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Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes

Robert Holmes
Agriculture Victoria

Project Number: VX99004

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Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes

Final Report

Horticulture Australia Limited

Project VX99004 (31st January 2003)

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Purpose: This project report:

1. clarifies regulatory requirements;
2. provides comparative performance data and describe the factors influencing the performance of sanitisers;
3. discusses the implications for washwater re-use and safe discharge with the objective of assisting growers and packers better manage postharvest sanitation.

This project was funded by the Victorian Department of Primary Industries, the AusVeg levy, Horticulture Australia Limited, Queensland Fruit and Vegetable Growers (QFVG)– Tomato Sectional Group Committee; Northern Victoria Fresh Tomato Growers Association (NVFTGA); Avis Chemicals Pty Ltd, Wobelea Pty Ltd and Bioteq Ltd.

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Media Summary

With globalisation of the fresh vegetable trade and consumer demand for clean, safe and fresh foods, there is strategic opportunity for Australian producers to capitalise on our clean and green image to access higher-value, discriminating markets. Growers generally recognise that a washing step can enhance the market quality of many fresh vegetables and in a national survey of 5000 vegetable growers, we found that sanitisers are now widely used. Sanitisers are used to reduce postharvest rot and reduce the concentrations of bacteria linked to human disease. When used properly, sanitising chemicals are safe for consumers and the environment. However, many growers indicated they wanted more information on the 'best practice' use of sanitisers and on alternative sanitisers to chlorine.

In response, we have conducted research to help achieve ‘best practice’ hygienic postharvest systems for vegetable and tomato growers. This research has shown the

- comparative effectiveness, safety and registration status of sanitisers;
- principles and practices of effective disinfection; and
- appropriate methods for safe disposal of used sanitisers.

Our research demonstrated that sanitisers destroy up to 99% of harmful bacteria on fresh produce and totally eliminate bacterial and fungal pathogens from wash water. However, the performance of washing systems depends on the quality of the supply water and the ability to control water quality in recirculated systems. In particular, growers and packers may need to consider controlling the temperature, pH and organic load in their wash systems.

Another aspect of the project was to identify alternatives to chlorine and formalin as water and surface disinfectants respectively. Chlorine dioxide, bromochlorine, ozone, peracetic acid, iodine, quaternary ammonia compounds, and non-chemical treatments were evaluated.

In partnership with manufacturers new hydrocooling and washing systems we developed and evaluated. For many vegetable types, rapid establishment of the cold chain greatly reduces the risk of rots and microbiological contamination.

Workshops were held in five states to encourage the adoption of `best practice’ for hygienic postharvest and growers have been informed of project developments via a newsletter and booklet.

Technical Summary

Growers, packers and other sectors of the vegetable industry recognise that producing fresh, clean and safe products is a priority if markets are to be maintained and developed. Quality assurance schemes have been introduced to help industry consistently manage quality. However, in the area of postharvest hygiene, a lack of knowledge of contamination control and inconsistent interpretation of the regulations dealing with postharvest chemicals has caused a great deal of confusion.

This project report:

1. clarifies regulatory requirements for postharvest chemicals;
2. provides comparative performance data and describe the factors influencing the performance of sanitisers and
3. discusses the implications for washwater re-use and safe discharge with the object of assisting growers and packers better manage postharvest hygiene.

Good Agricultural Practices for hygienic postharvest are described.

A nation-wide survey of growers and packers was conducted to ascertain the state of current practice, establish the main issues of concern and to identify gaps for research and extension. The survey established many growers were using sanitisers and some were using non-chemical controls for spoilage and food-borne diseases, however a large proportion were using unsuitable products and practices and others indicated they were dissatisfied with their controls.

A series of experiments were conducted with the objectives:

- To compare the efficacy of NRA registered and FSANZ approved sanitisers against a range of plant pathogens and *E. coli* in clean water and in water with a standardised organic and mineral content (TGA test).
- Investigate and describe the influences of water pH and temperature on sanitiser effectiveness.
- Compare products for the sanitisation of wood and metal surfaces.

In clean water, reductions of 4 to 6-$\log_{10}$ were achieved in less than 30 seconds in most pathogen sanitiser combinations. Fungi were more resistant to sanitisers than bacteria. In dirty water only peroxyacetic acid (2% v/v) and chlorine dioxide (2.5mg/L) were unaffected by the organic and mineral load and achieved greater than 4-$\log_{10}$ reductions of the more resistant organisms. Efficacy was also dependent on sanitiser concentration, contact time, pH and temperature.

As expected, surfaces were more difficult to sanitise than water. Peroxyacetic acid was the most effective on surfaces. Wood was found to deplete hypochlorite, bromochlorine and quaternary ammonium products. This depletion could be overcome by increasing the sanitiser concentration and the volume of sanitiser solution available to the surface. The addition of compatible surfactants to improve diffusion into microorganisms deserves further study.

Total aerobic (TA) counts on broccoli were reduced by approx 2 $\log_{10}$ following hydrocooling in 5-10 ppm bromochlorodimethyl hydantoin. TA counts were reduced by 90% on radish, spring onion and parsley and 70% on bok choy by washing for 6 min in 100ppm calcium hypochlorite or 15 – 20 ppm iodine. Reductions of coliforms on vegetables were generally above 75%.

The findings were communicated through: presentations at 2 international conferences, 8 workshops held in 6 states and at the Gatton Field Days; Six reports in industry journals, Two editions of the project newsletter and discussions with individual growers.

1 Introduction

Growers, packers and other sectors of the vegetable industry recognise that producing fresh, clean and safe products is a priority if markets are to be maintained and developed. Quality assurance schemes have been introduced to help industry consistently manage quality, however, the large number of schemes, and their diverse requirements has caused a great deal of confusion. This has especially been the case in the area of postharvest treatment, where auditors and customers have been inconsistent in their interpretation of the regulations dealing with postharvest washing.

There is some published knowledge on the influence of concentration, temperature, water pH, hardness and organic content on the power of sanitising agents. However, the impact of these parameters on the control of specific plant pathogens and human pathogens in wash water is poorly understood. Similarly, there is a scarcity of information on the ability of sanitisers to disinfest vegetable surfaces and contact surfaces such as harvesting and handling equipment.

This project report:

- clarifies regulatory requirements;
- provides comparative performance data and describe the factors influencing the performance of sanitisers and
- discusses the implications for washwater re-use and safe discharge with the object of assisting growers and packers better manage postharvest sanitation.

1.1 The Benefits of Washing Vegetables

The postharvest quality of many vegetable types can be improved by washing during preparation for market. The primary purposes of washing are to:

- remove soil, grit and other debris from the vegetables,
- reduce the occurrence of undesirable microbial contaminants and
- clean/sanitise wounds incurred during the harvesting process.

However, these aims are not achieved when the source water is not clean or when used wash water is recycled without appropriate treatment.

Soil and grit left adhering to vegetables is not appreciated by consumers. Washing can therefore enhance saleability. The soil and organic debris also harbour microorganisms including fungi and bacteria that can invade damaged tissues and cause severe rot during postharvest transport and storage (Table 1). The surface of vegetables and at times the internal tissues can also be contaminated by human pathogens (bacteria, viruses, nematodes and protozoans; Table 2). These may derive from the use of uncomposted animal manures or contaminated irrigation and wash water. There have been many outbreaks of disease in humans attributed to microbial contamination of fresh fruits and vegetables (Beuchat and Ryu, 1997, Little et al., 1997, Tauxe et al., 1997).

Table 1. Major postharvest pathogens of vegetables.

Fungi and protists	**Bacteria**
Alternaria spp *Botrytis cinerea* *Colletotrichum spp* *Fusarium spp* *Geotrichum candidum* *Mucor spp* *Penicillium spp* *Phytophthora spp* *Rhizopus spp* *Sclerotinia spp* *Stemphylium spp*	*Erwinia spp* *Xanthomonas campestris* *Pseudomonas spp*

Table 2. Human pathogens isolated from fresh vegetables (J Behrsing and R Premier, unpublished).

Bacteria	**Protozoans and viruses**
Bacillus cereus *Clostridium botulinum* *Listeria monocytogenes* *Salmonella spp.* *Escherichia coli* *Yersinia enterocolitica* *Camphylobacter jejuni* *Shigella spp.* *Staphylococcus aureus*	*Cryptosporidium* *Giardia* *Cyclospora* Hepatitis A Enteroviruses Norwalk virus Rotavirus

While postharvest washing is an important control point for microbial and chemical contamination, it can itself present a risk. Washwater rapidly accumulates soft rot organisms and possibly human pathogens if it is recirculated without sufficient treatment. To minimise contamination, farmers either use a continuous clean water source which may be cost prohibitive or, alternatively, employ an effective water treatment system using one of several classes of sanitising chemicals, heat, or UV irradiation. The discharge of water used for washing also has potential to spread plant disease or contaminate the environment with human pathogens and pesticides. Treatment of used wash water before disposal may therefore be desirable.

Hygienic postharvest practice is an effective strategy to minimise postharvest diseases of vegetables and this is usually achieved by sanitising produce and equipment (Coates and Johnson 1996). There are many sanitisers available, however, there are few objective guidelines to help determine which are the most appropriate for a particular purpose.

1.2 Project Objectives

Year 1

The objective of the first phase was to survey grower practices and establish gaps to help direct the research and extension activities. Experiments concentrated on the comparative efficacy of sanitisers against a range of plant pathogens and *E. coli* in clean water.

Year 2

In the second year, the research investigated the effects of water quality, pH and temperature on sanitiser effectiveness in water and compared sanitisers for disinfection of wood and metal surfaces. A protocol for Good Agricultural Practice for postharvest sanitation processes was described.

Year 3

Experiments in the third year evaluated sanitisers on produce surfaces. The regulatory status of sanitisers was documented and the project findings were delivered to vegetable growers, packers and processors through publications and workshops in five states.

Voluntary Contributor Projects

There were also 4 subprojects conducted for voluntary contributors

1. Efficacy of Phytoclean® on the viability of pathogenic bacteria and fungi (March 2000)
2. Evaluation of a vegetable hydrocooler and sanitation system (January 2001)
3. Alternatives to formalin for the disinfection of tomato stakes and trellising (2001-02)
4. Evaluation of the Iodoclean system for fresh vegetables (November 2002)

2 Investigations

2.1 Regulation of Sanitisers and their use

The international literature was searched to review the current status of biocides potentially suitable for the washing of vegetables and the decontamination of contact surfaces. In the 173 references found, most actives were considered too toxic to gain food approval in Australia. A few actives, however, which were not approved in Australia at the time were identified for further study. Throughout the life of the project there were many changes to registration status of sanitisers in Australia. At the beginning of this project (July 2000) there was only 1 product registered nationally (and a permit for a second in Queensland only) for vegetable washing. At the time of writing this report (January 2003) there were five products registered. In August 2002 the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) published a guide to help clarify whether a product used for postharvest treatment requires NRA registration and/or approval from Food Standards Australia New Zealand (FSANZ formerly ANZFA). This publication is referred to in the following two paragraphs. Quotation marks are used to indicate direct extracts from this publication. Growers, packers and processors now have a range of registered and approved chemicals that can be legally used for the washing of fresh vegetables and contact surfaces.

According to the National Registration Authority (Anon 2002) "The sale or supply of chemicals for use on harvested produce is controlled by two separate legislative codes – the Agvet Codes, regulated by the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) and the Food Standards Code, regulated by" FSANZ. "The NRA regulates the chemicals sold or supplied for the purpose of controlling a pest or disease on the harvested produce whereas" FSANZ "regulates the sale of treated food." The use of agricultural chemicals is controlled by state legislation.

Sanitisers and disinfectants for the postharvest washing of vegetables are considered to be an agricultural chemical product if "represented, imported, manufactured supplied or used as a means of directly or indirectly: Destroying, stupefying, repelling, inhibiting the feeding of, or preventing infestation by or attacks of, any pest in relation to a plant, place or thing;" The definition of 'pest' in the previous sentence includes "spoilage causing pathogens that may be on or contained within the produce, or develop on or within the produce during storage", but not human pathogens eg. *"Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella spp*." "Therefore, products used to control 'pests' that attack harvested produce, are considered to be agricultural chemicals and require registration under the Agvet Codes before they can be lawfully supplied, distributed or sold anywhere in Australia."

"To determine whether a product is a post harvest treatment requiring registration by the NRA, the following factors are taken into consideration:

- Intended uses,
- How the product is represented eg. label claims, advertising statements and
- Product composition, specifically the presence of constituents with known or implied antimicrobial and/or biocidal effects.

A product will not require NRA registration if it is specifically exempted from registration (see Table 3), or not defined as an agricultural chemical by the Agvet Codes, even if a constituent is recognised as a biocide." An example specific to postharvest treatment follows: "a product containing a recognised biocide such as calcium hypochlorite sold for the purpose of being used in packing shed with the label claim that it controls spoilage causing organisms on food would be an agricultural product and would require registration. However, a calcium hypochlorite product used only as a food processing aid or additive (as defined in the Food Standards Code, Standard 1.3.3) to control

organisms that do not contribute to the deterioration of the harvested produce but may be present on or in the produce would not require registration with the NRA."

Some Postharvest Treatments that DO require Registration with the NRA (Anon 2002)

Products claiming:

Sanitisation of fruits and vegetables, for use in food and beverage manufacturing industries (depending on target organisms, ie. is the target a 'pest')

Bactericides and/or fungicides used in washing and packing facilities (on produce)

Water disinfectants (for the control of plant pathogens).

Products that DO NOT require Registration with the NRA (Anon 2002)

Disinfectants for surfaces and equipment used in the food processing industry

Food Additives (require FSANZ approval)

Detergents

Postharvest Treatments that may require Registration with the NRA and approval from FSANZ

Products that claim control of both plant and human pathogens.

Conclusion

Growers and QA auditors should be aware of the legislation quoted above (Anon 2002) and should note that chemicals supplied for postharvest washing of vegetables, which claim to **control spoilage organisms,** either, on the label or in the advertising material, are required to be registered with the NRA.

There are now many general-purpose sanitisers excluded from the requirements of NRA approval (exempt). These may be suitable for use on foods or for washing down equipment if they are approved for that purpose by Food Standards Australia New Zealand (see website http://www.foodstandards.gov.au/foodstandardscode/).

In Victoria only, "control-of-use legislation" means that off label uses of registered products may be legal.

Table 3 NRA registered vegetable sanitisers and exempt active ingredients for which there is a FSANZ Maximum Permitted Level (MPL).

Active ingredient	**NRA Registration?**	**Application rate**	**FSANZ MPL**	**Product examples**
BCDMH	***Yes***	*5-15 mg/L*	*1 mg/kg available chlorine* *1 mg/kg inorganic bromine* *2 mg/kg dimethyl hydantoin*	*YM-FAB Nylate (Wobelea)*
Calcium hypochlorite	***Yes***	*40-80 mg/L*	*1 mg/kg available chlorine*	*YM-FAB Activ 8 (Wobelea)* *Hypochlor chlorine cartridge (Klorman)*
Calcium hypochlorite	*Exempt*		*1 mg/kg available chlorine*	
Chlorine dioxide	***Yes***	*5 mg/L*	*I mg/kg chlorine dioxide* *I mg/kg sodium chlorite*	*Vibrex Horticare (Tecnica)*
Chlorine dioxide	*Exempt*		*1 mg/kg available chlorine*	*Oxine*
Hydrogen peroxide	*Exempt*		*5mg/kg*	*Virkon S*
Iodine	***Yes***	*3-30 mg/kg*	*NA*	*Biomax A iodine granules (Bioteq)*
Ozone	*Exempt*		*GMP*	
Peroxyacetic acid	*Exempt*		*GMP*	*Peratec 5* *Tsunami*
Sodium Hypochlorite	*Exempt*		*1 mg/kg available chlorine*	

GMP – Good Manufacturing Practice
NA – Not available at time of publication

2.2 Survey of Grower Practice and GAP analysis

With assistance from Vegetable Industry Development Officers in Victoria, Queensland, Tasmania, South Australia and West Australia, a survey form was mailed to about 5,000 growers and packers nationwide (see appendix 1 for survey form). Over 400 survey responses were received and this response rate is considered more than satisfactory for this survey method.

