pathogen resistance is on the increase from pesticide overuse (Eckert 1991). Pesticide residues in the environment and on food products are an increasing concern for human health (Paoletti & Pimentel 2000). Non-chemical control measures, such as biological control of *Fusarium* rot in squash, have been attempted using yeast and have shown some promise (Cheah & Marshall 1995).

Harvested squash fruit are mechanically washed to quickly remove soil and other debris adhered to fruit surfaces. Washing agents are generally used in other export crops such as asparagus to enhance the removal of soil and debris, and act as sanitizers. A preliminary study was carried out evaluate two washing additives on the incidence of rots, as well as to develop a suitable technique and gain experience in enumerating bacteria, yeasts and filamentous fungi on the surfaces of squash fruit in storage.

MATERIALS AND METHODS

Commercially grown buttercup squash fruit (cv. Delica) were hand picked in April during the 1997 growing season. Sufficient mature fruit for export was supplied by

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Morgen Laurensen and Linton Horticulture for the trial. Two hundred fruit per treatment, replicated three times, were either left untreated, washed with water or washed with one of two commercial washing agents (1% Veggi-Wash and 1% Y14258, an experimental formulation supplied by the Tender Tips Company, Levin). Fruit were hand-washed in a large vessel using a scrubbing brush in all treatments, except in the unwashed treatment. The wash water was changed twice to reduce micro-organism load. The washed fruit was allowed to air-dry before placing them in uncovered well ventilated wooden export bins. The wooden bins were placed side by side in a well-ventilated shed. The temperature in the shed varied between 10-20°C and relative humidity ranged between 50-75% during the course of the trial.

Incidence of rots in fruit

After 7 weeks in storage, squash fruit were assessed in each of the treatment bins by counting lesions between 10 and 90 mm on the surface of fruit.

Treatment and preparation of sample for microbial enumeration assay

After 7 weeks, five fruit showing no lesions, were taken at random from each wash treatment for assay of microbial populations. Each fruit was placed in a sterile autoclavable plastic bag (305x203 mm) containing 1.5 litre sterile laboratory prepared phosphate buffer (0.005 M, pH 7.0) and 0.005% Tween 80. The sampled fruit was returned to the laboratory where they were held at $1\pm0.5^{\circ}\text{C}$ until the assay was carried out within 24 h.

The bags containing the buffer and fruit were shaken on a rotary shaker (Chiltern Scientific Orbital Shaker) at 500 rpm for 10 min and were then placed in an ultrasonic water bath (Ultrasonic Power Cooperation Braun-Sonic 1000L) having an output of 200 ± 10 watts and frequency 20 kHz for a further 10 min (Chand-Goyal & Spotts 1996). The fruit in each bag was turned occasionally in the ultrasonic water bath with a long sterile spatula to ensure more complete removal of surface microflora. After washing, a 2 ml sample of the buffer from each bag was removed and vortexed. Ten-fold dilutions to 1×10^{-4} were then made in phosphate buffer. All sampled fruit were weighed after surface washing.

Microbial enumeration

The plate-dilution frequency technique described in Harris & Sommers (1968) was modified and used for the enumeration of micro-organisms. Yeasts, fungi and bacteria were recovered by spreading 0.1 ml aliquots of each dilution onto duplicate plates of selective agar media. Potato sucrose agar was prepared and amended with chloramphenicol (250 mg/litre; Sigma) and rose bengal (100 mg/litre; Sigma) was used for filamentous fungi and yeasts. Nutrient-broth yeast extract agar supplemented with cycloheximide (50 mg/litre; Sigma) was used for bacteria. Plates were incubated at $20\pm1^{\circ}\text{C}$ for 2-4 days depending on whether bacteria or fungi/yeast counts were being made. When the colonies were visible, the number of bacteria, yeast and filamentous fungal colony forming units (cfu) were recorded. Colonies were separated into filamentous fungal colonies or mucoid yeasts and bacteria on the basis of their macroscopic characteristics.

Determination of surface area of squash fruit

Surface area of squash fruit was estimated using the mass area relationship:

$$A = 0.02169 + 0.03423 M$$

where A=fruit surface area (m^2) and M=weight (kg) ($r=0.99$, $P<0.0001$; B. Cooke, unpubl. data). The standard errors of the constant and slope estimates are 0.0018 and 0.0008 respectively. This equation was used to estimate the number of colony forming units per unit area of fruit surface.

