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Managing bacterial breakdown in washed potatoes

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SA Research &
Development Institute

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FINAL REPORT

Horticulture Australia Project

PT 98007

Managing Bacterial Breakdown in Washed Potatoes

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HAL Project PT 98007

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This report is the result of 4 years investigation into managing tuber soft rot in washed potatoes. Studies were undertaken at Commercial washing plants to determine where soft rot originates and develops. Studies were also undertaken at the Plant Research Centre to develop and evaluate methods to control soft rot in washed tubers.

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Monday, 2 June 2003

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INDUSTRY SUMMARY

Investigations were undertaken at Commercial washing plants to determine where bacterial soft rot originates and develops. Studies were also undertaken at the Plant Research Centre to develop and evaluate methods to control bacterial soft rot in washed tubers.

Four washing plants in South Australia were monitored for levels of *Erwinia* in tuber wash water and tuber soft rot. The main findings were:

- Three *Erwinia* bacterium were shown to caused rotting of tubers in the field and storage in South Australia, namely *E. carotovora* subsp. *carotovora* (75% of isolates), *E. carotovora* subsp. *atroseptica* (21% of isolates) and *E.chrysanthemi* (1.8% of isolates).
- The average incidence of tubers contaminated with *Erwinia* in the field was 26% (range 0 – 78%) and with an average severity of 0.7 (range 0 – 3.5).
- *Erwinia* were found throughout the washing plants. In general 57% of all water samples had levels within the range of 10^3 to 10^4 *Erwinia* colony forming units per millilitre (cfu/ml).
- Most of the washing plants reused wash water, with surplus water collected from the washing plant into a series of settling ponds and re-used in the main washing areas of the washing plant. *Erwinia* were detected in all untreated recycled pond water and 86% of the pond water samples had *Erwinia* levels at 10^3 cfu/ml or higher.
- In the washing plant most tubers were first infected when they were immersed in water in the initial washing tank and tumbler region. Average initial wash soft rot incidence and severity levels were 64% and 1.9, respectively whereas the soft rot incidence and severity in the tumbler were 88.6% and 2.9, respectively.
- Levels of *Erwinia* in the initial washing tank and tumbler water increased rapidly from 0 to 10^3 cfu/ml within 15 minutes of washing time.

- Maintaining a low population of *Erwinia* in the wash water (less than 10^2 cfu/ml) reduced tuber soft rot. Regular renewal of wash water with water free of *Erwinia* will maintain low levels of *Erwinia.*
- Sanitising agents such as Oxine®, Nylate®, Klorman®, Liquid Pool Chlorine, Sporekill®, Proxitane® reduced *Erwinia* in water. However rates 10 to 250 times greater were needed to achieve the same level of control in wash water which was high in soil and organic matter. Most importantly before any of these treatments are applied to recycled pond water the quality of the water must be improved by removing the majority of the soil and organic matter.
- Ultra violet irradiation controlled *Erwinia* in recycled pond water. As with the sanitisers, quality of water must be improved before application.
- Exposing infected tubers for 60 seconds microwave on a high setting at 850Watt controlled soft rot.
- Air drying infected tubers with hot air at 45 - 50°C for 60 seconds or an air knife also controlled soft rot but was not as successful as microwave irradiation.
- Maintaining tubers in a dry condition before packaging helped minimise soft rot.

Main conclusions

- The main contributor to soft rot infection was infected wash water
- The level of rot on tubers entering the washing plant did not have a significant effect on the development of soft rot in the washing process
- Using clean water reduced soft rot
- Once infection had occurred in the washing process, only microwave, ultraviolet irradiation or heated air drying or drying with an air knives reduced soft rot before packing

TECHNICAL SUMMARY

Isolations from water and tubers collected from commercial potato washing plants showed that three *Erwinia* bacterium *Erwinia carotovora* subsp. *carotovora* (Ecc), *Erwinia carotovora* subsp. *atroseptica* (Eca) and *Erwinia chrysanthemi* (Echrys) caused tuber soft rot in South Australia. Fifty six South Australian isolates were sent to Scotland for identification, 77% of these *Erwinia* isolates were sourced from tubers, 18% from wash water samples and 5% from potato stems. Of the 43 *Erwinia* isolated from tubers 88.4% were identified as Ecc and 11.6% as Eca. Of the 10 isolates sourced from water samples 40% were identified as Ecc, 40% as Eca, 10% as E.chrys and 10% as other *Erwinia* species. All 3 isolates recovered from potato stems were identified as Eca.

Four washing plants in South Australia were monitored to determine the level of *Erwinia* in wash water samples and to determine where tubers were becoming infected in the washing production line. Tubers were sampled from the main areas of the washing plant: the field bins, initial wash, tumbler, underneath final rinse sprays bars and from the end line. Collected tubers were induced to rot by maintaining them at 100% relative humidity for 3 days at 25°C.

After incubation tubers were assessed for incidence and severity of tuber soft rot on a scale from 0 to 5, where 0 = no signs of soft rot, 1 = lenticel infection only, 2 = less then ¼ surface area (SA) affected with soft rot, 3 = ¼ - ½ SA affected with soft rot, 4 = ½ - ¾ SA affected with soft rot and 5 = greater than ¾ SA affected by soft rot. Tubers were often contaminated in the field, with 73% of all field bin samples having a low level of rot, with the average incidence and severity of field bin tubers being 26% and 0.7 respectively. However most tubers became infected when they were immersed in water in the initial wash and tumbler region. Average initial wash soft rot incidences and severity were 72% and 2.3, respectively. Average incidence and severity of soft rot in tubers selected from the tumbler region were 87% and 2.8, respectively. Levels of rot at the end line remained similar to those of the tumbler with an incidence of 75% and severity of 2.0.

The practice of hosing down or wetting field bin tubers prior to entering the washing line also increased the levels of infection. When tubers were hosed down for a short period, a small increase in incidence and severity was observed, from 30% and 0.75 to 45% and 1.3. However when tubers were sprinkled for up to several hours with untreated pond water, soft rot incidence and severity levels were significantly increased, from 4.3% and 0.1 before wetting to 96% and 3.9 after wetting.

When tubers were immersed in water infected with *Erwinia*, the level of rot increases with the concentration of *Erwinia*. Tubers dipped in water only, 10 or 10^2 *Erwinia* cfu/ml resulted in tuber soft rot severity increasing from near zero levels to 2. Soft rot severities were further increased when inoculum levels were increased to 10^3 and 10^4 *Erwinia* cfu/ml to severity of 3.4 and 4.4 respectively.

To determine levels of *Erwinia* in the water used in the washing process, water samples were tested by placing onto a *Erwinia* selective media, crystal violet pectate (CVP) (Perombelon MCM and van der Wolf, 1998), and incubating at 27°C for 24-48 hours. The number of colonies on the plates were assessed and calculated as a number of colony forming units (cfu/ml) of water tested. *Erwinia* were found throughout the washing plants with levels ranging from 10^3 and 10^4 cfu/ml in 57% of all water samples.

Most of the washing plants re-used wash water, which is collected from the washing plant to a series of settling ponds. *Erwinia* was detected in all untreated recycled pond water at levels that ranged from 10 to 10^5 cfu/ml, however in 86% of the sampling occasions levels were at 10^3 and 10^4 cfu/ml. In 87% of sampling occasions recycled pond water re-entered the washing plant areas at 10^3 and 10^4 cfu/ml, indicating that *Erwinia* levels were not reduced after storage in the ponds. Pond water was used in the main washing areas of the washing plants such as the initial wash and tumbler wash water. Levels of *Erwinia* in the initial washing areas were at 10^4 cfu/ml or higher

on 68% of sampling times. Similar levels were found in the tumbler region on 55% of sampling occasions.

To determine the changes in *Erwinia* populations in tumbler wash water, two commercial washing plants were monitored as tubers were being washed. In the first washing plant, the tumbler was filled with untreated recycled pond water with an initial *Erwinia* level of 5.0×10^3 cfu/ml. After 30 minutes of washing the levels increased to 10^4 cfu/ml and were maintained at this concentration for the remainder of the testing (1 hour).

In the second washing plant, the tumbler was initially filled with clean bore water. However within a 15 minute period of washing, the *Erwinia* levels increased from 0 to 10^3 cfu/ml. The levels of *Erwinia* fluctuated between 10 and 10^2 cfu/ml over the 2 hour period of measurements. This may have been as a result of chlorinated water from the final rinse sprayers located just after the tumbler pouring back into the tumbler wash water and reducing the levels of *Erwinia*. After 1.5 hours most of the tumbler wash water was renewed with clean bore water. *Erwinia* levels before renewal were at 10^2 cfu/ml and immediately after renewal dropped to near zero levels.

Further sampling at this site evaluated the effect of Sporekill (Didecyl dimethyl ammonium chloride) at 500-900 ppm added to the tumbler wash water. Sporekill had an immediate effect on *Erwinia* levels, dropping them to near zero, however within 15 minutes after application populations rapidly increased from near zero levels to 10^3 cfu/ml. *Erwinia* levels again fluctuated over the following hour, possibly related to the chlorinated run off from the final rinse sprayers as previously mentioned.

In summary, replenishing tumbler water with bore water was as effective as treating wash water with Sporekill at maintaining low *Erwinia* populations in wash water.

In vitro studies were undertaken to evaluate various chemicals for the control of *Erwinia* in water. The first series of experiments used *Erwinia* levels at 10^3 and 10^4 cfu/ml suspended in demineralised water and exposed to various concentrations of Oxine, ChlorDox, Klorman, Nylate, Liquid Pool Chlorine, Proxitane, Sporekill, Citric acid and Metabisulphite for 5 minutes. Most of the chemicals trialed controlled *Erwinia* suspended in demineralised water at concentration of less than 1ppm. Proxitane, Sporekill, Citric acid and Metabisulphite required concentrations of 10, 100, 100 000 and 100 000 ppm respectively to kill *Erwinia* in demineralised water.

This experiment was repeated with all chemicals except citric acid and metabisulphite on initial wash water (a natural source of *Erwinia*) collected from a commercial potato washing plant. When the chemicals were added to the initial wash water, which contained varying levels of soil and organic matter, concentrations need to be 10 to 250 times greater to achieve the same level of control. Before any of these treatments could be applied in a commercial situation the quality of recycled pond water must be improved.

Ultra violet (UV) irradiation was also investigated as a means of disinfecting recycled pond water. This technique used an ultra violet unit called UVTA LC 50-EB where recycled pond water flowed through the inside of parallel lengths of advanced fluropolymer tubing (AFP), located on the outside of the AFP tubing were the UV lamps which applied a concentrated dose of UV at 254 nanometres (nM) which worked as a powerful germicide, killing bacteria, viruses, algae and other micro-organisms. The UV treatments were trialed on recycled pond water at two commercial washing plants. The quality of the recycled pond water was also assessed by measuring the turbidity of the water. Five different flow rates were investigated namely 15 l/min, 20 l/min, 25 l/min, 30 l/min and 35 l/min. For experiment 1 all UV treatments significantly reduced *Erwinia* levels in the pond water, and at the flow rate of 15 l/min *Erwinia* was completely reduced. For experiment 2 none of the UV treatments were effective. This was probably due to the higher levels of soil colloids and organic matter in the water. Turbidity levels in experiment 1 were

between 36 and 52% (in comparison Adelaide mains water has a turbidity of 87%) and the UV treatments were effective in reducing *Erwinia* levels. However in experiment 2 the turbidity levels were between 2 to 6% and the UV treatments had no effect. These results indicated the importance of improving the quality of wash water before the application of UV treatments.

The use of filtration units to disinfect water was also investigated. Sand and rock wool filtration units were evaluated as these are widely used in the hydroponic nursery industries to reduce pathogenic organisms in recycled water. Either demineralised water artificially inoculated with *Erwinia* at levels at 10^5 cfu/ml, or naturally infected potato wash water was added to the filtration units. The potato wash water was collected from the initial wash of a commercial washing plant, and contained varying levels of soil and organic matter with *Erwinia* at 10^4 cfu/ml. To prevent clogging of the filters, a flocculating agent, aluminium sulphite, was added to reduce the soil content of the initial wash water.

Filtration of inoculated demineralised water at a flow rate of 300 l/hr/m^2 for rock wool and sand filtration systems reduced *Erwinia* levels from 10^5 to 10^3 *Erwinia* cfu/ml at 2 hours after inoculation. By 72 hours after inoculation *Erwinia* levels were at 10 cfu/ml. Filtration of inoculated demineralised water at a flow rate of 100 l/hr/m^2 reduced *Erwinia* levels from 10^5 to 10^2 and 10 cfu/ml for sand and rock wool systems, respectively. When potato wash water at 10^4 cfu/ml was added to the filtration systems *Erwinia* levels were reduced to 10^2 cfu/ml and finally to 10 cfu/ml at 72 hours after inoculation.

Although both filtration units reduced populations of *Erwinia* in the water, it was concluded that this technology is unlikely to be adapted by the washed potato industry, as the volume of water used in the washing plant is too great for these systems.

While effective at reducing levels of *Erwinia* in the water, sanitisers applied to tubers during the washing process were generally not effective in reducing soft rot. Only ChlorDox applied at 200ppm as a tumbler spray significantly reduced soft rot levels.

In overseas studies it was reported that when the tuber temperature was higher than that of the wash water, the resulting disease severity was higher. However in numerous similar experiments, we could not reproduce these increases in severity. The level of infection was lower at lower temperatures, but was not dependant on the temperature differentials. Tuber and wash water temperatures measured in commercial washing plants generally showed that the tuber temperature was less than the wash water temperature by at least 2.6 to 3.5°C.

Washed tubers can show symptoms of soft rot either in transit to the market places or on the supermarket shelves. An experiment was undertaken to test the effect of different storage temperatures on the rate of development of tuber soft rot. Soft rot infected tubers were stored in plastic bags with a water soaked sponge at temperatures of 4, 10, 20 and 25°C for up to 14 days. Infected tubers held at either 4 or 10°C failed to develop rot over the 14 days. Infected tubers held at 20°C showed lenticel infection only until day 7, the soft rot severity increasing to 2.8 by day 11 and to 3.5 by day 14. Infected tubers held at 25°C showed lenticel infection until day 4. By day 7 high levels of rot was observed, with the severity of 3.0 which increasing to 5.0 by day 11.

The susceptibility of 15 tuber cultivars were evaluated by artificially inoculating tuber slices and scoring the amount of rotted tuber flesh after 48 hours incubation. All tubers were susceptible to *Erwinia*.

Further experiments were undertaken to determine whether chemical and physical treatments would reduce soft rot when applied to infected tubers. These experiments were undertaken using

a purpose built mini washing plant and artificially inoculated tubers. The mini washing plant replicated the main tuber washing processes found in a commercial situation, consisting of a dumping area, a tumbler, rinsing sprays, rollers, a drying area and a collection area. The sanitisers used were ChlorDox, Proxitane and Metabisulphite applied as either a tumbler or final rinse spray and air drying treatments with heated air or air knives or the application of ultraviolet or microwave irradiation which were applied to infected tubers. After the application of the various treatments tubers were induced to rot and then visually assessed for the incidence and severity of tuber soft rot.

The most successful treatment was exposing infected tubers for 60 seconds to microwave irradiation for 60 seconds on a high setting. Air drying infected tubers with heated air at 45 – 50°C for 60 seconds or an air knife or exposing infected tubers to black light (365nM) for 30 or 60 seconds or UV light (254nM) for 30 seconds also reduced soft rot but were not as successful as the microwave irradiation treatment.

RECOMMENDATIONS

Extension / Adoption

- To control soft rot, populations of *Erwinia* bacteria in wash water must be reduced to levels of 10^2 cfu/ml or less. This can be achieved by:
  - Regular renewal of wash water with clean water, free of *Erwinia*.
  - Applying sanitisers or UV irradiation to recycled pond water before it re-enters the washing plant. These treatments may not be effective unless levels of soil colloids and organic matter in the wash water are reduced. This can be achieved by the use of settling ponds, filtering or other techniques to separate the major soil particles from the wash water, as well as adding a flocculating agent to reduce the turbidity.

- Control of soft rot is more difficult once tubers are infected in the washing process. Post infection tuber treatments such as ultraviolet light, microwave or drying with heated air or an air knife helped reduce soft rot before packaging.

- Packed washed tubers need to be stored below 10^0C to prevent tubers rot developing.

- Tubers should be stored and maintained in a dry condition to prevent development of tuber rot. Changes in storage temperatures can cause condensation inside plastic packaging, which may encourage development of rot.

Directions for future work

- Investigate the use of ultrasonic technology to help reduce the turbidity of recycled pond water.
- Investigate the use of ultrasonic technology to air dry washed tubers before packaging.

- Investigate the efficacy of commercial application of ultraviolet and microwave irradiation to reduce soft rot in washed tubers.
- Continue to evaluate new sanitisers and other new technologies to improve water quality.
- Continue to evaluate new sanitisers and other new methods of reducing post harvest soft rot in infected washed tubers.

TECHNOLOGY TRANSFER

The results of this work have been presented in many forms. We established close collaborations with the washing plant managers and the results of many experiments were regularly conveyed verbally. In addition several meetings and inspections of the research facilities were held with the washing plant managers, including a meeting with washing plant operators in WA. Six editions of the newsletter "Exposing *Erwinia*" were produced, summarising the main findings. These were sent directly to washing plant operators and other Industry personnel. The newsletter was also placed on the SARDI website http://www.sardi.sa.gov.au/hort/pathology, and generated considerable interest locally and overseas. Copies of the newsletter are presented in Appendix 3.

The results of this work have also been presented to industry via four articles in Potato Australia, and to scientific colleagues at four conferences: the 12th Australasian Plant Pathology Conference, Canberra 27th - 30th September, 1999; the Australian Potato Research, Development and Technology Transfer Conference, Adelaide 31st July to 3rd August 2000; the Triennial Conference of European Association for Potato Research Hamburg Germany 14-19 July 2002; and the 8th International Congress of Plant Pathology (incorporating the 14th Australasian Plant Pathology Conference) Christchurch New Zealand 2nd-7th February, 2003. Copies of these articles, abstracts and posters are also presented in Appendix 3.

TECHNICAL REPORT

General Experimental Methods

Isolating *Erwinia* from tubers

Most of the *Erwinia* used in this project were recovered from naturally infected tubers. Tubers were washed thoroughly under running tap water to remove adhering soil then dried with paper towels. Tuber surfaces were sterilised by spraying with ethanol and flaming, before removing small pieces (approximately 0.1gm) of tissue from the margin of rotted flesh. The pieces were placed in 2ml of sterile water within a sterile *petri* dish, teased out and left for 5 to 15 minutes to allow bacteria to diffuse into the water. A sterile inoculating loop was used to streak the bacterial suspension onto Crystal Violet Pectate (CVP) media (Perombelon, M.C.M & Burnett, E.M., 1991). Inoculated plates dishes were dried for 15 minutes in a laminar flow unit before they were incubated at 27°C for up to 48 hours.

Maintenance of *Erwinia*

Erwinia was collected from typical colonies on CVP and transferred by streaking onto Nutrient Agar (NA) plates with a 100*ul* loop. Single colonies of *Erwinia* were then replated onto NA.

Short term storage

Erwinia isolates were stored on NA in *petri* dishes sealed with parafilm at 27°C in the dark. For periods of up to a year *Erwinia* isolates were stored on NA slopes in the dark at room temperature.

Long term storage

Erwinia isolates kept for long term storage were frozen at -80°C. *Erwinia* isolates were prepared by plating out a loopful of pure fresh bacterium (<72hr) onto NA and added to 10ml of nutrient broth in a 100ml Schott bottle. The broth was placed in an incubator shaker at 27°C for

approximately 18hr. 1ml of the broth culture was mixed with 1ml of a freezing media (Appendix) and 0.5ml aliquots were added to 2ml eppendorfs and placed in the $^{-}$80°C freezer.

Before use frozen cultures were thawed to room temperature and streaked onto NA plates and incubated at 27°C for 48 hr.

Quantifying *Erwinia* from water samples

Water samples were collected in sterilised 1000ml Schott bottles and taken to the laboratory in an esky chilled with ice. The water was serially diluted with 1ml of sample water added to 9ml of sterile water repeated for at least 3 dilution series and then plated onto CVP media by spreading 100*ul* of the dilution over the CVP plate. Each dilution was replicated at least three times. The plates were air dried in the laminar flow before being incubated at 27°C for 48 hours in the dark.

Erwinia causes sunken pits in CVP, so levels of *Erwinia* could be determined by counting the number of these pits in the media.

Identifying soft rot organisms

Erwinia isolates were recovered from rotted tuber tissue, tuber stems and bases and from water collected from commercial potato washing plants as shown in Table 1 of the Appendix. Approximately 56 isolates were forwarded to Dr. I. Toth and Beth Hyman at the Scottish Crop Research Institute, Invergowie, Dundee, United Kingdom, for identification. They used a combination of biochemical tests, PCR amplification with De Boers primers ECA1f/ECA2r (DeBoer & Ward 1985 Phytopathology, 85 854 - 858), which are specific for Eca and a new method based on PCR amplification using 16 primers L1/G1 (Jensen et al, 1993, Appl. And Envir. Micro. 59, 945 - 952). Of the 56 isolates tested 77% of these *Erwinia* isolates were sourced from tubers, 18% from wash water samples and 5% from potato stems. Of the 43 isolates from tubers 88.4% were identified as *E. carotovora* subsp. *carotovora* (Ecc) and 11.6% were identified

as *E. carotovora* subsp. *atroseptica* (Eca). Of the 10 water isolates, 40% were identified as Ecc, 40% as Eca, 10% as *E. chrysanthemi* (Echrys) and 10% as another *Erwinia* species. Only 3 isolates were sourced from potato stems and these were all Eca.

Inoculating techniques

Several techniques were used to artificially inoculate tubers.

The first technique used *Erwinia* grown *in-vitro* on NA. A mixture of four 24 hour old Ecc isolates, recovered from tubers, were suspended in sterile distilled water. The bacterial suspension was diluted and adjusted to the desired level using a Jenway 6100 Spectrophotometre which at an absorbance of 1.0 at 590nm was equivalent to approximately 10^8 cfu/ml of *Erwinia*. Concentrations ranging from 10^4 to 10^6 cfu/ml were used to inoculate batches of 20 tubers by immersing them in 20L of the bacterial suspension for 15 minutes.

In the second technique the inoculum was prepared from rotted tuber flesh. Tubers were washed free of soil, surface sterilised by immersion in 10% solution of sodium hypochlorite for 10 minutes, rinsed twice in demineralised water and then towel dried. Tubers were then sprayed with alcohol and flamed before they were cut into 10mm slices with a sterile knife. The slices were placed into humid trays with moistened wipes covered with a paper towel and inoculated by streaking a 1*ul* loopful of 24 hour old culture of Ecc over the cut surface. After incubation for 48 hours in the tray enclosed in a plastic bag, the rotted tissue was mixed at the rate of 1gm of rotted flesh to 10L of demineralised water with a hand held blender. After 30secs of mixing the suspension was left for 10 minutes to allow the bacteria to disperse into the water, and the suspension diluted to the required concentration as previously described.

Batches of freshly harvested tubers were inoculated by dipping for 15 minutes in 20L of suspension containing 10^4 *Erwinia* cfu/ml.

