Hort Innovation

Final Report

Australian citrus postharvest science program

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Content

Summary

The consistent supply of high quality citrus fruit requires a whole of chain approach which needs to be supported with innovative postharvest technologies and information. There are a number of significant challenges to consistently and efficiently deliver high quality, safe and nutritious fruit to domestic and export markets. This Program addressed three major challenges; (1) postharvest decay control, (2) managing fruit quality through the supply chain, (3) postharvest treatments to reduce MRLs. These outputs were research-based with the aim of controlling postharvest decay and maintaining fruit quality through the supply chain where MRLs are not a barrier to market access. The results showed there are significant opportunities for the effective use of new postharvest fungicides and new alternative methods for the control of postharvest decay (green and blue mould). New fungicide chemistries and new formulations were assessed and showed significant potential for the control postharvest decay. The Program also showed there maybe opportunities for the postharvest control of anthracnose, which can contribute to significant out-turn problems in wetter growing regions, such as Queensland. The results of the two seasons of packinghouse surveys showed there were significant issues with poor postharvest sanitation and the development of technical resistance to common postharvest fungicides in some packinghouses around Australia. An active extension program to address these issues was conducted with presentations at regional and national citrus forums, articles in major grower / packer magazines. Sanitation is essential to reduce the number of decay-causing spores and reduce the risk of postharvest decay and development of fungicide resistance to postharvest fungicides. The Program assessed a range of current and new sanitation systems for their application to the Australian citrus industry. The results identified some new water and coolroom sanitation systems which may have application to industry.

While decay is the primary cause of postharvest losses, it can be successfully managed with good postharvest practices and fungicides. However there are a range of other fruit quality issues which impact the ability of the citrus industry to successfully market fruit into both domestic and export markets. These include; (1) chilling injury, (2) development of off-flavours, (3) and adoption of new technologies to improve fruit quality management during storage. These issues were addressed with a series of postharvest storage trials. The results demonstrated opportunities to reduce chilling injury through greater understanding of preharvest factors that contribute to the disorder and the potential application of postharvest treatments to minimise the development of symptoms. These need more work to assess their efficacies over different growing seasons. A review of the potential postharvest quality effects of low dose irradiation as a market access treatment for export was also conducted and showed low dose irradiation has a place in the market access tool box, where high value export markets allow this treatment.

To minimise the risk of MRLs, a postharvest trial illustrated that any postharvest washing at treatment temperatures of 20 or 40°C reduced the preharvest orchard chemical residues in lemon fruit. This is a good result for industry and shows the benefits of postharvest washing and handling to reduce the potential of orchard residues. However it is critical to minimise chemical use to ensure MRLs are not an issue.

A range of extension materials were developed and extended to industry through regular contributions to industry newsletters, citrus postharvest web pages, and with regular presentations and workshops in all major growing regions around Australia. Recommendations for future work and outcomes are made.

Keywords

Citrus, orange, mandarin, quality, storage, postharvest, supply chain, export, decay

Introduction

The consistent supply of high quality and safe citrus fruit onto domestic and export markets requires a whole of chain approach supported by innovative postharvest technologies and information to meet and exceed consumer and regulatory requirements. With record exports and solid returns on many markets, there is a need to optimise this market development to ensure continued access and profitability of the Australian citrus industry. However there are a number of significant and real challenges to consistently and efficiently deliver high quality, safe and nutritious fruit to domestic and export markets.

The major postharvest problem for the marketing of citrus on the both the domestic and export market is decay caused by blue and green mould. This decay is currently controlled with postharvest fungicides. Whilst fungicides are usually effective, the development of technical resistance can lead to reduced efficacy and fruit breakdown. This can have significant consequences, particularly in export markets where transport and storage times can be long. Correct management of postharvest fungicides is critical to maintaining their efficacy, as well as ensuring that chemical residues are not a barrier to market access.

The development of new fungicide chemistries and sanitisers are opening up new avenues of controlling postharvest decay in citrus through improved decay control and reducing fungicide resistance risk management. For example the introduction of 'Chairman' (with active ingredients fludioxonil and propiconazole) was introduced in 2018 giving growers greater options for postharvest decay control. However there is growing demand for reduced MRL fruit where this Program is working towards postharvest solutions to meeting the Australian citrus industry's 'ultra-low' residue goals. Alternative and non-chemical control of postharvest decay is important to give industry options to manage decay. For example there are a range of low toxicity chemical treatments such as generally regarded as safe (GRAS) compounds (e.g. sodium carbonate salts etc.), and natural compounds (e.g. essential oils, plant extracts etc.). These suites of options are in different stages of development and require assessment to measure their efficacy and value to industry. In addition to decay management, postharvest sanitisers and washing treatments can improve the appearance of the fruit, and also remove pre-harvest fungicides /contamination from the fruit surface.

To maintain its position as a provider of premium citrus, and extract maximum value from export markets, it is essential that the Australian citrus industry is at the forefront of the latest developments and technology. This Program partnered with key research agencies such as Institut Valencià d'Investigacions Agràries (IVIA) in València, Spain. Dr Lluis Palou at IVIA is the world's leading authority in citrus postharvest decay and was a key member of the Program. Dr Palou helped guide the research and gave insightful up-to-date information on current development in postharvest in Spain.

The continued success of the Australian citrus industry relies on the delivery and adoption of relevant and timely information. Therefore the development and extension of up-to-date postharvest resources (e.g. regular articles in '*Australian Citrus News'*, continuation of '*The Packer'* newsletter etc.) and Program updates at grower forums are essential to ensure industry are using best practice and meet retailers / consumers expectations.

Methodology

This Program conducted a series of postharvest storage trials and assessments:

1. Postharvest decay control
2. Managing fruit quality through the supply chain
3. Postharvest treatments to reduce MRLs

Each of these different trials had specific methodologies which are reported for each experiment, but the *General Methods* for each of these trials are listed below:

Postharvest decay assessment

Green mould (*Penicillium digitatum* (Pers.: Fr.) Sacc.) and blue mould (*P. italicum* Wehmer.) isolates (fungicide-sensitive) were cultured for 1 – 2 weeks on potato dextrose agar at 25 °C. Both were isolated from infected navel oranges from citrus packinghouses in Griffith, NSW. Conidia were then harvested from both species by adding 5mL of sterile, de-ionized water containing 0.05% Triton X-100 to the petri dish. Conidia then were rubbed with a sterile glass rod, and conidia suspension was passed through two layers of cheese cloth. The suspension was diluted with water to an inoculum density of 1×10^6 conidia / mL unless stated otherwise. Inoculation was done by dipping a steel rod with a 1- mm wide and 2 –mm long tip into the inoculum suspension and making a single puncture in each fruit with the rod (Palou et al., 2001). Navel and Valencia oranges were inoculated with *Penicillium* inoculum 24 hours before treatments. In laboratory experiments, fruit were immersed in 10 L of each solution. After treatment, the fruit were not rinsed, packed into cavity trays, stored for up to 10 days at 20 °C and 95% relative humidity and then the number of decayed fruit was counted and the diameter of the infection measured (mm).

Measurement of fruit quality

Calyx senescence assessment Calyx senescence was visually evaluated with changes in colour of the calyxes from green to brown and scored according to Li et al. (2016) using a 5-point scale where 1 = green, 2 = slightly yellow, 3 = moderately yellow, 4 = totally yellow and 5 = brown and the mean score of all fruit in the sample then calculated.

Calyx abscission Calyx abscission was assessed by the presence or absence of the calyx of the fruit. Calyxes which had abscised during storage were counted on each assessment day and the results expressed as percentage calyx abscission.

Chilling injury assessment Chilling injury (CI) was assessed using a 4 point scale where 1 = normal (no pitting symptoms), 2 = slight pitting (a few scattered pits), 3 = moderate pitting (up to 30% surface covering), 4 = severe pitting (>30% surface covering), with results expressed as a percentages of the total number of fruit evaluated in the experiment.

Weight loss Weight loss of the fruit was assessed using an electronic balance (Model Kern & Sohn GmbH, D-72336, Balingen, Germany), where fruit weight of each treatment unit was recorded each assessment day. Weight change was expressed as a percentage value determined by deducting the initial weights (W_1) from the final weights (W_2) divided by the initial weights and multiplied by hundred percent (%).

Fruit firmness A texture analyser (Lloyd Instrument LTD, Fareham, UK) was used to determine fruit firmness. The maximum force (N) was measured by compressing the fruit in the equatorial zone between two flat surfaces closing together at the rate of 1 mm min^{-1} to a depth of 2 mm. The average of two reading points from each side of the fruit was recorded.

Respiration rate Fruit respiration was measured as carbon dioxide (CO_2) production (mL CO_2 kg^{-1} h^{-1}). Fruit were sealed into an airtight 2 L glass jars fitted with a septum for different incubation times to accumulate respiratory gases. CO_2 concentration in the jar was determined by withdrawing a 1 mL gas sample from the headspace and injecting into a gas chromatograph (Gow-Mac, Bridgewater, NJ, USA) fitted with two stainless steel columns (60 cm × 1 mm i.d.) connected in series. Operating temperatures for the detector, injector, and column were 110 °C, 50 °C and 110 °C respectively. The carrier gas used was high purity argon (BOC Gases, Sydney, NSW,

Australia) at a flow rate of 25 mL min^{-1}. Respiration rate was calculated according to the method of Li et al., (2016).

Internal fruit quality assessments

TSS Total soluble solids (TSS) also known as soluble solids content (SSC) were measured on the juice and determined using a digital refractometer (Atago Co., Ltd., Tokyo Atago, Japan) and expressed as °Brix.