The principal findings were:

- 73% of respondents wash or handle at least some of their produce in water.
- 27% add a sanitiser to their water (mainly chlorine-based agents).
- 80% believe they need to clean handling equipment, however, only
- 50% believe they have an effective method for cleaning dirty surfaces.
- Most respondents requested to be placed on the mailing list for the project newsletter to receive more information on the topic.

Chemical sanitisers used by the respondents fall into the following categories:

- Registered with the National Registration Authority.
- Approved as food processing aids by FSANZ and approved food ingredients eg vinegar (2 respondents)
- exempt from NRA registration (eg chlorine dioxide manufactured on site from two non-agricultural chemicals)
- detergents and sanitisers used in the food industry to clean food contact surfaces (Listed in 'Draft Australian Standard - Guide to cleaning and sanitising plant and equipment in the food industry').
- products in none of the above categories which have no permitted residue under the FSANZ Food Standards Code.
- A small number of growers use non-chemical cleaning methods for equipment eg. vacuuming (2 respondents) and steaming (2 respondents).

A large number of growers acknowledge the need to clean handling equipment. However, only half of the respondents believe they have an effective method for cleaning surfaces. This is considered a gap in current practices and research was planned to investigate effective methods for cleaning dirty surfaces.

2.3 Sanitiser Evaluations

2.3.1 Introduction

A series of experiments were conducted with the objectives:

- To compare the efficacy of sanitisers against a range of plant pathogens and *E. coli* in clean water and in water with a standardised organic and mineral content (TGA test).
- Investigate and describe the influences of water pH and temperature on sanitiser effectiveness.
- Compare sanitisers for the sanitisation of wood and metal surfaces.
- Describe a protocol for Good Agricultural Practice for postharvest sanitation.

2.3.2 Materials and Methods

Pathogen cultures

The following plant pathogenic fungi and bacteria were used as test organisms: *Mucor* spp, *Penicillium* spp, *Geotrichum candidum*, *Xanthomonas campestris* pv *campestris*, *Pseudomonas syringae* pv. *syringae*, *Clavibacter michiganensis* subsp. *michiganensis*. *E. coli*, a common indicator of faecal contamination was also used, to represent human pathogenic bacteria.

Fungi were maintained at 21°C on Potato Dextrose Agar (PDA). Stock inoculum was prepared by washing five to ten day old cultures with sterile purified water. Concentrations were counted using a haemocytometer and adjusted to approximately $1x10^6$ spores/ml.

Bacteria were maintained at 21°C on Nutrient Agar (NA). Cell suspensions were prepared from three to five day old cultures, enumerated by absorbance (Hach 2010 spectrophotometer) and adjusted to achieve approximately $1x10^6$ cells/ml.

Sanitisers

Sanitisers chosen for evaluation include the active ingredients of the registered products; bromo-chloro-dimethyl hydantoin (BCDMH) and calcium hypochlorite ($Ca(OCl)_2$); the exempt active, chlorine dioxide (ClO_2) and the Food Standards approved actives peroxyacetic acid (PAA) and benzalkonium chloride (QAC). Concentrations of active ingredients were determined by spectrophotometry (Hach).

Sanitisation of clean and dirty water

Sanitiser efficacy tests were adapted from the published methods of the Association of Official Analytical Chemists (AOAC 1984) and the Therapeutic Goods Administration (Graham 1978). Inoculum (1 millilitre of cell/ spore suspension) was added to 99ml of sanitiser solutions at various concentrations. After 30, 60, 90, 120 and 240 seconds, 0.1ml of this solution was extracted and added to a microcentrifuge tube containing 0.9ml of deactivator solution (0.1N sodium thiosulphate and 10% v/v Ecoteric T80). The control was sterile de-ionised water (SDW) in place of the sanitiser and was extracted at 240 seconds only. A sample of the reacted product (0.1 ml) was spread-plated onto NA for bacteria or PDA for fungi. The procedure was repeated 3 times for each sanitiser. Plates were incubated and colonies counted after 72 hours, except for *M. piriformis* that was counted after 24 hours and *C. michiganensis* that was counted after 5 days.

Sanitisers were trialed at half, single and double 'label' rates. All treatments were duplicated in 'dirty water' containing a standard water hardness and 5% inactivated baker's yeast (Graham, 1978). pH was buffered at 5.5, 7.0 and 8.5 with 0.2M NaH_2PO_4 and 0.2M Na_2HPO_4. All reactions were conducted at 4°C, 20°C and 30°C.

Handling equipment surface disinfestation

The national grower survey on sanitation identified a need for an effective method for cleaning surfaces and handling equipment. Three substrates were used; aluminium, and smooth-planed wood (*Pinus radiata*) and rough-sawn wood (*Eucalyptus camaldulensis*). Materials were cut into 5 x 5cm coupons. Wood was autoclaved, whereas metal was surface sterilised with 70% ethanol. Metal coupons were inoculated on one face (25 cm^2) with 100µl of $1x10^4$ cells/ml spread with a glass rod. Evaluations were conducted against the following fungi and bacteria *Clavibacter michiganensis* (Bacterial canker), *Geotrichum candidum* (Sour rot), *Mucor piriformis* (Mucor rot), *Xanthomonas campestris* (Bacterial spot, soft rot), *Pseudomonas syringae* (Bacterial speck, soft rot), *Penicillium expansum* (Blue mould rot) and *Escherichia coli* (a food safety indicator). Three coupons were placed in stainless steel trays containing 150ml sanitiser and removed after 1, 5 and 20 minutes. The metal was directly plated onto NA or PDA plates that were flooded with 1ml of the deactivator. Plates were air dried for 30 minutes and then incubated at 21°C. Wood coupons were inoculated on one face with 200µl of $1x10^4$ cells/ml and pressed onto plates flooded with 2ml of the deactivator.

Resulting colonies were counted after approximately 72 hours. Differences in the efficacy of the sanitisers were analysed by ANOVA (Genstat 5 for Windows, Lawes Agricultural Trust, Rothamsted for Windows).

2.3.3 Results and Discussion

Sanitiser effectiveness in clean and dirty water

In clean water, reductions of 4 to 6-$\log_{10}$ were achieved in less than 30 seconds in most pathogen sanitiser combinations. Fungi were more resistant to sanitisers than bacteria (Tables 4a-4d). Gram-positive bacteria eg *C. michiganensis* , are likely to be more susceptible to some sanitisers than Gram-negative species (Prince *et al.* 1993). However, the experimental design did not allow statistical comparisons between species and we were therefore unable to establish this. In dirty water (TGA test), where kill rates were slower, increasing the concentration of sanitiser was required to achieve better than 4-$\log_{10}$ reductions (Tables 5a-5d; Figure 1, *Geotrichum* and BCDMH data shown). Dirt decreased the rate of pathogen reduction at the lower concentrations of BCDMH, calcium hypochlorite and peroxyacetic acid (Figure 2, *C. m. michiganensis* and BCDMH data shown). The performance of chlorine dioxide (2.5 mg/L) was unaffected by the TGA conditions. In dirty water, 6-$\log_{10}$ reductions were achieved within 4 minutes for all organisms (except *Mucor*) at 60mg/L of hypochlorite, 2.5mg/L of chlorine dioxide, 2% v/v peroxyacetic acid and 10mg/L of BCDMH. Only peroxyacetic acid (2% v/v) and chlorine dioxide (2.5mg/L) achieved greater than 4-$\log_{10}$ reductions of *Mucor* in dirty water.

In both clean and dirty water, sanitiser efficacy was proportionate to sanitiser concentration. While many sanitisers reduced pathogen counts more rapidly at double the label rate, most performed adequately at the label rate. The sanitisers that did not achieve better than 2-$\log_{10}$ reductions in dirty water within 4 minutes contact time were calcium hypochlorite against *Mucor* and *G. candidum*, BCDMH against *Mucor* and Peroxyacetic acid against *Mucor.* As increasing contact time beyond 4 minutes would be impractical, increased amounts of sanitiser would need to be added to overcome the demand of the water hardness and organic load. Dirty water contains substances that interfere with chlorination and bromochlorination for example ammonia, amino acids and calcium carbonate (Bessems, 1998,White, 1999). These substances create a 'chlorine demand' and only once this initial demand is met does free available chlorine (the main biocidal compound) occur. Chlorinating until the chlorine demand is satisfied is known as ‘breakpoint chlorination’ (Dychdala, 1977). Sanitation systems that automatically deliver hypochlorites (including BCDMH) would be expected to maintain effective levels of the sanitiser above the chlorine demand of the water. Alternatively, water can be treated to reduce impurities before the sanitiser is added, or a sanitiser, which is more affective in dirty water, could be used. Increasing concentrations of sanitisers without prior water treatment can prove costly and lead to increased corrosion, pollution or worker discomfort. The extent of these problems would depend on the ‘chlorine’ demand of the source water.

Current data from researchers, manufacturers and regulators indicate approximately 50mg/L of free available chlorine (from calcium hypochlorite), 5mg/L of chlorine dioxide, 0.5% peroxyacetic acid and 5-10mg/L free chlorine equivalents (fce, from BCDMH) were effective rates in clean wash water.

When hypochlorites are dissolved in water they dissociate into two main compounds, hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-). The relative abundance of each compound depends on the pH of the water. At low pH, hypochlorous acid, the more biocidal product, predominates (White 1999). In this study, as expected (Segall 1968), hypochlorite-based sanitisers were more effective at low pH (Tables 6a – 6d, Figure 3). This indicates that in some instances, acidification of alkaline wash water, (eg. using citric acid) could improve the efficiency of chlorination. At the concentrations used, chlorine dioxide and peroxyacetic acid performed well over the 5.5 to 8.5 pH range, however, both are known to be more biocidal at low pH (White 1999).

The performance of sanitisers was greatest at the higher temperature, as expected (Sabaa-Srur *et al.* 1993). Kill rates at 20°C and 30°C were similar, however, at 4°C kill rates were significantly lower. For example the time required for BCDMH to completely kill *Mucor* was 60, 90 and 240 seconds at 30, 20 and 4°C respectively (Table 7, Figure 4). This demonstrates that in cold water eg, in hydrocoolers, the contact time needs to be prolonged.

Table 4a. Effect of exposure time and concentration of hypochlorite (free available chlorine, fac) on the survival of pathogens (cfu/ml) in clean water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***Calcium hypochlorite concentration (mg/L fac)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor sp.	*0*					*22.0^{d}*
	5	*19.0^{cd}*	*17.3^{cd}*	*16.3^{cd}*	*15^{c}*	*6.0^{ab}*
	10	*21.3^{d}*	*17.7^{cd}*	*14.3^{c}*	*13.7^{bc}*	*9.0^{b}*
lsd 5.1	*20*	*16.0^{c}*	*18.3^{cd}*	*16.0^{c}*	*14.0^{b}*	*3.7^{a}*
Geotrichum candidum	*0*					*43.3^{d}*
	5	*36.3^{c}*	*29.7^{bc}*	*33.7^{bc}*	*28.7^{b}*	*11.0^{a}*
	10	*34.3^{c}*	*33.0^{bc}*	*35.3^{c}*	*24.0^{b}*	*9.7^{a}*
lsd 9.8	*20*	*32.3^{bc}*	*26.3^{b}*	*31.0^{bc}*	*24.3^{b}*	*3.3^{a}*
E. coli	*0*					*497*
	5	*0*	*0*	*0*	*0*	*0*
	10	*0*	*0*	*0*	*0*	*0*
lsd n/a	*20*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*862.3*
	5	*0*	*0*	*0*	*0*	*0*
	10	*0*	*0*	*0*	*0*	*0*
lsd n/a	*20*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*222.7*
	5	*29.7*	*0*	*0*	*0*	*0*
	10	*0*	*0*	*0*	*0*	*0*
lsd n/a	*20*	*0*	*0*	*0*	*0*	*0*

Table 4b. Effect of exposure time and concentration of chlorine dioxide on the survival of pathogens (cfu/ml) in clean water. For species where all values are zero, a lsd is not applicable (n/a).

Species	***Chlorine dioxide concentration (mg/L)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor sp.	*0*					*33.3*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Geotrichum candidum	*0*					*48.3*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
E. coli	*0*					*401.7*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*455.3*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*841*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*

Table 4c. Effect of exposure time and concentration of peracetic acid on the survival of pathogens (cfu/ml) in clean water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***Peracetic acid concentration (%)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor sp.	*0*					61.7^{e}
	0.5	42.3^{cd}	48.0^{d}	46.7^{d}	46.0^{d}	43.3^{cd}
	1	43.3^{cd}	44.3^{cd}	48.3^{d}	38.0^{cd}	20.3^{b}
lsd 12.3	*2*	48.3^{d}	42.3^{cd}	32.7^{c}	17.0^{b}	0^{a}
Geotrichum candidum	*0*					75.0^{cd}
	0.5	94.3^{d}	100.0^{d}	104.7^{d}	82.7^{cd}	62.0^{c}
	1	93.7^{d}	76.3^{cd}	61.3^{c}	37.3^{b}	2.7^{a}
lsd 21.8	*2*	63.3^{c}	15.3^{a}	3.3^{a}	0^{a}	0^{a}
E. coli	*0*					*844.3*
	0.5	*405.3*	*28.7*	*0*	*0*	*0*
	1	*14.3*	*0*	*0*	*0*	*0*
lsd n/a	*2*	*1*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*757.7*
	0.5	*262.7*	*58.3*	*0*	*0*	*0*
	1	*53.3*	*0*	*0*	*0*	*0*
lsd n/a	*2*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*291*
	0.5	*0*	*0*	*0*	*0*	*0*
	1	*0*	*0*	*0*	*0*	*0*
lsd n/a	*2*	*0*	*0*	*0*	*0*	*0*

Table 4d. Effect of exposure time and concentration of BCDMH (free chlorine equivalents) on the survival of pathogens (cfu/ml) in clean water. For species where all values are zero, a lsd is not applicable (n/a).