The third most reliable technique for inoculating tubers was the use of natural inoculum collected from commercial washing plants. 60 - 80L of wash water was collected from the initial wash area of a washing plant 24 hours prior to inoculation. The wash water was usually at 10^4 cfu/ml and if lower levels of inoculum were required in experiments the wash water was diluted with tap water. Initially freshly harvested tubers were inoculated under vacuum, adapted from the method described by Bain and Perombelon (1988). Batches of 20 to 30 tubers were placed inside a 44 gallon drum filled with wash water which was sealed and placed under a vacuum pressure of 10 - 20 kpa for 15 minutes. However it was found that an equivalent inoculation efficiency could be achieved without vacuum, so freshly harvested tubers were inoculated by placing them in 150L of the wash water for a period of 15 minutes. The level of *Erwinia* in the wash water was determined by plating out onto CVP, as previously described.

Inducing tuber soft rot

Two techniques were evaluated as methods of inducing rot in tubers.

The first method used tubers individually wrapped with pre moistened sterile filter paper and then wrapped with parafilm. Batches of 6 tubers were placed into a plastic trays enclosed within a plastic bag and Nitrogen gas was added into the plastic bag to provide anaerobic conditions. The tubers were held at room temperature in the dark for 96 hours and then visually assessed for tuber soft rot lesions. High levels of rot were achieved with this technique but the method was very time consuming.

The second and most successful and efficient method used to induce tubers to rot involved placing batches of 20 to 30 tubers within a nylon onion bag and incubating them for 72 hours in a dark humid tent at approximately 25°C and 100% relative humidity (Lund and Kelman, 1977). A black plastic tent was built in the greenhouse, containing two Defensor atomiser humidifiers with

a humidification capacity of up to 3 l/hr. The inside temperature was generally 25°C but varied between 18°C in winter and 28°C in summer. The use of black plastic ensured dark conditions.

After incubation tubers were removed from the tent and placed on a bench in the glasshouse to air dry. Severity was assessed a 0 to 5 scale where 0 = no obvious infection, 1 = lenticel infection only, 2 = < 25% tuber rotted, 3 = 26% to 50% of tuber rotted, 4 = 51% to 75% of tuber rotted and 5 = > 75% of tuber rotted.

Description of Washing Plants

Commercial

Washing plants in South Australia are based on having separate areas of operation: a dump area where the tubers are received, a washing area with initial wash and a tumbler washer unit, a rinsing / drying area, and a sorting and packing area. The variation in the size of these areas and the machinery involved in each area vary between each washing plant, but are all based on similar systems.

In South Australia tubers are usually harvested into 1 or 4 tonne wooden or heavy duty plastic bins and transported to the washing plant into the receival area. Within 24 hours of harvest the tubers are gradually added to the washing line. To assist in soil removal from the tubers some sheds hose down the field bin tubers with recycled, treated recycled or bore water before they are added to the washing line.

Dirt, rocks and plant pieces adhering to the tubers surface are removed in an initial wash by either dipping tubers into a large tank filled with recycled water or sprayed with overhead spray bars with recycled or bore water as they are moved along a conveyor belt.

A conveyor belt moves tubers into a tumbler consisting of a perforated stainless steel mesh barrel 5-10 m long by 100cm diameter enclosed within a tank half filled with recycled or bore water. The barrel rotates on its axis causing the tubers to gently rub against one another to remove any remaining soil. The degree of abrasion is adjusted by lowering or raising the water level and in some cases adjusting the angle of the perforated barrel. For example if the tubers need to be more aggressively washed the incline of the barrel is increased and the water level lowered.

The tubers are then lifted onto a conveyor belt which passes under final rinse sprayers, which uses either fresh bore, mains water or a combination of fresh water and water treated with a sanitising agent. Tubers are moved over sponge rollers or brushes to reduce surface moisture before being graded and bagged. One washing plant incorporated a fan just after the final rinsing sprayers to assist with air drying the tubers before packaging.

The time taken for tubers to travel through each section of the washing plant was measured for three commercial washing plants. Tubers travel through the initial washing section of the plant ranged between 1 to 58 minutes, 1 to 10 minutes in the tumbler, 2 seconds to 1 minute underneath final rinsing sprayers and between 1 to 2 minutes from the final rinse bars to point of packaging. Actual times in each section are shown in Appendix 2, Table 2.

Experimental

Because of the difficulty in conducting experiments in commercial situations a "mini" washing plant was constructed to incorporate the main washing processes involved in commercial plants (Figure 1 and 2). The mini washing plant consisted of a dumping area, a tumbler, rinsing sprayers (3 bars with 4 hydraulic fan spraying nozzles on each bar 5cm apart, situated approximately 35cm above the rollers), a drying area and a collection area. The rotation speed of the tumbler was adjusted to 8 revolutions / minute, which was similar to the speed used in commercial plants.

Water levels in the tumbler area of the mini washer were adjusted so that tubers were partially covered with water. The water depth in the mini washer was 5cm above the base of the tumbler bars. The mini washer was used to apply different chemical and physical treatments to soft rot infected tubers to investigate the best way to manage bacterial breakdown in washed tubers.

The time tubers spent in the tumbler and the rotation speed were adjusted to be as close as possible to the commercial situation.

Figure 1 Mini Potato Washing Plant - Plant Research Centre

Figure 2 Mini Potato Washing Plant – control panel.

Levels of Erwinia and soft rot in South Australian Potato Washing Plants

A survey of four commercial washing plants was undertaken to determine

- The level of *Erwinia* in wash water from the different areas of the washing process and
- Where the tubers became infected along the production line

Erwinia **levels in water samples**

Water samples were collected from various points in the washing plant to determine the level of Erwinia contamination of water in the washing plant.

Methods

Water samples were collected from different washing plants from the recycled pond water, initial washing areas, the tumbler and the final rinse. Most of the washing plants re-used wash water which is collected from the washing plant and transferred to a series of settling out ponds referred to as recycled pond water. Bore water used in the washing line at some plants was also collected and tested for *Erwinia* levels.

Four different washing plants were sampled at various times corresponding to summer, autumn, winter and spring periods. At each sampling site at least 500ml was collected and assessed for levels of *Erwinia* as previously described.

Results and Discussion

Bore water was sampled on a number of occasions and no *Erwinia* was detected. In general 57% of all water samples had levels within the range of 10^3 to 10^4 *Erwinia* cfu/ml. On 15% of the sampling occasions *Erwinia* was not detected and in 7% of sampling occasions levels of 10^5 cfu/ml were found (Appendix 2, Table 3).

Most of the washing plants re-used wash water and *Erwinia* was detected in all untreated recycled pond water. In general levels of greater than 10^3 cfu/ml was found in 64.7% of the water samples (Figure 3). The levels in the recycled pond water ranged from 10 to 10^5 cfu/ml and on 86% of sampling occasions levels were at 10^3 and 10^4 cfu/ml (Figure 4). On 87.5% of the sampled occasions it was shown that untreated pond water exited the pond area and re-entered the washing plant still contaminated with 10^3 to 10^4 *Erwinia* cfu/ml, highlighting the fact that cycling water through settling ponds without treatment did not reduce the *Erwinia* population. Water from the recycle ponds are used in the initial wash and tumbler regions of the washing plant.

In the washing plants that did not treat their wash water, *Erwinia* levels were at 10^4 cfu/ml in the initial wash areas on 68% of sampling occasions (Figure 5). *Erwinia* levels in the tumbler region were 10^4 cfu/ml on 55% of sampling occasions (Figure 5).

In the washing plant that treated/sanitised the recycled pond water and then partially renewed water within the initial wash and tumbler regions on a regular basis, *Erwinia* levels were considerably lower. For example levels in the initial wash water ranged from 10 to 10^2 cfu/ml in 71.3% of the samples (Figure 6) and 71.3% of sampling times *Erwinia* numbers in the tumbler water ranged from 0 to 10 cfu/ml. This concluded that treating water before use and regular renewal with clean water maintained low levels of *Erwinia* in wash water.

Only one washing plant chemically treated water used in the initial wash and tumbler area.
Full details of *Erwinia* levels in wash water for four South Australian washing plants are shown in Appendix 2, Table 3.

Figure 3 Levels of *Erwinia* (0 to 10^6 cfu/ml) in water samples collected from all areas of the four washing plants, 1999 – 2002.

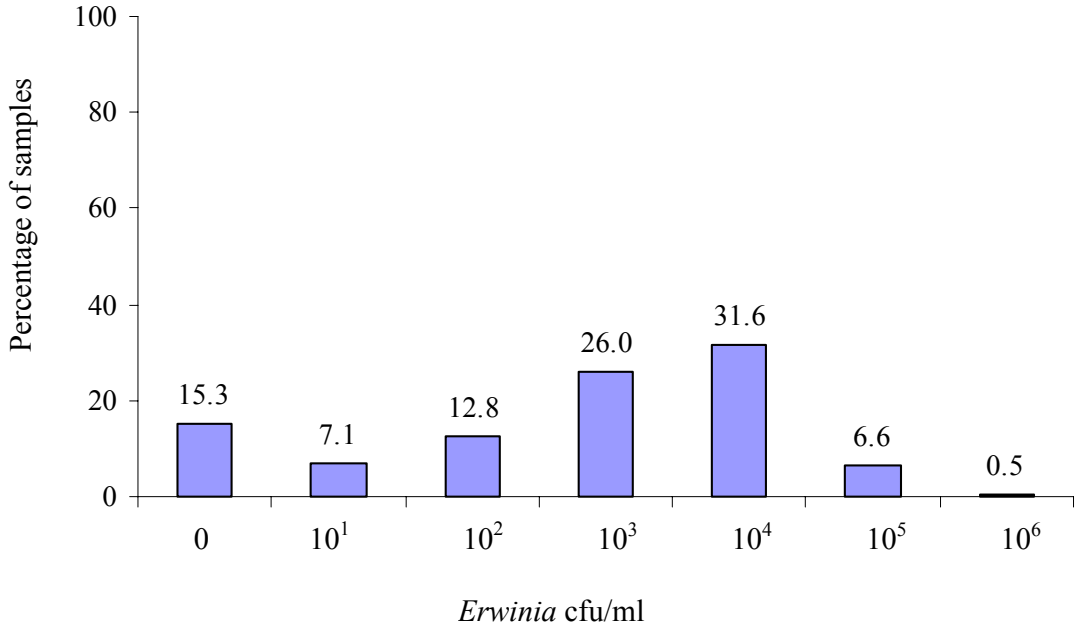


Figure 4 Levels of *Erwinia* (10^1 to 10^5 cfu/ml) in recycled pond water collected upon entry and exit from the ponds, 1999 - 2002

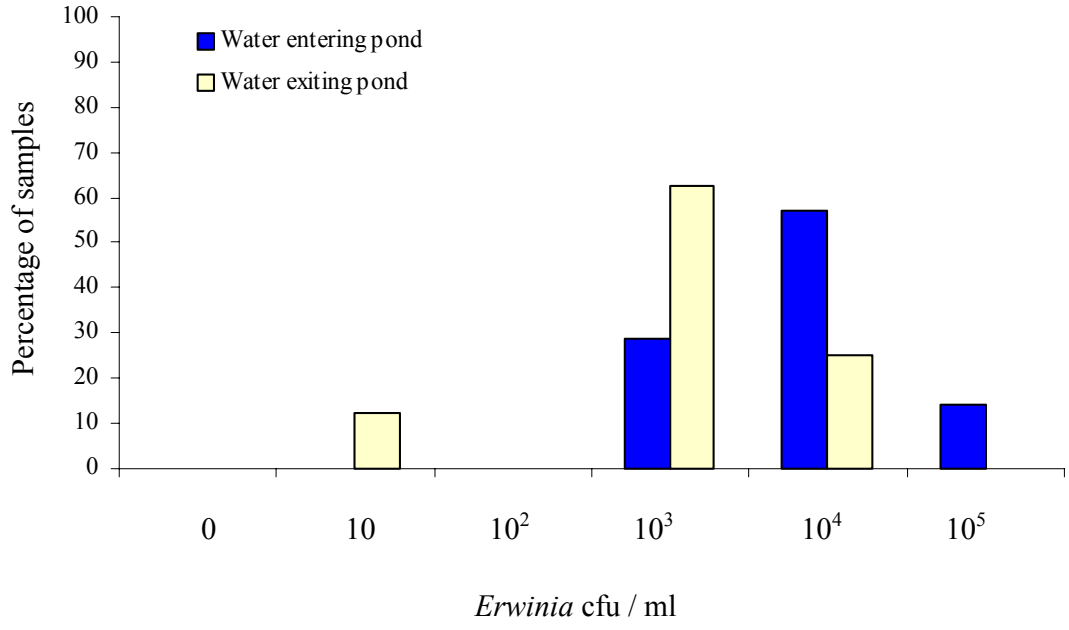


Figure 5 Levels of *Erwinia* (0 to 10^6 cfu/ml), in untreated water samples collected from initial wash and tumbler, 1999 – 2002.

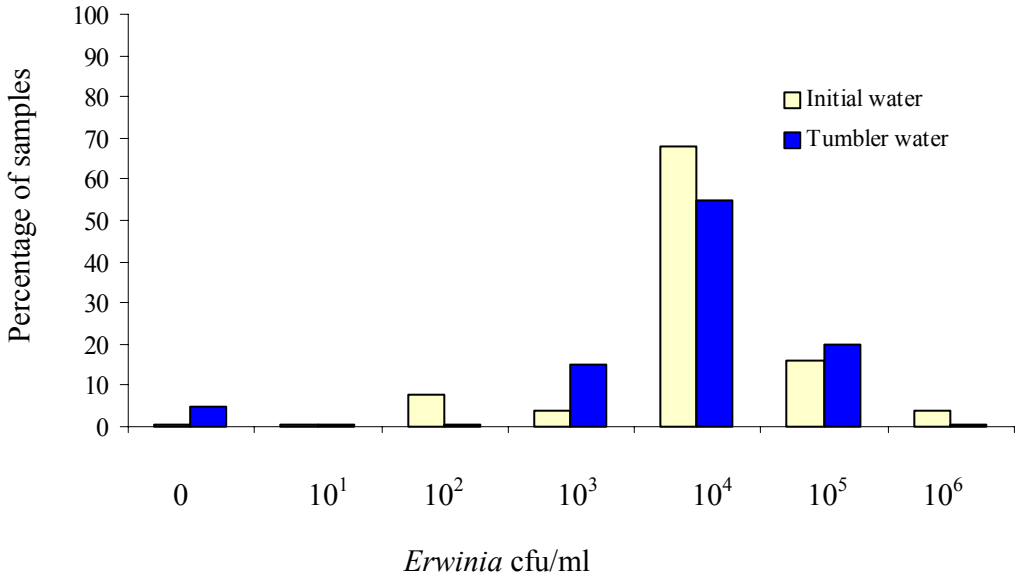
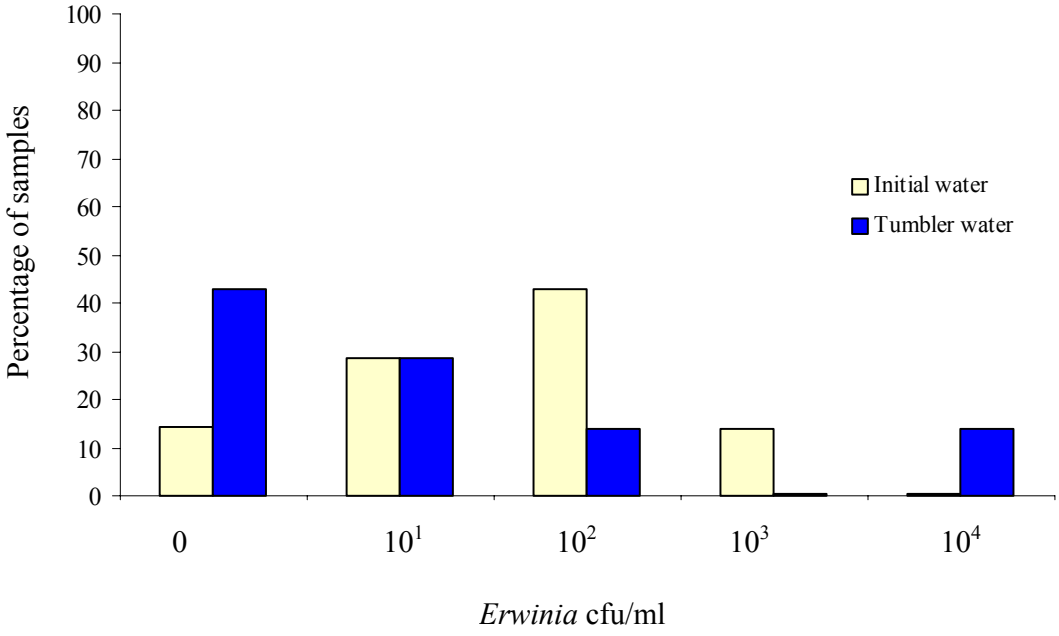


Figure 6 Levels of *Erwinia* (0 to 10^4 cfu/ml) in treated water samples collected from initial wash and tumbler areas, Washing Plant 2, 1999 – 2002.



Erwinia levels within tumbler wash water

To determine the changes in *Erwinia* levels during washing, water samples were collected from the tumbler area of two commercial washing plants. In the second washing plant wash water was sampled before and after the tumbler was treated with Sporekill (Didecyl dimethyl ammonium chloride) and again when the tumbler wash water was renewed with fresh bore water and Sporekill added at the beginning of the wash period and 30 minutes later.

Methods

Water samples were collected beneath the tumbler area every 10 to 15 minutes and the levels of *Erwinia* determined as previously described. In the first washing plant water samples were collected over 90 minutes, whereas in the second washing plant samples were collected at various times on three separate occasions.

In the second washing plant the water was renewed with fresh bore water after 90 minutes, and tumbler water sampled on a further 3 occasions. Another sample period commenced in this washing plant after Sporekill at 500 – 900ppm was added to the tumbler immediately prior to sampling and again 30 minutes later. A further sampling period was undertaken following the addition of Sporekill after the tumbler was replaced with fresh bore water and a further addition of Sporekill 30 minutes later.

Results and Discussion

Water used in the tumbler water at the first washing plant was sourced from untreated recycled pond water. The first sampling took placing before washing had commenced and the *Erwinia* level was at 5.0×10^3 cfu/ml (Figure 7a). Within 30 minutes of washing the *Erwinia* level increased to 2.6×10^4 cfu/ml and remained around this level for the remainder of the sampling period (Figure 7a).

Erwinia levels in the tumbler of the second washing plant where bore water was used was 360 cfu/ml at the start of washing and within 15 minutes the level increased to 2.3 x 10^3 cfu/ml (Figure 7b). The levels of *Erwinia* gradually declined over the 2 hour period. This was unexpected, as previous measurements showed that levels remain high. The drop may have been as a result of overflow of chlorinated water from the final rinse sprayers, located just after the tumbler. When the tumbler water was renewed with bore water, the levels of *Erwinia* were reduced to near zero (Figure 7b).

Further sampling from the same washing plant where Sporekill was added to the tumbler showed that on the two occasions where Sporekill was added *Erwinia* levels increased from 160 to 2.8 x 10^3 cfu/ml and from 0 to 3.5 x 10^3 cfu/ml within 10 – 15 minutes afterwards (Figure 8).

In the samples where the tumbler water was completely renewed with fresh bore water and Sporekill was added low levels of *Erwinia* were recorded for the first 30 minutes of washing. However within 15 minutes of the second addition of Sporekill, the level of *Erwinia* increased to 3.3 x 10^2 cfu/ml (Figure 9).

In summary, in untreated wash water in the tumbler *Erwinia* levels remained high at around 10^3 to 10^4 cfu/ml and did not decline over time.

The addition of Sporekill had no significant long term effect on reducing *Erwinia* levels in the tumbler wash water.

Figure 7a *Erwinia* levels in water collected from the tumbler area of a commercial washing plant during washing, washing plant 1

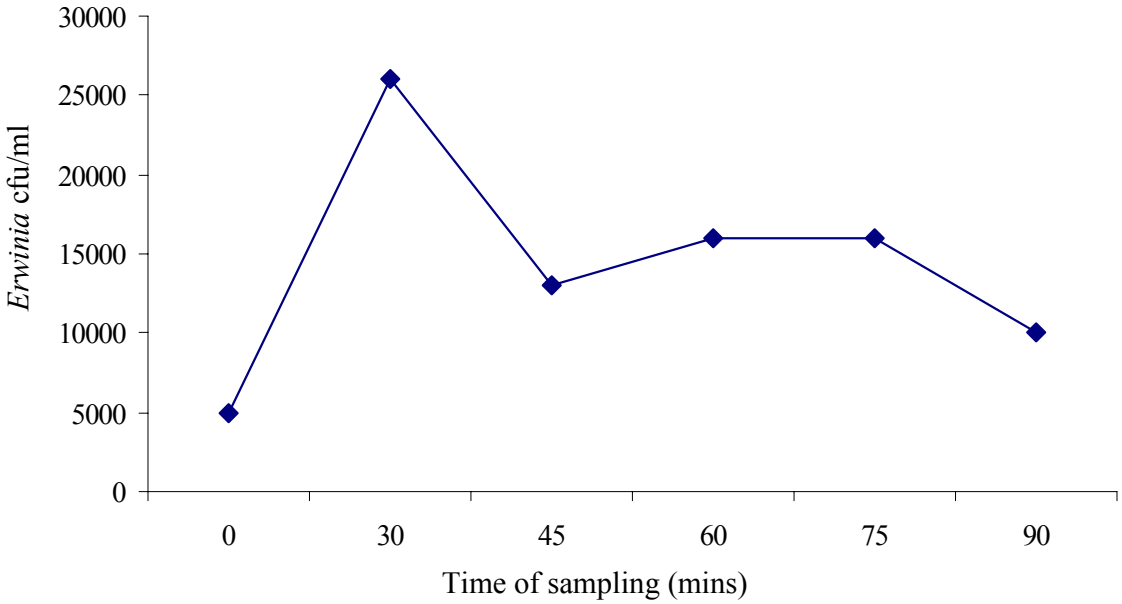


Figure 7b *Erwinia* levels in water collected from the tumbler area of a commercial washing plant during washing, washing plant 2.