Titratable acidity Titratable acidity (TA) was determined by titrating 5 mL of the juice with 0.1 M NaOH to pH 8.2 with an automatic titrator (Mettler Toledo, Greifensee, Switzerland) and data were expressed as percentage citric acid.

Ethanol content For ethanol accumulation, 10 mL of juice was transferred into a 20 mL glass vial with crimp-top caps sealed with silicone septa. The sealed sample was then incubated in a water bath of 30 °C for 10 min before analysis. Ethanol accumulation was determined by headspace analysis using a gas chromatograph (Model 580, Gow-Mac, Bethlehem, PA, USA) with a flame ionization detector and a column (Carbwax, Gow-Mac, Bethleham, PA, USA). The injector was set at 190 °C, the column at 68 °C, the detector (FID) at 190 °C with gas flow rates of 30, 30 and 300 mL min^{-1} for nitrogen, hydrogen and air respectively. After incubating the samples, 1 mL of the headspace gas sample was drawn from the vials and injected into the GC. Ethanol accumulation was calculated and expressed as g L^{-1}.

Statistical analysis

A range of statistical techniques were used in each study. These are described in each study, but in general the statistical procedures were performed using Genstat, R or SPSS for Microsoft version. ANOVA, analysis of variance was conducted and least significant differences (LSD) at P=0.05 for means were calculated to determine significant differences between treatments.

Outputs

The Program had major four outputs / research outcomes; (1) postharvest decay control, (2) managing fruit quality through the supply chain, (3) postharvest treatments to reduce MRLs and (4) extension resources. The first three outputs were research based with the aim of controlling postharvest decay and maintaining fruit quality through the supply chain where MRLs are not a barrier to market access. The extension resources developed and extended in the Program are listed in *Section 4*.

Summary of Program outputs

1. Postharvest decay control

- 1.1 Postharvest decay control
  - i. Postharvest fungicides
  - ii. Alternative chemical decay control
  - iii. Assessment of potential postharvest fungicides to manage anthracnose (*Colletotrichum gleosporioides*) in Imperial mandarins
- 1.2 Fungicide resistance survey and management,
- 1.3 Improved use of sanitising treatments,
  - i. Effect of sanitisers on postharvest decay
  - ii. Comparative efficacy of new electrolysed water sanitiser system
  - iii. Evaluation of alternative sanitation treatments
- 1.4 Physical treatments to manage postharvest decay

2. Managing fruit quality through the supply chain

- 2.1 Chilling injury (pre-harvest and postharvest treatments)
- 2.2 Minimising the development of off-flavours in mandarins in export markets
- 2.3 New approaches to maintain fruit quality during storage
- 2.4 Review of irradiation as a market access treatment on fruit quality

3. Postharvest treatments to reduce MRLs

4. Extension resources

1. Postharvest decay control

Introduction

Postharvest decay is the major problem for the storage and marketing of citrus both on domestic and export markets. The major postharvest rots that develop on citrus are green and blue mould (caused by the fungus *Penicillium*) and sour rot (caused by *Galactomyces citri-aurantii*). While the control of decay after harvest requires an integrated approach (handling, sanitation, storage conditions and fungicides), the correct use of fungicides and sanitisers are essential for the successful storage and export of citrus.

This Section assessed a number of approaches to postharvest decay control: (1) fungicides, (2) sanitisers and (3) physical treatments. Each of these different and complimentary approaches were assessed against the major decay causing fungi, green and blue mould (*Penicillium digitatum* and *P. italicum)*.

1.1 Postharvest fungicides

1.1 Effect of time of fungicide dipping time on green mould development

Experiment 1. A range of commercial fungicides; fludioxonil ('Scholar'), 'Chairman' (fludioxonil + propiconazole), thiabendazole (TBZ, 'Vorlon' or 'Tecto'), and imazalil ('Magnate' or 'Fungaflor') were applied either 16 hours before infection with green mould, or + 6, 12, 24 or 48 hours after green mould infection. The results presented in Figure 1 shows that each fungicide had some protective effect when applied 16 h prior to green mould inoculation (Pre-). But the efficacy of the fungicides was highlighted when applied within 24 hours after infection. Delaying treatment of fruit with fludioxonil and 'Chairman' fungicides 48 hours after infection reduced their efficacy. (Figure 1).

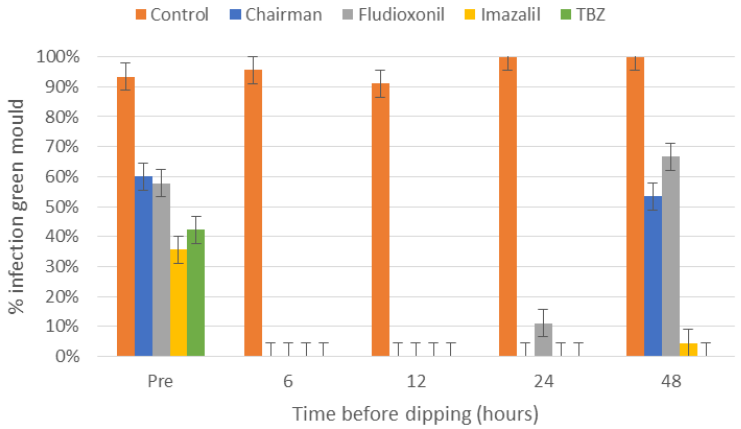


Figure 1. Effect of different postharvest fungicides at different dipping treatment times before and after initial infection on the subsequent % of green mould fruit.

Experiment 2. The effect of delayed postharvest fungicide application was further examined in this experiment. Green and blue mould infected oranges were treated with imazalil, fludioxonil, 'Chairman' (fludioxonil and propiconazole), 'Philabuster' (imazalil and pyrimethanil) at either 6, 12, 24, 48 or 72 hours after infection. The results show the effectiveness of the different postharvest fungicides was reduced when applied 24 hours after infection for both green (Figure 2) and blue mould (Figure 3).

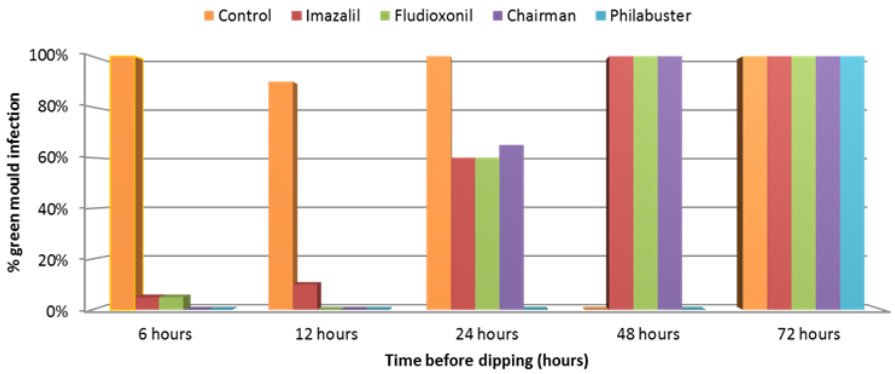


Figure 2. Effect of different postharvest fungicides at different dipping treatment times after initial infection on the subsequent % of green mould fruit.

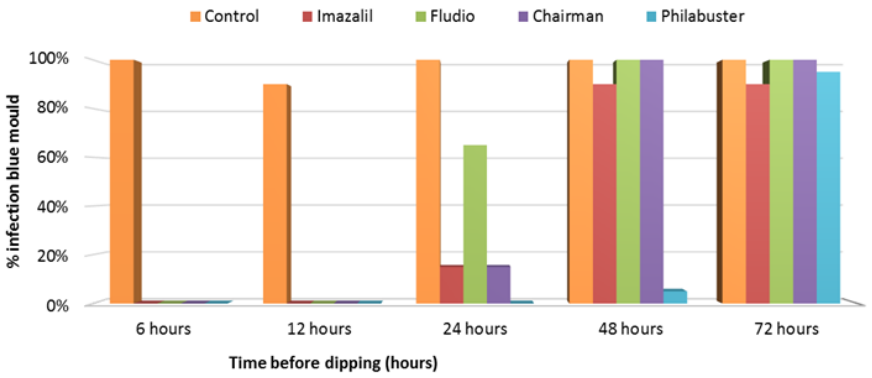


Figure 3. Effect of different postharvest fungicides at different dipping treatment times after initial infection on the subsequent % of blue mould fruit.

Conclusion The efficacy of the application of postharvest fungicides is dependent on their application time after infection. It is recommended that these postharvest fungicides are applied within 24 hours of harvest. This was a focus of extension articles and presentations (*Australian Citrus News*, Spring 2017).

1.2 Alternative chemical decay control

The current synthetic postharvest fungicides used for the control of postharvest decay are effective, but with growing consumer and regulatory barriers to the use of chemical residues there is a need to find other chemical and non-chemical treatments to control postharvest decay. This section assessed a range of new chemistries or new formulations of chemical treatments such as:

(1) improved use of salts,
(2) effects of pH,
(3) assessment of ortho-phenylphenol (OPP),
(4) assessment of 'Cerafruta',
(5) assessment of 'Propirly™' and
(6) novel treatments (plant extracts) to control postharvest decay.

Non-chemical / physical treatments to control postharvest decay are explored in Section 4.