Species	***BCDMH concentration (mg/L)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor sp.	*0*					*15.7*
	2.5	*11.7*	*1.3*	*0*	*0*	*0*
	5	*1.3*	*1*	*2.7*	*0.7*	*0*
lsd n/a	*10*	*0.7*	*0*	*0*	*0*	*0*
Geotrichum candidum	*0*					*24*
	2.5	*1.3*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
E. coli	*0*					*178.3*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*595.7*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*48*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*

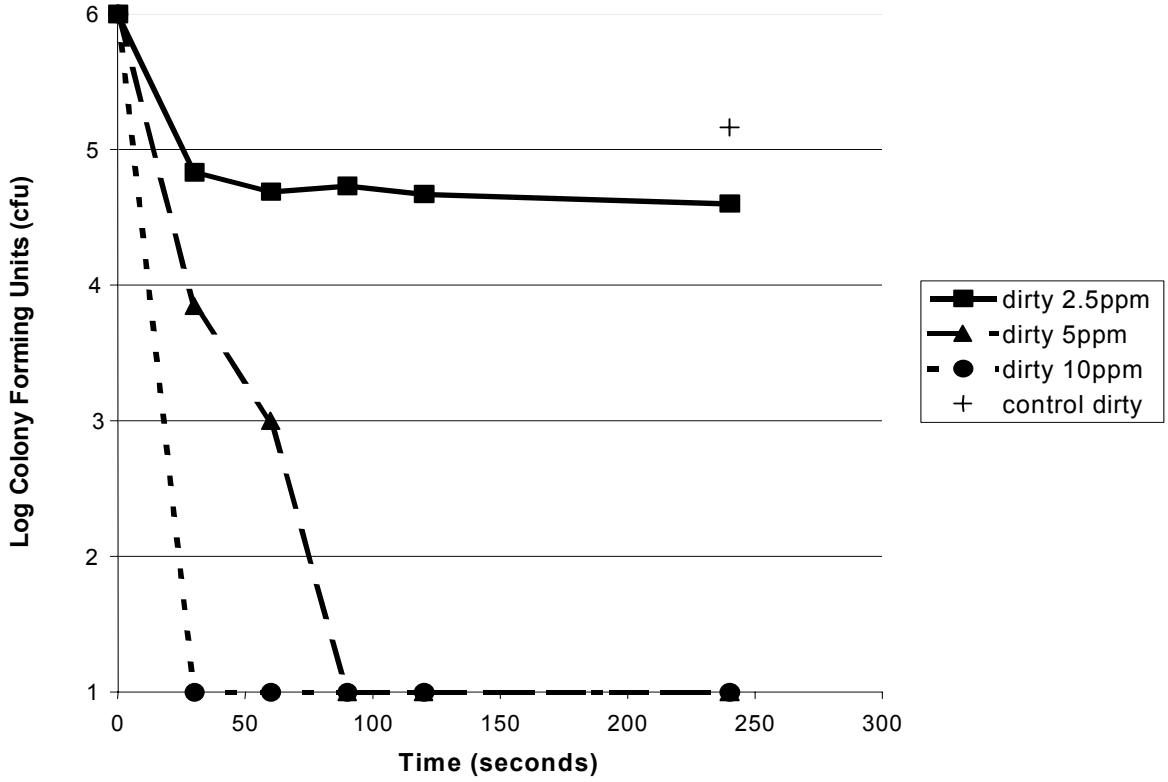

Figure 1: Effect of exposure time and sanitiser concentration on the efficacy of BCDMH (fce) against *G. candidum* in dirty water

Table 5a. Effect of exposure time and concentration of hypochlorite (free available chlorine, fac) on the survival of pathogens (cfu/ml) in dirty water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***Calcium hypochlorite concentration fac(mg/L)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor sp.	*0*					*14.3^{b}*
	5	*12.3 ^{b}*	*11.7^{b}*	*10.7^{ab}*	*12.0^{b}*	*9.0^{ab}*
	10	*11.7^{b}*	*10.3^{ab}*	*13.7^{b}*	*13.7^{b}*	*8.7^{ab}*
lsd 5.1	*20*	*14.3^{b}*	*9.7^{ab}*	*12.0^{b}*	*9.0^{ab}*	*5.0^{a}*
Geotrichum candidum	*0*					*16.0^{c}*
	5	*10.7^{b}*	*9.0^{b}*	*9.7^{b}*	*8.0^{b}*	*7.3^{b}*
	10	*9.3^{b}*	*4.7^{ab}*	*5.0^{ab}*	*8.7^{b}*	*5.0^{ab}*
lsd 5.9	*20*	*10.3^{b}*	*10.3^{b}*	*7.0^{b}*	*8.0^{b}*	*0.3^{a}*
E. coli	*0*					*178.7*
	5	*0*	*0*	*0*	*0*	*0*
	10	*0*	*0*	*0*	*0*	*0*
lsd n/a	*20*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*311.3*
	5	*0*	*0*	*0*	*0*	*0*
	10	*0*	*0*	*0*	*0*	*0*
lsd n/a	*20*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*163*
	5		*0*	*0*	*0*	*0*
	10	*0*	*0*	*0*	*0*	*0*
lsd n/a	*20*	*0*	*0*	*0*	*0*	*0*

Table 5b. Effect of exposure time and concentration of chlorine dioxide on the survival of pathogens (cfu/ml) in dirty water. For species where all values are zero, a lsd is not applicable (n/a).

Species	***Chlorine dioxide concentration (mg/L)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor *sp.*	*0*					*17.7*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Geotrichum candidum	*0*					*48.3*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
E. coli	*0*					*266.3*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*397.3*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*418.7*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*

Table 5c. Effect of exposure time and concentration of peracetic acid on the survival of pathogens (cfu/ml) in dirty water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***Peracetic acid concentration (%)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor sp.	*0*					42.7^{e}
	0.5	36.7^{de}	36.3^{de}	36.7^{de}	33.0^{d}	32.3^{d}
	1	37.7^{de}	36.3^{de}	34.0^{d}	29.67^{d}	15.7^{bc}
lsd 8.7	*2*	42.7^{e}	24.7^{cd}	18.3^{c}	9.00^{b}	0^{a}
Geotrichum candidum	*0*					54.7^{d}
	0.5	62.7^{d}	63.0^{d}	56.3^{d}	48.3^{c}	42.3^{c}
	1	53.7^{cd}	55.7^{d}	39.7^{c}	22.3^{b}	0.3^{a}
lsd 14.1	*2*	34.0^{b}	13.7^{a}	2.0^{a}	0^{a}	0^{a}
E. coli	*0*					*570.3*
	0.5	*292.3*	*91.3*	*0.3*	*0*	*0*
	1	*48.0*	*0.6*	*0*	*0*	*0*
lsd n/a	*2*	*1*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*571.3*
	0.5	*416.0*	*197.0*	*17.7*	*0*	*0*
	1	*193.0*	*0*	*0*	*0*	*0*
lsd n/a	*2*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*272.0*
	0.5	*0*	*0*	*0*	*0*	*0*
	1	*0*	*0*	*0*	*0*	*0*
lsd n/a	*2*	*0*	*0*	*0*	*0*	*0*

Table 5d. Effect of exposure time and concentration of BCDMH (free chlorine equivalents) on the survival of pathogens (cfu/ml) in dirty water. For species where all values are zero, a lsd is not applicable (n/a).

Species	***BCDMH concentration (mg/L)***	***Exposure time (seconds)***				
		30	***60***	***90***	***120***	***240***
Mucor sp.	*0*					*9.0*
	2.5	*10.7*	*6.3*	*5.3*	*5.0*	*7.0*
	5	*5.0*	*4.7*	*6.3*	*11.0*	*6.7*
lsd n/a	*10*	*7.5*	*6.0*	*5.0*	*5.0*	*6.0*
Geotrichum candidum	*0*					*145*
	2.5	*67.7*	*49.0*	*54.7*	*46.7*	*40.3*
	5	*7.3*	*1.3*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
E. coli	*0*					*86.7*
	2.5	*0*	*0*	*0*	*0*	*0*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*0*					*276*
	2.5	*38.3*	*11.0*	*4.7*	*2.3*	*0.3*
	5	*0*	*0*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*0*					*33.7*
	2.5	*7.3*	*1.3*	*0*	*0*	*0*
	5	*2.7*	*0.3*	*0*	*0*	*0*
lsd n/a	*10*	*0*	*0*	*0*	*0*	*0*

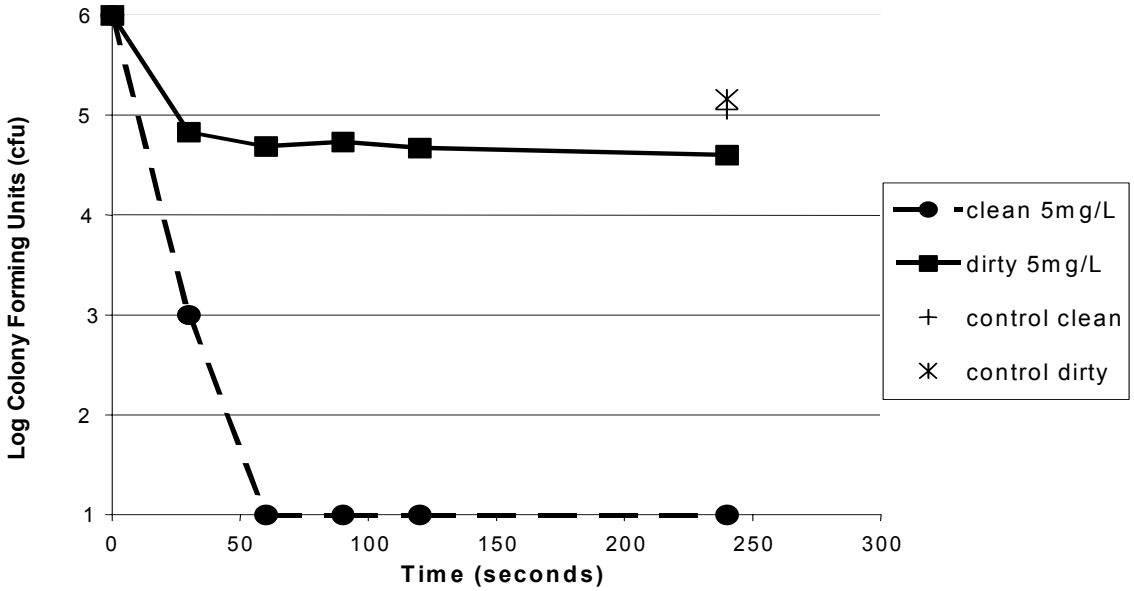

Figure 2: Effect of dirty water on the efficacy of BCDMH (5 mg/L fac) against *C. m. michiganensis*

Table 6a. Effect of pH and exposure time for calcium hypochlorite (30 ppm free available chlorine) on the survival of pathogens (cfu/ml) in water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***pH Water***	***pH Calcium hypochlorite***	***Exposure time (seconds)***				
			30	***60***	***90***	***120***	***240***
Mucor sp.	*5.5* *7* *8.5*						*46* *52* *48*
		5.5	*26.3^{c}*	*18.0^{bc}*	*4.7^{ab}*	*0.33^{a}*	*0^{a}*
		7	*40.0^{d}*	*24.7^{c}*	*3.7^{ab}*	*0^{a}*	*0^{a}*
lsd 9.6		*8.5*	*46.3^{d}*	*31.3^{c}*	*11.7^{b}*	*2.0^{a}*	*0^{a}*
Geotrichum candidum	*5.5* *7* *8.5*						*77* *81* *81*
		5.5	*10.3^{b}*	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
		7	*36.0^{c}*	*1.3^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
lsd 4.7		*8.5*	*63.7^{d}*	*40.0^{c}*	*15.3^{b}*	*0^{a}*	*0^{a}*
E. coli	*5.5* *7* *8.5*						*257* *163* *284*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*5.5* *7* *8.5*						*165* *190* *189*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*5.5* *7* *8.5*						*44* *53* *42*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*

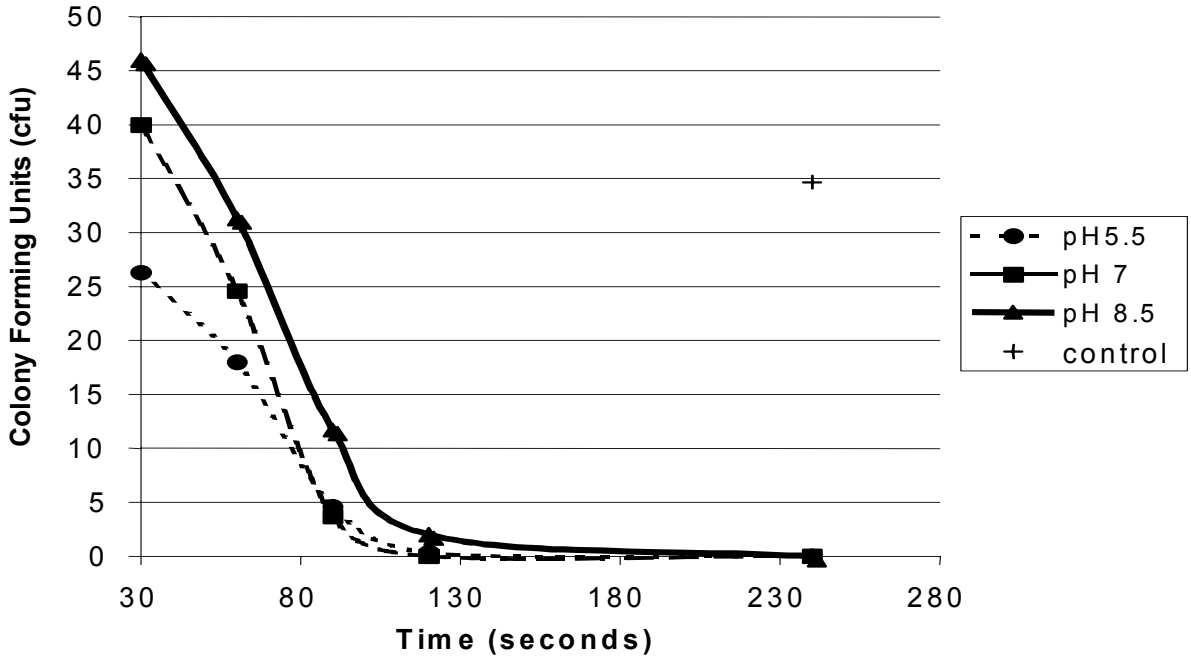

Figure 3. Effect of pH on the efficacy of calcium hypochlorite (30 ppm) against *Mucor* sp.

Table 6b. Effect of pH and exposure time for chlorine dioxide (5ppm) on the survival of pathogens (cfu/ml) in water. For species where all values are zero, a lsd is not applicable (n/a).

Species	***pH Water***	***pH chlorine dioxide***	***Exposure time (seconds)***				
			30	***60***	***90***	***120***	***240***
Mucor sp.	*5.5* *7* *8.5*						*20* *20* *23*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
Geotrichum candidum	*5.5* *7* *8.5*						*79* *77* *105*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
E. coli	*5.5* *7* *8.5*						*224* *46* *76*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*5.5* *7* *8.5*						*155* *161* *190*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*5.5* *7* *8.5*						*39* *36* *44*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*

Table 6c. Effect of pH and exposure time for peracetic acid (1%) on the survival of pathogens (cfu/ml) in water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***pH Water***	***pH Peracetic acid***	***Exposure time (seconds)***				
			30	***60***	***90***	***120***	***240***
Mucor sp.	*5.5* *7* *8.5*						*53* *47* *30*
		5.5	*32.0^{cd}*	*27.0^{cd}*	*23.7^{c}*	*12.3^{b}*	*0.7^{a}*
		7	*44.3^{de}*	*32.3^{cd}*	*27.7^{cd}*	*23.3^{c}*	*2.0^{ab}*
lsd 10.7		*8.5*	*10.3^{b}*	*10.3^{b}*	*7.0^{b}*	*8.0^{b}*	*0.3^{a}*
Geotrichum candidum	*5.5* *7* *8.5*						*84* *72* *97*
		5.5	*67.3^{cd}*	*61.3^{cd}*	*48.3^{bc}*	*41.3^{bc}*	*7.3^{a}*
		7	*73.7^{d}*	*75.7^{d}*	*54.7^{c}*	*39.0^{b}*	*3.0^{a}*
lsd 14.3		*8.5*	*71.3^{d}*	*77.3^{d}*	*65.0^{cd}*	*58.3^{cd}*	*7.7^{a}*
E coli	*5.5* *7* *8.5*						*149* *123* *127*
		5.5	*37.3*	*2.3*	*0*	*0*	*0*
		7	*45.3*	*0.3*	*0*	*0*	*0*
lsd n/a		*8.5*	*45.7*	*0.7*	*0*	*0*	*0*
Clavibacter michiganensis	*5.5* *7* *8.5*						*242* *272* *233*
		5.5	*70.7*	*1.3*	*0*	*0*	*0*
		7	*111.7*	*3.3*	*0*	*0*	*0*
lsd n/a		*8.5*	*90.5*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*5.5* *7* *8.5*						*77* *79* *79*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*7.7*	*0.3*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*

