Hort Innovation

Final Report

Managing microbial food safety risks in the Australian citrus industry

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Delivery partner:

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Managing microbial food safety risks in the Australian citrus industry (CT20005)

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Public summary

The project (CT20005) was aimed at mitigating microbial food safety risks associated with the production, postharvest handling and supply of citrus to consumers in domestic and export markets. To remain competitive in the export markets it is imperative that the Australian citrus industry has the capacity to consistently supply safe fruit. Due to the inedible peel, citrus fruit presents as a relatively lower microbial food safety risk to consumers. However, any detection of microbial contaminants on the fruit could lead to product recall and trigger a non-tariff barrier in the export markets, thus posing a serious 'Trade Risk'. This investment delivered a national snapshot of the industry's current food safety practices and identified potential gaps to be addressed. The project followed a whole-of-chain approach to identify potential hotspots for microbial contamination and cross-contamination along the supply chain. Each link within the supply chain was examined, starting with field production and postharvest processing through to retail by collecting fruit and environmental samples (2,257) to detect the target foodborne bacterial pathogens (*Listeria monocytogenes*, *Salmonella* species and Shiga-toxin *Escherichia coli,* STEC). Associating industry practice with microbial risk mapping proved to be an effective strategy towards microbial risk identification and development of targeted interventions to manage these risks.

The project achieved remarkable success in engaging more than 50 citrus packers throughout Australia, representing a substantial network of over 750 growers. Through microbial mapping analysis along the supply chain, it was revealed that *Listeria monocytogenes* emerged as the primary environmental pathogen of concern, with *Salmonella* and STEC posing minor risks. Insights derived from the prevalence and distribution data indicate that *Listeria monocytogenes* was present in citrus orchard soils, which could lead to the potential contamination of fruit on trees through dust storms and harvest equipment. The subsequent whole genome sequencing of Listeria isolates confirmed that harvested fruit served as the primary carrier of the pathogen from the field into packing facilities. The pathogen established itself in the postharvest processing equipment and premises, including harvest bins, conveyor belts, brushes, and eventually reached cool rooms via forklifts and workers' movements.

The industry has robust microbial food safety preventative controls during both production and postharvest handling operations. However, continuous improvement and change of some practices were recommended during fruit production, harvest and postharvest operations. The project's effectiveness in garnering significant industry interest has played a pivotal role in raising awareness about microbial risks. Based on the current industry practice, microbial risk mapping and available scientific literature, Best Practice Guidance was developed as a technical resource to achieve the microbial risk management objectives. Several technical presentations and articles were delivered through various channels of communication and engagement such as on-site technical visits, workshops, forums, field days, meetings and conferences. The on-site visits were the most effective method to enhance the skills and knowledge of growers, packers and the key staff involved in quality assurance.

In conclusion, the project's success was dependent not only on its ability to engage citrus growers and packers but also in uncovering novel insights into the prevalence, distribution, and pathways of microbial contamination. The identified gaps in industry practices serve as opportunities for improvement and addressing them will contribute significantly to safeguard the food safety record of the citrus industry. The project outcomes contribute to the Citrus Fund's Strategic Investment Plan (2022-2016) which outlines monitoring and managing food safety risks to maintain consumer confidence in Australian citrus as a strategic priority (1.6) to achieve sustained market access and trade growth. The project achievements align with the Hort Innovation Strategy (2024-2026; Priorities 1.3 and 2.1) to enhance and safeguard supply through research strengthening industry's readiness for existing and emerging threats which could impact local and global demand.

Technical summary

This project's aim was to identify and manage microbial food safety risks associated with citrus production and postharvest supply of fruit. Benchmarking industry practice is a crucial step to evaluate the effectiveness of current food safety practices, along with a comprehensive mapping process to determine the nature and magnitude of microbial hazards present in the supply chain. This project collected industry practice data to generate a national snapshot of the industry's current food safety practices and identify potential gaps to be addressed. Microbial risk mapping process involved the collection of fruit and environmental samples (2,257) from citrus orchards, harvest, and postharvest operations to detect the target foodborne bacterial pathogens (*Listeria monocytogenes*, *Salmonella* species and Shiga-toxin *Escherichia coli*).

It was revealed that *Listeria monocytogenes* emerged as the primary environmental pathogen of concern, with *Salmonella* and STEC posing minor risks. Insights derived from the prevalence and distribution data indicate that *Listeria monocytogenes* is present in citrus orchard soils, suggesting potential contamination of fruit on trees through dust storms. The whole genome sequencing (WGS) of Listeria isolates confirmed that harvested fruit served as the primary carrier of the pathogen from the field into packing facilities. The pathogen establishes itself in the postharvest processes, including harvest bins, conveyor belts, brushes, and eventually reaches cool rooms via forklifts and worker movements.

Experiments were performed under simulated supply chain conditions to analyse and understand the potential survival of *Salmonella* and *Listeria monocytogenes* on various types and varieties of citrus fruit. It was discovered that regardless of the citrus type and cultivar, these pathogens did not multiply on the fruit surface during simulated cold phytosanitary treatments and shelf-life periods however they still could survive these conditions. These observations highlight a need for preventing microbial contamination and cross-contamination of fruit in the first place and not be reliant upon potential pathogen die-off during supply chain conditions.

Based on the current industry practice, microbial risk mapping and available scientific literature, Best Practice Guidance was developed to identify critical points and practices and manage food safety risks effectively. A variety of engagement methods were deployed in this project, including on-site technical visits, workshops, forums, meetings and conferences. In conclusion, scientific evidence and microbial data generated in this project provided the foundation for microbial risk management in the citrus industry. Overlaying industry practice data with microbial risk analysis offered novel insights into the microbial pathogen contamination and transmission patterns and routes. Further investigations are warranted to answer unresolved questions on the prevalence and distribution of environmental pathogens in citrus production systems as influenced by agronomic practices, geographical landscapes, and extreme weather conditions.

Keywords

Citrus; Listeria; Salmonella; Food Safety; Microbial risks; Postharvest.

Introduction

In 2021-2022, the Australian citrus industry produced 760,000 tonnes of fruit valued at $910 million and almost a third of it was exported to major Asian markets valued at $451.2 million (Anonymous, 2023). Oranges and mandarins were the major citrus fruit exported and contributed ~$260 million and ~$180 million to the total export value, respectively. The success of citrus export is built on free trade agreements with Asian countries and a clean, green, and safe reputation of the industry. 'Healthy, nutritious and safe' is the unique selling point that the Australian citrus industry promotes in its marketing campaigns. However, the industry needs to be proactive in maintaining the confidence of consumers, regulators and trading partners in the quality and safety of their fruit to ensure the market access is retained, and new markets are created.

The NSW Department of Primary Industries (NSW DPI) proposed a 'Food Safety Shield' concept to assist the citrus industry in developing and understanding food safety controls and strategies required to manage physical, chemical and microbial hazards. Adoption of on-farm quality assurance programs forms the first line of defence and is the foundation of the food safety shield against all types of hazards. The second line of defence is targeted towards chemical risks as the domestic and export markets have maximum residues limits (MRLs) for preharvest and postharvest chemical usage. The industry has ongoing R&D investments to manage insects, pests and diseases with integrated approaches, to find alternative chemicals and other control measures. Similarly, a citrus postharvest program provides the industry with solutions to manage shelf-life, supply chain disease-related issues and fungicide resistance. Until 2017-18, the citrus industry had been a signatory to the National Residue Survey program which provided verification of the chemical risk management. From 2018 onwards, the citrus agrichemical and export MRL program (CT18001) has been supporting the industry in compliance with the regulatory MRL limits set both in Australia and overseas. This program is responsible for a residue monitoring program and is conducting trials on residue testing. The current project was undertaken to strengthen microbial food safety preventative controls in the citrus industry.

In 1999 a Salmonella outbreak linked to unpasteurised orange juice in South Australia led to a ruling by the Federal Court that citrus growers who supplied the fruit to the processor had breached their contracts to supply 'safe' fruit, and the liability for the loss and damage was found to rest with the growers (Rajapakse, 2016). The *Salmonella* Typhimurium (phage type 135a) strain involved in the outbreak was traced back to the fruit in the packing shed and in particular the fungicide and wax solutions which were applied to fruit. Prevention of this outbreak was possible with certain measures including the use of a sanitiser in wash and fungicide tanks as well as a regular change of the water in the fungicide tank (Rajapakse, 2016). This historical example highlights the microbial food safety risks posed by fresh produce including citrus. Similar examples of salmonellosis outbreaks linked to citrus has been reported from other countries (Parish, 1998).

Environmental pathogens such as *Listeria monocytogenes*, *Salmonella a*nd pathogenic *Escherichia coli* are widely prevalent in natural conditions (Strawn et al., 2013). Among these top-three pathogens, *Listeria monocytogenes* has been reported to the most widely prevalent one in fresh produce production landscapes, particularly in soils and water (Strawn et al., 2013; Weller et al, 2015). These pathogens are capable of transferring to fruit through various routes and their transmission may be increased by extreme weather conditions such as duststorms, flooding and heat waves (Singh, 2023). Once these pathogens enter the processing facilities with fruit, machinery and workers; these can establish and harbour in processing equipment and premises. *Listeria monocytogenes* has been reported in the citrus packing sheds in California (Suslow et al., 2019). The research has shown that *Listeria monocytogenes* attach to the citrus fruit surface more strongly than *Salmonella* and *E. coli* O157:H7 making it harder to kill during fruit washing and sanitisation process (Martínez-Gonzáles et al., 2011). Postharvest washing and sanitisation treatments are known to be the hotspot for cross-contamination of fruit if the sanitisation of wash water, fungicides and waxes is not maintained (Kanetic et al., 2008; Harris 2019; Adaskaveg et al., 2021; Sheng et al., 2023). The bacterial pathogens are capable of surviving in waxing solutions for extended periods and also on the fruit surfaces (Sheng et al., 2023). If these pathogens could persist through the supply chain conditions, these could be detected by regulatory authorities in the export markets and could also be transferred from the fruit to consumer during hand peeling (Jung et al., 2017) or juicing (Martínez-Gonzáles et al., 2011). It is therefore of utmost importance that everyone involved in the supply chain must understand their responsibility to ensure microbial food safety.

This project (CT20005) was aimed at providing an additional layer of defence to the food safety shield. It was the first-of-its-kind investment to bolster microbial risk management in the citrus industry. Microbial food safety risks for fresh fruit, including citrus, are real, and an understanding of the sources and routes of microbial contamination is the first step towards managing such risks. Postharvest washing and sanitisation do not eliminate the microbial hazards from the fruit if the contamination has already occurred in the field. Furthermore, the microbial pathogens (*Listeria monocytogenes*, *Salmonella* species, noroviruses), if present in the packing house environment, can contaminate the fruit during postharvest processing. The growers' perception of citrus fruit being 'low-risk' or 'no-risk' to consumers requires a change in their attitude towards managing microbial contaminant risks.

In 2020, citrus fruit (oranges, limes and lemons) were recalled in California due to the presence of *Listeria monocytogenes* on the fruit surface (US FDA 2020). It triggered international regulatory authorities to consider '*Listeria monocytogenes*' as the highest microbiological analytical priority for citrus. To remain competitive in export markets it is imperative that the Australian citrus industry has the capacity to consistently supply safe and nutritious fruit. Due to the inedible peel, citrus fruit presents as a relatively lower microbial food safety risk to consumers. However, any detection of microbial contaminants on the fruit poses a potential "trade risk" and could trigger a non-tariff barrier. Given the current volatility in export markets due to various geopolitical reasons this risk is significant. In managing food safety risks prevention is seen as a better approach than cure. Preventative and proactive investments are recognised as cost-effective as the consequences of dealing with, and recovering from, a food safety crisis for an export-oriented industry is enormous.

To mitigate microbial food safety risks to the citrus industry, the current project was undertaken with the following objectives:

- To assess the current food safety practices of the citrus industry and identify potential gaps
- To conduct microbial food safety risk mapping along the supply chain with a focus on citrus packing sheds
- To understand the survival and persistence of foodborne bacterial pathogens (*Salmonella* and *Listeria monocytogenes*) on fruit in the supply chain
- To develop and deliver a best practice guide to the industry to improve food safety practices
- To disseminate citrus microbial food safety information and resources to the citrus industry.

Methodology

A co-innovation systems approach was adopted that incorporates science, extension and adoption strategies designed to involve all stakeholders. A project reference group (PRG) with representatives from the citrus industry, Hort Innovation, and NSW DPI was formed to review the project progress and provide strategic direction. The following project activities were undertaken to achieve the abovementioned project objectives:

Project activity 1: Analyse the Australian citrus supply chain to identify microbial food safety risks and deliver a national snapshot of the industry's current food safety practices.

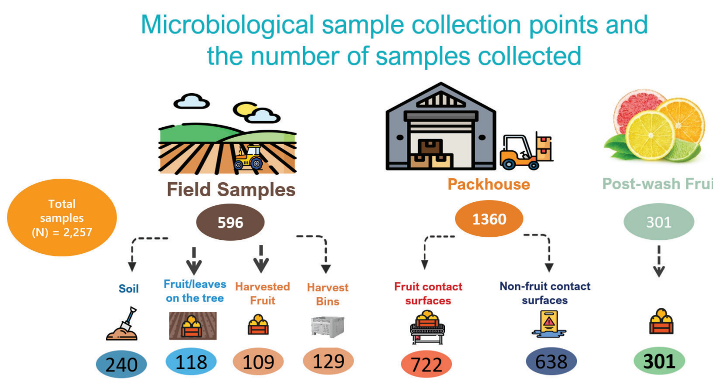
Microbial contamination can occur anywhere along the supply chain. However, the highest chance of contamination occurs in the field and at the packhouse. Our knowledge and experience in traceback investigations and root-cause analysis reinforced the hypothesis that cross-contamination in the packhouses could lead to major market failures. This project focused on the major production regions supplying all types of citrus, including oranges, mandarins, limes, lemons and grapefruit. A total of 56 citrus packers were visited, including 24 in NSW, 13 in Qld, 8 in SA, 6 in Vic, 4 in WA and 1 in

NT. The project team reviewed current practices and critical control points in the growing and postharvest processing of fruit. Each grower/packer visited during this activity who shared their practices received a summary of observations, food safety results from microbiological test results (project activity 2) and potential gaps in both on-farm and postharvest best practice and recommendations for improvement. The collated data was analysed to determine the industry trends and a national snapshot of the practices along with recommendations for continuous food safety improvement was generated.

Project activity 2: Map microbial risks along the supply chain starting from field production through postharvest processing, distribution and retail.

Mapping potential microbial contamination and cross-contamination hot spots was the aim of this project activity. Fruit and environmental samples were collected from all citrus-growing regions in Australia and various stages of the supply chain (growers, packers, and retailers). A total of 2,257 fruit and environmental samples were collected from citrus orchards (field samples = 596), packhouses (1,360) and retail (post-wash fruit = 301). The collected samples were enriched and analysed employing real-time polymerase chain reaction (RT-PCR) assays for three target pathogens – *Listeria monocytogenes*, *Salmonella* species, and *E. coli O157:H7*. The assays used for RT-PCR were approved for commercial use by the Association of Official Analytical Collaboration (AOAC) International. Following any positive detection, confirmatory culturing and assays were completed as well as consultation with the grower/packer for traceback investigation. The investigations were then subjected to root-cause analysis for the grower's education. Based on the microbial risk mapping, potential hotspots for contamination and cross-contamination were determined in the citrus supply chain.

During the microbial risk mapping, *Listeria monocytogenes* emerged as the major pathogen of concern. Therefore, some isolates of *Listeria* were subjected to whole-genome sequencing (WGS) to understand the diversity of bacteria present in various environments and regions. The WGS was undertaken by Agriculture Victoria Research (AVR). An in-depth analysis of the isolates occurring in the environment enabled mapping of the transmission route of the pathogen from field to the packing houses.



Project activity 3: Generate experimental evidence to analyse the potential survival of foodborne bacterial pathogens on various types and varieties of citrus fruit under simulated conditions.

Survival and persistence of foodborne bacterial pathogens on the fruit surface are influenced by several factors. In this project, supply chain conditions for export and domestic markets were simulated. For export, cold treatment is a commercial phytosanitary treatment accepted by major markets (e.g., China and Japan). In 2019, in the fruit fly sensitive export markets, 60% of fruit of the 200,000 tonnes exported was treated with cold disinfestation (CT15012 Final report). A review of the current phytosanitary requirements revealed that a generic storage temperature of 3°C or below for 21 days will meet on-shore and in-transit treatment requirements for all citrus types, export markets and interstate phytosanitary protocols. To simulate the supply chain conditions, we investigated the potential survival of *Salmonella* and *Listeria monocytogenes* on the surface of oranges (Navel, Valencia and Bellamy), mandarins (Afourer and Imperial), and lemons. The fruit inoculated with low and high populations of these bacteria were stored under a simulated generic cold treatment (3°C or below for 21 days) and were evaluated at weekly intervals immediately after storage and simulated shelf-life conditions (20°C for 4 days). Further details on the methodology are presented in Appendix III.

Project activity 4: Develop a best practice guide based on the knowledge of potential sources and routes of contamination.

In this project, a Best Practice Guidance for Microbial Food Safety Management in the Citrus Industry has been developed with a major focus on food safety risks and their management during citrus production, harvest and postharvest handling. This guide applied food safety principles to the citrus production and postharvest processes and aimed to enhance food safety knowledge across the supply chain allowing a better understanding of the sources and routes of microbial contamination. Industry practice review (project activity 1), microbial risk mapping (project activity 2), experimental evidence (project activity 3), literature review, technical consultation with citrus growers, R&D providers, retailers and other stakeholders along the supply chain provided the foundational information for the best practice guide. The critical information summary from each section of the guide has been condensed into infographics for easy and quick uptake of the information. The dissemination of the guidance to the industry has been/will be undertaken through multiple channels of communication, including articles in the Australian Citrus News and uploading the guide on the websites of Hort Innovation, NSW DPI, Citrus Australia and other organisations. The guide will be released to the industry during the Australian Citrus Congress 2024 to be held in March 2024 on the Sunshine Coast for broader publicity, followed by promotion of the guide through horticulture media portals.

Project activity 5: Deliver food safety extension through appropriate channels including workshops, forums, meetings and industry communications channels

Dissemination and adoption of best practice in the industry was the overarching long-term goal of this project. The NSW DPI being the lead agency for citrus R&D within the National Horticulture Research Network (NHRN) has an extensive network of researchers, extensionists and professional relationships with national and regional peak industry bodies, state government agencies and consultants. This network was deployed for engagement with citrus growers and packers in the major citrus production regions of Australia and to disseminate project information to the beneficiaries. Some of the extension methods used include one-on-one consultation (on-site technical visits), technical presentations in industry meetings, workshops and conferences, participation and discussions in open/field days, exhibition of project information at major events, and food safety helpdesk services. The industry practice and microbiological survey reports to the citrus packers were instrumental in establishing functional and trusted relationships. All industry data and information were handled with the utmost care to avoid any commercial confidentiality and privacy breaches.

Project activity 6: Identify opportunities for future R&D investment

This project established strong linkages with stakeholders and as a result developed sound knowledge of the emerging challenges and opportunities that the industry will face in the future. The project leader in consultation with the industry representatives identified future R&D opportunities to ensure the food safety outcomes for the industry are met. This report contains recommendations for future R&D to mitigate food safety risks for the citrus industry.

Results and discussion

Project activity 1: Analyse the Australian citrus supply chain to identify microbial food safety risks and deliver a national snapshot of the industry's current food safety practices.

Production and postharvest practices determine the microbial quality of fruit. In this project, the major focus was on analysing the food safety practices at harvest and postharvest stages in the citrus supply chain. A total of 56 citrus packers were visited across Australia but the practice data was collected from 51 packing sheds which were operational during the visit. The collection of practice information was evidence-based, hence data from operational sites was used for analysing the industry practice snapshot and trends.

Citrus grower/packer profiles: All citrus packers (N=51) had compliance with a food safety certification scheme with FreshCare being the most common one. ***Majority of the citrus packers (37/51) exported fruit to various markets including, China, Japan, South Korea, Thailand, Indonesia, Canada, USA, India, New Zealand, and Vietnam***. The citrus packers engaged in this project represented a diverse group of citrus types produced and packed in their facilities: oranges (34/51), mandarins and tangelos (33/51), lemons (31/51), limes (19/51), and grapefruit (18/51).

The high-level information on industry practices is presented below:

Citrus fruit production: As described above, all citrus growers/packers who participated in this project had a food safety scheme certification in place. Preharvest sources and routes of microbial contamination in citrus orchards were investigated through in-field observations and analysing growers' practices (N=51). The use of organic amendments in the orchard soil health management was common (41/51) and most growers used the word "compost" that contained animal products. The timing of application of organic amendments was mainly after the fruit harvest using the band application method under the tree trunk. A high level of awareness on the presence of microbial contaminants in raw animal manures was observed among growers. However, the knowledge of transfer route of microbial pathogens from the environment to fruit was not common. It was observed that some citrus production regions were highly prone to dust storms and were located in the vicinity of commercial livestock operations. The livestock operations were hypothesised to be the primary contributor to the microbial contamination of surface water sources and could be contributing to the long-distance dispersal of pathogens with dust storms. However, these are preliminary observations and need data and evidence to dissect the microbial contamination threats with the coexistence of horticulture and livestock industries. The use of perennial windbreaks in some citrus orchards was intended to improve fruit production and quality. ***However, the use of perennial windbreaks and other agronomic practices aiding in dust suppression on farms will be the best practice to mitigate microbial contamination of fruit in the first place.***

The high levels of prevalence of Listeria monocytogenes in citrus orchard soils, particularly under and near tree trunk was observed in this study (Project activity 2). The year-around moisture in this zone combined with high organic matter from soil amendments and leaf litter along with low or no exposure to the UV from sunshine are speculated to be the main reasons for the higher prevalence of Listeria in this zone. Tree skirting as part of the integrated pest management for citrus orchards could also minimise the risk of Listeria survival under the trees due to increased sunshine exposure.

Drip irrigation was the most common irrigation method across all production regions, minimising the possibility of microbial contamination of fruit through water directly. The contaminated irrigation water can introduce microbial pathogens in orchard soils and it has been reported that irrigation water could be one of the major carriers of Listeria in fresh produce production in the field (Weller et al., 2015). On the other hand, the use of contaminated water for chemical spray applications, especially before fruit harvest, could lead to microbial contamination of fruit directly. In addition to the microbial risk, the water sources contaminated with blue-green algae (BGA) could contain BGA toxins which could be transferred to the fruit. The persistence and diffusion of BGA toxins on and into citrus fruit are currently unknown. The growers' awareness of microbial contamination via chemical spray applications and BGA chemical hazards could be raised in the future.

Wildlife incursion in citrus orchards was commonly reported by growers along with the awareness of risks these animals pose to microbial food safety. It was observed that growers undertake measures such as fencing and netting to minimise the wildlife incursion into their farms.

In conclusion, organic soil amendments, dust storm-mediated pathogen dispersal, water used in the chemical spray application, irrigation water and wildlife incursions emerged as the major sources and routes of microbial contamination during citrus fruit production. The best practice guide (project activity 4) addresses the strategies to mitigate microbial contamination risks due to these factors.

Harvesting: The use of plastic harvest bins (51/51) was common across all citrus production regions. The plastic harvest bins are easier to clean and sanitise compared to wooden bins. Only one citrus grower/packer was observed to be using some wooden bins for postharvest fruit handling. The wider adoption of plastic harvest bins is a good industry practice to minimise microbial food safety risks. However, harvest bin hygiene was a major issue as only 20% citrus packers were rated to have acceptable levels of hygiene, that is no signs of excess soil/organic matter in bins. It was commonly observed that harvest bins were placed on the ground during fruit harvesting operations in citrus orchards. The harvest of fruit in winter coincides with a significant amount of rainfall and wet conditions. As a result, harvest bins often get muddy during their contact with the ground. Once these harvest bins are stacked during fruit transportation to the packing house or for temporary storage of fruit, the soil/mud gets transferred to the inside causing widespread soiling of fruit and harvest bins. Since the environmental pathogens such as *Listeria monocytogenes* live in soils, it is potentially the main reason for microbial contamination of harvest bins and fruit during harvesting operations. This has been identified as the major food safety practice gap during harvesting operations. Some citrus growers place harvest bins on trailers to avoid ground contact which is the best practice to minimise microbial contamination linked to soil.

Best practice recommendations to mitigate microbial food safety risks associated with harvesting operation are trailer mounting of harvest bins, thus avoiding ground contact, and cleaning and sanitisation of harvest bins after each use. Some citrus packers have installed and started using automated cleaning and sanitisation systems for harvest bins and adopted this practice.

Postharvest handling

Pre-sorting fungicide drenching: Postharvest fungicide drenching was a common practice (39/51) to minimise fungal rots in the supply chain. This operation uses a large quantity of fungicide in recirculation mode for drenching of citrus fruit in harvest bins. Since this is the first wash/drench step, there is a significant organic load in the drench solutions, presenting a risk of cross-contamination of fruit with bacterial pathogens. Harvest bins which are generally placed on the ground during fruit harvest were observed to be introducing organic matter in the drench solution potentially affecting the fungicide efficacy and transferring the pathogens such as *Listeria monocytogenes* into the drench solution. The fungicide solutions generally don't have bactericidal effects and bacterial pathogens such as *Salmonella* and *Listeria monocytogenes* can survive in most fungicide solutions (Harris, 2019; Adaskaveg et al., 2021). The frequency of fungicide solution change also varied from daily to weekly with topping up the most common practice.

This postharvest operation was identified as the major risk of cross-contamination of fruit with bacterial pathogens. Some packers (7/51) were using a sanitiser in the drench fungicide solution to mitigate bacterial pathogen risks. Six out of seven packers who used sanitisers mixed the sanitiser with fungicide. The most common fungicide used in drenching was thiabendazole and chlorine was the common sanitiser used in combination. There was only one packing shed which had a two-step treatment system in which fruit first received a sanitiser drench (chlorine) followed by a fungicide drench. This is the best practice example to mitigate cross-contamination risks during drenching operations.

Immediately after harvesting, citrus fruit should be drenched with a sanitiser followed by drenching with a fungicide. This 'two-step' drenching operation minimises cross-contamination risks and maximises the efficacy of both types of chemicals. Their concentrations should be monitored regularly along with changes in the frequency of replacing drenching solutions adjusted according to the organic matter load on the fruit. Mixing of fungicides and sanitisers is not recommended unless the manufacturers have suggested their compatibility with each other.

Postharvest processing

Cool chain: Following postharvest fungicide drenching, fruit were generally held in cool rooms until further processing. If degreening was to be undertaken, the fruit were held in degreening rooms maintained at the required temperature, humidity and ethylene conditions. Occasionally, fruit in harvest bins, before and after drenching, were observed to be stored under ambient conditions (inside and outside the sheds) which is not ideal from the postharvest and food safety perspectives. **Maintaining cool chain after harvest is critical to maximise food safety, storage and transportation potential of fruit with minimum quality and fungal decay losses.**

Fruit receival: The fruit receival area in the packing shed is the gateway for fruit (and environmental pathogens) to enter the processing facility. The harvested fruit comes with significant amount of organic matter and debris from the field. It was observed that the fruit receival area was the weakest link in the food safety chain of the citrus industry. Most packers neglected this area for regular cleaning and sanitisation. As a result, the high number of Listeria detections were recorded in the fruit receival areas. As most citrus packing facilities are not designed with proper drainage for regular cleaning, this is a challenging aspect. **It is recommended to clean and sanitise the fruit receival area after each shift so that the pathogen transfer continuum is interrupted.**

Fruit dumping: At the commencement of postharvest processing, fruit are either dumped on a conveyor belt or rollers (dry dumping) or in a large capacity water tank or flume (wet dumping). Dry dumping (45/51) is a common industry practice which is favourable to minimise microbial cross-contamination risks posed by water flumes. Four out of six wet dumping operations used a sanitiser in the dump tank or flumes. However, maintaining an optimum sanitiser concentration in large quantities of water could be a challenge. The wet dumping process is believed to reduce the impact damage on fruit during dumping. On the other hand, dry dumping could cause minor injuries and cracks in the fruit during the dumping process leading to the potential pathways for internationalisation of bacterial and fungal pathogens during subsequent processing steps, causing higher levels of fruit breakdown in the supply chain. **Overall, dry dumping with careful unloading of fruit onto the conveyor belt is relatively lower risk than wet dumping in large volumes of water.**

Pre-wash sorting: Approximately 10% citrus packers (5/51) were observed to sort out damaged and rotten fruit before washing and sanitisation process. The mouldy and damaged fruit were often observed to be undergoing washing and sanitisation process leading to increased microbial burden on wash water and fungicide tanks and wastage of resources used in their processing. The lack of provision for sorting in packing lines design and layout was observed. **Eliminating rotten and damaged fruit before washing and sanitisation process is a cost-effective strategy to minimise mould spore load in the wash water and fungicide tanks and packing sheds. It would lead to improved food safety outcomes for the citrus packers.**

Brushing and washing: Fruit washing and brushing is a critical postharvest operation to improve microbial and appearance quality of fruit. Flatbed brushes were most commonly used along with spray bar washing systems with several packers (26/51) claiming a high pressure washing (not properly defined water pressure) system in place. Ideally, all brush rollers should be under the sanitised wash water for self-cleaning and sanitising purposes. However, the distribution of wash water spray nozzles was observed to be not covering all brush rollers. The hygiene of brushes is critically important to minimise cross-contamination risks. *Listeria monocytogenes* was detected in several samples collected from brushes in the washing areas and also the brushes used for final finishing of waxed fruit. Once brushes get contaminated with bacterial pathogens, these are very difficult to clean and sanitise. The complete replacement of contaminated brush rollers is the practical solution. **It is recommended that all brush rollers should remain covered under the sanitised wash water along with daily cleaning and sanitisation schedule at the end of the shift to remove organic matter and debris from the core and sides of the rollers.**

Postharvest water is an important element in microbial food safety of citrus fruit. The citrus packers use a variety of postharvest water sources as follows:

- Surface water: 26/51 (50.9%) (River/creek-13; channel 5; dam 4; rainwater 4)
- Town water: 21/51 (41.2%)
- Ground water: 6/51 (11.8%)
- No wash water: 1/51; Some packers use more than one water sources.

Surface water from various sources (rivers, creeks, channels, dam and rainwater harvesting) was the most common for citrus packers located at remote locations. The microbiological quality of surface water changes dramatically depending upon the season and weather events. However, 58% (15/26) citrus packers who use surface water have installed water filtration and treatment systems to improve water quality for postharvest usage. On the other hand, town water, which is considered the safest, is widely used in the packing operations (41.2%) located in larger towns. **These statistics highlight that there is need for increasing the adoption of water filtration and treatment systems in the packing operations who are dependent upon surface water sources.**

Postharvest wash water sanitisers: The presence of a sanitiser in the wash water is critical to kill bacterial pathogens. The bactericidal potential of the sanitiser is dependent upon its type, concentration, contact time and other factors such as organic load and pH. Citrus packers who used town water added sanitisers during the washing process through automated systems or manually. Some citrus packers who used surface water from rivers and creeks injected small doses of sanitisers after filtration step in large capacity water storage tanks which were further connected to the washing lines. This practice allows extended duration of sanitiser contact with wash water, leading to proper disinfection process.

The application of sanitiser in wash water was widely adopted (49/51) in the industry, two packers were not using any sanitiser in the wash water – one was a small-scale operation, another was a large-scale packer supplying to domestic and export markets. Recirculation of wash water without a filtration step was found to be a common practice (40/51). Considering most operations used a sanitiser in the wash water, it reduced the risk of cross-contamination. **It is recommended to treat the wash water to drinking quality before it should be used or re-used for fruit washing to mitigate the microbial contamination risks to the lowest level. Alternately, single use wash water (run-to-waste) system is recommended, but it may not be an environmentally sustainable option.**

Among 49 sanitiser-users in the postharvest wash water, peroxyacetic acid (PAA) was the most popular (19/49) followed by calcium hypochlorite (13/49), chlorine dioxide (7/49), sodium hypochlorite (3/49), chloro-bromo dimethylhydantoin (3/49), and chlorocyanurates (4/49). Most citrus packers using PAA, chlorine dioxide and chloro-bromo dimethylhydantoin as sanitisers used automated dosing systems (59%) for injection of the sanitiser into wash water. However, sodium/calcium hypochlorite and chlorocyanurates users adopted the manual addition of sanitiser in wash water tanks.

Each sanitiser has its own merits and demerits due to highly oxidative nature. **It is recommended that the industry should avoid the use of chlorine-based sanitisers due to their negative impact on the environment.** The selection of PAA in postharvest wash water is recommended due to its efficacy, low environmental impact, and its action is least influenced by the presence of organic matter. Other alternative options such as electrolysed water, cold plasma and ozone should be explored to meet sustainability requirements with minimal environmental impact.

Automation of sanitiser dosing and its digital monitoring and logging are strongly recommended to eliminate human error. Furthermore, verification of sanitiser concentration should be conducted at regular intervals using digital measurement tools. The use of test strips in measuring sanitiser concentrations and pH should be avoided. It was observed that target and actual concentrations of sanitisers varied greatly among citrus packing operations. Most operations did not achieve the target sanitiser concentrations and were dependent upon test strips results for verification. **Achieving recommended levels of sanitiser concentrations is critical to miminise microbial food safety risks associated with the postharvest wash water.**

Understanding basic facts about the chemistry of postharvest sanitisers and chemicals is critical for their successful use without compromising efficacies. Some citrus packers were using two types of sanitisers in the postharvest washing line in a sequential manner. For example, fruit were first washed with water containing chlorine (effective at neutral/slightly acidic pH ≤7.0) followed by washing with water containing PAA (effective at low pH (≤3.5). The higher pH of chlorine in residual wash water on the fruit surface could potentially impact the efficacy of PAA and could also increase the pH of PAA solution tank as the time progresses during processing. It is therefore recommended to consider 'pH' factor in adopting such practices. Another similar example is the use of sodium bicarbonate (1-2%; pH 8.5 to 9.0) solution before washing the fruit with PAA. Some citrus packers were observed to be applying sodium bicarbonate solution before fruit washing for postharvest disease control and argued its role in wound repair on the fruit surface. If the application of sodium bicarbonate is to be integrated before fruit washing, a few wash water spray bars should be added for rinsing the fruit before these roll into the sanitised wash water zone, especially if the PAA is being used. Similarly, the impact of residual wash water pH on the subsequent fungicide treatment should be considered. The use of air blast/knives in removing excess moisture from the fruit surface during such conflicting pH scenarios may also be adopted.

Postharvest fungicides: The application of postharvest fungicides is a common practice to minimise fungal decay and rots. Postharvest fungicide applications can be divided into low- and high-volume systems based on the volume of fungicide solution used per tonnage of fruit. Low-volume systems include controlled droplet and air-nozzle applicators, whereas high-volume systems include flooders, drenchers, or dip tanks. Low-volume systems have an advantage with no disposal issues because all of the prepared solutions are applied to the fruit. These systems often use high concentrations of a postharvest fungicide in a fruit coating (i.e., wax) and rely on moving brushes to distribute it over the fruit surface with little to no run-off. High-volume application systems outperform low-volume systems in decay control efficacy because they provide better fungicide coverage (Adaskaveg et al., 2021).

It was observed that most citrus packers (43/51) used at least one postharvest fungicide during processing. Some small-scale lime and lemon packers (8/51) did not use any postharvest fungicide. Out of 43 fungicide-users, 31 adopted low-volume spray application, 6 adopted high-volume fungicide tank immersion method and 3 adopted drench/sheet application, while 7 packers used a combination of low and high-volume application methods. Most packers (29/43) applied cold fungicides, but 14 packers applied hot fungicide treatment. Imazalil was the most common fungicide (33/43), followed by thiabendazole (24/43), guazatine (16/43), fludoxionil (12/43) and others (6/43). Twenty-one packers out of 43 used at least 2 postharvest fungicides, 8 packers used at least 3 fungicides and 4 packers used 4 fungicide options. None of the packers shared if they were adding a sanitiser to the fungicide tank for sanitisation of fungicide solutions. This could be largely attributed to the uncertainty about the compatibility of these products.

From a food safety perspective, recirculation of fungicide solutions is a cross-contamination opportunity for bacterial pathogens which can survive in fungicide tanks. Our experimental data showed that *Listeria monocytogenes* and *Salmonella* could survive in most fungicide solutions currently registered for postharvest applications in the citrus industry. Fungicide solutions have to be effectively sanitised using sanitisers such as sodium or calcium hypochlorite or PAA. Sanitisers for recirculating fungicide systems need to be: (1) compatible with fungicides; (2) not phytotoxic to the fruit; (3) odour-free and non-irritant to workers; (4) fast-acting and able to kill human pathogens within a short time period of ideally less than 30 s (although a 4–5 min dwell time may be acceptable); and (5) should cause a > 5-log-reduction in colony forming units (cfu)/ml (i.e., a 99.999% reduction). Unfortunately, some fungicides are incompatible with these sanitisers. The active ingredient breaks down, and subsequently, performance of the fungicide is lost. The manufacturers recommendations should therefore be followed while mixing different products. **Imazalil, thiabendazole, fludioxonil, azoxystrobin, and pyrimethanil are compatible with PAA. Thiabendazole, fludioxonil, and azoxystrobin are compatible with sodium hypochlorite while imazalil and pyrimethanil are incompatible with sodium hypochlorite (Adaskaveg et al., 2021). It is a practical recommendation to frequently clean and sanitise fungicide tanks to remove bacterial contamination.**

Postharvest waxing: The application of wax is a standard industry practice for postharvest benefits. It was observed that 46 out of 51 packers used postharvest waxes in their operations. Carnauba was the most popular (29/46) waxing material used in the industry, followed by shellac (17/46) and composite waxes (12/46). The low-volume applicators were used for waxing the fruit, followed by drying step. The majority of citrus packers (30/46) used waxing material containing imazalil fungicide which was intended to provide residual protection against fungal pathogens in the supply chain. It could be speculated that waxing on the fruit surface would create a moisture-rich zone between the fruit surface and waxy top layer. This moisture-rich layer would be conducive to the survival of foodborne bacterial pathogens on the fruit surface. *Listeria monocytogenes* and *Salmonella* have been reported to survive in wax solutions for extended periods (Sheng et al., 2023). **It is recommended that conveyor belts/rollers connecting washing and waxing zones should be cleaned and**

sanitised with utmost care to avoid any contamination opportunities between these two steps. Also, the importance of washing and sanitisation step is reiterated as a control mechanism.

Some citrus packers use overhead brushes for giving a finishing touch to the waxed fruit. It was observed that these brushes were occasionally contaminated with *Listeria monocytogenes* which could lead to cross-contamination of fruit. It is recommended that these overhead brushes should be avoided. The application of waxes should also be undertaken according to the rates recommended for various types of fruit. Some citrus packers were either using higher rates of wax application or they were not properly cleaning and sanitising the post-waxing processing areas, leading to excessive deposits of waxes on the conveyor belts and rollers. Bacterial pathogens detected in these waxy deposits suggesting that it could be another hotspot for cross-contamination of washed, sanitised and waxed fruit ready for shipping. **The use of food-grade detergents is strongly recommended to remove excessive waxes from the processing equipment.**

Cool rooms: The processed fruit is held in the cool rooms which are generally maintained at or below 5°C. The cooler conditions are not congenial for bacterial growth, but *Listeria monocytogenes* can survive and grow under cooler conditions. It was observed that cool room hygiene is an issue across the industry. Only 65% citrus packers maintained their cool rooms in acceptable conditions while the rest were below average due to the presence of organic matter, rotten fruit, and dirty floors and walls. *Listeria monocytogenes* was regularly detected in samples collected from cool room floors and walls. The workers and forklift movements could have spread pathogens from fruit receival/processing areas to cool rooms and vice-versa. **It is therefore recommended that packers use proper cleaning and sanitisation strategies to maintain cool room hygiene.**

Packhouse hygiene: It is essential that a high level of hygiene in the packhouse is adhered to for minimization of postharvest fungal rots and for the improvement of food safety outcomes. Observations show 59% of packhouses maintained overall hygiene at acceptable level which reflected the cleanliness of packhouse floors (receival and processed fruit areas), cool rooms, processing equipment and premises. The concept of environmental monitoring and management programs (EMP) is novice for the citrus industry. However, several packers have contacted the project team to develop their EMPs. Regular cleaning and sanitisation of citrus packhouses is not widely practiced. Only 18 out of 51 packers used a packhouse cleaning chemical while the use of sanitisers in the equipment and premises hygiene was slightly more (24/51). **There is a significant opportunity to uplift the hygiene of packhouse through proper cleaning and sanitisation options, tools and processes. It is recommended that the industry should be aiming to popularise the EMPs among citrus packers and highlight the benefits linked to EMP costs.**

Project activity 2: Map microbial risks along the supply chain starting from field production through postharvest processing, distribution and retail.

In this project, fruit and environmental samples were collected from all citrus-growing regions in Australia and various stages of the supply chain (growers, packers, and retailers). A total of 2,257 samples were collected from citrus orchards (field samples = 596), packhouses (1,360) and retail (post-wash fruit = 301) and were analysed for three target pathogens – *Listeria monocytogenes*, *Salmonella* species, and Shiga-toxin E. coli (STEC; *E. coli O157:H7*).

Listeria monocytogenes was identified as the principal environmental pathogen of concern due to its higher levels of detection, followed by STEC and Salmonella. Based on the positive detections, STEC and Salmonella could be considered a minor microbial risk in the citrus supply chain. However, the detection of STEC in orchard soil samples (7.9%) indicate the potential use of animal products from livestock operations such as cattle feedlots and dairy. Salmonella was only detected in environmental samples from the packhouse premises (non-fruit contact surfaces).

Listeria monocytogenes was the major environmental pathogen detected in 2,257 microbial samples which were collected from citrus orchard soils, harvest bins, harvested fruit and packing shed equipment and premises. The highest prevalence of *Listeria monocytogenes* was observed in orchard soils (40.8%) followed by packing shed equipment and premises, pre-wash fruit and harvest bins. Based on these datasets, harvest bins, fruit receival areas and cool rooms were identified as the hotspots for microbial contamination and cross-contamination.

The detection of *Listeria monocytogenes* was much higher in soil samples collected from under the tree trunks compared to the sample from mid-row soils. It could be speculated that higher moisture levels in the root zone of citrus trees throughout the year, abundant organic matter from soil amendments and leaf litter and no or low exposure to UV light from sunshine could be contributing to the higher prevalence of this pathogens in orchard soils. The prevalence of *Listeria monocytogenes* in soil samples varied greatly from 3 to 72% among 8 citrus orchards. Further investigation is required to determine contributory factors or practices leading to the higher prevalence of Listeria in citrus orchard soils and their transmission and management options. In the interim, it is recommended not to use raw animal manures or partially composted soil amendments in citrus orchards and monitor the microbiological quality of irrigation water. The use of dust suppression agronomic practices along with perennial windbreaks and netting could mitigate the transfer of pathogens on to the fruit on trees from dust storms.