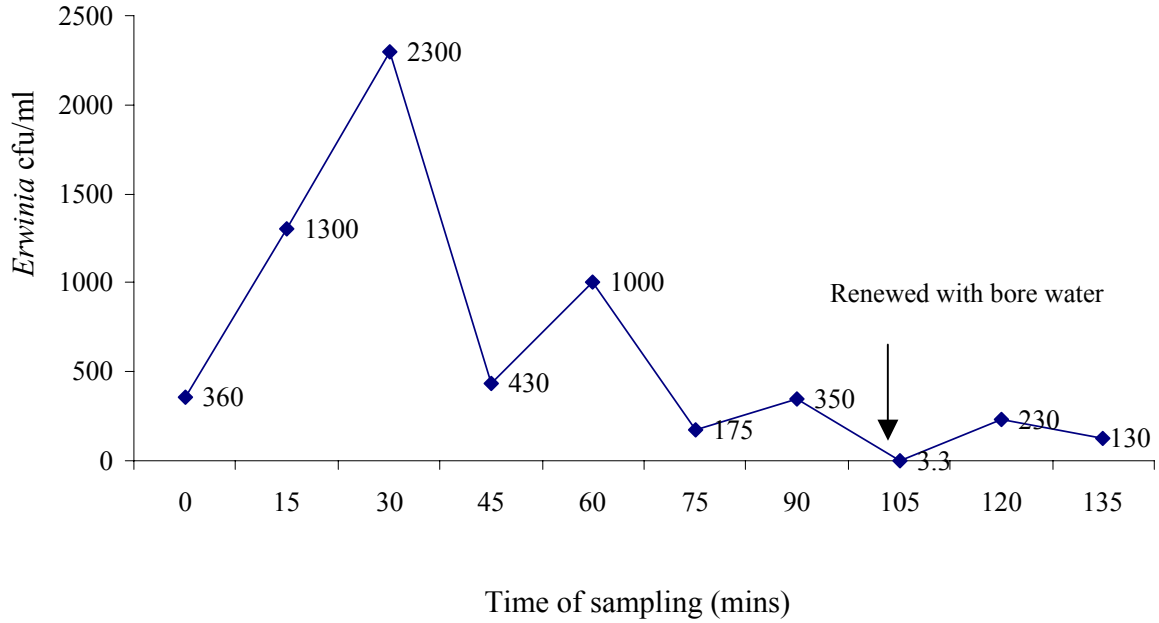


Figure 8 *Erwinia* levels in water collected from the tumbler of a commercial washing plant after the addition of Sporekill (500 – 900ppm), washing plant 2.

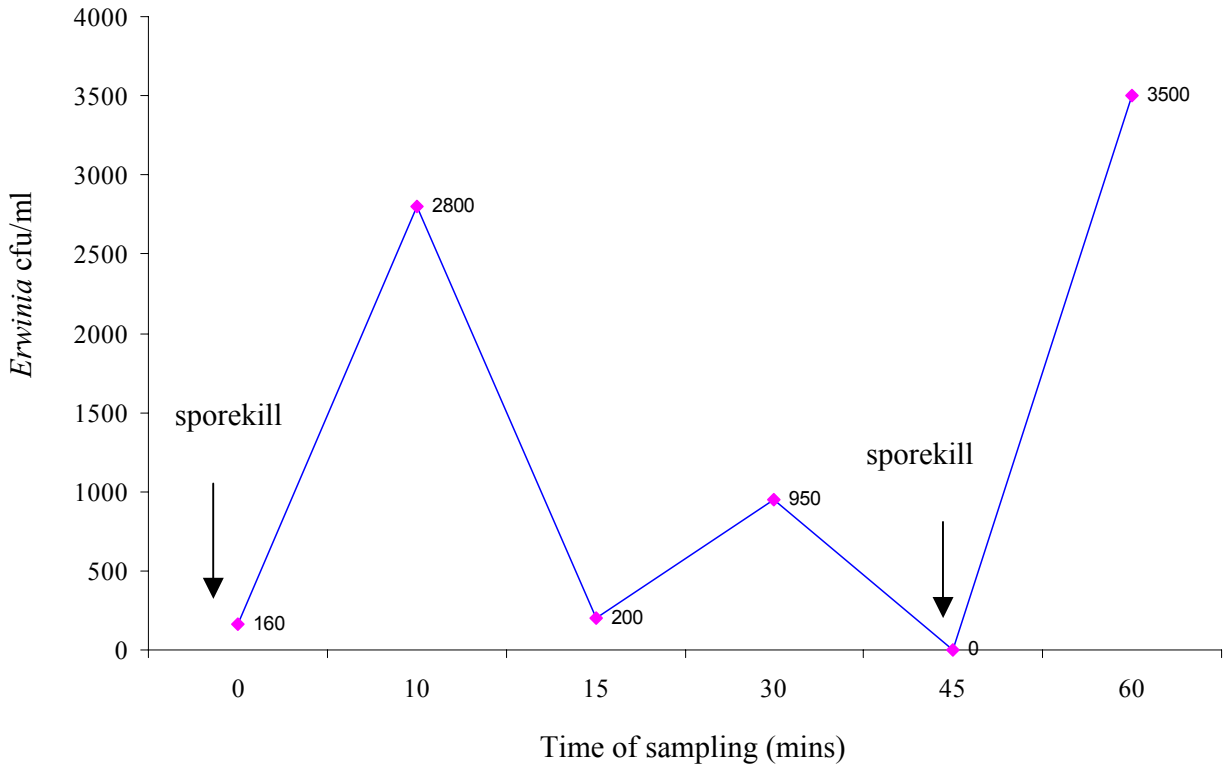
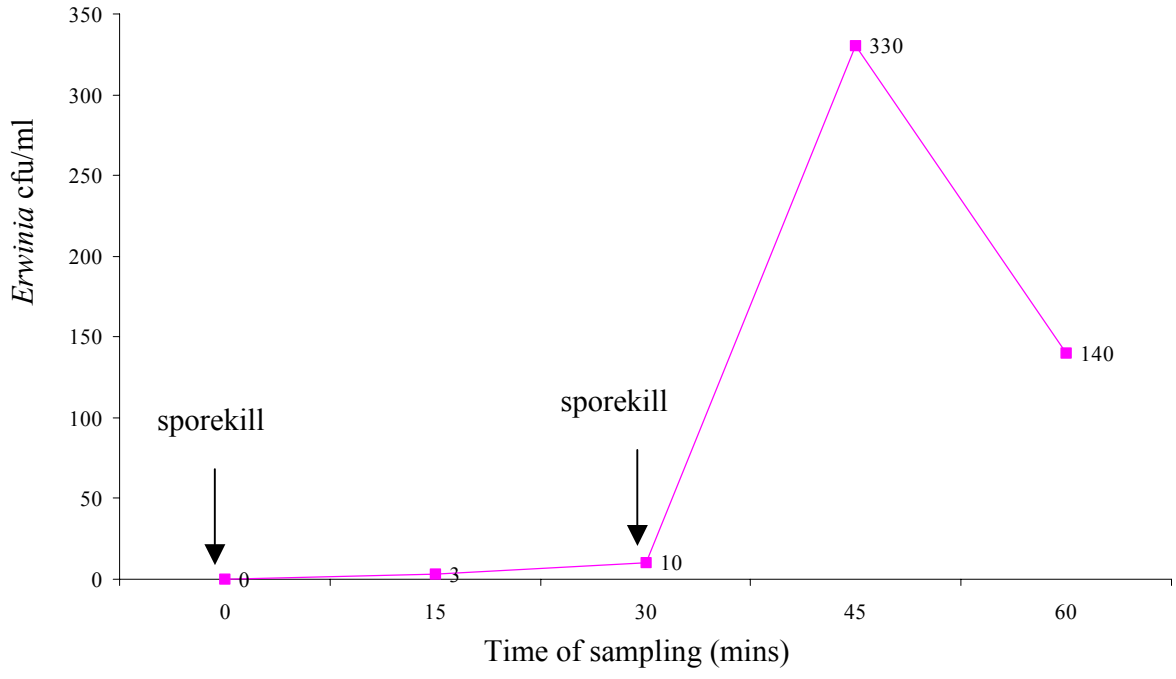


Figure 9 *Erwinia* levels in water collected from the tumbler area of a commercial washing plant and the addition of Sporekill (500-900ppm), washing plant 4.



Incidence and severity of tuber soft rot

Tubers were collected from various points on the washing plants to determine the point on the washing line where tubers became infected with *Erwinia*.

Methods

Tubers were collected from the field bins, field bins after tubers were sprayed with water, the initial wash area, the tumbler, after the final rinse and just before the tubers were packed. At each washing plant and sampling time between 20 - 30 tubers were collected from each site. Batches of tubers were placed in nylon onion bags and transferred to the laboratory within 1 hour of collection, where they were induced to rot and incidence and severity of rotting assessed using the methods previously described.

Results and Discussion

Many tubers were contaminated with *Erwinia* in the field, as on 73% of the sampling occasions field bin tubers developed soft rot when placed in conditions conducive to disease development (Figure 10). However in these samples the severity was low and most samples had severity less than 1. When the field bin data was categorised into different seasons the results over all the districts and varieties showed that incidence and severity of soft rot was highest in autumn and winter and lowest in spring (Figure 11).

Most tubers became infected with soft rot once they were immersed in water. The mean incidence and severity of field bin tubers was 26% and 0.7 respectively, however once the tubers were immersed into wash water in the initial washing area, average incidence and severity increased to 72% and 2.3 respectively. The average soft rot increased further when the tubers left the tumbler region, with incidence and severity of 87% and 2.8 respectively. By the end line the tubers had an average incidence and severity of 74% and 2.0 respectively (Figure 12).

Tubers that did not develop rot when removed from the field bins developed rot after commencing the washing process. For example in 35% and 50% of Coliban and Red La Soda tubers respectively, rot developed when they were sampled from the initial wash areas, whereas no rot developed in the same batch of tubers sampled from the field bins.

The incidence and severity of tubers collected from the end line were categorised into different seasons and showed that a slightly higher average incidence level was observed in summer however the severity level was higher in spring (11&12).

In general these results showed that once tubers were washed the incidence and severity of rotting increased.

Figure 10 Percentage of field bin samples in each category of tuber rot after incubation, 1999 – 2002.

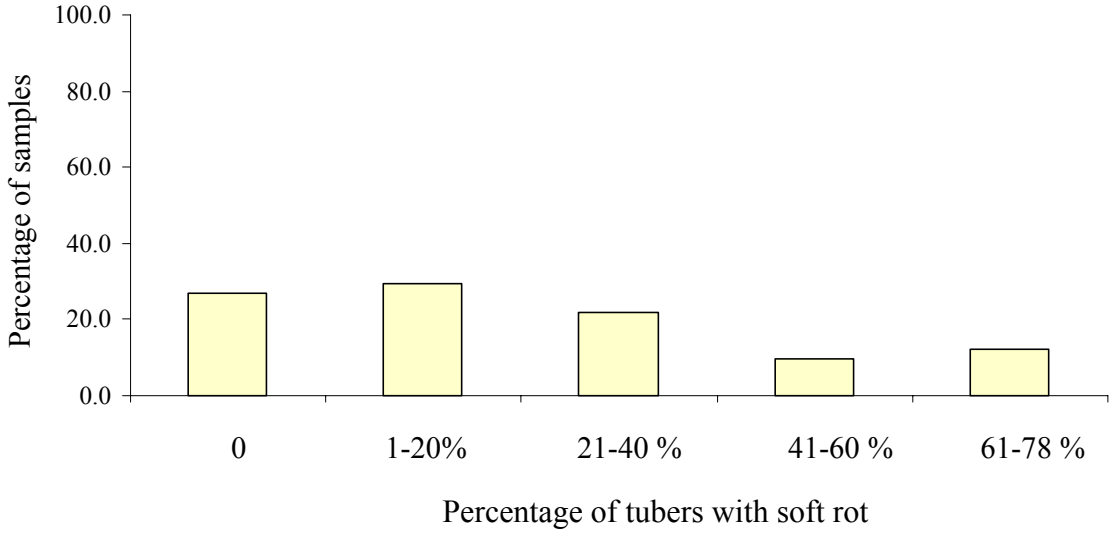


Figure 11 Incidence and severity of soft rot in tubers collected from the field bins from four washing plants during various seasons, 1999 - 2002

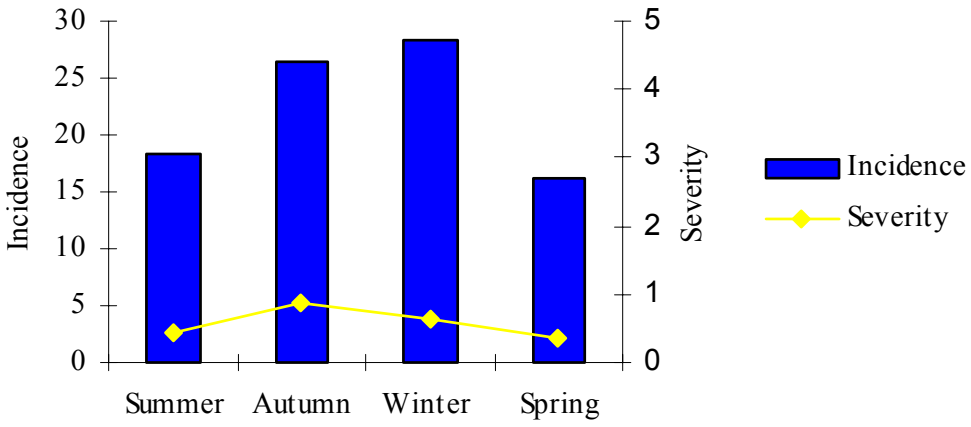


Figure 12 Incidence and severity of soft rot in tubers collected from the different areas on the washing line from 4 washing plants, 1999 – 2002.

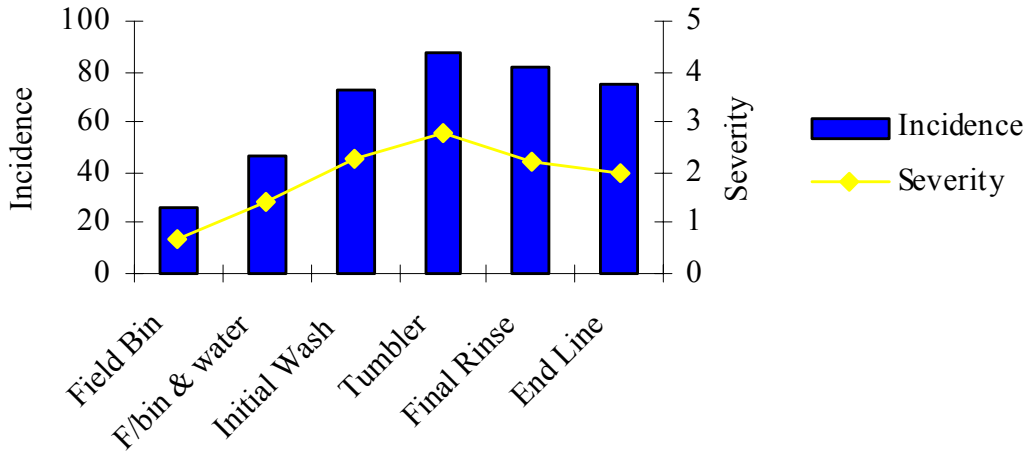
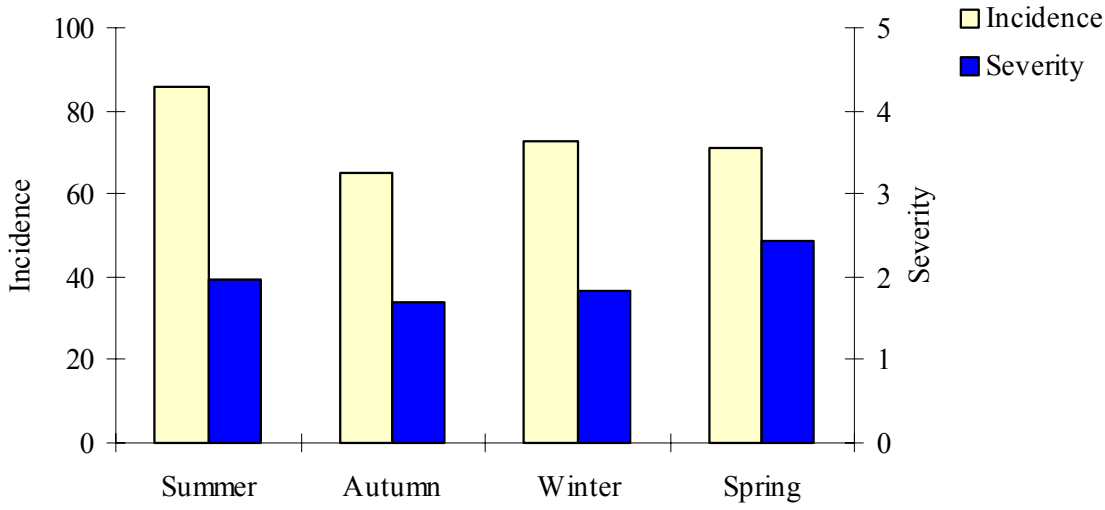


Figure 13 Incidence and severity of soft rot in tubers collected from the end of the washing line during various seasons 1999 –2002.



Efficacy of sanitisers and other treatments on Erwinia

A number of sanitisers and other chemicals shown in Table 1 were evaluated against *Erwinia* in two different situations.

The first series of experiments investigated the effect of the sanitisers on the viability of *Erwinia* suspended in demineralised water. Another series of experiments evaluated the sanitisers applied to wash water containing *Erwinia* and different levels of suspended soil that had been washed from tubers.

In the section titled "Control of soft rot in the mini tuber washing plant" a number of sanitisers were applied as sprays in the tumbler area and as final rinses on artificially inoculated tubers.

Ultra violet (UV) irradiation was investigated on recycled potato wash water collected directly from pond water from a commercial washing plant.

Finally a number of substances were investigated to determine the viability of *Erwinia* suspended in demineralised water. These included, brine solution, fruit cordials, collodial silver and other materials (Table 4).

Efficacy of sanitisers in water

Methods

Demineralised water

In these experiments a mixture of 4 Ecc isolates were used. There were two techniques used to prepare inoculum in demineralised water. The first technique used a bacterial suspension, the second used rotted tuber tissue as inoculum, both techniques were described in the inoculation section. Inoculum levels in these experiments ranged from 10^2 - 10^6 *Erwinia* cfu/ml.

The sanitisers and chemicals investigated are listed in Table 1. Each chemical was prepared as a stock solution of 1000ppm active ingredient (ai) for Nylate, Klorman, Liquid Pool Chlorine, Proxitane and Sporekill. A stock solution of 4000ppm ai was used for the Oxine/ChlorDox products.

Different quantities of stock solution were added to the bacterial suspension to give the desired chemical concentration. After a contact time of 5 minutes a aliquot of the chemical/bacterial suspensions was plated onto CVP, air dried in the laminar flow and incubated at 27°C for 48 hours before the levels of *Erwinia* were determined. Control plates of inoculated demineralised water only were also plated out to check the level of *Erwinia* in the water. Inoculum levels ranged from 10^2 - 10^6 *Erwinia* cfu/ml.

Table 1 Sanitisers and chemicals evaluated against *Erwinia.*

Trade Name	**Active Ingredient**
Oxine/ ChlorDox	Chlorine dioxide
Nylate	Bromo chloro dimethyl hydantion
Klorman	Calcium hypochlorite
Vitasan 4	Benzalkonium chloride
Proxitane	Peroxyacetic acid, Hydrogen peroxide and Acetic acid
Liquid Pool Chlorine	Sodium Hypochlorite
Sporekill	Didecyl dimethyl ammonium chloride
Sodium Metabisulphite	Sodium metabisulphite
Citric acid	2 hydroxy-1,2,3- Propanetricarboxylic acid

Potato wash water

Sanitisers were also evaluated in wash water containing a natural source of inoculum and suspended soil. Wash water was collected on the day of each experiment. Different quantities of stock solution of sanitisers were added to the wash water to give a range of concentrations. After a contact time of 5 minutes plated out onto CVP as previously described, air dried in the laminar flow and incubated for 48 hours. Control plates of wash water only were also plated out to determine the initial level of *Erwinia* in the wash water. The inoculum levels in the potato wash water ranged from 10^2 to 10^5 cfu/ml but were generally at 10^4 cfu/ml.

Results and Discussion

All sanitisers killed *Erwinia* when added to inoculated demineralised water (Table 2). Oxine / ChlorDox and Klorman at 0.1ppm, Nylate and Liquid Pool Chlorine at 1.0 ppm, Proxitane and Sporekill at 10 and 100ppm respectively and Citric acid and Metabisulphite at 100 000ppm controlled *Erwinia* in demineralised water. However when the sanitisers were added to potato wash water with varying levels of soil and organic matter in the water concentrations of the chemicals needed to be increased from 10 to 250 ppm greater to achieve the same kill (Table 2).

Table 2 Efficacy of sanitisers against *Erwinia* in demineralised and tuber wash water.

Sanitisers	**Demineralised water**	**Potato Wash Water**
	Lowest concentration (ppm) required to kill *Erwinia* in water	
Oxine / ChlorDox	0.1 / 0.1	25
Nylate	1.0	15 – 30
Klorman	0.1	50
Proxitane	10	50
Liquid Pool Chlorine	1.0	50 – 100
Sporekill	100	1000
Citric Acid	100 000	Not tested
Metabisulphite	100 000	Not tested

Efficacy of ultraviolet irradiation (UV) in potato wash water

UV was investigated as a means of disinfecting recycled pond water. UV irradiation is a powerful germicide, killing bacteria, viruses, algae and other micro-organisms. These experiments were carried out at two commercial washing plants where recycled water was treated with a ultra violet unit called UVTA LC 50-EB (Ultraviolet Technology of Australasia).

Methods

Recycled pond water was pumped directly into the unit through the inside of parallel lengths of advanced fluropolymer tubing (AFP), located on the outside of the AFP tubing. The UV lamps applied a concentrated dose of UV irradiation at 254 nanometres (nM).

Five flow rates were evaluated 15 l/min, 20 l/min, 25 l/min,30 l/min and 35 l/min. Similar flow rates were trialed in the second experiment, but using 10 l/min and not 35 l/min. Samples of water were collected pre and post treatment and plated out onto CVP to detect *Erwinia* as previously described. During the sampling period the quality of water was also determined by measuring the transmission using UVTA Spectrometer at 254 nanometres and level of total dissolved solids (TDS) using a Myron TDS meter. The transmission of reverse osmosis water is 100%, so the higher the transmission value, the cleaner the water. However TDS is lower with clean water, for example Adelaide mains water has a TDS of 550.

Results and Discussion

For experiment 1 transmission recordings of the recycled pond water were 52%, 44% and 36%, and the recordings for the TDS were 1795 and 1747.

In the second experiment transmission levels were between 2 – 6% and TDS at 1920, indicating the water was much more turbid than in Experiment 1.

The UV treatments for Experiment 1 significantly reduced *Erwinia* levels at all flow rates. The initial *Erwinia* levels in the recycled pond water for experiment 1 was 2000 cfu/ml. The higher flow rates achieved similar kill rates reducing the *Erwinia* populations to 30 cfu/ml and the lowest flow rate of 15 l/min reduced bacteria to below detectable levels (Table 3).

In Experiment 2 the UV treatment failed to reduce the level of *Erwinia* for all the flow rates tested (Table 3).

While UV was extremely efficient in reducing *Erwinia* levels these results highlight the need for the wash water to be of a better quality before the application of UV light. In experiment 1 the transmission of the water ranged from 36 to 52% and was successful at reducing *Erwinia* in the water. However when a similar water source with a transmission level of 2% was trialed, UV sanitation failed to work. Improving the quality of water is achievable for the washing plant situations. Separation of potato plant debris and larger soil particles from the water would be required, followed by a flocculation/filtration treatment to improve turbidity and therefore transmission.