Statistical analysis

Analysis of variance was carried out on the percent incidence of rots and estimated number of micro-organisms using the statistical package Genstat 5. The estimated most probable numbers (MPN) of micro-organisms in the washing solution were divided by the surface area of the fruit, square root transformed and then analysed to meet the assumptions of the analysis of variance procedure. Back-transformed means are included in brackets. For the purpose of discussion back-transformed numbers are used.

RESULTS

Incidence of rots

After 7 weeks in storage, 38% of the unwashed fruit had lesions on their surfaces (Table 1). The commercial washing agents Veggi-Wash (22%) and Y14258 (31%) had fewer fruit with lesions ($P < 0.05$) than unwashed fruit. However, the smallest number of lesions (12%) were found on fruit washed with water only ($P < 0.05$).

Enumeration of surface micro-organisms

The estimated microbial numbers on the surfaces of unwashed squash fruit after 7 weeks of storage were 4×10^5 , 5×10^3 and 2×10^5 cfu/cm² bacteria, filamentous fungi and yeast respectively. The various washing treatments imposed did not affect bacterial or fungal populations ($P > 0.05$) (Table 1). However, yeast populations were reduced ($P < 0.05$) on fruit washed with Veggi-Wash. There was a 5-fold reduction compared with the other treatments, between which the populations were not different ($P > 0.05$). In the water treatment, high yeast populations were observed.

TABLE 1: Mean incidence of fruit rots and mean surface microbial populations 7 weeks after different washing treatments. Data presented are the square root of colony forming units per cm² fruit surface. Back-transformed means are included in parenthesis.

Wash treatment	% squash fruit showing 10-90 mm lesions	Microbial population on squash surface ($\sqrt{\text{cfu/cm}^2}$)		
		Bacteria	Filamentous Fungi	Yeasts
Unwashed	38	650 (4×10^5)	72 (5×10^3)	450 (2×10^5)
Water only	12	667 (5×10^5)	100 (1×10^4)	771 (6×10^5)
Veggi-Wash	22	665 (5×10^5)	59 (4×10^3)	121 (2×10^4)
Y14258 ¹	31	661 (5×10^5)	58 (4×10^3)	596 (4×10^5)
LSD ($P < 0.05$)	6.9	NS	NS	432

¹Experimental formulation

DISCUSSION

Squash picked from the field have soil and debris adhered to their surfaces. If not properly removed this will encourage rots during storage. The two commercial washing agents reduced the incidence of rots on squash in storage but were still higher than that generally found in transit and storage (Eckert 1991). Only the water wash reduced lesions to a level that was not significantly different from 10%.

Populations of micro-organisms showed little difference between washing treatments. This was surprising, given the large differences between treatments in the percentage of lesions formed during storage. It is possible that fruit treated with washing agents may have had waxes removed from the surface of fruit predisposing surface tissue to rot pathogens (Kolattukudy 1980), which would explain why lesions were higher than for squash washed with water only. In the case of the unwashed squash having higher lesions than the washed fruit, despite similar numbers of surface micro-organisms, the soil and debris on the surface of the fruit may have contributed to increased lesion development.

The literature is scant on the microbial populations on fruit surface in storage. Spurr (1994) estimated micro-organism populations at harvest on aerial fruit surfaces to fluctuate between 1×10^5 and 1×10^7 cfu/cm². The microbial populations on the surfaces of squash fruit after 7 weeks in storage were greater than most types of aerial borne fruit probably because squash rest on the soil surface. Our estimated populations of bacteria, yeasts

and fungi on unwashed squash fruit 7 weeks in storage were generally 10-fold higher than those found in aerial fruit such as grapefruit. Chun & McDonald (1987) estimated microbial populations of $2\text{-}5 \times 10^4$, $2\text{-}5 \times 10^3$ and $6 \times 10^3\text{-}1 \times 10^4$ cfu/cm² for bacteria, yeasts, and fungi, on the grapefruit. The total microbial population of unwashed fruit in Table 1 (6.5×10^5) falls within the range estimated by Spurr (1994) but shows a different spectrum from that on grapefruit. While bacteria were 10 times higher in this study, filamentous fungi at 5×10^3 cfu/cm² were somewhat lower than the $6 \times 10^3\text{-}1 \times 10^4$ cfu/cm² on grapefruit and yeast were dramatically higher (2×10^5) than the grapefruit yeast populations ($2\text{-}5 \times 10^3$).

Further research is needed to determine population dynamics of micro-organisms in squash over a growing season in the field, at harvest and in different conditions of storage. In particular, the relationship between numbers and different types of micro-organisms and of incidence of rots requires urgent attention.

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