1.2.1 Improved use of salts

Alternatives to synthetic chemical fungicides are required to maintain decay control with no chemical residues. This section explores different postharvest salt treatments to control postharvest decay. Organic and inorganic salts are widely used in the food industry; they are common food additive which also have a broad spectrum of activity against bacteria and fungi. They are classified as 'generally regarded as safe' (GRAS) compounds for many applications under European and North America regulations. In addition to their consistent antimicrobial activity, they are inexpensive, readily available, with favourable safety profile for humans and the environment and suitable for commercial postharvest handling practices.

Sodium bicarbonate (SBC) and sodium carbonate have been well studied in the control of postharvest decay in citrus with SBC used commercially in many citrus packinghouses around Australia. Research has shown that SBC treatment at room temperature and dip times of 60 and 150 seconds decreased the incidence of green and blue mould in Clementine mandarins by 40% (Palou et al., 2002). Corresponding work showed similar results with sodium carbonate. However, while SBC had a significant effect at controlling green mould, it also had negative effects on quality including increased water loss and increase in rind deformation and internal maturity. But, this work was conducted at 45 °C with relatively long treatment times of 150 seconds (Larrigaudière et al., 2002). The aim of this study is to examine the effects of SBC at shorter treatment times at higher treatment temperatures to assess if higher SBC treatment temperatures in combination with high pressure washing (Figure 4) are more effective in its control of green and blue mould.

Figure 4. Treating green and blue mould inoculated Navel oranges in dipping bucket (left), or high pressure washer (right).

a. Effect of dipping temperature (20 to 60 °C) of 3% SBC in high pressure washer on mould growth

Methods Navel oranges artificially inoculated with green and blue mould were treated with 3% SBC in a custom built high pressure washer at; 20, 40, 45, 50, 55 and 60 °C for either 30 or 60 seconds. A total of 20 fruit per treatment unit were treated, and each treatment was independently replicated three times. After treatment, the levels of decay were recorded after 7 days storage at 25 °C.

Results The results presented in Figures 5 – 8 show that increasing treatment temperature from 20 °C to 40 °C did not result in any significant differences in the level of infection or growth of the green or blue mould. However higher treatment temperatures (≥ 45°C) resulted in reduced incidence of infection and lower growth rates of green and blue mould development. In this experiment there was no difference in the different durations of treatment for both green and blue mould, i.e. there was no difference between 30 second or 60 second treatment time, except at 50 °C for green mould infection and growth.

The hot water treatments (without SBC) also resulted in lower infection and decay growth above 45 °C. This result is already used by industry and was further explored in Section 4 ('*Physical treatments to manage postharvest decay*').

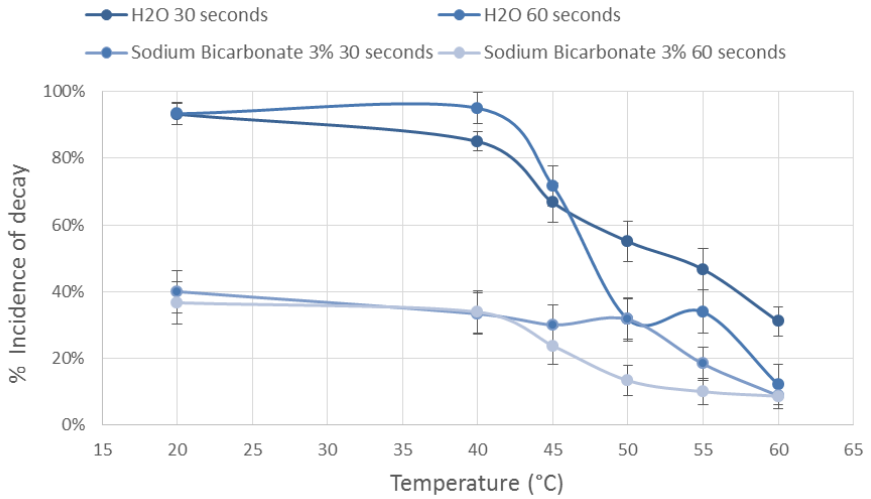


Figure 5. Incidence of green mould decay (%) in Navel oranges following high pressure washing in water or 3% SBC at 20, 40, 45, 50, 55 and 60 °C for either 30 or 60 seconds. *Bars are standard errors around the mean (n=3).*

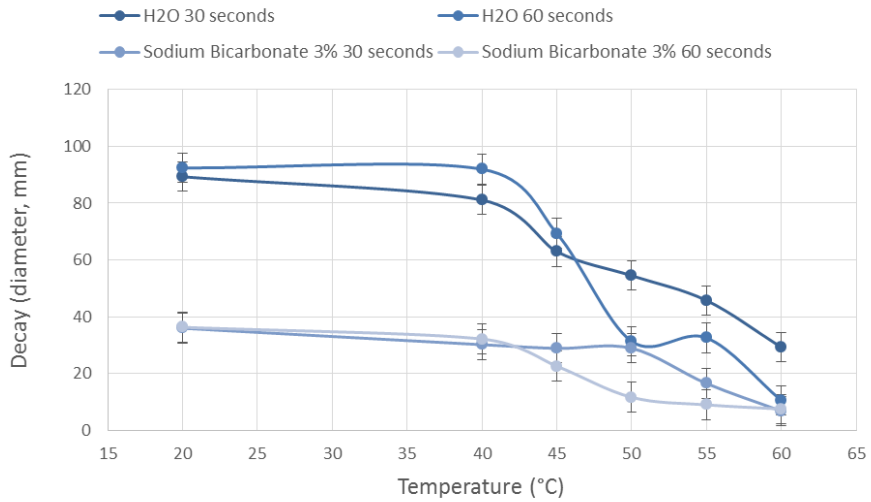


Figure 6. Size of green mould decay infection (diameter, mm) in Navel oranges following high pressure washing in water or 3% SBC at 20, 40, 45, 50, 55 and 60 °C for either 30 or 60 seconds.
Bars are standard errors around the mean (n=3).

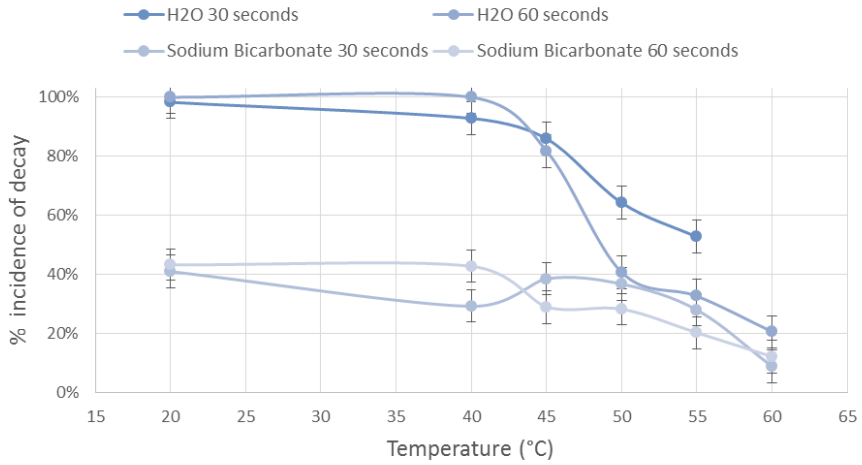


Figure 7. Incidence of blue mould decay (%) in Navel oranges following high pressure washing in water or 3% SBC at 20, 40, 45, 50, 55 and 60 °C for either 30 or 60 seconds.
Bars are standard errors around the mean (n=3).

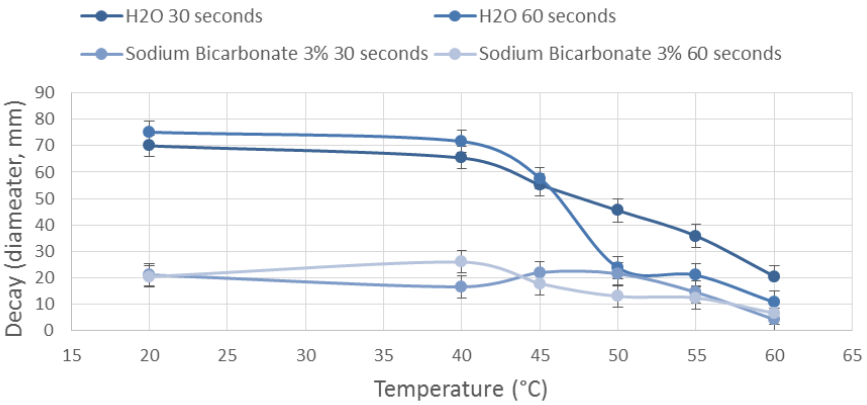


Figure 8. Size of blue mould decay infection (diameter, mm) in Navel oranges following high pressure washing in water or 3% SBC at 20, 40, 45, 50, 55 and 60 °C for either 30 or 60 seconds.
Bars are standard errors around the mean (n=3).