Table 3 Viability of *Erwinia* in recycled pond water after exposure to UV at various flow rates

	***Erwinia* colony forming units/ml**	**Turbidity and Total dissolved solids**
Experiment 1		
Pre UV Treatment	2.0×10^3	Transmission 36 – 52%
Post UV Treatment		TDS 1795 and 1747
15 l/min	0	
20 l/min	7.6	
25 l/min	33	
30 l/min	32	
Experiment 2		
Pre UV Treatment	6.0×10^3	Transmission 2 – 6%
Post UV Treatment		TDS 1920
10 l/min	2.0×10^3	
15 l/min	1.0×10^3	
20 l/min	3.0×10^3	
25 l/min	4.0×10^3	

Efficacy of brine solutions, fruit cordials, colloidal silver and other materials

Several novel treatments were investigated as a means of reducing *Erwinia* populations suspended in water.

As raspberry cordial has been used to purify drinking water, several cordials were tested for efficacy against *Erwinia*. Several preservatives, some known to be ingredients of these cordials, were evaluated, along with salt, colloidal silver solution and the biological agents "Bokashi", "Kasugamycin" and "Effective Micro-organisms." As *Erwinia* is an anaerobic bacterium, compressed air was passed through the suspension to determine if increased oxygen would reduce *Erwinia* levels.

Methods

A suspension of *Erwinia* in demineralised water was prepared either from Ecc cultures or rotten tuber tissue as previously described. Treatments and concentrations used are outlined in Table 4. Treatments were adjusted to the various concentrations and added to the bacterial suspension after 5 minutes contact time the chemical/bacterial suspensions were plated onto CVP, air dried in the laminar flow and incubated at 27°C for 48 hours to determine the levels of *Erwinia.* Control plates of inoculated demineralised water were also plated out to check the initial level of *Erwinia* in the demineralised water. Inoculum levels ranged from 10^3 - 10^5 *Erwinia* cfu/ml.

Results and Discussion

Of the three different cordials tested only the Cottees and Dick Smiths cordials killed *Erwinia* in water when used at 100 000ppm and above (Table 4).

The preservatives Sodium metabisulphite, Citric acid and Tartaric acid killed *Erwinia* when used at concentrations from 50 000ppm (Table 4).

None of the biological treatments tested, the salt, colloidal silver or the compressed air reduced the levels of *Erwinia*.

There were a number of substances tested that were successful in killing *Erwinia* however these products required very high concentrations before they were effective, making them uneconomical to use in a commercial situation.

Table 4 Evaluation of substances to control *Erwinia* in clean water

Treatment	**Trialed rates (ppm)**	**+/- *Erwinia* killed**
Cascade Black Currant cordial	100 000, 200 000	No control
Cottees' Raspberry cordial	1000, 10 000, 100 000	Killed at 100 000 ppm
Cottees' Orange cordial	50 000, 100 000, 200 000	Killed at 200 000 ppm
Dick Smiths Raspberry cordial	10 000, 100 000, 200 000	Killed at 200 000 ppm
Citric acid	10 000, 50 000, 100 000, 200 000	Killed from 50 000 ppm
Compressed Air	-	No control
Colloidal Silver	10, 100, 1000, 10 000, 100 000	No control from 10–10000ppm Some reduction at 100 000
Bokashi		No control – population increased after 24 hours contact
Effective micro-organisms (EM)– neat	1 part Em to 20 water	No control
(EM)	1 part Em to 20 water	No control
Kasugamycin	10, 50, 100, 1000	No control
Salt (sodium chloride)	100 000, 150 000, 200 000	No control
Sodium bicarbonate	100 000, 200 000	No control
Sodium metabisulphite	10, 100, 1 000, 2 000, 50 000, 100 000, 200 000	Total kill from 50 000 ppm
Tartaric acid	100 000	Total kill

Control of soft rot in the mini washing plant

In commercial washing plants, sanitisers were usually added in the final rinse spray after the tubers had passed through the tumbler section. Many tubers are already infected by this stage, so the aim of these experiments was to determine which sanitiser/s or treatments could minimise the severity of soft rot on tubers already infected with soft rot. Infected tubers were sanitised at either the tumbler or final rinsing stage of the mini washing plant, or treated with an air drying treatment just prior to packaging.

Evaluation of tumbler sanitation

Sanitisers were applied as a spray within the tumbler to determine if applying a sanitiser in this area of the washing plant could minimise tuber soft rot.

Methods

16 experiments were undertaken to determine the effect of sanitisers applied to inoculated tubers in the tumbler section. Treatments and application rates are outlined in Table 5.

Freshly harvested tubers were collected directly from a field bin from a commercial washing plant and inoculated with wash water collected from the washing plant on the same day. The sanitisers were applied through five flat fan spray jets sited on a bar that was inserted into the tumbler which sprayed the rotating tubers for approximately seven minutes. After being the treated, the tubers passed over nylon brushes to the packing point, where they were bagged and induced to rot.

Raw data was analysed by Biometrics SA using an ordinal scale response (Appendix 3).

Table 5 Treatments, application rates and cultivars tested in evaluation of sanitisers in the tumbler

Sanitisers	**Active ingredient**	**Rates (ppm)**	**No. Experiments**	**Cultivar**
ChlorDox	Chlorine dioxide	50 and 100	5	Coliban, Nadine, Red La Soda
		100 and 200	4	Desiree, Pontiac, Coliban
Proxitane	Peroxyacetic acid hydrogen peroxide acetic acid	50	2	Coliban, Desiree
Citric Acid	2 hydroxy-1,2,3 propanetricarboxylic acid	1000 – 10 000	2	Coliban
Metabisulphite	Sodium metabisulphite	1000	1	Coliban
		10 000	2	Coliban, Coliban
Water	Used in all experiments as a control			

Results and Discussion

After inoculated tubers were treated with water in the tumbler, on average 89% of the tubers developed soft rot with an average severity of 3.1 (range 0.7 to 4.2).

ChlorDox applied at 50 ppm reduced soft rot in 3 out of 5 occasions (Figure 14) however this reduction was not statistically significant (Table 6). ChlorDox applied at 100 ppm reduced tuber soft rot in 8 of the 9 experiments (Figures 14&15). This was shown to be significantly lower than the tumbler rinse of water only (Table 6). In 3 out of 4 experiments ChlorDox at 200 ppm also reduced the level of rot significantly compared to water alone (Figure 15).

The use of 100 ppm Proxitane as a tumbler spray produced variable results, on 2 out of 4 occasions it reduced tuber soft rot, and on the other occasions it either had no effect or increased the level of rot (Figure 16).

Neither Citric acid or Metabisulphite reduced the level of soft rot (Figures 17 and 18). When Metabisulphite was used, a strong sulphur smell occurred, making the use of this chemical unpleasant and possibly of becoming an occupational health and safety issue.

Conclusion

Only the higher rate of ChlorDox at 100 ppm had any significant effect on the level of soft rot when applied as a spray in the tumbler (Table 6). ChlorDox and the other chemicals killed *Erwinia* bacterium suspended in water, however they were less successful treating tubers infected with *Erwinia*.

Table 6 Significant differences between treatments applied in sprays within the tumbler

Treatments	*
Water	B
Citric acid 1000	BC
Citric acid 10 000	BC
Proxitane 50	BC
ChlorDox 50	BC
ChlorDox 100	C

* Treatments with the same letter are not significantly different from one another

Figure 14 Severity of tuber soft rot developing in inoculated tubers sprayed with 50 or 100ppm ChlorDox in the tumbler, 2001 – 2002.

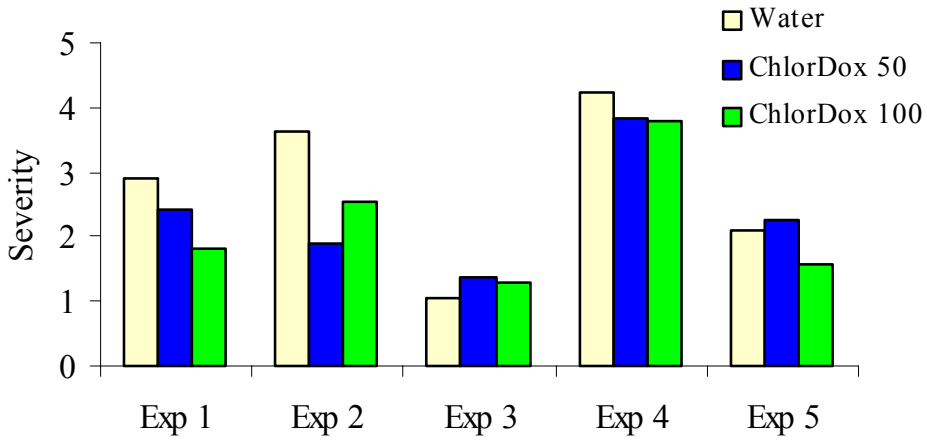


Figure 15 Severity of tuber soft rot developing on inoculated tubers sprayed with 100 or 200 ppm ChlorDox in the tumbler, 2001 – 2002.

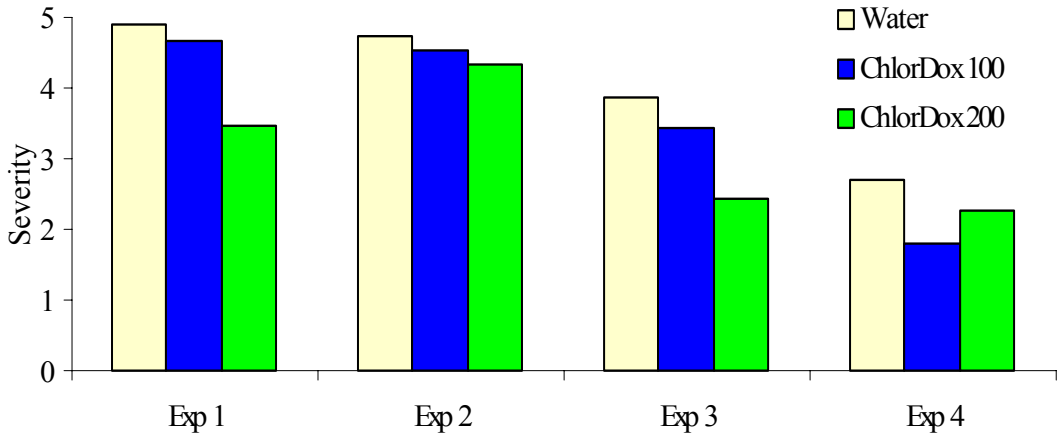


Figure 16 Severity of tuber soft rot developing on inoculated tubers sprayed with 50 or 100ppm Proxitane in the tumbler, 2001 - 2002

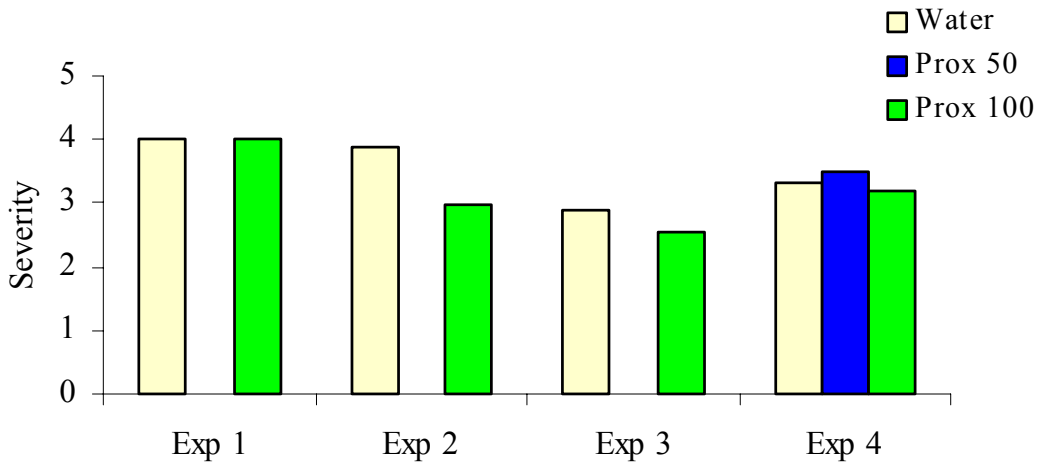


Figure 17 Severity of tuber soft rot on inoculated tubers sprayed with 10^3 or 10^4 ppm Citric Acid (CA) in the tumbler, 2001 – 2002.

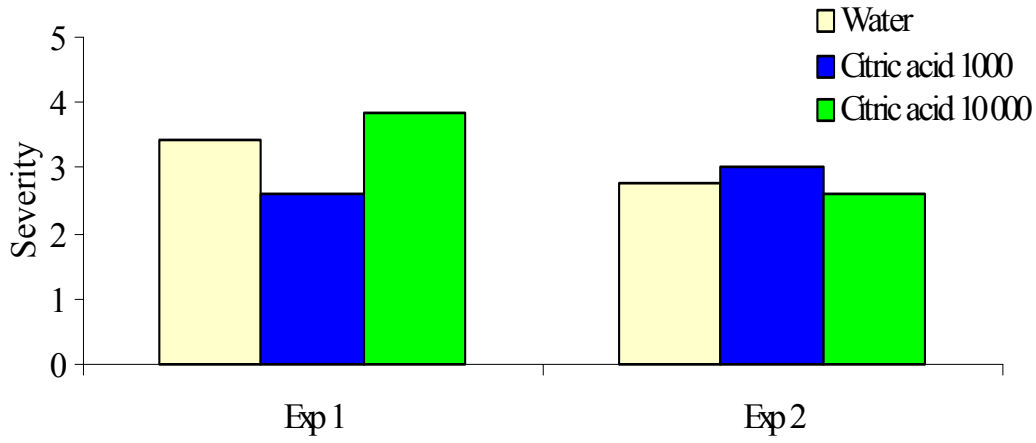
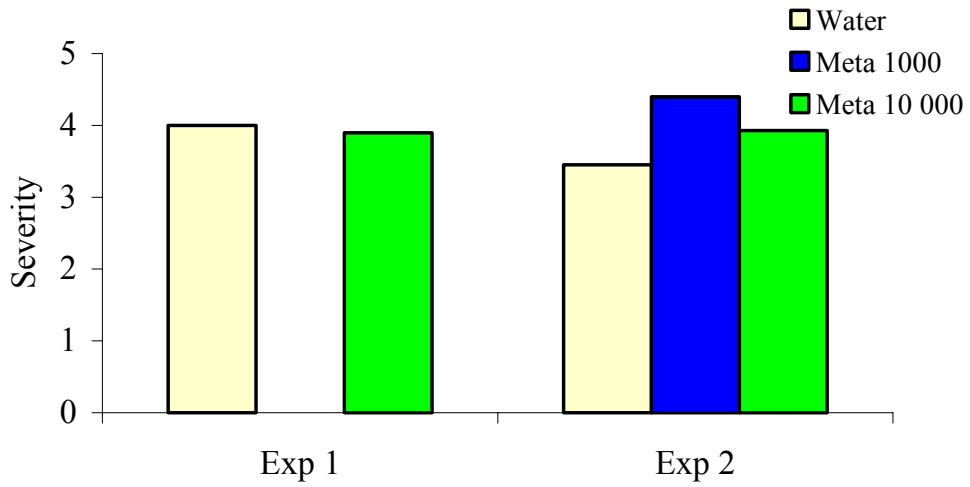


Figure 18 Severity of tuber soft rot on inoculated tubers sprayed with 10^3 or 10^4 ppm Metabisulphite in the tumbler, 2001 – 2002.



Evaluation of final rinse sanitation

Most washing plants in South Australia add a sanitiser as one of the final rinse sprays and this was investigated to determine if adding sanitisers as a final rinse would reduce tuber soft rot.

Methods

Freshly harvested tubers were collected directly from a field bin at a commercial washing plant and inoculated as previously described with wash water collected from the washing plant on the same day. After seven minutes in the tumbler the tubers were sprayed with the sanitisers using four flat fan spray jets at 140kpa/20psi sited on a bar over the conveyor between the tumbler and packing line. The treated tubers were immediately placed into onion bags without rinsing and induced to rot in the misting tent for three days before each tuber was individually assessed for visual signs of rotting, as previously described.

Nine experiments were conducted evaluating various sanitisers, the treatments and rates are outlined in Table 7. Raw data was analysed by Biometrics SA using an ordinal-scaled response (Appendix 3).

Table 7 Treatments, rates and cultivars used in evaluating sanitisers added to the final rinse

Sanitiser	Active ingredient	Rates (ppm)	No. Experiments	Cultivar
ChlorDox	Chlorine Dioxide	50 and 100	5	Nadine, Coliban, Red La Soda
ChlorDox	Chlorine Dioxide	100 and 200	2	Desiree
Proxitane	Peroxyacetic acid hydrogen peroxide acetic acid	100	1	Desiree
Metabisulphite	Sodium metabisulphite	10 000	1	Desiree
Water	Used in all experiments as a control			

Results and Discussion

In all experiments 70 – 100% of the inoculated untreated tubers developed soft rot with an average severity of 2.2 and ranging from 1.5 to 3.5.

ChlorDox applied at 50 ppm resulted in similar or slightly higher soft rot severity compared with those tubers which were treated with a final rinse of water only (Figure 19). The higher rate of ChlorDox 100 ppm in the same experiments reduced soft rot in 2 out of 5 occasions, however the differences were not statistically significant (Table 8). Since the higher rate of ChlorDox had some effect, ChlorDox applied at 100 and 200 ppm was investigated on two more occasions, however for both experiments neither rate of ChlorDox reduced tuber soft rot compared with the final rinse of water only (Figure 20).

Proxitane and Metabisulphite were assessed on one occasion. The Metabisulphite treatments did not control soft rot as the severity of rot was similar to that developing in the control. While Proxitane slightly reduced the level of rotting compared with water alone, with severities of 3.6 and 4.3 respectively, this effect was not considered great enough to continue further testing.

In summary none of the sanitisers reduced tuber soft rot when evaluated as a final rinse.

Table 8 Effect of ChlorDox at 50 and 100ppm applied as a final rinse to infected tubers

Final rinse treatments	*
Control water	A
ChlorDox 50 ppm	A
ChlorDox 100 ppm	A

* Treatments with the same letter are not significantly different from one another

Figure 19 Severity of tuber soft rot after tubers were inoculated, tumbled and treated with a final rinsing spray of either water or ChlorDox at 50 or 100 ppm, 2001 – 2002.

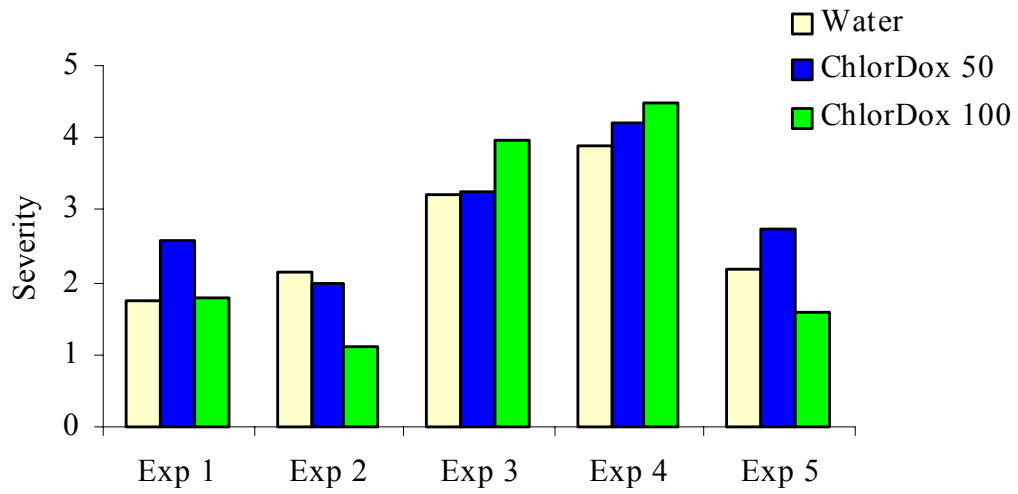
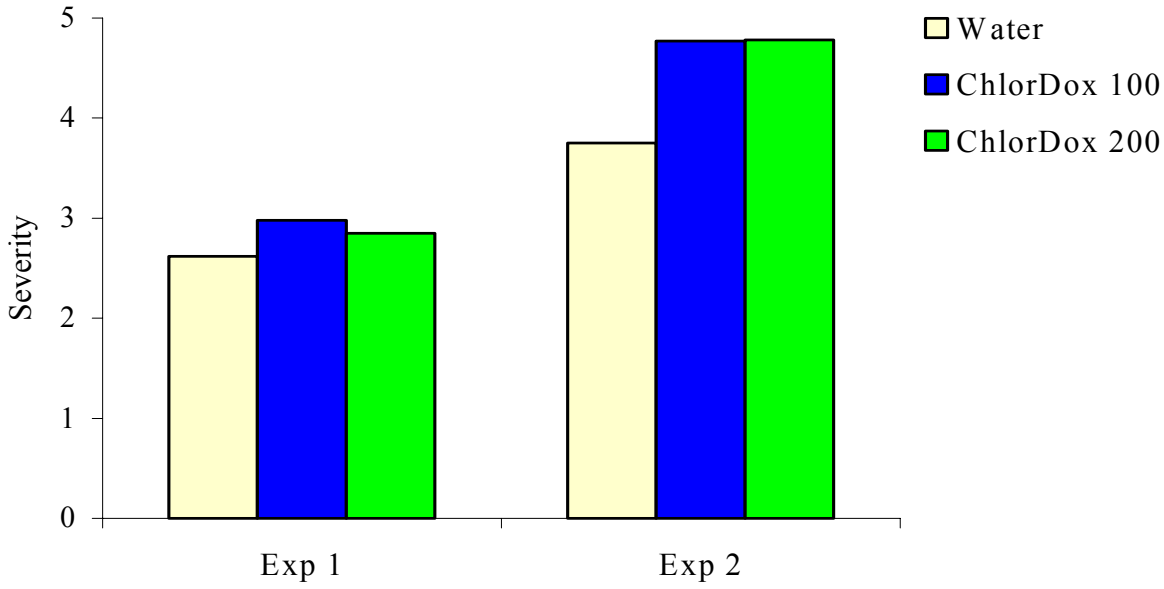


Figure 20 Severity of tuber soft rot after tubers were inoculated, tumbled and treated with either water or higher rates of ChlorDox at 100 or 200ppm applied as a final rinse, 2001 – 2002.



Immersion versus spraying tubers in the tumbler area

In commercial washing plants tubers are usually partially immersed in water within the tumbler. Our work found that once the tubers were immersed in water containing *Erwinia* the potential for post harvest decay was increased. Using the mini washing plant, two methods of washing potatoes were compared. These were immersing the tubers in wash water in the tumbler and washing tubers with overhead water sprays sited within the tumbler.

Methods

Tubers were collected from the field bin 24 hours before treatment and were not inoculated. For the immersion treatment fresh mains water was added to the tumbler until approximately 5 cm appeared above the base of the tumbler bars. For the spray treatment no water was added to the tumbler, water was sprayed onto tubers from 5 fan spray jets sited on a bar inserted into the tumbler, approximately 50cm above the tubers. All tubers were tumbled for a period of 7 minutes before being passed over the nylon brushes. Tubers were induced to rot as previously described and then assessed for incidence and severity of soft rot. These experiments were trialed on 4 occasions using the cultivar Coliban.