Harvested fruit appears to be the principal carrier of environmental pathogens from the field to the packing facilities. Harvest bins showed the presence of this pathogen and could lead to cross-contamination issues if not cleaned and sanitised properly after each use. In the packhouses, the fruit receival area was identified as the major hotspot for pathogen entry, establishment and transfer into the processing zone. The conveyor belts, floors and drains in this area often showed higher detection of *Listeria monocytogenes* which could be due to constant wet conditions along with high organic matter and leaf litter. For example, conveyor belts/rollers before fruit washing zone showed 20 positive detections (15%) out of 133 samples collected, highlighting the potential cross-contamination risks. The packing facilities with poor hygiene in this area tended to show higher detection of this pathogen. Hence, the key to management is regular cleaning and sanitisation after each shift.

Observations revealed that the pre-processing zone (before washing and sanitisation) had the highest detection levels (17.7%) of *Listeria monocytogenes*, followed by processing zone (13.2%) and post-processing zone (9.0%). Fruit contact surfaces such as conveyor belts, brushes and rollers in the processing zone showed detection of *Listeria monocytogenes*, indicating the transfer of this pathogen from the field with fruit and harvesting equipment to the processing equipment and premises. The whole-genome sequencing of Listeria isolates confirmed the proposed transmission route.

Postharvest processing premises showed the detection of Listeria on non-fruit contact surfaces such as floors, walls and drains. It was commonly observed that packing facilities did not have proper drainage system, leading to difficulties in maintaining cleanliness and sanitation of processing floors. Similarly, detection of Listeria on cool room floors and walls were observed supporting the known surviving nature of this pathogen under cooler conditions. As stated earlier, the distribution of the same strain type on the fruit, postharvest equipment and premises clearly indicates the pathogen transfer continuum and its interruption is required as part of the food safety management.

Agriculture Victoria Research (AVR) conducted whole genome sequencing (WGS) and bioinformatic analysis of some *Listeria monocytogenes* isolates that were collected by NSW DPI from citrus fruit and environmental samples. The analysis of isolates showed the presence of 14 strains of *L. monocytogenes*. The major strain types (ST) that were found include ST1, ST2, ST224, ST324, ST323 and ST240. ST1 was the widely prevalent strain. No clustering pattern of strain type according to the region or the sample type was observed. Phenotypic studies were not conducted and are required to confirm the resistance of the isolates to antibiotics. Further work incorporating isolates would provide a broader scope of the diversity of *L. monocytogenes* isolates that exist within the citrus industry.

Project activity 3: Generate experimental evidence to analyse the potential survival of foodborne bacterial pathogens on various types and varieties of citrus fruit under simulated conditions.

Survival and persistence of foodborne bacterial pathogens on the fruit surface are influenced by several factors including postharvest supply chain conditions. The fate of major pathogens such as *Salmonella* and *Listeria monocytogenes* on the citrus fruit surfaces are unknown during the cold phytosanitary treatments (≤3.0°C for 3 to 4 weeks) and domestic supply chain (4-5°C for 2 to 3 weeks), plus simulated shelf-life conditions at 20°C for 3 to 4 days. Eighteen experiments were conducted on different citrus types (3 cultivars of oranges, 2 cultivars of mandarins and 1 cultivar of lemon) under both export and domestic supply chain simulations.

Oranges: Populations of both pathogens, *Salmonella* and *Listeria monocytogenes*, on the orange (Navel, Valencia and Bellamy) fruit surfaces decreased by 1- to 2-log as the storage duration progressed at both temperatures (≤3.0°C for 3 to 4 weeks or 4-5°C for 2 to 3 weeks). A further reduction in the population was observed during simulated shelf-life studies, except the *Listeria monocytogenes* on Navel oranges surfaces after 4 weeks of cold storage. The increase in population during shelf-life studies of Navel oranges could be attributed to the chilling injury induced damage to the fruit surface, oozing out nutrients from the flavedo tissue for the growth of *Listeria monocytogenes*. In a nutshell, the orange fruit surface was not supportive for multiplication and growth of both pathogens. However, both pathogens survived the cold treatment and domestic supply chain and shelf-life conditions.

Mandarins: *Listeria monocytogenes* population on Imperial mandarins showed a 3-4 log reduction over 4 weeks at ≤3.0°C and a further 2-4 log reduction in the population was observed during shelf-life simulation. The survival of *Salmonella* on Imperial mandarins was slightly better than *Listeria monocytogenes* with 1 to 3 log reduction over 4 weeks cold treatment simulation. The trend of *Salmonella* population decline by 1-2 log was observed except after 1 and 2 weeks of storage at 2.8°C. Similarly, Afourer mandarins' surface did not allow growth of both pathogens but allowed their survival for three weeks at 2.8°C. During the domestic supply simulation trial, Afourer mandarins showed 3 to 4 log reduction in both pathogens during 2 weeks storage at 4°C but their population increased by 2-3 log during shelf-life conditions.

Lemons: Survival and persistence of both pathogens showed a declining trend in their populations for 2 weeks storage at ≤3.0°C and during shelf-life conditions. Like oranges and mandarins, lemons also showed a similar trend. The higher population (10^6 to 10^8 CFU/mL) of pathogens tended to survive better on the fruit surface compared to the lower populations (10^3 to 10^5 CFU/mL). Sheng et al (2023) also reported the survival of *Listeria monocytogenes* on lemon fruit surfaces.

Project activity 4: Develop a best practice guide based on the knowledge of potential sources and routes of contamination.

In this project, a Best Practice Guide for Microbial Food Safety Management in the Citrus Industry has been developed with a major focus on food safety risks and their management during citrus production, postharvest handling and distribution. The guide is aimed to fill the industry practice gaps identified in this project and to overall maintain microbial food safety management in the industry based on scientific evidence and microbial surveillance data. The guide is heavily pictorial along with illustrations and summaries of food safety control measures to ensure it is practical, relevant and informative to the end users (APPENDIX IV).

Project activity 5: Deliver food safety extension through appropriate channels including workshops, forums, meetings and industry communications channels

This project was a great success in terms of engagement with citrus growers and packers and other supply chain participants. Due to the export focused nature of the industry, there was a high level of appetite for new knowledge and its integration in the food safety practices. It is conservatively estimated that the project activities encompassing industry practice review, microbial risk mapping and outreach covered 74 citrus packers, representing approximately 900 citrus growers across Australia. Fifty-one citrus packers were visited on-site to review their fruit production, harvest and postharvest practices along with the collection of microbiological samples. The positive response from the growers and packers enabled the success of this project in understanding microbial hazards present in the supply chain and developing intervention strategies to mitigate the risks.

In addition to the one-on-one consultation, technical presentations on citrus microbial food safety management were delivered at important industry events like national and regional forums, scientific conferences in Australia and overseas. The project objectives and outcomes were widely disseminated to the industry through technical articles, workshops, conference exhibitions, industry consultants and extension agents. There were approximately 33 technical enquiries from the citrus growers, packers and consultants which were addressed by the food safety helpdesk service. Overall, the level of success of the extension and outreach objective was much more than anticipated at project planning stage.

Project activity 6: Identify opportunities for future R&D investment

Future R&D investment is recommended to address the following unresolved questions:

- Understand how preharvest practices (e.g., soil amendments, irrigation and orchard management) influence the introduction, survival and persistence of *Listeria* during citrus production and its transmission onto the fruit surface.
- Expand the application of whole-genome sequencing to get better insights into the diversity, distribution and transmission of *Listeria monocytogenes* and also conduct phenotyping for antimicrobial resistance.
- Determine if Listeria isolates collected from the farms or packing facilities have developed or are developing sanitiser resistance.
- Implement the best practice guide and objectively measure the microbial food safety performance of the citrus industry.
- Hands-on workshops for citrus packers and QA staff for further food safety training and education.
- Continue microbial food safety outreach and support to the citrus industry through a food safety helpdesk service and other extension methods.

Outputs

Table 1 Output summary

Output	Description	Detail
Project management documentation including a	These outputs were developed to ensure	The project management documents including M&E plan were submitted to Hort Innovation at Milestone 102. These

program logic and monitoring and evaluation (M&E) plan with linkage to Hort Innovation and industry/fund objectives; a project risk register; and a project IP register; and a stakeholder engagement plan.	the project meets its objectives, delivers anticipated benefits to the industry by optimally utilising resources.	were approved and subsequently used for project management purposes, including their usage for reporting to the Project Reference Group (PRG) and Hort Innovation.
Project reference group (PRG) terms of reference (ToR) and meetings	This document was designed for the PRG members to define their roles and responsibilities in the project governance.	The ToR for PRG was submitted to Hort Innovation at Milestone 102 and a copy was provided to the PRG members for their nomination acceptance to the group. Three PRG meetings were virtually held during the project duration on 21/12/2021, 03/05/2022 and 15/11/2022.The minutes of the meetings were submitted to Hort Innovation with corresponding milestone reports.
Milestone reports	Six-monthly milestone reports were prepared to report on the project progress as planned.	Three milestone reports (MS102, MS103, and MS104) were submitted to Hort Innovation in Nov 2021, May 2022 and Nov 2022. These reports were aligned with the project monitoring and evaluation plan.
A project brochure	A project brochure was developed highlighting the need for R&D, project objectives and expected outcomes.	The project brochure was a critical document to achieve grower/packer/stakeholder engagement and was submitted to Hort Innovation at MS 102. It explained the basic questions about the project needs, potential benefits of participation in the project and assurance of confidentiality and privacy in the written form.
Review industry practice	Assessment of current food safety practices adopted by the citrus industry was conducted in 51 citrus packing operations across Australia.	A national snapshot of the food safety practices was generated along with gaps in the industry practice identified. The industry practice data was linked to the microbial risk mapping process to deepen the understanding of microbial contamination and cross-contamination (APPENDIX I).
Microbial risk mapping	A database of 2,257 samples (target 2,000) collected from different parts of the citrus supply chain was developed. A total of 74 citrus packers were captured in this process.	The microbial risk mapping involved the detection of target pathogens (*Salmonella* species, *Listeria monocytogenes* and Shiga-toxin *E. coli* O157:H7) to determine the nature and magnitude of microbial hazards in the citrus supply chain. The prevalence and distribution of primary environmental pathogen was understood in detail and associated with potential sources and routes of microbial contamination (APPENDIX II).
Whole-genome sequencing (WGS) analysis	Some isolates of *Listeria* collected during microbial risk mapping were subjected to WGS and bioinformatics analysis.	The strain typing and diversity analysis of Listeria isolates was employed to determine the transmission patterns/route of this pathogen from citrus fields to the packing facilities. The novel insights into the pathogen continuum were achieved with the WGS data. Application of WGS data in the microbial risk mapping and industry practice analysis was achieved (APPENDIX II).
Survival and persistence of foodborne bacterial pathogens	Supply chain participants including growers and packers	The evidence was provided by conducting 18 experiments on different types of citrus types (3 cultivars of oranges, 2 cultivars of mandarins and 1 cultivar of lemons)

on the citrus fruit surfaces	were the target audience for this output.	demonstrating that *Salmonella* and *Listeria monocytogenes* could survive the cold phytosanitary treatment (≤3.0°C for 3 to 4 weeks) and domestic supply chain conditions (≤4.0°C for 2 to 3 weeks) followed by shelf-life simulation conditions (APPENDIX III).
Best Practice Guidance for Microbial Food Safety Management in the Citrus Industry	Guidance for microbial risk management has been developed with a major focus on citrus production and postharvest handling.	This guidance assimilates data from industry practice, microbial risk mapping, WGS combined with literature review and international best practice. This resource is designed to be highly practical, and easy to follow, highlighting the areas for food safety improvements in the industry (APPENDIX IV).
Project presentations at industry events such as conferences, workshops and forums	Technical presentations were delivered to a range of audience including growers, packers, exporters, supermarkets, researchers and regulators.	The presentations were delivered at the following: • Riverina Citrus Packers Field Day, Griffith (9 Dec 2021 afternoon) • Griffith and District Growers Association Meeting, Griffith NSW (9 Dec 2021 evening) • Citrus Tech Forum, Sunshine Coast Qld (8-9 March 2022) • International Postharvest Unlimited Conference, Wageningen, Netherlands (15-17 May 2023) • Citrus SIAP presentation (24 May 2023) • Fresh Produce Safety Conference, Sydney (11 Aug 2023) • Dareton Citrus Industry Field Day, Dareton, NSW (13 Sep 2023) (APPENDIX V)
Project exhibitions	As part of the stakeholder engagement plan, project posters were displayed in leading industry conferences and exhibitions.	Project posters were displayed, brochures distributed to relevant stakeholders and engagement with diverse stakeholders was achieved at the following industry exhibitions: • Citrus Tech Forum, Sunshine Coast Qld (8-9 March 2022) • Hort Connections, Brisbane (June 2022) • Hort Connections, Adelaide (June 2023) (APPENDIX V)
Technical articles	Technical articles targeted at citrus growers and packers were written and published.	Singh SP (2021). 'Seek and Destroy' the Bugs in Packhouses. *Australian Citrus News* Autumn Edition 2021. Singh SP (2022). Improving food safety practices to safeguard exports. *Citrus News* Summer 2022. Singh SP (2023). Managing microbial food safety risks. *Australian Citrus News* Summer edition 2023 (submitted) (APPENDIX V)
One-on-one consultation	More than 50 citrus packers were visited on site to understand their food safety practices and collect microbiological samples.	This extension method was the most effective in food safety education and training. Growers and packers, especially QA staff, were provided food safety knowledge and asked to identify the gaps in practice on site. Informal trainings on sanitisers' digital monitoring, microbial sample collection and developing EMPS were provided. The photographic evidence of these activities is provided in APPENDIX V.
Industry survey reports	Growers/packers who participated in the project by sharing information and offering microbial sample collection were	More than 50 individual business-specific reports were provided to citrus packers, showing the microbiological survey results, and providing them with technical advice to improve food safety practices. These customised reports were highly valuable to the packers in their food safety management plans A sample copy of the report is provided

	provided with survey reports.	in the APPENDIX V.
Food safety helpdesk	All supply chain stakeholders were offered a food safety helpdesk service.	Approximately 33 technical enquiries on citrus microbial food safety were received from various stakeholders. The packers asked several technical questions on advice on processing steps, postharvest chemical incompatibilities, fruit washing and sanitisation, packhouse hygiene, environmental monitoring plans, responding to microbiological test results and improving packhouse design. The service was also availed by horticultural consultants in addressing their client inquiries. A few snapshots of the technical enquiries and their responses are provided as evidence in APPENDIX V.

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Citrus Fund Outcome 1 The Australian citrus industry has increased profitability, efficiency, and sustainability by protecting the production base through innovative R&D, biosecurity preparedness and responsiveness, sustainable BMPs and superior varieties. **Project outcome** Current food safety practices in the Australian citrus supply chain benchmarked and increased knowledge of the potential survival of foodborne pathogens on citrus fruit.	**Citrus fund outcome 1**: Strategy 6. Monitor and manage food safety risks to maintain consumer confidence in Australian citrus **Citrus Fund KPI:** Food safety practices in the supply chain are benchmarked and practice change is monitored **Project KPI**: The project achieved engagement with 51 citrus packers in all major citrus production regions against the target of 50 packers. The growers/packers were briefed the project need, objectives and outcomes which led them to participate by sharing their food safety practices. **Citrus Fund KPI:** Increased knowledge of the potential survival of foodborne pathogens on citrus fruit **Project KPI:** Understand the potential survival of pathogens on different types of citrus types and varieties. These experiments were conducted as planned on oranges, mandarins and lemons.	The major practice gaps were identified and communicated to individual businesses. The collated and anonymised industry practice data was used for deriving industry-wide trends, thus generating a national snapshot of the industry practice. The insights into the survival and persistence of pathogens on the citrus fruit surfaces during postharvest phytosanitary treatments in export and domestic supply chain showed that the microbial risks are real and significant. The experimental data is a powerful tool in driving practice change in the industry. Bridging these gaps with best practice interventions will directly contribute to achieving microbial food safety at the industry level.	The success of this project was dependent on the grower/packer's engagement on highly sensitive topic of microbial food safety. The level of engagement clearly indicates that stakeholders were keen to get their food safety practices reviewed and verified by an independent team of experts and were interested in risk mitigation. The verbal and written feedback was received from growers/packers about the benefits that they received and practice changes (APPENDIX I)
Project outcome	**Citrus fund outcome 1**: Strategy 6. Monitor and manage food safety risks to maintain	Citrus growers and packers participated in this project and allowed the	Determination of the nature and magnitude of microbial hazards is

Microbial food safety risks in the Australian citrus supply chain identified and mapped, enabling controls to be put in place to mitigate food safety incidents.	consumer confidence in Australian citrus **Citrus Fund KPI:** Identification and mapping of microbial food safety risks in the supply chain enabling controls to be put in place to mitigate food safety incidents. **Project KPI:** A total of 2,257 microbiological samples (target =2,000) were collected from citrus orchards, packhouses and retail representing all major citrus types in different production regions. The survey reports were communicated to the participating growers and packers leading to further interest in microbial food safety. *Listeria monocytogenes* emerged as the primary pathogen of concern. Some of its isolates were subjected to whole-genome sequencing (WGS) for strain typing, diversity analysis and identification of virulence, antibiotic resistance and stress survival genes in these isolates.	microbiological sample collection from their orchards, harvest and postharvest operations across all major citrus production regions of Australia. Hotspots for contamination and cross-contamination were determined across the supply chain. The microbial risk mapping data was overlaid with corresponding industry practice information to develop a data-based decision and information package. The application of WGS provide novel insights into the pathogen transmission patterns and routes from the field to packing facilities, resulting a better understanding of the microbial contamination process.	the first step towards risk mitigation process. The insights provided from this project will strengthen the industry's preparedness to interrupt the pathogen continuum from the environment to the fruit. The targeted interventions to manage such risks are only possible due to the availability of fundamental information provided by this project. Citrus growers and packers showed deep interest in microbial risks linked to Listeria in their businesses. The technical enquiries and post-presentation discussions were often received. Further to it, the citrus packers reviewed/developed the environmental monitoring plans (APPENDIX II).
Project outcome Increase in the knowledge, attitudes, skills, aspirations of the citrus industry supply chain with regards to microbial food safety risks and demonstrated use of developed resources.	**Citrus fund outcome 1**: Strategy 6. Monitor and manage food safety risks to maintain consumer confidence in Australian citrus **Citrus Fund KPI:** Increased knowledge of the potential survival of foodborne pathogens on citrus fruit **Project KPI:** The survey reports were communicated to the participating growers and packers leading to further interest in microbial food safety. Three technical resources – benchmarking industry practice snapshot, microbial risk mapping and best practice guidance-developed in this project will be complementary in improving microbial risk management.	The growers/packers devoted their significant time and resources to participate in this project. Collecting practice data and microbial samples from their orchards and facilities is a time-consuming and invasive process in terms of confidentiality and privacy. The project team successfully engaged with all participants and provided them with information and knowledge that can be translated into a new practice or change in the practice. When enquired about the	Independent review of industry practice combined with microbial analysis at business level demonstrated participants how their practices influence the prevalence and distribution of environmental pathogens. It was a great learning experience for them along with informal training opportunities for critical food safety elements such as fruit washing and sanitisation, environmental management plans. The follow-up enquiries were another evidence

	Communication of pathogen survival on the citrus fruit surfaces remained an ongoing project activity and future industry conversations.	formatting of best practice guidance, many suggested that illustration-based and pictorial resources would be more helpful and easy to follow.	of aspiration for new knowledge and information. The increase in the knowledge, skills and aspirations was evident from the growers/packers interest in the project outputs. Some have sent written feedback on their satisfaction with the new knowledge and latest information on the microbial food safety management.
Project outcome Opportunities and gaps for future R&D communicated to industry and Hort Innovation.	**Project KPI:** Based on this project outputs, opportunities and gaps for future R&D were identified.	Considering a high level of grower/packer engagement in this project along with the application of science and data led to the identification of several R&D opportunities that exist. PRG contributed to the strategic direction of the project and supported R&D recommendations.	Technical enquiries and analysis of the complexity of industry issues facing the near future will drive and define the future R&D needs. Future R&D recommendations have been developed and outlined in results section 3.6.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
Effectiveness 1. To what extent has the project achieved its expected outcomes?	*Has the project team conducted technical visits to citrus growers and packers in the major citrus production regions? Has the project team consulted with at least 50 citrus packers across Australia to benchmark the key food safety practices?* ***Yes, the project team has conducted technical visits to 51 packers in all citrus production regions, including Riverina, Murray, Riverland, Burnett, Far North Queensland, WA and NT.*** Has the project team conducted microbiological surveillance by collecting and analysing at least 2,000 samples for target foodborne bacterial pathogens and mapped the food safety risks along the supply chain? Have all activities, KPIs and outputs been achieved? ***Yes, the project team has completed microbiological surveillance by collecting and***	The project scope could be extended to cover 30 more citrus packers, increasing the outreach to all exporting packhouses. Engage citrus growers in different regions to understand how preharvest practices (e.g., soil amendments and orchard management) influence the introduction, survival and persistence of *Listeria* during citrus production and its transmission onto the fruit surface. Expand the whole-genome sequencing to all isolates to get better insights into the diversity, distribution and transmission of *Listeria monocytogenes*. Determine if Listeria isolates collected from the farms or packing facilities have developed or developing sanitiser

	analysing 2,257 samples. All activities, KPIs and outputs have been achieved.	resistance.
Relevance 2. How relevant was the project to the needs of intended beneficiaries?	*Did target beneficiaries participate in the project and had access to technical information and advice as proposed? To what extent has the project met the needs of Australian citrus industry? Are there any gaps or additional opportunities for research?* ***Yes, target beneficiaries participated in the project as planned and were provided technical information and business-specific advice. The project activities encompassed all foreseeable industry needs on this theme. However, there are further R&D opportunities to understand and mitigate food safety risks.***	Implement the Best Practice Guide, achieve practice change and monitor objective microbiological performance indicators.
Process appropriateness 3. How well have intended beneficiaries been engaged in the project?	*Has the project team engaged with proposed number of citrus growers/packers and other stakeholders?* ***Yes, the project team engaged the proposed number (N=50 citrus packers, representing approximately 750 growers. Overall, 74 packers were captured in the process, representing approximately 900 growers.***	Aiming for 100% citrus packer engagement, representing 95% citrus growers.
Process appropriateness 4. To what extent were engagement processes appropriate to the target audience/s of the project?	*Did the project engage with industry levy payers through appropriate methods? How appropriate were the project activities and outputs for achieving its intended outcomes? How appropriate were the management and governance processes and the resources used for delivering the project?* ***A variety of engagement methods were adopted including one-on-one consultation, on-site visits, e-communication, face-to-face technical presentations, workshops, open/field days, conferences, exhibitions, technical articles, and food safety helpdesk service.*** ***Project activities and outputs were designed to achieve the desired outcomes. A PRG was formed and met three times at six-monthly intervals to review the project progress. NSW DPI's project management systems ensured all resources were allocated to the project in accordance with the R&D contract.***	Continue microbial food safety awareness and engagement with the industry with regular updates on the latest knowledge and information on management. Further investment is required to expand the WGS work to gain novel insights into the pathogen transmission at preharvest level.
Efficiency 5. What efforts did the project make to improve efficiency?	*Did the project identify opportunities to improve efficiencies? (including implementation of new technology, technique, practices, workflow etc.)* ***The project was implemented efficiently to optimise the use of allocated resources. However, there are always opportunities to improve such as the use of remote/virtual tools***	Opportunities exist to improve project efficiency by incorporating virtual means of communication such as podcasts and webinars.

	in site visits and grower engagement. The limitation of good quality internet coverage is an ongoing challenge in some regional towns, impeding the use of virtual tools.	

Recommendations

The following recommendations are based on the R&D conducted in this project:

- Organic soil amendments, dust storm-mediated pathogen dispersal, irrigation water, water used in the chemical spray application and wildlife incursions emerged as the major sources and routes of microbial contamination during citrus fruit production.
- Citrus orchards in proximity to livestock operations and high wildlife pressures should revisit their food safety plans to understand the pathogen load and dispersal pathways affecting their operations, especially during extreme weather events such as duststorms, floods, and bushfires.
- Raw animal manures or partially composted manures in citrus orchards. Dust-suppression agronomic practices including the use of perennial windbreaks is recommended. Spray water quality should be assessed before its application.
- Trailer mounting of harvest bins, avoiding ground contact, is the best practice to mitigate microbial food safety risks. Cleaning and sanitisation of harvest bins after each use are the best practice recommendations to mitigate microbial food safety risks associated with harvesting.
- Immediately after harvesting, citrus fruit should be drenched with a sanitiser followed by drenching with a fungicide. This 'two-step' drenching operation will minimise the cross-contamination risks and maximise the efficacy of both types of chemicals. Their concentrations should be monitored regularly along with the changes in the frequency of replacing drenching solutions adjusted according to the organic matter load on the fruit.
- Maintaining cool chain after harvest is critical to maximise food safety, storage and transportation potential of fruit with minimum quality and fungal decay losses.
- Clean and sanitise the fruit receival area after each shift so that environmental pathogen transfer continuum is interrupted. Dry dumping with careful unloading of fruit onto the conveyor belt is relatively lower risk than wet dumping in large volumes of water.
- Eliminating/sorting out rotten, mouldy and damaged fruit before washing and sanitisation process is a cost-effective strategy to minimise mould spore load in the fungicide tanks/packing shed and improve the food safety outcomes.
- All brush rollers should remain covered under the sanitised wash water along with daily cleaning and sanitisation schedule at the end of the shift to remove organic matter and debris from the core and sides of the rollers.
- Treat the wash water to drinking quality before it is used/re-used for fruit washing to mitigate the microbial contamination risks to the lowest level. Alternately, single use wash water (run-to-waste) system is recommended, but it may not be an environmentally sustainable option.
- The citrus packers should be avoiding the use of chlorine-based sanitisers due to their negative impact on the environment. The selection of PAA in postharvest wash water is recommended due to its efficacy, low environmental impact, and its action is least influenced by the presence of organic matter. Other alternative options such as electrolysed water, cold plasma and ozone should be explored to meet sustainability requirements with minimal environmental impact.
- Automation of sanitiser dosing and its digital monitoring and logging are strongly recommended to eliminate human errors. Furthermore, verification of sanitiser concentration should be conducted at regular intervals using digital measurement tools.
- Sanitisers for recirculating fungicide systems must be compatible with fungicides, not phytotoxic to the fruit, odour-free and non-irritant to workers, fast-acting and kill human pathogens within a short time period of ideally less than 30 s (although a 4–5 min dwell time may be acceptable), should cause a > 5-log-reduction in colony forming units (cfu)/ml (i a 99.999% reduction).
- Frequent cleaning and sanitisation of fungicide tanks to remove bacterial contamination and compatibility of sanitisers and fungicides should be confirmed before mixing them in fungicide tanks.
- Conveyor belts/rollers connecting washing and waxing zones should be cleaned and sanitised with utmost care to avoid any contamination opportunities between two steps. Also, the importance of washing and sanitisation step is reiterated as a control mechanism.
- There is significant opportunity to uplift the hygiene of packhouses through proper cleaning and sanitisation tools and processes. It is recommended that the industry should be aiming to popularise the EMPs among citrus

packers and highlight the benefits linked to the EMP costs.

References

- Adaskaveg, J.E., H. Förster, D. Chen, and K.A. Nguyen. 2021. Integration of postharvest fungicides and fruit sanitation treatments to optimize decay control and address food safety concerns. In: Spadaro, D., Droby, S., Gullino, M.L. (eds) Postharvest Pathology. Plant Pathology in the 21st Century, vol 11. Springer, Cham.
- Anonymous. 2023. Australian Horticulture Statistics Handbook 2021/22. Horticulture Innovation Australia Limited. https://www.horticulture.com.au/globalassets/hort-innovation/australian-horticulture-statistics-handbook/ahsh-2021-22-fruit-r3.pdf
- Harris, L.J. 2019. Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus. Final project report, Centre for Produce Safety, USA.
- Jung, J., L.M. Friedrich, M.D. Danyluk, and D.W. Schaffner. 2017. Quantification of transfer of Salmonella from citrus fruits to peel, edible portion, and gloved hands during hand peeling. J. Food Prot. 80: 933–939.
- Kanetis, L., H. Forster, and J. E. Adaskaveg. 2008. Optimizing efficacy of new postharvest fungicides and evaluation of sanitizing agents for managing citrus green mold. Plant Dis. 92:261-269.
- Martínez-Gonzáles, N.E., L. Martínez-Chávez, C. Martínez-Cárdenas, and A. Castillo. 2011. Comparison of different washing treatments for reducing pathogens on orange surfaces and for preventing the transfer of bacterial pathogens to fresh-squeezed orange juice. J Food Prot. 74(10):1684-1691.
- Pao, S. and G. E. Brown. 1998. Reduction of microorganisms on citrus fruit surfaces during packinghouse processing. J. Food Prot. 61:903-906.
- Parish, M. E. 1998. Coliforms, *Escherichia coli*, and *Salmonella* serovars associated with a citrus processing facility implicated in a salmonellosis outbreak. J. Food Prot. 61:280-284.
- Rajapakse, P.J. 2016. Contamination of food and drinks: product liability in Australia. Deakin Law Review 21:45-70.
- Sheng, L., H. Wang, L.J. Harris, and L. Wang. 2023. Survival of *Listeria monocytogenes* and *Salmonella* in citrus storage waxes or on lemons held under common commercial storage conditions. Food Microbiol. 115:104339.
- Singh, S.P. 2023. Flooding adversely affects fresh produce safety. Microbiology Australia 44(4), 185–189.
- Strawn, L.K., E.D. Fortes, E.A. Bihn, K.K. Nightingale, Y.T. Gröhn, R.W. Worobo, M. Wiedmann, and P.W. Bergholz. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Appl Environ Microbiol. 79(2):588-600.
- Suslow, T., A. Sbodio, and J. Pinzon-Avila. 2019. Resolving postharvest harborage sites of Listeria protects Zone 1 surfaces. Final project report, Centre for Produce Safety, USA.
- US FDA 2020. Product recall: lemons, Valencia oranges and items containing fresh lemon. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/product-recall-lemons-valencia-oranges-and-items-containing-fresh-lemon (Accessed on 31 August 2023).
- Weller D., M. Wiedmann, and L.K. Strawn. 2015. Irrigation is significantly associated with an increased prevalence of listeria monocytogenes in produce production environments in New York State. J Food Prot. 78(6):1132-1141.

Intellectual property

No project IP or commercialisation to report.

Acknowledgements

All citrus growers and packers who participated in the project are acknowledged for their contributions through sharing of food safety practices and allowing microbial sampling from orchards and packhouses. We are thankful to Citrus Australia, regional peak industry bodies and several other organisations who assisted in the grower/packer engagement. Thanks to Agriculture Victoria Research team for collaborating and conducting whole-genome sequencing analysis. All project team members, collaborators and supporters are acknowledged for their hard work and dedication to the project delivery.

Appendices

I. Analysis of the Australian citrus supply chain – benchmark the industry practice.

II. Mapping microbial risks along the supply chain starting from field production through postharvest processing.
III. Survival and persistence of foodborne pathogens on the citrus fruit surfaces during simulated supply chain conditions
IV. Best practice guidance for microbial food safety management in the citrus industry
V. Citrus microbial food safety outreach and extension

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APPENDIX I

Analysis of the Australian citrus supply chain to identify microbial food safety risks and deliver a national snapshot of the industry's current food safety practices.

Benchmark the industry practice: Methodology

Microbial contamination can occur anywhere along the supply chain. However, the highest chance of contamination occurs in the field and at the packhouse. Our knowledge and experience in traceback investigations and root-cause analysis reinforced the hypothesis that cross-contamination in the packhouses could lead to major market failures.

This project focused on the major production regions supply all types of citrus, including oranges, mandarins, limes, lemons and grapefruit. A total of 56 citrus packers were visited, including 24 in NSW, 13 in Qld, 8 in SA, 6 in Vic, 4 in WA and 1 in NT. The project team reviewed current practices and critical control points in the growing and postharvest processing of fruit.

Each grower/packer visited during this activity who shared their practices received a summary of observations, food safety results from microbiological test results (project activity 2) and potential gaps in both on-farm and postharvest best practice and recommendations for improvement, enabling them to take corrective action(s). The collated data was analysed to determine industry trends and a national snapshot of the practices along with recommendations for continuous food safety improvement.

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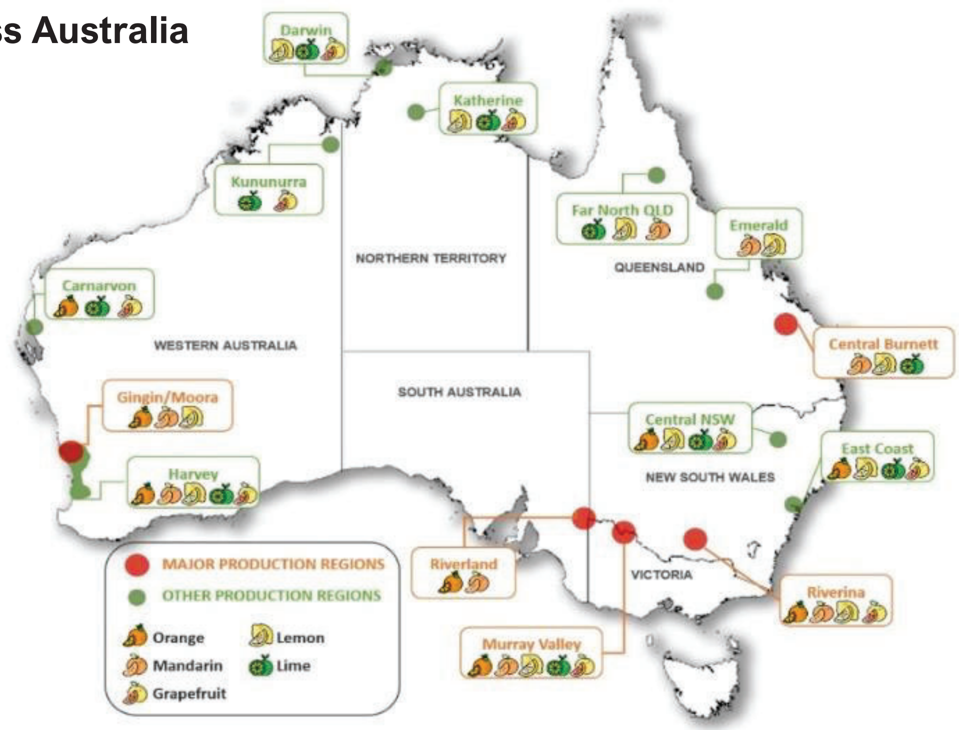
Benchmark the industry practice: Methodology

Technical site visits to 56 citrus packers across Australia

- NSW (24)
- Qld (13)
- SA (8)
- Vic (6)
- WA (4)
- NT (1)



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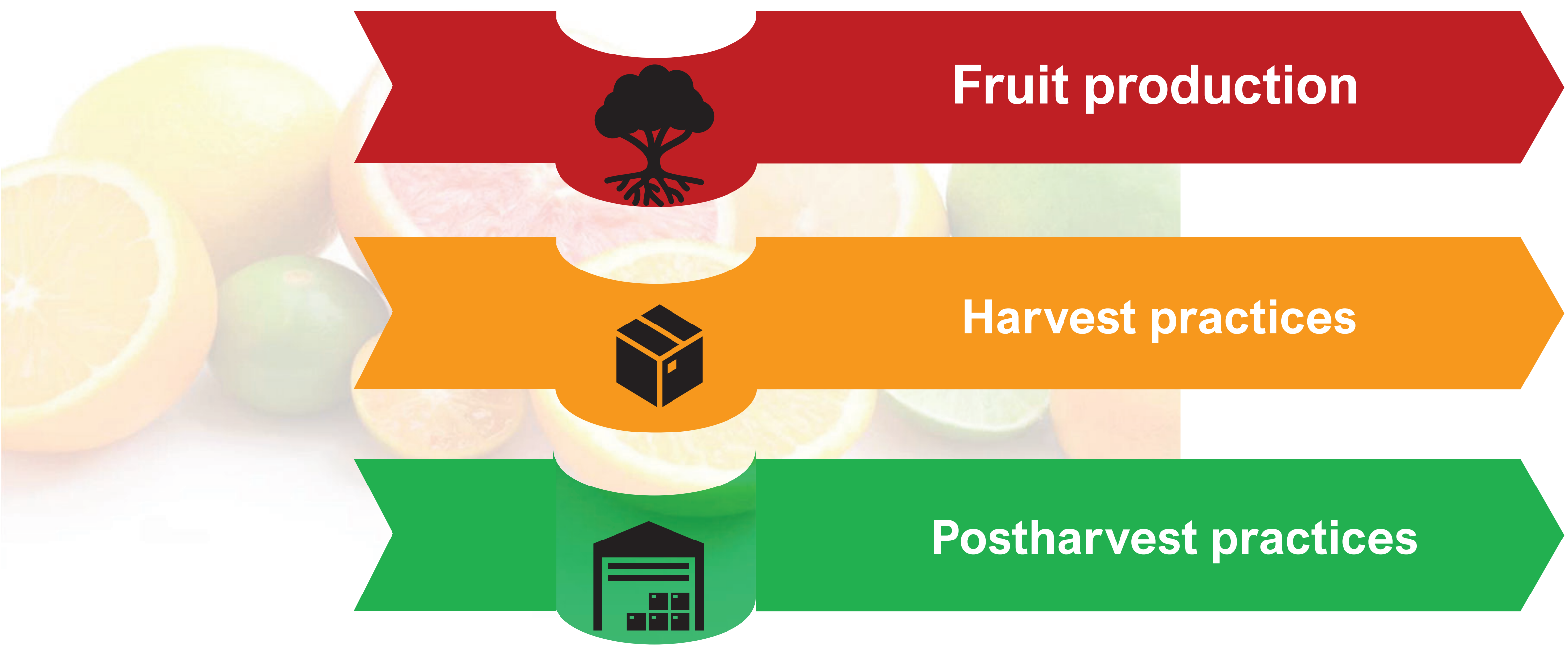
Benchmark the industry practice: Results



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Benchmark the industry practice: Results

Production and postharvest practices determine the microbial quality of fruit. In this project, the major focus was on analysing the food safety practices at harvest and postharvest stages in the citrus supply chain. A total of 56 citrus packers were visited across Australia but the practice data was collected from 51 packing sheds which were operational during the visit. The collection of practice information was evidence-based, hence data from operational sites was used for analysing the industry practice snapshot and trends.

Citrus grower/packer profiles: All citrus packers (N=51) had compliance with a food safety certification scheme with FreshCare being the most common one.

Majority of the citrus packers (37/51) exported fruit to various markets including, China, Japan, South Korea, Thailand, Indonesia, Canada, USA, India, New Zealand, and Vietnam.

The citrus packers engaged in this project represented a diverse group of citrus types produced and packed in their facilities: oranges (34/51), mandarins and tangelos (33/51), lemons (31/51), limes (19/51), and grapefruit (18/51).

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Benchmark the industry practice: Results

Citrus fruit production

All citrus growers/packers who participated in this project had a food safety scheme certification in place.

Preharvest sources and routes of microbial contamination in citrus orchards were investigated through in-field observations and analysing growers' practices (N=51). The use of organic amendments in the orchard soil health management is common (41/51) and most growers used the word "compost" that contained animal products.

The timing of application of organic amendments was mainly after the fruit harvest using the band application method under the tree trunk. A high level of awareness on the presence of microbial contaminants in raw animal manures was observed among growers. However, the transfer route of microbial pathogens from the environment to fruit was not a common knowledge.

Benchmark the industry practice: Results

Citrus fruit production

It was observed that some citrus production regions are highly prone to dust storms and are located in the vicinity of commercial livestock operations. The livestock operations were hypothesized to be the primary contributor to the microbial contamination of surface water sources and could be contributing to the long-distance dispersal of pathogens with dust storms. However, these are preliminary observations and need data and evidence to dissect the microbial contamination threats with the coexistence of horticulture and livestock industries.

The use of perennial windbreaks in some citrus orchards was observed to improve fruit production and quality. However, the use of perennial windbreaks and other agronomic practices aiding in dust suppression on farms will be the best practice to mitigate microbial contamination of fruit in the first place.

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Benchmark the industry practice: Results

Citrus fruit production

The high levels of prevalence of Listeria monocytogenes in citrus orchard soils, particularly under and near tree trunk has been observed in this study (Project activity 2).

The year-around moisture in this zone combined with high organic matter from soil amendments and leaf litter along with low or no exposure to the UV from sunshine are speculated to be the main reasons for the prevalence of Listeria in this zone.

Tree skirting as part of the integrated pest management for citrus orchards could also minimise the risk of Listeria survival under the trees due to increased exposure to sunshine.

Benchmark the industry practice: Results

Citrus fruit production

Drip irrigation was the most common irrigation method across all production regions which minimise the possibility of microbial contamination of fruit through water. However, the use of contaminated water for chemical spray applications, especially before fruit harvest, could lead to microbial contamination of fruit.

In addition to the microbial risk, the water sources contaminated with blue-green algae (BGA) could contain BGA toxins which could be transferred to the fruit. The persistence and diffusion of BGA toxins on and into citrus fruit are currently unknown. The growers' awareness of the microbial contamination via chemical spray applications and BGA chemical hazards could be raised in the future.

Wildlife incursion in the citrus orchards was commonly reported by growers along with the awareness of the risks these animals pose to microbial food safety. It was observed that growers undertake measures to minimise the wildlife incursion into their farms. However, discussions on wildlife management were not very open considering the sensitivities around animal welfare issues raised by the communities.

Benchmark the industry practice: Results

Citrus Fruit Production: Microbial food safety risks

Organic soil amendments, dust storm-mediated pathogen dispersal, water used in the chemical spray application and wildlife incursions emerged as the major sources and routes of microbial contamination during citrus fruit production.

Benchmark the industry practice: Results

Harvesting

The use of plastic harvest bins (51/51) is common across all citrus production regions. The plastic harvest bins are easier to clean and sanitise compared to wooden bins. Only one citrus grower/packer was observed to be using some wooden bins for postharvest fruit handling.

It was commonly observed that harvest bins were placed on the ground during fruit harvesting operations in citrus orchards. The fruit harvesting in winters often coincides with significant amount of rainfall and wet conditions. As a results, the harvest bins often get muddy during their contact with ground. Once these harvest bins are stacked during fruit transportation to packing house or for temporary storage of fruit, the soil/mud gets transferred to the inside causing widespread soiling of fruit and harvest bins.

Since the environmental pathogens such as Listeria monocytogenes live in soils, it is potentially the main reason for microbial contamination of harvest bins and fruit during harvesting operations. This has been identified as the major food safety practice gap during harvesting operations. Some citrus growers place harvest bins on trailers to avoid ground contact which is the best practice to minimise microbial contamination linked to soil.

Benchmark the industry practice: Results

Harvesting: Microbial food safety risks

Trailer mounting of harvest bins, thus avoiding ground contact, and cleaning and sanitisation of harvest bins after each use are the best practice recommendations to mitigate microbial food safety risks associated with harvesting.

Benchmark the industry practice: Results

Postharvest handling

Pre-sorting fungicide drenching

Postharvest fungicide drenching is a common practice (39/51) to minimise fungal rots in the supply chain. The drenching operation uses a large quantity of fungicide in recirculation mode for drenching of citrus fruit in harvest bins. Since this is the first wash/drench step, there is significant organic load in the drench solutions, and it also presents a risk of cross-contamination of fruit with bacterial pathogens. The harvest bins which are generally placed on the ground during fruit harvest were observed to be introducing more organic matter in the drench solution potentially affecting the fungicide efficacy and transferring the pathogens as *Listeria monocytogenes* into the drench solution.