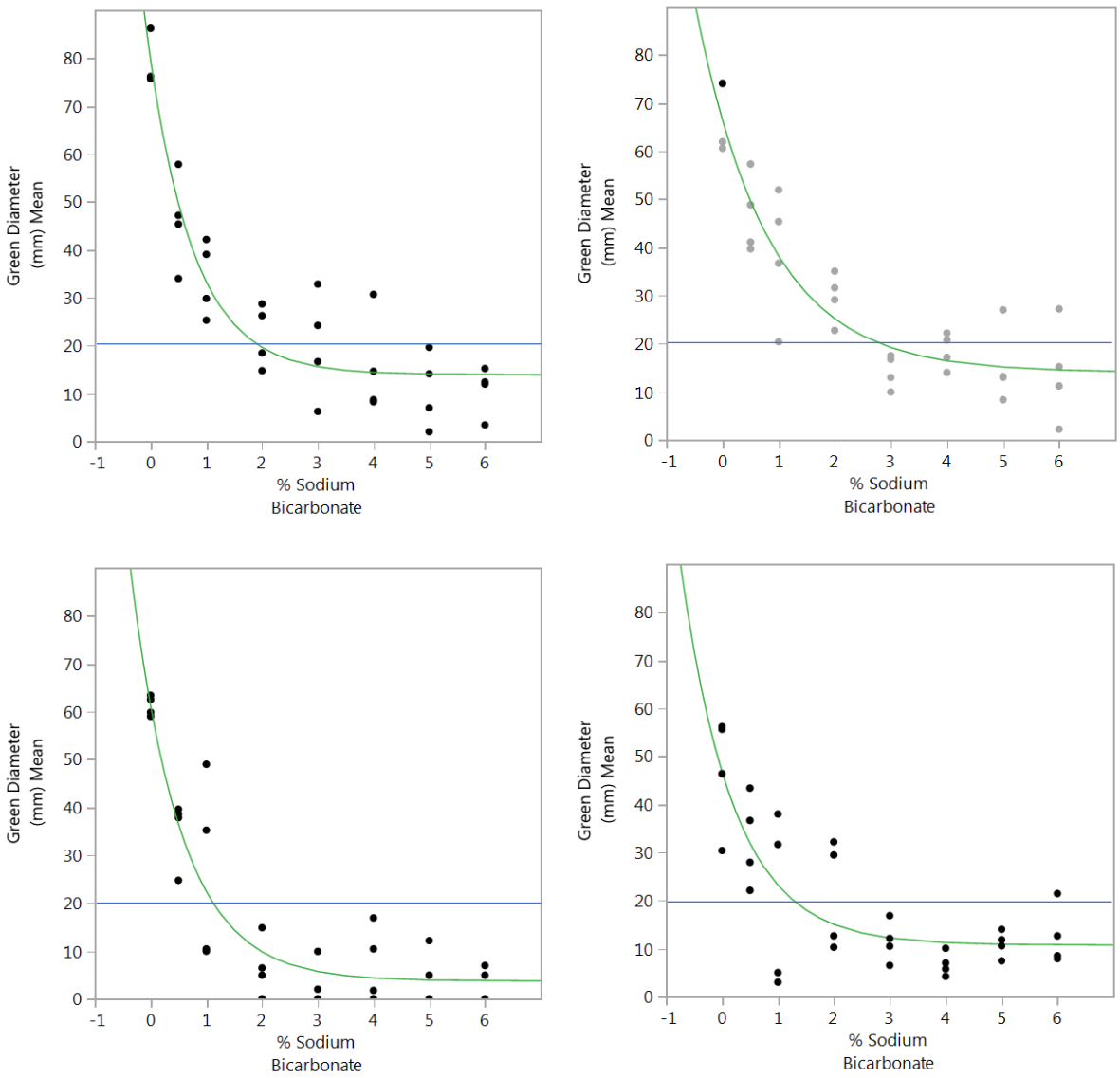
b. Effect of different concentrations of SBC in high pressure washer and dip at 20 and 40 °C on green mould

Methods Navel oranges which had been inoculated 24 hours previously with green mould spores were treated with a range of SBC concentrations (0, 0.5, 1, 2, 3, 4, 5 and 6% SBC) at either (20 or 40 °C) in a high pressure washer or in dip tank for 60 seconds. A total of 20 fruit per treatment unit were treated, and each treatment was replicated four times. After treatment, the levels of decay were recorded after 7 days storage at 25 °C.

Results The results presented in Figure 9 show the response of the green mould growth to increasing SBC concentration were similar, where increasing SBC resulted in lower green mould growth. The results showed that the growth of green mould following SBC treatment with high pressure washing was lower than SBC treatment as a dip. The high pressure washing with brushing (Figure 4) reduced the growth of the green mould.

A. Dip Tank at 20 °C

B. Dip Tank at 40 °C



C. High Pressure Wash at 20 °C

D. High Pressure Wash at 40 °C

Figure 9. Effect of SBC concentration (0, 0.5, 1, 2, 3, 4, 5 and 6% SBC) and treatment temperature (20 °C (left), or 40 °C (right)) dipped in tank (top) or treated with high pressure washer (lower) on green mould growth after 7 days storage at 25 °C. Black dots represent the mean for each treatment replicate (n = 20 fruit) and the green line represents equations that best fit the data (below).

Equations for SBC responses at different temperatures and treatment methods:

A. Dip Tank at 20 °C	y = 13.9 + 65.9 x exp (-1.22 x %SBC)	r^2 = 0.89
B. Dip Tank at 40 °C	y = 14.0 + 52.4 x exp (-0.77 x %SBC)	r^2 = 0.85
C. High Pressure Wash at 20 °C	y = 3.7 + 57.5 x exp (-1.12 x %SBC)	r^2 = 0.86
D. High Pressure Wash at 40 °C	y = 10.7 + 36.1 x exp (-1.06 x %SBC)	r^2 = 0.65

Conclusion SBC is a salt that is commercially used in some citrus packinghouses. The results of these trials showed its beneficial effects on reducing the growth of green mould following treatment at different temperatures, where longer treatment times and temperatures resulted in lower decay growth. Increasing SBC concentration resulted in reduced growth, but its increased effectiveness was marginal above 3%. Treatment of green mould infected orange with SBC in a high pressure washer increased its effectiveness as compared to a simple dip. SBC treatment is inexpensive and can be a useful tool in the management of fungicide resistant isolates, which have become particularly problematic but more work on its effects on fruit quality need to be conducted.

1.2.2 Effects of pH

pH is a scale used to specify how acidic or basic a water-based solution is. Acidic solutions have a lower pH, while basic solutions have a higher pH. Many postharvest dip treatments used as sanitisers or fungicides have a broad pH range. For example, the pH of peroxyacetic acid ('Tsunami') is around pH 4, whilst the pH of sodium ortho-phenylphenol (SOPP) is around pH 8.5. To examine the effect of just the pH only, this experiment examined the effect of different pH of the dip solutions on the growth of green and blue mould infected oranges.

Methods Green and blue mould infected oranges were treated with different pH solutions (pH 3, 5, 7, 9 and 11). A total of 20 fruit were dipped in each treatment unit which was independently replicated 4 times. The data was analysed by one-way analysis of variance with the Tukey-Kramer test to determine significant differences.

Results There was no significant difference in the results from the different pH solutions on both the rate of infection and growth of green and blue mould in oranges (Figure 10).

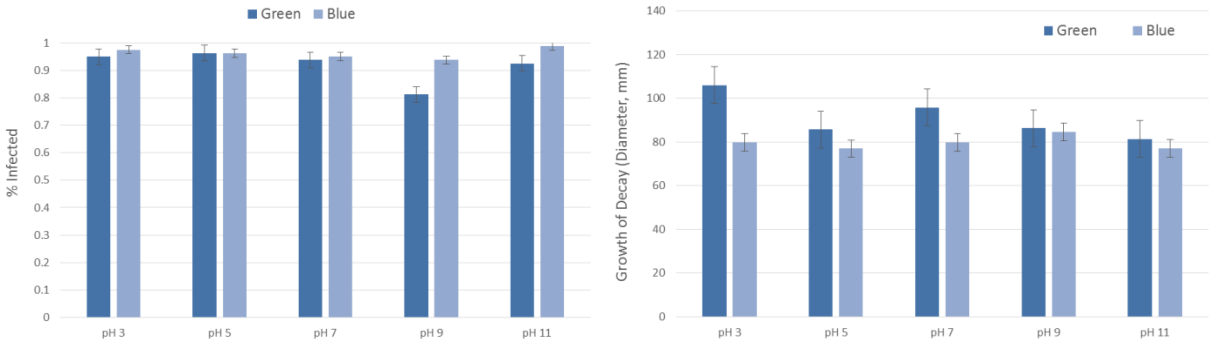


Figure 10. Effect of pH on the level of fruit infected with green and blue mould (left), and the growth of the green and blue mould (mm) (right). *Bars are standard deviations around the mean (n=4).*

Conclusion pH did not have any significant effect on the infection and growth of green and blue mould in infected oranges. These dips were conducted on fruit that had been infected with green and blue mould spores for 24 hours.

1.2.3 Assessment of OPP

The current form of sodium ortho-phenylphenol (SOPP) is registered in Australia as 'Preventol® ON' for the postharvest control of blue mould. The main activity of SOPP comes from direct contact with fungal spores on fruit or in solution and as a residue on fruit surfaces. Due to its widespread antimicrobial effects (it works on a wider range of pathogens than most chemical fungicides) it has seen resurgence in the USA where postharvest fungicide resistance is a problem (Cunningham, 2005). However SOPP needs to be maintained in its highly alkaline state for greatest effectiveness. Lower pH of the current form of SOPP has been known to adversely damage the skin of fruit causing phyto toxocity. While currently registered in Australia, industry use of SOPP has lost interest due to corrosive issues and the risk of fruit damage. However a new formulation 'Ortocil' from 'Citrosol' in Spain is

claimed to be more stable and does not cause rind damage with no corrosion to the packinghouse equipment. 'Ortocil' is a new formulation of ortho-phenylphenol (OPP) that is registered in Spain for the control of Penicillium sp. and is currently being used in major packinghouses in Spain, Egypt and Peru. This trial assessed the efficacy of 'Ortocil' in the control of green and blue mould.