Table 6d. Effect of pH and exposure time for BCDMH (5 ppm free chlorine equivalents) on the survival of pathogens (cfu/ml) in water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***pH Water***	***pH BCDMH***	***Exposure time (seconds)***				
			30	***60***	***90***	***120***	***240***
Mucor sp.	*5.5*						*57*
	7						*69*
	8.5						*37*
		5.5	*10.0^{b}*	*1.0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
		7	*28.0^{c}*	*7.3^{a}*	*0.7^{a}*	*1.0^{a}*	*0^{a}*
lsd 7.9		*8.5*	*32.7^{a}*	*8.0^{a}*	*0.7^{a}*	*0^{a}*	*0^{a}*
Geotrichum candidum	*5.5*						*67*
	7						*71*
	8.5						*83*
		5.5	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
		7	*27.4^{b}*	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
lsd 4.5		*8.5*	*58.0^{c}*	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
E. coli	*5.5*						*161*
	7						*150*
	8.5						*113*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*5.5*						*289*
	7						*205*
	8.5						*203*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*5.5*						*59*
	7						*39*
	8.5						*67*
		5.5	*0*	*0*	*0*	*0*	*0*
		7	*0*	*0*	*0*	*0*	*0*
lsd n/a		*8.5*	*0*	*0*	*0*	*0*	*0*

Table 7. Effect of temperature and exposure time for BCDMH (5 ppm free chlorine equivalents) on the survival of pathogens (cfu/ml) in water. For species where all values are zero, a lsd is not applicable (n/a). Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***Water Temp. (°C)***	***Sanitiser Temp. (°C)***	***Exposure time (seconds)***				
			30	***60***	***90***	***120***	***240***
Mucor sp.	*4*						*57.3*
	20						*52.0*
	30						*75.3*
		4	*69.3^{e}*	*39.0^{d}*	*24.0^{c}*	*9.7^{b}*	*0.3^{a}*
		20	*17.7^{c}*	*1.7^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
lsd 7.6		*30*	*2.0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
Geotrichum candidum	*4*						*103*
	20						*120*
	30						*92*
		4	*60.33^{c}*	*24.0^{b}*	*4.5^{a}*	*0.33^{a}*	*0^{a}*
		20	*27.4^{b}*	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
lsd 12.4		*30*	*58.0^{c}*	*0^{a}*	*0^{a}*	*0^{a}*	*0^{a}*
E. coli	*4*						*1080*
	20						*1090*
	30						*1210*
		4	*0*	*0*	*0*	*0*	*0*
		20	*0*	*0*	*0*	*0*	*0*
lsd n/a		*30*	*0*	*0*	*0*	*0*	*0*
Clavibacter michiganensis	*4*						*3420*
	20						*4250*
	30						*3960*
		4	*0*	*0*	*0*	*0*	*0*
		20	*0*	*0*	*0*	*0*	*0*
lsd n/a		*30*	*0*	*0*	*0*	*0*	*0*
Pseudomonas syringae	*4*						*1710*
	20						*1560*
	30						*1160*
		4	*0*	*0*	*0*	*0*	*0*
		20	*0*	*0*	*0*	*0*	*0*
lsd n/a		*30*	*0*	*0*	*0*	*0*	*0*

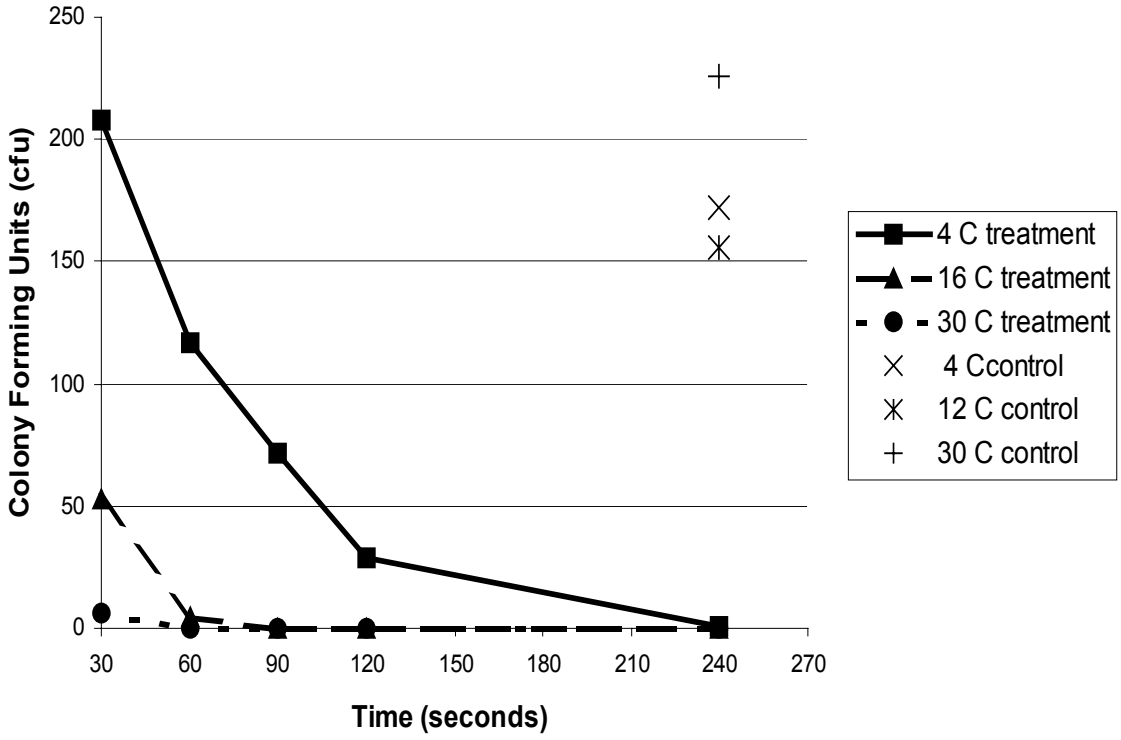

Figure 4. Effect of temperature on efficacy of BCDMH (5 ppm) against *Mucor* sp.

Sanitiser effectiveness on surfaces

Peroxyacetic acid and calcium hypochlorite were the best performing sanitisers against *E. coli* on an aluminium surface, and chlorine dioxide was the poorest (at their recommended label rates). There was no significant difference between water and chlorine dioxide (Table 8, Figure 5).

Peroxyacetic acid was the only sanitiser to achieve better than a 5 $\log_{10}$ reduction against *E. coli* on smooth *P. radiata*. There was no significant difference between chlorine dioxide and water, whereas, BCDMH, calcium hypochlorite and benzylkonium chloride displayed similar sanitising properties at 'label' rates (Figure 6). Peroxyacetic acid was the best sanitiser against *C. michiganensis* on rough-saw*n E. camaldulensis*. Other sanitisers gave similar kill rates, with chlorine dioxide showing the lowest efficacy (Table 9, Table 10).

As expected (Gibson et al. 1995), surfaces were more difficult to sanitise than water. In some cases, ten times the concentration of disinfectant is required to disinfect surfaces compared to water (Van Klingeren et al. 1998). In general, sanitisers had similar performance on wood and aluminium. Peroxyacetic acid was the most effective on surfaces, whereas chlorine dioxide (which was the most effective in water tests) performed poorly. Wood was found to be the more reactive surface, and in some instances sanitisers become ineffective within 5 minutes of contact (Figure 7). We expect this could be overcome by increasing the sanitiser concentration, or the volume of sanitiser solution available to the surface. As with the suspension test, fungi were found more resistant than bacteria to sanitisers on surfaces.

The reductions of *E. coli* achieved on wood and metal surfaces (1–5 $\log_{10}$) are similar to the 1–4 $\log_{10}$ reductions achieved by BCDMH on broccoli after 30 minutes (Harrup and Holmes, unpublished).

Smaller reductions (1.7–2.8 $\log_{10}$) were achieved by calcium hypochlorite on broccoli and lettuce when the contact time was 30 seconds (Behrsing *et al.* 2000).

The efficacy of sanitisers is related to the rate of diffusion of the active agent through the cell wall (White 1999). Therefore, the addition of suitable surfactants to reduce the surface tension on the cell wall could enhance surface sanitation (Kostenbauder 1977). This aspect deserves further study.

Table 8. Efficacy of sanitisers on the survival of common spoilage organisms and *E. coli* on an aluminium surface Tabulated values are square root transformed means of cfu/cm^2. Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***Sanitiser***	***Exposure time (minutes)***		
		1	***5***	***20***
Mucor sp.	*Distilled water*	1.61^{ab}	1.96^{ab}	1.22^{ab}
	Chlorine dioxide	3.21^{b}	1.41^{ab}	1.28^{ab}
	BCDMH	0.94^{a}	0.67^{a}	1.52^{ab}
	Benzylkonium chloride	0.91^{a}	0.33^{a}	0^{a}
	Calcium hypochlorite	0.47^{a}	0^{a}	0.67^{a}
lsd 2.00	*Peracetic acid*	0^{a}	0^{a}	0^{a}
Geotrichum candidum	*Distilled water*	12.0^{b}	1.33^{a}	2.0^{a}
	Chlorine dioxide	3.21^{b}	1.41^{ab}	1.28^{ab}
	BCDMH	2.0^{a}	0.67^{a}	0.33^{a}
	Benzylkonium chloride	1.67^{a}	0^{a}	0^{a}
	Calcium hypochlorite	1.0^{a}	0^{a}	0^{a}
lsd 3.54	*Peracetic acid*	0^{a}	0^{a}	0^{a}
E. coli	*Distilled water*	35.19^{c}	28.35^{c}	22.94^{bc}
	Chlorine dioxide	32.35^{c}	20.7^{bc}	17.05^{b}
	BCDMH	16.7^{b}	7.5^{a}	0.67^{a}
	Benzylkonium chloride	2.1^{a}	2.15^{a}	0^{a}
	Calcium hypochlorite	0^{a}	0^{a}	0^{a}
lsd 7.8	*Peracetic acid*	0^{a}	0^{a}	0^{a}
Clavibacter michiganensis	*Distilled water*	24.0^{c}	19.3^{b}	50.2^{d}
	Chlorine dioxide	1.3^{a}	10.0^{ab}	13.0^{ab}
	BCDMH	0^{a}	0.3^{a}	0^{ab}
	Benzylkonium chloride	0^{a}	0^{a}	0^{a}
	Calcium hypochlorite	0^{a}	1.2^{a}	0^{a}
lsd 15.7	*Peracetic acid*	0^{a}	0^{a}	0^{a}
Pseudomonas syringae	*Distilled water*	11.5^{c}	6.2^{b}	2.24^{ab}
	Chlorine dioxide	4.45^{b}	1.69^{ab}	0.33^{a}
	BCDMH	2.48^{ab}	0^{a}	0.33^{a}
	Benzylkonium chloride	1.33^{a}	0^{a}	0.33^{a}
	Calcium hypochlorite	0.82^{a}	1.25^{a}	0.58^{a}
lsd 2.99	*Peracetic acid*	0^{a}	0^{a}	0^{a}

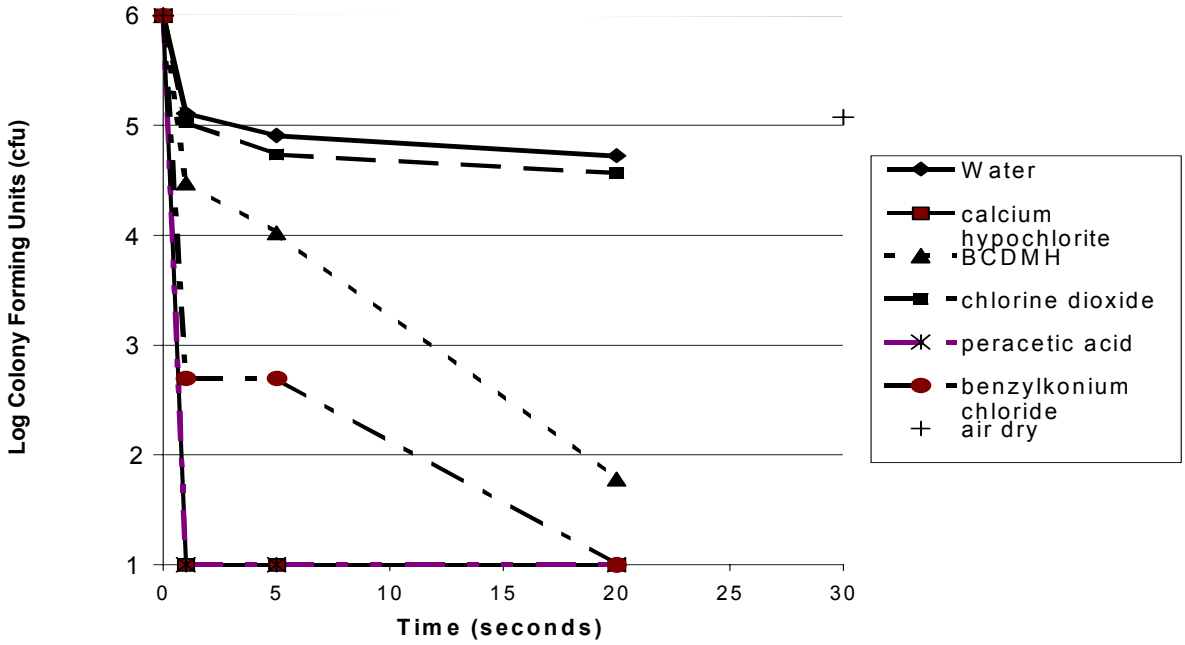

Figure 5. Efficacy of selected sanitisers against *Escherichia coli* on an aluminium surface. Sanitiser concentrations were 30 ppm fac, 5 ppm fce, 5 ppm ClO_2, 500 ppm PAA, 1000 ppm QAC.

Table 9. Efficacy of sanitisers on the survival of common spoilage organisms and *E coli* on a smooth wood surface. Tabulated values are square root transformed means of cfu/cm^2. Numbers with the same letter within a species are not significantly different ($p<0.05$).

Species	***Sanitiser***	***Exposure time (minutes)***		
		1	**5**	***20***
Mucor sp.	*Distilled water*	*9.37^{d}*	*10.45^{d}*	*10.16^{d}*
	Chlorine dioxide (5ppm)	*10.04^{d}*	*9.79^{d}*	*8.82^{cd}*
	BCDMH (5ppm)	*8.59^{cd}*	*8.92^{cd}*	*8.18^{cd}*
	Benzylkonium chloride (1000 ppm)	*10.44^{d}*	*9.95^{d}*	*7.22^{c}*
lsd 1.88	*Calcium hypochlorite (30ppm)*	*2.0^{b}*	*0.8^{ab}*	*0.67^{ab}*
	Peracetic acid (500ppm)	*0^{a}*	*0^{a}*	*0^{a}*
Geotrichum candidum	*Distilled water*	*8.11^{c}*	*8.71^{c}*	*6.57^{c}*
	Chlorine dioxide (5ppm)	*7.14^{c}*	*8.14^{c}*	*7.72^{c}*
	BCDMH (5ppm)	*4.66^{bc}*	*3.19^{b}*	*7.13^{c}*
	Benzylkonium chloride (1000 ppm)	*1.41^{ab}*	*3.71^{b}*	*0.67^{ab}*
lsd 2.58	*Calcium hypochlorite (30ppm)*	*2.81^{b}*	*0.91^{ab}*	*0^{a}*
	Peracetic acid (500ppm)	*0^{a}*	*0^{a}*	*0^{a}*
E. coli	*Distilled water*	*33.56^{d}*	*44.01^{e}*	*40.9^{e}*
	Chlorine dioxide (5ppm)	*29.69^{d}*	*29.54^{d}*	*25.24^{cd}*
	BCDMH (5ppm)	*20.6^{c}*	*15.98^{bc}*	*8.98^{b}*
	Benzylkonium chloride (1000 ppm)	*9.07^{b}*	*6.79^{ab}*	*9.23^{b}*
lsd 7.05	*Calcium hypochlorite (30ppm)*	*3.05^{ab}*	*1.88^{a}*	*2.23^{ab}*
	Peracetic acid (500ppm)	*0^{a}*	*0^{a}*	*0^{a}*
Clavibacter michiganensis	*Distilled water*	*16.2^{b}*	*8.27^{ab}*	*11.8^{b}*
	Chlorine dioxide (5ppm)	*4.96^{ab}*	*3.26^{ab}*	*0.03^{a}*
	BCDMH (5ppm)	*2.34^{a}*	*0.67^{a}*	*0^{a}*
	Benzylkonium chloride (1000 ppm)	*2.54^{a}*	*1.0^{a}*	*0^{a}*
lsd 9.64	*Calcium hypochlorite (30ppm)*	*0.47^{a}*	*1.2^{a}*	*0^{a}*
	Peracetic acid (500ppm)	*0^{a}*	*0^{a}*	*0^{a}*
Pseudomonas syringae	*Distilled water*	*10.43^{b}*	*1.97^{a}*	*2.45^{a}*
	Chlorine dioxide (5ppm)	*2.37^{a}*	*0.82^{a}*	*0^{a}*
	BCDMH (5ppm)	*1.11^{a}*	*0^{a}*	*0^{a}*
	Benzylkonium chloride (1000 ppm)	*0^{a}*	*0^{a}*	*0^{a}*
lsd 2.99	*Calcium hypochlorite (30ppm)*	*0^{a}*	*0^{a}*	*0^{a}*
	Peracetic acid (500ppm)	*0^{a}*	*0^{a}*	*0^{a}*