The fungicide solutions generally don't have bactericidal effects and bacterial pathogens such as Salmonella and Listeria monocytogenes can survive in most fungicide solutions. The frequency of fungicide solution change also varied from daily to weekly with topping up the most common practice. This postharvest operation was identified as the major risk of cross-contamination of fruit with bacterial pathogens.

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Benchmark the industry practice: Results

Postharvest handling

Pre-sorting fungicide drenching

Some packers (7/51) were also using a sanitiser in the drench solution to mitigate bacterial pathogen risks.

Six out of seven packers who used sanitisers mixed the sanitiser with fungicide. The most common fungicide used in drenching was thiabendazole and chlorine was the common sanitiser used in combination.

There was only one packing shed which had a dual treatment system in which fruit first received a sanitiser drench (chlorine) followed by a fungicide drench. This is the best practice example to mitigate cross-contamination risks during drenching operations.

Benchmark the industry practice: Results

Postharvest: pre-sorting drenching

Immediately after harvesting, citrus fruit should be drenched with a sanitiser followed by drenching with a fungicide. This ‘two-step’ drenching operation will minimise the cross-contamination risks and maximise the efficacy of both types of chemicals. Their concentrations should be monitored regularly along with the changes in the frequency of drenching solutions adjusted according to the organic matter load on the fruit.

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Benchmark the industry practice: Results

Postharvest handling

Cool chain

Following postharvest fungicide drenching, fruit were generally held in cool rooms until further processing. If degreening was to be undertaken, the fruit were held in degreening rooms maintained at the required temperature, humidity and ethylene conditions.

Occasionally, fruit in harvest bins, before and after drenching, were observed to be stored under ambient conditions (inside and outside the sheds) which is not ideal from the postharvest and food safety perspectives.

Postharvest: cool chain

Maintaining cool chain after harvest is critical to maximise food safety, storage and transportation potential of fruit with minimum quality and fungal decay losses.

Benchmark the industry practice: Results

Postharvest handling

Fruit receival area

The fruit receival area in the packing shed is the gateway for fruit (and environmental pathogens) to enter the processing facility. The harvested fruit comes with significant amount of organic matter and debris from the field. It was observed that the fruit receival area was the weakest link in the food safety chain of the citrus industry.

Most packers neglected this area for regular cleaning and sanitisation. As a result, the higher number of Listeria detections were recorded in the fruit receival areas compared to other sampling locations. As most citrus packing facilities are not designed with proper drainage for regular cleaning, this is a challenging aspect.

Postharvest: fruit receival area

Clean and sanitise the fruit receival area after each shift so that environmental pathogen transfer continuum is interrupted.

Benchmark the industry practice: Results

Postharvest handling

Fruit dumping

At the commencement of postharvest processing, fruit are either dumped on a conveyor belt (dry dumping) or in a large capacity water tank or flume (wet dumping). Drying dumping (45/51) is a common industry practice which is favourable to minimise microbial cross-contamination risks posed by water flumes. Four out of six citrus packers who used wet dumping used a sanitiser in the dump tank or flumes. However, maintaining an optimum sanitiser concentration in large quantities of water could be a challenge.

The wet dumping process is believed to reduce the impact damage on fruit during dumping. On the other hand, dry dumping could cause minor injuries and cracks in the fruit during dumping process leading to the potential pathways for internationalisation of bacterial and fungal pathogens during subsequent processing steps, causing higher levels of fruit breakdown in the supply chain.

Postharvest: fruit dumping

Dry dumping with careful unloading of fruit onto the conveyor belt is relatively lower risk than wet dumping in large volumes of water.

Benchmark the industry practice: Results

Postharvest handling

Pre-wash sorting

Pre-wash sorting: A few citrus packers (5/51) were observed to sort out unwanted fruit before washing and sanitisation process. Damaged and rotten fruit were often observed to be undergoing washing and sanitisation process leading to increased microbial burden on the packing system and wastage of resources used in their processing. The lack of provision for sorting workers in packing lines design and layout was observed.

Postharvest: pre-wash sorting

Eliminating/sorting out rotten and damaged fruit before washing and sanitisation process is a cost-effective strategy to minimise mould spore load in the fungicide tanks/packing shed and improve the food safety outcomes.

Benchmark the industry practice: Results

Postharvest handling

Postharvest water

Postharvest water is an important element in microbial food safety of citrus fruit. The citrus packers use a variety of postharvest water sources as follows:

- Surface water: 26/51 (50.9%) (River/creek-13; channel 5; dam 4; rainwater 4)
- Town water: 21/51 (41.2%)
- Ground water: 6/51 (11.8%)
- No wash water: 1/51; Some packers use more than 1 water sources.

Surface water from various sources (rivers, creeks, channels, dam and rainwater harvesting) is the most common for citrus packers located at remote locations. The microbiological quality of surface water changes dramatically depending upon the season and weather events. However, 58% (15/26) citrus packers who use surface water have installed water filtration and treatment systems to improve water quality for postharvest usage. On the other hand, town water, which is considered the safest, is widely used in the packing operations (41.2%) located in larger towns. These statistics highlight that there is need for increasing the adoption of water filtration and treatment systems in the packing operations who are dependent upon surface water sources.

Benchmark the industry practice: Results

Postharvest handling

Brushing and washing

Flatbed brushes were most commonly used along with spray bar washing systems with several packers (26/51) claiming a high pressure washing (not properly defined water pressure) system in place. Ideally, all brush rollers should be under the sanitised wash water for self-cleaning purposes. However, the distribution of wash water spray nozzles was observed to be not covering all brush rollers. The hygiene of brushes is critically important to minimise cross-contamination risks. *Listeria monocytogenes* was detected in several samples collected from brushes in the washing areas and also the brushes used for the final finishing of waxed fruit. Once brushes get contaminated with bacteria pathogens, these are very difficult to clean and sanitise. The complete replacement of contaminated brush rollers is the practical solution.

Postharvest: brushing and washing

All brush rollers should remain covered under the sanitised wash water along with daily cleaning and sanitisation schedule at the end of the shift to remove organic matter and debris from the core and sides of the rollers.

Benchmark the industry practice: Results

Postharvest handling

Postharvest wash water sanitisers
The presence of a sanitiser in the wash water is critical to kill bacterial pathogens. The bactericidal potential of the sanitiser is dependent upon its type, concentration, contact time and other factors such as organic load.

Citrus packers who use town water add sanitisers during the washing process through automated systems or manually. Some citrus packers who use surface water from rivers and creeks inject small doses of sanitisers after filtration step in large capacity water storage tanks which are further connected to the washing lines. This practice allows extended duration of sanitiser contact with wash water, leading to proper disinfection process.

The application of sanitiser in wash water was widely adopted (49/51) in the industry, two packers were not using any sanitiser in the wash water – one was a small-scale operation, another was a large-scale packer supplying to domestic and export markets.

Benchmark the industry practice: Results

Postharvest handling

Postharvest wash water sanitisers

Recirculation of wash water is a common practice (40/51), most medium to large-scale operations recirculate wash water without a filtration step. Considering most operations used a sanitiser in the wash water, it reduces the risk of cross-contamination.

Postharvest: wash water

Treat the wash water to drinking quality before it should be used/re-used for fruit washing to mitigate the microbial contamination risks to the lowest level. Alternately, single use wash water (run-to-waste) system is recommended, but it may not be an environmentally sustainable option.

Benchmark the industry practice: Results

Postharvest handling

Postharvest wash water sanitisers

Among 49 sanitiser-users in the postharvest wash water, peroxyacetic acid (PAA) is the most popular (19/49) followed by calcium hypochlorite (13/49), chlorine dioxide (7/49), sodium hypochlorite (3/49), chloro-bromo dimethylhydantoin (3/49), chlorocyanurates (4/49).

Most citrus packers using PAA, chlorine dioxide and chloro-bromo dimethylhydantoin as sanitisers used automated dosing systems (59%) for injection of the sanitiser into wash water. However, many of sodium/calcium hypochlorite and chlorocyanurates users adopted manual addition of sanitiser in wash water tanks.

Each sanitiser has its own merits and demerits due to its highly oxidative nature. It is recommended that the industry should be avoiding the use of chlorine-based sanitisers due to their negative impact on the environment. The selection of PAA in postharvest wash water is recommended due to its efficacy, low environmental impact, and its action is least influenced by the presence of organic matter. Other alternative options such as electrolysed water and ozone should be explored to meet sustainability requirements with minimal environmental impact.

Benchmark the industry practice: Results

Postharvest handling

Postharvest wash water sanitisers

Postharvest: wash water sanitisers

The citrus packers should be avoiding the use of chlorine-based sanitisers due to their negative impact on the environment. The selection of PAA in postharvest wash water is recommended due to its efficacy, low environmental impact, and its action is least influenced by the presence of organic matter. Other alternative options such as electrolysed water, cold plasma and ozone should be explored to meet sustainability requirements with minimal environmental impact.

Benchmark the industry practice: Results

Postharvest handling

Postharvest wash water sanitisers

Automation of sanitiser dosing and its digital monitoring and logging are strongly recommended to eliminate the human errors. Furthermore, verification of sanitiser concentration should be conducted at regular intervals using digital measurement tools.

The use of test strips in measuring sanitiser concentrations and pH should be avoided. It was observed that target and actual concentrations of sanitisers varied greatly among the citrus packing operations.

Most operations did not achieve the target sanitiser concentrations and were dependent upon the test strips results for verification. Achieving recommended levels of sanitiser concentrations is critical to miminise microbial food safety risks associated with the postharvest wash water.

Benchmark the industry practice: Results

Postharvest handling

Postharvest wash water sanitisers

Understanding basic facts about the chemistry of postharvest sanitisers and chemicals is critical to successfully using them without compromising efficacies.

Some citrus packers were using two types of sanitisers in the postharvest washing line in a sequential manner. For example, the fruit were first washed with water containing chlorine (effective at neutral/slightly acidic pH ≤7.0) followed by washing with water containing PAA (effective at low pH (≤3.5).

The higher pH of chlorine in residual wash water on the fruit surface could potentially impact the efficacy of PAA and could also increase the pH of PAA solution tank as the time progresses during processing. It is therefore recommended to consider 'pH' factor in adopting such practices.

Benchmark the industry practice: Results

Postharvest handling

Postharvest wash water sanitisers

Another similar example is the use of sodium bicarbonate (1-2%; pH 8.5 to 9.0) solution before washing the fruit with PAA. Some citrus packers were observed to be applying sodium bicarbonate solution before fruit washing for postharvest disease control and argued its role in wound repair on the fruit surface.

If the application of sodium bicarbonate is to be integrated before fruit washing, a few wash water spray bars should be added for rinsing the fruit before these roll into the sanitised wash water zone, especially if the PAA is being used.

Similarly, the impact of residual wash water pH on the subsequent fungicide treatment should be considered. The use of air blast/knives in removing excess moisture from the fruit surface during such conflicting pH scenarios may also be adopted.

Benchmark the industry practice: Results

Postharvest handling

Postharvest fungicides

Most citrus packers (43/51) used at least one postharvest fungicide during processing.

Some small-scale lime and lemon packers (8/51) did not use any postharvest fungicide.

Out of 43 fungicide-users, 31 adopted spray application, 6 adopted fungicide tank immersion method and 3 adopted sheet application, while 7 packers used a combination application method.

Benchmark the industry practice: Results

Postharvest handling

Postharvest fungicides

Most packers (29/43) applied cold fungicides, but 14 packers applied hot fungicide treatment.

Imazalil was the most commonly used fungicide (33/43), followed by thiabendazole (24/43), guazatine (16/43), fludoxionil (12/43) and others (6/43).

Twenty-one packers out of 43 used at least 2 postharvest fungicides, 8 packers used at least 3 fungicides and 4 packers used 4 fungicide options.

None of the packers shared if they were adding a sanitiser to the fungicide tank for sanitisation of fungicide solutions. This could be largely attributed to the uncertainty about the compatibility of these products.

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Benchmark the industry practice: Results

Postharvest handling

Postharvest fungicides

From the food safety perspective, recirculation of fungicide solutions is a cross-contamination opportunity for bacterial pathogens which can survive in fungicide tanks. Our experimental data showed that *Listeria monocytogenes* and *Salmonella* could survive in most fungicide solutions currently registered for postharvest applications in the Australian citrus industry.

To meet safety standards, fungicide solutions have to be effectively sanitised using sanitisers such as sodium or calcium hypochlorite or PAA. Sanitisers for recirculating fungicide systems need to be:

- compatible with fungicides
- not phytotoxic to the fruit
- odour-free and non-irritant to workers
- fast-acting and kill human pathogens within a short time period of ideally less than 30 s (although a 4–5 min dwell time may be acceptable)
- should cause a > 5-log-reduction in colony forming units (cfu)/ml (i.e., a 99.999% reduction).

Benchmark the industry practice: Results

Postharvest handling

Postharvest fungicides

Unfortunately, some fungicides are incompatible with these sanitisers. The active ingredient breaks down, and subsequently, performance of the fungicide is lost. The manufacturers recommendations should therefore be followed while mixing different products.

Imazalil, thiabendazole, fludioxonil, azoxystrobin, and pyrimethanil are compatible with PAA. Thiabendazole, fludioxonil, and azoxystrobin are compatible with sodium hypochlorite while imazalil and pyrimethanil are incompatible with sodium hypochlorite (Adaskaveg et al., 2021).

Postharvest: fungicides

Frequently clean and sanitise fungicide tanks to remove bacterial contamination and compatibility of sanitisers and fungicides should be confirmed before mixing them in fungicide tanks.

Benchmark the industry practice: Results

Postharvest handling

Postharvest waxing

It was observed that 46 out of 51 packers used postharvest waxes in their operations.

Carnauba was the most popular (29/46) waxing material used in the industry, followed by shellac (17/46) and composite waxes (12/46).

The low-volume applicators were used for waxing the fruit, followed by drying step.

Majority of the citrus packers (30/46) used waxing material containing imazalil fungicide which is intended to provide residual protection against fungal pathogens in the supply chain. It could be speculated that waxing on the fruit surface would create a moisture-rich micro layer between the fruit surface and waxy top layer. This moisture-rich layer would be conducive to the survival of foodborne bacterial pathogens on the fruit surface.

Benchmark the industry practice: Results

Postharvest handling

Postharvest waxing

Some citrus packers use overhead brushes for giving a finishing touch to the waxed fruit. It was observed that these brushes were occasionally contaminated with *Listeria monocytogenes* which could lead to cross-contamination of fruit. It is recommended that these overhead brushes should be avoided.

The application of waxes should also be undertaken according to the rates recommended for various types of fruit. Some citrus packers were either using higher rates of wax application or they were not properly cleaning and sanitising the post-waxing equipment, leading to the excessive deposits of waxes on the conveyor belts and rollers.

The bacterial pathogens were detected in these waxy deposits, suggesting it could be another hotspot for cross-contamination of washed, sanitised and waxed fruit ready for shipping. The use of food-grade detergents is strongly recommended to remove excessive waxes from the processing equipment.

Benchmark the industry practice: Results

Postharvest: waxing

Conveyor belts/rollers connecting washing and waxing zones should be cleaned and sanitised with utmost care to avoid any contamination opportunities between two steps. Also, the importance of washing and sanitisation step is reiterated as a control mechanism.

Benchmark the industry practice: Results

Postharvest handling

Cool rooms

The processed fruit is held in the cool rooms which are generally maintained at or below 5°C. The cooler conditions are not congenial for bacterial growth but *Listeria monocytogenes* can survive and grow under cooler conditions.

It was observed that cool room hygiene is an issue across the industry. Only 65% citrus packers maintained their cool rooms in acceptable conditions while the rest were below average due to the presence of organic matter, rotten fruit, and dirty floors and walls.

Listeria monocytogenes was regularly detected in samples collected from cool room floors and walls. The workers and forklifts' movement could have spread the pathogens from fruit receival/processing areas to cool rooms and vice-versa. It is therefore recommended that packers use proper cleaning and sanitisation strategies to maintain cool room hygiene.

Benchmark the industry practice: Results

Postharvest handling

Packhouse hygiene

It was observed that 59% of packhouses maintained overall hygiene at acceptable level which reflected the cleanliness of packhouse floors (receival and processed fruit areas), cool rooms, processing equipment and premises. The concept of environmental monitoring and management programs (EMP) is novice for the citrus industry. However, several packers have contacted the project team to develop their EMPs. Regular cleaning and sanitisation of citrus packhouses is not widely practiced. Only 18 out of 51 packers used a packhouse cleaning chemical while the use of sanitisers in the equipment and premises hygiene was slightly more (24/51).

Postharvest: packhouse hygiene

There is significant opportunity to uplift the hygiene of packhouse through proper cleaning and sanitisation tools and processes. It is recommended that the industry should be aiming to popularise the EMPs among citrus packers and highlight the benefits linked to EMP costs.

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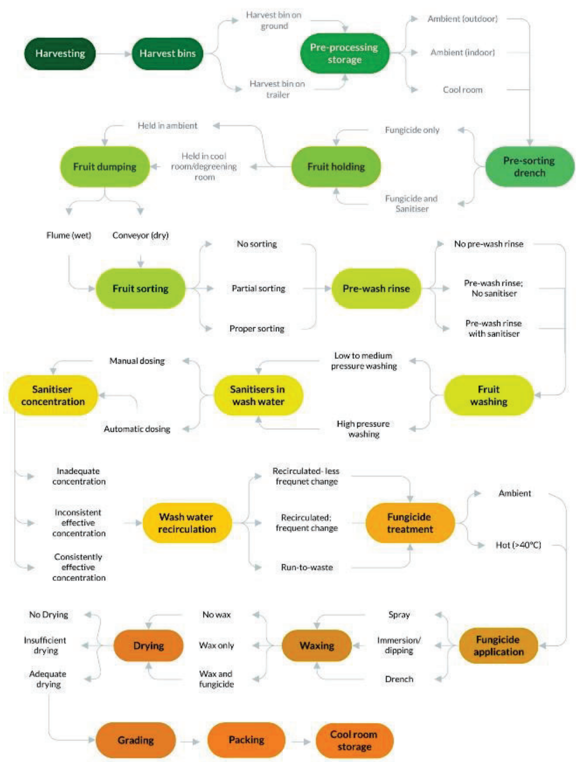
A Summary of Citrus Harvest and Postharvest Workflow



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Major Gaps Identified in the Microbial Food Safety Management Practices in the Citrus Industry

- Harvest bins hygiene
- Neglected cleaning and sanitisation of the fruit receival area
- Recirculation of wash water with inadequate levels of sanitisers
- Low level of knowledge about the interactions among postharvest chemicals
- Erratic adoption of automating dosing and monitoring of wash water sanitisers
- Cool room sanitation and lack of proper drains in the packing sheds
- Inadequate use of packhouse cleaning and sanitisation chemicals
- Environmental monitoring programs are missing.

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APPENDIX II

Mapping microbial risks along the supply chain starting from field production through postharvest processing, and retail

Mapping the microbial risks- Summary

- Microbial risk mapping process showed that citrus fruit pose a relatively lower food safety risk to consumers, but the detection of environmental pathogens such as *Listeria monocytogenes* on the fruit surface could pose a serious "trade-risk" in export markets.
- *Salmonella* and Shiga-toxin producing *E. coli* (STEC) did not present a significant risk. However, the detection level of STEC in orchard soils could potentially be linked to the use of raw animal manures received as byproducts from livestock operations.
- *Listeria monocytogenes* was the major environmental pathogen detected in 2,257 microbial samples collected from citrus orchard soils, fruit/leaves on trees, harvest bins, harvested fruit and packing shed equipment and premises.
- The highest prevalence of *Listeria monocytogenes* was observed in orchard soils (40.8%) followed by packing shed equipment and premises, pre-wash fruit and harvest bins.
- Harvest bins, fruit receival areas, wash water and fungicide tanks, brushes and cool rooms were identified as the hotspots for microbial contamination and cross-contamination.
- Whole-genome sequencing of some *Listeria monocytogenes* isolates elucidated the transmission of *Listeria* from the field to the packing facilities, indicating the fruit being the principal carrier of environmental pathogens.

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Mapping the microbial risks along the citrus supply chain

- To determine the nature and magnitude of microbial hazards across the supply chain
- To understand the prevalence and distribution of the major environmental pathogens during production and postharvest systems
- To establish the pathogen transfer routes and mechanisms along the supply chain.

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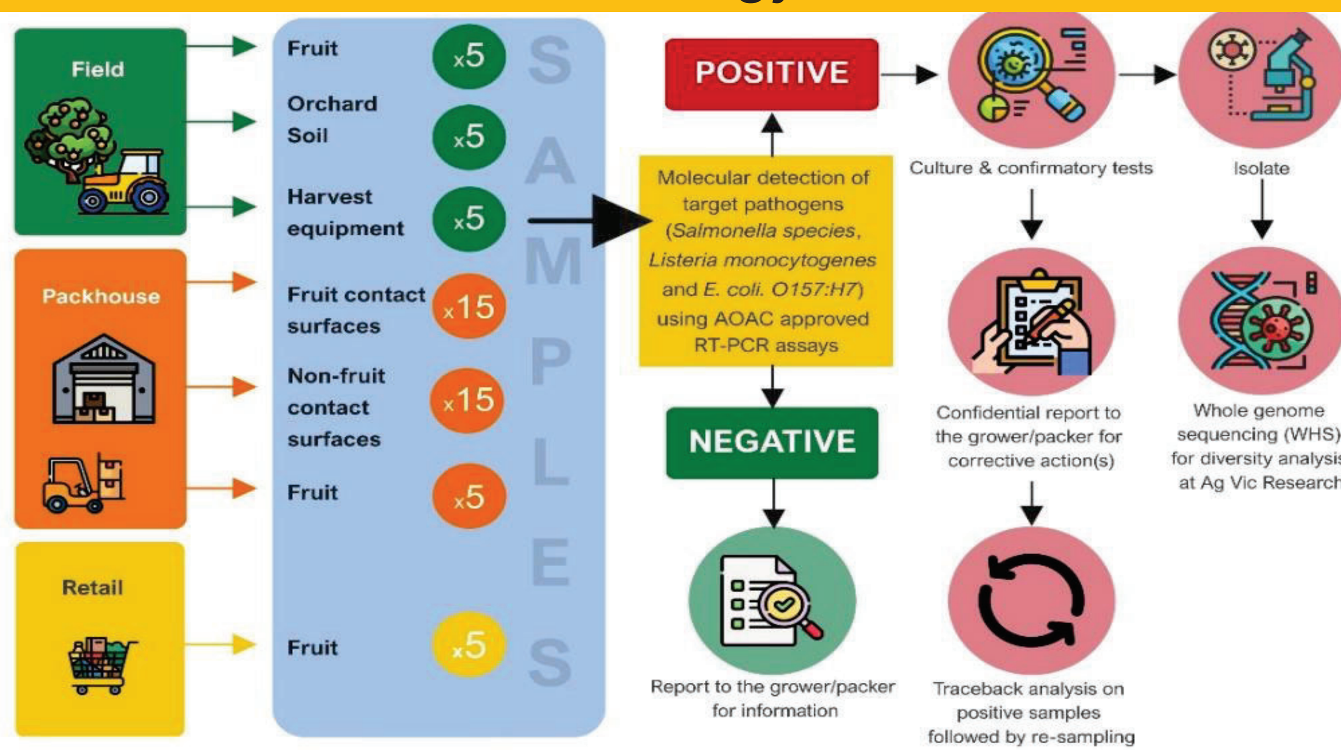
Mapping the microbial risks- Methodology

An overview of the sample collection plan



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Mapping the microbial risks- Methodology

Citrus orchard sample collection

- Leaf/fruit swab (For each sample 5x trees, for each tree swab 10x leaves/fruit, swab covering all aspects of tree randomly)
- Soil drag swab - under the tree 30 cm away from the tree trunk (for each sample, 5 trees
- Soil drag swab - between rows (for each sample, drag swab in both direction opposite 5 trees)

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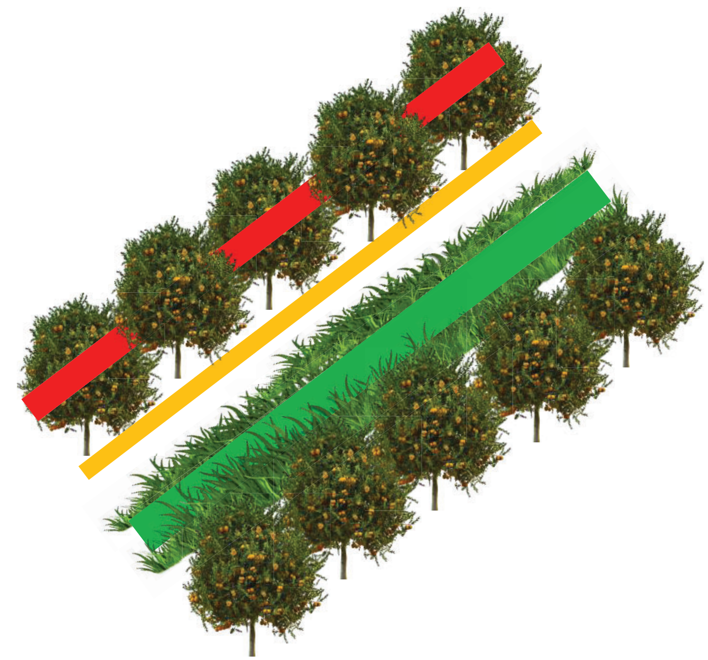
Mapping the microbial risks- Methodology

Citrus orchard sample collection

Red line - Leaf/fruit swabs
Yellow line - Soil drag under tree (30cm away from trunk)
Green line - Soil drag (middle) between rows



Eight citrus orchards were selected in NSW and WA

- 6 orange orchards
- 1 mandarin orchard
- 1 lemon orchard

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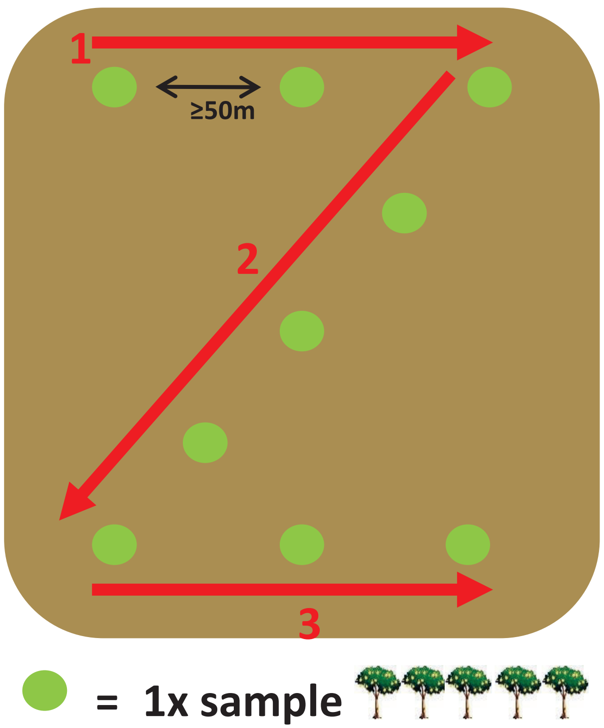
Mapping the microbial risks- Methodology



Citrus orchard sample collection: Z-configuration followed for sample collection avoiding outer 1 row of trees

Examples of aerial maps of two citrus orchards with sample collection points

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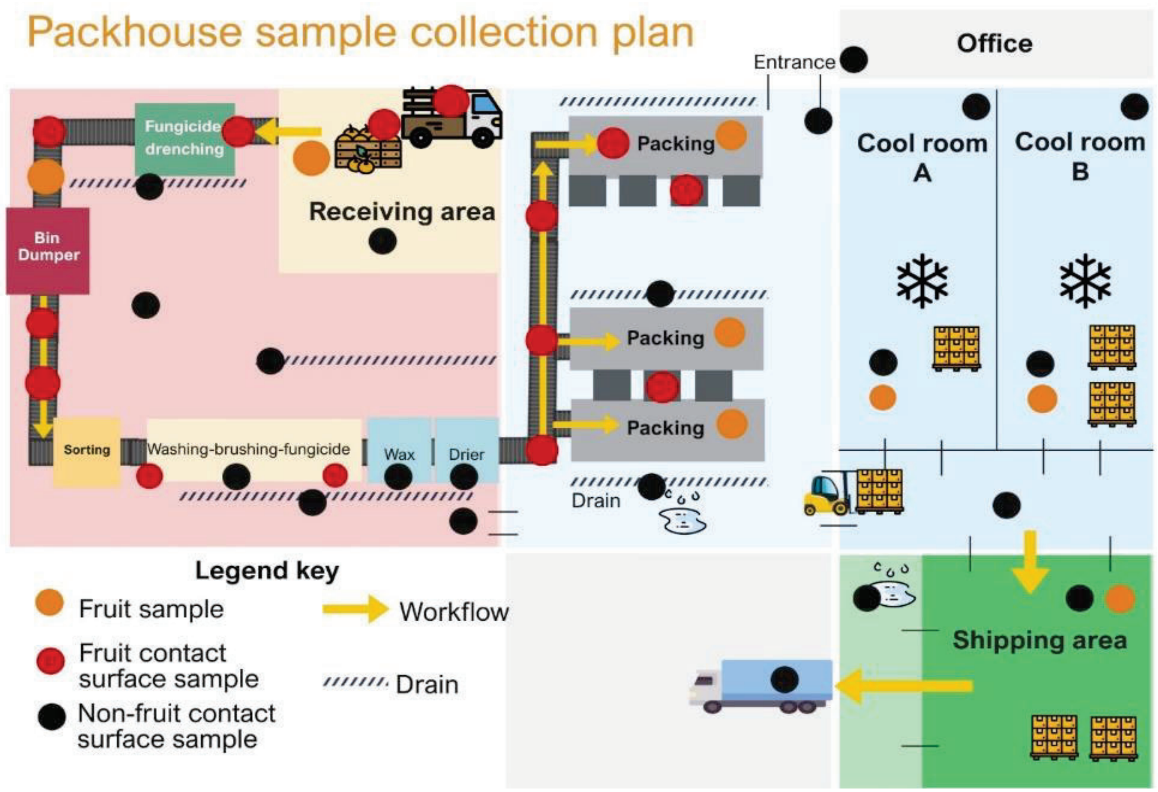
Mapping the microbial risks- Methodology



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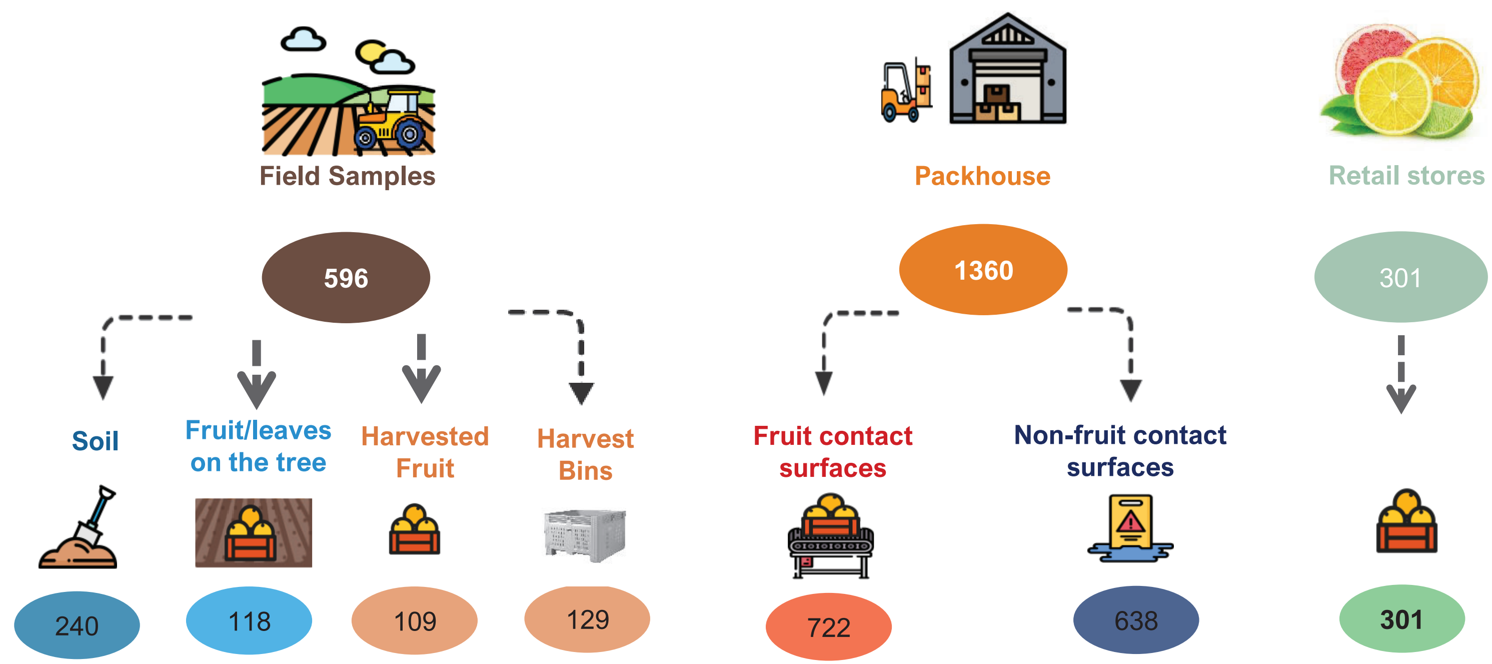
Mapping the microbial risks- Methodology

Microbiological sample collection points and the number of samples collected



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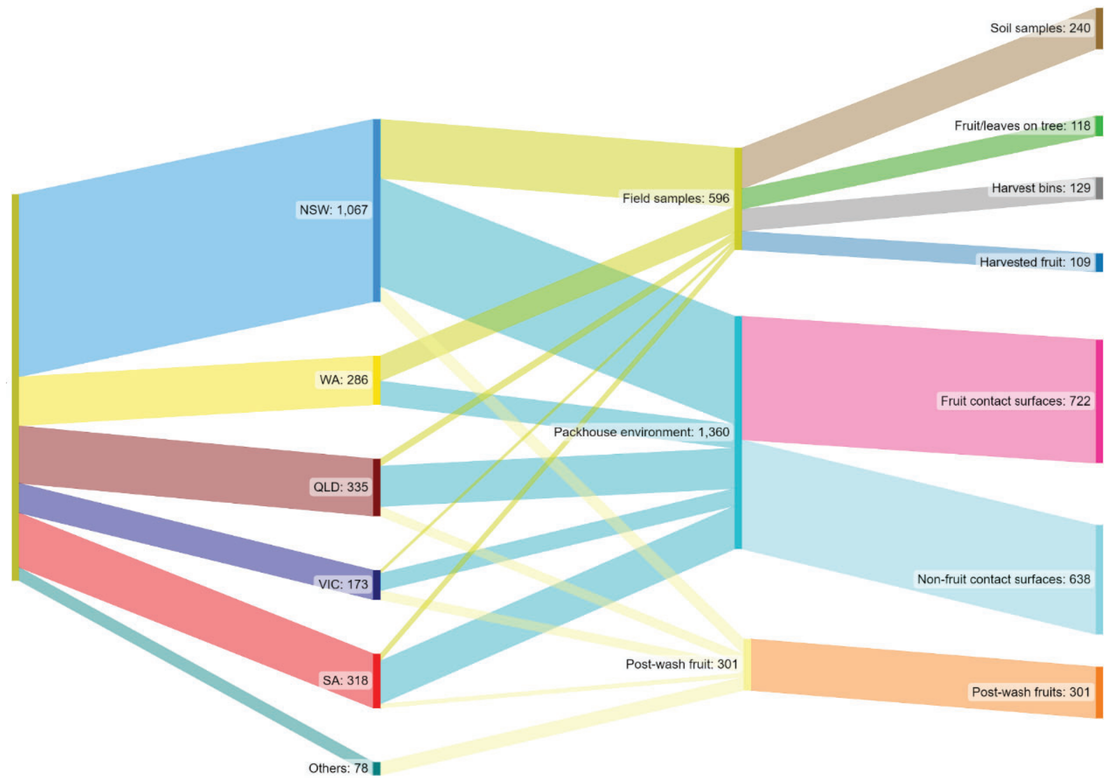
Mapping the microbial risks- Methodology

Total samples collected = 2,257



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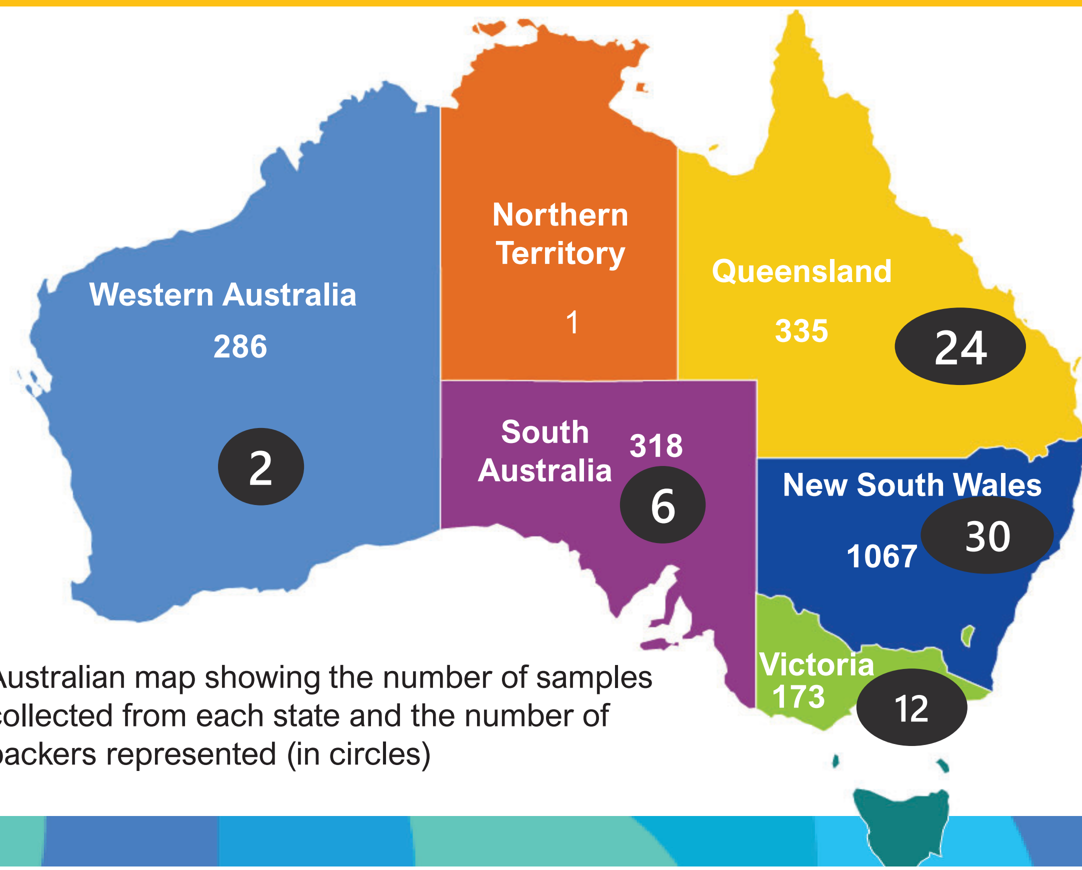
Mapping the microbial risks- Methodology

Total number of citrus packers (field/packhouse + retail) represented in the sampling = 75

Total number of citrus packers visited = 51

Total number of samples collected = 2,257 (target 2,000)



Australian map showing the number of samples collected from each state and the number of packers represented (in circles)

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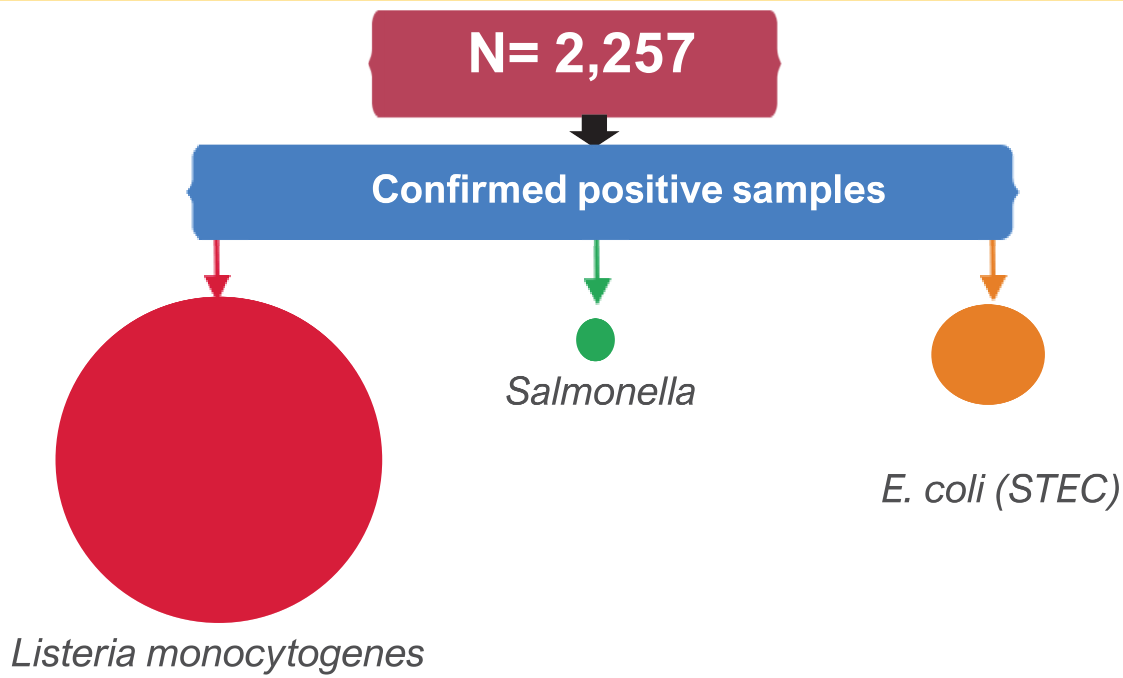
Mapping the microbial risks- Results



The size of the circle for each pathogen approximately represents its prevalence.