Methods Green and blue mould infected oranges were treated with 'Ortocil' at two rates; 1% 'Ortocil' (1,000 ppm OPP) and 2% 'Ortocil' (2,000 ppm OPP) which were compared to standard thiabendazole (TBZ) fungicide treatment and untreated water control. In addition, all treatments were conducted at either 20 °C or 40 °C. There was 20 fruit in each treatment unit which were independently replicated four times. The data was analysed by one-way analysis of variance with the Tukey-Kramer test to determine significant differences.

Results The effects of the different postharvest treatments on the growth of green and blue mould are presented in Figure 11. The results show that the 2% 'Ortocil' solution treatment was more effective than 1% 'Ortocil' solution treatment. There was no difference in the different dip temperature treatments on decay growth (Figure 12). Both 'Ortocil' solution treatments were equivalent to the TBZ treatment, but there was some decay growth in the 1% 'Ortocil' solution treatment. The pH of the 'Ortocil' dips were pH 6.4.

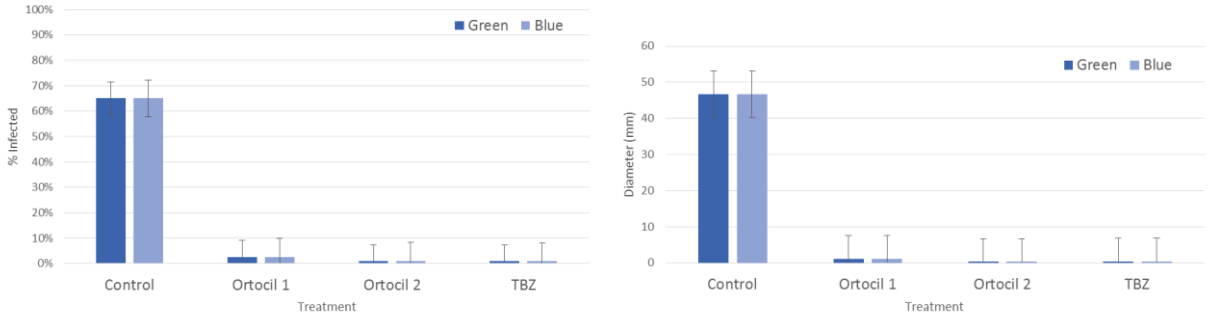


Figure 11. Effect of 'Ortocil' at 1% OPP ('Ortocil 1') and 2% OPP (Ortocil 2'), and thiabendazole (TBZ) at 20 °C on the infection rate and growth of green and blue mould. *Bars are standard deviations around the mean (n=4).*

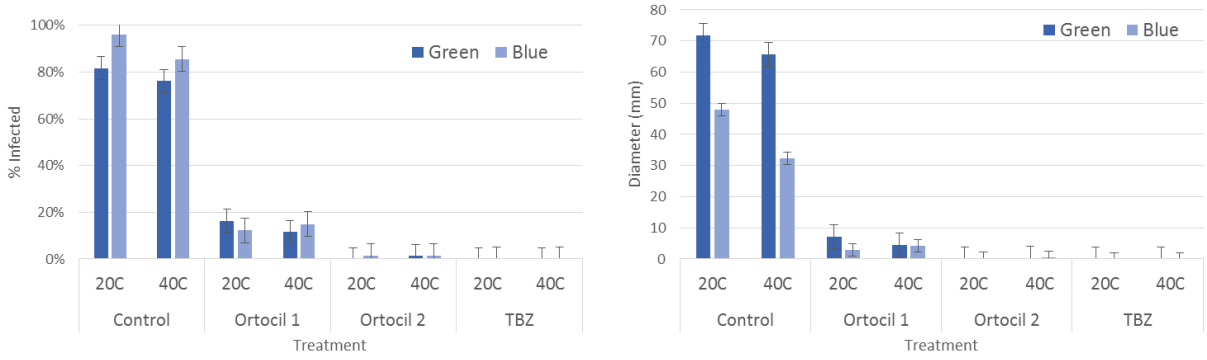


Figure 12. Effect of 'Ortocil' at 1% OPP ('Ortocil 1') and 2% OPP ('Ortocil 2'), and thiabendazole (TBZ) at two treatment dip temperatures (20 °C and 40 °C) on the infection rate and growth of green and blue mould. *Bars are standard deviations around the mean (n=4).*

Conclusion SOPP is currently registered in Australia as 'Preventol® ON' for the control of blue mould in citrus but this formulation can have issues with management and handling in the packingline and can also cause phyto toxicity. 'Ortocil' is a new formulation of OPP which is available in other citrus markets. In these experiments, this new OPP formulation showed good decay control and more work should be conducted to examine is wider efficacy in larger scale trials.

1.2.4 Assessment of 'Cerafruta'

'Cerafruta' is marketed by 'Ceradis' and contains an active ingredient with broad antifungal activity which is used in the food industry as a preservative. It has been used for decades on dairy products and is considered by the European Food Safety Authority as a food additive (E235). It is able to inhibit growth of fungi by specifically binding to ergosterol present in fungal cell membranes and has broad antifungal active against molds and yeast but not bacteria. It is extensively used worldwide including in Australia, especially in the cheese and sausage industries. It has Food Standards Australia New Zealand (FSANZ) approval for use as a food additive in the cheese and meat industries. In addition to food applications, it has also been used therapeutically (via topical application) to treat several types of fungal infections of the eye. The emergence of antimicrobial resistance, particularly through misuse of antibiotics, has been a major human health issue in recent years. Therefore the use of antimicrobial agents in foods will therefore, will need to be examined in light of any potential for antimicrobial resistance.

Onay et al. (2014) showed that postharvest application of natamycin on infected lemons reduced blue mould growth by 50% after 4 weeks and 80% decay of green mould after 2 weeks storage. They also conducted postharvest storage studies on fruit quality and concluded natamycin had some potential for postharvest decay control (Onay et al., 2014). A commercial formulation 'Cerafruta', was assessed for efficacy on green and blue mould.

Methods Green and blue mould infected oranges were treated with 'Cerafruta' and were compared to standard thiabendazole (TBZ) fungicide treatment and untreated water control. In the first experiment, these treatments were conducted at either 20 °C or 40 °C. There was 20 fruit in each treatment unit which were independently replicated 4 times. The data was analysed by one-way analysis of variance with the Tukey-Kramer test to determine significant differences. In a second experiment 'Cerafruta' was applied either 16 hours before infection with green mould, or + 6, 12, 24 or 48 hours after infection with green mould, and the levels of green mould were assessed.

Results The results of the first experiment are presented in Figures 13 and 14 and show that the 'Cerafruta' treatment had a significant effect on lowering the infection and growth rate of green and blue mould, as compared to the untreated control fruit. There was no effect of the different dip temperatures (20 °C and 40 °C) on the growth of blue and green mould with 'Cerafruta' treatment. The results of the second experiment are presented in Figure 15 and shows that the application of 'Cerafruta' was improved with a shorter time between inoculation / infection with green mould spores and 'Cerafruta' dip treatment.

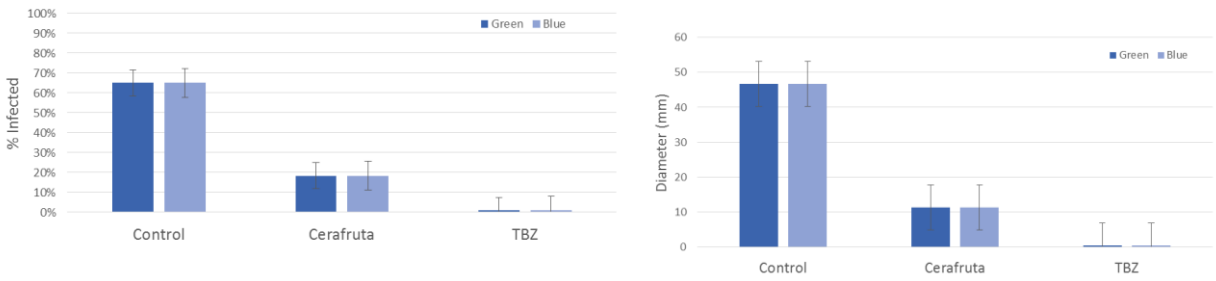


Figure 13. Effect of 'Cerafruta' treatment at 20 °C on the infection rate and growth of green and blue mould. *Bars are standard deviations around the mean (n=4).*

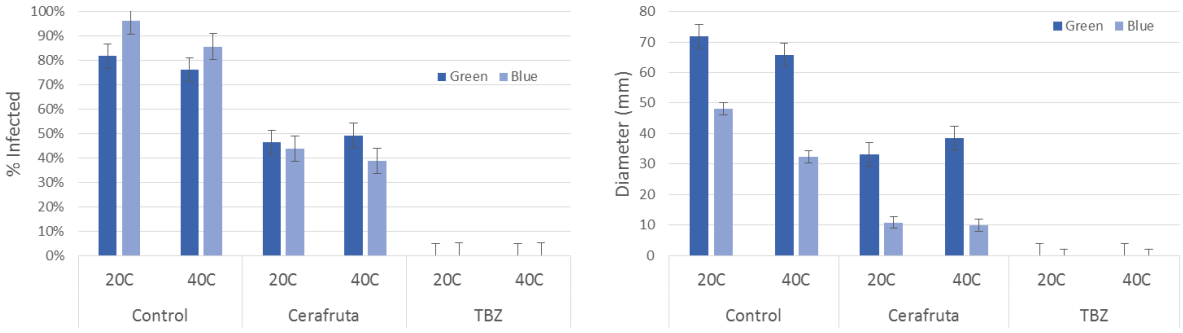


Figure 14. Effect of 'Cerafruta' at two treatment dip temperatures (20 °C and 40 °C) on the infection rate and growth of green and blue mould. *Bars are standard deviations around the mean (n=4).*

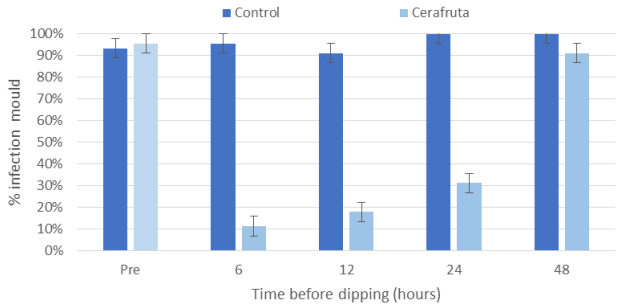


Figure 15. Effect of different timings on the effectiveness of 'Cerafruta' treatment on green mould inoculated oranges.