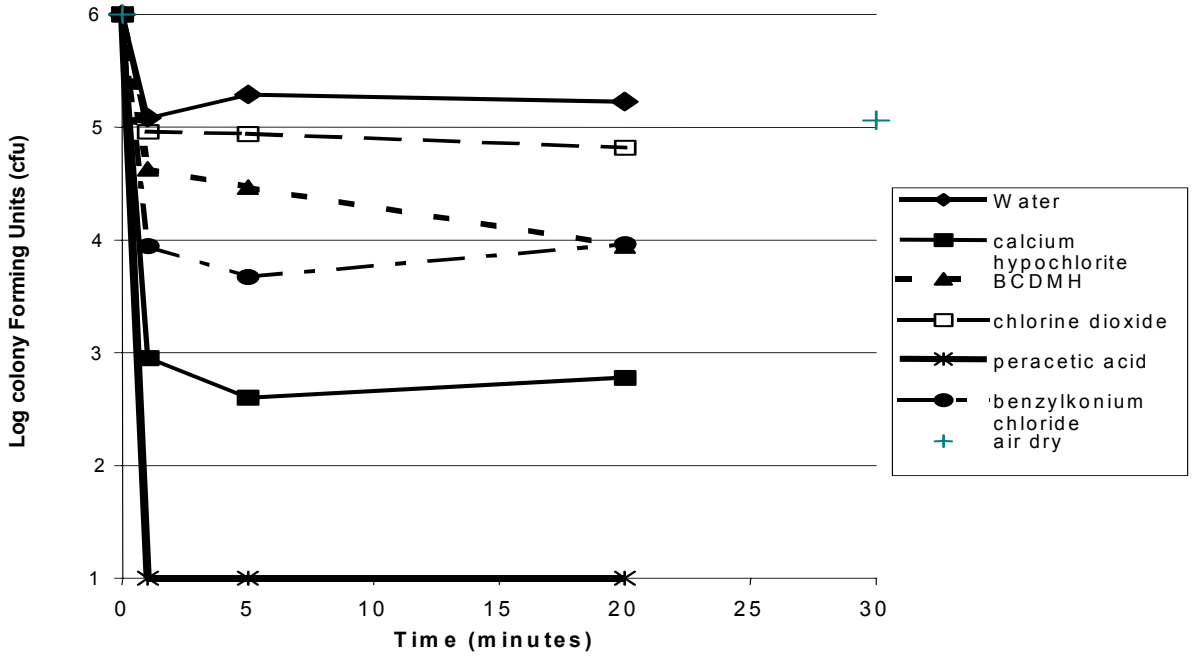

Figure 6. The efficacy of selected sanitisers against *Escherichia coli* on a smooth wood surface. Sanitiser concentrations were 30 ppm fac, 5 ppm fce, 5 ppm ClO_2 , 500 ppm PAA, 1000 ppm QAC.

Table 10. Efficacy of sanitisers against *C. michiganensis* on a rough-sawn *E. camaldulensis* surface. Tabulated values are square root transformed means of cfu/cm^2. Numbers with the same letter within a species are not significantly different ($p<0.05$).

Clavibacter michiganensis	***Sanitiser***	***Exposure time (minutes)***		
		1	***5***	***20***
lsd 12.2	*Distilled water*	*43.9*	*45.5*	*22.1*
	Chlorine dioxide (5ppm)	*30.6^{c}*	*21.5^{bc}*	*11.9^{ab}*
	BCDMH (5ppm)	*13.7^{b}*	*12.7^{b}*	*3.3^{ab}*
	Benzylkonium chloride (1000 ppm)	*12.4^{b}*	*12.9^{b}*	*6.2^{ab}*
	Calcium hypochlorite (30ppm)	*7.4^{ab}*	*5.8^{ab}*	*3.5^{ab}*
	Peracetic acid (500ppm)	*0.4^{a}*	*0.2^{a}*	*0.1^{a}*

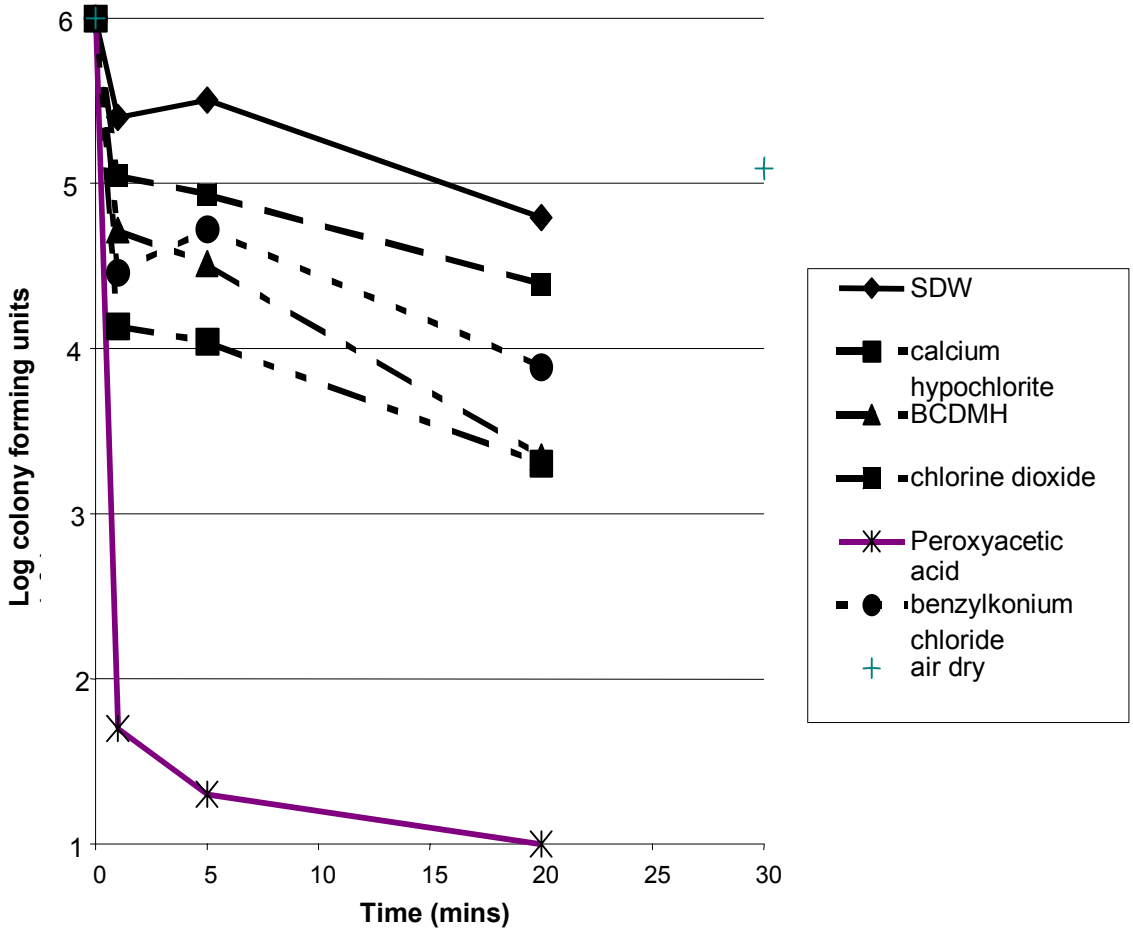

Figure 7. The efficacy of selected sanitisers against *Clavibacter michiganensis* subsp. *michiganensis* on a rough-sawn wood surface. Sanitiser concentrations were 30 ppm fac, 5 ppm fce, 5 ppm ClO_2, 500 ppm PAA, 1000 ppm QAC. SDW is sterile distilled water.

Safe discharge of Vegetable washwater

Hamilton and Mebalds (2000) reported water quality deteriorated rapidly with the throughput of carrots. Concentrations of *E. coli,* yeasts and moulds increased by an average of $\log_{10}$ and the frequency of detection of plant pathogens also increased markedly (Table 11). Other research (Morgan 2001) found higher than normal sanitiser concentrations were required to sanitise potato wash water because of the accumulation of organic materials. Thus, used washwater will contain nutrients, potentially harmful microbes, agrochemicals and chlorination by-products which may need to be controlled before the water can be released or re-used. The probability of this being necessary is high on rootcrops and where processes such as peeling are employed.

Table 11. The effect of washing carrots on the quality and pathogen concentration of washwater (Hamilton and Mebalds, 2000)

Parameter	*source water*	*waste water*
Turbidity (NTU)	*62.5*	*195.2*
Biochemical oxygen demand (mg/L)	*7.7*	*29.6*
Total reactive phosphorus-P (mg/L)	*1.79*	*32.7*
E. coli (cfu/100mL)	*44*	*555*
Yeasts and moulds (cfu/100mL)	*41,591*	*418,409*
Alternaria alternata (% of samples)	*20%*	*52%*
Fusarium oxysporum (% of samples)	*12%*	*64%*
Mucor sp. (% of samples)	*16%*	*48%*
Pythium sp. (% of samples)	*0%*	*8%*

The *E. coli* concentrations detected in used washwater exceed the class A reclaimed water levels recommended for vegetable to be eaten raw (EPA Vic 2002 a). This is indicative that the used washwater is unsuitable for direct reuse on farm. The Victorian EPA suggests the 'best practice' disinfection process for water with this level of *E. coli* could be detention lagoons with an algal management plan (EPA Vic 2002b). Added to this, the presence of plant pathogens suggests a possible biological hazard if the water was reused for crop irrigation or carrot washing, without treatment. Mebalds and Hamilton (2002) have discussed how constructed wetlands can be employed to manage these hazards.

Adverse environmental impacts are possible from the presence of sanitisers and by-products. De-chlorination eliminates the free and combined chlorine residual but will not reduce and may increase the concentrations of more residual and toxic by-products. The dynamics of these chemicals in constructed wetlands should be investigated.

Where various types of vegetables are washed in the same system, water treatment or replacement will be needed to prevent cross-contamination between heavily contaminated vegetables (eg root vegetables which are usually cooked) and cleaner salad vegetables. Wash water contaminated with human pathogens has been shown to infiltrate fresh cut lettuce and tomatoes through the stomata and wound sites (Zhuang *et al.* 1995, Seo and Frank 1999).

Good Agricultural Practices for Hygienic Postharvest

- Wash vegetables only where there is a proven advantage
- Remove or trim off rotted plant parts before washing to minimise contaminating the wash water and remove trimmings from the grading/packing line as soon as possible
- Do not mix rotting produce with intact produce during harvest, handling or storage
- Clean and sanitise harvest, grading and packing equipment (eg harvest bins Figure 8)
- Test source water (and sanitise if contaminated) and sanitise recirculated wash and hydro-cooling water.
- Maintain handling equipment so that mechanical damage to produce is minimised.
- Encourage personal hygiene – provide clean toilet and hand washing facilities
- Cool chain reduces spoilage, but check for chilling injury in susceptible cultivars
- Monitor critical control points
- Refer to Guidelines for On-Farm Food Safety (Agriculture Fisheries and Forestry-Australia 2001)

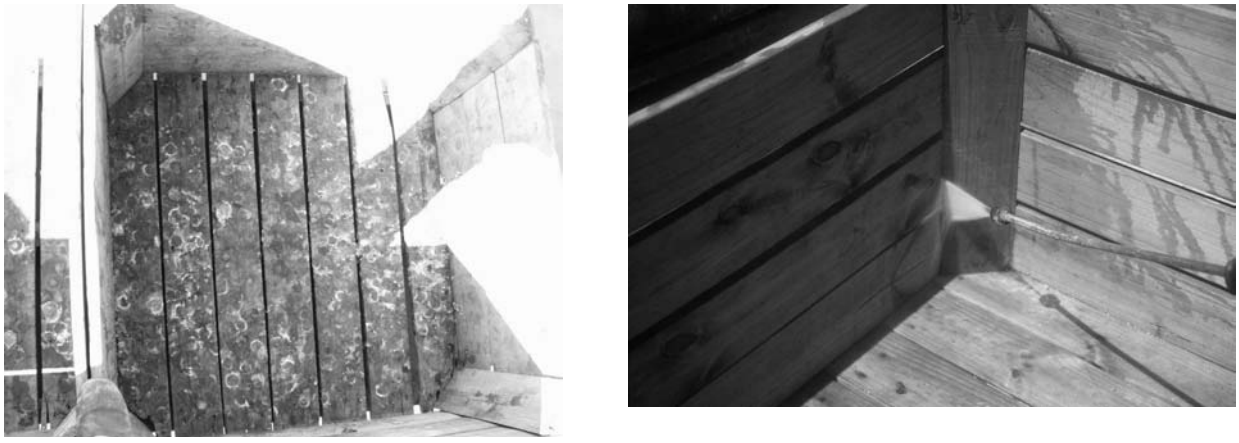

Figure 8 A tomato bin heavily contaminated by rot pathogens (left) and decontamination using a hot water pressure cleaner (right). A pressure cleaner delivering water at a temperature above 72°C at the bin surface is very effective against most postharvest pathogens.

Description of an effective Water Sanitisation System

- Automatic monitoring and dispensing of active ingredient (a.i.) to be maintained label rate eg by measuring oxidation/reduction potential (ORP) and pH
- pH adjustment or buffering
- On-line data recording (a.i., ORP, pH, temperature) for quality assurance audits
- Controlled temperature appropriate to commodity type
- Filtration system for primary treatment

- Recirculate water to reduce water consumption and costs
- Retaining tank for dechlorination and detoxification before disposal
- Regular manual monitoring to confirm effective operation

2.4 Voluntary Contributor Projects

2.4.1 Efficacy of Phytoclean® on the viability of pathogenic bacteria and fungi

Introduction

Quaternary ammonium compounds are cationic (positively charged) surface-active agents. They have been used for many years for a variety of purposes including disinfectants (e.g. Phytoclean®), antibiotics (e.g. Cepacol®), herbicides (e.g. Paraquat®) and even hair-care products (e.g. guar hyroxypropyltrimonium chloride—Selinger, 1989). Chemically, a quaternary ammonium compound is a modification of the ammonium ion, the hydrogen ions being replaced by organic groups. At least one of these groups is a long, water repellent hydrocarbon chain. Molecules of a quaternary ammonium compound arrange themselves in a layer by attaching to the surface (e.g. cell surface) via this hydrocarbon chain.

In general, Gram-positive bacteria are far more susceptible to quaternary ammonium compounds than Gram-negative species. They have also proven to be effective against certain viruses (e.g. hepatitis B virus—Prince *et al.* 1993).

Quaternary ammonium compounds can be neutralised by soaps and anionic detergents. Colloidal or particulate organic matter can also greatly reduce (up to 3 or 4 fold reduction) the efficacy of quaternary ammonium compounds (Gardener and Peel, 1998).