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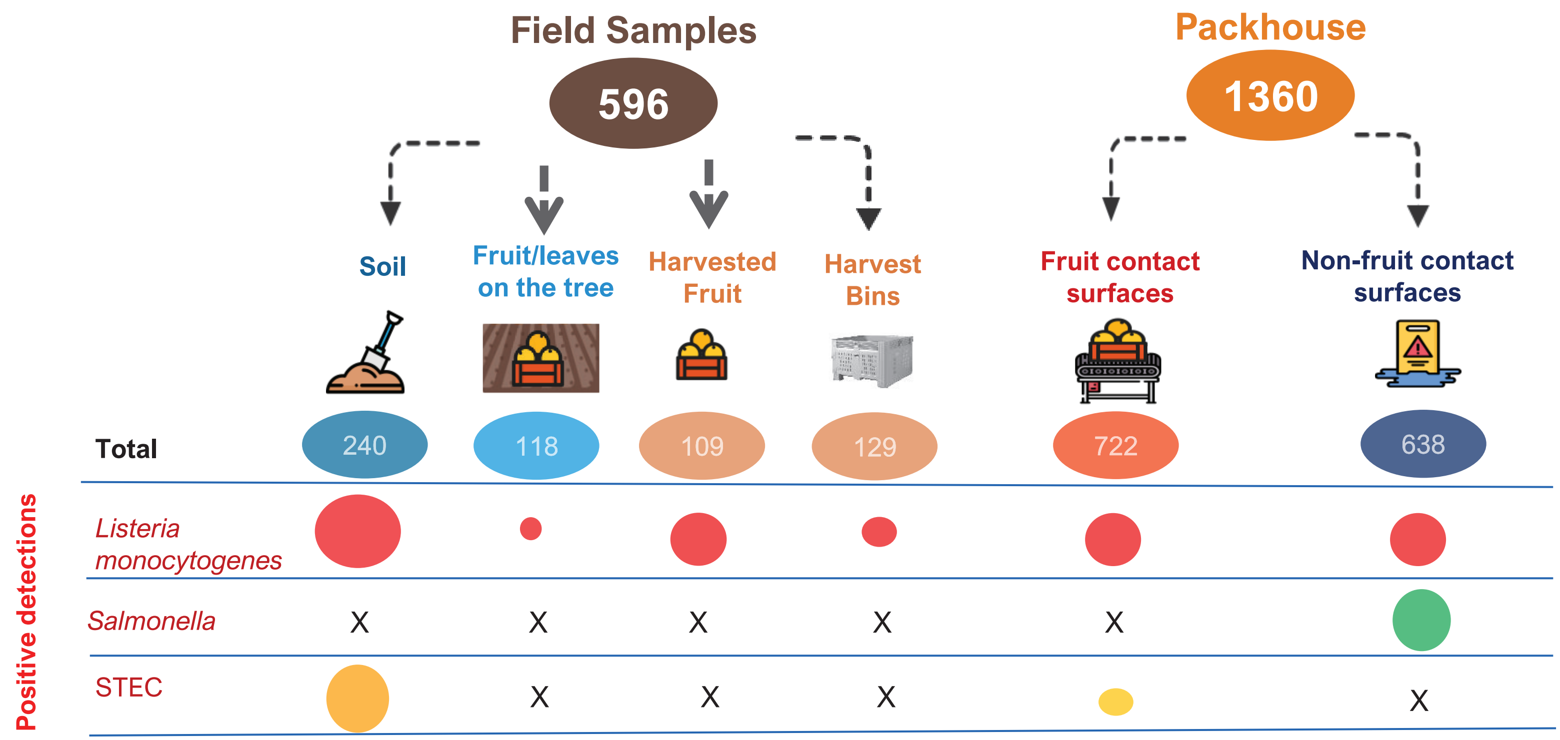
Mapping the microbial risks- Results



The size of the circle for each pathogen approximately represents their prevalence. X = no detection

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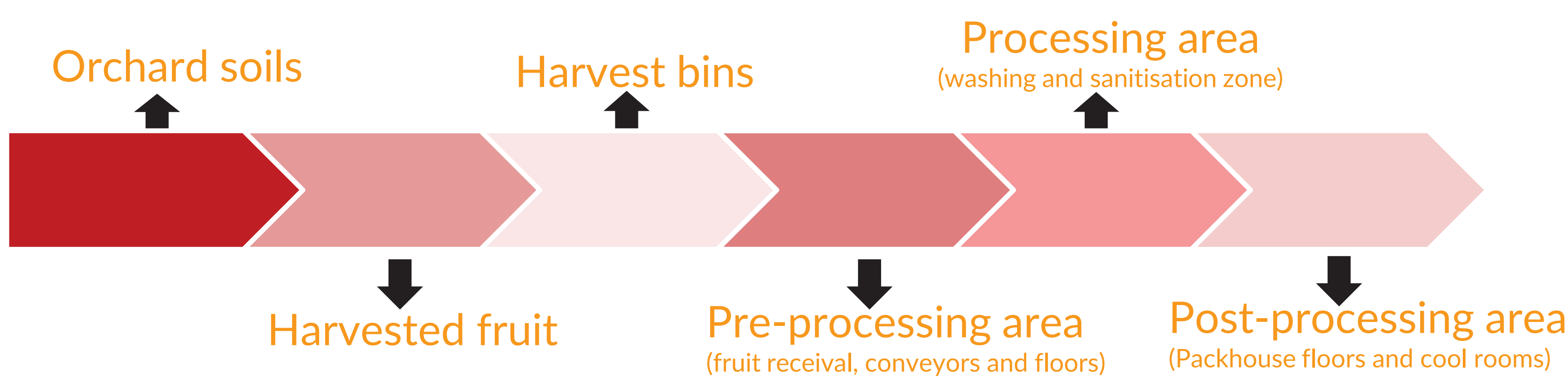
Mapping the microbial risks- Results

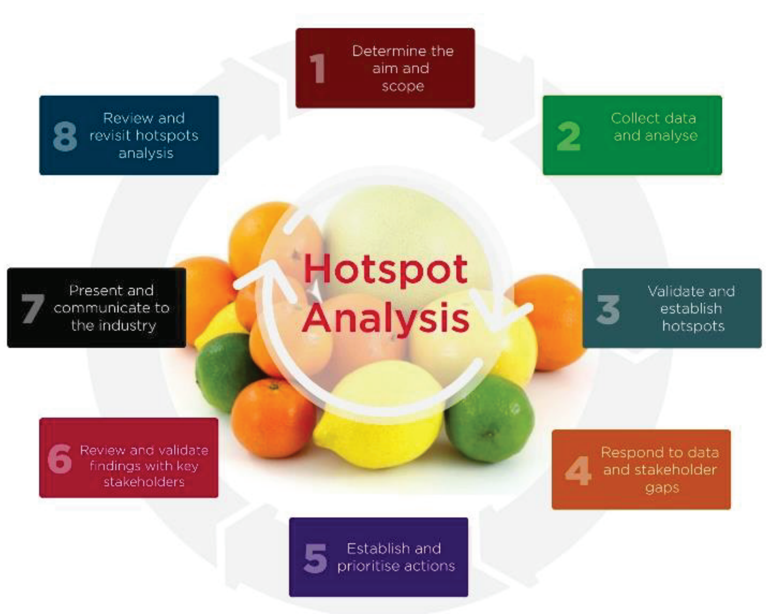
Prevalence and distribution of *Listeria monocytogenes* in the citrus supply chain

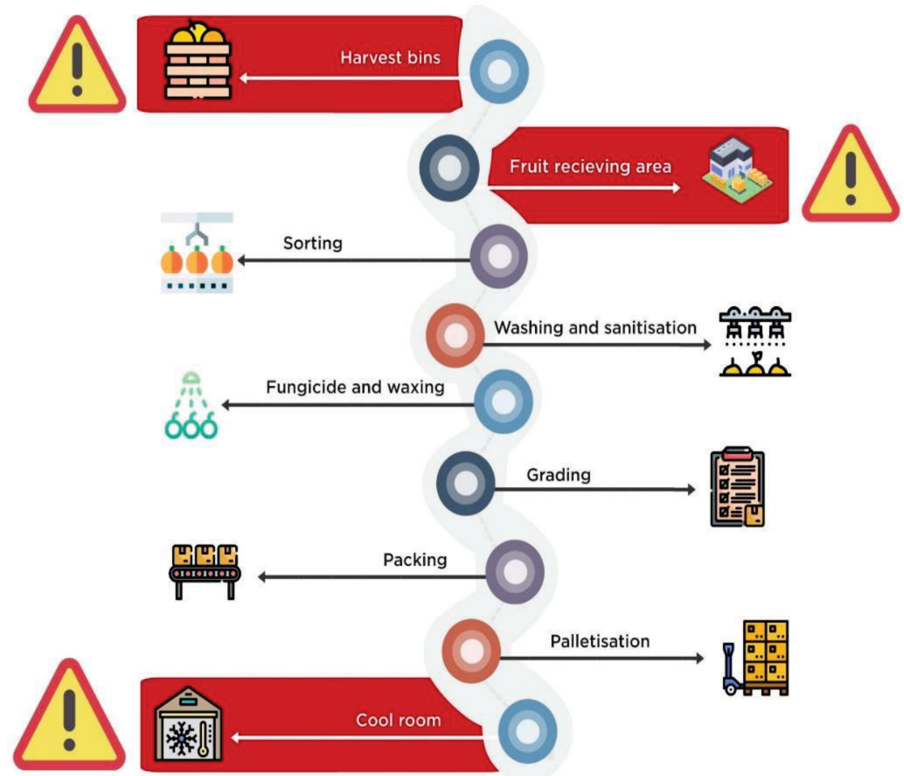


The microbial risk map shows the prevalence of *Listeria monocytogenes* (deeper the shade, higher was the prevalence) along the supply chain from field production to retail .

Mapping the microbial risks- Results

Listeria monocytogenes hotspots





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Mapping the microbial risks- Results

Whole-genome sequencing (WGS) of the *Listeria monocytogenes* isolates

- Persistence and transmission of pathogen
- Genetic diversity of isolates
- Virulence and antimicrobial resistance genes

WGS elucidated the transmission route of pathogen strains

The presence of same strain type on different types of samples (pre-wash fruit, conveyor belt, receival floor and drain and on fungicide application rollers) indicated the transmission of pathogen from field to the processing equipment and premises.

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Mapping the microbial risks- Summary

- Microbial risk mapping process showed that citrus fruit pose a relatively lower food safety risk to consumers, but the detection of environmental pathogens such as *Listeria monocytogenes* on the fruit surface could pose a serious "trade-risk" in export markets.
- *Salmonella* and Shiga-toxin producing *E. coli* (STEC) did not present a significant risk. However, the detection level of STEC in orchard soils could potentially be linked to the use of raw animal manures received as byproducts from livestock operations.
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Mapping the microbial risks- Results

What is still unresolved?

- There are still unanswered questions on the contributory factors or practices leading to the higher prevalence of Listeria in citrus orchard soils and their transmission and management options.
- Preharvest orchard factors and practices hold the key to managing Listeria contamination risks at the first point in the supply chain.
- A preliminary study was undertaken on 8 citrus orchards in NSW and WA and showed that *Listeria monocytogenes* was detected in 80 soil drag samples out of 187 field samples and all farms had varied prevalence levels ranging from 3.3 to 72.0%. However, this trial did not establish the cause-effect relationships due to limited time and resources.

APPENDIX III

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Survival and persistence of foodborne bacterial pathogens on the citrus fruit surfaces during simulated supply chain conditions

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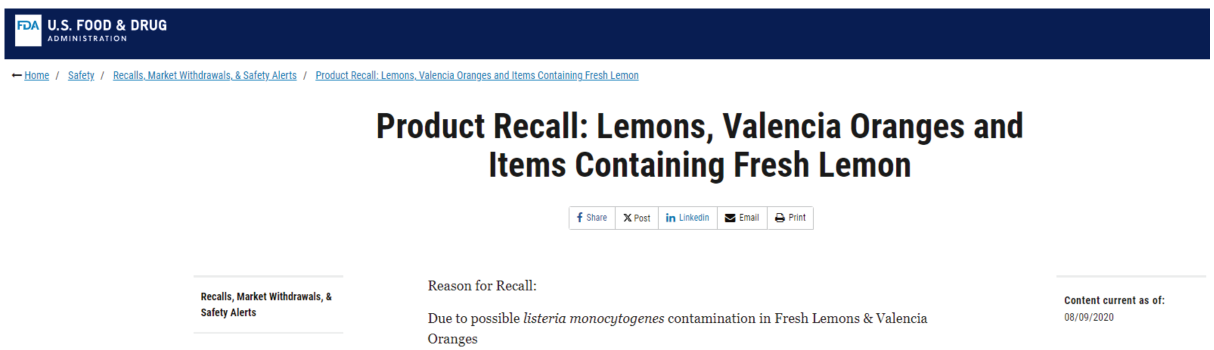
Citrus product recalls in the USA in 2020

Due to possible *Listeria monocytogenes* contamination in fresh lemons, limes and Valencia oranges


U.S. FOOD & DRUG ADMINISTRATION

Home / Safety / Recalls, Market Withdrawals, & Safety Alerts / Product Recall: Lemons, Valencia Oranges and Items Containing Fresh Lemon

Product Recall: Lemons, Valencia Oranges and Items Containing Fresh Lemon

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Recalls, Market Withdrawals, & Safety Alerts

Reason for Recall:

Due to possible *listeria monocytogenes* contamination in Fresh Lemons & Valencia Oranges

Content current as of:
08/09/2020

Source: Product Recall: Lemons, Valencia Oranges and Items Containing Fresh Lemon | FDA

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Listeria monocytogenes has emerged

as a microbial contaminant of concern for the citrus industry

Commodity	Country of origin	Contaminant	Analytical Priority
Oranges	United States	Listeria monocytogenes	High
Limes	United States	Listeria monocytogenes	High
Lemons	United States	Listeria monocytogenes	High
Oranges	Tunisia	Fungal moulds and yeasts	Very low
Mandarins (inc clementines & similar hybrids)	Morocco	Fungal moulds and yeasts	Very low
Limes	Thailand	Salmonella (unspecified or other spp)	Very low
Lemons	Argentina	Fungal moulds and yeasts	Very low

Showing 1 - 7 of 7 result(s).

- High: Issues within the last 12 months
- Medium: Issues within the last 2 years
- Low: Issues within the last 3 years
- Very low: Previous history of issues (more than 3 years ago)

Source: HorizonScan, FERA, UK

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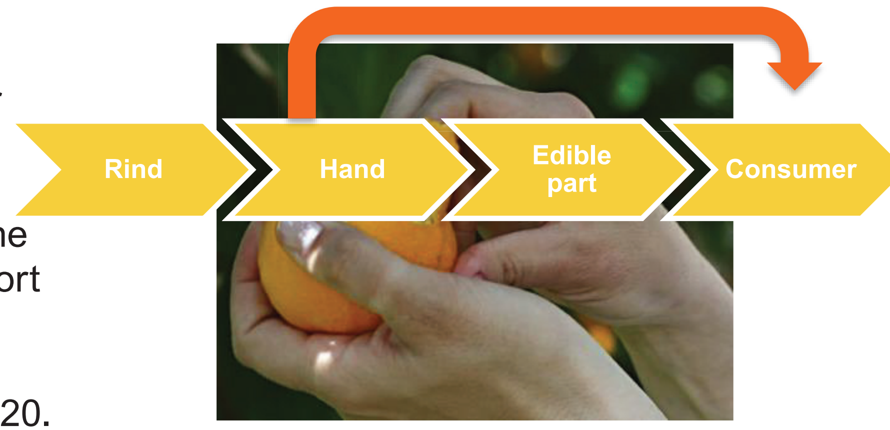
Foodborne bacterial pathogens present on the surface of fresh citrus fruits can be transferred to the edible portion of the fruit or to the hands of the person doing the peeling.

Due to the inedible peel, citrus fruit poses a relatively lower microbial risk to consumers.

Detection of pathogens (e.g. *Listeria monocytogenes*) on the citrus fruit surface could be a serious ‘TRADE RISK’ in export markets.

Oranges and mandarins export value ~$0.5 billion in 2019-20.

Understanding the survival and persistence behaviour of foodborne bacterial pathogens on the citrus fruit surfaces is critical to inform the supply chain best practice guidance.



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Survival and persistence of bacterial pathogens- Summary

The postharvest export chain simulation trials showed that surfaces of citrus fruit (oranges, mandarins, and lemons) did not support the multiplication of environmental pathogens (*Listeria monocytogenes* and *Salmonella*) during 3-4 weeks of cold treatment (2.8-3.0°C), which is the major phytosanitary treatment for citrus export to several markets such as China and Japan.

Both pathogens could survive on the fruit surfaces of all types of citrus with a slight reduction in their populations during export simulation trials. The experimental datasets support the hypothesis that these pathogens are capable of surviving and persisting on the fruit surface during export shipments subjected to cold phytosanitary treatments.

Preventing contamination and cross-contamination of fruit with bacterial pathogens during production and postharvest handling is critical to ensure safe supply of fruit to export markets.

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Survival and persistence of bacterial pathogens- Methodology

The following experiments were conducted to understand the survival and persistence behaviour of bacterial pathogens on different types of citrus fruit surfaces:

1. Survival of *Listeria monocytogenes* on Navel oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation
2. Survival of *Salmonella* Typhimurium on Navel oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation
3. Survival of *Listeria monocytogenes* on Valencia oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation
4. Survival of *Salmonella* Typhimurium on Valencia oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation
5. Survival of *Listeria monocytogenes* on Bellamy oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation
6. Survival of *Salmonella* Typhimurium on Bellamy oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation
7. Survival of *Listeria monocytogenes* on Bellamy oranges after 0 and 3 weeks at 2.8°C as a simulation of the phytosanitary cold treatment for export markets
8. Survival of *Salmonella* Typhimurium on Bellamy oranges after 0 and 3 weeks at 2.8°C as a simulation of the phytosanitary cold treatment for export markets

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Survival and persistence of bacterial pathogens- Methodology

9. Survival of *Listeria monocytogenes* on Bellamy oranges after 0 and 2 weeks at 4°C followed by shelf-life as a simulation of domestic supply
10. Survival of *Salmonella* Typhimurium on Bellamy oranges after 0 and 2 weeks at 4°C followed by shelf-life as a simulation of domestic supply
11. Survival of *Listeria monocytogenes* on Imperial mandarins after 0-4 weeks at 2.8°C followed by shelf-life simulation
12. Survival of *Salmonella* Typhimurium on Imperial mandarins after 0-4 weeks at 2.8°C followed by shelf-life simulation
13. Survival of *Listeria monocytogenes* on Afourer mandarins after 0 and 3 weeks at 2.8°C followed by shelf-life simulation
14. Survival of *Salmonella* Typhimurium on Afourer mandarins after 0 and 3 weeks at 2.8°C followed by shelf-life simulation
15. Survival of *Listeria monocytogenes* on Afourer mandarins after 0 and 2 weeks at 4°C followed by shelf-life simulation (domestic supply chain simulation)
16. Survival of *Salmonella* Typhimurium on Afourer mandarins after 0 and 2 weeks at 4°C followed by shelf-life simulation (domestic supply chain simulation)
17. Survival of *Listeria monocytogenes* on lemons after 0-2 weeks at 2.8°C followed by shelf-life simulation
18. Survival of *Salmonella* Typhimurium on lemons after 0-2 weeks at 2.8°C followed by shelf-life simulation

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Survival and persistence of bacterial pathogens- Methodology

Fruit preparation ➡ Fruit inoculation ➡ Cold treatment ➡ Shelf-life simulation

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Survival and persistence of bacterial pathogens- Methodology

Fruit preparation

Fresh fruit were either harvested from NSW DPI research station at Somersby or from commercial citrus orchards or supermarkets, stored in the laboratory overnight and the next day they were washed in 0.1% Tween-20 and dipped in 100 ppm chlorine (~pH 7.0) for 2 minutes. The fruit were then dried with tissue paper and placed in the BSC II to be inoculated.

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Survival and persistence of bacterial pathogens- Methodology

Inoculation preparation

Listeria monocytogenes: The bacterial stock isolate was maintained at -20°C until inoculum preparation. A 10-µL suspension of *L. monocytogenes (*IFM1011 Batch:RM1800; IFM quality services) was transferred to 25 mL of brain heart infusion (BHI) using a sterile loop and incubated overnight at 37°C. The inoculum was serial diluted in BHI, plated on Listeria selective Oxford Agar Base (OXOID) and incubated at 37°C for 24 h to determine starting concentration. Two concentrations of inoculum were used for the inoculation (10^7 and 10^5 CFU/ml). For the concentration of 10^7 CFU/mL, 200µL of the overnight culture were transferred in 9,800µL of BHI to achieve a concentration of 2×10^8 CFU/mL, whereas for the 10^5 CFU/mL 100µL of the overnight culture were transferred in 9,900µL of BHI. The same inoculum was used for all time points within a treatment.

Salmonella: The bacterial stock isolate was maintained at -20ºC until inoculum preparation. A 10-µL suspension of *Salmonella* Typhimurium (IFM2310 Batch: RM1880 (IFM quality services) ATCC Ref.: 14028) was transferred to 25 mL of brain heart infusion (BHI) using a sterile loop and incubated overnight at 37ºC. The inoculum was serial diluted in BHI, plated on xylose lysine deoxycholate (XLD) agar (Difco) and incubated at 37ºC for 24 h to determine starting concentration. Two concentrations of inoculum were used for the inoculation (10^7 and 10^5 CFU/ml). For the concentration of 10^7 CFU/mL 160µL of the overnight culture were transferred in 7,840µL of BHI to achieve a concentration of 2×10^8 CFU/mL, whereas for the 10^5 CFU/mL 16µL of the overnight culture were transferred in 7,984µL of BHI. The same inoculum was used for all time points within a treatment.

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Survival and persistence of bacterial pathogens- Methodology

Fruit inoculation

The fruit marked with a permanent marker to indicate the spot of inoculation. Each spot was inoculated with five 10-μL drops of inoculum, spread in a 2 cm circle using a sterile spreader and dried in a biosafety cabinet for 30 minutes at ambient temperature. This process was repeated for all fruits including the negative controls (3 replications of 5 fruit per population, per storage condition (0, 1, 2, 3, and 4 weeks followed by 4 days of storage in ambient conditions per week). For the negative controls, the oranges were inoculated with five 10-μL drops of BHI.

Department of Primary Industries

Survival and persistence of bacterial pathogens- Methodology

Storage preparation and conditions

The inoculated fruit were placed on trays with spaced individual holders and then in plastic boxes with ventilation holes on the sides for better airflow. Each box was holding ten fruit per inoculum concentration (0, 10^7 and 10^5 CFU/mL) and three different stacks were made with one box of each (one per replication, 12 stacks in total). The stacks were randomly placed in the cool room on plastic trays. The fruit were stored at 2.5-2.8˚C for 1, 2, 3, and 4 weeks and at 20°C for 5 days after they've been stored for the required weeks. On day 0, 15 fruits from each inoculum concentration (including negative controls) were kept for enumeration.

Bacterial enumeration

Each fruit was placed in a sterile stomacher bag with 10 mL of Dey-Engley neutralising buffer and massaged manually for 30 seconds to dislodge the bacteria. 1 mL from each bag was transferred in 1.5mL tubes to be used for spiral plating (Whitley WASP Touch) on Listeria selective Oxford Agar Base for *Listeria monocytogenes* (OXOID) and xylose lysine deoxycholate (XLD) agar for Salmonella. The plates were incubated at 37°C overnight and enumeration was performed with the Protocol3 automated colony counter. The results were recorded in log10 per fruit.

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Survival and persistence of bacterial pathogens- Methodology

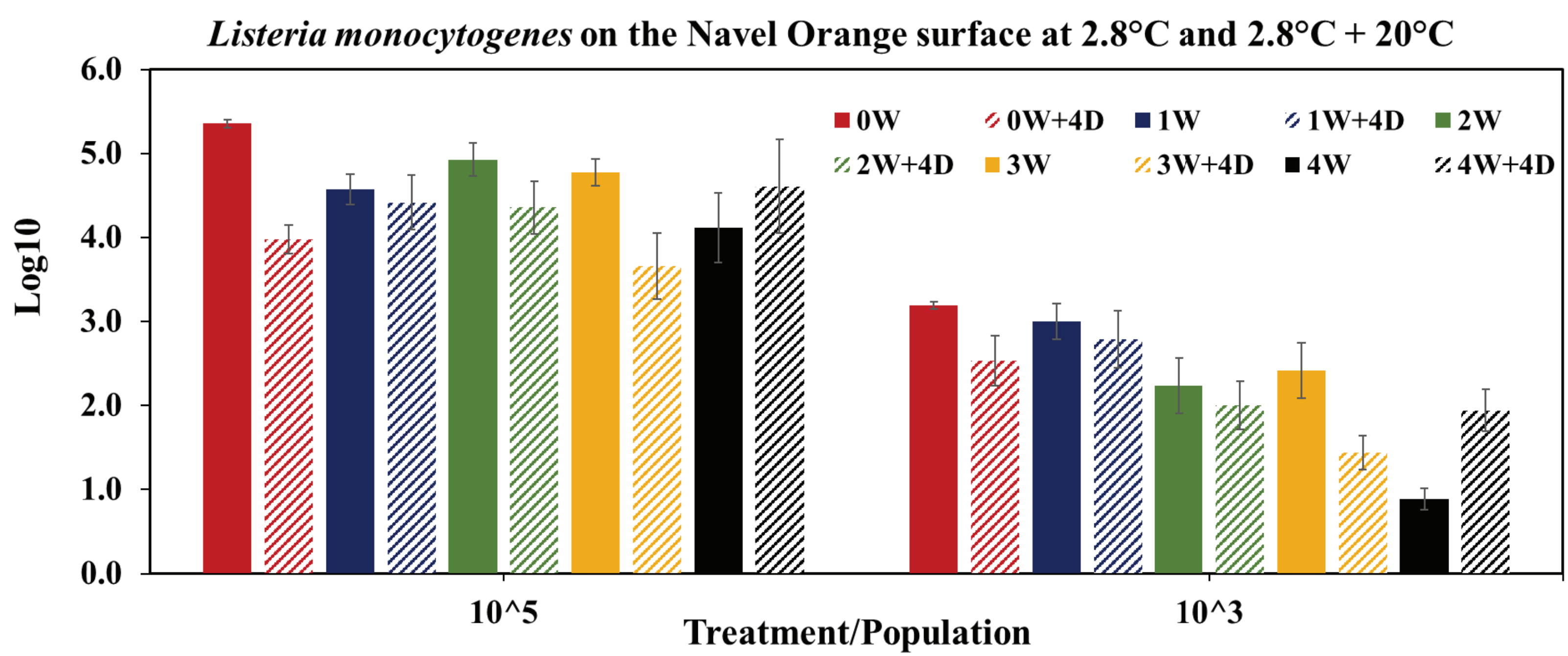
1. Survival of *Listeria monocytogenes* on Navel oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation

- The fruit were harvested from Somersby on 8 Sep 2021.
- The fruit were inoculated with *Listeria monocytogenes* using 2 different populations (10^5 and 10^3 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI. All oranges were stored in the cool room at 2.8°C for 1, 2, 3 and 4 weeks and then for 4 days at 20°C after each week.

Department of Primary Industries

Survival and persistence of bacterial pathogens- Results

The storage duration and the storage temperature significantly affected the survival of *Listeria monocytogenes* on Navel oranges



Department of Primary Industries

Survival and persistence of bacterial pathogens- Methodology

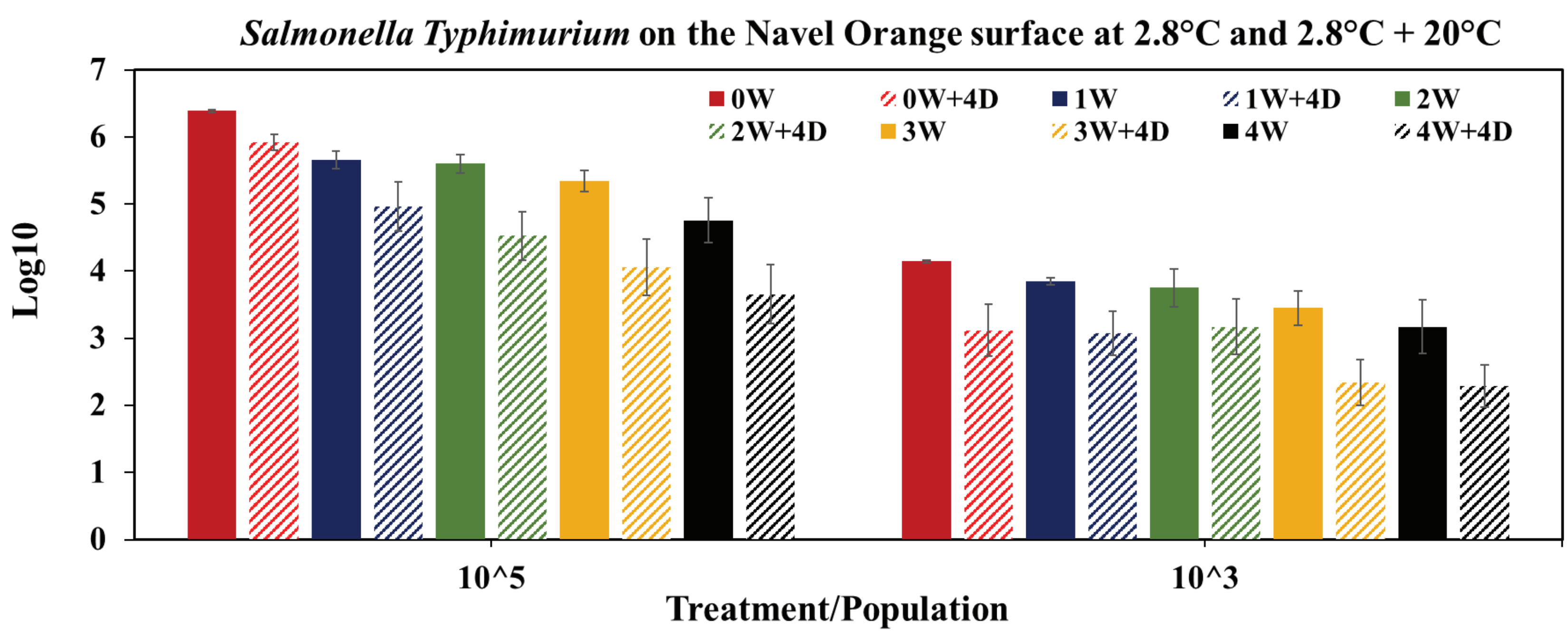
2. Survival of *Salmonella* Typhimurium on Navel oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation

- The fruit were harvested from Somersby on 30 Sep 2021
- The fruit were inoculated with *Salmonella typhimurium* using 2 different populations (10^5 and 10^3 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI
- All oranges were stored in the cool room at 2.8°C for 1, 2, 3 and 4 weeks and then for 4 days at 22°C after each week.

Department of Primary Industries

Survival and persistence of bacterial pathogens- Results

The storage duration and the storage temperature significantly affected the survival of *Salmonella Typhimurium* on Navel oranges



Department of Primary Industries

Survival and persistence of bacterial pathogens- Methodology

3. Survival of *Listeria monocytogenes* on Valencia oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation

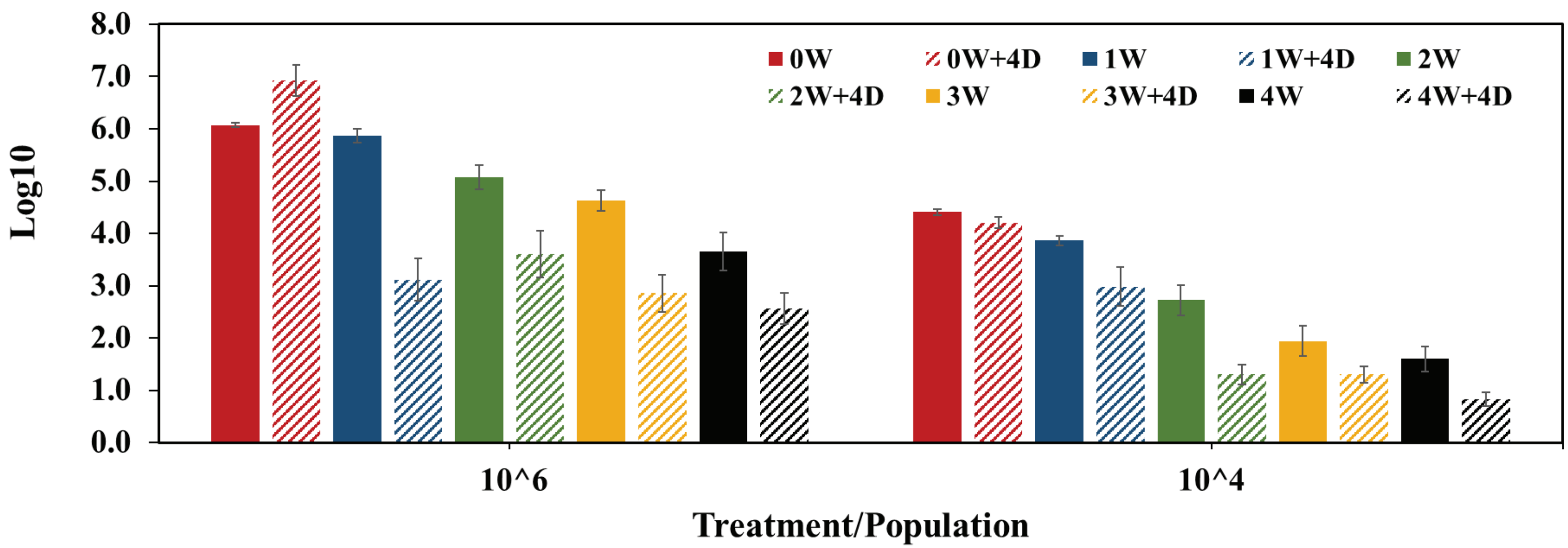
- The fruit were harvested from Somersby on 26 October 2021.
- The fruit were inoculated with *Listeria monocytogenes* using 2 different populations (10^6 and 10^4 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI
- All oranges were stored in the cool room at 2.8°C for 1, 2, 3 and 4 weeks and then for 4 days at 22°C after each week.

Department of Primary Industries

Survival and persistence of bacterial pathogens- Results

The storage duration and the storage temperature significantly affected the survival of *Listeria monocytogenes* on Navel oranges.

Listeria monocytogenes on the Valencia Orange surface at 2.8°C and 2.8°C + 20°C



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Survival and persistence of bacterial pathogens- Methodology

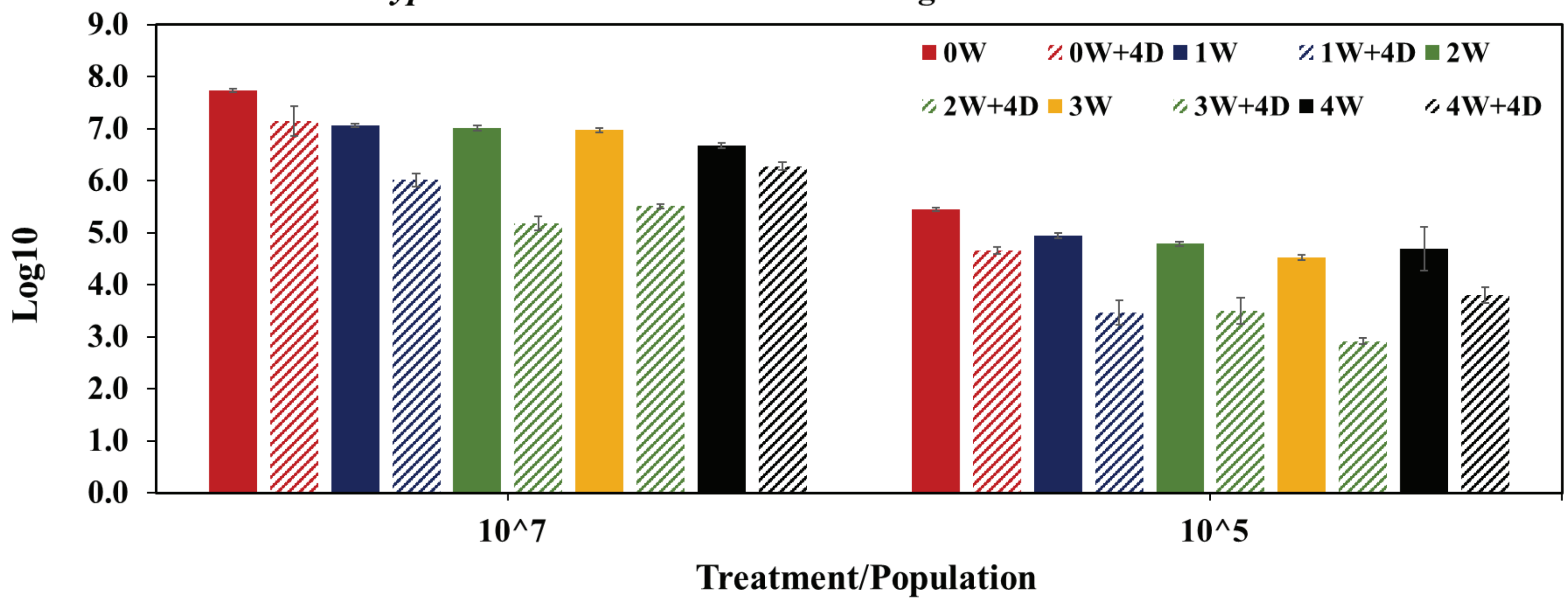
4. Survival of *Salmonella Typhimurium* on Valencia oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation

- The fruit were harvested from Somersby on 26 October 2021
- The fruit were inoculated with *Salmonella typhimurium* using 2 different populations (10^7 and 10^5 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI
- All oranges were stored in the cool room at 2.8 °C for 1, 2, 3 and 4 weeks and then for 4 days at 22 °C after each week.

Survival and persistence of bacterial pathogens- Results

The storage duration and the storage temperature significantly affected the survival of Salmonella Typhimurium on Valencia oranges.

Salmonella typhimurium **on the Valencia Orange surface at 2.8°C and 2.8°C + 20°C**



Survival and persistence of bacterial pathogens- Methodology

5. Survival of *Listeria monocytogenes* on Bellamy (Navel) oranges after 0-4 weeks at 2.8 ^{o}C followed by shelf-life simulation

- The fruit were harvested from Somersby on 3 August 2022.
- The fruit were inoculated with *Listeria monocytogenes* using 2 different populations (10^8 and 10^6 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI.
- All oranges were stored in the cool room at 2.8 ^{o}C for 1, 2, 3 and 4 weeks and then for 4 days at 20 ^{o}C after each week.

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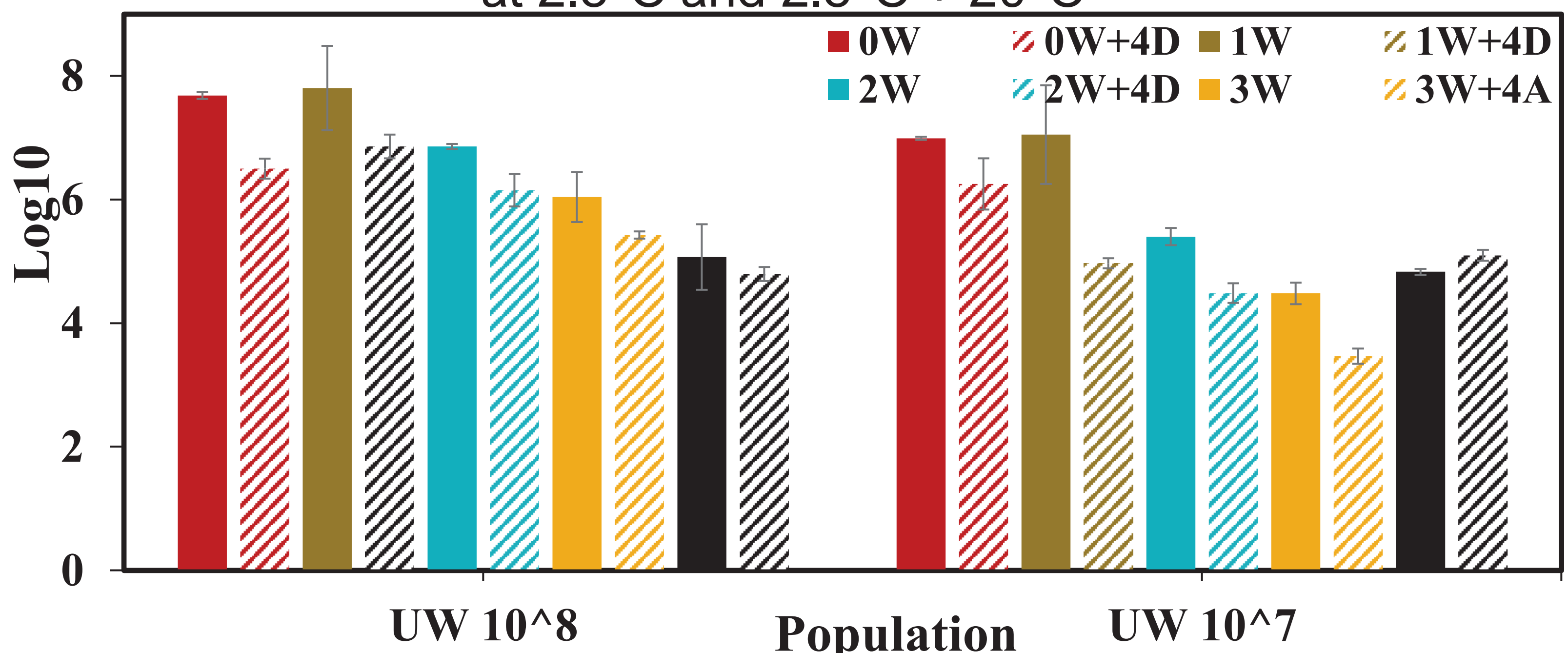
Survival and persistence of bacterial pathogens- Results

Listeria monocytogenes on the Bellamy Orange surface
at 2.8°C and 2.8°C + 20°C

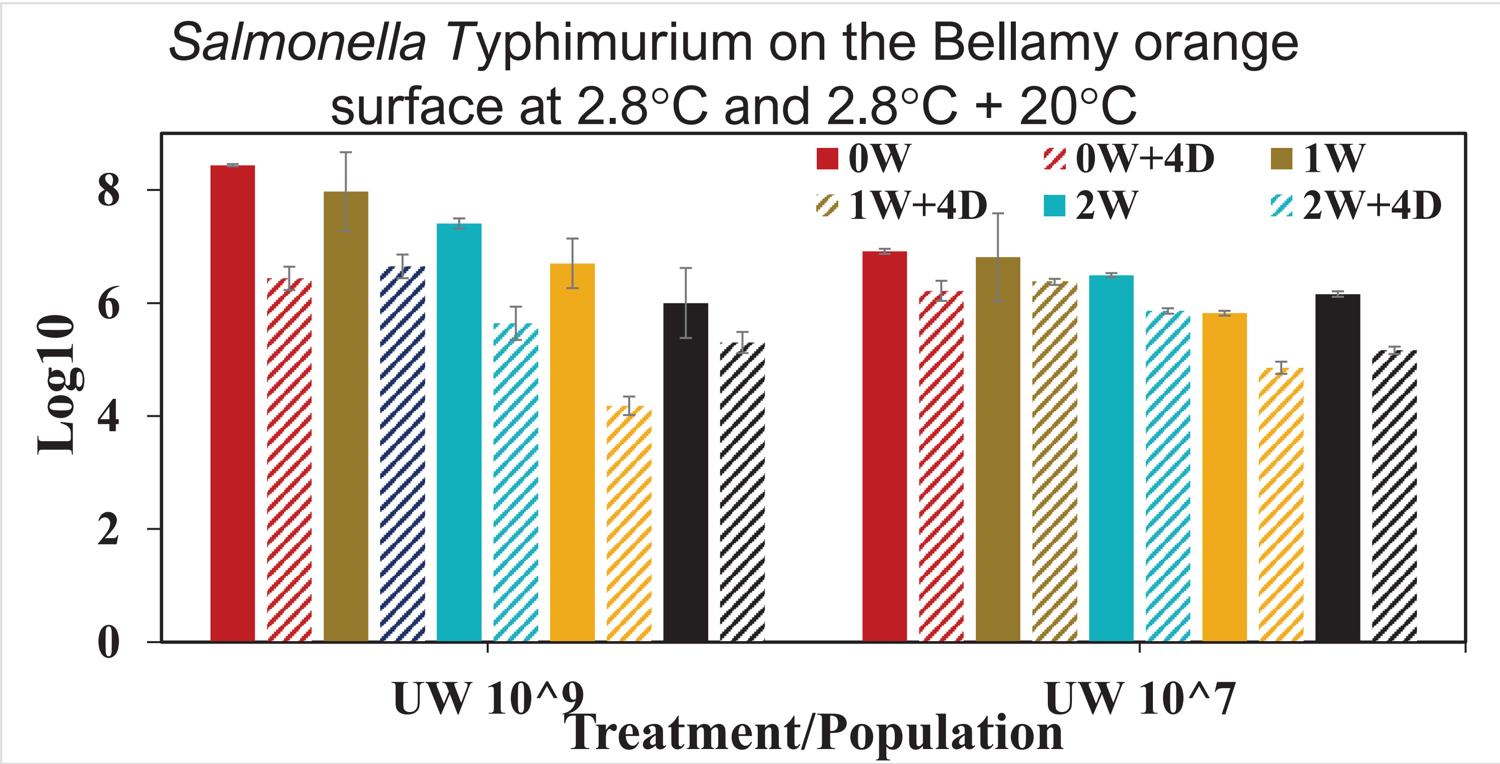


Survival and persistence of bacterial pathogens- Methodology

6. Survival of *Salmonella* Typhimurium on Bellamy oranges after 0-4 weeks at 2.8°C followed by shelf-life simulation

- The fruit were harvested from Somersby on 3 August 2022.
- The fruit were inoculated with *Salmonella typhimurium* using 2 different populations (10^9 and 10^7 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI.
- All oranges were stored in the cool room at 2.8°C for 1, 2, 3 and 4 weeks and then for 4 days at 20°C after each week.