Conclusion The postharvest application of 'Cerafruta' reduced the infection and growth rate of green and blue mould in oranges. While not as effective as TBZ fungicide, it reduced green and blue mould infection and growth. The active ingredient of 'Cerafruta' is approved by FSANZ as a food additive, but it may have some regulatory barriers to its use in postharvest horticulture. In a recent review Dalhoff and Levy (2015) conclude that surface treatment with these chemicals are not of safety concern, however homogeneous mixture with food is, whereby resistance selective pressures may put the efficacy of polyenes as a class of life-saving antifungals at risk. Therefore close regulatory scrutiny will be required for potential registration as a postharvest treatment for the citrus industry.

1.2.5 Assessment of 'Propirly'

'Propirly 270 EC' is a contact and systemic emulsion concentrate fungicide containing the active ingredients propiconazole (120 g /L) and pyrimethanil (150 g /L). It is registered for use and is recommended in the South African citrus industry and marketed by ICA International Chemicals. It contains two postharvest fungicides which are already used in Australia, but in different combinations and products. This trial assessed the efficacy of 'Propirly' on the growth of green and blue mould in oranges. The activity of this new fungicide was compared to current fungicides, thiabendazole and 'Philabuster' (containing the active ingredients imazalil and pyrimethanil).

Methods Navel oranges were inoculated with green mould spores (as per *General Methods*). Fruit were then dipped in fungicide solutions at the following times at 20 °C: (1) dip at pre-inoculation (16 hours before inoculation), (2) 6 hours after inoculation, (3) 12 hours after inoculation, (4) 24 hours after inoculation and (5) 48 hours after inoculation. The fungicides assessed in this trial were; thiabendazole, 'Philabuster' and 'Propirly'. Dips were all at the recommended label rates. These treatments were compared to water-dip treated fruit (control

fruit). Each treatment was replicated three times where 15 fruit used for each replicate. The growth of the green mould from each of the inoculation point were assessed after 7 days at 20 °C.

Results In this preliminary experiment, 'Propirly' provided good control of green mould in Navel oranges for up to 48 hours after infection. Similarly, TBZ and 'Philabuster' also gave good control for up to 48 hours after infection (Figure 16).

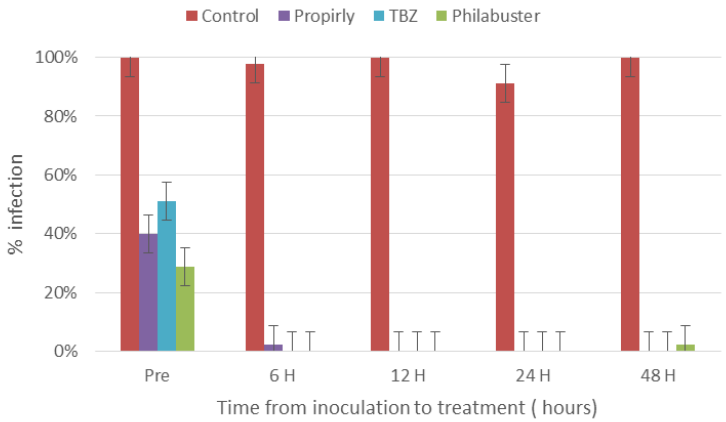


Figure 16. Effect of and 'Propirly', 'Philabuster' and thiabendazole (TBZ), with different dipping treatment times after initial infection on the subsequent number of fruit infected with green mould.
Bars are standard deviations around the mean (n=3)

Conclusion The new fungicide formulation 'Propirly' containing the active ingredients propiconazole (120 g/L) and pyrimethanil (150 g/L) was effective at controlling green and blue mould growth in oranges. Both of these different active ingredients are registered and used in Australia, but not in this combination. 'Propirly' contains propiconazole which has a proven ability to also control sour rot (McKay et al., 2012). If registered in Australia, the introduction of fungicide would give another decay control option for the Australian citrus industry.

1.2.6 Novel treatments to control postharvest decay

Plant extracts contain a range of secondary metabolites which may be involved in plant defense mechanisms such as decay control. Among the different classes of secondary metabolites, polyphenols have an important contribution to the antifungal activities of the plant extracts. A large amount of citrus by-products are annually generated by the juice industry and are a good source of polyphenols (Papoutsis et al., 2017). Phenolic compounds of citrus peel include phenolic acids and flavonoids which are usually found in conjugated forms.

Methods Lemon waste including peel, membranes and seeds were collected from a local commercial juicing factory on the NSW Central Coast. After seed removal, the remaining peels and membranes with a moisture content of 85% were freeze-dried for 48 hours as described by Papoutsis et al. (2017). The dried by-product was then ground using a commercial blender and the powder passed through a 1.40-mm mesh sieve and was used for the extraction. The effect of two lemon by-product aqueous extracts at different concentrations (14, 7, 3.5 and 1 mg mL $^{-}$1) were tested against the *in vitro* growth of *Alternaria alternata*. Prior to extraction, one batch of by-product was dehydrated by freeze-drying (untreated by-product), while the other batch was treated by microwave irradiation in conjunction with freeze-drying (microwave-treated by-product). High-performance liquid chromatography (HPLC) was employed for the identification of individual phenolic compounds with potent antifungal activities.

Results Both lemon by-product aqueous extracts inhibited the mycelial growth and suppressed the spore germination of the fungus in a concentration-dependent manner. In general, the extracts obtained from the microwave-treated lemon by-product displayed enhanced antifungal activity than those obtained from the untreated one. Scanning electron microscopy revealed that both lemon by-product extracts affected the hyphal morphology of the fungus (Figure 17). The antifungal activity of the extracts was attributed to their phenolic acid and ascorbic acid contents.

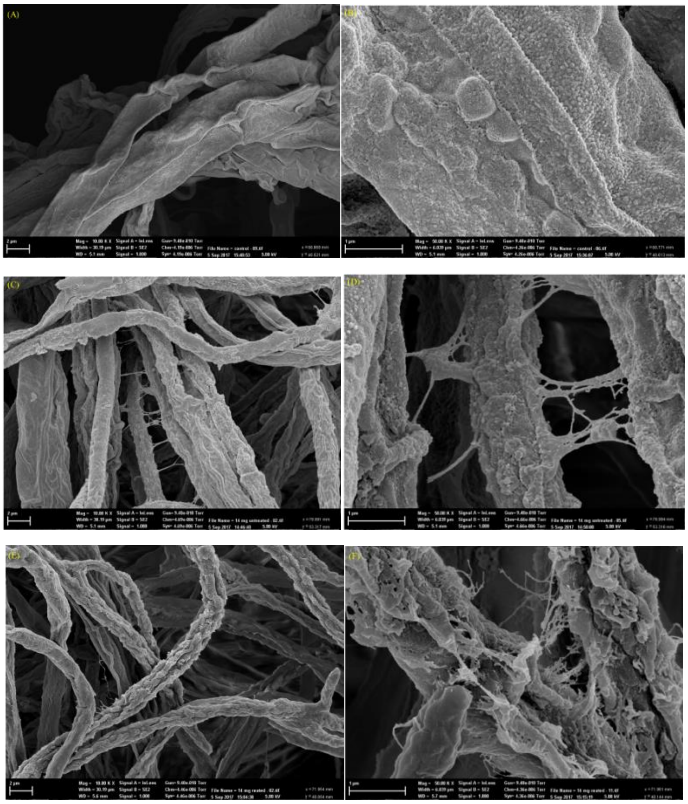


Figure 17. Scanning electron micrographs of *Alternaria alternata* hyphae. (A, B) Hyphae of untreated fungus (control); (C, D) Hyphae of fungus grown on ¼ PDA incorporated with extract (14 mg/ml) obtained from untreated lemon pomace; (E, F) Hyphae of fungus grown on PDA incorporated with extract (14 mg/ml) obtained from microwave treated lemon pomace. (Papoutsis et al., 2018)

This experiment was supported by the University of Newcastle and Australian Research Council (ARC) Training Centre for Food and Beverage Supply Chain Optimisation (IC140100032). This research was also supported by the Australian Citrus Postharvest Science Program (Horticulture Innovation. CT 15010) and acknowledged in the research paper.

This experiment was published in an international peer-reviewed paper:

Papoutsis K., Vuong Q.V., Tesoriero L., Pristijono P., Stathopoulos C.E., Gkountina S., Lidbetter F., Bowyer M.C., Scarlett C.J., Golding J.B. (2018) Microwave irradiation enhances the in vitro antifungal activity of citrus by-product aqueous extracts against *Alternaria alternata. International Journal of Food Science and Technology.* 53, 1510-1517.