Results and Discussion

The results were variable, as in experiments 1 and 2 the severity of soft rot was reduced when tubers were sprayed rather than immersed, whereas in experiments 3 and 4 no differences were observed between the two treatments (Figure 21). A severe disadvantage with the spray washing process was the development of cracking on the tubers surface. For example 10 – 50% of the sprayed tubers cracking compared to 5 – 20% cracked tubers that were washed by immersion. In these experiment the increased damage to the tubers did not result in the development of higher levels or increased severity of soft rot. Spraying in the tumbler is an unlikely choice for the washed potato industry because of increased damage caused to the tubers.

Effect of salted water versus fresh water in the tumbler

Our work has shown that once tubers are immersed in wash water containing *Erwinia* the potential for soft rot increases. We investigated the use of sodium chloride in the wash water as means of slowing down or preventing the uptake of *Erwinia* into tubers that were immersed in water in the tumbler.

Methods

Field bin tubers were collected 24 hours prior to the experiment. Tubers were not inoculated prior to tumbling. Tubers were either added to the tumbler filled with water alone, or filled with volumes of water and salt (sodium chloride) added at 10 g/L. The salt was dissolved in hot water first before being added to the water. Tubers were tumbled for a period of 7 minutes before they were passed over nylon brushes to remove excess water and incubated for 3 days at high humidity before being assessed for soft rot. These experiments were repeated on five occasions.

Results and Discussion

The use of salt water in the tumbler gave mixed results. In two of the five experiments the salted water slightly reduced soft rot (Figure 22), however on the other 3 occasions salt water slightly increased the severity of soft rot. These results suggest that salt water did not prevent infiltration of the bacteria. They also confirm our previous studies that showed that sodium chloride solutions did not effect *Erwinia*.

Figure 21 Effect of immersing or spraying tubers in the tumbler on the severity of tuber soft rot.

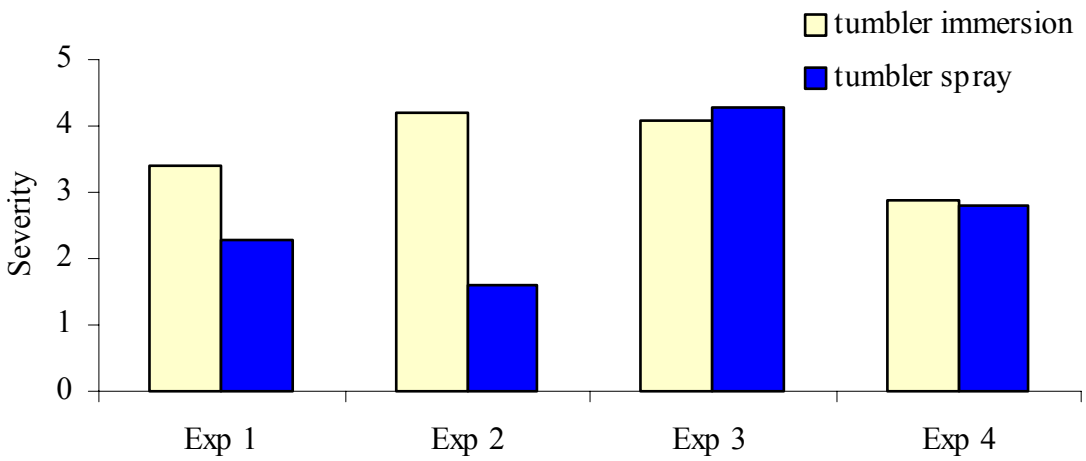
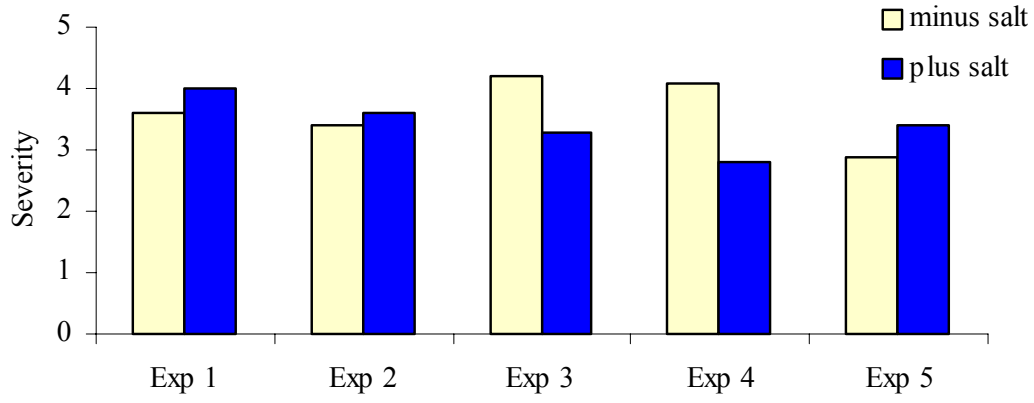


Figure 22 Effect of soft rot on tubers tumbled with or with out salt ($NaCl_2$ at 10g/L) water.



Air drying

Different methods of air drying were tested on inoculated tubers after washing and just prior to packaging. An air blower was installed on the mini washing plant so air could be delivered at either at ambient temperature or heated to temperatures ranging from 45 - 55°C. An air knife was also evaluated as a means of drying tubers. An air knife uses a narrow band of high velocity air to remove water films, a technique commonly used to dry objects before labelling in manufacturing industries. In our experiments the air knife consisted of a stainless steel tear shaped manifold that was 400mm wide x 190mm, with a basal gap of 1.2mm. The air knife was attached to a Rotron Regenerative Blower which forced air at 140 – 150 m/sec or 220 – 230 m/sec at a temperature of 30 - 35°C through a narrow gap at the base of the manifold. The methods of air drying evaluated were:

- Air blower at ambient temperature
- Air blower with heated air (45 - 55°C)
- Air knife with heated air (30 - 35°C)

Methods

Seven experiments were undertaken where air was applied at either ambient temperatures or heated, and applied to inoculated tubers for different lengths of time. Three experiments investigated heated air applied to tubers inoculated at two levels of inoculum to simulate high and low levels of infected tubers. The inoculum was obtained from naturally infected wash water as previously described, and the *Erwinia* levels were determined after inoculation. The low inoculum was a 1:10 dilution of the initial strength. In the first of these experiments the initial inoculum level was at 10^5 cfu/ml and the low level 10^4 cfu/ml. The other two experiments used initial inoculum at only 10 cfu/ml, so the low inoculum was effectively zero. After inoculation the tubers were tumbled, passed over nylon brushes to remove excess water then exposed to the air treatments. These experiments are summarised in Table 9.

Eight experiments were conducted using an air knife (Table 9). Inoculated tubers were again tumbled and passed over nylon brushes to remove excess water before travelling either once or twice under the air knife. In all experiments tubers were induced to rot immediately after they emerged from the air treatments and visually assessed for soft rot, as previously described.

Raw data was analysed by Biometrics SA using an ordinal scale response (McCullagh, P. and Nelder J.A. 1989)

Table 9 Air drying treatments and cultivars used in soft rot experiments

Air drying treatments	**No. of Experiments**	**Potato Cultivar**
Ambient temp or heated air 60 secs	4	Desiree, Nadine, Shine
Heated air 10 or 30 secs	1	Coliban
Heated air 60,90 or 120 secs	2	Coliban, Desiree
Heated air with high level or 1/10 the inoculum level	3	Nadine, Coliban
Air knife 140 –150 m/secs	4	Coliban
Air knife 220 - 230 m/secs	4	Desiree, Nadine

Results and Discussion

When the tubers were inoculated and tumbled soft rot developed on averaged in 93% of tubers (range 35 – 100%) with an average severity of 3.2 (range 1.0 – 4.7).

Air drying tubers for 60 seconds with ambient air or heated air at 45 – 55°C generally reduced tuber soft rot (Figure 23 and Table 10). However in one experiment air drying at ambient temperatures increased severity of tuber soft rot. For 2 out of 3 experiments drying with heated

air for 60 seconds resulted in a greater reduction in soft rot severity, than drying with ambient air (Figure 23).

Since drying with heated air showed encouraging results, varying times of air drying were further investigated. Results showed that 10 seconds of heated air drying failed to reduce tuber soft rot, whereas 30 seconds of heated air gave variable results (Figure 24). When heated air drying times were increased to 60, 90 and 120 seconds, all three times reduced the severity of soft rot (Figure 25).

It was concluded that heated air needed to be applied for at least 30 seconds preferably 60 seconds to reduce tuber soft rot. In two experiments the pulp temperatures of 110 – 200gm tubers treated with heated air for 60 seconds were monitored. No significant increases in temperature were found with less than a 0.3°C rise observed following the treatment.

When the inoculum level was reduced from 10^5 to 10^4 cfu/ml, the heated air treatment reduced the soft rot severities from 4.9 to 2.3 (Figure 26). Where the initial inoculum level was reduced to *Erwinia* levels of 10 cfu/ml or less the results showed that heated air for 60 seconds was more effective at the lower level of inoculum (Figure 26).

When analysed statistically, the results showed that the combination of the lowered inoculum level and heated air drying for 30 seconds was the most successful treatment (Table 10).

In the eight experiments evaluating the air knife, incidence of tubers with soft rot was slightly reduced using the higher velocity (Figure 27). Overall there was little difference in the level of rot between the single and double passes under the air knife at the same velocity (Figures 27, 28 & 29).

All air knife treatments significantly reduced tuber soft rot severity compared to no air drying (Table 11). All air knife treatments except for the single pass at the lower velocity gave equivalent control of soft rot to 60 seconds of heated air drying (Table 11).

Before the air drying treatments were applied to the tubers they were passed over nylon brushes over a distance of 1 to 2.5 metres where most of the water droplets on the tubers surface were removed. However none of the air drying treatments completely dried out the tuber surface.

Conclusions

All air treatments except the 10 seconds of hot air lowered the severity of soft rot compared to no air drying. The best treatments were heated air drying for at least 60 seconds, particularly when levels of inoculum were low. The air knife treatments with either one or two passes at 220-230m/sec or 2 passes at 140-150m/sec also provided good levels of control.

Air drying at ambient temperatures was not as effective as heated air in reducing soft rot.

In all experiments the tubers surface were never completely dry after the air treatments. Time from washing to packaging is relatively short for all surveyed washing plants, which would not allow any of the techniques used in these experiments to completely dry the tubers. However partial air drying was the most successful treatment in reducing soft rot. Further work needs to be done to test whether completely drying the tuber surface would further improve the control and not have any detrimental effects on the tuber appearance and hence affect marketability.

Figure 23 Efficacy of ambient or heated air drying for 60 seconds on the severity of tuber soft rot on inoculated and washed tubers, 2001 - 2002.

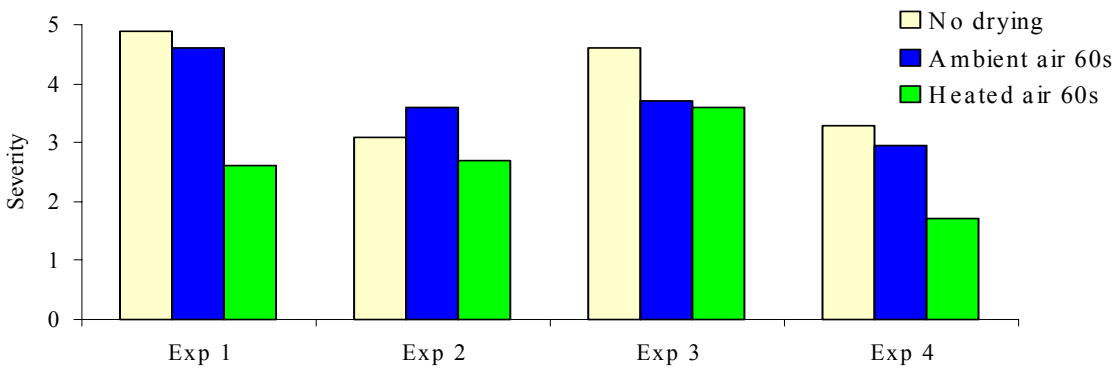


Figure 24 Efficacy of air drying tubers with heated air for 10 or 30 seconds on soft rot severity, 2001 – 2002.

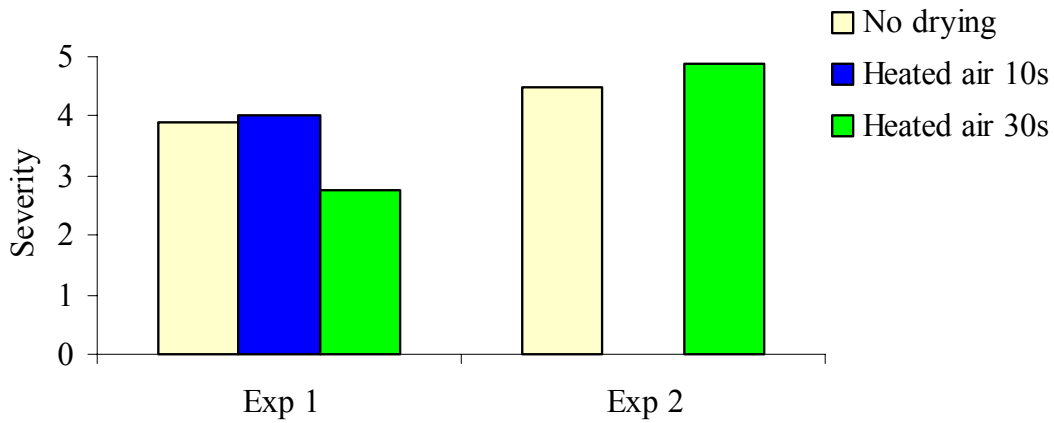


Figure 25 Efficacy of different exposure lengths of hot air drying on the severity of tuber soft rot on inoculated and washed tubers, 2001 – 2002.

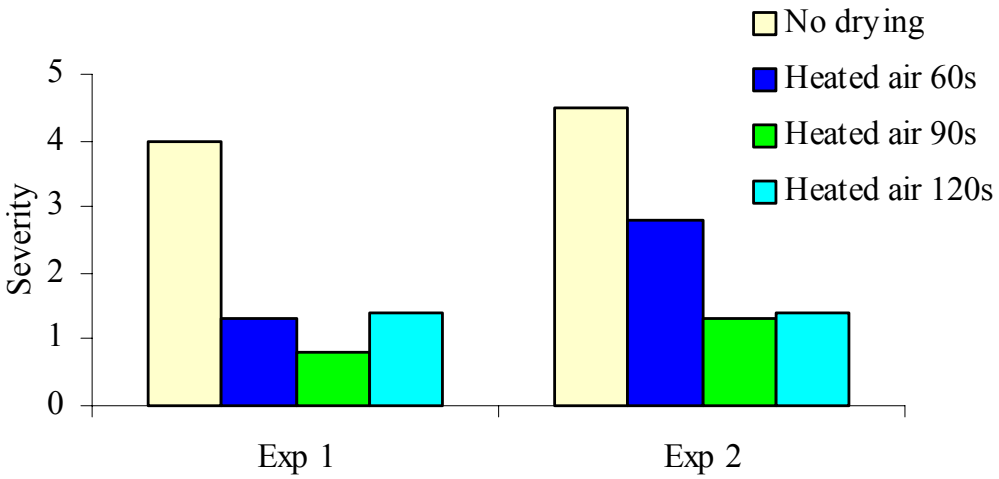
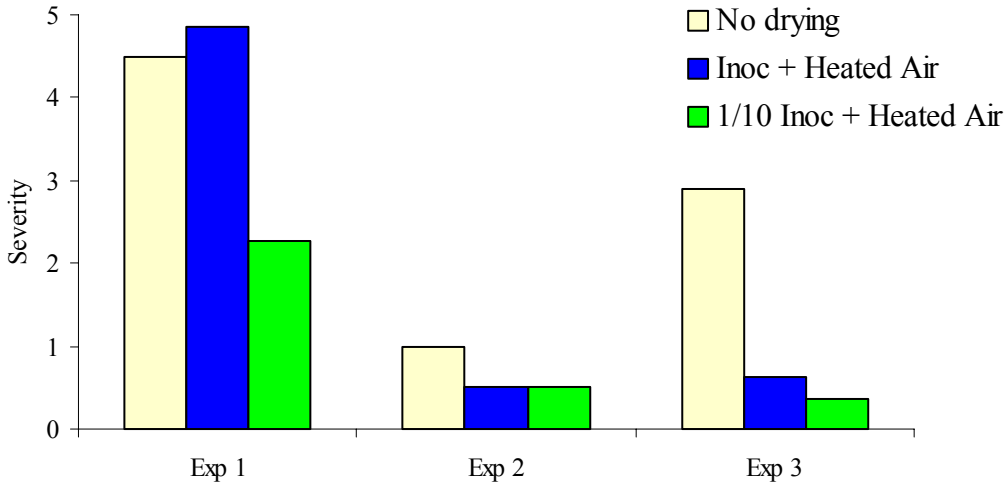


Figure 26 Efficacy of hot air and inoculum level on severity of tuber soft rot on inoculated and washed tubers, 2001 - 2002.



Exp 1: The control (no drying) was inoculated but not washed, and the heated air treatments were for 30 seconds only. The initial inoculum level was 10^5 and the 1/10 levels 10^4 cfu/ml.

Exp2&3: The control (no drying) was inoculated and washed, and heated air treatments were for 60 seconds. The initial inoculum level was 10 cfu/ml and the 1/10 level was near 0.

Table 10 Significant differences between air drying treatments based on severity of rot (Appendix 3).

Treatments	*
I + T only	A
I + T + Hot air 10s	A
I + T + ambient air 60s	B
I + T + Hot air 60s	C
1/10 I + T + Hot air 60s	CD
I + T + Hot Air 30s	CDE
I + T Hot Air 120s	DE
I + T Hot Air 90s	E
1/10I + T Hot Air 30s	F

* Treatments with the same letter are not significantly different from one another

Figure 27 Effect of either heated air or air knife drying treatments on the severity of tuber soft rot on inoculated and washed tubers (Air knife flow rate 140 - 150m/sec), 2001 – 2002.

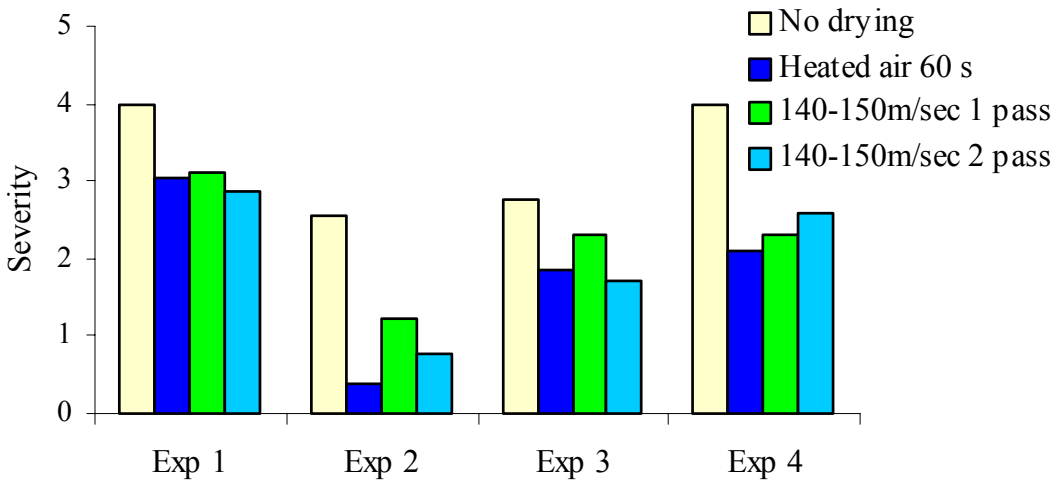


Figure 28 Effect of either heated air or air knife drying treatments on the incidence of tuber soft rot on inoculated and washed tubers (Air knife flow rate 220-230 m/sec), 2002.

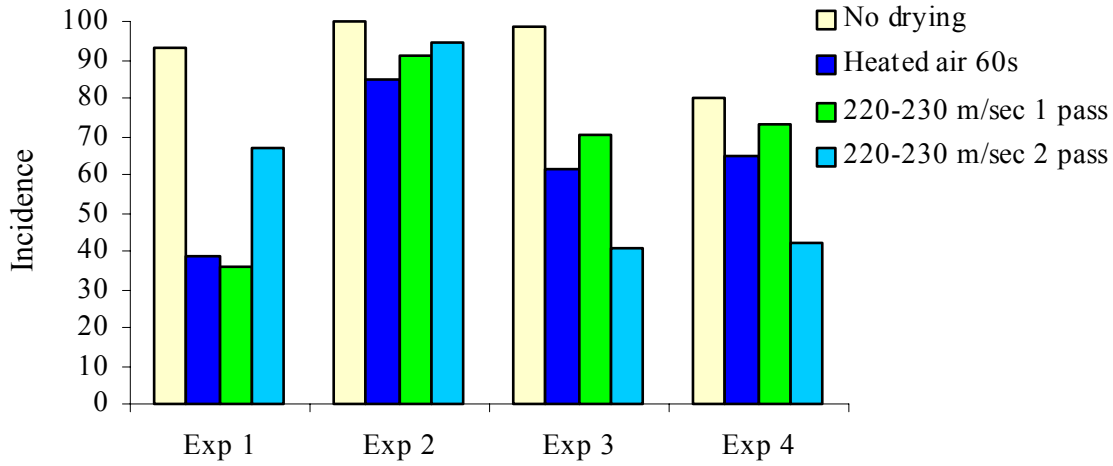


Figure 29 Effect of either heated air or air knife drying treatments on the severity of tuber soft rot on inoculated and washed tubers (Air knife flow rate 220-230m/sec), 2002.

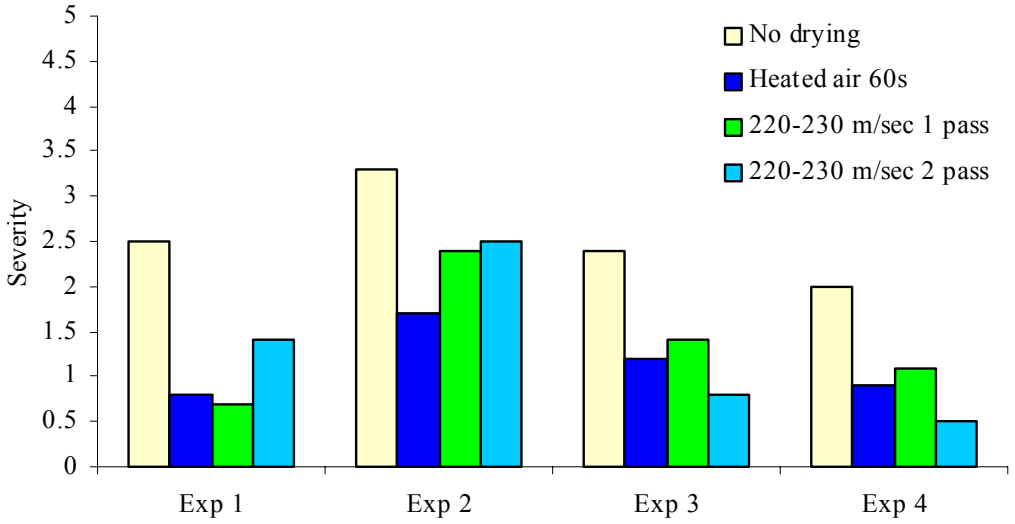


Table 11 Severity of soft rot after air drying treatments before packaging

Treatments	*
I + T only	A
I + T An 400 1 pass	B
I + T AN 400 2 pass	BC
I + T AN 200 1 pass	BC
I + T AN 200 2 pass	BC
I + T Hot Air 60s	C

* Treatments with the same letter are not significantly different from one another

Other Experiments

Effect of inoculum levels on the incidence and severity of soft rot

Water samples collected from four washing plants showed that *Erwinia* levels between 10^3 – 10^4 cfu/ml were found. Four experiments were conducted to test what effect different concentrations of *Erwinia* had on the development of tuber soft rot.