Survival and persistence of bacterial pathogens- Results



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Survival and persistence of bacterial pathogens- Methodology

7. Survival of *Listeria monocytogenes* on Bellamy oranges after 0 and 3 weeks at 2.8°C as a simulation of the phytosanitary cold treatment for export markets

- The fruit were harvested from Somersby on 28 June 2022.
- The fruit were inoculated with *Listeria monocytogenes* using 2 different populations (10^5 and 10^4 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI.
- All oranges were stored in the cool room at 2.8 °C for 0 and 3 weeks.

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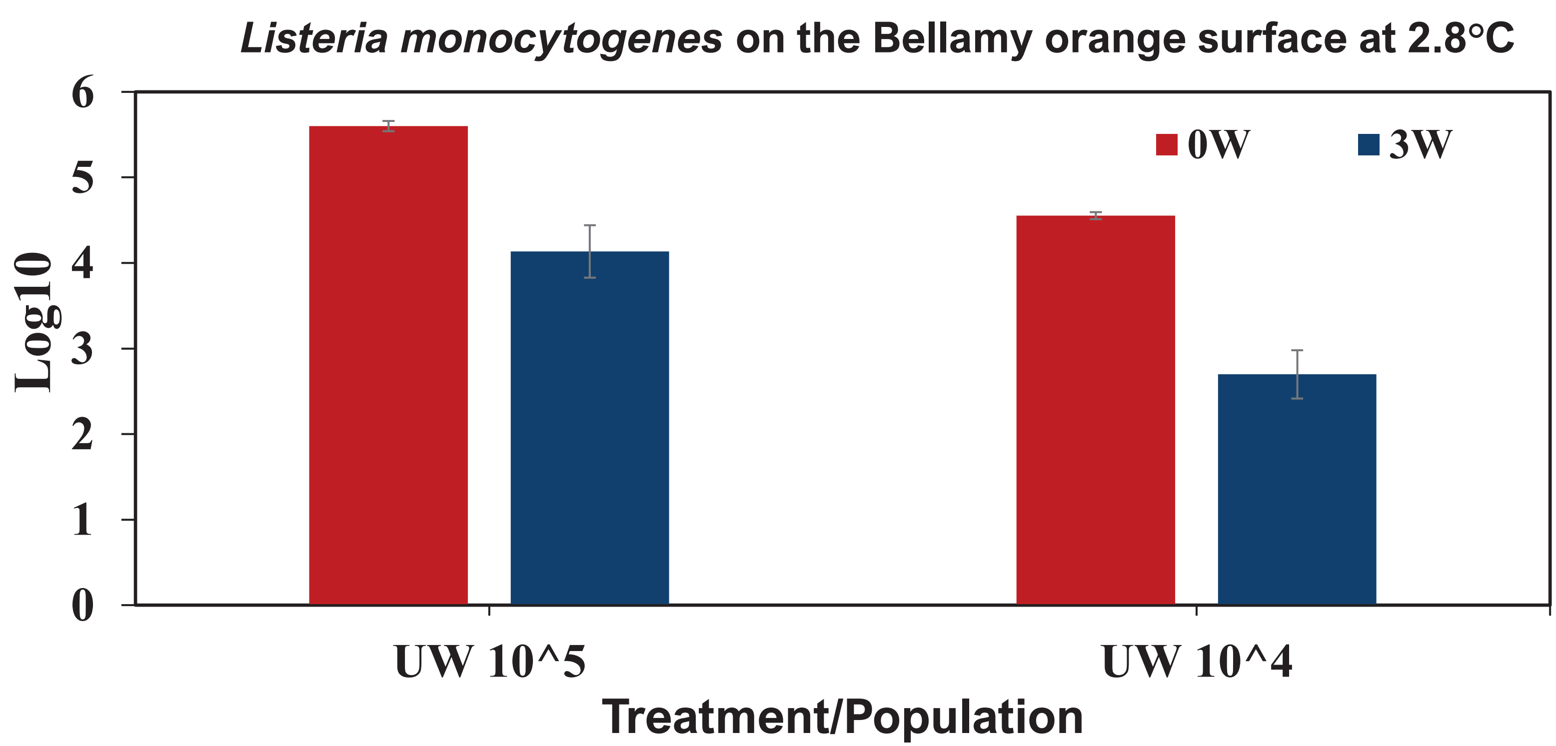
Survival and persistence of bacterial pathogens- Results



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Survival and persistence of bacterial pathogens- Methodology

8. Survival of *Salmonella Typhimurium* on Bellamy oranges after 0 and 3 weeks at 2.8 °C as a simulation of the phytosanitary cold treatment for export markets

- The fruit were harvested from Somersby on 28 June 2022.
- The fruit were inoculated with *Salmonella* Typhimurium using 2 different populations (10^8 and 10^6 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI.
- All oranges were stored in the cool room at 2.8 °C for 0 and 3 weeks.

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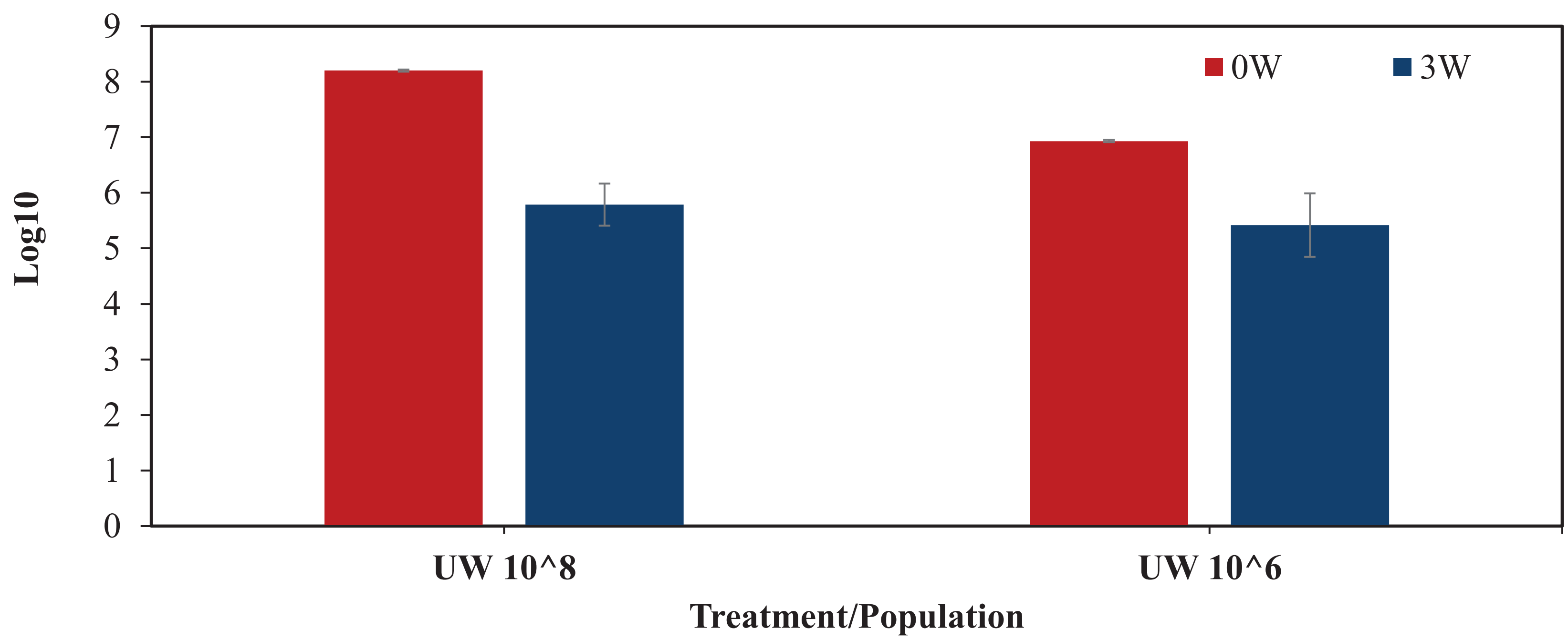
Survival and persistence of bacterial pathogens- Results

***Salmonella* Typhimurium on the Bellamy orange surface at 2.8°C**



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Survival and persistence of bacterial pathogens- Methodology

9. Survival of *Listeria monocytogenes* on Bellamy oranges after 0 and 2 weeks at 4°C followed by shelf-life as a simulation of domestic supply

- The fruit were harvested from Somersby on 28 June 2022.
- The fruit were inoculated with *Listeria monocytogenes* using one population (10^7 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI.
- All oranges were stored in the cool room at 4 °C for 0 and 2 weeks followed by a 4 day storage at 20 °C.

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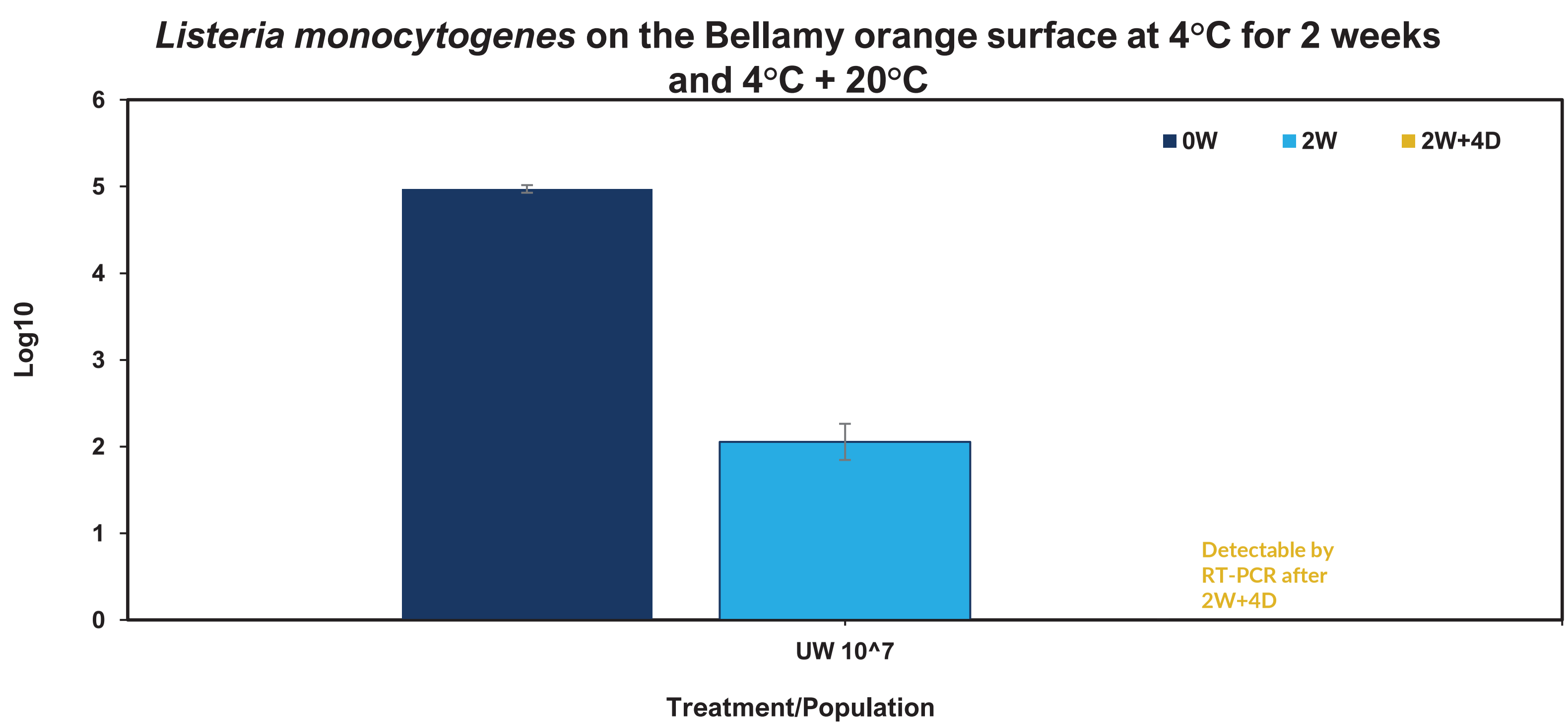
Survival and persistence of bacterial pathogens- Results



Survival and persistence of bacterial pathogens- Methodology

10. Survival of *Salmonella Typhimurium* on Bellamy oranges after 0 and 2 weeks at 4°C followed by shelf-life as a simulation of domestic market supply

- The fruit were harvested from Somersby on 28 June 2022.
- The fruit were inoculated with *Salmonella typhimurium* using one population (10^7 CFU/mL made in BHI).
- Negative control oranges were inoculated with BHI.
- All oranges were stored in the cool room at 4 °C for 0 and 2 weeks followed by a 4 day storage at 20 °C.

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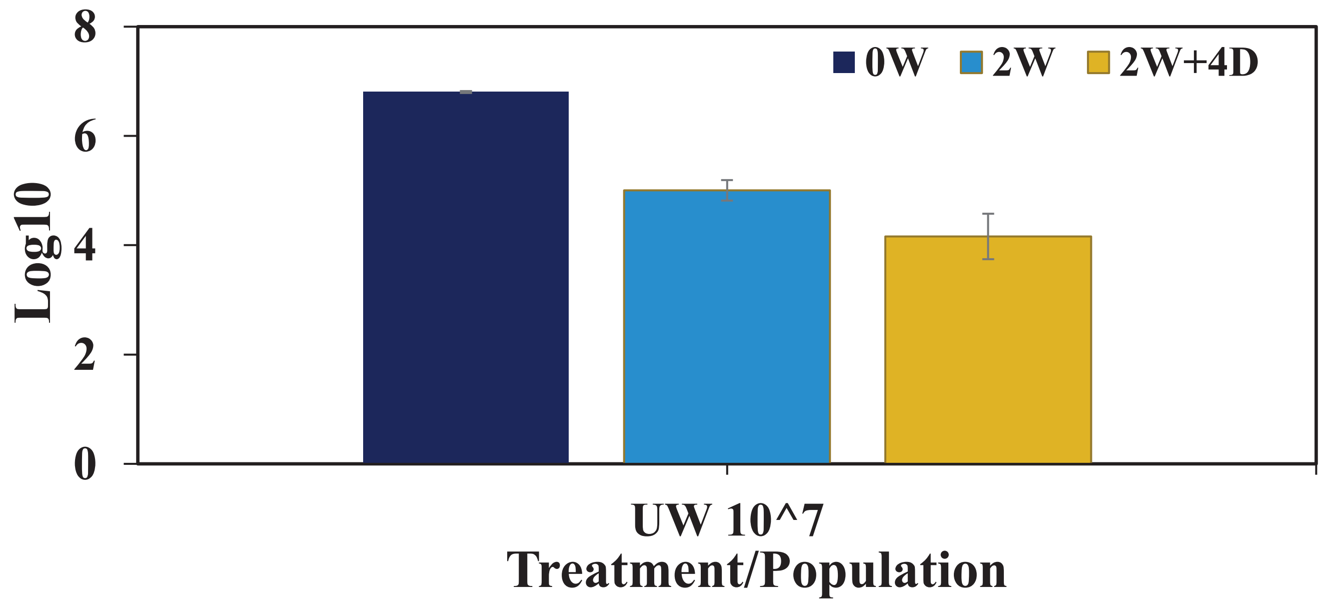
Survival and persistence of bacterial pathogens- Results

***Salmonella* Typhimurium on the Bellamy orange surface at 4°C and 4°C + 20°C**



Mandarins

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Survival and persistence of bacterial pathogens- Methodology

11. Survival of *Listeria monocytogenes* on Imperial mandarins after 0-4 weeks at 2.8 ^{o}C followed by shelf-life simulation

- The mandarins were purchased from the Sydney markets on 20/05/2022.
- The fruit were inoculated with *Listeria monocytogenes* using 2 different populations (10^8 and 10^6 CFU/mL made in BHI).
- Negative control mandarins were inoculated with BHI.
- All mandarins were stored in the cool room at 2.8 ^{o}C for 1, 2, 3 and 4 weeks and then for 4 days at 20 ^{o}C after each week.

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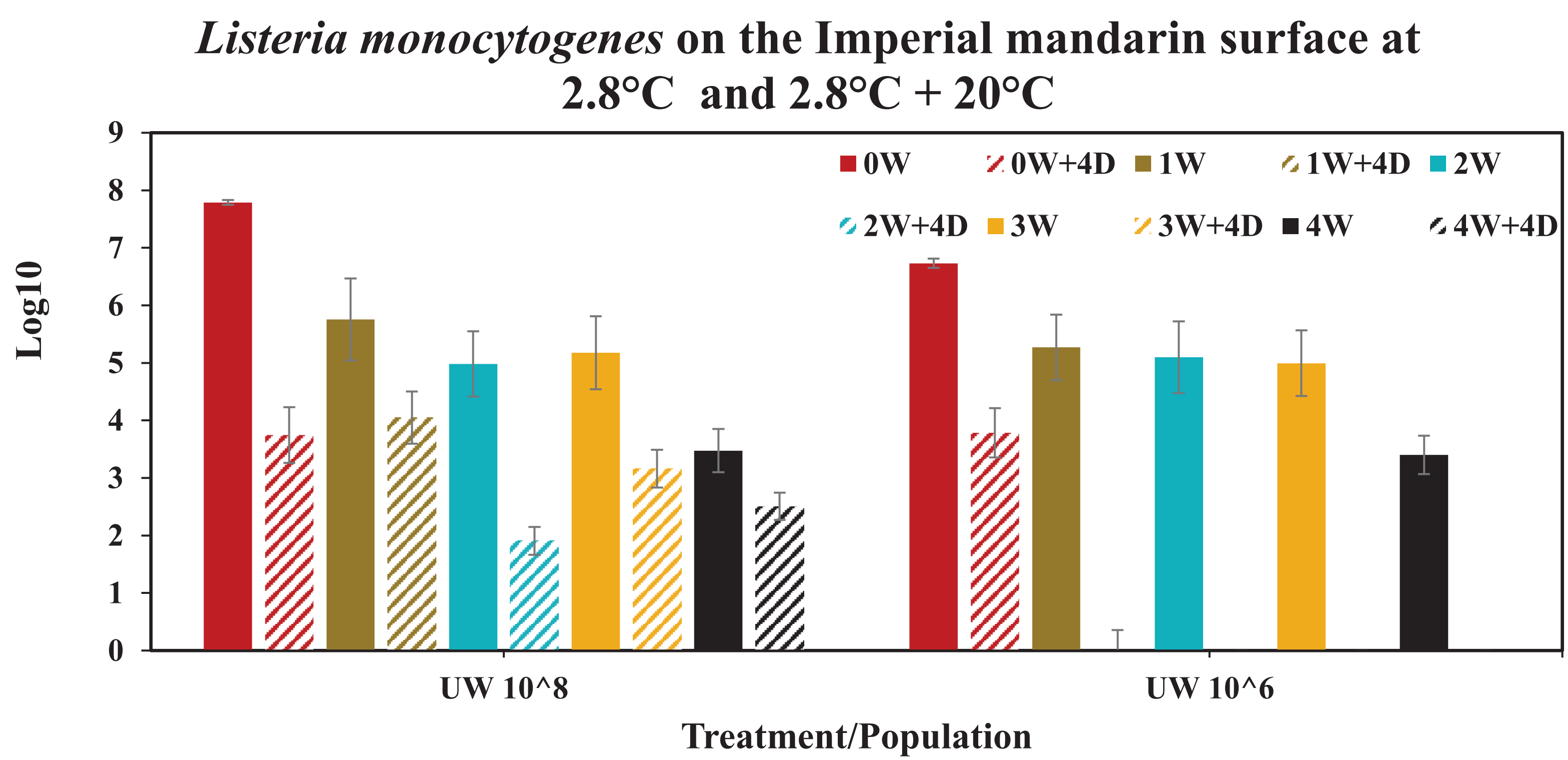
Survival and persistence of bacterial pathogens- Results



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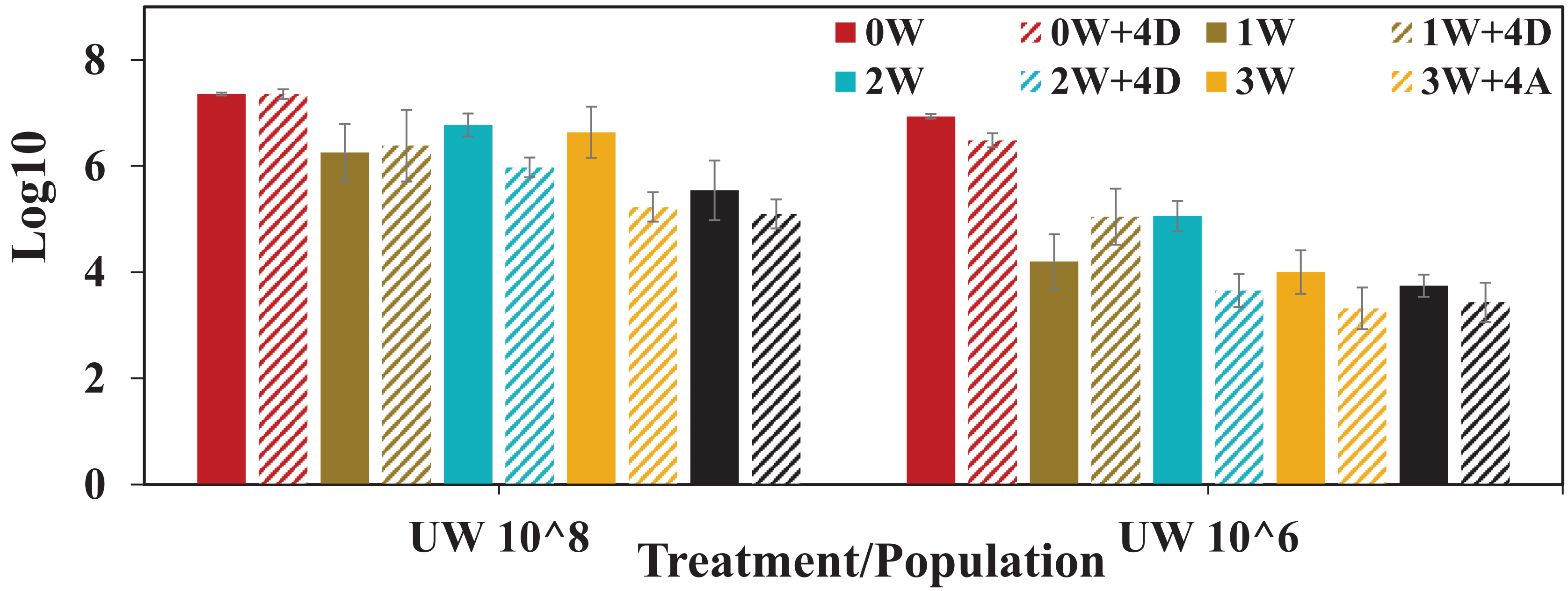
Survival and persistence of bacterial pathogens- Methodology

12. Survival of *Salmonella Typhimurium* on Imperial mandarins after 0-4 weeks at 2.8°C followed by shelf-life

- The mandarins were purchased from the Sydney markets on 20/05/2022.
- The fruit were inoculated with *Salmonella typhimurium* using 2 different populations (10^8 and 10^6 CFU/mL made in BHI).
- Negative control mandarins were inoculated with BHI.
- All mandarins were stored in the cool room at 2.8 °C for 1, 2, 3 and 4 weeks and then for 4 days at 20 °C after each week.

Survival and persistence of bacterial pathogens- Results

Salmonella Typhimurium on the Imperial mandarin surface at 2.8°C and 2.8°C + 20°C



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Survival and persistence of bacterial pathogens- Methodology

13. Survival of *Listeria monocytogenes* on Afourer mandarins after 0 and 3 weeks at 2.8 °C

- The fruit were bought from Woolworths on 20/07/2022 (harvested on 11/07/2022 by Agriexchange Pty Ltd Renmark supplied by Vitor marketing Pty, Renmark)
- The fruit were inoculated with *Listeria monocytogenes* using 2 different populations (10^8 and 10^7 CFU/mL made in BHI).
- Negative control mandarins were inoculated with BHI
- All mandarins were stored in the cool room at 2.8°C for 0 and 3 weeks.

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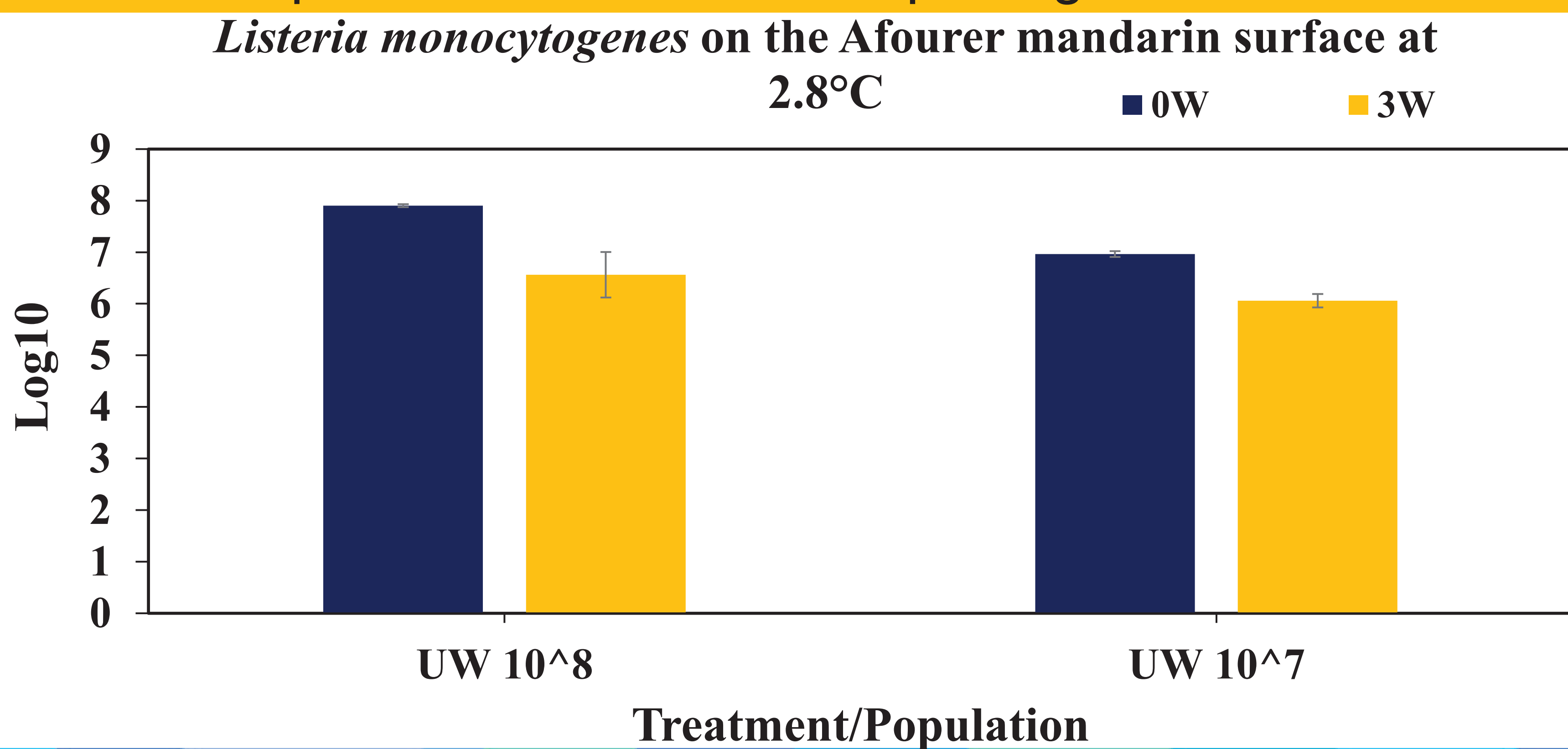
Survival and persistence of bacterial pathogens- Results



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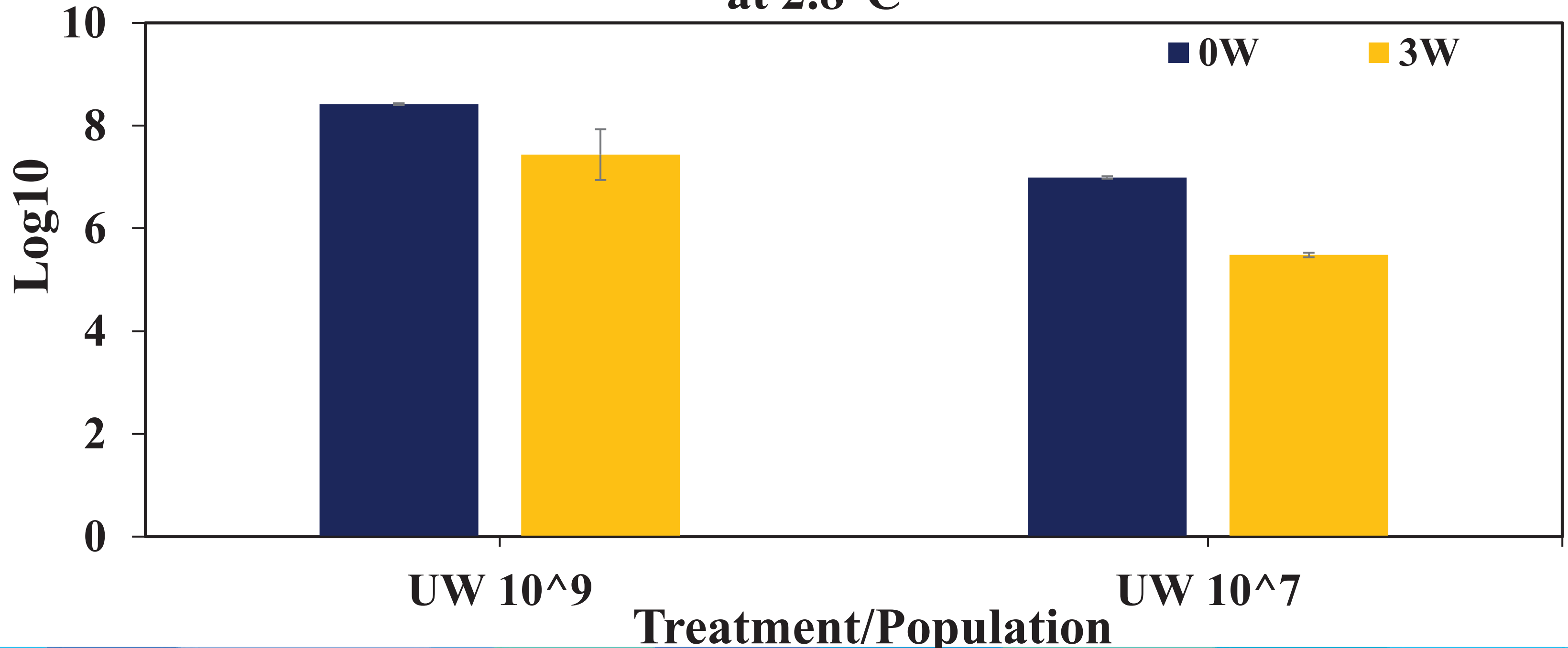
Survival and persistence of bacterial pathogens- Methodology

14. Survival of *Salmonella* Typhimurium on Afourer mandarins after 0 and 3 weeks in 2.8 ^{o}C

- The fruit were bought from Woolworths on 20/07/2022 (harvested on 11/07/2022 by Agriexchange Pty Ltd Renmark supplied by Vitor marketing Pty, Renmark)
- The fruit were inoculated with *Salmonella typhimurium* using 2 different populations (10^8 and 10^6 CFU/mL made in BHI).
- Negative control mandarins were inoculated with BHI.
- All mandarins were stored in the cool room at 2.8 ^{o}C for 0 and 3 weeks.

Survival and persistence of bacterial pathogens- Results

Salmonella typhimurium **on the Afourer mandarin surface at 2.8°C**



Survival and persistence of bacterial pathogens- Methodology

15. Survival of *Listeria monocytogenes* on Afourer mandarins after 0 and 2 weeks in 4°C followed by shelf-life simulation

- The mandarins were bought from Woolworths on 20/07/2022 (harvested on 11/07/2022 by Agriexchange Pty Ltd Renmark supplied by Vitor marketing Pty, Renmark, SA)
- The fruit were inoculated with *Listeria monocytogenes* using one population (10^7 CFU/mL made in BHI).
- Negative control mandarins were inoculated with BHI.
- All mandarins were stored in the cool room at 4°C for 0 and 2 weeks followed by a 4 day storage at 20°C

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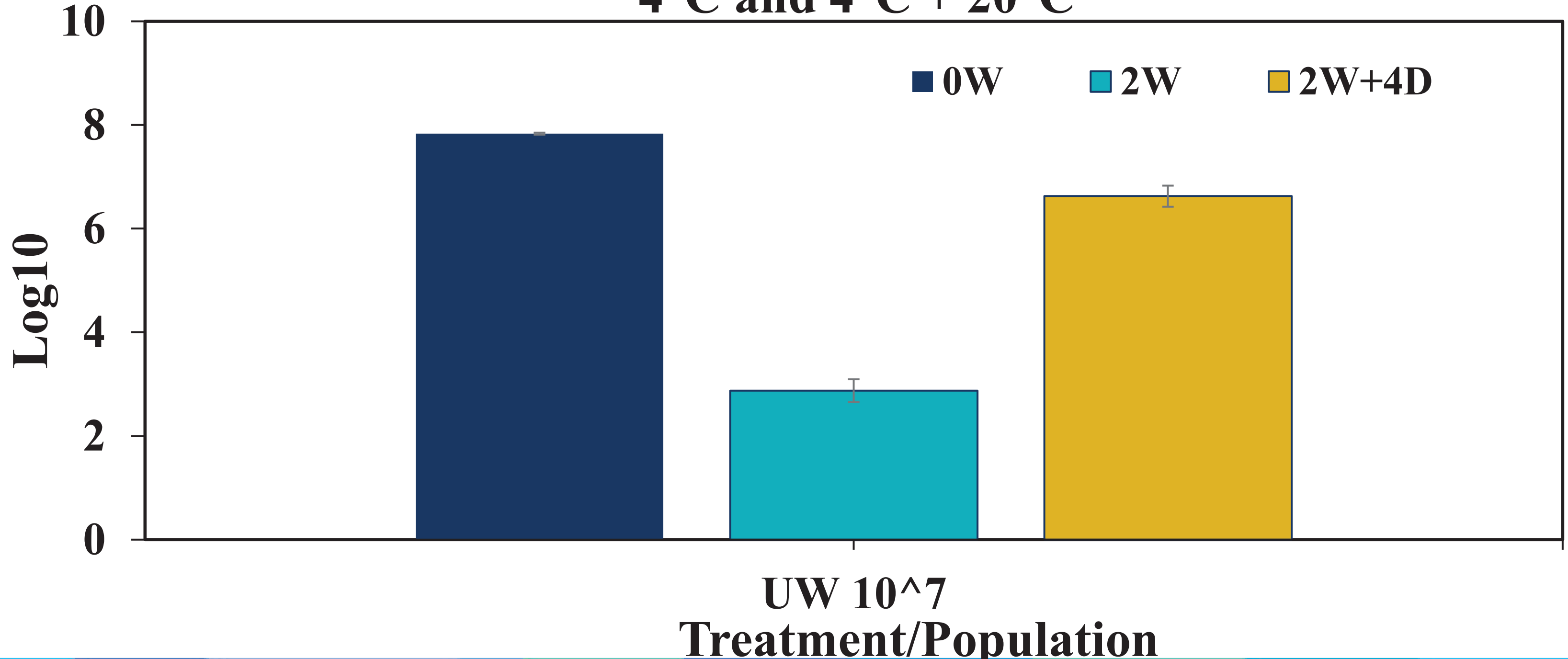
Survival and persistence of bacterial pathogens- Results

Listeria monocytogenes **on the Afourer mandarin surface at 4°C and 4°C + 20°C**



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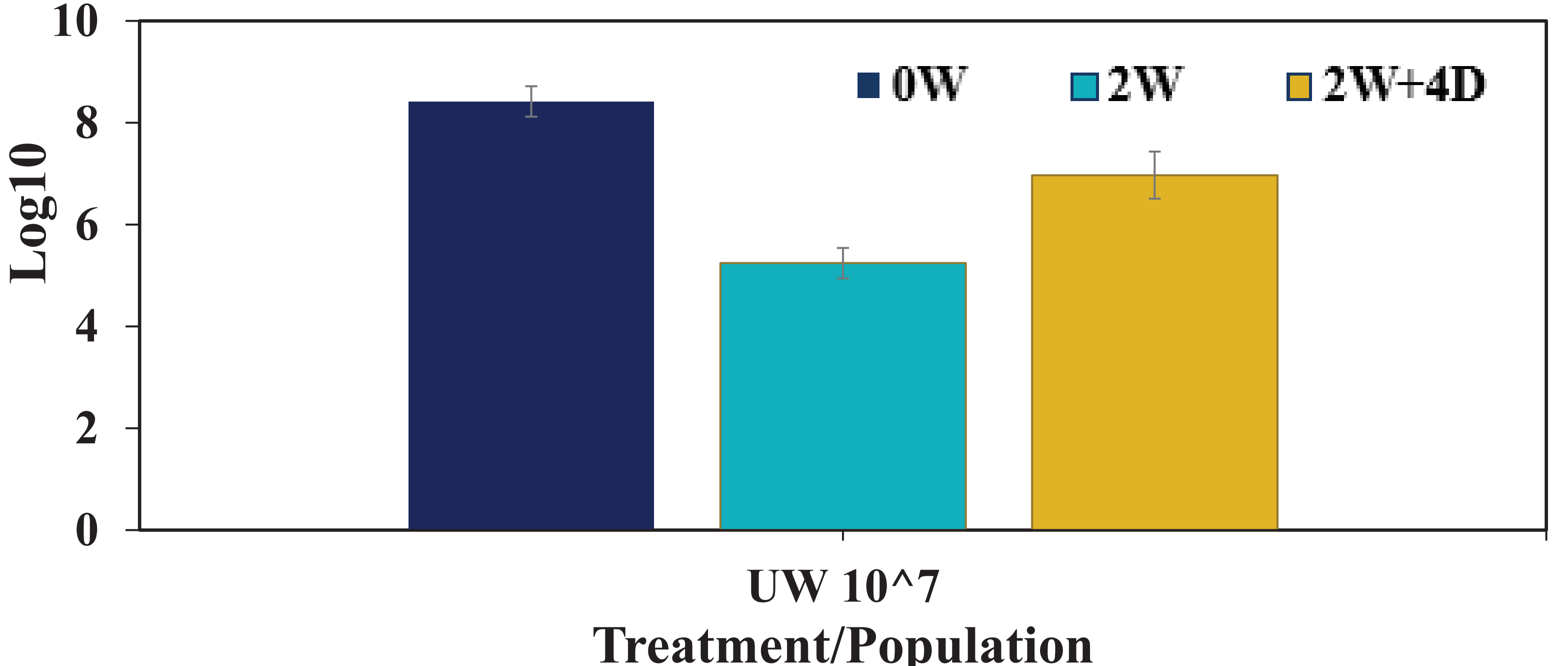
Survival and persistence of bacterial pathogens- Methodology

16. Survival of *Salmonella* Typhimurium on Afourer mandarins after 0 and 2 weeks at 4°C followed by shelf-life simulation

- The mandarins were bought from Woolworths on 20/07/2022 (harvested on 11/07/2022 by Agriexchange Pty Ltd Renmark supplied by Vitor marketing Pty, Renmark)
- The fruit were inoculated with *Salmonella typhimurium* using one population (10^9 CFU/mL made in BHI).
- Negative control mandarins were inoculated with BHI.
- All mandarins were stored in the cool room at 4 °C for 0 and 2 weeks followed by a 4 day storage at 20 °C.

Survival and persistence of bacterial pathogens- Results

***Salmonella* Typhimurium on the Afourer mandarin surface at 4°C and 4°C + 20°C**



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Survival and persistence of bacterial pathogens

Lemons

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Survival and persistence of bacterial pathogens- Methodology

17. Survival of *Listeria monocytogenes* on lemons after 0-2 weeks at 2.8°C followed by shelf-life simulation

- The lemons were purchased from East Coast Citrus on 16/02/2022
- The fruit were inoculated with *Listeria monocytogenes* using 2 different populations (10^8 and 10^6 CFU/mL made in BHI).
- Negative control lemons were inoculated with BHI.
- All lemons were stored in the cool room at 2.8°C for 1, and 2 weeks and then for 4 days at 20°C after each week.