The aim of this research was to evaluate the effectiveness of the quaternary ammonium compound Phytoclean® against five post-harvest pathogens—two fungi (*Mucor piriformis* and *Penicillium expansum*) and three bacteria (*Xanthomonas campestris, Pseudomonas syringae*—both Gram negative— and *Clavibacter michiganensis*—Gram positive). *M. piriformis* and *P. expansum* cause post-harvest rotting of pome and stone fruits. *X. campestris*, *P. syringae* and *C. michiganensis* are also important post-harvest pathogens and are the causative agents of bacterial spot, bacterial speck and bacterial canker in tomatoes (Snowden 1991). *P. syringae* has a very wide host range, causing blight or canker on many important horticultural crops.

Materials and Methods

Cultures of *Penicillium expansum* and *Mucor piriformis* were maintained on potato dextrose agar (PDA) at 21°C. Fresh cultures were prepared, by sub-culturing stock plate, every three weeks. Spore suspensions of approximately $1x10^6$ spores/ml were prepared by flooding the culture plate with sterile ultra-filtered deionised water (SFDW) and agitating with a glass rod. The concentration of spores in this suspension was counted directly using a haemocytometer, and adjustments were made by adding the appropriate volume of SFDW to achieve the desired spore concentration.

Cultures of *Clavibacter michiganensis* (batch # OR/1984/1 from tomato)*, Pseudomonas syringae* (batch # MC/118/10 from cherry) and *Xanthomonas campestris* (batch # AG/7/27 from tomato) were maintained at 21°C on nutrient agar (NA). Bacterial cell suspensions of approximately $1x10^6$ spores /ml were prepared as per the technique described for fungal spores. However, bacterial cells were too small to enumerate with a haemocytometer, and consequently suspension concentrations were estimated via a turbidity method. For *C. michiganensis* and *P. syringae* this simply involved a visual estimation. When turbidity first appeared through an 11.0mm path length in a glass container, the solution was assumed to contain approximately $1x10^6$ cells /ml. This method did not work for *X. campestris* as visual turbidity was not reached at concentrations well in excess of $1x10^6$ /ml. Instead, turbidity was measured across serial dilutions of a cell suspension on a spectrophotometer at a wavelength of 400nm. These suspensions were also plated out on NA, and hence, a standard curve was developed relating optical density to cell concentration.

Clean water trials

One millilitre of the bacterial cell / fungal spore suspension was added to 99 ml of a 2% Phytoclean® solution. At 30, 60, 90, 12 and 240 seconds 1 ml of this solution was extracted and added to a vial containing 9 ml of inactivator solution (1.4% v/v sodium thiosulphate & 10% v/v Tween 80). The inactivator solution quenched the disinfectant activity of the Phytoclean. One millilitre of this solution was spread-plated onto nutrient agar for bacteria and potato dextrose agar for fungi. The same procedure was followed for the control with the exception that the 2% Phytoclean solution was replaced with SFDW. The control was added to the inactivator 240 seconds after the addition of the sterile distilled water. The entire procedure was replicated three times for each species. Each experimental unit, a culture plate, was duplicated (i.e. two plates inoculated) for consistency checking.

Dirty water trials

'Dirty water' (i.e. water containing an organic loading) was prepared as per the methods described for the Therapeutic Goods Administration Disinfectant Test (Graham 1978). This method uses inactivated baker's yeast for the organic loading. The methods used for these trials were identical to those used for the *in situ* trials with the exception that cell/spore suspensions were prepared with dirty water rather than SFDW. As per the *in situ* trial, the entire procedure was replicated three times for each species.

Surface trials

The efficacy of Phytoclean® was also tested on a non-porous (aluminium) surface. Six blocks of aluminium ($27cm^2$)—1 control, 5 treatments—were dipped in a 10^6 spore/cell suspension for 30 seconds and allowed to dry for 30 minutes in a laminar flow cabinet. The treatment blocks were then dipped in a 2% Phytoclean solution for varying lengths of time (30, 60, 90, 120 or 240 seconds) and the control block was dipped in sterile distilled water for 240 seconds. The blocks were then pressed directly onto an agar plate onto which 1.8 ml of inactivator had previously been added. As per the aforementioned trials, the entire procedure was replicated three times for each species.

Differences in efficacy between exposure times were analysed by analysis of variance. Most of the data required logarithmic or square root transformation before analysis. In cases where complete kill—or near complete kill—was achieved by the shortest treatment, no statistical analyses were conducted.

Results and Discussion

In vitro tests revealed that all three bacteria were more susceptible to Phytoclean® than the two fungal species. None of the three bacterial species exhibited growth after any of the treatments. Conversely, complete kill was not reached at the longest treatment (240 sec) for *M. piriformis*. *P. expansum* was less tolerant, complete kill being attained by 240 sec (Table 12). For one of the bacterial species, *C. michiganensis*, growth on the control plates was very low, and hence cautious assessment of the efficacy of Phytoclean against this pathogen, under these conditions, needs to be made.

Table 12: Viability (cfu per 0.2 ml – mean from 3 replicates) of bacteria and fungi exposed to 2% Phytoclean® *in vitro*.

		Exposure time (sec)				
Species	***control***	***30***	***60***	***90***	***120***	***240***
M. piriformis	165^{a}	48^{b}	54^{b}	39^{b}	32^{b}	8^{c}
P. expansum	281^{a}	39^{b}	15^{c}	5^{c}	4^{c}	0^{d}
C. michiganensis	*15*	*0*	*0*	*0*	*0*	*0*
P. syringae	*351*	*0*	*0*	*0*	*0*	*0*
X. campestris	*1357*	*0*	*0*	*0*	*0*	*0*

For each species (row), exposure times with the same letter are not significantly different from each other ($p<0.05$).

Dirty water trials

The addition of organic loading appeared to have no impact on the efficacy of Phytoclean® against any of the three bacterial species; no growth was recorded after any of the exposure times. However, efficacy appeared to increase against *M. piriformis* and diminish against *P. expansum* (Table 13) when compared to the *in situ* tests. The fact that *C. michiganensis* did not grow even after the 30 second exposure time may add some weight to the previous *in situ* observations where the control sample size was low.

Table 13: Viability (cfu per 0.2 ml – mean from 3 replicates) of bacteria exposed to 2% Phytoclean® in dirty water (inactivated yeast).

		Exposure time (sec)				
Species	***control***	***30***	***60***	***90***	***120***	***240***
M. pyriform	282^{a}	165^{b}	18^{c}	0^{c}	0^{c}	0^{c}
P. expansum	335^{a}	141^{b}	93^{bc}	46^{cd}	20^{de}	7^{e}
C. michiganensis	*46*	*0*	*0*	*0*	*0*	*0*
P. syringae	*418*	*0*	*0*	*0*	*0*	*0*
X. campestris	*50*	*0*	*0*	*0*	*0*	*0*

For each species (row), exposure times with the same letter are not significantly different from each other ($p=0.05$).

Surface trials

In general, the action of Phytoclean® was less effective on an aluminium surface than it was in clean or dirty water. Complete kill was not reached for either fungal species even after the maximum exposure time of 240 seconds. Complete kill was observed for all bacterial species by 120 seconds. Whilst no growth was recorded after any of the treatments for *X. campestris*, this result needs to be treated with caution as there was limited growth on the controls.

Table 14: Viability (cfu per 0.2 ml – mean from 3 replicates) of fungi and bacteria exposed to 2% Phytoclean® on aluminium.

		Exposure time (sec)				
Species	***control***	***30***	***60***	***90***	***120***	***240***
M. piriformis	122^{a}	116^{a}	123^{a}	55^{a}	80^{a}	48^{a}
P. expansum	130^{a}	159^{a}	89^{ab}	83^{ab}	70^{bc}	34^{c}
C. michiganensis	220^{a}	99^{b}	72^{b}	5^{c}	0^{c}	0^{c}
P. syringae	*1722*	*0*	*19*	*2*	*0*	*0*
X. campestris	*31*	*0*	*0*	*0*	*0*	*0*

For each species (row), values with the same letter are not significantly different from each other ($p<0.05$).

Conclusion

Phytoclean® was effective in controlling the bacterial pathogens *Xanthomonas campestris, Pseudomonas syringae* and *Clavibacter michiganensis in situ*, in 'dirty water' and on aluminium surfaces. There were no clear differences in the susceptibility of the Gram positive species (*C. michiganensis*) and the Gram negative species (*X. campestris* and *P. syringae*). The two fungal species investigated, *M. piriformis* and *P. expansum*, were generally more tolerant to Phytoclean® than the bacteria. Whilst substantial reductions were achieved for these species *in situ* and in 'dirty water', control was poor on an aluminium surface. Pressure cleaning, which is able to remove fungal and bacterial material from surfaces, would be a useful adjunct to the disinfectant. Cells once removed from the surface would be more prone to the action of the disinfectant.

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2.4.2 Development of a transportable, sanitised hydrocooling system for vegetables - Ym Fab Postharvest Chemicals

Introduction

The main purposes of this evaluation were:

To determine the time required to cool broccoli from a field temperature of 28°C to 2°C.

To determine populations of Total Aerobic (TA) microorganisms and *E. coli* on broccoli and in the wash water during the washing/cooling cycle.

Results and Discussion

The core temperature of broccoli heads was reduced from 28°C to 2°C within 36 to 75 minutes (Figure 9). The average time taken for the entire load to reach the required temperature was less than 60 minutes. This experiment used several vegetable types, including, broccoli, celery, parsnips and drumhead cabbage. Improved cooling rates could be expected on a uniform load of broccoli due to its greater surface area to volume ratio. The variability between cooling rates of individual heads may have been due to the position and the variation in the diameter of the broccoli stalks.

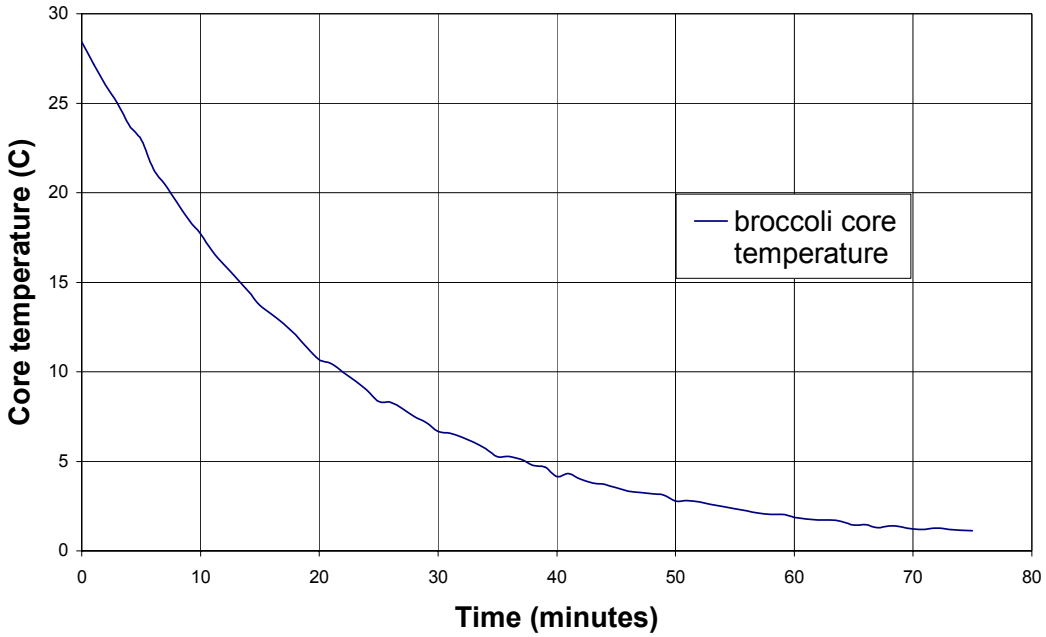


Figure 9 The average cooling curve for broccoli heads measured at the thickest part of the stalk.

Water samples for microbial analysis were taken from the recirculated water stream immediately before it was showered onto the produce. There were no coliforms or *E. coli* recovered from the water samples at 0, 10, 20, 30 and 60 minutes after the start of the washing cycle, indicating the wash water was effectively sanitised. The TA count was very low. These organisms were probably derived from the dirty produce, as they tended to increase over time (Table 15).

Table 15: Concentration of Total Aerobic microorganisms in the wash water. Nylate was deactivated in the samples using 0.1N of sodium thiosulphate.

Time (minutes)	***Total Aerobic Counts (cfu/ml)***
0	*310*
10	*816*
20	*900*
30	*1056*
60	*900*

In an experiment where broccoli was intentionally inoculated with a non-pathogenic strain of *E. coli* before washing in the hydrocooler, a reduction of approx 2 $\log_{10}$ was achieved (mean counts before washing 1.5×10^6 ; mean counts after washing 4.1×10^4). Other research (Behrsing *et al*. 2000) demonstrated a 2 $\log_{10}$ reduction of *E. coli* on broccoli using a chlorine dip (50-100ppm).

2.4.3 Alternatives to Formalin for sanitising tomato stakes -Northern Victorian Fresh Tomato Growers Association

Introduction

Bacterial canker of tomato, caused by *C. m. michiganensis* reduces tomato yield and quality (Jones et al. 1991, Hausebeck 1999, Ioannou 2000, and Medina-Mora 2000). The sources of inocula for this disease are seed, weeds, transplants, soil, plant debris, and wooden stakes (Strider, 1969, Jones, 1991, Blancard, 1997, Reid, 1999). Transmission of the disease occurs via machinery, workers, rain splash, contaminated equipment, overhead irrigation, wounds and cultural practices such as pruning and leaf removal (Blancard, 1997). Symptoms include systemic wilt, 'bird's eye' lesions on fruit, dead leaflets and spreading necrosis of the plant (Jones, 1997). To prevent disease, specialists recommend using certified seed and transplants, crop rotation and other hygienic practices such as the sanitisation of equipment.

There are no products registered with the NRA for the sanitisation of equipment against *C. m. michiganensis*. Formalin has been used 'off label' for the disinfestation of wooden stakes and polyethylene irrigation tubing, however this is not registered and its use creates a hazardous work environment.

Figure 9 Dipping tomato stakes to minimise the carryover of the bacterial canker pathogen.

Objectives

- Review the local and international knowledge on the transmission of the bacterial canker pathogen on tools, trellising materials and machinery.
- Review overseas recommendations on the sanitation of these surfaces for local applicability (eg suitability and registration status).
- Monitor the concentration of formalin in 'dip tanks' over time and Identify safe disposal methods for formalin.
- Conduct lab and field trials on the effectiveness of sanitisers against fungi and bacteria including *Clavibacter* on stakes.
- Promote the findings in Red Gold News and other venues appropriate for industry.

Methods

Hydrated Petrifilm™ Total Aerobic plates were pressed against tomato stakes before and after dipping in tanks containing various sanitiser treatments for 3 minutes. Plates were incubated at 20°C for 48 hours before enumeration. Merckoquant® Formaldehyde-Test analytical test strips and reagent were used to determine formaldehyde concentrations in the formalin solutions before and after dipping. Other sanitiser concentrations were determined by Hach methods.

Results and Discussion

Recommendations for the disinfestation of *C. m. michiganensis* from wooden stakes and end posts are:

0.5% calcium hypochlorite for 30 minutes (Reid, 1999)

1% bleach solution (sodium hypochlorite) dip for 24 hours or steam sterilisation (Jones, 1991)

2% formalin (Fullelove 1992, Blancard 1997, DPI Qld. 1998)

copper hydroxide and streptomycin - no concentrations or contact times mentioned for wood (Hazzard and Wick, 1997)

Some Victorian growers were aware of the routes of disease transmission and had implemented the following strategies. Wooden stakes were dipped in formalin, bleach, peroxygens, heat treated or steamed. Trellis wire was dipped in diesel oil to stop corrosion, secateurs dipped in methylated spirits and footbaths with detergents were in place at the end of each row on some farms. Machinery was cleaned with pressure hoses and a foaming disinfectant (ie. Tandem) between crops and before it was taken into new land. The run-off water and soil from the washing process was contained (on one farm). Some growers had considered gloves and overalls for pickers, which can be replaced or washed regularly.