Methods

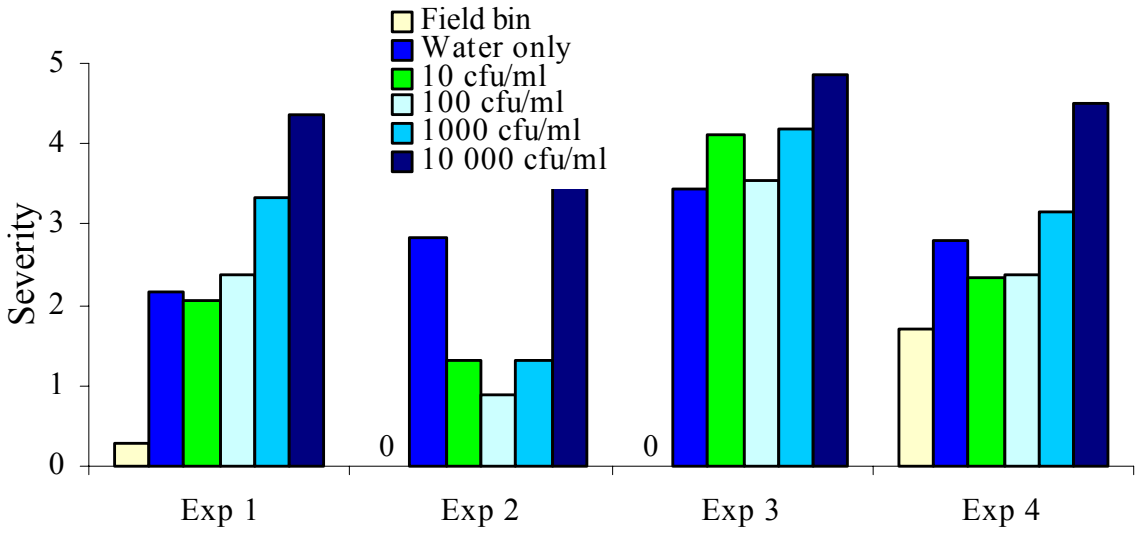
One day prior to the experiment approximately 80L of wash water was collected from the initial washing area of one of the washing plants, providing a natural source of *Erwinia*. Freshly harvested tubers were collected at the same time. The treatments included in these experiments were field bin tubers not dipped in water, tubers dipped in tap water only and tubers dipped in *Erwinia* levels of 10, 10^2, 10^3 and 10^4 cfu/ml. Each treatment consisted of 3 replicates each with 10 tubers, placed into nylon onion bags. On the day of the experiment the wash water was diluted to the desired levels with tap water, to make up a final volume of 20L. Each different level was plated out onto CVP media to confirm the *Erwinia* level. Dipping time was 15 minutes and after this the tubers were induced to rot and then assessed for incidence and severity as previously described.

Results and Discussion

Overall the results showed that once tubers were immersed in water the severity of tuber soft rot increased significantly. All field bin tubers which had not come into contact with water had very low severity of soft rot. In these experiments the severity ranged from 0 – 1.7 (Figure 30). In Experiment 1 severity in the field bin started with 0.3 and this increased to 2.2 once the tubers were immersed in water (Figure 30). Levels of tuber rotting were similar when tubers were immersed in water only or in *Erwinia* levels of 10^2 cfu/ml. When the inoculum levels were increased to 10^3 and 10^4 cfu/ml, severity also greatly increased to 3.4 and 4.4 respectively (Figure

30). Similar trends were observed in the other three experiments (Figure 30). Overall the use of inoculum levels of 10^4 cfu/ml resulted in the highest severity of rot and tubers dipped in lower concentrations developed less severe rot.

Figure 30 Severity of tuber soft rot after dipping freshly harvested tubers into various levels of *Erwinia*.



Effect of tuber and wash water temperature on the incidence and severity of soft rot.

Monitoring the levels of soft rot in washing plants showed that all wash water used had high levels of *Erwinia* present in the water and once tubers were immersed in this water the levels of tuber soft rot increased dramatically. Bartz (1984) showed that tomato fruits with a negative temperature difference between the water and the fruit (water temperature – fruit temperature) resulted in greater water uptake and more bacterial infiltration into the tomato. Infiltration of wash water into potatoes is most likely to enter into the tuber primarily through natural openings such as lenticels, cracks bruises etc (Burton, WG and Spraag WT 1950). Bartz (1984) also investigated whether a negative temperature difference between water and tuber temperature had an affected on the post harvest rot of potatoes. The general finding was that when tuber temperatures were higher than that of water containing the bacterium, disease severity was higher. We conducted numerous experiments to determine whether differences in tuber and wash water temperatures influenced the incidence and severity of soft rot.

Methods

Tubers and wash water were collected from commercial potato washing plants and incubated for 24 hours at various temperatures ranging from 5, 10, 15, 20 and 25°C. At least 7 experiments were undertaken where the tuber temperature was at least 5°C less than, greater than or the same as the water temperature. Tubers were inoculated by immersing them for 15 minutes in wash water containing a natural source of *Erwinia* bacterium and then immediately placing them into the misting tent for 4 days rather than the usual 3 days to encourage more rot development.

Results and Discussion

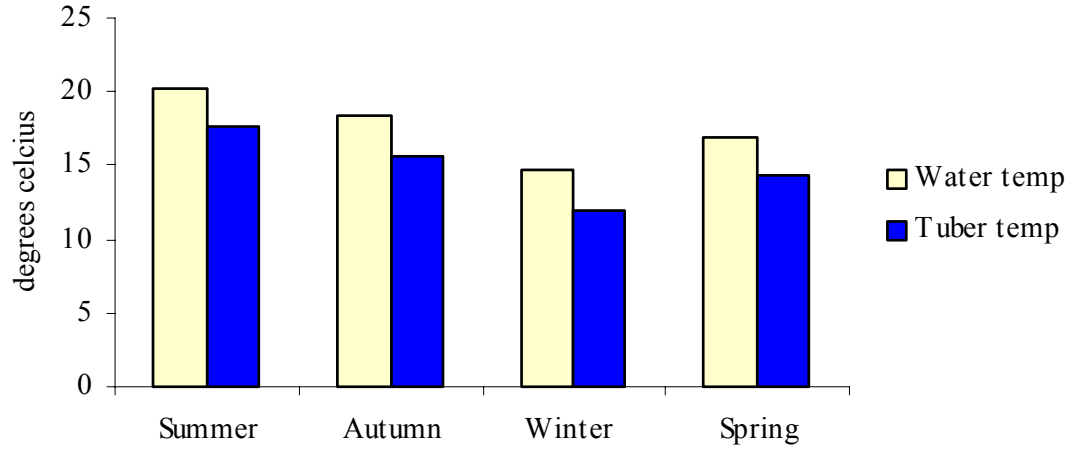
In most cases there was little difference between treatments for both soft rot incidence and severity (Appendix 2 Table 6). High tuber temperatures did not increase soft rot severity as shown by the studies undertaken by Bartz, (1984). The reason for this are difficult to explain. One possibility may be that the cultivars used in South Australia were different to those used by

Bartz. For example cultivars Early Gem and Russet Burbank were used in the USA studies whereas we used Rubylou, Nadine and Coliban. When tuber and water sample temperature were measured in the washing plants in most cases the tuber temperatures were less than the wash water temperature by at least 2.6 to 3.5°C (Figure 31 and Table 12). This suggests that temperature effect of infiltration may not be contributing to high levels of soft rot in South Australia.

Table 12 Average tuber and water temperatures from four commercial potato washing plants in South Australia, 1999 – 2002.

Season	**Tuber temperatures** **Average (Range)**	**Water temperatures** **Average (Range)**	**Water temperature minus tuber temperature**
Summer	17.7 (6.2 – 22.9)	20.1 (10.2 – 25)	-3.5
Autumn	15.6 (10.6 – 18.9)	18.3 (14 – 20)	-2.8
Winter	11.9 (9.0 – 18.9)	14.7 (9 – 20)	-2.6
Spring	14.3 (10.9 – 16.6)	16.9 (13.5 – 21)	-3.1

Figure 31 Average temperatures of water and tubers taken from four commercial washing plants in South Australia, 1999 - 2002



Effect of storage temperatures on post harvest decay

Washed tubers can become infected with soft rot either in transit to the market places or on the supermarket shelves. Tuber temperatures from 5 – 25°C were observed in tubers collected from the washing line throughout our survey. Potatoes are generally washed, packaged and immediately placed into refrigeration at 4°C. The time spent under refrigeration varies from a few minutes to hours, depending on the time in refrigeration and transport. The potatoes are either packaged into plastic bags, woven nylon, hessian bags or cardboard boxes. One experiment was undertaken to test the effect of different storage temperatures on the rate of tuber soft rot development.

Methods

Field bin tubers and wash water containing a natural source of *Erwinia* were collected from a washing plant prior to the day of the experiment. Infected tubers were bagged in a plastic bag with no holes and included a water soaked sponge, to provide high humidity. Storage temperatures of 4,10, 20 and 25°C were used and tubers where held at these temperatures for periods that ranged from 3 to 14 days. Tubers were assessed on day 3, 4, 7, 11 and 14 after inoculation.

Results and Discussion

The infected tubers held at either 4 or 10°C failed to develop rot over the 14 days (Figure 32 & 33). Infected tubers held at 20°C showed lenticel infection only until day 7, then the soft rot severity increased to 2.8 by day 11 and this increased further to 3.5 by day 14 (Figure 32 & 33). Infected tubers maintained at 25°C showed lenticel infection until day 4, high levels of rot were observed from day 7 with a severity of 3.0 which increased to 5.0 by day 11 (Figures 32 & 33).

Figure 32 Development of tuber soft rot in infected tubers stored in plastic bags and incubated at various temperatures for 14 days.

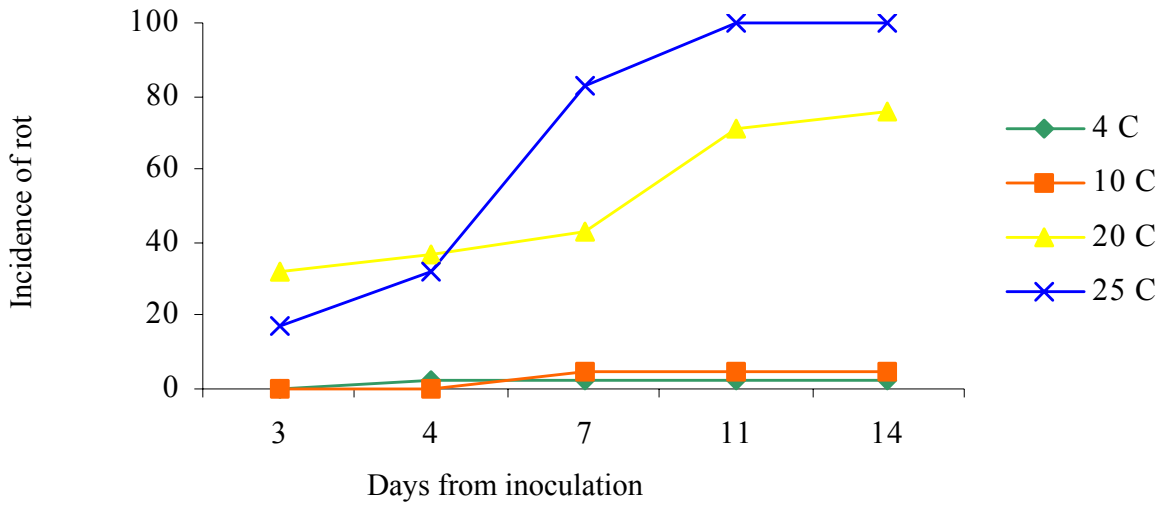
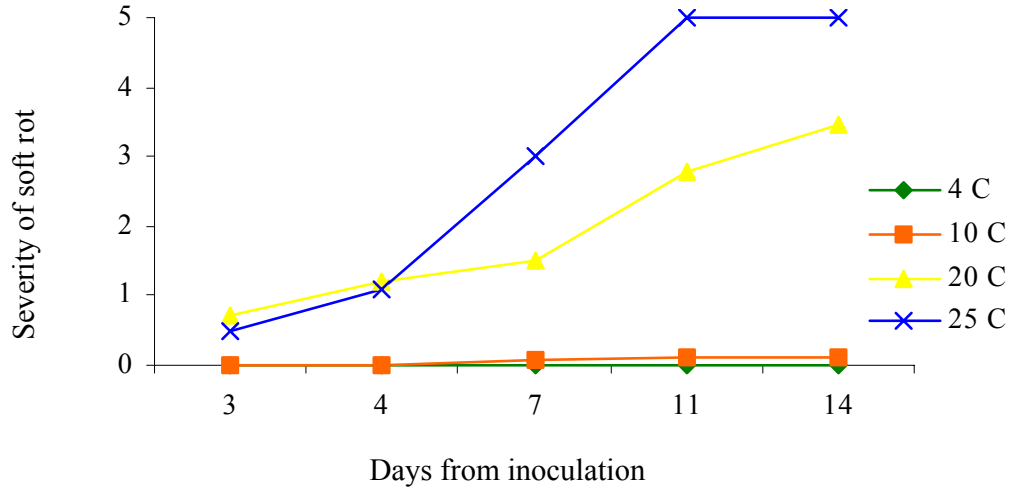


Figure 33 Development of tuber soft rot in infected tubers stored in plastic bags and incubated at various temperatures for 14 days.



Cultivar susceptibility to soft rot

Several cultivars were tested for their susceptibility to *Erwinia carotovora* subsp. *carotovora*.

Methods

Tubers were collected from a variety trial on two separate occasions and stored at 4°C until used. Healthy undamaged tubers were washed and surface sterilised by soaking for 10 minutes in a sodium hypochlorite solution (0.05% w/v available chlorine), rinsed twice in distilled water and allowed to air dry. Slices of approximately 5 – 10 mm were cut at right angles to a line from the apical to the stem end of the tuber and were used in the experiment (Method A). In Method B, tubers were sliced thinly at 5 to 10 mm and then a 25 mm core borer was used (Bartz, JA 1999), to achieve more evenly sized tuber pieces. Tuber slices were placed in petri dishes lined with filter paper and 3 ml of sterile water. The varieties used are shown in Table 13a & b.

Tuber slices were inoculated with *Erwinia* by spreading 100*ul* of a bacterial suspension onto the cut surface. Control slices were inoculated with 100*ul* of sterile water. There were four replicates per bacterial dilution. The levels of *Erwinia* inoculum ranged from 10^2 to 10^5 cfu/ml for method A and 10 to 10^3 cfu/ml for method B. Inoculated tuber sliced were incubated at 27°C for 48 hours and then assessed for visible rot using a 0 to 4 rating system, where 0 = no signs of rotting, 1 = trace rot < 5%, 2 = 5 – 20%, 3 = 20 – 50% and 4 > 50%.

Results and Discussion

The results showed that all the varieties tested were infected with *Erwinia* (Table 13a&b). There were a number of varieties that required a slightly higher concentration of bacteria to induce the same level of rot as most of the other tuber varieties. These slightly less susceptible varieties included Fontenot P, Pontiac, Winter Gem, MacRusset, Red La Soda and White Rhino (Table 13a&b). The more susceptible varieties requiring very low concentrations of bacteria to induce a

high level of rot included Atlantic, Coliban, Dynamite P, Shine, Bison and Crispa (Table 13a&b). The variety Coliban, which is one of the most popular washed potato varieties in South Australia, showed that it was susceptible at low concentrations of Erwinia.

Soft rot was also observed on the control slices in method B but not method A, possibly introduced from the cutting process.

Table 13a Susceptibility of potato cultivars to *Erwinia carotovora* subsp. *carotovora* using testing method A

		concentration *Erwinia* cfu/ml				
Variety	**Control**	**10**	**10^2**	**10^3**	**10^4**	**10^5**
		mean severity per tuber slice				
Atlantic	0	-	3.5	4.0	4.0	4.0
Coliban	0	-	2.8	4.0	4.0	4.0
Dynamite P	0	-	2.8	4.0	4.0	4.0
Fontenot P	0	-	0.3	1.3	3.0	3.3
MacRusset	0	-	1.0	0.3	3.0	4.0
Pontiac	0	-	0.8	1.0	3.5	2.0
Ruby Lou	0	-	0.8	3.0	3.5	4.0
Shine	0	-	2.3	2.5	4.0	4.0
Winter Gem	0	-	0.5	0.8	2.8	4.0

Severity rating: 0=no signs of rotting, 1=trace rot, 2=5-20% slice rotted, 3=20-50% slice rotted, 4=>50% slice rotted

- = not tested

Table 13b Susceptibility of potato cultivars to *Erwinia carotovora* subsp. *carotovora* using testing method B

		concentration *Erwinia* cfu/ml				
Variety	**Control**	**10**	**10^2**	**10^3**	**10^4**	**10^5**
		mean severity per tuber slice				
Bison	2.0	2.3	1.8	4.0	-	-
Coliban	1.8	2.8	3.3	4.0	-	-
Crispa	1.3	2.0	3.0	4.0	-	-
MacRusset	0.3	0	1.8	3.0	-	-
Pontiac	0.3	0.8	1.3	2.8	-	-
87-13-3	0	1.3	3.5	3.5	-	-
Atlantic	0	2.0	3.0	4.0	-	-
Coliban	0.5	2.0	3.8	4.0	-	-
Red La Soda	0	0.3	1.3	0	-	-
Ruby Lou	0	0.5	2.0	3.5	-	-
Shine	0.3	2.0	2.0	3.0	-	-
White Rhino	0.5	0.3	2.0	2.0	-	-
85-2-1	2.3	1.5	3.0	3.8	-	-

Severity rating: 0=no signs of rotting, 1=trace rot, 2=5-20% slice rotted, 3=20-50% slice rotted, 4=>50% slice rotted

- = not tested

Effect of sand and rock wool filtration on *Erwinia* levels in recycled water

Two filtration techniques widely used in the hydroponic nursery industries to reduce pathogenic organisms in recycled water were investigated as a means of reducing *Erwinia* bacterium in wash water. The two filtration mediums used in these experiments were sand and rock wool.

Experiments were conducted to test the efficiency of both filter types by passing either artificially inoculated demineralised water or naturally infected potato wash water through these units using water flow rates of either 100 or 300 l/hr/m^2.

Methods

The sand filtrating unit consists of housing, water layer, filter bed, drainage system and a flow control. The housing for the sand filtration units consisted of a heavy duty black plastic rain water tank 180cm tall and 88cm in diameter (Figure 34). Recycled water was continuously pumped into a water layer approximately 20cm deep at the top of the rain water tank above the filter bed. This water moved downwards through a filter bed of uniform fine particle washed white sand approximately 100cm deep, with a permeable piece of material to separated the fine sand from the first gravel layer to prevent sand from getting into the filter outlet. Two 30cm layers of gravel were used, the top layer consisting of small stones of 8-16 mm diameter and the second layer larger stones at 16-32 mm in diameter. A flow regulating tap was inserted near the base of the rain water tank, which drained into a holding tank. Water was pumped continually from the holding tank to the top of the filter tank at a flow rate of either 100 l/hr/m^2 or 300 l/hr/m^2. The total water used in the sand filtration system was estimated at 1100 l.

The rock wool filtration unit used a smaller tank standing 85 cm in height, 54 cm in diameter, filled with rock wool with a 25 cm water head at the top. Similar to the sand system, recycled water was pumped from a holding tank into the top of the filter tank and a flow regulated tap

inserted at the base of the tank drained the filtered water back into the holding tank. Total water volume was estimated at 600 l.

Table 14 Estimated time taken for water within the two filtration types to complete one cycle through the unit

Filtration type	Estimated time taken to complete one cycle
Sand Filter	
100 l/hr/m^2	11-12 hr per cycle
300 l/hr/ m^2	3.6 hr per cycle
Rock wool filter	
100 l/hr/m^2	4 hrs per cycle
300 l/hr/ m^2	1.25 hrs per cycle

Figure 34 Sand filtration system – Plant Research Centre

The demineralised water was inoculated either with a bacterial suspension of *Erwinia* or with rotten tuber tissue as previously described. Inoculum levels ranged from 10^3 to 10^7 cfu/ml.

Tuber wash water known to be infected with *Erwinia* was collected from the initial wash water of a commercial washing plant with 10^4 cfu/ml *Erwinia*. The soil and organic matter was removed from the wash water to prevent clogging of the filters. Alum was added to the tuber wash water at a rate of 200 mg/L and agitated for 15 minutes and then left while the soil and organic matter settled out to the bottom of the container.

The layer of water on top of the filter medium was removed and replaced with the inoculated water or wash water collected from the commercial washing plant. 115L was added to the sand filtration unit and 41L added to the rock wool filtration unit. The pump was then turned on returning recycled water back into the tank. Water samples were taken from the tap at the base of the filter tanks every 15 minutes for the first hour and then hourly up to 7 hours and again at 24, 48 and 72 hours after the pump was started. The levels of *Erwinia* was determined by plating onto *Erwinia* selective media, (CVP).

Results and Discussion

Filtration at 100l/hr/m^2

The average initial population of the inoculated demineralised water was at 10^5 *Erwinia* cfu/ml. The estimated time taken to complete one cycle for the sand filter and rock wool filtration units at 100 l/hr/m^2 was 11 – 12 hrs per cycle and 4 hrs per cycle respectively (Table 14). The first *Erwinia* was detected at the tap 3 hours after inoculation, *Erwinia* levels were reduced considerably from 10^5 cfu/ml to 10^2 within the first 7 hours for the sand filter system and from 10^5 cfu/ml to 10 cfu/ml in the rock wool system. At 24 hours after inoculation levels were at 20 and 1 *Erwinia* cfu/ml for the sand and rock wool filtration systems respectively. By 72 hours after inoculation no *Erwinia* was detected (Figure 35).

The rock wool filtration system at 100 l/hr/m^2 was 10 fold more effective than the sand filtration at reducing initial levels of *Erwinia* (Figure 35).