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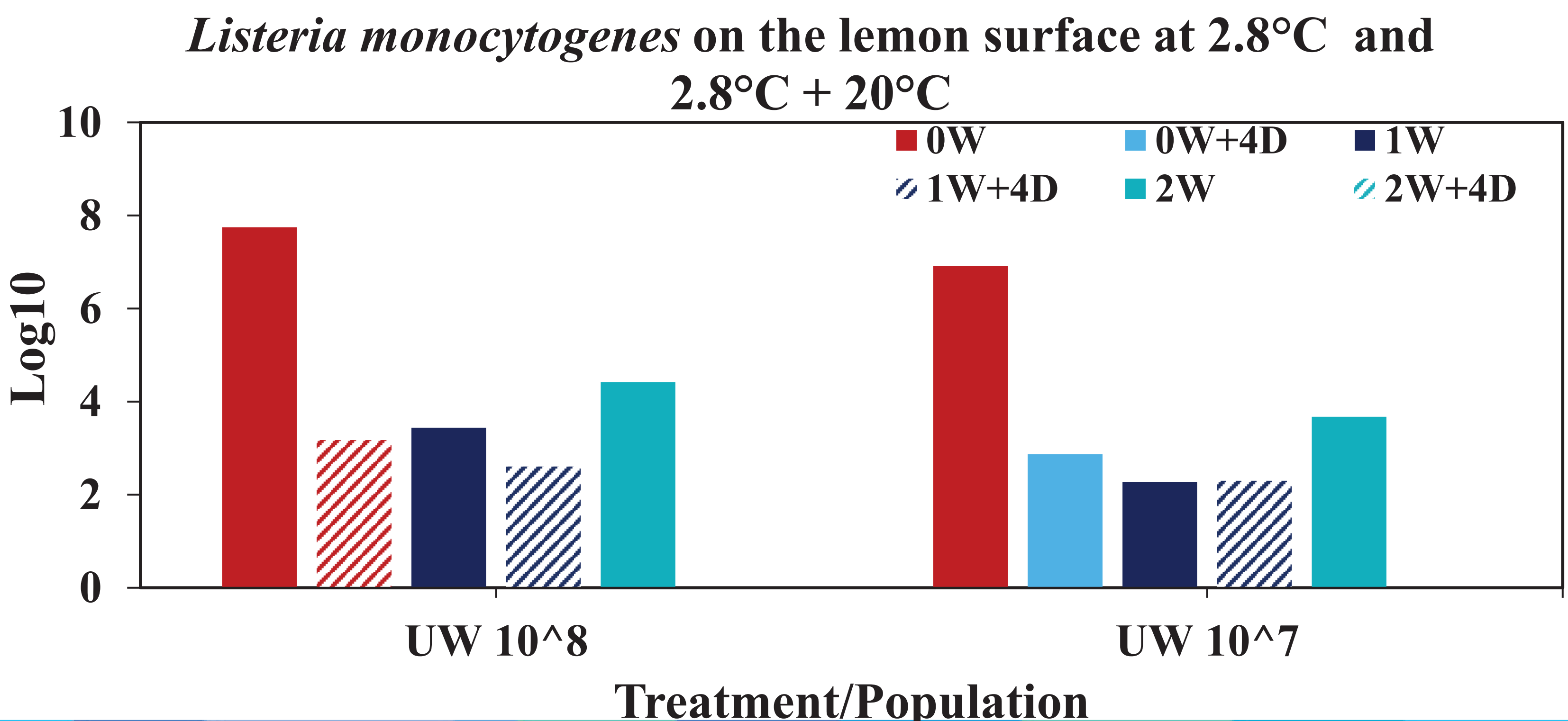
Survival and persistence of bacterial pathogens- Results



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Survival and persistence of bacterial pathogens- Methodology

18. Survival of *Salmonella* Typhimurium on Lemons after 0-2 weeks at 2.8°C followed by shelf-life simulation

- The lemons were purchased from East Coast Citrus on 16/02/2022
- The fruit were inoculated with *Salmonella typhimurium* using 2 different populations (10^8 and 10^6 CFU/mL made in BHI).
- Negative control lemons were inoculated with BHI.
- All lemons were stored in the cool room at 2.8 °C for 1, and 2 weeks and then for 4 days at 20 °C after each week.

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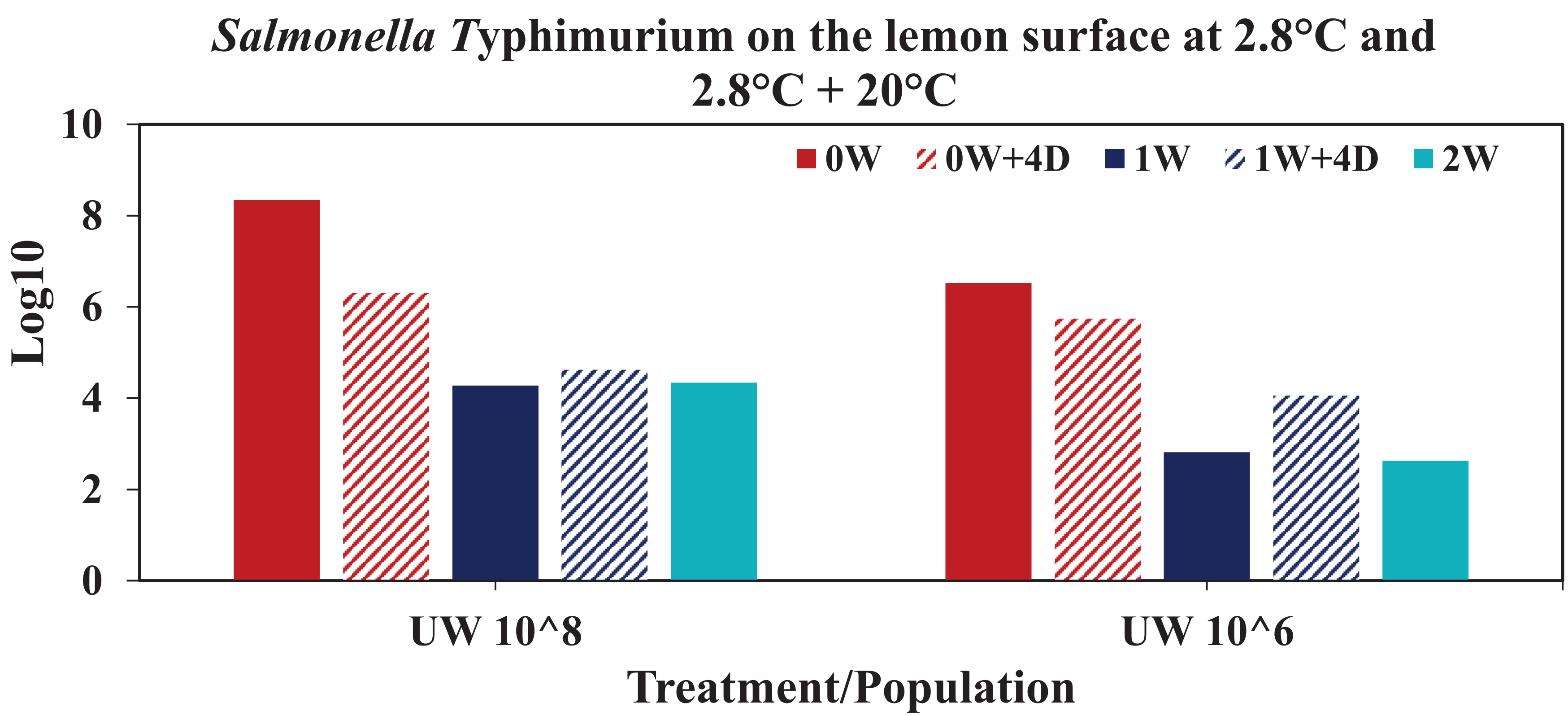
Survival and persistence of bacterial pathogens- Results



APPENDIX IV

Best Practice Guidance for Managing Microbial Food Safety Risks in the Citrus Industry

Sukhvinder Pal (SP) Singh, PhD

Senior Research Scientist

First edition 2023

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Microbial food safety risks to consumers and industry

In 2021-2022, the Australian citrus industry produced 760,000 tonnes of fruit valued at $910 millions and almost a third of it was exported to major Asian markets valued at $451.2 millions (Anonymous, 2023). The success of citrus export is built on the free trade agreements with importing countries and a clean, green, and safe reputation of the industry. However, the industry needs to be proactive in maintaining the confidence of consumers, regulators and trading partners in the quality and safety of fruit to ensure the market access is retained, and new markets are created.

To remain competitive in the export markets it is imperative that the Australian citrus industry has the capacity to consistently supply safe fruit, with the aim of zero product recalls.

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Microbial food safety risks to consumers and industry

Due to the inedible peel, citrus fruit poses a relatively lower microbial food safety risk to consumers. However, foodborne pathogens present on the surface of fresh citrus can be transferred to the edible portion of the fruit or juice or to the hands of the consumer during peeling/cutting.

Secondly, detection of pathogens (e.g. *Listeria monocytogenes*) on the citrus fruit surface by regulatory authorities could lead to product recalls and reputational losses for the industry and could be a serious 'TRADE RISK' in export markets.

Like any other food product, citrus fruit could be a carrier of foodborne pathogens capable of causing consumer illness and market failure.

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Microbial food safety risks to consumers and industry

A historical perspective

In 1999 a Salmonella outbreak affecting 507 consumers in South Australia was linked to the unpasteurised orange juice. Based on the investigation reports, the Federal Court ruled that citrus growers who supplied the fruit to the processor had breached their contracts to supply 'safe' fruit, and the liability for the loss and damage was found to rest with the growers (as cited in Rajapakse 2016). *Salmonella* Typhimurium (phage type 135a) strain involved in the outbreak was traced back to the fruit in the packing shed and in particular the fungicide and wax solutions which were applied to fruit.

Prevention of this outbreak was possible with certain measures including the use of a sanitiser in wash and fungicide tanks as well as a regular change of the water in the fungicide tank (Rajapakse, 2016). This historical example refreshes the microbial food safety risks posed by fresh produce including citrus. Similar examples of salmonellosis outbreaks linked to citrus juice products have been reported from other countries (Parish 1998).

It is not only an ethical or moral responsibility but a legal obligation to supply "safe" fruit which is a food product.

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Microbial Risks

Fungal

Penicillium
Alternaria

Bacterial

Salmonella species
Listeria monocytogenes
Shiga-toxin *E. coli (STEC)*
Campylobacter

Viral

Noroviruses
Hepatitis A

Parasitic

Cryptosporidium
Cyclospora

Bacteria, viruses and parasites can cause illness among humans. These generally originate from animals and humans and enter the fruit supply chain at production and postharvest stages.

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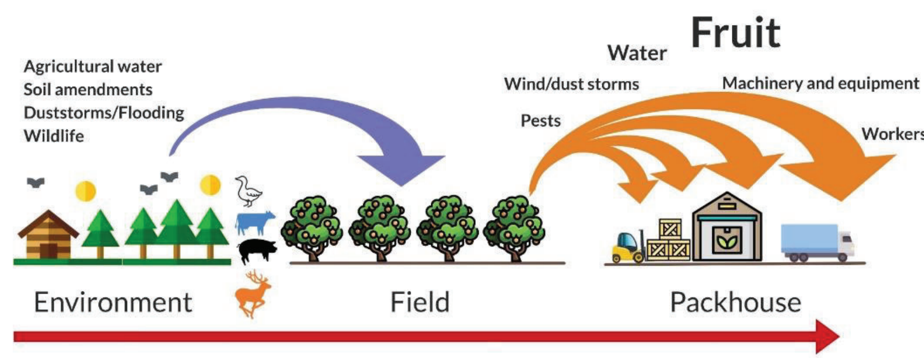
Microbial pathogen continuum

Environmental pathogens such as *Listeria monocytogenes*, *Salmonella and* pathogenic *Escherichia coli* are widely prevalent in the natural conditions (Strawn et al., 2013).

Among these pathogens, *Listeria monocytogenes* has been reported to the most prevalent one in fresh produce production landscapes, particularly in soils and water (Strawn et al., 2013; Weller et al, 2015).

These pathogens are capable of transferring to fruit through various routes as shown in the figure on the right and their transmission is aggravated by extreme weather conditions such as duststorms, flooding and heat waves (Singh, 2023).

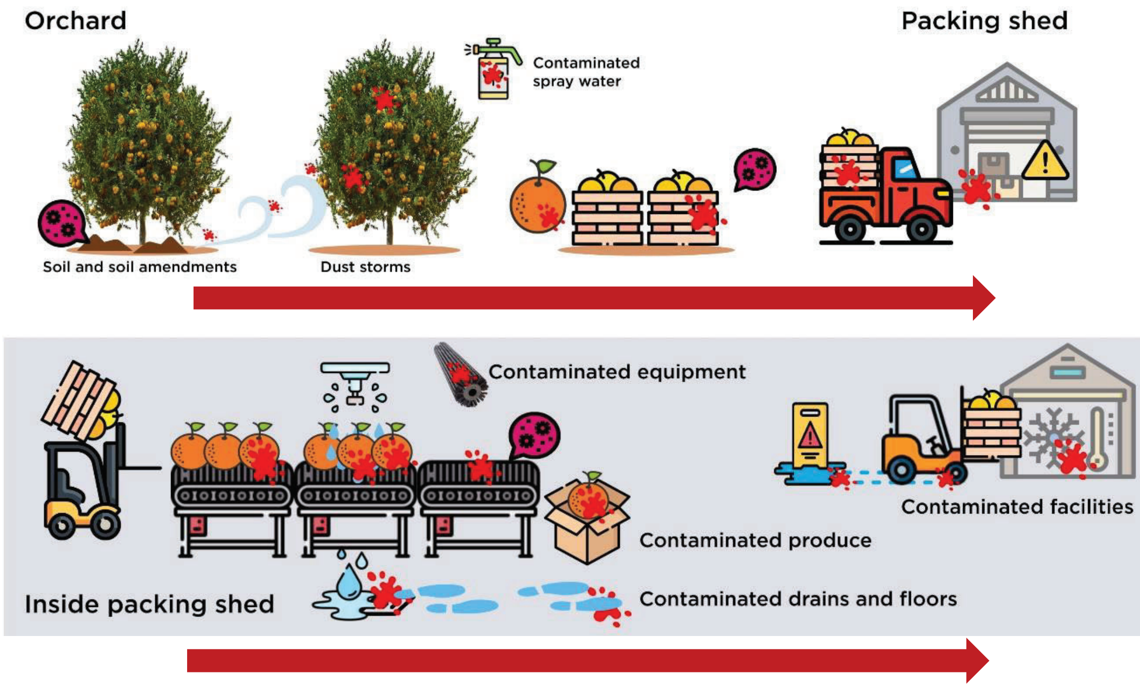


Microbial pathogen continuum

Microbes rule the world. Never underestimate the power of microbes.

Department of Primary Industries

Microbial pathogen continuum



The proposed microbial continuum is based on the citrus industry data, evidence and validated by whole-genome sequencing tools.

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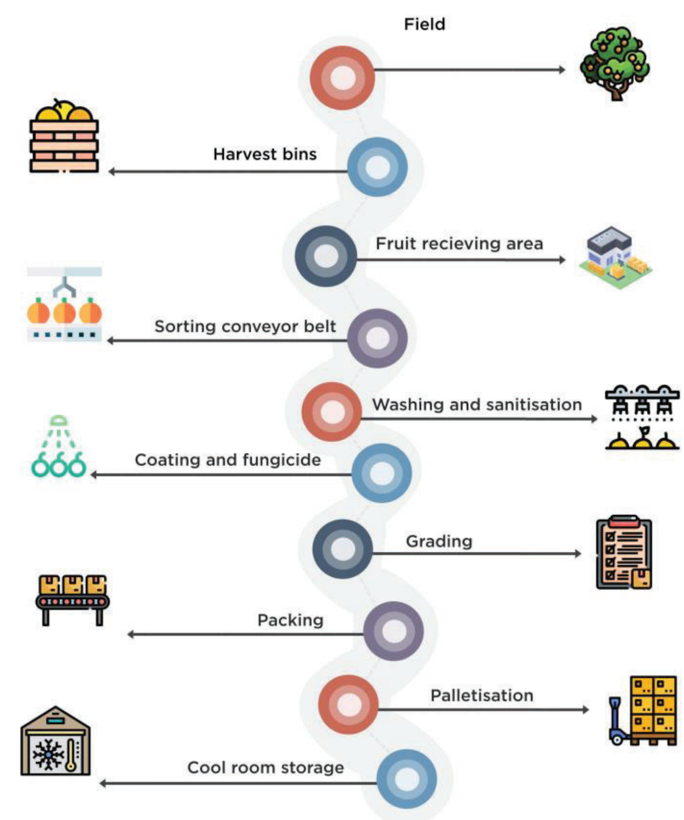
Microbial risk mapping

Our research showed that *Listeria monocytogenes was* the primary environmental pathogen of concern in the supply chain.

Shiga-toxin E. coli (STEC) was ranked second in terms of prevalence that was mainly restricted to soil samples. *Salmonella* was prevalent at very minor levels in environmental samples from packhouse premises.

The microbial risk mapping data was based on 2,257 microbiological samples collected from 51 citrus packing sheds representing over 750 growers across Australia.



Knowing the nature, quantum and location of pathogens is critical to design and apply food safety interventions.

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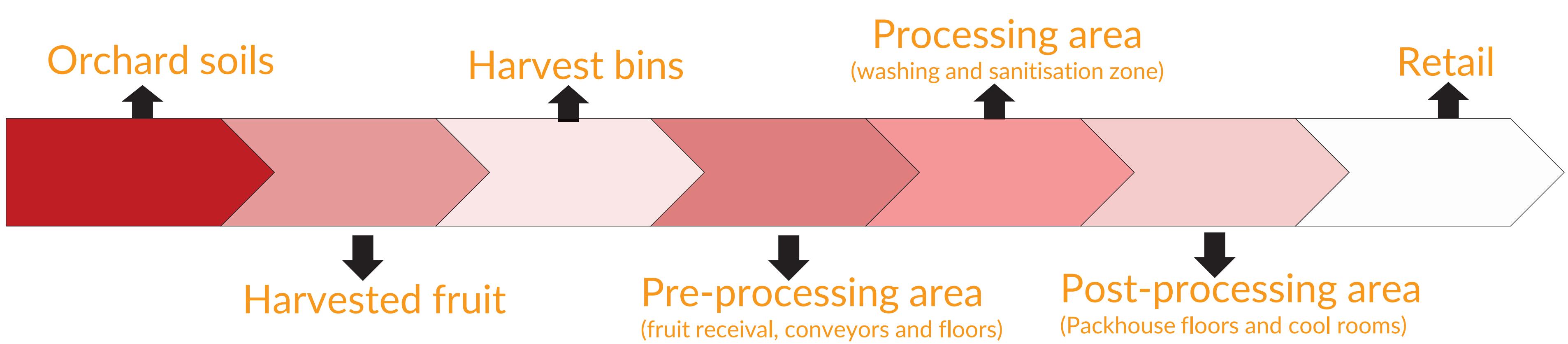
Microbial risk mapping

Prevalence and distribution of *Listeria monocytogenes* in the citrus supply chain



The microbial risk map shows the prevalence of *Listeria monocytogenes* (deeper the red shade, higher the prevalence) along the supply chain from field production to retail .

The darker regions on the microbial risk map require significant attention of citrus growers and packers.

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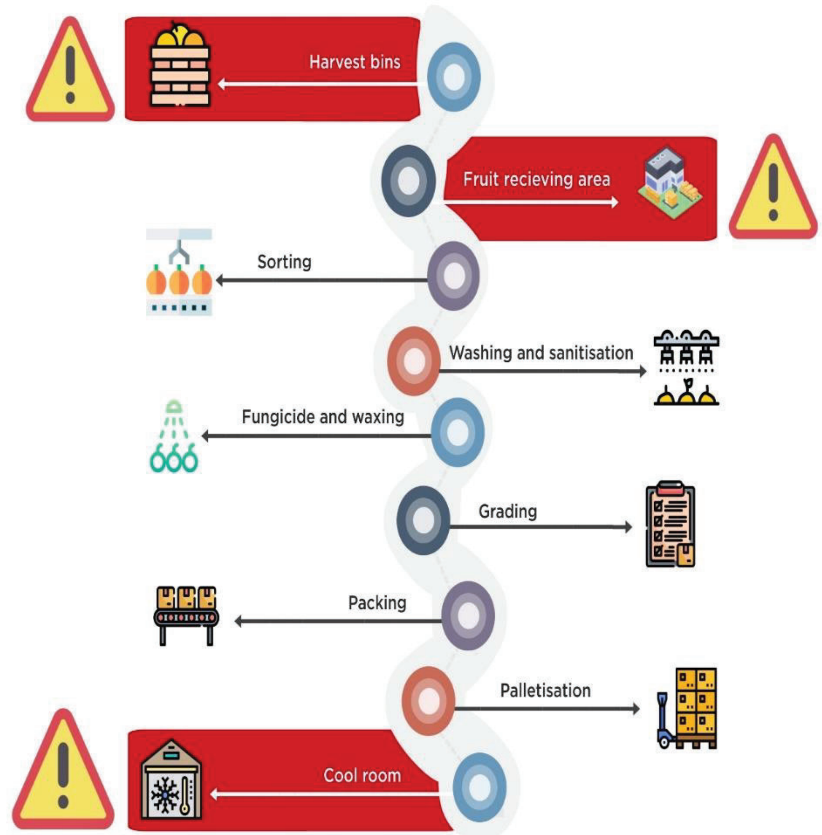
Microbial risk mapping

The following hotspots for microbial contamination and cross-contamination have been identified:

- Harvest bins
- Fruit receival area
- Brush rollers
- Wash water tanks/flumes
- Fungicide and waxing solutions
- Cool rooms



Allocation of cleaning and sanitisation resources to hotspots will provide long-term dividends to citrus packers.

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Industry practice gaps- highlights

- Harvest bins hygiene need improvement
- Neglected cleaning and sanitisation of the fruit receival area
- Recirculation of wash water with inadequate levels of sanitisers
- Determination of fungicide solution change frequency is subjective
- Erratic adoption of automated sanitiser dosing and monitoring in wash water
- Verification of fruit sanitisation process using digital tools
- Low level of knowledge about the interactions among postharvest treatments
- Lack of proper drains in the packing sheds and cool room sanitation
- Inadequate packhouse cleaning and sanitisation protocols
- Environmental monitoring programs need to be developed and implemented consistently.

Find and mind the gaps; it will end the crisis before it begins.

Best Practice Guidance- Objectives

The objectives of this guidance is

- To raise awareness of microbial food safety risks associated with citrus fruit production and supply
- To enable benchmarking food safety practices in citrus production and postharvest handling allowing identification and filling of potential practice gaps
- To understand the nature and magnitude of microbial food safety risks associated with citrus production, harvest and postharvest operations
- To enhance food safety skills and knowledge of citrus growers, packers and other supply chain participants.

Find and mind the gaps; it will end the crisis before it begins.

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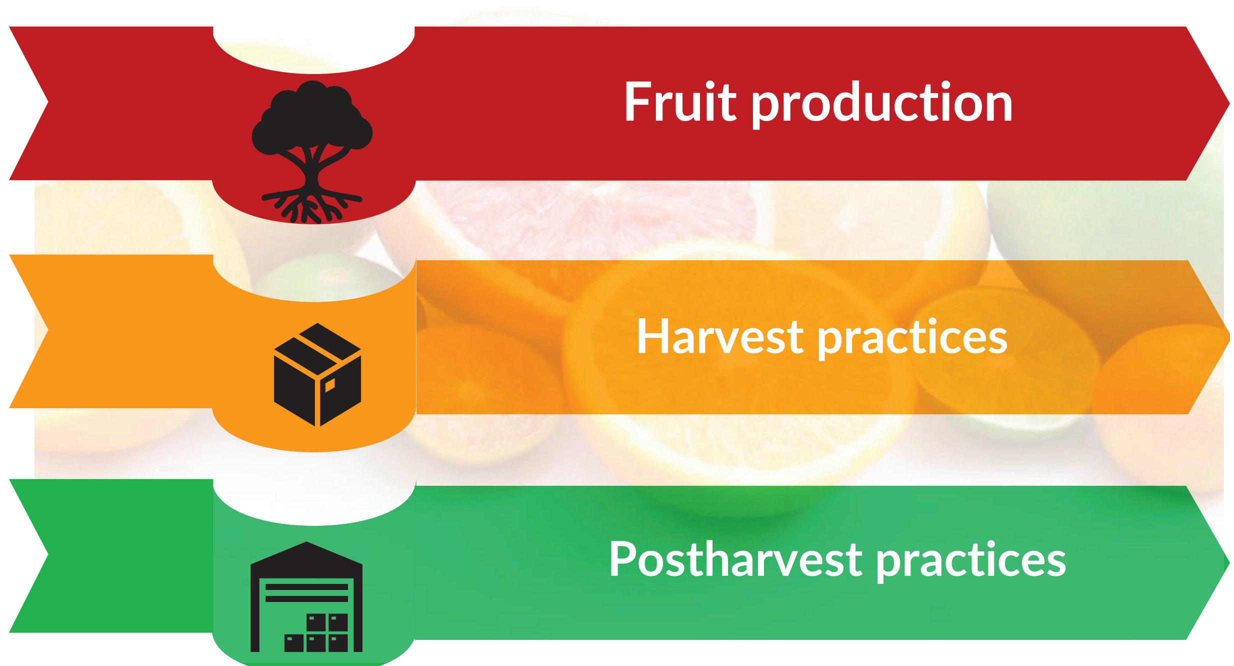
Best Practice Guidance- Scope and contents

The adoption of volunteer food safety schemes in the industry is widespread. As a result, this guidance is a highly targeted technical resource to address critical food safety practice gaps which could be further filled.

The guidance is divided into three parts:

- Fruit production
- Harvest practices
- Postharvest practices



Food safety certificate is based on the snapshot of evidence provided on the day of audit. But food safety is based on the movie of best practice undertaken every day.

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Best Practice Guidance- Literature review

- *Listeria monocytogenes* has been reported in the citrus packing sheds in California (Suslow et al., 2019). The prevalence and distribution patterns found in California were similar to Australian conditions.
- *Listeria monocytogenes* attach to the citrus fruit surface more strongly than *Salmonella* and *E. coli* O157:H7 making it harder to kill during fruit washing and sanitisation process (Martínez-Gonzáles et al., 2011).
- Postharvest washing and sanitisation treatments are known to be the hotspot for cross-contamination of fruit if the sanitisation of wash water, fungicides and waxes is not maintained (Kanetic et al., 2008; Harris 2019; Adaskaveg et al., 2021; Sheng et al., 2023).
- The bacterial pathogens are capable of surviving in waxing solutions for extended periods and also on the fruit surfaces (Sheng et al., 2023).

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Best Practice Guidance- Fruit Production

Listeria monocytogenes is ubiquitously present in soils and is also shed by cattle and sheep suffering from listeriosis infection. It is also found in contaminated irrigation water (Weller et al., 2015). Our research showed that most *Listeria* strains were isolated from orchard soils under tree canopy (30 cm away from tree trunk).

Its persistence and survival varied from orchard to orchard presumably influenced by the following factors:

- Organic soil amendments
- Irrigation water quality
- Leaf litter under tree
- High soil moisture throughout the year
- No exposure to UV under tree

Don’t apply raw/untreated or partially composted animal manures in citrus orchards.

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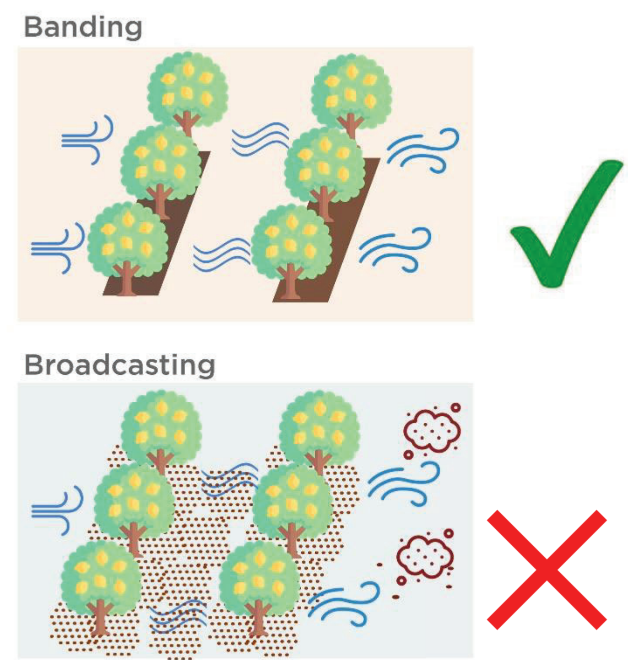
Best Practice Guidance- Fruit Production



The use of organic amendments in the orchard soil health management is common.

Composts should be sourced from suppliers along with a certification of composts' compliance with Australian Standards.

The timing and method of application of organic amendments can play an important role in mitigating microbial food safety risks.

Growers are aware of the presence of microbial contaminants risks in raw animal manures. However, the knowledge of transfer route of microbial pathogens from the environment to fruit is uncommon.

The timing of application compost application should be restricted to after fruit harvest and band application method should be adopted instead of broadcasting.

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Best Practice Guidance- Fruit Production

Drip irrigation is the most common irrigation method across all production regions which minimise the possibility of microbial contamination of fruit through water directly.

The contaminated irrigation water can introduce microbial pathogens in orchard soils and it has been reported that it could be one of the major carriers of Listeria in fresh produce production in the field (Weller et al., 2015).

Monitor microbiological quality of irrigation water. Know the water source and potential upstream contamination.

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Best Practice Guidance- Fruit Production

The use of contaminated water for chemical spray applications, especially before fruit harvest, could lead to microbial contamination of fruit directly. In addition to the microbial risk, the water sources contaminated with blue-green algae (BGA) could contain BGA toxins which could be transferred to the fruit.

The persistence and diffusion of BGA toxins on and into citrus fruit are currently unknown. Be aware of the microbial contamination via chemical spray applications and BGA chemical hazards.

Monitor microbiological quality of chemical spray application, especially 1-4 weeks before fruit harvest.

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Best Practice Guidance- Fruit Production

Wildlife incursion in the citrus orchards is commonly reported by growers along with the awareness of the risks these animals pose to microbial food safety. Growers undertake measures to minimise the wildlife incursion into their farms and water sources.

Fencing and barriers are the most animal-friendly measures to restrict access of wildlife into production fields.

Natural disasters such as floods and bushfires can displace wildlife towards production fields and water sources. Consider these weather events' impact on your production fields.

Assess and manage the microbial food safety risks posed by wildlife in your region.

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Best Practice Guidance- Fruit Production

Photo: Shane Burgess, NSW DPI

Dust storms are powerful vehicle for microbial pathogen dispersal and large-scale contamination of food production and water systems.

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Best Practice Guidance- Fruit Production

Dust-suppression agronomic practices by managing inter-row sod culture is recommended to minimise the transfer of Listeria from soils to fruit.

Windbreaks and orchard netting could also contribute to minimising dust loads on the fruit surface.

Perennial windbreaks can reduce the dust-storm associated microbial contamination to some extent.

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Best Practice Guidance- Fruit Production

Coexistence of citrus orchards and livestock

The livestock operations (cattle feedlots, poultry, piggery and dairy) could be the primary contributor to the microbial contamination of surface water sources and a source of the long-distance dispersal of pathogens with dust storms.

Easy and inexpensive supply of animal byproducts from intensive operations could lead to microbial contamination of fruit production fields and water sources.

Adoption of buffer zones, run-off management, windbreaks and monitoring water sources are practical and effective ways to mitigate microbial risks.

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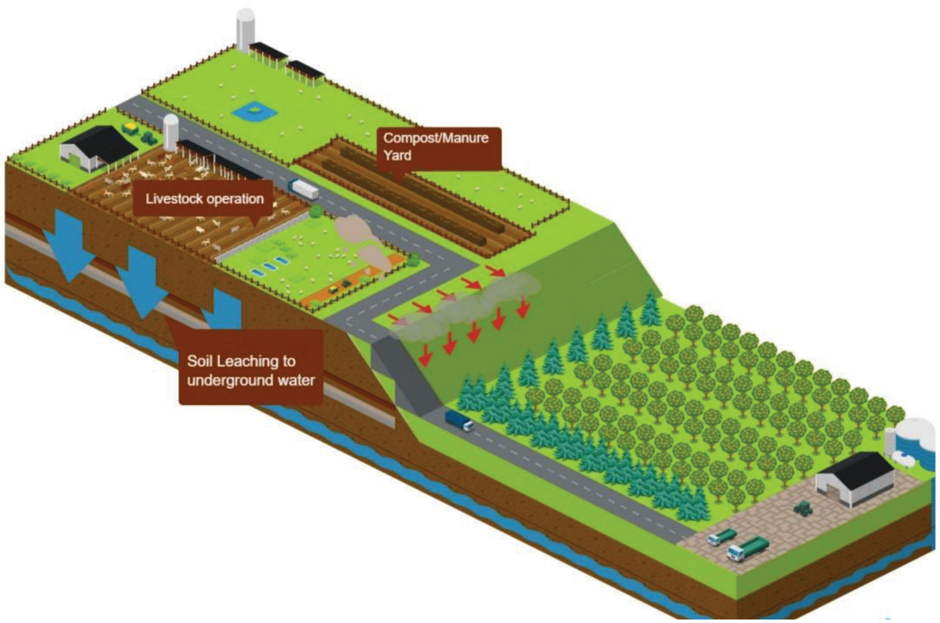
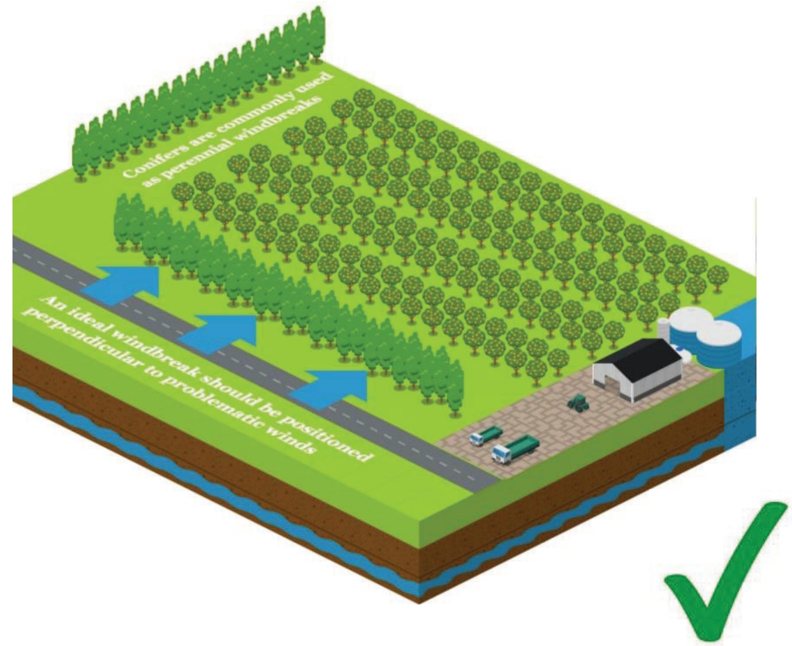
Best Practice Guidance- Fruit Production





Citrus growers should consider microbial risks associated with neighbourhood livestock operations and upstream water contamination sources.

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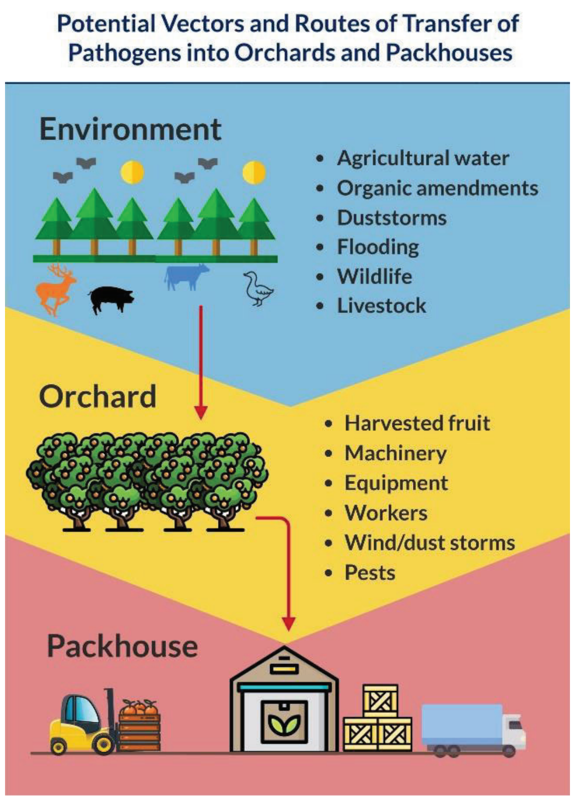
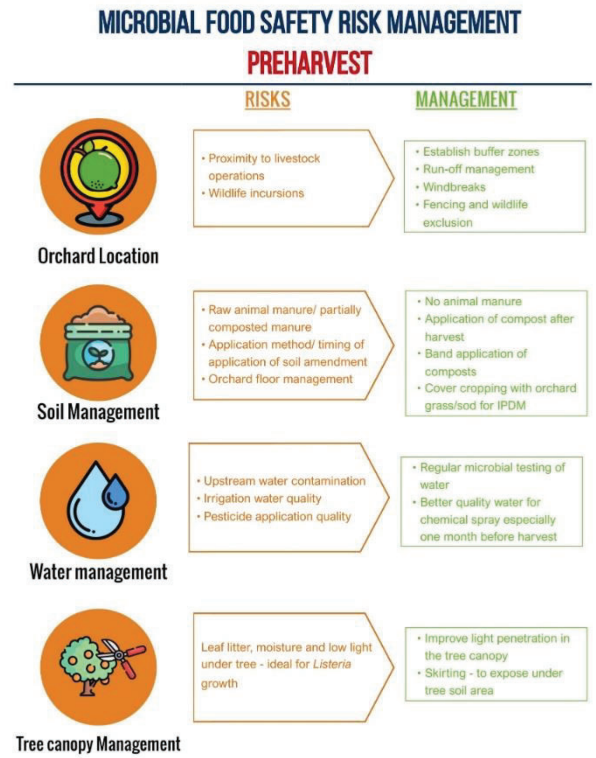
Best Practice Guidance





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Best Practice Guidance- Harvest

The use of plastic harvest bins is a standard industry practice. It was observed that harvest bins were placed on the ground during fruit harvesting operations which often coincide with a significant amount of rainfall and wet conditions. As a result, the harvest bins often get muddy during their contact with ground.

Avoid placing harvest bins on the ground during fruit harvest operations.

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Best Practice Guidance- Harvest

Once the harvest bins lifted from the orchard floor are stacked during fruit transportation to packhouse or for temporary storage of fruit, the soil/mud gets transferred to the inside causing widespread soiling of fruit and harvest bins.

Since the environmental pathogens such as *Listeria monocytogenes* live in soils, it is potentially the main reason for microbial contamination of harvest bins and fruit during harvesting operations. This has been identified as the major food safety practice gap during harvesting operations.

Stacking muddy harvest bins leads to the transfer of soil and organic matter inside the bins, soiling the fruit.

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Best Practice Guidance- Harvest

Placement of harvest bins on the ground attracts soil and debris.

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Best Practice Guidance- Harvest

Trailer mounting is strongly recommended to avoiding ground contact of harvest bins.

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Best Practice Guidance- Harvest

Cleaning and sanitisation are two different steps. Cleaning involves the use of food-grade detergents in removing excessive dirt and extraneous matter from the bins. Sanitisation is the second step to disinfect the bins with a food grade sanitiser.

Some citrus packers have installed and started using automated cleaning and sanitisation systems for harvest bins and adopted this practice.

Clean and sanitise harvest bins after each use. Separate bins should be used for handling fruit before and after processing.

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Best Practice Guidance

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Best Practice Guidance- Postharvest

Postharvest fungicide drenching

Pre-wash drenching is a common practice to minimise fungal rots in the supply chain. The drenching operation uses a large quantity of fungicide in recirculation mode for drenching of citrus fruit in harvest bins. Since this is the first wash/drench step, there is significant organic load in the drench solutions.

Postharvest fungicide drenching was identified as the major risk of cross-contamination of fruit with bacterial pathogens.

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Best Practice Guidance- Postharvest

Postharvest fungicide drenching

The harvest bins which are generally placed on the ground during fruit harvest introduce more organic matter in the drench solution potentially affecting the fungicide efficacy and transferring bacterial pathogens into the drench solution. The fungicide solutions generally don't have bactericidal effects and bacterial pathogens such as *Salmonella* and *Listeria monocytogenes* can survive in most fungicide solutions (Harris, 2019; Adaskaveg et al., 2021).

Postharvest fungicide drenching was identified as the major risk of cross-contamination of fruit with bacterial pathogens.

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Best Practice Guidance- Postharvest

Frequency of fungicide drench solution should be adjusted according to the organic matter load anticipated during each lot of harvest.

To minimise cross-contamination risks due to bacterial pathogens, sanitisers should be used to sanitise fungicide solutions if both were compatible with each other.

Mixing of fungicides and sanitisers is not recommended unless the manufacturers have suggested their compatibility with each other.

Determine the frequency of fungicide solution change based on the organic load accumulated rather than time-bound.

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Best Practice Guidance- Postharvest

A citrus packer in Murray region uses a two-step drenching system in which fruit first receives a sanitiser drench followed by a fungicide drench.

This is the best practice example to mitigate cross-contamination risks during drenching operations, allowing the use of incompatible sanitisers and fungicides.

This ‘two-step’ drenching operation will minimise cross-contamination risks and maximise the efficacy of both sanitisers and fungicides.

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Best Practice Guidance- Postharvest

Cool chain

Following postharvest fungicide drenching, fruit are generally held in cool rooms until further processing. If degreening is required, the fruit are held in degreening rooms maintained at the required temperature, humidity and ethylene conditions.

Occasionally, fruit in harvest bins, before and after drenching, were observed to be stored under ambient conditions (inside and outside the sheds) which is not ideal from the postharvest and food safety perspectives.

Maintaining cool chain after harvest is critical to maximise food safety, storage and transportation potential of fruit with minimum quality and fungal decay.

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Best Practice Guidance- Postharvest

Fruit receival area

The fruit receival area in the packing shed is the gateway for fruit (and environmental pathogens) to enter the processing facility. The harvested fruit comes with significant amount of organic matter and debris from the field.

Bin tip platforms are commonly covered with porous padding material to minimise impact damage to fruit. These porous surfaces provide harbourage sites to pathogens like Listeria.

Clean and sanitise the fruit receival area after each shift so that environmental pathogen transfer continuum is interrupted. Fruit receival area has been identified as the weakest link in the food safety management chain.

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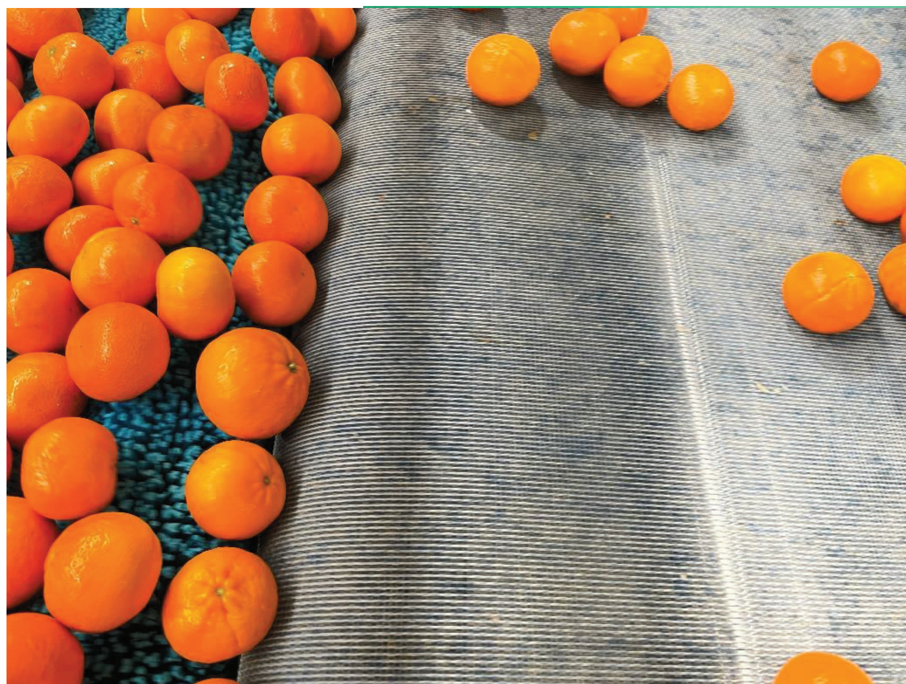
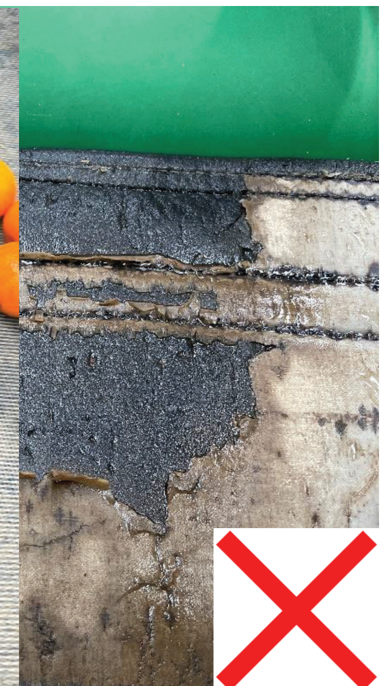
Best Practice Guidance- Postharvest

Fruit receival area

Bin tip platforms are commonly covered with porous padding material to minimise impact damage to fruit. These porous surfaces provide harbourage sites to pathogens like Listeria.