In laboratory evaluations 1% peroxyacetic acid and 0.5% formaldehyde very effectively eliminated *Clavibacter* from tomato stakes in less than one minute. Other sanitisers at the low range of label rates were less effective, however, their effectiveness increased over time and higher concentrations or extended contact times are expected to give improved results. Both calcium hypochlorite and BCDMH (Nylate) at relatively low rates reduced *Clavibacter* concentrations to very low levels (Figure 10).

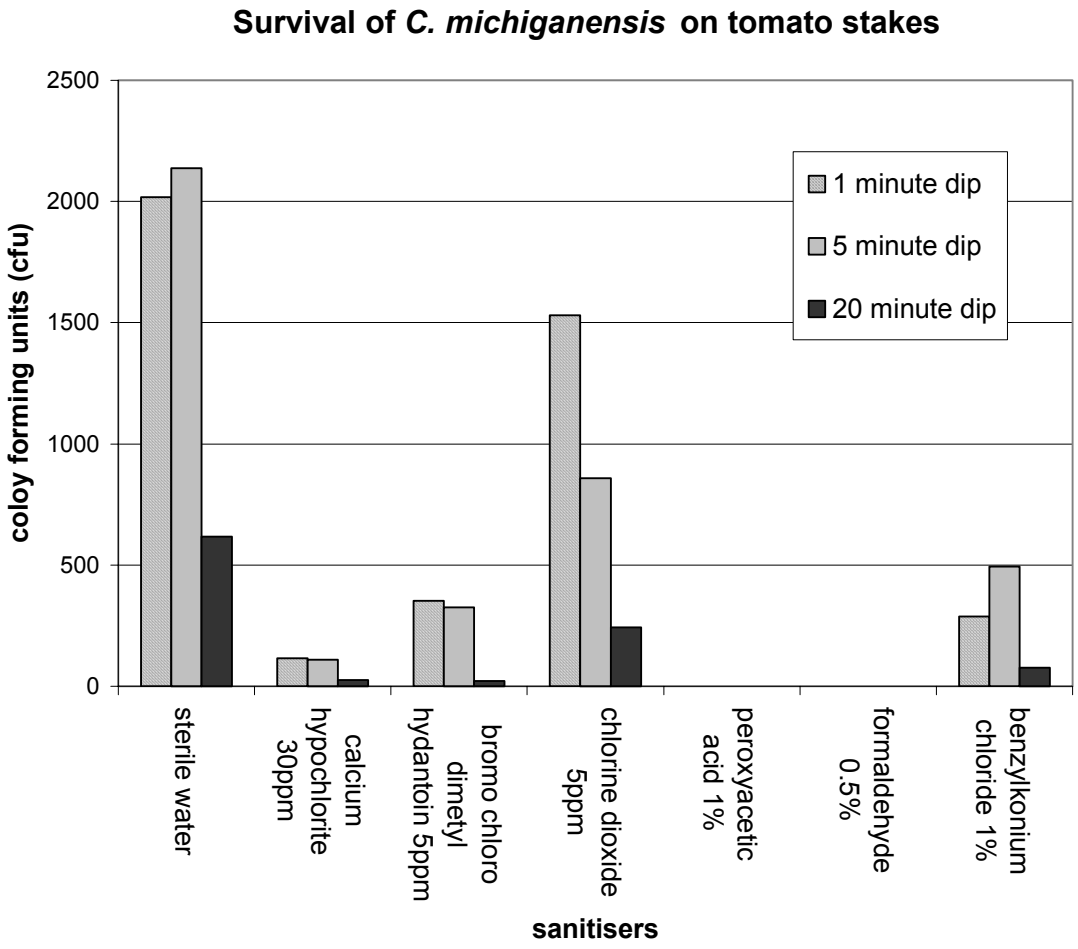


Figure 10 Effectiveness of sanitisers against *Clavibacter* on tomato stakes at 20°C

In field trials, formalin, peracetic acid and a quaternary ammonium compound (Phytoclean®) reduced total aerobic counts to zero (Table 16). On formalin dipped stakes, counts increased from 0 to 5 CFU/cm^2 after 24 hours field exposure and on chlorine dipped stakes increased from 3 to 14 CFU/cm^2 after 48 hours field exposure. This recolonisation, however, is likely to involve the prevailing saprophytic microflora rather than pathogens that derive from contact with diseased plants. The performance of hypochlorites and BCDMH could be enhanced by removal of soil before dipping, pH adjustment, mechanical agitation, using higher rates and longer contact times.

Table 16: Total Aerobic Counts (CFU/cm^2) on tomato stakes before and after dipping

Sanitiser	***Before dipping***	***After dipping***
Water	*18.5*	*19.3*
Formalin (15g/L formaldehyde)	*18.5*	*0*
Peracetic acid (2.5g/L)	*16.1*	*0*
Benzylkonium chloride (1%)	*19.8*	*0*
Sodium hypochlorite (1g/L)	*31.6*	*2.9*

The concentration of formalin was tested in two growers dip tanks. The amount of formaldehyde in these solutions was15g/L and 30g/L. There was no detectable change in the concentration of formalin after dipping several tonnes of stakes. High concentrations of formaldehyde should be treated before disposal. The current method of disposal is to flood the unused formalin solution onto land. A deactivator (Formalex™) is available however this has not been used by tomato farmers to date. Alternatively, Lofty and Rashed (2002) discuss the consumption of formaldehyde by sodium sulphite.

Conclusion

Dipping of stakes in sanitisers is about one tenth of the replacement cost and should be encouraged as a preplanting field hygiene practice. However, precautions should be taken to prevent poisoning where formalin is used and to prevent environmental contamination. Simple test methods are available to determine if sanitiser concentrations become depleted during use so that topping up rates are based on informed decisions. A 1 minute dip in 1% Peratec 5 and a 20 minute dip in 30ppm calcium hypochlorite 5ppm BCDMH or 1% Phytoclean® were effective against *Clavibacter*.

2.4.4 Evaluation of vegetable washing systems – Bioteq Ltd

Aim

Compare the efficacy of calcium hypochlorite and BIOMAX A iodine as delivered by the IODOCLEAN Iodine Management System for the reduction of microbial contaminants on four types of fresh vegetables.

Materials and Methods

Experiments were conducted at a vegetable farm using washing equipment already in place. The washing plant consisted of a wire mesh conveyor belt running below a series of pressure nozzles delivering non-recirculated mains water. There were four rows of nozzles above the conveyor and two rows at the side of the conveyor directing water horizontally. Bunched produce was placed in a single layer on the conveyor and oriented so that the root systems (if present) were proximal to the nozzles along the side of the conveyor. At the end of the conveyor, the vegetables fell into a tank of sanitised water (Figure 11). For the experiment there were two washing tanks (each containing approx 800 litres of water) in parallel at the end of the conveyor. The water in one tank was manually dosed with granular calcium hypochlorite, achieving an initial free chlorine concentration of 80-100 µg/ml. There was however undissolved chemical in the tank and it is not known how much the concentration may have varied during the experiment. It is likely that the residual undissolved chemical in the tank due to overdosing, acted as a reservoir which recharged the system somewhat as chlorine levels were depleted by the produce. The water in the other tank was sanitised with an Iodoclean system (Bioteq Ltd, Australia), automatically maintaining approx 15 - 20µg/ml iodine throughout the experiment. Equal amounts of vegetables were transferred from the end of the conveyor into each washing tank.

Vegetables used for the evaluations were; 1) spring onions, which had been pulled with the root systems intact and bunched before washing, 2) flat leaved parsley, cut from the root system and bunched before washing, 3) intact radish plants, bunched before washing and 4) Shanghai bok choy heads, cut from the root system and bunched before washing in threes and fours. Vegetables were run over the washing plant in the above order and the water was changed and re-dosed with sanitisers between the parsley and radish. Approx 200kg of each vegetable type was put through each washing tank.

Figure 11 The washing plant used in the experiments. Vegetables were placed on the conveyor where they were pressure washed with fresh water. At the end of the conveyor, vegetables fell into a tank of sanitised water. For the experiments there were two tanks, one containing calcium hypochlorite and the other iodine.

Produce Sampling

Samples (6, approx 20 g) of each vegetable type were removed from the end of the conveyor and from the sanitised water tanks 1, 6 and 16 minutes after vegetables were first added. Because of the continual input and removal of vegetables, bunches resided in the tank for a maximum of 3-5 minutes. Immediately after removal vegetables were placed in sterile polyethylene bags and held on ice for up to 4 hours. Sub-samples (25g) of each were placed into stomacher bags containing 250mL of 0.1% of bacteriological peptone and homogenised in a stomacher for 2 minutes. Duplicate aliquots of each (1mL) were be pipetted onto Total Aerobic Petrifilm™ and *E. coli*/Coliform Petrifilm™. Aerobic count plates and *E. coli*/Coliform count plates were be incubated at 20°C for 48 hours and 37°C for 24 hours, respectively. Resulting colony counts (CFU/g) were transformed ($\log_{10}$) and statistically analysed by ANOVA (Genstat for Windows).

Water Sampling

Six replicate water samples (100mL) were removed from both tanks before vegetables were added and then one, six and sixteen minutes after vegetables were added and placed in bottles containing 1N sodium thiosulphate (2.5g/100ml), to stop further disinfestation. Bottles were refrigerated for 20-24 hours and duplicate aliquots were pipetted onto Total Aerobic Petrifilm™ and *E. coli*/Coliform Petrifilm™. Enumeration and statistical methods were the same as for the produce samples.

Results and Discussion

The average initial total aerobic (TA) counts were approx 6.6×10^4 CFU/g of radish, 5.0×10^4 CFU/g of spring onion, 1.3×10^4 CFU/g of bok choy and 4.0×10^3 CFU/g of parsley. The comparatively low level on parsley may be attributed to it being cut from the plant well above soil level and the absence of petiole bases and axils which would retain soil. Initial coliform counts did not parallel the TA counts. Average initial coliform counts were 1.6×10^2 CFU/g of radish, 4.3×10^3 CFU/g of spring onion, 2.0×10^2 CFU/g of bok choy and 5.0×10^2 CFU/g of parsley.

Irrespective of the sanitiser used, sanitised water washing reduced the microbial contamination levels of all the vegetables. It should be noted that vegetables had received a prior wash in mains water (which could achieve a 1.5 log reduction, Behrsing *et al.*2000), before the sanitised water therefore total reductions are expected to be greater than those recorded here. Reductions in TA counts were greater than 90% for radish, spring onion and parsley and 70% on bok choy within 6 minutes exposure. Reductions in coliforms were above 75% except on bok choy where there was no reduction. Lower reductions on bok choy may be attributable to the protected axils retaining soil. Soil in the axils was evident after washing.

The two sanitisers performed similarly, except that;

- TA counts on iodine washed radish were lower than chlorine washed after 1 min exposure;
- TA counts on chlorine washed spring onion and parsley were lower than iodine washed, 16 min after the vegetables were first added;
- Coliform counts on chlorine washed bok choy were lower than iodine washed, after 1 min exposure and 16 min after vegetables were first added; and
- Coliform counts on iodine washed parsley were lower than chlorine washed, after 1 min exposure and 16 min after vegetables were first added (Figure 12).

E .coli was uncommon and sporadic, except on radish and its occurrence was not statistically compared. *E .coli* was isolated from about half the radish samples at up to 22 CFU/g. It was less

commonly isolated from the sanitised water samples, being typically found at 3 CFU/ml in about 7% of samples.

Total aerobic (TA) counts in the wash water increased rapidly within the first minute after produce was added. In most situations, TA counts then decreased or remained static during the addition of more produce. Counts were similar in both sanitisers, except during the washing of spring onions and parsley. In spring onion wash water contamination levels tended to rise over the monitoring period of 16 min. TA and coliform counts were lower in the iodine tank at 6 minutes than in the chlorine tank. This indicates that both sanitised water systems and more so the chlorine system had insufficient capacity to meet the 'chlorine demand' created by a throughput of 200 kg of spring onions in 16 minutes. Also during the washing of parsley TA and coliform counts were lower in the iodine tank than the chlorine tank at 1 minute (Figure 13).

Conclusions

These results indicate very similar sanitiser performance of iodine and chlorine despite initial chlorine levels of 80 -100ppm being up to 5 times higher than the iodine levels used of 15-20 ppm. There were differences in effectiveness of sanitisers on different types of vegetables, with the best results being for spring onions with log reductions between 0.8 and 1.05 and the least effective results being for bok choy with log reductions between -0.2 and 0.6. The better results for the spring onions could be due to the smoother surface, cleaner water used initially in the treatment tank and lack of soil on the produce. The less effective results for the bok choy would be largely due to the soil retained on them after treatment.

Given that the iodine was being dosed at one end of the wash tank and the sampling taken from the other end of the wash tank dosing iodine at multiple points in the tank may further improve performance of the Iodoclean system.

Figure 12 Total aerobic (TA, left column) and coliform counts (right column) on four vegetable types before washing and after 1, 6 and 16 minutes exposure in water sanitised with calcium hypochlorite (Cl) or iodine (I). Error bars are lsd of means ($p < 0.05$).