Filtration at 300 l/hr/m^2

The average initial *Erwinia* level was at 10^5 cfu/ml. The estimated time taken for the water within the sand and rock wool filtration systems at 300l/hr/m^2 was 3.6 and 1.25 hrs per cycle respectively (Table 14). Neither filtration unit was as effective at reducing *Erwinia* when the flow rate was increased to 300 l/hr/m^2. *Erwinia* was first detected at the sampling 15 minutes after inoculation. At 2 hours after inoculation, the levels of *Erwinia* had been reduced from 10^5 to 10^3 cfu/ml (Figure 36). The level of *Erwinia* gradually decreased over time, by 72 hours after inoculation the sand filtration unit had 83 cfu/ml (Figure 36). As the cycle was completed in approximately 4 hours, it would have been expected that the level would be lower as the water had passed through the filter 2-3 times more. However with this faster flow rate channelling of the sand can occur, allowing water to flow down the channels and remove any filtering effect of the sand.

The rock wool filtration system at 300 l/hr/m^2 had *Erwinia* detected at the first sampling. The highest level was detected at 2 hours after inoculation at 10^3 cfu/ml reduced from 10^5 cfu/ml. The *Erwinia* levels stayed at 10^3 cfu/ml until 6 hours after inoculation. At 24 hours the *Erwinia* level was at 10^2 cfu/ml and by 48 hours had reached near zero levels (Figure 36).

Filtered Potato wash water at 100 l/hr/m^2

The flocculated potato wash water was trialed in both filtration units. The initial level of *Erwinia* in the sand filtration unit was at 10^4 cfu/ml and was reduced to 10^2 cfu/ml by 2 hours after inoculation and to levels of 10 cfu/ml at 72 hours after inoculation (Figure 37).

The rock wool filter was more effective at reducing the *Erwinia* population. The average initial level was at 10^4 cfu/ml which was reduced to 10^2 cfu/ml 4 hours after inoculation and levels were at 10 cfu/ml by 72 hours after inoculation (Figure 37).

Although the filter systems reduced the amount of *Erwinia* in the water, this technology is unlikely to be adopted by the washed potato industry. In one of the commercial washing plants, water is pumped to the washing plant at a rate of 30 l/sec and operates 9 hours/day, therefore approximate water usage per day is 972 000 l. This volume is much too great for this system to manage so no further investigations were undertaken.

Figure 35 *Erwinia* levels detected in filtrate from either a sand or rock wool filtration unit (Flow rate 100l/hr/m^2)

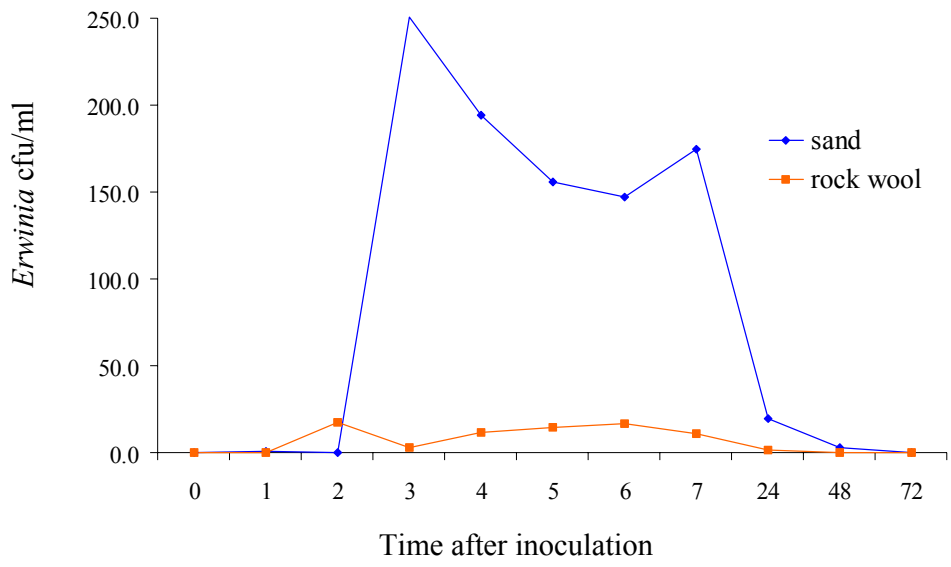


Figure 36 *Erwinia* levels detected in filtrate from either a sand or rockwool filtration unit (Flow rate 300 l/hr/m^2).

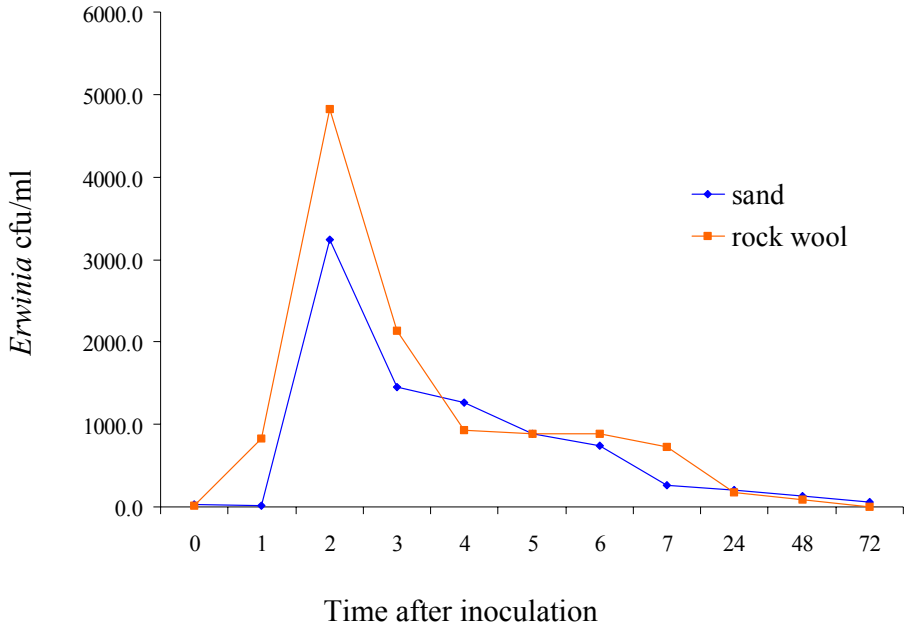
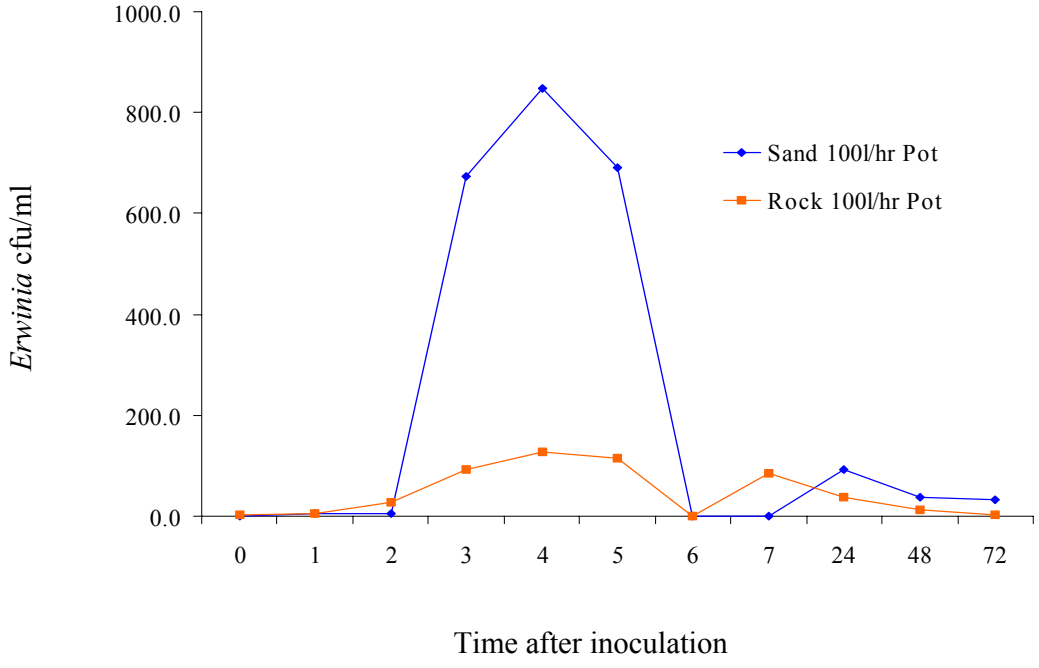


Figure 37 *Erwinia* levels detected in filtrate from either a sand or rock wool filtration unit (Flow rate 100 l/hr/m^2)– initial *Erwinia* source potato wash water



Effect of ultraviolet and microwave radiation on tuber soft rot

Ultraviolet light is an effective method of disinfecting liquids, and was used with success in this report to disinfect recycled pond water. It has a strong germicidal property in the range of 200-280 nM wavelength, therefore ultraviolet irradiation was applied to *Erwinia* infected tubers to determine whether it could control soft rot.

Microwave technology is used in the food processing industries already, for example to irradiate spices and seasonings found in processed foods (USA), electromagnetic microwave irradiation for the industrial rice disinfection processes, continuous drying of inorganic salts and hydro thermic treatments of mustard seed. It is investigated in this report to determine if it could control soft rot on infected tubers.

Methods

Tubers were inoculated with naturally infected water as previously described, and exposed for 30 or 60 seconds to UV light at either 254 nM wavelength (UV light) or at 365 nM wavelength (Black light).

Inoculated tubers were also exposed to microwave (MW) irradiation at either 30 or 60 seconds on a high setting, to determine if this method also controlled soft rot. The microwave used in these experiments was a Sharp Carousel microwave oven model R9320 with an output of power at 650 W and Frequency of 2450 MHz.

These experiments are summarised in Table 15.

All tubers were artificially inoculated, tumbled and then passed over nylon brushes to remove water droplets, before they were exposed to either the ultraviolet light or microwave irradiation.

For both the ultraviolet lights and microwave treatments, when half of the total exposure time had lapsed each tuber was rotated by hand to ensure that the tubers were subject to an even exposure of the treatments. After the treatments were applied the tubers were induced to rot as previously described and then visually assessed for severity of soft rot.

Table 15 Ultra violet light, Black light and Microwave irradiation treatments and cultivars evaluated to control soft rot.

Treatment	No. Experiments	Cultivar
UV light 30 seconds	7	Coliban, Desiree
UV light 60 seconds	4	Coliban, Desiree
Black light 30 seconds	7	Coliban, Desiree
Black light 60 seconds	3	Coliban
Microwave – (H) 30seconds	5	Coliban, Desiree
(M) 30 seconds	1	Coliban
Microwave - (H) 60 seconds	3	Coliban

Results and Discussion

After inoculation and washing an average of 98% (range 90-100) of tubers were rotted with an average severity of 3.8 (range 2.5 – 4.9). These experiments were conducted on eight separate dates and on three occasions inoculation failed to induce rotting in some replicates. In these cases the data for all treatments were omitted.

The average incidence of soft rot for tubers treated with 30 or 60 seconds at both UV wavelengths were only marginally reduced when compared to tubers not exposed to UV with an average incidence of 98%, as shown in figures 38 and 40. However in most experiments (6 out of 7) ultraviolet light at 254 nM for 30 seconds reduced tuber soft rot severity when compared to the

control (Figure 39). When exposure to the ultraviolet irradiation was increased from 30 to 60 seconds no further improvements in disease control was observed (Figure 39).

Exposure of tubers to Black light irradiation also decreased the severity of tuber soft rot in most experiments but increasing exposure from 30 to 60 seconds did not increase the level of control (Figure 41).

When the severity of the ultraviolet and black light treatments were statistically analysed the results showed that all treatments except the ultraviolet light for 60 seconds significantly reduced soft rot, when compared to tubers which were not treated with UV or black light (Table 16). The most successful treatments were black light at 30 and 60 seconds and ultraviolet light exposure at 30 seconds (Table 16). Although the most effective germicidal range is considered to be 200-280 nM wavelength, in these investigations we found that the black light treatments at 365 nM performed as well as or better than the treatments of UV light at 254 nM. In fact it was shown UV light for 60 seconds had no control on soft rot (Table 16).

Figures 42 and 43 show that most of the microwave treatments reduced both incidence and severity of soft rot when compared to the rot that developed in tubers which were not exposed to microwave. When the exposure times were increased from 30 to 60 seconds the level of rot was also markedly decreased.

Statistical analysis showed that soft rot severity for all microwave treatments were significantly better than untreated tubers (Table 17). The most successful treatment was the microwave for 60 seconds treatment.

Conclusions

Exposing tubers to microwave irradiation for 60 seconds significantly reduced both the incidence and the severity of tuber soft rot. This control was achieved with a domestic microwave appliance, suggesting that further evaluation is needed to evaluate efficiency and cost effectiveness when the treatment is applied on a large scale. Other successful treatments included microwave for 30 seconds, exposure to black light for either 30 or 60 seconds and ultraviolet light exposure for 30 seconds.

Table 17 Soft rot severity of inoculated tubers treated with UV light

Treatments	*
Control – No UV	A
Ultraviolet light 60s	A
Black Light 30s	C
Black Light 60s	C
Ultraviolet light 30s	C

* Treatments with the same letter are not significantly different from one another

Table 18 Soft rot severity of inoculated tubers treated with microwave irradiation

Treatment	*
I + T only	B
I + T microwave 30s H	C
I + T microwave 60s H	D

* Treatments with the same letter are not significantly different from one another

Figure 38 Incidence of soft rot after exposure to 30 or 60 seconds of ultra violet light at 254 nM, 2002.

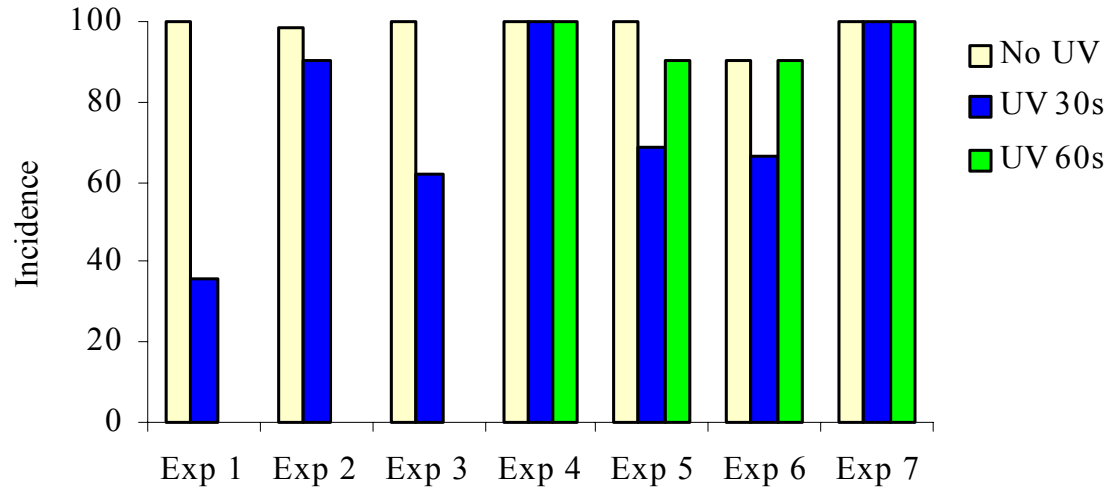


Figure 39 Effect on soft rot severity after 30 or 60 seconds exposure to ultraviolet light irradiation, 2002.

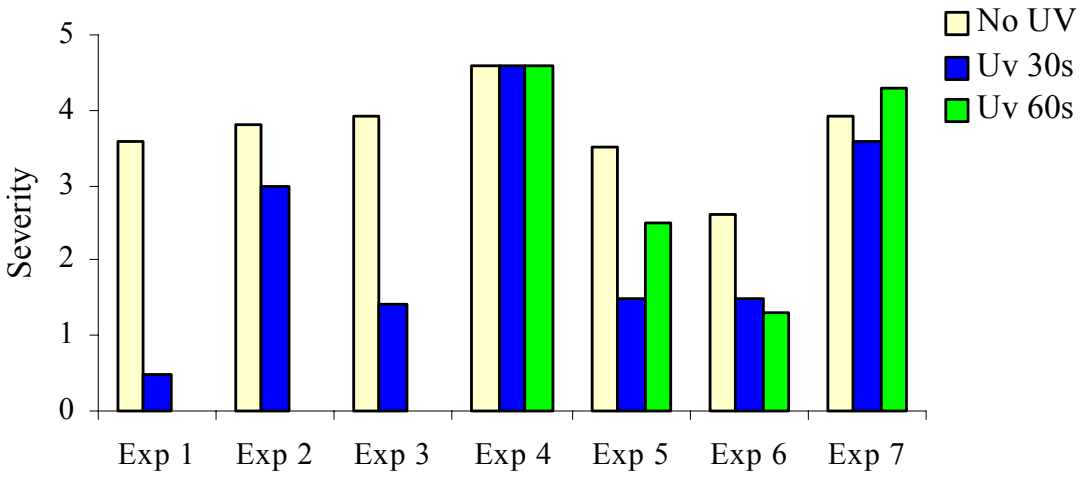


Figure 40 Incidence of soft rot after 30 or 60 seconds exposure to black light(BL), 2002.

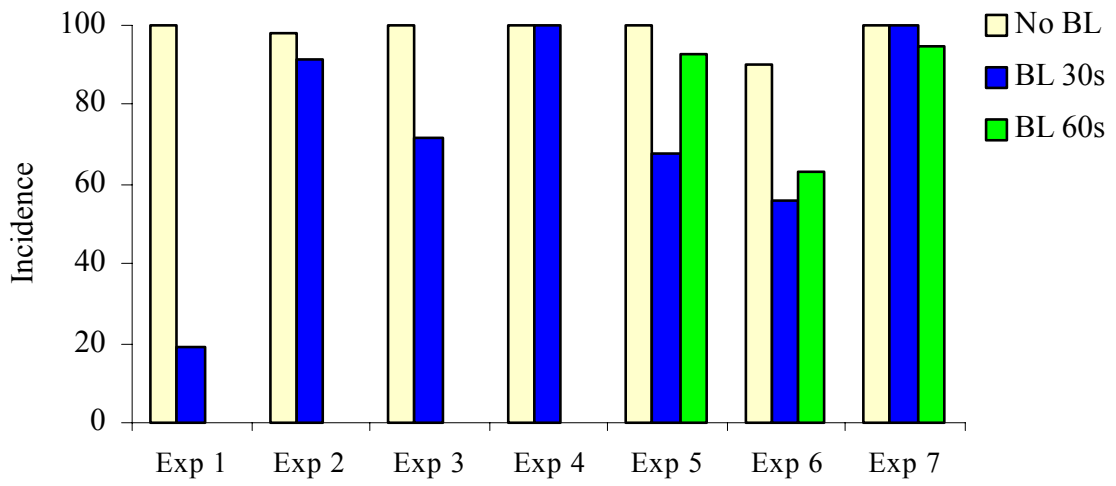


Figure 41 Effect of 30 or 60 seconds exposure to black light irradiation on the severity of tuber soft rot, 2002.

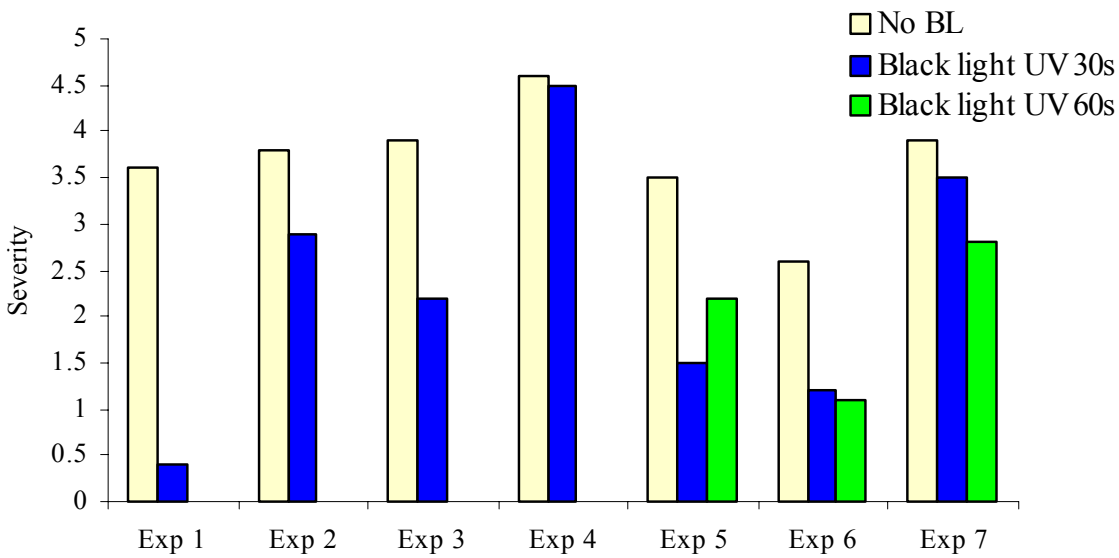


Figure 42 Incidence of soft rot after 30 or 60 seconds exposure to microwave irradiation, 2002.

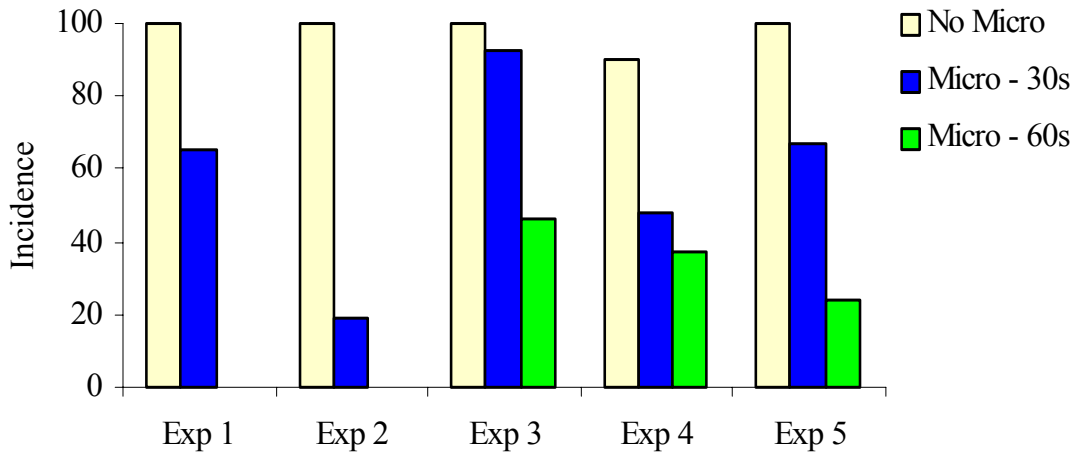
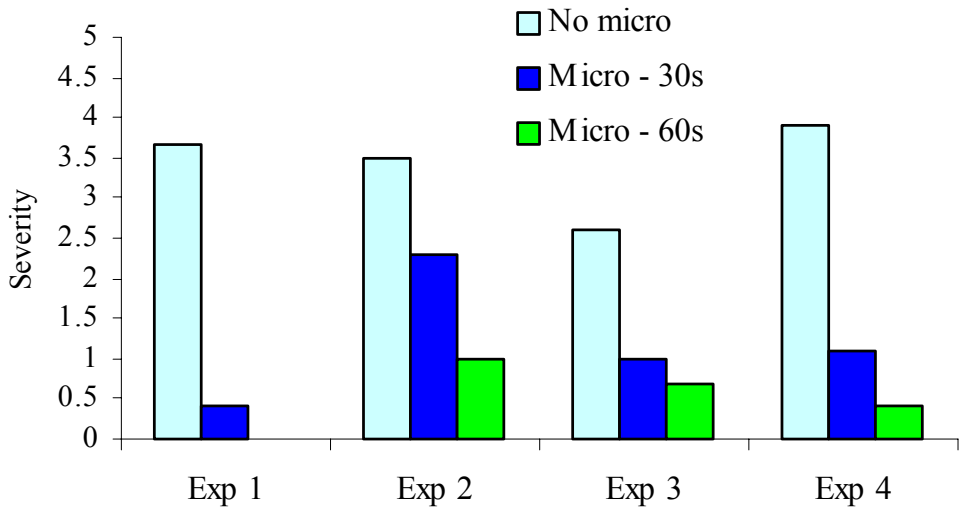


Figure 43 Effect on soft rot severity after 30 or 60 seconds exposure to microwave irradiation, 2002.