Regular maintenance of packing line equipment should be undertaken to replace damaged surfaces.

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Best Practice Guidance- Postharvest

Fruit receival area

Some packers neglect this area for regular cleaning and sanitisation due to labour, water and chemical costs. As a result, the higher number of Listeria detections were recorded in the fruit receival areas compared to other sampling locations.

As most citrus packing facilities are not designed with proper drainage for regular cleaning, this is a challenging aspect. Regular wet conditions along with high organic load are congenial for pathogen survival and growth.

Clean and sanitise the fruit receival area after each shift so that environmental pathogen transfer continuum is interrupted. Fruit receival area has been identified as the weakest link in the food safety management chain.

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Best Practice Guidance- Postharvest

Fruit dumping

Drying dumping is a common industry practice which is favourable to minimise microbial cross-contamination risks posed by water flumes. The citrus packers who use wet dumping method must use a sanitiser in the dump tank or flumes. However, maintaining an optimum sanitiser concentration in large quantities of water could be a challenge. Water change should be conducted frequently and more frequently during higher organic load.

Dry dumping with careful unloading of fruit onto the conveyor belt is relatively lower risk than wet dumping in large volumes of water or water flumes.

Best Practice Guidance- Postharvest

Fruit dumping

The wet dumping process is believed to reduce the impact damage on fruit during dumping. On the other hand, dry dumping could cause minor injuries and cracks in the fruit during dumping process leading to the potential pathways for internationalisation of bacterial and fungal pathogens during subsequent processing steps, causing higher levels of fruit breakdown in the supply chain.

Maintain optimum levels of sanitisers in dump tanks and water flumes to prevent cross-contamination.

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Best Practice Guidance- Postharvest

Fruit dumping

If harvest bins are placed on ground in orchards, these are highly likely to transfer significant loads of soil and organic matter.

The packers who use wet dumping method involving immersion of harvest bins in the water tank will increase the risk of cross-contamination many folds.

Trailer-mounting of harvest bins are a must if these are immersed in the water tank during fruit dumping.

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Best Practice Guidance- Postharvest

Pre-wash sorting

Pre-wash sorting is not a common practice. Damaged and rotten fruit also go through washing and sanitisation process leading to increased microbial burden on the packing system and wastage of resources used in their processing.

The lack of provision for sorting workers in packing lines design and layout is common.

Eliminating/sorting out rotten and damaged fruit before washing and sanitisation process is a cost-effective strategy to minimise mould spore load in the fungicide tanks/packing shed and improve the food safety outcomes.

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Best Practice Guidance- Postharvest

Postharvest water

Postharvest water is an important element in microbial food safety of citrus fruit.

The citrus packers use a variety of postharvest water sources. Based on the practice data, it is estimated that ~51% citrus packers use surface water (river, creek, channel, dam, rainwater) for postharvest operations while the use of town water and ground water is 41% and 10%, respectively.

Drinking quality water is recommended for postharvest applications in citrus packing sheds.

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Best Practice Guidance- Postharvest

Postharvest water

Surface water from various sources (rivers, creeks, channels, dam and rainwater harvesting) is the most common for citrus packers located at remote locations. The microbiological quality of surface water changes dramatically depending upon the season and weather events.

More than half of citrus packers who use surface water have installed water filtration and treatment systems to improve water quality for postharvest usage. On the other hand, town water, which is considered the safest, is widely used in the packing operations (41%) located in larger towns.

Adoption of water filtration and treatment systems is recommended in the packing operations which are dependent upon surface water sources.

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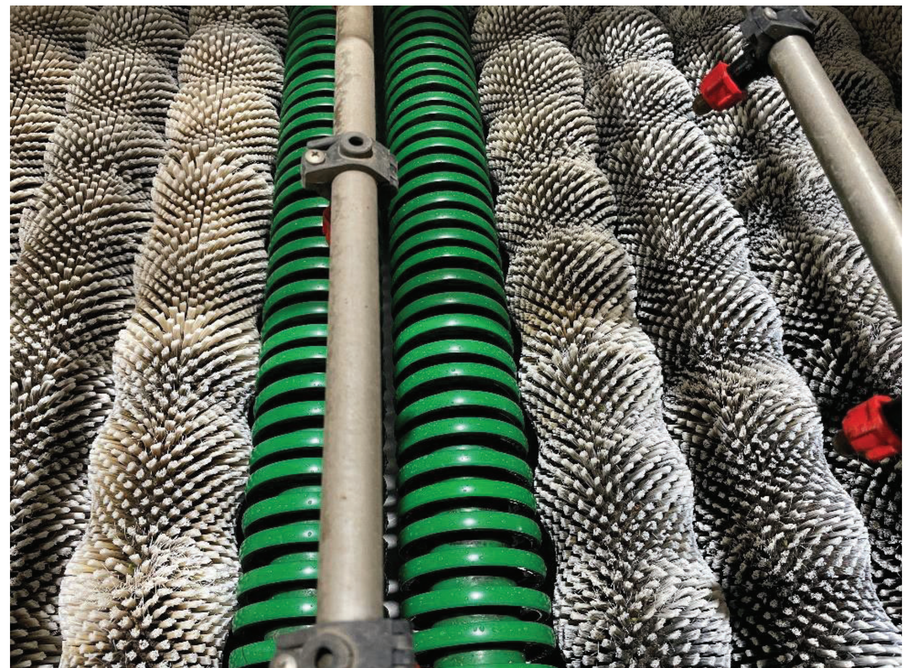
Best Practice Guidance- Postharvest

Brushing and washing

Flatbed brushes are most commonly used along with spray bar washing. Ideally, all brush rollers should be under the sanitised wash water for self-cleaning purposes. The hygiene of brushes is important to minimise cross-contamination risks. *Listeria monocytogenes* has been detected in several samples collected from brushes in the washing areas. Once brushes get contaminated with bacterial pathogens, these are very difficult to clean and sanitise. The complete replacement of contaminated brush rollers is the practical solution.



All brush rollers should remain covered under the sanitised wash water along with daily cleaning and sanitisation schedule at the end of the shift to remove organic matter and debris from the core and sides of the rollers.

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Best Practice Guidance- Postharvest

Pre-wash rinsing step is recommended to minimise microbial load on recirculatory wash water systems.

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Best Practice Guidance- Postharvest

Postharvest wash water sanitisers

Recirculation of wash water is a common practice.

Most postharvest operations recirculate wash water without a filtration step, but the use of sanitisers is common.

Maintaining optimum concentrations of sanitiser and frequent water change are recommended to minimise cross-contamination risks.

Treat the wash water to drinking quality before it is used/re-used for fruit washing to mitigate microbial contamination risks to the lowest level. Alternately, single use wash water (run-to-waste) system is recommended, but it may not be an environmentally sustainable option.

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Best Practice Guidance- Postharvest

Postharvest wash water sanitisers

Peroxyacetic acid (PAA) is the most popular sanitiser in the industry followed by calcium hypochlorite, chlorine dioxide and others such as sodium hypochlorite, chloro-bromo dimethylhydantoin, and chlorocyanurates.

Most citrus packers using PAA, chlorine dioxide and chloro-bromo dimethylhydantoin as sanitisers use automated dosing systems for injection of the sanitiser into wash water. However, many of sodium/calcium hypochlorite and chlorocyanurates users manually add sanitisers in wash water tanks.

Department of Primary Industries

Best Practice Guidance- Postharvest

Postharvest wash water sanitisers

Each sanitiser has its own merits and demerits due to its highly oxidative nature. It is recommended that the industry should be avoiding the use of chlorine-based sanitisers due to their negative impact on the environment.

The selection of PAA in postharvest wash water is recommended due to its efficacy, low environmental impact, and its action is least influenced by the presence of organic matter.

Other alternative options such as electrolysed water and ozone should be explored to meet sustainability requirements with minimal environmental impact.

Department of Primary Industries

Best Practice Guidance- Postharvest

Postharvest wash water sanitisers

Automation of sanitiser dosing and its digital monitoring and logging are recommended to eliminate the human errors. Furthermore, verification of sanitiser concentration should be conducted at regular intervals using digital measurement tools.

The use of test strips in measuring sanitiser concentrations and pH should be avoided.

Automated sanitiser dosing and monitoring systems are highly recommended to remove human errors.

Department of Primary Industries

Best Practice Guidance- Postharvest

Most operations did not achieve the target sanitiser concentrations and were dependent upon the test strips results for verification.

Achieving recommended levels of sanitiser concentrations is critical to miminise microbial food safety risks associated with the postharvest wash water.

Automated sanitiser dosing and monitoring systems are highly recommended to remove human errors.

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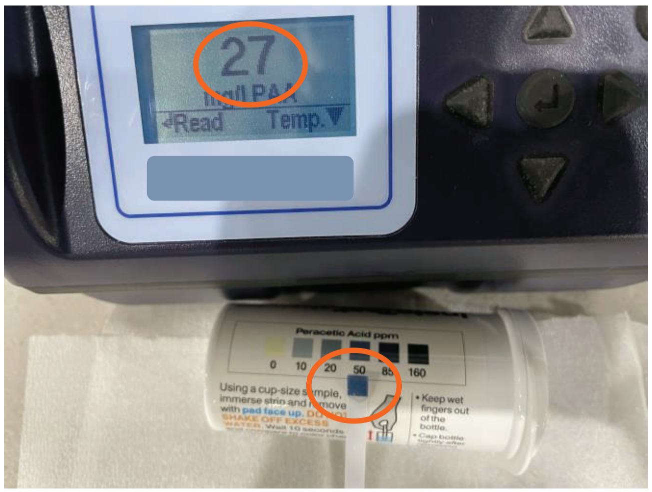
Best Practice Guidance- Postharvest



Peracetic acid

Target conc.: 80ppm
Actual conc: 50 ppm (test strip)
27 ppm (digital meter)

Iodine

Target conc.: 2 ppm
Actual conc: 2 ppm (test strip)
Oxidation reduction potential (ORP) = 620 mV (sub-optimal)

Chlorine-bromine

Target ORP: 500 mV
Actual ORP: 365 mV (sub-optimal)

Achieving target concentrations of sanitisers is critical to the sanitisation process. Sub-optimal sanitiser concentrations and a lack of digital verification of these concentrations are key concerns.

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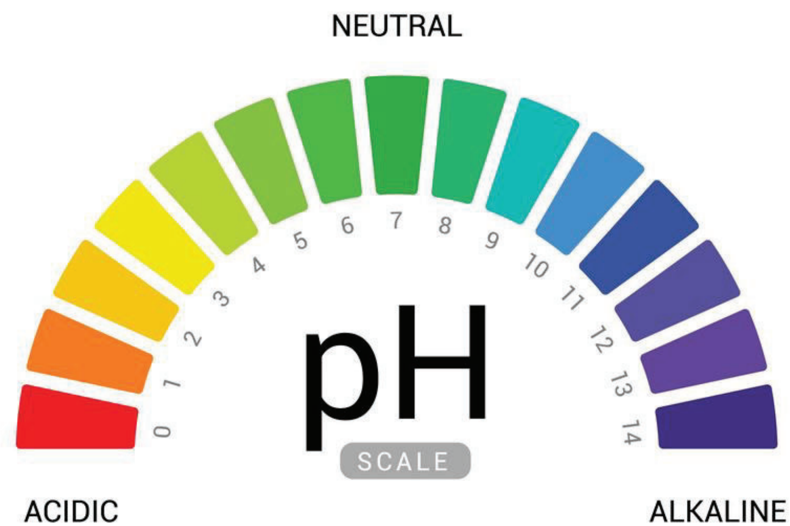
Best Practice Guidance- Postharvest

Postharvest chemical interactions

Understanding basic facts about the chemistry of postharvest sanitisers and chemicals is critical to successfully using them without compromising efficacies. Some citrus packers use two types of sanitisers in the postharvest washing line in a sequential manner. For example, the fruit are first washed with water containing chlorine (effective at neutral/slightly acidic pH ≤7.0) followed by washing with water containing PAA (effective at low pH (≤3.5).

The higher pH of chlorine in residual wash water on the fruit surface could potentially impact the efficacy of PAA by increasing pH of PAA solution as the time progresses during processing.



Consider the 'pH' factor in adopting postharvest chemical treatments such as sanitisers, fungicides and other compounds such as sodium bicarbonates.

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Best Practice Guidance- Postharvest

Postharvest chemical interactions

Some citrus packers treat fruit with sodium bicarbonate solution (1-2%; pH 8.5 to 9.0) before fruit washing for postharvest disease control and argue its role in wound repair. If sodium bicarbonate is to be applied before fruit washing, a rinsing step should be added between bicarbonate and sanitised wash water treatments, especially if the PAA is being used.

Similarly, the impact of residual wash water pH on the subsequent fungicide treatment should be considered. The use of air blast/knives in removing excess moisture from the fruit surface during such conflicting pH scenarios may also be adopted.

Department of Primary Industries

Best Practice Guidance- Postharvest

Postharvest fungicides

Fungicides are commonly used for pre-washing drenching, post-washing treatment and waxing.

Approximately 25% citrus packers use high-volume drenching system for fungicide applications, ~60% use fungicide spray application and ~15% employ a combination of both methods. Two-third of packers also use a fungicide in wax.

Cold fungicide application is common, but one-third packers use a hot fungicide treatment.

Sanitisation of high-volume drench system could be a challenge. Frequency of fungicide change is a critical step.

Department of Primary Industries

Best Practice Guidance- Postharvest

Postharvest fungicides

Imazalil was the most commonly used fungicide, followed by thiabendazole, guazatine, fludoxionil and others.

Almost a half of citrus packers use two postharvest fungicides, ~19% packers use three fungicides and ~10% packers use four fungicide options.

The use of a sanitiser for sanitisation of fungicide solutions is uncommon. This could largely be attributed to the uncertainty about the compatibility of these products.

Fungicide solutions are meant for killing/inactivating moulds and generally don't have bactericidal effects.

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Best Practice Guidance- Postharvest

Postharvest fungicides

From a food safety perspective, recirculation of fungicide solutions is a cross-contamination opportunity for bacterial pathogens which can survive in fungicide tanks. Fungicide solutions have to be effectively sanitised using sanitisers.

Sanitisers for recirculating fungicide systems need to be compatible with fungicides; not phytotoxic to the fruit; odour-free and non-irritant to workers; fast-acting and kill human pathogens within a short time period of ideally less than 30 s (although a 4–5 min dwell time may be acceptable); should cause a > 5-log-reduction in colony forming units (cfu)/ml (Adaskaveg et al., 2021).

Listeria monocytogenes and *Salmonella* could survive in most fungicide solutions currently registered for postharvest applications in the citrus industry.

Best Practice Guidance- Postharvest

Fungicides and sanitisers compatibility

Unfortunately, some fungicides are incompatible with sanitisers. The active ingredient breaks down, and subsequently, performance of the fungicide is lost. The manufacturers recommendations should therefore be followed while mixing different products.

Imazalil, thiabendazole, fludioxonil, azoxystrobin, and pyrimethanil are compatible with PAA. Thiabendazole, fludioxonil, and azoxystrobin are compatible with sodium hypochlorite while imazalil and pyrimethanil are incompatible with sodium hypochlorite (Adaskaveg et al., 2021).

Frequently clean and sanitise fungicide tanks to remove bacterial contamination and compatibility of sanitisers and fungicides should be confirmed before mixing them in fungicide tanks.

Department of Primary Industries

Best Practice Guidance- Postharvest

Postharvest waxing

Carnauba is the most popular waxing material followed by shellac and composite waxes. The use of shellac and composite waxes is common in oranges and lemons while carnauba is mainly used on mandarins. The low-volume applicators are used for waxing the fruit, followed by drying step.

Majority of the citrus packers use wax containing imazalil fungicide which is intended to provide residual protection against fungal pathogens in the supply chain. Waxing on the fruit surface creates a moisture-rich microlayer between the fruit surface and waxy top layer. The moisture-rich layer would be conducive to the survival of foodborne bacterial pathogens on the fruit surface.

Bacterial pathogens are capable of surviving in wax solutions (Sheng et al., 2023).

Department of Primary Industries

Best Practice Guidance- Postharvest

Postharvest waxing

The application of waxes should also be undertaken according to the rates recommended for various types of fruit.

Some citrus packers either use higher rates of wax application or they don’t not properly clean and sanitise the post-waxing equipment, leading to excessive deposits of waxes on the conveyor belts and rollers.

The bacterial pathogens have been detected in waxy deposits, suggesting it could be another hotspot for cross-contamination of washed, sanitised and waxed fruit ready for shipping.

The use of overhead brushes for finishing the waxed fruit should be avoided or these should be regularly cleaned with food-grade detergents and sanitised.

Department of Primary Industries

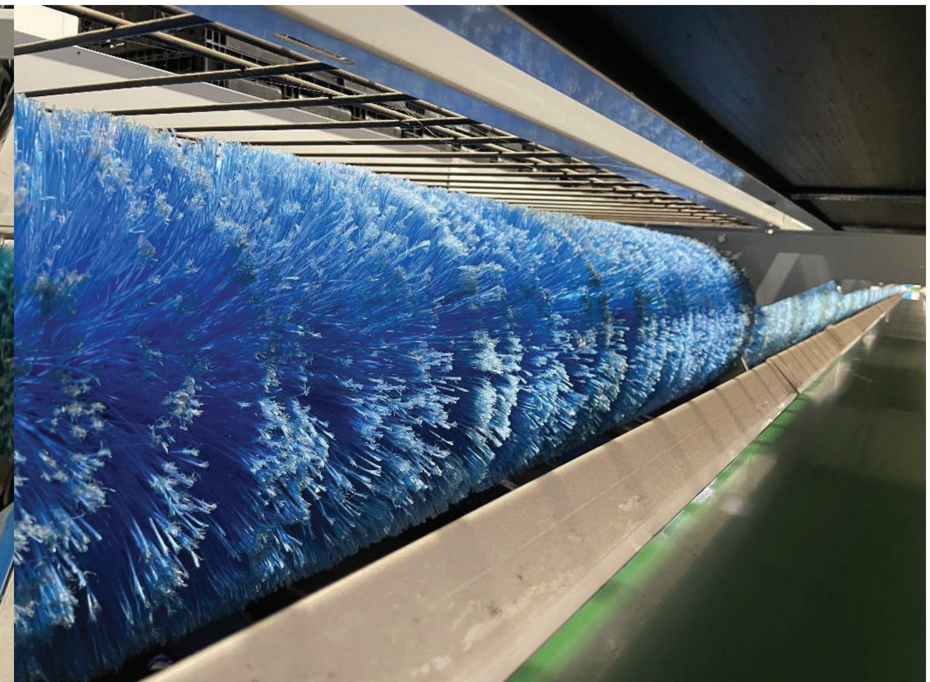
Best Practice Guidance- Postharvest

Post-waxing brush rollers should be regularly cleaned with food-grade detergents to avoid deposition of excessive waxes. Pathogens can survive in these waxy deposits.

Best Practice Guidance- Postharvest

Postharvest waxing

Some citrus packers use overhead brushes for giving a finishing touch to the waxed fruit. These finishing brushes could get contaminated with *Listeria monocytogenes* leading to cross-contamination of fruit.



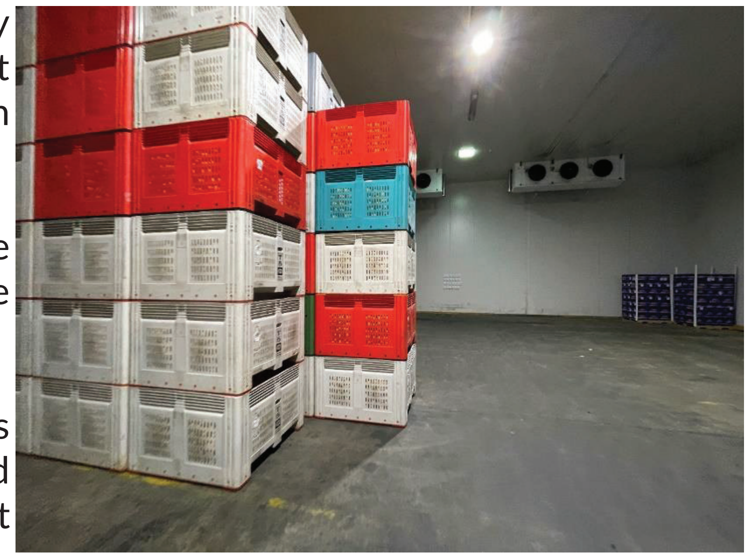
The use of overhead brushes for finishing touch to waxed fruit should be avoided. Alternatively, clean these brushes with food-grade detergents and sanitise regularly to avoid the risk of cross-contamination.

Department of Primary Industries

Best Practice Guidance- Postharvest

Cool rooms

The processed fruit is held in the cool rooms which are generally maintained at or below 5°C. The cooler conditions are not congenial for bacterial growth but *Listeria monocytogenes* can survive and grow under cooler conditions.



Only 65% citrus packers maintained their cool rooms in acceptable conditions while the rest were below average due to the presence of organic matter, rotten fruit, and dirty floors and walls.

Listeria monocytogenes has been regularly detected in samples collected from cool room floors and walls. The workers and forklifts movements can spread the pathogens from fruit receival/processing areas to cool rooms and vice-versa.

Listeria monocytogenes can grow and multiply under cool conditions.

Department of Primary Industries

Best Practice Guidance- Postharvest

Packhouse hygiene

Develop and implement postharvest environmental monitoring and management programs (EMPs).

Uplift the hygiene of packhouse through proper cleaning and sanitisation tools and processes. EMP's benefits outweigh its costs.

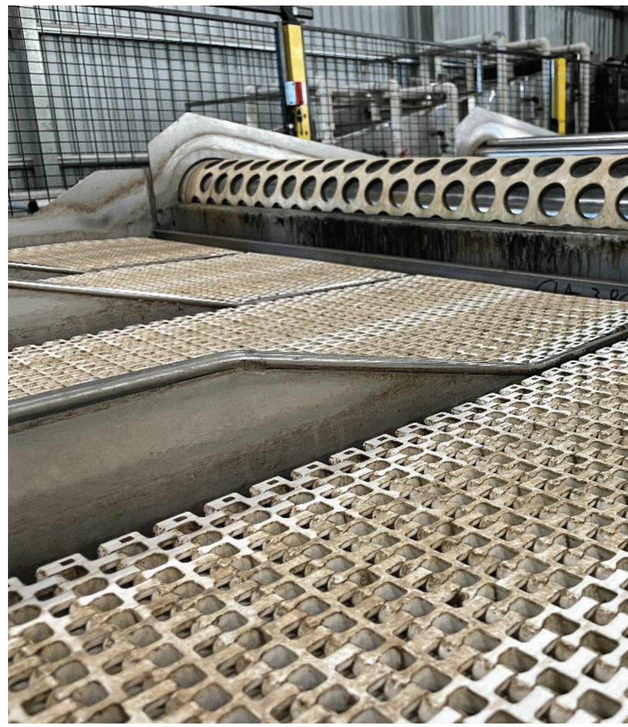
Department of Primary Industries

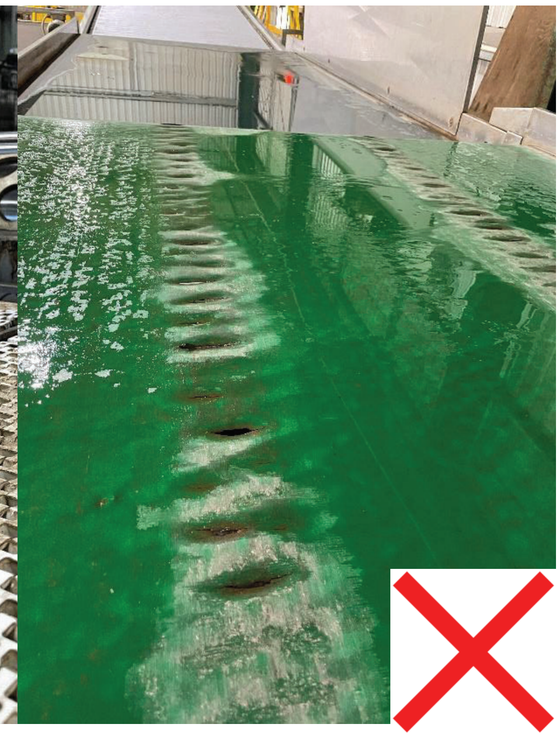
Best Practice Guidance- Postharvest

Packhouse hygiene

Packhouse equipment design and material should be easy to clean and sanitise.

The presence of crevices and cracks and netting and porous material attract and provide harbourage sites to environmental pathogens such as Listeria.





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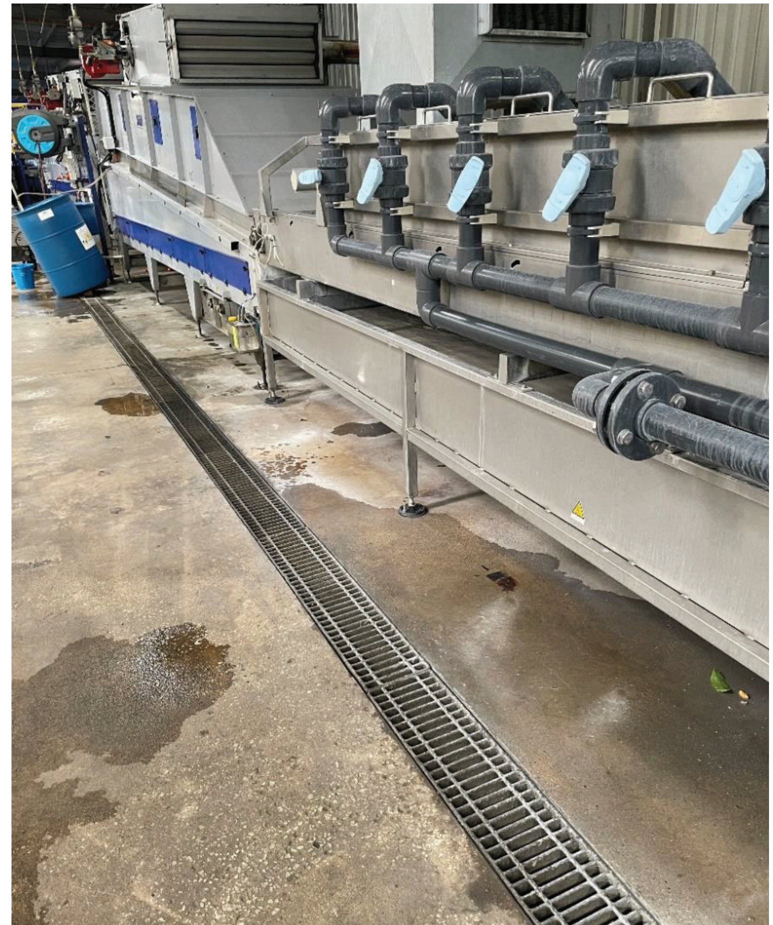
Best Practice Guidance- Postharvest

Packhouse hygiene

Drainage is an essential component of packhouse design. Older packhouse facilities don't have proper provisions for drainage. It limits the ability to clean and sanitise packhouse equipment and floors allowing rapid run-off and disposal of wastewater. The constant wet conditions in packhouses provide an ideal environment for pathogen growth and survival.

Effective cleaning and sanitisation of drains is equally important to avoid the spreading of pathogens from drains to other areas.



Proper drainage is critical to packhouse hygiene.

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Best Practice Guidance- Postharvest

Packhouse Environmental Management Program (PEMP)

1 Packhouse layout and design

2 Postharvest processing flow

3 Equipment design

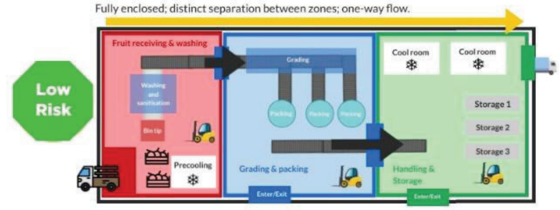
4 Cleaning and sanitisation

5 Environmental monitoring

Packhouse Layout, Design and Processing Flow

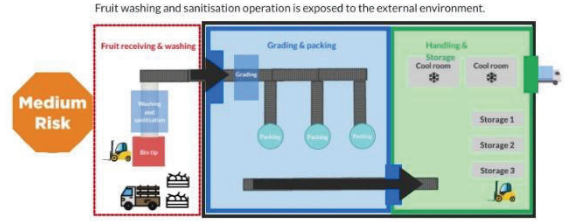
Fully enclosed packhouse

Fully enclosed; distinct separation between zones; one-way flow.



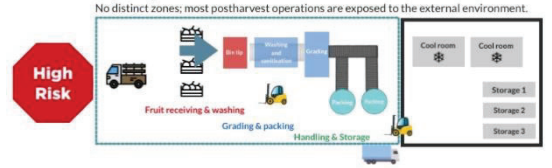
Semi-enclosed packhouse

Fruit washing and sanitisation operation is exposed to the external environment.



Open packhouse

No distinct zones; most postharvest operations are exposed to the external environment.



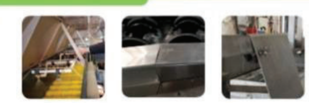
Packhouse Identification Zones

Zone 1: Fruit contact surfaces

- Conveyor belts
- Brushes - flatbed and overhead
- Hollow conveyor rollers
- Grading and sorting cups
- Packing tables
- Curtains in drying tunnels
- Harvest bins

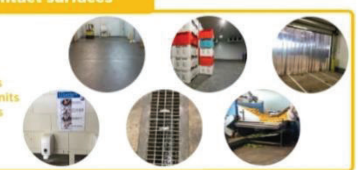
Zone 2: Near-fruit contact surfaces

- Conveyor belts undersides
- Hollow rollers
- Weld seams
- Electrical cords
- Equipment stand/legs
- Metal cracks
- Bolt threads



Zone 3: Non-fruit contact surfaces

- Cracks in flooring
- Improper drains
- Rubber mats
- Plastic curtain strips
- Entrance.exit roll-up doors
- Drip pans under cooling units
- Cool room floors and walls
- Wash water/dump tanks
- Fruit waste areas
- Handwash stations



Zone 4: Outside packhouse area

- Locker rooms
- Cafeteria
- Hallways
- Loading dock
- Maintenance areas
- Offices

Department of Primary Industries

Best Practice Guidance- Postharvest

SEEK & DESTROY the BUGS

Why?

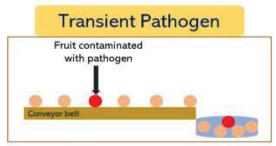
- To prevent transient pathogens from becoming entrenched, forming biofilms and spreading
- To verify the effectiveness of cleaning and sanitation measures
- To determine and undertake corrective action

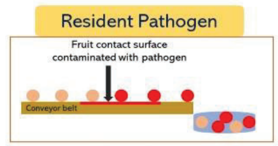
How?

1. **Target pathogens**
   - *Listeria species or L. monocytogenes*
   - *Salmonella* species
   - *Escherichia coli or E.coli*
2. **How frequent?**
   - One size does not fit all
   - Consider processing volumes and packhouse risk history
     - weekly testing for large operations (>20 tonnes/shift); bi-weekly for medium scale (3-20 tonnes/shift); and monthly for small packhouses (up to 3 tonnes/shift)
   - Increase the frequency in the event of a positive detection and weather events (dust storms, flooding)
3. **How many samples and from where?**
   - Determine on the basis of trends & risk history
   - Minimum 5-7 swabs from each zone
   - Minimum 5 processed fruit samples
4. **When to sample?**
   - Immediately after packhouse sanitation and prior to processing
   - During fruit processing, 2-4 hours from the start of the shift
   - After packhouse cleaning, but prior to sanitation
5. **How to sample ?**
   - Train QA staff in aseptic techniques
   - Follow the sampling schedule as per food safety plan
   - Use sponge swabs for sampling larger areas and Q-tip swabs for hard to reach niches
   - Avoid compositing samples when targeting harbourage sites.
   - Accurate labelling and documentation
   - Pack samples with frozen gel-ice packs to maintain 0-4°C during transportation
6. **Microbiological testing**
   - Contact an accredited laboratory service provider to select sampling swabs and testing
   - Test reports to be documented for anlaysing trends and record-keeping
   - Corrective actions to be undertaken in response to a positive detection

Understanding the residency of pathogens

Transient vs Resident Pathogens





Transient isolate is a one off isolate which is not detected after repeated swabbing (a minimum of 3 negative results in a row). This detection indicates that good manufacturing practices including cleaning and sanitisation are being implemented effectively. Pathogens such as Listeria may be introduced occasionally through incoming fruit and it is essential that best practices are used to keep occasional presence under control. Once introduced and not controlled a transient pathogen can take up residence.

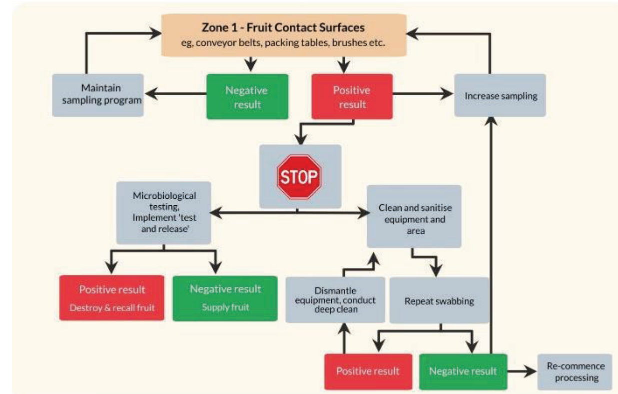
Resident isolate is an isolate that is repeatedly detected. This can indicate a failing implementing best practices in particular cleaning and sanitisation or an undiscovered niche where a pathogen has established a habourage site. There is a high chance that the site is continually re-contaminating the facility, increasing the potential for fruit to be contaminated. Processing should be stopped until repeated negative results can be produced. Aggressive corrective measures need to be implemented to seek out and eliminate resident isolates and assess the factors that allowed the site to be established.

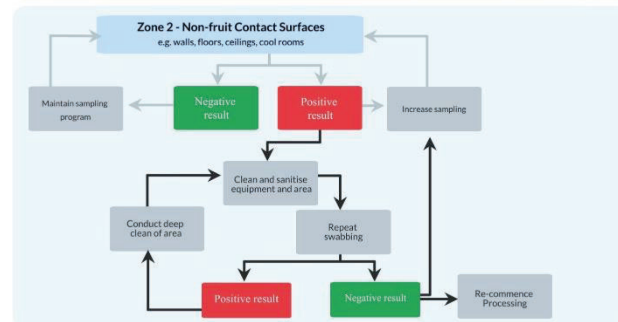
First detection vs. second detection

Occasional isolates can be brought in to the facility through incoming produce, however these must be prevented from continuing through the packhouse and taking up residence along the process. It can be expected that most detected isolates will be transient, from one location and not actually the source location. However, it is critical that this initial detection is not dismissed as a one off but is aggressively followed by testing to ensure it is not a resident isolate. If repeated detection occurs, an intensified response such as dismantling of equipment, deep cleaning and line shut downs are warranted.

It is crucial that after first detection, the seek and destroy methodology is employed to identify niches and prevent habourage sites and future product contamination.

Responding to microbiological test results





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Best Practice Guidance- Postharvest

Packhouse Equipment Design

Examples of bad equipment design

Best Practice

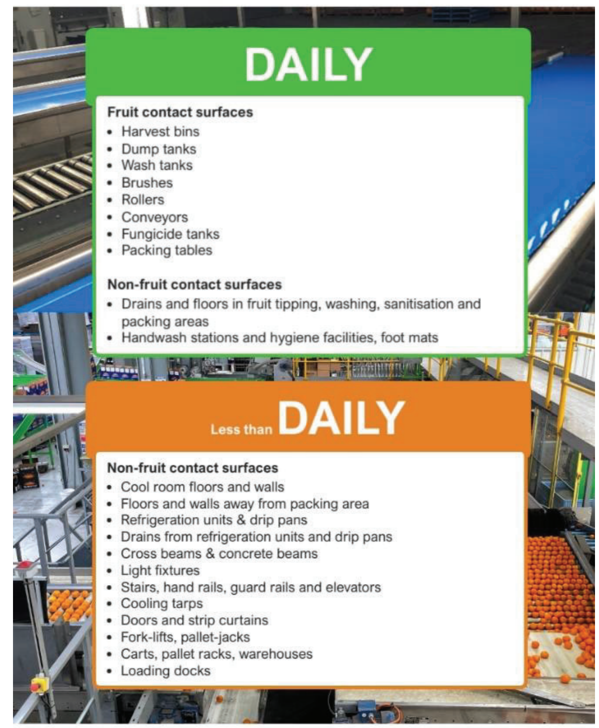
Equipment design should:

- be cleaning and sanitisation friendly
- be easy to disassemble and reassemble.
- be such as to have minimal areas for habouring bacterial growth
- consider sanitary materials and laminations

Equipment should:

- NOT have porous surfaces
- NOT have metal cracks
- NOT have sharp edges
- NOT have seams with metal or plastic lacing
- NOT have hollow rollers

Cleaning and Sanitisation Schedule



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Cleaning and Sanitising

The terms "cleaning" and "sanitising" are often misunderstood and used interchangeably, resulting in the misinterpretation of recommendations - it is important to understand their differences.

Cleaning is the physical removal of soils such as dust, dirt, plant material, food residue or grim from the surface using products such as soap (or detergent) and water. While cleaning removes soils from a surface leaving them visually clean, it doesn't actually kill bacteria. However, by removing soils, pathogen numbers are lowered and subsequent sanitising is more effective.

Cleaning is the crucial first step in the process.
In order to kill and remove pathogens, sanitising will need to be performed after cleaning.

Sanitising lowers the number of bacteria on surfaces or objects to a safe level, as judged by public health standards or requirements.

Sanitisers are only intended to kill bacteria.

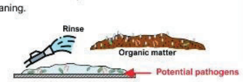
Note: Not all sanitisers are food-grade and approved for use in food contact areas. Always check chemical manufacturers guidelines and instructions for use.

Multi-step process

In the same way that cleaning does not kill pathogens, sanitising do not clean surfaces. It is critical that a surface is cleaned first, removing any soil that could influence the efficacy of a sanitiser. Pathogens hide under soils, essentially being protected from sanitisers.

1 Clean
Pre-rinse target surface with water to loosen and remove soil and debris. Apply detergent and scrub the surface properly. Mechanical force, concentration, time and temperature are four factors influencing cleaning efficacy.

2 Rinse and inspect
Perform a post-rinse step to remove detergent and remaining soil and debris loosened by washing. Rinse equipment and other surfaces from top to bottom. Conduct a visual inspection to assess effectiveness of cleaning.



3 Sanitise
Use approved chemicals sanitisers at recommended concentrations and contact times for effective sanitisation. A proper cleaning step ensures interaction of sanitiser with potential pathogens present on surface.

4 Validate and verify
Validate your cleaning and sanitisation schedule as per the food safety plan. May use adenosine triphosphate (ATP) swab analysis to verify the surface has been effectively cleaned and sanitised.

Note: ATP is a cleaning verification system that counts the number of living cells on a sample you take from a surface. By testing the ATP levels on a surface, you can determine the effectiveness of your cleaning and sanitisation.

Cleaning and sanitising: Things to consider

Dwell time - The 'wet dwell' time varies between products depending on their registration, so it is important to follow recommended times. If products are not given enough time to work, pathogens are left behind and processes efficacy is reduced.

Drying - ensuring surfaces after cleaning and sanitisation are dry to prevent the growth of pathogens e.g. avoiding pooling water.

Avoiding aerosols - avoid creating aerosols through high pressure washing and vigourous scrubbing that could lead to movement of soil and pathogens to clean and sanitised areas.

Effectiveness of cleaning and sanitisation process - validating and verifying your cleaning and sanitisation process with the use of ATP and pathogen microbiological testing.

Portable water - It is important that water is potable (microbiologically fit for human consumption) and is properly monitored regularly for any potential harmful chemicals or microorganisms.

Drainage - should be designed to be easily cleaned and maintained. Drainage should be installed to support effective wet cleaning of processing areas. Floor channels should be open, shallow and easy to clean and connected to drain outside the processing area. Floor drains are potential hot spots *Listeria monocytogenes*.

Surface finish - cleanability of product contact surface relates to roughness or surface finish of product. Rougher surfaces are more difficult to clean. Surfaces should be smooth, continous and free from cracks, crevices, scratches and pits that can retain soil and microorganisms.

Design principle - consider all features of equipment including joints, fasteners, internal angles, bearings, controls, doors and panels.

Ease of dismantling - equipment should be dismantled as far as it is possible or practical to make all surfaces that microorganism could attach accessible for cleaning and sanitisation.

Hazards from cleaning and sanitising

Actions that may result in microbiological contamination
- Cleaning from low-to-high risk areas (i.e. areas more likely to be contaminated to cleaner zones)
- Using contaminated equipment for cleaning such as brushes and clothes
- Using incorrect chemicals or at wrong dilutions
- Movement of dust during sweeping or dry cleaning
- Creating aerosols by pressure spraying, floor scrubbing.

Actions that may result in microbiological survivor
- Not adequately cleaning surfaces prior to sanitising
- Inadequate type, concentration or contact time of sanitiser used.

Actions that may result in microbiological growth
- Ineffective cleaning and disinfection including incorrect frequency
- Failure to remove food debris thoroughly/frequently

Actions that may result in chemical contamination
- Use of wrong chemicals
- Use of chemicals at wrong concentrations
- Failure to rinse properly
- Incorrect storage of chemicals including storage containers used and storage location.

Appointed trained personnel with the responsibility of cleaning and sanitising the packhouse environment.