1.3 Assessment of potential postharvest fungicides to manage anthracnose (*Colletotrichum gleosporioides*) in Imperial mandarins

While the major storage problem for citrus is green and blue mould, other postharvest conditions / decay issues can occur. Green and blue mould are wound pathogens and only infect fruit in an open wound which are generally caused with harvest and handling, but there are a range of latent diseases which can cause significant problems, particularly in wetter growing areas. Gas burn and anthracnose can be a major problem with Imperial mandarins in the market. Fruit are harvested, degreened and packed with no symptoms, but develop unsaleable dark patches on the skin during storage and marketing. Anthracnose caused by *Colletotrichum gloeosporioides* is believed to be the major problem for Queensland Imperial mandarins. Anthracnose grows on dead wood in the trees and spreads with rain splash onto the fruit. After the spores germinate on the fruit, they form a resting stage which allows them to remain dormant until an injury or degreening. The disease can be troublesome on early season fruit and in fruit that are degreened for over 24 hours.

'Graduate A+' is a postharvest fungicide which contains azoxystrobin and fludioxonil, and is registered for the control of some postharvest decay fungi in avocados. It is registered for the control of side rot caused by anthracnose (*Colletotrichum* spp.) and *Botryosphaeriaceous* fungi) and stem end rots (*Lasiodiplodia* sp., *Fusicoccum* spp., *Neofusicoccum* spp., *Colletotrichum* spp. and other *Botryosphaeriaceous* fungi). Its efficacy against anthracnose (*Colletotrichum* spp.) could be useful for Imperial mandarins. The presence of fludioxonil is already proven to control green and blue mould in citrus and is already registered and used in the Australian citrus industry, so this fungicide could be useful for Australian growers. In addition, other fungicides such as prochloraz, pyraclostrobin and benomyl (banned) have shown some efficacy against anthracnose. This experiment assessed the efficacy of a range of different postharvest fungicides for their potential against latent infections (anthracnose and stem end rot, etc.) in Imperial mandarins. The fungicides assessed in this experiment were; (1) 'Graduate A+' (active ingredient (a.i.) azoxystrobin and fludioxonil), (2) 'Chairman' (a.i. fludioxonil and propiconazole), (3) 'Cabrio' (a.i. pyraclostrobin), (4) 'Sportak' (a.i. prochloraz), (5) 'Tecto' (a.i. thiabendazole) which were compared to (6) control fruit with no treatment and (7) control fruit with a water dip. These fungicides (except 'Chairman' and 'Tecto') are not registered for use on citrus, but were trialed as a potential alternative for the postharvest management of anthracnose.

Methods Imperial mandarins with a history of susceptibility for *Colletotrichum* and stem end rot in previous seasons were obtained from a commercial grower in Queensland in May 2019. The fruit were harvested directly from with tree with no postharvest degreening or fungicide treatments and transported to NSW Department of Primary Industries at Ourimbah. Upon arrival, the fruit were dipped with: (1) 'Graduate A+' (a.i. azoxystrobin + fludioxonil), (2) 'Chairman' (a.i. fludioxonil and propiconazole), (3) 'Cabrio' (a.i. pyraclostrobin), (4) 'Sportak' (a.i. prochloraz), (5) 'Tecto' (a.i. thiabendazole) which were compared to (6) control fruit with no treatment and (7) control fruit with a water dip. After postharvest dipping, the fruit were allowed to dry and then degreened for either one day or four days (4 ppm ethylene at 25 ^{o}C and 70% RH). The treatment unit was 100 mandarin fruit and each treatment was independently treated four times, i.e. four replicates. Fruit were stored at 20 ^{o}C for four weeks. A separate sample of fruit was treated with a semi-commercial washer / brusher (four replicates) to examine if brushing induced increased damage. After storage, fruit were subjectively scored for anthracnose: 1 = no damage- good sound fruit; 2 = some detected – trace; 3 = <10% of fruit affected; 4 = 10 – 25% of fruit affected; and 5 = >25% of fruit affected (Figure 18).

Figure 18. Scoring standards for anthracnose - 1 = no damage (left fruit); 2 = some symptoms detected; 3 = <10% of fruit affected (middle fruit); 4 = 10 – 25% of fruit affected; and 5 = >25% of fruit affected (far right fruit) (left image) and assessment of mandarins for anthracnose symptoms after storage (right).

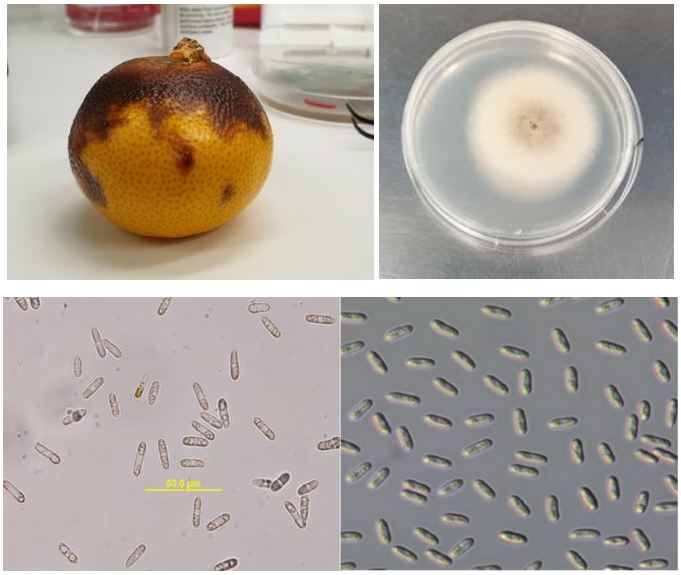


Figure 19. Typical severe symptoms of anthracnose damage on Imperial mandarins (top left). *Colletotrichum gloeosporioides* was isolated from this fruit and grown on PDA agar plates (top right), and the conidia (asexual chlamydospore) isolated from infected fruit (lower left) is compared to known *Colletotrichum gloeosporioides* conidia in citrus (*from* Rhaiem and Taylor, 2016) (lower right).

Results *Colletotrichum gloeosporioides* was isolated from the damaged peel tissue (Figure 19).

Effect of degreening The Imperial mandarins used in this trial were sourced from a commercial grower in Queensland on an orchard block with a history of postharvest skin damage after harvest. The fruit used in this trial were commercial maturity (10.8% TSS and 0.81% TA) with some semi-green colour in the peel. All Imperial mandarins at this time of year in Queensland (May) are treated with ethylene treatment to degreen the peel. The results showed that the length of degreening time significantly affected both the incidence of anthracnose symptoms observed in the fruit and the severity of the symptoms (Tables 1 and 2). Mandarins degreened for four days had higher incidence of fruit with anthracnose symptoms along with more severe symptoms of damage. Indeed the number of fruit with anthracnose damage was double in fruit degreened for four days (52% of fruit) when compared with fruit degreened for only one day (23% of fruit) (Table 1). Fruit degreened for four days were also more severely damaged (Table 2).

A side batch of this fruit were not degreened, but put over a high pressure washer with brushes for one minute. After storage, 71% of all mandarins had some visible anthracnose damage and the symptoms were significantly greater than observed in the non-brushed (and degreened) fruit.

Table 1. Effect of the duration of ethylene degreening on the incidence of anthracnose in Imperial mandarins (*averaged across all treatments*)

Treatment	Anthracnose infection (%)
1 day degreening	23 a
4 days degreening	52 b
brushed and washed	71 b

Table 2. Effect of the duration of ethylene degreening on anthracnose disease scores in Imperial mandarins (*averaged across all treatments*)

Treatment	Anthracnose severity score
1 day degreening	0.35 a
4 days degreening	0.96 b
brushed and washed	1.45 c

Treatments with the same letter indicate these treatments are statistically similar (p<0.05)
(0 = no damage, 1 = trace damage; 2 = <10% of fruit affected;
3 = 10 – 25% of fruit affected; and 4 = >25% of fruit affected)

Effect of fungicide treatments Anthracnose grows on dead wood in the trees and spreads with rain splash onto the fruit. After the spores germinate on the fruit, they form a resting stage which allows them to remain dormant until an injury or degreening. As with most storage trials with postharvest latent infections, such as anthracnose, there was a large level of variation in the expression of disease incidence and severity within and between treatments. However there was a significant interaction of the different fungicide treatment and degreening duration. The incidence (levels) of anthracnose infection for each of the fungicides and ethylene degreening durations is presented in Figures 20 and 21 and Table 3. The results show that after both one and four days degreening treatment, all the different postharvest fungicides had lower numbers of fruit with anthracnose symptoms, but due the inherent variability of the disease, these differences were not statistically different. However fruit treated with 'Cabrio' and degreened for four days had significantly lower numbers of fruit with anthracnose symptoms, than control fruit. In addition fruit treated with 'Graduate A+' and 'Cabrio' had significantly lower numbers of fruit with anthracnose symptoms than fruit dipped in water. An example of the anthracnose symptoms in Imperial mandarins treated with water (left) and 'Graduate A+' (right) before four days of degreening are presented in Figure 20 and show that the 'Graduate A+' fungicide treatment had lower incidence of fruit with anthracnose symptoms which also had less severe symptoms.

Figure 20. Anthracnose symptoms in Imperial mandarins after four days degreening and four weeks in storage at 20°C. Control fruit treated with water (left) had 72 % of the total fruit population showing some anthracnose symptoms with an average of severity score of 1.4), whereas fruit treated with 'Graduate A+' fungicide before degreening (right) had a total of 34% of the fruit that were infected with an average severity score of 0.5. (note this is only one replicate of the storage trail).