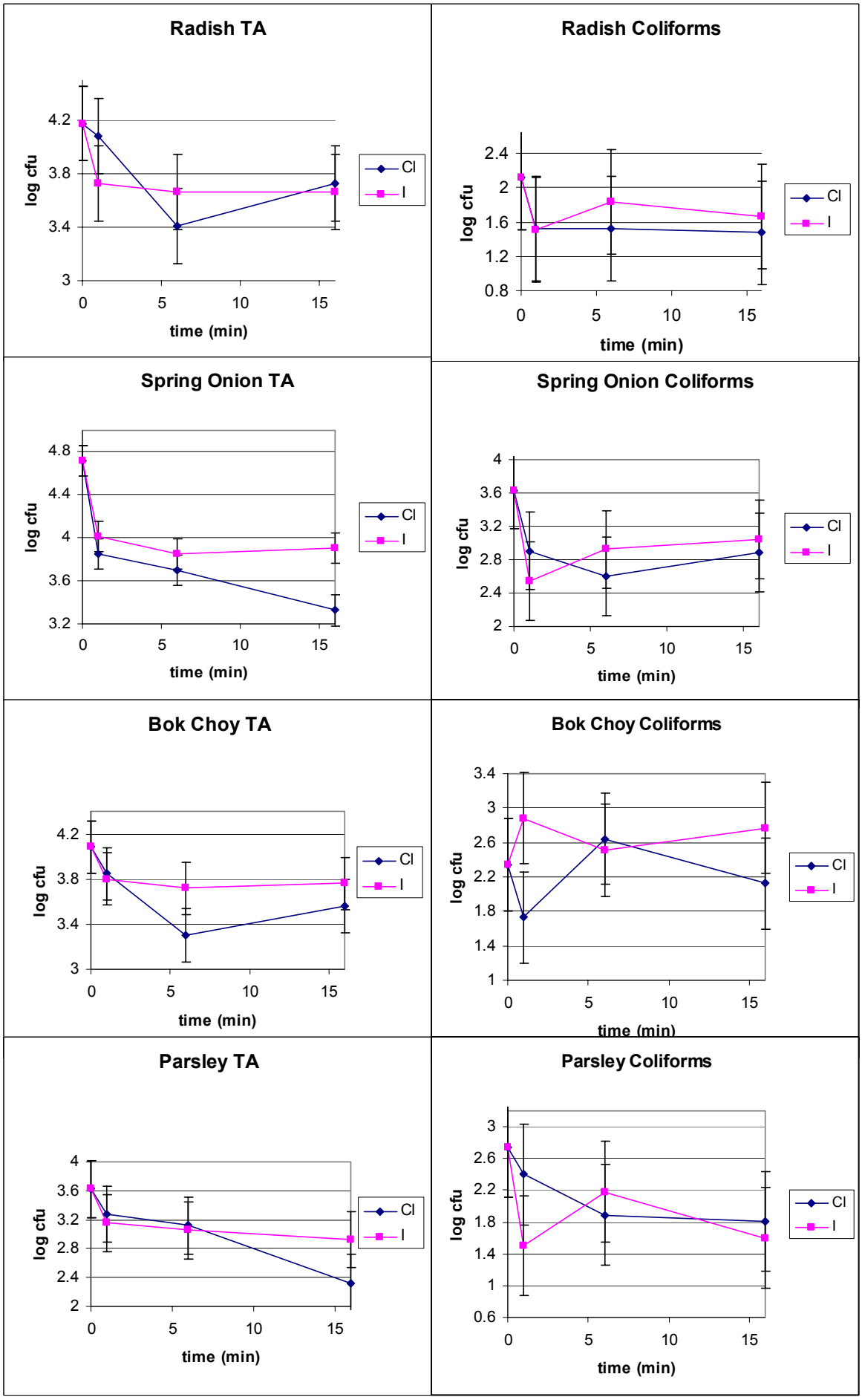
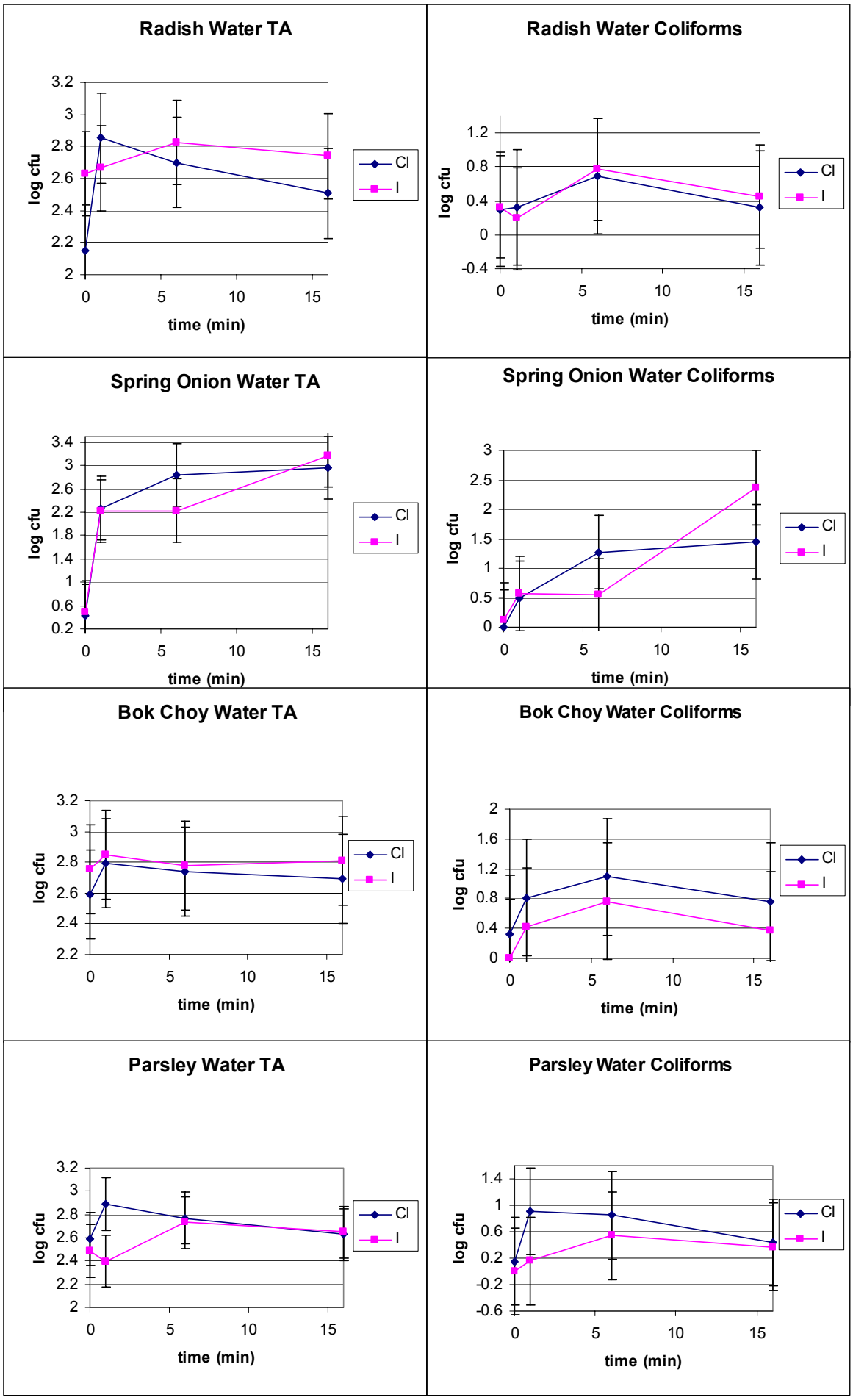


Figure 13 Total aerobic (TA, left column) and coliform counts (right column) in water sanitised with calcium hypochlorite (Cl) or iodine (I)., before and during the washing of four types of vegetables. Error bars are lsd of means ($p < 0.05$).



3 Technology Transfer

An extension plan was developed in consultation with Victorian Industry Development Officer; Patrick Ulloa and former Vegcheque Team Leader Sarah Barry. The primary targets for the information were vegetable and tomato growers and packers. Annual newsletters with the combined outcomes of projects VX99004 and VG99005 were distributed to over 400 growers and packers, including those who responded to the initial survey. Numerous articles were published throughout the life of the project. The culmination of the technology transfer activities was a series of hands-on workshops and field days presented around the country with assistance from the vegetable IDOs.

Outcomes of the investigations were also extended to other sectors of industry and the research and regulatory communities through two international conferences.

Conferences:

Harrup, P., Holmes, R., Hamilton, A., Mebalds, M., and Premier, R. (2001) Sanitary Washing of Vegetables, In*: Postharvest Handling of Fresh Vegetables*, Ed: O'Hare, T. *et al.* Workshop Proceedings in Beijing, People's Republic of China, May 2001;

Harrup, P. (2001) Clean and Safe Handling Systems for Fresh Vegetables, At: *International Freshcut Industry Conference and Workshop*, Werribee, October, 2001;

Reports and Industry Publications:

Harrup, P.G. and Holmes. (2000) Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes. In: *WA Grower* – September, 2000.

Harrup, P. (2001) Clean and Safe Handling of Vegetables, media release to *Southern Farmer* and *Good Fruit and Vegetables.*

Harrup, P. and Holmes, R. (2001) *Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes, Progress Report 1 – NVFTGA*

Harrup, P.G. and Holmes. (2001) An Evaluation of a Prototype Broccoli Hydrocooler. (Confidential Report to Wobelea – YM FAB Postharvest Chemicals)

Harrup, P. and Holmes, R. (2002) *Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes, Progress Report 2 – NVFTGA*

Harrup, P. and Holmes, R. (2000-02) *Awareness articles in: Vegetable Matters, Swan Hill Summer Fruits, Access to Asian Vegetables (RIRDC), Bundaberg Fruit and Vegetables, Vegetable Platters.*

Project newsletters:

Hamilton, A., Holmes, R., Mebalds, M. and Harrup, P (2000) *In the Wash*, Vol. 1 (Project Newsletter circulated to Vegetable and Tomato Growers)

Harrup, P., Hamilton, A., Mebalds, M., Holmes, R. and Jacka, L. (2001) *In the Wash*, Ed. No 2 - October, 2001 (Project Newsletter circulated to Vegetable and Tomato Growers).

Grower workshops:

The workshops were developed to deliver project outcomes in collaboration with the Quality Wash Water project (VG99005). Workshops consisted of presentations, a question time of approximately 30 minutes and demonstrations on water quality testing (using water brought along to the workshops by the participants) and on chlorination, sanitiser monitoring and factors influencing chlorine demand. A comprehensive field day handbook was given to all participants and further copies were given to state vegetable IDOs for distribution.

Workshops were held during 2002 at Clyde, Victoria on 26 April, Virginia, South Australia on 26 June, Yanco NSW on 20 June, Cowra, NSW on 30 July, Bundaberg Queensland on 18 June and Perth WA on 4 October.

A more comprehensive postharvest course was presented to a major retail chain and their distribution company.

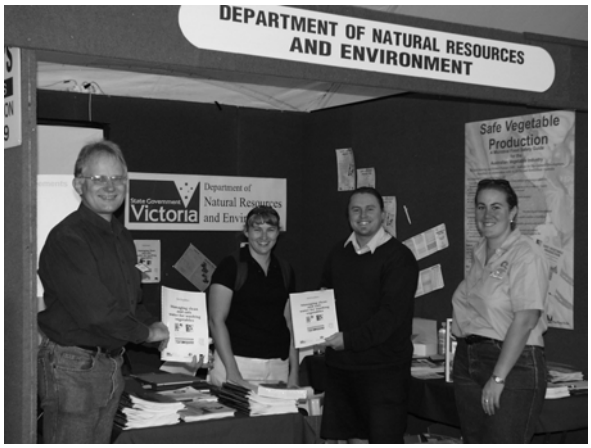
Posters and presentations were delivered at Gatton Field Days Gatton, Qld. on 7, 8 and 9 May 2002 Field day notes were distributed to interested vegetable growers and the project outcomes discussed.

Sunraysia growers were visited individually.

The project was exhibited at the *Great Australian Science Show,* Melbourne, 2002

VX99004 Project scientist Paul Harrup and VG99004 Project leader Martin Mebalds at the Virginia Workshop

Paul Harrup testing effluent from a carrot washing plant



(l-r) Martin Mebalds, NSW IDO Allison Anderson, Paul Harrup and Qld IDO Julia Telford at the Gatton Field Days.

Robert Holmes and Paul Harrup conducting a postharvest hygiene session for a distribution company and retail chain.

Paul Harrup demonstrating the concept of chlorine demand

Paul Harrup and Sally-Anne Henderson discussing sanitisation with a carrot packer in Sunraysia.

4 Recommendations

Regulation of sanitisers

Many growers have been confused about which sanitisers are legal to use in on-farm packing operations. To some degree QA auditors and retail chains have contributed to this confusion by at times recognising only some of the legal sanitisers. Growers and auditors need to be aware that chemicals supplied for postharvest washing, which claim to control spoilage organisms, either on the label or in the advertising material, are required to be registered with the NRA. There are now 5 sanitisers for the postharvest washing of vegetables registered with the NRA. There are also many general-purpose sanitisers excluded from the requirements of NRA approval (exempt). These may be suitable for use on foods or for washing down equipment if they are approved for that purpose by Food Standards Australia New Zealand.

We recommend that further work is done to increase the awareness by all sectors of the vegetable industry of the NRA and FSANZ regulations

Selection of appropriate sanitisers

In clean water, pathogens were reduced by 4 to 6-$\log_{10}$ by most sanitisers. Fungi were more resistant to sanitisers than bacteria. In dirty water, only peroxyacetic acid (2% v/v) and chlorine dioxide (2.5mg/L) achieved greater than 4-$\log_{10}$ reductions of the most resistant fungus. BCDMH and calcium hypochlorite are the most cost-effective actives to sanitise relatively clean wash water. Chlorine dioxide is a more appropriate water sanitiser for the washing of dirty vegetables or vegetables which contribute a high organic load, such as brush-polished carrots. Prewashing dirty vegetables in unsanitised water before a rinse in sanitised water will be an option for some growers, depending on water availability. However prolonged soaking of vegetables in dirty and unsanitised water to remove soil increases the risk of rots. This risk is highest where the produce is warmer than the water or the produce sinks in the water and is therefore more subject to infiltration by contaminated water. A newly registered water treatment system, which uses iodine as the sanitiser, was highly effective in relatively clean water. We are not yet able to make any judgement of it's cost efficiency.

All sanitisers were affected by pH in the range 5.5 to 8.5. Growers may therefore need information and products to assist pH control.

Surfaces were more difficult to sanitise than water and proved reactive, depleting sanitiser levels. Peroxyacetic acid (which is used at a high concentration of 1%) was the most effective on surfaces and especially superior on wood. Other sanitisers can be used but because of the depletion, larger volumes are required if used at label rates. Non chemical alternatives should be developed and evaluated such as solar pasteurisation and biofiltration of water and heat treatments for contact surfaces.

The addition of surfactants to reduce the surface tension on the microbial cell wall, improving sanitiser uptake, deserves further study. For sanitisers depleted by organic and mineral load, flocculation followed by filtration is an option to improve economy of treatment. The technologies and their cost effectiveness should be investigated with the objective to assist growers reduce the consumption of both water and sanitiser. For some sanitisers methods for pH management need investigation.

The use of formalin for sanitising tomato stakes

Dipping of stakes in sanitisers is about one tenth of the replacement cost and should be encouraged as a preplanting field hygiene practice. However, precautions should be taken to prevent poisoning where formalin is used and to prevent environmental contamination. Simple test methods are available to determine if sanitiser concentrations become depleted during use so that topping up rates are based on informed decisions. A 1 minute dip in 1% Peratec 5 and a 20 minute dip in 30ppm calcium hypochlorite 5ppm BCDMH or 1% Phytoclean® was effective against *Clavibacter*.

Recycling of used wash water

More work is required to develop water treatment technologies to enable the efficient use and safe reuse of washwater, minimising water consumption and water discharge. Constructed wetlands may be functional, however many farms especially periurban farms do not have the required space.

General recommendations for hygienic postharvest

- Wash vegetables only where there is a proven advantage.
- Remove or trim off rotted plant parts before washing to minimise contaminating the wash water and remove trimmings from the grading/packing line as soon as possible.
- Do not mix rotting produce with intact produce during harvest, handling or storage.
- Clean and sanitise harvest, grading and packing equipment.
- Test source water (and sanitise if contaminated) and sanitise recirculated wash and hydro-cooling water.
- Maintain handling equipment so that mechanical damage to produce is minimised.
- Encourage personal hygiene – provide clean toilet and hand washing facilities.
- Cool chain reduces spoilage, but check for chilling injury in susceptible cultivars.
- Monitor critical control points.

Refer to Guidelines for On-Farm Food Safety (Agriculture Fisheries and Forestry-Australia 2001)

Effectiveness of technology transfer

We estimate that 480 growers, packers and others attended the workshops and a further 200 received the field day handbooks on "Managing clean and safe water for washing vegetables". This is less than one tenth of the industry. We anticipate that some messages will diffuse further, however there will be a need for advisers to maintain an awareness of hygienic postharvest principles and methods to assist industry in the future. Many advisers need enhanced capabilities and resources to enable them to be effective consultants on these topics.

Other issues

The industry would benefit from a rapid test for microbial contamination. Conventional tests (such as those used in this study) take several days and may not give an accurate assessment of the food safety status of stored vegetables. For example, *E. coli* is very sensitive to sanitisers and therefore testing produce which has been sanitised, for the presence of this bacterium, may overlook the presence of other hazardous microorganisms.

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Appendix 1

AGRICULTURE VICTORIA

A business of the Department of Natural Resources and Environment

This is a new research project funded by the national vegetable levy

HRDC

If this project is relevant to your business, please answer the following survey.

Project: Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes

Aim of the Project: To develop effective ways of cleaning produce and handling equipment in a way that is safe for consumers and cost effective to growers.

Activities to be undertaken: Laboratory and farm testing of different water and surface disinfectants and application systems.

	Yes	No
Do you wash, hydrocool or use water in handling produce?	☐	☐
Do you add chemicals to water when washing produce?	☐	☐

If 'yes', what is the tradename?……

	Yes	No
Do you regularly clean harvesting or grading equipment?	☐	☐
Do you have an effective method for cleaning surfaces	☐	☐

or equipment? High pressure/ hot water/ chemical/ other

If yes, please describe……

What vegetables do you grow? Broccoli/ carrots / tomatoes / sweet corn / celery / Asian vegetables / Brussels sprouts / other, please describe……

	Yes	No
Do you wish to receive our free newsletter on this topic?	☐	☐

Please send your completed survey using the prepaid envelope by 30/6/00 — no stamp required

Contact Details (or attach business card)

Name:

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Phone: Mobile:

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