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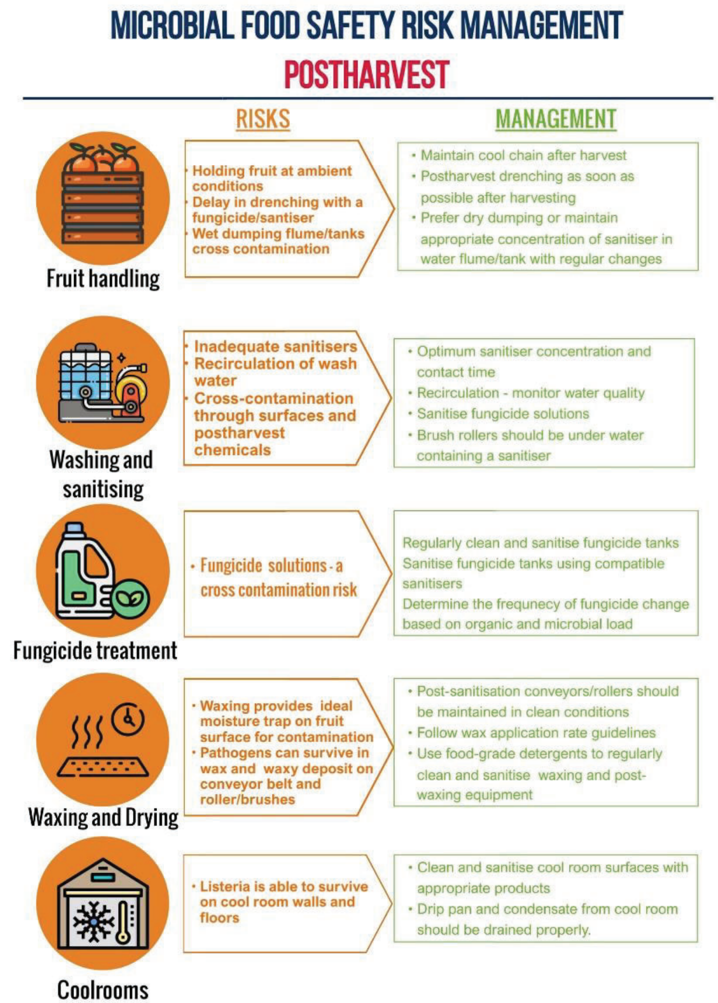
Best Practice Guidance



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Best Practice Guidance: Summary

Best Practice Guidance- References

- Adaskaveg, J.E., H. Förster, D. Chen, and K.A. Nguyen. 2021. Integration of postharvest fungicides and fruit sanitation treatments to optimize decay control and address food safety concerns. In: Spadaro, D., Droby, S., Gullino, M.L. (eds) Postharvest Pathology. Plant Pathology in the 21st Century, vol 11. Springer, Cham.
- Anonymous. 2023. Australian Horticulture Statistics Handbook 2021/22. Horticulture Innovation Australia Limited. https://www.horticulture.com.au/globalassets/hort-innovation/australian-horticulture-statistics-handbook/ahsh-2021-22-fruit-r3.pdf
- Harris, L.J. 2019. Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus. Final project report, Centre for Produce Safety, USA.
- Jung, J., L.M. Friedrich, M.D. Danyluk, and D.W. Schaffner. 2017. Quantification of transfer of Salmonella from citrus fruits to peel, edible portion, and gloved hands during hand peeling. J. Food Prot. 80: 933–939.
- Martínez-Gonzáles, N.E., L. Martínez-Chávez, C. Martínez-Cárdenas, and A. Castillo. 2011. Comparison of different washing treatments for reducing pathogens on orange surfaces and for preventing the transfer of bacterial pathogens to fresh-squeezed orange juice. J Food Prot. 74(10):1684-1691.
- Parish, M. E. 1998. Coliforms, Escherichia coli, and Salmonella serovars associated with a citrus processing facility implicated in a salmonellosis outbreak. J. Food Prot. 61:280-284.
- Rajapakse, P.J. 2016. Contamination of food and drinks: product liability in Australia. Deakin Law Review 21:45-70.
- Sheng, L., H. Wang, L.J. Harris, and L. Wang. 2023. Survival of Listeria monocytogenes and Salmonella in citrus storage waxes or on lemons held under common commercial storage conditions. Food Microbiol. 115:104339.
- Singh, S.P. 2023. Flooding adversely affects fresh produce safety. Microbiology Australia 44(4), 185–189.
- Strawn, L.K., E.D. Fortes, E.A. Bihn, K.K. Nightingale, Y.T. Gröhn, R.W. Worobo, M. Wiedmann, and P.W. Bergholz. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Appl Environ Microbiol. 79(2):588-600.
- Suslow, T., A. Sbodio, and J. Pinzon-Avila. 2019. Resolving postharvest harborage sites of Listeria protects Zone 1 surfaces. Final project report, Centre for Produce Safety, USA.
- Weller D., M. Wiedmann, and L.K. Strawn. 2015. Irrigation is significantly associated with an increased prevalence of listeria monocytogenes in produce production environments in New York State. J Food Prot. 78(6):1132-1141.

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Acknowledgements

Thanks to the citrus growers and packers for their contributions to guidance.

Hort Innovation CITRUS FUND

This project has been funded by Hort Innovation using the citrus research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

APPENDIX V

NSW GOVERNMENT | Department of Primary Industries

Citrus Microbial Food Safety Outreach and Extension

NSW GOVERNMENT | Department of Primary Industries

Citrus microbial food safety outreach and extension- Summary

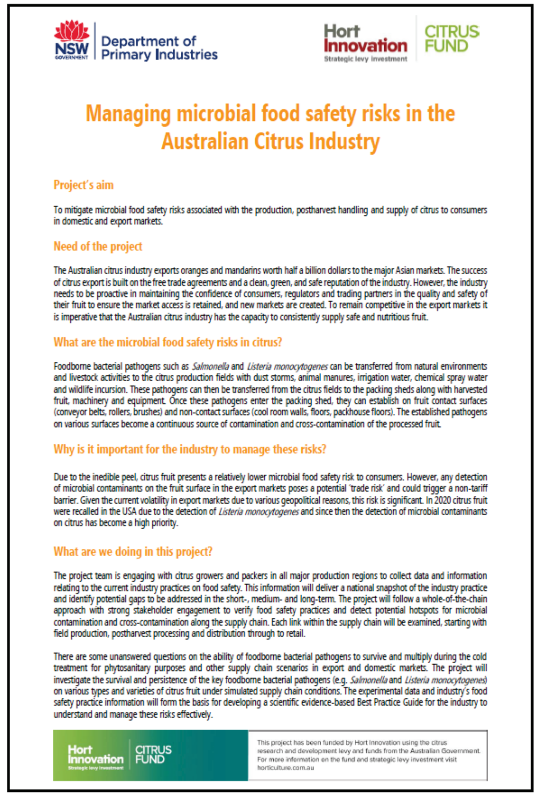
This project was a great success in terms of engagement with citrus growers/packers and other supply chain participants. Due to the export focused nature of the industry, there was a high level of appetite for new knowledge and its integration in the food safety practices. It is conservatively estimated that the project activities encompassing industry practice review, microbial risk mapping and outreach covered 74 citrus packers, representing approximately 900 citrus growers across Australia. 51 Citrus packers were visited on-site to review their fruit production, harvest and postharvest practices along with the collection of microbiological samples. The unprecedented positive response from the growers and packers enabled the success of this project in understanding the microbial hazards present in the supply chain and developing intervention strategies to mitigate the risks. In addition to the one-on-one consultation, technical presentations on citrus microbial food safety management were delivered at important industry events like national and regional forums, scientific conferences in Australia and overseas. The project objectives and outcomes were widely disseminated to the industry through technical articles, workshops, conference exhibitions, industry consultants and extension agents. The food safety helpdesk service addressed approximately 33 technical enquiries from citrus growers, packers and consultants. Overall, the level of success of the extension and outreach objective was much more than the anticipated at project planning stage.

Department of Primary Industries

Citrus microbial food safety outreach and extension

Project brochure

- Stakeholder engagement
- Project need, objectives and outcomes
- Benefits of project participation
- Confidentiality and privacy assurance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Hort Innovation Strategic levy investment | CITRUS FUND

Managing microbial food safety risks in the Australian Citrus Industry

Project's aim

To mitigate microbial food safety risks associated with the production, postharvest handling and supply of citrus to consumers in domestic and export markets.

Need of the project

The Australian citrus industry exports oranges and mandarins worth half a billion dollars to the major Asian markets. The success of citrus export is built on the free trade agreements and a clean, green, and safe reputation of the industry. However, the industry needs to be proactive in maintaining the confidence of consumers, regulators and trading partners in the quality and safety of their fruit to ensure the market access is retained, and new markets are created. To remain competitive in the export markets it is imperative that the Australian citrus industry has the capacity to consistently supply safe and nutritious fruit.

What are the microbial food safety risks in citrus?

Foodborne bacterial pathogens such as *Salmonella* and *Listeria monocytogenes* can be transferred from natural environments and livestock activities to the citrus production fields with dust storms, animal manures, irrigation water, chemical spray water and wildlife incursion. These pathogens can then be transferred from the citrus fields to the packing sheds along with harvested fruit, machinery and equipment. Once these pathogens enter the packing shed, they can establish on fruit contact surfaces (conveyor belts, rollers, brushes) and non-contact surfaces (cool room walls, floors, packhouse floors). The established pathogens on various surfaces become a continuous source of contamination and cross-contamination of the processed fruit.

Why is it important for the industry to manage these risks?

Due to the inedible peel, citrus fruit presents a relatively lower microbial food safety risk to consumers. However, any detection of microbial contaminants on the fruit surface in the export markets poses a potential 'trade risk' and could trigger a non-tariff barrier. Given the current volatility in export markets due to various geopolitical reasons, this risk is significant. In 2020 citrus fruit were recalled in the USA due to the detection of *Listeria monocytogenes* and since then the detection of microbial contaminants on citrus has become a high priority.

What are we doing in this project?

The project team is engaging with citrus growers and packers in all major production regions to collect data and information relating to the current industry practices on food safety. This information will deliver a national snapshot of the industry practice and identify potential gaps to be addressed in the short-, medium- and long-term. The project will follow a whole-of-the-chain approach with strong stakeholder engagement to verify food safety practices and detect potential hotspots for microbial contamination and cross-contamination along the supply chain. Each link within the supply chain will be examined, starting with field production, postharvest processing and distribution through to retail.

There are some unanswered questions on the ability of foodborne bacterial pathogens to survive and multiply during the cold treatment for phytosanitary purposes and other supply chain scenarios in export and domestic markets. The project will investigate the survival and persistence of the key foodborne bacterial pathogens (e.g. *Salmonella* and *Listeria monocytogenes*) on various types and varieties of citrus fruit under simulated supply chain conditions. The experimental data and industry's food safety practice information will form the basis for developing a scientific evidence-based Best Practice Guide for the industry to understand and manage these risks effectively.

Hort Innovation Strategic levy investment | CITRUS FUND

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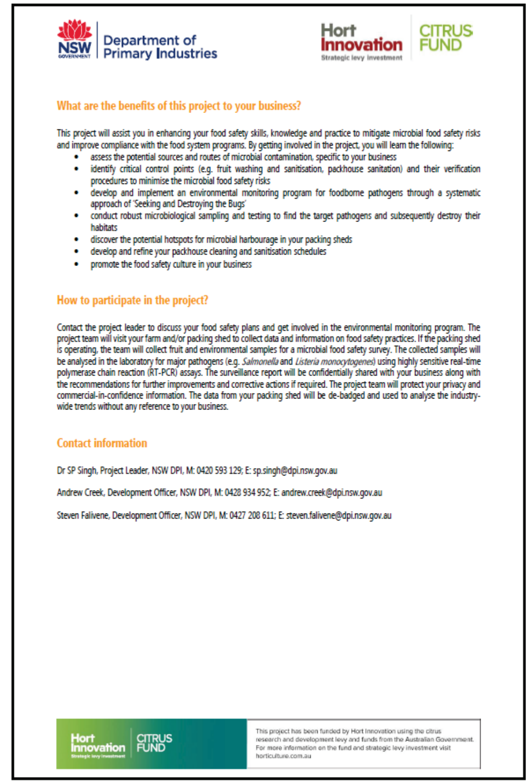
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What are the benefits of this project to your business?

This project will assist you in enhancing your food safety skills, knowledge and practice to mitigate microbial food safety risks and improve compliance with the food system programs. By getting involved in the project, you will learn the following:

- assess the potential sources and routes of microbial contamination, specific to your business
- identify critical control points (e.g. fruit washing and sanitisation, packhouse sanitation) and their verification procedures to minimise the microbial food safety risks
- develop and implement an environmental monitoring program for foodborne pathogens through a systematic approach of 'Seeking and Destroying the Bugs'
- conduct robust microbiological sampling and testing to find the target pathogens and subsequently destroy their habitats
- discover the potential hotspots for microbial harbourage in your packing sheds
- develop and refine your packhouse cleaning and sanitisation schedules
- promote the food safety culture in your business

How to participate in the project?

Contact the project leader to discuss your food safety plans and get involved in the environmental monitoring program. The project team will visit your farm and/or packing shed to collect data and information on food safety practices. If the packing shed is operating, the team will collect fruit and environmental samples for a microbial food safety survey. The collected samples will be analysed in the laboratory for major pathogens (e.g. *Salmonella* and *Listeria monocytogenes*) using highly sensitive real-time polymerase chain reaction (RT-PCR) assays. The surveillance report will be confidentially shared with your business along with the recommendations for further improvements and corrective actions if required. The project team will protect your privacy and commercial-in-confidence information. The data from your packing shed will be de-badged and used to analyse the industry-wide trends without any reference to your business.

Contact information

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Citrus microbial food safety outreach and extension

Technical presentations were delivered at the following events/meetings:

- Griffith and District Growers Association Meeting, Griffith NSW (9 Dec 2021 evening)
- Riverina Citrus Packers Field Day, Griffith (9 Dec 2021 afternoon)
- Australian Citrus Tech Forum, Sunshine Coast Qld (8-9 March 2022)
- International Postharvest Unlimited Conference, Wageningen, Netherlands (15-17 May 2023)
- Citrus SIAP presentation (virtual; 24 May 2023)
- Fresh Produce Safety Conference, Sydney (11 Aug 2023)
- Dareton Citrus Industry Field Day, Dareton, NSW (13 Sep 2023).

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Citrus microbial food safety outreach and extension

Griffith and District Growers Association Meeting (9 Dec 2021)

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Citrus microbial food safety outreach and extension

Riverina Citrus Packers Field Day (9 Dec 2021)

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Citrus microbial food safety outreach and extension

Exhibition of project information, objectives and outcomes and stakeholder engagement:

- Australian Citrus Tech Forum, Sunshine Coast Qld (8-9 March 2022)
- Hort Connections, Brisbane (June 2022)
- Hort Connections, Adelaide (June 2023)

Citrus Tech Forum 2022
Sunshine Coast, Qld

Hort Connections 2022
Brisbane, Qld

Hort Connections 2023
Adelaide, SA

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Citrus microbial food safety outreach and extension

Technical articles:

- Singh SP (2021). ‘Seek and destroy’ the bugs in packhouses. Australian Citrus News Autumn Edition 2021.
- Singh SP (2022). Improving food safety practices to safeguard exports. Citrus News Summer 2022.
- Singh SP (2023). Managing microbial food safety risks. Australian Citrus News Summer edition 2023 (submitted)

Citrus microbial food safety outreach and extension

AUSTRALIAN Citrus News
AUTUMN 2021

'Seek and destroy' the bugs in packhouses

Foodborne bacterial pathogens have recently emerged as a challenge for the tree fruit industries.

In August 2020, citrus fruit (orange, limes and lemons) were recalled in the USA for contamination due to Listeria monocytogenes.

Since then the citrus fruit have been scrutinised for microbial food safety risks at an international level.

The presence of pathogen on the citrus fruit due to inedible peel poses relatively lower risk to the consumers.

However, the detection of any such pathogen in the export markets surveillance could trigger a major market access issue for the Australian citrus industry.

The industry therefore needs to remain vigilant and follow a preventative approach - "End the crisis before it begins".

Environmental pathogens, such as Listeria monocytogenes and Salmonella species, can be present in soil and water.

They can be transferred on to the citrus fruit in the field through various sources and routes such as dust storms, wildlife activity, soil amendments, irrigation water and water used for chemical sprays (**Figure 1**).

Good agricultural practices include preventative control measures to minimise the risk of microbial contamination in the field through quality control of preharvest water, wildlife management and windbreaks etc.

Listeriosis, is a rare but serious disease caused by the bacteria Listeria monocytogenes. Some people are unaffected from eating small amounts of the bacteria whilst others who do get sick may require hospitalisation and it may lead to death.

However, despite these preventative measures, microbial contamination of fruit can still occur in the field. The pathogens can survive in soil, water or on the fruit for reasonably long durations, depending upon the environmental conditions.

Pathogens such as Listeria may be introduced in the packhouse occasionally through incoming fruit.

It is essential that best practices are used to keep occasional presence under control. A contaminated fruit can cause cross-contamination of the harvest bins, conveyor belts, washing brushes and other fruit contact surfaces.

Once introduced in the packhouse and not controlled, a transient pathogen can take up residence on the fruit contact surfaces as well as non-contact surfaces such as floors, walls, cool rooms (**Figure 2**).

Resident pathogens indicate a failure in implementing best practices in particular cleaning and sanitisation or an undiscovered niche where a pathogen has established a harbourage site.

There is a high chance that the site is continually re-contaminating the facility and increasing the potential for fruit to be contaminated.

Aggressive corrective measures need to be implemented to seek out and eliminate resident pathogens and assess the factors that allowed the site to be established.

We recommend that citrus growers and packers follow a 'Seek and Destroy' approach in managing the risk posed by environmental pathogens. There are three objectives of this approach:

- To prevent transient pathogens from becoming entrenched, forming biofilms and spreading in the facility
- To verify the effectiveness of cleaning and sanitation measures
- To determine and undertake corrective action

Most citrus growing and packing operations are compliant with the food safety certification programs.

Figure 1. *Potential sources and routes of microbial contamination in the citrus fruit*

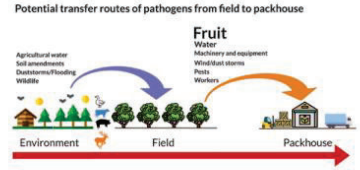
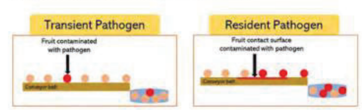


Figure 2. *A transient pathogen enters the packhouse with a contaminated fruit and can become a resident pathogen if cleaning and sanitisation schedules are not effective.*



Microbiological testing of the facility, water and fruit samples are required to achieve certification.

The general practice is to collect a swab test and hope for a negative test report.

However, packers must search for the pathogens in their facilities through appropriate testing in order to destroy them.

When a test report for a target pathogen such as Listeria monocytogenes is positive, a thorough investigation and re-testing can then determine whether the pathogen was transient at the time of sampling or has become a resident.

Cleaning and sanitisation of a packhouse is a critical strategy that can remove the transient pathogens.

The procedure entails two steps: 1) cleaning involves removing the soil and debris from the surfaces followed by 2) sanitising with approved reagents at recommended concentrations.

We recommend that each packhouse should have a standard operating procedure for cleaning and sanitisation to ensure these are effective.

To develop and implement a packhouse environmental monitoring program (PEMP), the citrus packers must ask the following questions on microbiological testing:

1. What are the target pathogens?
2. How frequently should sampling be conducted?
3. How many samples and from where?
4. When to sample and how to sample?
5. What will a response to a positive test result be?

The Horticulture Food Safety Team of the NSW Department of Primary Industries has developed practical resources for PEMP.

The team can assist you in developing and implementing the PEMP plan for your citrus packhouse while maintaining your commercial-in-confidentiality and privacy.

Dr SP Singh is Research Horticulturist-Food Safety with NSW DPI. You can contact Dr Singh on 0420 593 129 or email sp.singh@dpi.nsw.gov.au

This article is a contribution from NSW DPI's 'Safe Citrus' initiative.

Department of Primary Industries

Citrus microbial food safety outreach and extension

Improving food safety practices to safeguard exports

13/01/2021

A new project focused on food safety in citrus aims to mitigate microbial food safety risks associated with the production, postharvest handling and supply of citrus to consumers in domestic and export markets.

The project will be led by Dr SP Singh and the NSW DPI.

The Australian citrus industry exports oranges and mandarins worth half a billion dollars to the major Asian markets. The success of citrus export is built on the free trade agreements and a clean, green, and safe reputation of the industry.

"'Healthy, nutritious and safe' is the unique selling point that the Australian citrus industry promotes in its marketing campaigns," Dr Singh said.

"However, the industry needs to be proactive in maintaining the confidence of consumers, regulators and trading partners in the quality and safety of their fruit to ensure the market access is retained, and new markets are created.

"To remain competitive in the export markets it is imperative that the Australian citrus industry has the capacity to consistently supply safe and nutritious fruit.

"Due to the inedible peel, citrus fruit presents a relatively lower microbial food safety risk to consumers.

"However, any detection of microbial contaminants on the fruit poses a potential 'trade risk' and could trigger a non-tariff barrier. Given the current volatility in export markets due to various geopolitical reasons, this risk is significant."

Dr Singh and his team will engage with citrus growers and packers in all major production regions to collect data and information relating to the current industry practices on food safety.

This information will deliver a national snapshot of the industry practice and identify potential gaps to be addressed in the short-, medium- and long-term.

The project will follow a whole-of the-chain approach with strong stakeholder engagement to verify food safety practices and detect potential hotspots for microbial contamination and cross-contamination along the supply chain.

Each link within the supply chain will be examined, starting with field production, postharvest processing and distribution through to retail.

With the voluntary participation of citrus growers and packers, fruit and environmental samples will be collected from all primary production and supply regions to detect the target foodborne bacterial pathogens.

"I encourage citrus growers and packers to contact me to confidentially discuss their food safety practices and participate in the project to ensure the robust preventative control measures are in place to minimise the food safety risks to consumers and industry," Dr Singh said.

'Washington Navel' oranges being inoculated with Listeria monocytogenes for a simulated supply chain experiment in the laboratory.

Dr Singh said there are some unanswered questions on the ability of foodborne bacterial pathogens to survive and multiply during the cold treatment for phytosanitary purposes and other supply chain scenarios in export and domestic markets.

The project will investigate the survival and persistence of the key foodborne bacterial pathogens (e.g. Salmonella and Listeria monocytogenes) on various types and varieties of citrus fruit under simulated supply chain conditions.

The influence of critical postharvest conditions (e.g. storage temperature, cold phytosanitary treatment and shelf-life) affecting the pathogen survival will be investigated.

"The experimental data and industry's food safety practice information will form the basis for developing a scientific evidence-based Best Practice Guide for the industry to understand and manage these risks effectively," Dr Singh said.

The information pack and Best Practice guidance will be delivered through various channels of communication such as workshops, forums, meetings and industry magazines to enhance the skills and knowledge of growers, packers and the key staff involved in the supply chain.

"The outcomes of this project will enhance the industry's food safety capacity and influence the practice to mitigate microbial food safety risks."

More information

This article is a contribution from the project 'Managing microbial food safety risks in the Australian Citrus Industry (CT20005)'. You can contact Dr SP Singh on 0420 593 129 or email sp.singh@dpi.nsw.gov.au

Hort Innovation Strategic levy investment | CITRUS FUND

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Citrus microbial food safety outreach and extension

Managing microbial food safety risks

Dr S.P. Singh, Senior Research Scientist, NSW Department of Primary Industries

Environmental pathogens such as *Listeria monocytogenes* and *Salmonella* are often linked to fresh produce contamination. Due to the inedible peel, citrus fruit presents a relatively lower microbial food safety risk to consumers. However, any detection of microbial contaminants on the fruit surface in the export markets could pose a potential 'trade risk' and trigger a non-tariff barrier. This risk is significant given the current volatility in export markets due to various geopolitical reasons. In 2020 citrus fruit were recalled in the USA due to the detection of *Listeria monocytogenes*, and since then, the detection of microbial contaminants on citrus has become a high analytical priority.

How can microbial contamination occur on citrus fruit?

Microbial contamination can occur anywhere along the supply chain. However, the highest probability of contamination is in the field and at the packhouse. Foodborne bacterial pathogens such as *Listeria monocytogenes* can be transferred from natural environments and livestock activities to the citrus production fields with dust storms, animal manures, irrigation water, flooding and runoff from adjacent livestock operations and wildlife intrusion. Weather events such as dust storms and pesticide spray applications using contaminated water have the potential to carry and transfer microbial contaminants onto the fruit on trees. The transmission of pathogens from the field to the packing sheds occurs through the contaminated fruit, harvest bins, machinery and equipment. Once these pathogens enter the packing shed, they can establish on the fruit contact surfaces (conveyor belts, rollers, and brushes) and non-contact surfaces (packhouse floors, drains, and cool room walls and floors). The established pathogens on various surfaces become a continuous source of contamination and cross-contamination of the processed fruit.

What did we do?

In the current project (CT20005), one of the objectives is to map potential microbial contamination and cross-contamination sources and routes in the citrus supply chain. Between August 2021 and November 2022, we visited 51 citrus packing sheds across Australia to review their food safety practices and collect microbiological samples from various locations in the packing sheds and farms. Approximately 75% of these packing sheds were exporting fruit to Asian markets. All major citrus production regions were covered in this project, including Riverina, Murray, Riverland, Bundaberg, Gayndah, Mundubbera, Mareeba, Harvey, Gingin, and Katherine. These citrus packing operations handled and supplied a range of citrus fruit, including oranges, mandarins, grapefruit, limes and lemons. In addition to the sample collection from farms and packing sheds, the microbiological samples of citrus fruit were also collected from supermarkets at regular intervals.

So far, the project team has collected ~1850 microbiological samples from various stages in the citrus supply chain, with a major focus on environmental samples from the packing sheds. The samples were analysed for foodborne bacterial pathogens such as *Listeria monocytogenes*, *Salmonella* species and *E. coli* O157:H7. The analysis was conducted following internationally approved RT-PCR assays in the laboratory. The data were subjected to a hotspot analysis to define the key areas of contamination and cross-contamination.

What did we find?

Salmonella and E. coli O157:H7 were rarely detected in environmental samples collected from various stages in the citrus supply chain. The majority of the positive detections of *Listeria monocytogenes* were from harvest bins, fruit receival areas and cool rooms. These spots have been identified as hotspots for contamination and cross-contamination of citrus fruit with *Listeria monocytogenes* (Figure 1).

Figure 1. Listeria hotspots for microbial contamination and cross-contamination

Based on the data and scientific evidence of industry practice, we propose the following transmission routes of microbial contamination in citrus production and postharvest handling:

- *Listeria monocytogenes* is naturally present in soil and is also shed by animals suffering from listeriosis. Weather events such as dust storms can spread microbial contaminants onto the fruits on the tree (preharvest).
- As horticulture industries co-exist with livestock operations, the potential for transfer of these pathogens via weather events, irrigation water and runoff is real. Using contaminated water for irrigation and pesticide spray application could be another transmission route.
- Detection of *Listeria monocytogenes* on the harvested fruit indicated that it could attach to the fruit and survive preharvest weather conditions such as rainfall, UV and temperature.
- At harvesting, contaminated fruit can spread contamination onto the harvest bins. Once harvest bins are contaminated, these could become a source of cross-contamination.
- Harvest bins are generally placed on the ground during picking operations (Figure 2). The wet conditions during harvesting season, especially in heavy soils, lead to mudding of the harvest bin feet. When these bins are stacked, this can contaminate the fruit in the harvest bin below (Figure 3).
- The fruit receival area (bin top platform, conveyor belts, floor and drains) was established as the gateway for the entry of *Listeria monocytogenes* into the packing sheds through contaminated fruit (Figure 4).
- Occasionally the pathogens were detected in/on the processing machinery (conveyor rollers/belts, brushes).
- After the fruit waxing step, deposition of residual wax on the conveyor belts/roller and grading machinery is common (Figure 5). Several research studies have shown a higher prevalence of Listeria species in the waxing area in fruit packing operations, indicating that wax residues could increase the persistence of pathogens.
- Dry brushes in the fruit washing zone or the grading area (for polishing) are an important reservoir for pathogens (Figure 6). They are very difficult to clean and sanitise once they are contaminated.
- No provision of proper drains in several packing operations was observed. This could lead to the spreading of pathogens across the premises with workers' and forklifts' movements.
- The detection of Listeria on cool room walls and floors indicate that pathogen can survive and thrive at <5°C.

How to manage the microbial risks associated with Listeria?

In this project, we are developing a best practice guide to manage microbial food safety risks in citrus production and postharvest handling. The guide will be released before June 2023. In the interim, we recommend the following to mitigate microbial food safety risks:

- Don't apply raw/untreated manure in the citrus production fields. Raw manures are a leading source of microbial contamination.
- Harvest bins must be cleaned and sanitised after each use. Avoid placing harvest bins on the ground in the citrus orchards. The placement of harvest bins on trailers is the best practice to minimise the soiling of harvest bins.
- The fruit receival area must be cleaned and sanitised at the end of each shift. Cleaning and sanitisation daily can mitigate the risk of the establishment of pathogens in this area. Once the pathogen forms a biofilm for its protection from harsh weather and cleaning, it can become a continuous source of contamination.
- The pathogen can easily migrate from the fruit receival area to the other processing equipment with the fruit. Therefore, cleaning and sanitising the fruit receival area is an opportunity to push the pathogens out of the packing shed regularly.
- Postharvest wash water must contain a food-grade sanitiser (or disinfectant) such as peracetic acid or chlorine to sanitise wash water and improve the efficacy of the washing and sanitisation process.
- Follow the "Seek and Destroy" approach to minimise the risk of environmental pathogens such as Listeria. The microbiological testing should be aimed at seeking/finding the harbourage sites of this pathogen so it can be destroyed.
- Chemical degreasers are recommended to minimise wax deposits on fruit contact surfaces (rollers and brushes) and to reduce the risk of pathogens getting trapped in the waxy layer causing cross-contamination.
- All fruit contact surfaces such as harvest bins, conveyor belts/rollers and brushes should be cleaned with food-grade detergents, followed by the application of sanitisers/disinfectants.
- Fruit contact surfaces are most commonly implicated in cross-contamination risks. In the 2014 caramel apple listeriosis outbreak in the USA, polishing brushes were one of the fruit contact surfaces from which *Listeria monocytogenes* was isolated.

What were the services delivered to citrus growers and packers?

Grower/packer engagement in this project has exceeded our expectations and targets. Many citrus growers and packers have availed of the Food Safety Helpdesk service to address technical enquiries on microbial food safety. Those who participated in this project received business-specific technical reports on the microbiological status of their operation and recommendations to improve the practice. The reports also identified contamination points, critical control points (e.g. fruit washing and sanitisation, packhouse sanitation) and their verification procedures to minimise the microbial food safety risks.

On-site training was provided to the Quality Assurance staff of several packing operations on postharvest washing and sanitisation processes, digital measurements and monitoring, and the development of environmental monitoring plans. Furthermore, technical resources were provided to improve packhouse hygiene by implementing a regular cleaning and sanitisation program.

The project team encourages growers and packers to participate in the project. For further information, please contact Dr S.P. Singh, sp.singh@dpi.nsw.gov.au; 0420 593 129

Acknowledgements

The project team would like to thank citrus growers and packers for sharing their food safety practice data and allowing us to collect samples. Support from other stakeholders, including Citrus Australia and E.E. Muirs, is greatly acknowledged. This article is a contribution of the project CT20005- Managing microbial food safety risks in the Australian citrus industry. The project has been funded by Hort Innovation using the citrus R&D levy and funds from the Australian Government.

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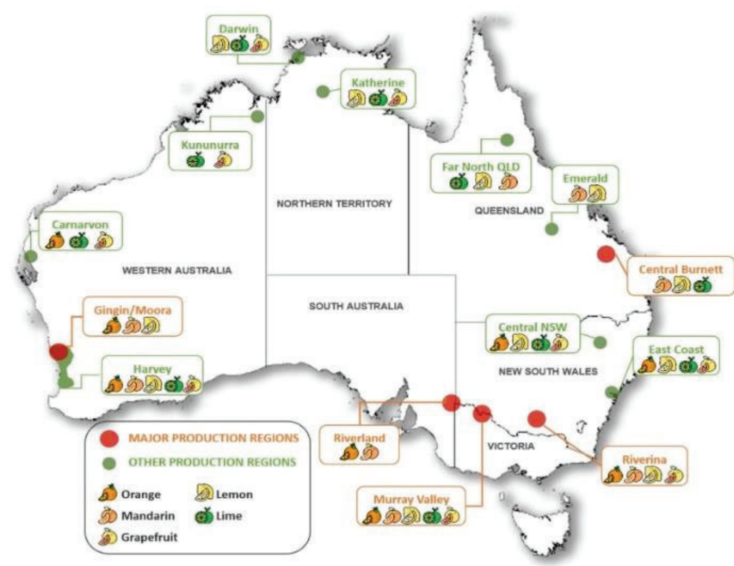
Citrus microbial food safety outreach and extension

One-on-one consultation: Technical on-site visits to 56 citrus packers in the following citrus production regions:

- NSW 24 (19 in Riverina, 4 in Murray and 1 Central Coast)
- Qld 13 (8 in Burnett and 5 in Far NQ)
- SA 8 (all in Riverland)
- Vic 6 (all in Murray)
- WA 4 (2 South of Perth and 2 North of Perth)
- NT 1 (Katherine)



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Citrus microbial food safety outreach and extension

One-on-one technical consultation visit to Burnett region, Qld

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Citrus microbial food safety outreach and extension



One-on-one technical consultation visit to Burnett region, Qld (on-site training)

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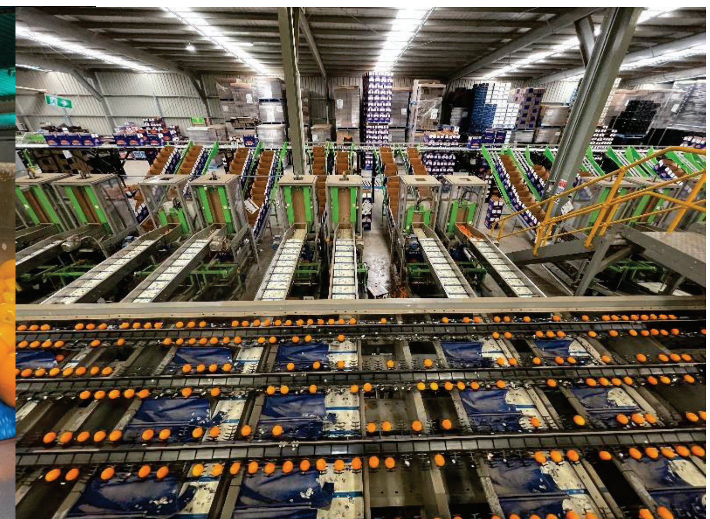
Citrus microbial food safety outreach and extension



One-on-one technical consultation visits to Murray region of Victoria and NSW

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One-on-one technical consultation visit to Riverland region of SA

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One-on-one technical consultation visit to Western Australia and Northern Territory

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One-on-one technical visits to lime and lemon growers/packers in Far North Queensland

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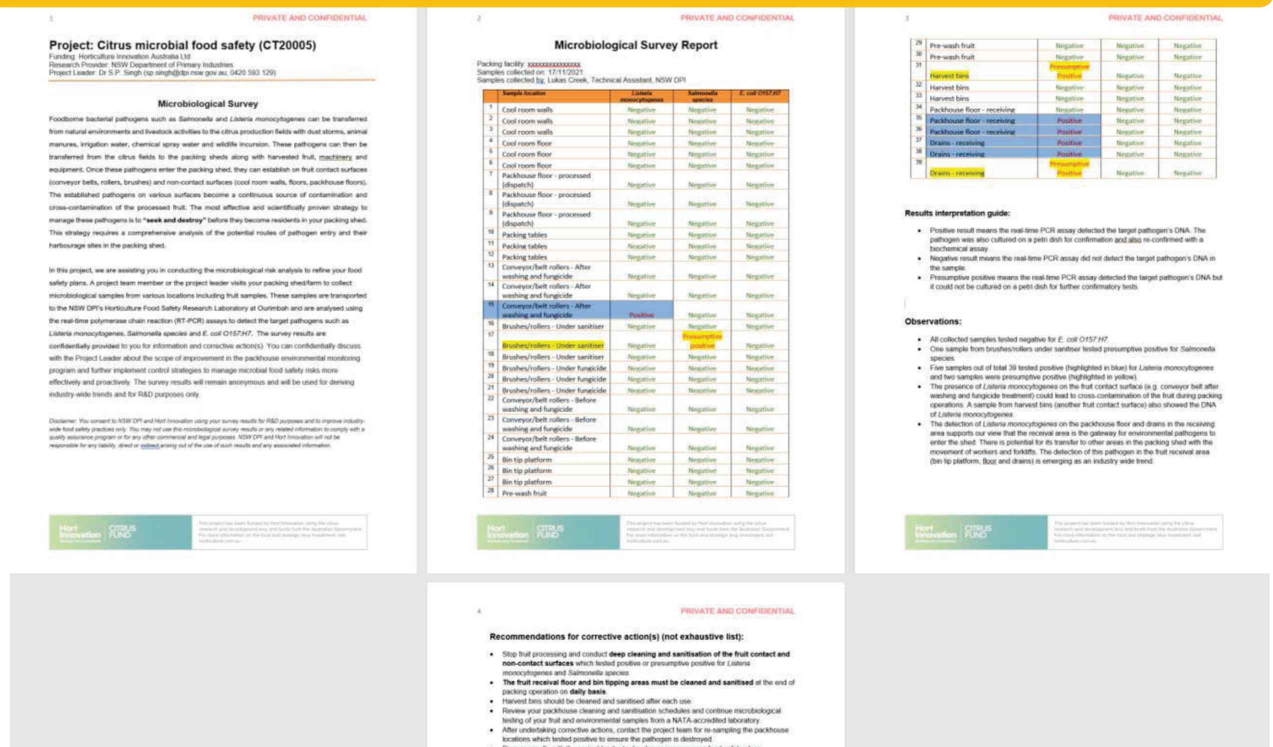
Citrus microbial food safety outreach and extension

Hort Innovation Project R&D Manager visited citrus growers/packers in the Riverina region

Citrus microbial food safety outreach and extension

Citrus microbiological survey report

- Project introduction
- Business details
- Sampling location
- Results (positive/negative)
- Result interpretation guide
- Practice observations
- Recommendations



A screenshot of a sample survey report sent to a citrus packer

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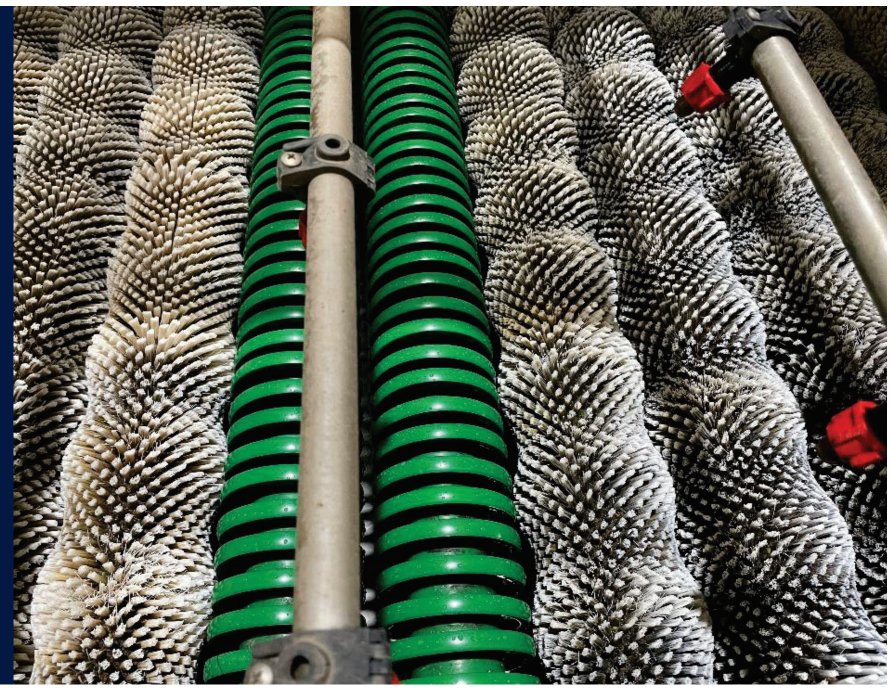
Citrus microbial food safety outreach and extension

Summary of a citrus packer case study involving traceback and root-cause analysis:

- 7 samples tested confirmed positive for *Listeria monocytogenes*
- Deep cleaning and sanitisation recommended for the positive sites
- Resampling from the positive sites
- 6 locations tested negatives and 1 location (brush rollers) again tested positive
- Listeria contamination was deep seated, and the packer replaced the brush rollers.
- Whole genome sequencing of *Listeria* isolates confirmed the story.



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Citrus microbial food safety outreach and extension

Food safety helpdesk service

You replied to this message on 14/10/2022 2:16 PM.

My apologies for the delayed reply on this.

Thank you for the detailed reports and follow up on this.

We have taken note of the couple of areas that could face improvement and have implemented some changes to our processes.

Of course, without further testing it is somewhat difficult to know if we have made effective improvements. Would it be possible to arrange another visit and testing program to see if we have made progress?

Thanks

Hope all is well? Was hoping you can point me in the right direction. We are packing citrus and recently had our Freshcare Supply Chain Audit. We need some assistance in drawing up an Env Management Plan. Our auditor mentioned Dr SP Singh and work he had done for Citrus Australia. Do you perhaps have his contact info?

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Citrus microbial food safety outreach and extension

Food safety helpdesk service

Hi SP,

Thank you for your time . It was interesting talking to you and spending some with you and getting another outlook from someone with your experience.
These test are a positive result and hope it is due to our regular cleaning regime. We can not sit back as it is an ongoing process.
Appreciate the attachment for sanitation.
I will keep in touch if I need further information.

SP,

Thank you for the results.

We have already started a new cleaning programme and will start washing bins and shed drainage.

Our shed will be shutting next week for the season. I will contact you soon to discuss some of the procedures/chemicals.

Regards,

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Citrus microbial food safety outreach and extension

Food safety helpdesk service

SP have I sent you this before. See below E-Coli is fine but the Coliforms are high. What is your take on this water sample.

See also below what I got from someone else:
Thermotolerant coliforms are typically spore-forming bacteria.

Bacterial spores can be resistant to freezing and high temperatures making them more difficult to eradicate.

As with most spores, higher CT values are relevant requiring either higher concentrations or longer contact times.

Analytical Results

Compound/Analyte	Method	LOR	Units	B1318098/1	B1318098/2	B1318098/3	B1318098/4
Escherichia coli	M8.5 - AS/NZS 4276.7	1	CFU/100 mL	<1	<1	9	<1
Thermotolerant Coliforms	M8.5 - AS/NZS 4276.7	1	CFU/100 mL	680	3200	10	<1

Hope you are well. E coli and colofirm results generally correspond. I suspect sediment/dirt in the water samples.

Water filteration, high sanitiser concentrations and longer contact time will help. Flumes seems to be running low on sanitiser conc.

Kind regards,
SP

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Citrus microbial food safety outreach and extension

Food safety helpdesk service

Thanks for email and sharing data.

Coliform count suggests that water filtration, treatment and sanitisation is not adequate. It could be faecal matter contamination or any other dirt or foreign matter in the water sample.

Since it's flume water, ask them to change it more frequently. However, the solution lies in having a prewash rinsing step (run to waste) so that extraneous matter from fruit surface could be removed before it goes to the flume / wash step where water is recycled and reused.

Hope this is useful. I'm currently in Europe for work, but available via email.

Kind regards
SP

Sent: Thursday, May 18, 2023 11:49:33 PM
To: SP Singh <sp.singh@dpi.nsw.gov.au>
Subject: Re: Citrus microbial food safety project (CT20005) update

Hi SP

Thanks for the very clear and concise report. You are truly a very solid and thorough scientist. A real asset to the Australian R&D community.