Soft rot in field bin tubers

A common practice in the washed potato industry is to hose down field bin tubers prior to entering the washing line. On other occasions field bin tubers are held overnight and misted for 6 to 12 hours. This is done to help soften the soil and *Rhizoctonia* sclerotes adhering to the tuber surface and to aid washing. Experiments was undertaken to determine if this practise increased the susceptibility of tubers to soft rot.

Methods

On nine occasions field bin tubers were collected before and after being hosed down, time of hosing ranged from minutes to approximately 2 hours. The water source in these experiments was untreated recycled pond water.

On another 2 occasions field bin tubers were collected pre and post hosing / misting. Misting took place overnight were tubers were misted for longer periods up to 12 hours. For the first 2 misting experiments field bin tubers were misted for 2 hours, intervals with 2 hours of no misting in between. Water source used on these occasion was untreated recycled pond water.

On the third occasion field bin tubers were misted continuously for a period of 7 hours with treated recycled pond water.

Collected tubers were taken back to the Plant Research Centre and induced to rot as previously described.

Results and Discussion

30% of field bin tubers from non wetted bins developed rot with a severity of 0.75. Once the tubers were hosed down for a short period of time a small increase in soft rot was observed at 45.5% incidence and a severity of 1.3.

When the field bins were wetted for 7 hours or more with untreated pond water a dramatic increase in soft rot developed. For example the initial incidence and severity increased from 4.3% and 0.1 to 96% and 3.9 incidence and severity respectively after the field tubers were wetted.

On one occasion where field bin tubers were wetted for 7 hours with treated pond water soft rot did not develop in the field bin tubers and did not increase after they were wetted.

These results show that where the tubers are infected in the field or infected water is use, misting or wetting tubers will increase the incidence and severity of rot.

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APPENDIX 1: MEDIA

Crystal Violet Pectate (CVP) medium

Ref: Perombelon and Van der Wolf (1998)

Tryptone	1.0g
Tri-sodium citrate	5.0g
$NaNO_3$	2.0g
$CaCl_2.2H_20$ (10% aqueous solution)	13.6ml
Crystal violet (0.075 % aqueous solution)	2.0ml
Demineralised water	to 1000ml
Agar	4g
Sodium Polypectate (Citrus colloids)	18g

Freezing medium

Ref: Perombelon and Van der Wolf (1998)

K2HPO4	12.6g
KH2PO4	3.6g
Sodium Citrate	0.9g
MgSO4.7H2O	0.18g
(NH)2SO4	0.18g
Glycerol (85%)	88.0g
Water	to 1000ml

APPENDIX 2: RAW DATA

Table 1 Location, host and *Erwinia* identification table.

Location	Isolation number	Host	Probable identification
Tuber Samples			
Finely, N.S.W.	1	Tuber	E chyr
Blighty, N.S.W.	2	Tuber	Ecc
Bolwara, N.S.W.	3	Tuber stem base	Eca
	4	Known negative	Pseudomonas Fluorescence
Blanchetown, S.A.	17	Tuber	Ecc
Blanchetown, S.A.	20	Tuber	Ecc
Blanchetown, S.A.	21	Tuber	Ecc
Blanchetown, S.A.	22	Tuber	Ecc
Blanchetown, S.A.	23	Tuber	Ecc
Blanchetown, S.A.	24	Tuber	Ecc
Gawler River, S.A.	5	Tuber	Ecc
Pinnaroo, S.A. 3	E26T	Tuber	Ecc
Pinnaroo, S.A. 3	E27T	Tuber	Ecc
Pinnaroo, S.A. 3	E28T	Tuber	Ecc
Pinnaroo, S.A. 3	E30T	Tuber	Ecc
Pinnaroo, S.A. 3	E31T	Tuber	Ecc
Pinnaroo, S.A. 3	E32T	Tuber	Ecc
Pinnaroo, S.A. 3	E33T	Tuber	Ecc
Pinnaroo, S.A. 3	E34T	Tuber	Ecc
Pinnaroo, S.A. 5	E35T	Tuber	Ecc
Pinnaroo, S.A. 5	E36T	Tuber	Ecc
Pinnaroo, S.A. 5	E37T	Tuber	Ecc
Pinnaroo, S.A. 5	E38T	Tuber	Ecc
Pinnaroo, S.A. 5	E39T	Tuber	?
Pinnaroo, S.A. 5	E40T	Tuber	Ecc
Pinnaroo, S.A.	E55T	Tuber	Ecc
Pinnaroo, S.A. 5	E41T	Water spot lenticel	Ecc
Pinnaroo, S.A. 5	E42T	Water spot eye	Ecc
Pinnaroo, S.A. 5	E43T	Puffy lenticel	Ecc
Pinnaroo, S.A. 5	E44T	Very dark lenticel	Ecc
Pinnaroo, S.A. 5	E45T	Lenticel	Ecc

Pinnaroo, S.A. 5	E46T	Dark spot on skin	Ecc
Pinnaroo, S.A. 5	E47T	Puffy lenticel	Ecc
Purnong S.A.	P	Tuber stem	Eca
Purnong S.A.	E	Tuber stem	Eca
Purnong S.A.	R	Tuber stem	Eca
Virginia, S.A.	8	Tuber	Eca
Virginia, S.A.	9	Tuber	Ecc
Virginia, S.A.	10	Tuber	Eca
Virginia, S.A.	12	Tuber	Eca
Virginia, S.A.	14	Tuber	Ecc
Virginia, S.A.	15	Tuber	Ecc
Virginia, S.A. Nicol	E53T	Tuber	Eca
Virginia, S.A. 2	E54T	Tuber	Ecc
Virginia, S.A.	13	Lenticel	Ecc
Virginia, S.A.	16	Lenticel	Eca
Waikerie, S.A.	E48T	Tuber	Ecc
Waikerie, S.A.	E49T	Tuber	Ecc
Waikerie, S.A.	E50T	Tuber	Ecc
Waikerie, S.A.	E51T	Tuber	Ecc
Waikerie, S.A.	E52T	Tuber	Ecc
Water samples			
Virginia, S.A. 1	E56W	Water-ponds	Eca
Virginia, S.A. 1	E57W	Water-ponds	Eca/Ecb
Virginia, S.A. 1	E65W	Water-ponds	Ecc
Virginia, S.A. 1	E66W	Water-ponds	Ecc
Virginia, S.A. 1	E67W	Water-ponds	Ecc
Virginia, S.A. 1	E68W	Water-ponds	Ecc
Virginia, S.A. 1	E58W	Water-initial wash	Eco
Virginia, S.A. 1	E59W	Water-initial wash	Eca
Virginia, S.A. 1	E60W	Water-tumbler	Eca
Virginia, S.A. 1	E61W	Water-tumbler	Contaminant
Virginia, S.A. 1	E62W	Water-final rinse	Contaminant
Virginia, S.A. 1	E63W	Water-after shed	E chry
Virginia, S.A. 1	E64W	Water-after shed	Dead

Table 2 Time (minutes) tubers traverse various sections of the washing plant.

Washing Plant	Initial wash	Tumbler	Final rinse	From final rinse to packaging point
		minutes		
1	1.1 – 6.5	1.2 – 7.4	1.1	1.1
2	1.0 – 6.1	6.0 – 10.3	0.6	2.2
3	3.0 – 58.0	2.3 – 5.5	0.02	1.1 – 1.3

Table 3 Level of *Erwinia* in water samples collected from recycled pond water and various sites on the washing plants

SAMPLING SITE	**SAMPLING TIME** *Erwinia* **colony forming units / ml (cfu/ml)**			
Washing Plant 1	**21/12/1998**	**25/05/1999**	**7/09/1999**	**20/10/2000**
After Shed	2.25×10^3	1.72×10^4	1.00×10^4	2.20×10^3
Pond 1	1.75×10^3	2.20×10^3	8.75×10^3	8.75×10^3
Pond 2*	Nt	3.75×10^3	4.25×10^3	3.00×10^3
Pond 3*	Nt	3.00×10^4	3.00×10^4	8.00×10^3
Initial Wash	2.25×10^4	1.45×10^5	3.25×10^4	1.90×10^4
Tumbler	1.75×10^3	4.50×10^5	3.25×10^3	5.50×10^4
Final Rinse	5.00×10^3	9.25×10^3	1.75×10^3	6.75×10^3
Washing Plant 2	**15/01/1999**	**6/04/1999**	**6/10/1999**	**17/02/2000**
After Shed	Nt	3.00×10^3	5.00×10^1	2.60×10^4
Pond 3	Nt	Nt	0	5.20×10^3
Pond 2	Nt	Nt	0	2.25×10^2
Pond 1*	Nt	Nt	0	0
Initial Wash	5.00×10^2	0.75×10^1	5.00×10^1	4.75×10^2
Tumbler	0	6.50×10^1	4.70×10^2	2.30×10^4
Final Rinse	0	0	0	17.5
Washing Plant 3	**14/12/1999 27/01/2000**	**16/03/1999**	**20/07/1999**	**6 & 8/09/1999**
After Shed	nt	5.75×10^4	5.24×10^4	3.75×10^3
Pond 1	7.30×10^2 *	6.00×10^3	1.57×10^4	7.50×10^3
Pond 2	7.50×10^2 *	7.25×10^3	1.00×10^4	2.25×10^3
Pond 3	2.50×10^2 *	5.00×10^2	7.75×10^3	3.25×10^3
Pond 4*	1.25×10^3 *	4.50×10^3	1.00×10^4	3.00×10^3
Pond 5	2.50×10^1 *	0	6.75×10^1	10
Initial Wash	5.00×10^2 *	5.00×10^5	3.75×10^4	8.00×10^4
Tumbler	2.50×10^3 *	8.75×10^4	4.50×10^5	4.25×10^4
Final Rinse	2.50×10^3 *	2.50×10^3	8.75×10^3	2.00×10^3
Initial Spray	nt	3.75×10^5	4.25×10^4	2.25×10^3
Tumbler Spray	nt	nt	2.30×10^5	5.00×10^4
Final Spray	nt	nt	2.50×10^4	4.75×10^3
Washing Plant 4	**29/07/1999**	**02/03/2000**	**25/09/2000**	**2/05/2001**
After Shed	1.75×10^3	6.00×10^4	5.00×10^4	2.10×10^5
Bore water	0	0	0	0
Initial Spray	1.75×10^4	-	1.25×10^4	3.00×10^4
Tumbler	6.75×10^4	4.25×10^4	1.00×10^4	1.00×10^5
Final Rinse	0	0	0	0
Pond 1	-	-	1.50×10^3	4.50×10^2
Pond 2	-	-	2.50	5.00×10^2
Pond 3	-	-	0	2.50×10^2

Table 3 continued

SAMPLING SITE	SAMPLING TIME *Erwinia* colony forming units / ml (cfu/ml)			
Washing Plant 1	**26/06/2000**	**18/04/2001**		
After Shed	2.40×10^4	2.00×10^4		
Pond 1	1.00×10^4	2.50×10^3		
Pond 2*	5.00×10^3	1.50×10^4		
Pond 3*	1.30×10^2	2.00×10^4		
Initial Wash	2.00×10^4	4.75×10^4		
Tumbler	6.70×10^4	3.00×10^4		
Final Rinse	8.00×10^3	2.00×10^4		
Washing Plant 2	**7/07/2000**	**3/04/2001**	**12/10/2001**	
After Shed	3.25×10^2	5.00×10^3	-	
Pond 3	8.50×10^3	4.80×10^2	3.6×10^2	
Pond 2	0	5.00×10^2	1.5×10^2	
Pond 1*	0	0	7.5	
Initial Wash	0	3.30×10^2	5.0×10^3	
Tumbler	20	0	0	
Final Rinse	2.75×10^2	0	2.25×10^2	
Washing Plant 3	**26/01/2000**	**2/04/2001**	**3/07/2001**	**16/10/2001**
After Shed	1.75×10^4	5.20×10^5	8.75×10^3	1.50×10^4
Pond 1	2.75×10^4	Same as above	4.50×10^4	6.00×10^3
Pond 2	2.50×10^3	1.90×10^5	5.75×10^4	1.25×10^4
Pond 3	3.50×10^3	4.00×10^4	9.25×10^4	1.50×10^4
Pond 4*	3.25×10^3	7.50×10^1	6.75×10^3	2.60×10^4
Pond 5	22.5	7.50×10^1	1.10×10^3	8.50×10^2
Initial Wash	1.20×10^4	1.50×10^6	9.75×10^4	2.00×10^5
Tumbler	6.50×10^4	1.30×10^5	1.75×10^5	1.50×10^4
Final Rinse	5.25×10^2	3.50×10^3	4.50×10^2	1.90×10^4
Initial Spray	1.75×10^2	6.00×10^2	2.75×10^4	1.75×10^4
Tumbler Spray	5.25×10^4	Same as other tumbler	Same as other tumbler	
Final Spray	3.25×10^3	4.75×10^4	Not in use	Not sampled
Washing Plant 4	**12/07/2001**	**22/01/2002**		
After Shed	2.75×10^4	3.0×10^5		
Bore water	0	0		
Initial Spray	1.50×10^4	2.0×10^4		
Tumbler	0	2.3×10^4		
Final Rinse	0	0		

- Water plated onto CVP media and number of colonies counted after 48 hours incubation at 27 degrees Celsius
- CVP media with more than 0.075% crystal violet solution
- Each dilution site was replicated by 4
- Nt = not tested

Table 4 Efficacy of various sanitisers in inoculated demineralised water with a contact time of 5 minutes

Chemical	Rate ppm	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6
Oxine	**0**	*6x10^4*	*2x10^5*	*2x10^5*	*5x10^6*	*5x10^3*	
	0.1	Nt	++	++	Nt	++	
	1.0	Nt	++	Nt	++	Nt	
	2.0	++	Nt	Nt	Nt	Nt	
	10.0	Nt	Nt	Nt	Nt	Nt	
	20.0	++	Nt	Nt	Nt	Nt	
ChlorDox	**0**	9x10^4	2x10^3				
	0.1	++	++				
	1.0	++	++				
	10	++	Nt				
Nylate	**0**	*6x10^4*	*2x10^5*	*2x10^5*	*8x10^4*	*5x10^6*	*2x10^3*
	0.1	Nt	++	++	+	-	+
	1.0	+	++	Nt	++	-	++
	10	++	Nt	Nt	Nt	Nt	Nt
Klorman	**0**	*6x10^4*	*2x10^5*	*2x10^5*	*8x10^4*	*2x10^3*	*5x10^3*
	0.1	Nt	++	++	++	++	++
	1.0	++	++	++	++	++	++
	10	++	Nt	Nt	Nt	Nt	Nt
Proxitane	**0**	*6x10^4*	*2x10^5*	*2x10^5*	*8x10^4*	*5x10^6*	*5x10^3*
	0.1	Nt	-	-	-	-	-
	1.0	-	-	-	-	-	++
	5.0	Nt	Nt	Nt	Nt	++	Nt
	10	++	Nt	++	++	++	Nt
Liquid Pool Chlorine	**0**	*2x10^5*	*2x10^5*	*8x10^4*	*5x10^3*	*2x10^6*	
	0.1	++	++	+	++	+	
	1.0	++	++	++	Nt	++	
	10	++	Nt	Nt	Nt	++	

Table 4 (cont) Efficacy of sanitisers exposed to various levels of *Erwinia* bacterium for 5 minutes in demineralised water

Chemical	Rate ppm	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6
Sporekill	**0**	$6x10^4$	$2x10^5$	$8x10^4$	$5x10^6$	$2x10^3$	
	1.0	-	-	-	Nt	Nt	
	10	-	-	Nt	++	Nt	
	20	Nt	Nt	Nt	++	Nt	
	100	++	++	++	Nt	++	
Meta-bisulphite	**0**	$7x10^4$	$5x10^4$	10^3	$2x10^3$		
	10	-	Nt	Nt	Nt		
	100	-	Nt	Nt	Nt		
	1000	-	Nt	Nt	Nt		
	2000	Nt	-	Nt	Nt		
	50000	Nt	Nt	Nt	++		
	100000	Nt	Nt	++	++		
	200000	Nt	Nt	++	Nt		
Citric Acid	**0**	$6x10^4$	$5x10^5$	10^3			
	10000	Nt	-	Nt			
	50000	-	Nt	Nt			
	100000	+	++	++			
Kasumin	**0**	$9x10^4$	$7x10^4$	$5x10^4$			
	10	-	Nt	Nt			
	50	Nt	Nt	-			
	100	-	-	-			
	1000	-	-	-			
Cottees Rasberry Cordial	**0**	$6x10^4$	$5x10^5$	10^3			
	1000	Nt	-	Nt			
	10000	Nt	-	Nt			
	50000	-	Nt	Nt			
	100000	+	++	++			

Table 4 (cont) Efficacy of sanitisers exposed to various levels of *Erwinia* bacterium for 5 minutes in demineralised water.

Chemical	Rate ppm	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6
Cottees	**0**	$6x10^4$					
Orange	**50000**	-					
Cordial	**100000**	-					
	200000	++					
DickSmith	**0**	$6x10^4$	$5x10^5$				
Rasberry	**10000**	Nt	-				
Cordial	**100000**	-	-				
	200000	-	++				
Salt Solution	**0**	$1x10^4$					
	100000	-					
	150000	-					
	200000	-					
Compressed	**0**	$1x10^3$					
Air	**5 minutes**	-					

Table 5 Efficacy of various sanitisers in potato wash water with a natural source of *Erwinia* contact time 5 minutes

Chemical	Rate ppm	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5
Oxine	**0**	*6.3x10^2*	*3.7x10^4*	*6.8x10^4*	*2.0x10^5*	*7.8x10^4*
	50	++	+	++	Nt	++
	100	Nt	+	++	++	++
	200	Nt	Nt	Nt	++	Nt
Nylate	**0**	*3.7x10^4*	*6.8x10^4*	*1.8x10^5*	*2.0x10^5*	*7.8x10^4*
	15	-	++	++	+	++
	30	-	++	++	+	++
	60	Nt	++	++	Nt	Nt
Klorman	**0**	3.7x10^4	6.8x10^4	1.8x10^5	2.0x10^5	7.8x10^4
	25	+	+	++	+	+
	50	+	++	++	+	+
Proxitane	**0**	*6.8x10^4*	*1.8x10^5*	*2.0x10^5*	*7.8x10^4*	
	50	++	+	++	++	
	100	++	+	++	++	
	150	++	++	Nt	Nt	
Liquid Pool Chlorine	**0**	*6.8x10^4*	*2.0x10^5*	*7.8x10^4*		
	50	++	Nt	+		
	100	Nt	+	++		
	500	++	Nt	Nt		
	1000	Nt	++	Nt		
	5000	++	Nt	Nt		
Sporekill	**0**	*2.0x10^5*	*7.8x10^4*			
	100	-	-			
	1000	++	++			
	1500	++	Nt			
Kasugamycin	**0**	8.8x10^4	6.5x10^4	4.5x10^4		
	10	-	Nt	nt		
	50	Nt	Nt	-		
	100	-	-	-		
	1000	-	-	-		

Table 6 Incidence and severity of soft rot developing in tubers which were at temperatures above and below the temperature of the inoculum wash water.

Exp & Date	Cv	Inoc level	Water temp	Tuber temp	Incidence %	Severity
Exp 1	Rubylou	10	15	5	10	0.2
			15	10	10	0.25
			15	15	5	0.1
			15	20	5	0.1
			15	25	0	0
Exp 2 12/10/01	Nadine	10^4	19.1	7.4	95	2.2
			19.1	10	96.3	2.5
			18.3	18.6	77.8	1.6
			18.3	24.2	78.6	1.6
Exp 3 26/10/01	Coliban	10^4	5	5	36.8	0.6
			10	16	94	3.2
			15	16	82.5	3.3
			25	15	86.45	3.4
Exp 4 6/11/01	Nadine	10^3	5	1.7	73	1.7
			15	1.7	83	2.7
			15	9.4	100	3.5
			15	15	100	3.5
			15	20	79	3.6
			15	24	100	4.9
			25	24	90	3.7
Exp 5 20/11/01	Nadine	10	10	5		
			15	5		
			20	5		
			10	10	93	3.9
			15	10	86	3.6
			20	10	56	2.2

Table 6 (cont) Incidence and severity of soft rot developing in tubers which were at temperatures above and below the temperature of the inoculum wash water.

Exp & Date	Cv	Inoc level	Water temp	Tuber temp	Incidence %	Severity
Exp 6	Nadine	10^3	10	5	90	1.4
27/11/01			15	5	50	0.5
			20	5	58	0.8
			10	10	31	0.4
			15	10	55	0.7
			20	10	100	2.1
Exp 7	Coliban	10^4	5	10	100	4.1
7/06/02			10	10	80	2.9
			15	10	66	2.8
			10	15	50	1.6
			15	15	70	2.6
			20	15	100	3.7
			15	20	70	2.8
			20	20	93	3.9
			25	20	73	2.8
			20	25	93	3.9
			25	25	63	2.4
			30	25	53	1.9

APPENDIX 3: TECHNOLOGY TRANSFER

1. Abstracts/posters of conference proceedings

- Australasian Plant Pathology Society, 12th Biennial Conference Canberra, 27-30 September 1999

- Australian Potato Research, Development and Technology Transfer Conference, Adelaide, 31st July to 3rd August 2000

- Triennial Conference of European Association for Potato Research, Hamburg, Germany, July 14 – 19 2002

- 8th International Congress of Plant Pathology (incorporating 14th biennial Australasian Plant Pathology Conference) Christchurch New Zealand 2nd-7th February, 2003

2. Potato Australia Articles

- September, 1999. "Managing bacteria breakdown in washed potatoes"
- September, 2000. "Managing bacteria breakdown in washed potatoes"
- September, 2001. "Managing bacteria breakdown in washed potatoes"
- September, 2002. "Managing bacteria breakdown in washed potatoes"

3. Exposing *Erwinia* Newsletters

- Issue 1 Introduction
- Issue 2 Survey Results
- Issue 3 Efficacy of sanitisers on inoculated demineralised water or potato wash water
- Issue 4 Effect of tumbler or final rinse sanitisers or air drying treatments on infected tubers

- Issue 5 Effect of ultraviolet and microwave irradiation on infected tubers
- Issue 6 Ultraviolet sanitation of naturally infected recycled potato wash water