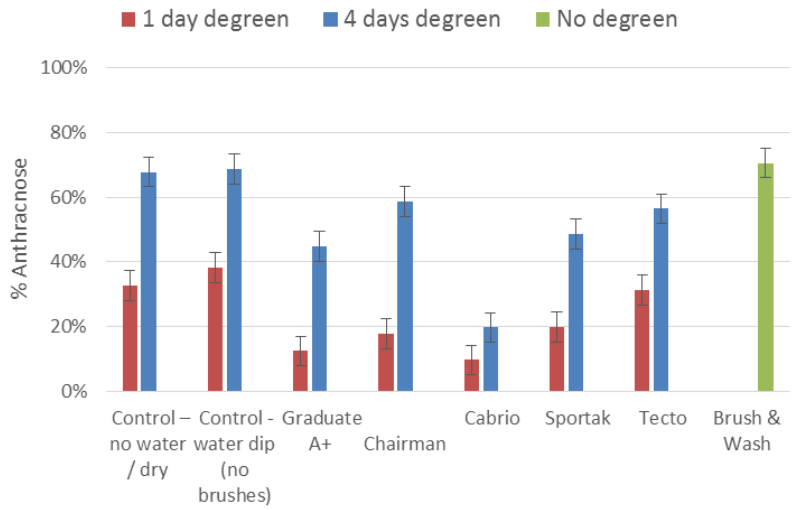


Figure 21. Percentage of Imperial mandarins with anthracnose symptoms following 4 weeks storage at 20 °C. *Bars are standard errors around the mean (n=4).*

Table 3. Average number of Imperial mandarins with anthracnose infection (%) following 4 weeks storage at 20 °C.

Treatment	Average anthracnose infection %
Brush and wash only	71 a
4 days degreen 2. Control - water dip (no brushes)	69 a
4 days degreen 1. Control – no water / dry	68 ab
4 days degreen 4. Chairman	59 abc
4 days degreen 7. Tecto	56 abc
4 days degreen 6. Sportak	49 abcd
4 days degreen 3. Graduate A+	45 bcd
1 day degreen 2. Control - water dip (no brushes)	38 cde
1 day degreen 1. Control – no water / dry	33 def
1 day degreen 7. Tecto	31 def
1 day degreen 6. Sportak	20 ef
4 days degreen 5. Cabrio	20 ef
1 day degreen 4. Chairman	18 ef
1 day degreen 3. Graduate A+	12 f
1 day degreen 5. Cabrio	10 f

Treatments with the same letter indicate these treatments are statistically similar (p<0.05)

The results presented in Figure 22 and Table 4 show that within the degreening times, all the postharvest fungicides tested in this experiment reduced the severity of the anthracnose symptoms, however these differences were often not statistically different. For example, there were no statistical differences in the severity of anthracnose symptoms following one day degreening treatment even though the levels of severity in 'Graduate A+' and 'Cabrio' (severity score 0.15-0.16) were much lower than the untreated control fruit (severity scores 0.57 / 0.58). However there were statistical differences observed in fruit that had been degreened for four days (Figure 22 and Table 4), where the postharvest application of 'Graduate A+' and 'Cabrio' significantly reduced the severity of anthracnose symptoms, as compared to the water control treatment in fruit that had been degreened for four days.

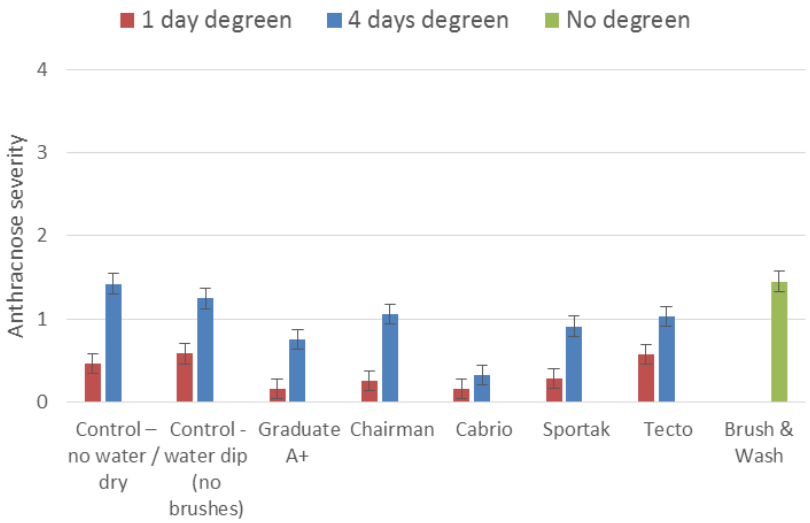


Figure 22. Severity score of anthracnose symptoms in Imperial mandarins following 4 weeks in storage at 20 °C. *Bars are standard errors around the mean (n=4).*

Table 4. Average anthracnose severity score of symptoms in Imperial mandarins following 4 weeks storage at 20 °C.

Treatment	Average anthracnose severity score
Brush and wash only	1.45a
4 days degreen 1. Control – no water / dry	1.42 a
4 days degreen 2. Control - water dip (no brushes)	1.24 ab
4 days degreen 4. 'Chairman'	1.06 abc
4 days degreen 7. 'Tecto'	1.03 abc
4 days degreen 6. 'Sportak'	0.91 abcd
4 days degreen 3. 'Graduate A+'	0.75 bcde
1 day degreen 2. Control - water dip (no brushes)	0.58 cde
1 day degreen 7. 'Tecto'	0.57 cde
1 day degreen 1. Control – no water / dry	0.46 cde
4 days degreen 5. 'Cabrio'	0.32 de
1 day degreen 6. 'Sportak'	0.28 e
1 day degreen 4. 'Chairman'	0.25 e
1 day degreen 3. 'Graduate A+'	0.16 e
1 day degreen 5. 'Cabrio'	0.15 e

Treatments with the same letter indicate these treatments are statistically similar (p<0.05)

Conclusions As with most storage trials with postharvest latent infections, such as anthracnose, there was a large level of variation in the expression of disease incidence and severity within and between treatments. This inherent variability made detecting statistical differences between the different treatments more difficult. The application of the postharvest fungicides; 'Graduate A+' (a.i. azoxystrobin + fludioxonil), 'Chairman' (a.i. fludioxonil and propiconazole), 'Cabrio' (a.i. pyraclostrobin), 'Sportak' (a.i. prochloraz) and 'Tecto' (a.i. thiabendazole) lowered the incidence and severity of anthracnose symptoms, but these differences were often not statistically different. However some significant differences were detected between the different degreening and postharvest fungicide treatments. The duration of the degreening time significantly affected both the incidence of anthracnose

symptoms observed in the fruit and the severity of the symptoms, where longer ethylene degreening (four days) resulted in a much higher incidence of fruit with anthracnose symptoms and greater area of peel with damage symptoms. This result is in agreement with general observations with anthracnose damage, whereby longer degreening times result in greater incidence of fruit with symptoms and more severe peel damage. These results also confirmed that physical brushing of the fruit also increased the incidence and severity of anthracnose and illustrate the need to reduce postharvest damage with softer brushes / rollers.

Of the different postharvest fungicides, treatment of the fruit 'Cabrio' and 'Graduate A+' resulted in significantly less fruit with anthracnose and lower severity of anthracnose symptoms after four days degreening. These results are encouraging and more work is recommended to continue this screening of fungicides as postharvest treatments to control anthracnose. However 'Cabrio' is only registered as a pre-harvest (field) application for the pathogen control, but 'Graduate A+' and 'Sportak' are already registered for use as a postharvest treatment in avocados. 'Tecto' is widely used in the citrus industry to control green and blue mould and stem end rot (*Phomopsis citri*), while 'Chairman' has been recently registered to control green and blue mould and sour rot (*Galactomyces citri-auranti*). The use of these already registered citrus postharvest fungicides may also have additional advantages in reducing anthracnose symptoms. More research work is required in this area.

2. Fungicide resistance survey and management

Introduction Postharvest fungicides are an important method to control the development of decay during handling and marketing. However postharvest fungicides can sometimes fail to work due to the development of resistance by the decay fungi to the fungicide. Fungicide resistance is a serious and important postharvest problem which needs to be actively managed in the packinghouses to minimise any potential losses.

Fungicide resistance can occur in packinghouses and cool rooms with poor hygiene and the ongoing use of the same postharvest fungicide. Resistance arises when a single decay fungi spore that is resistant to a fungicide multiplies. The continued selection of these resistant spores can happen when the same fungicide is used and these spores can multiply and full resistance develops (Figure 23).

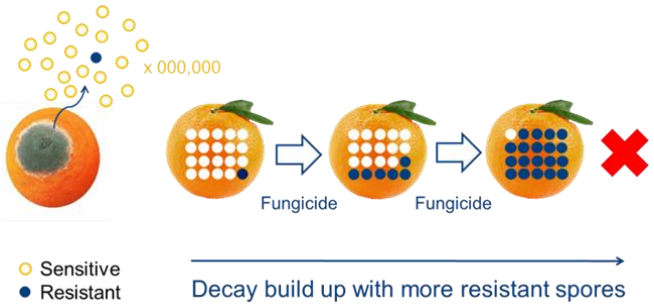


Figure 23. Selection of resistant decay spores with continual exposure to fungicides with the same mode of action leads to the development of resistant decay.

Packinghouse surveys to assess the sanitation and presence of technical resistance to postharvest fungicides in decay causing fungi

Methods Surveys to assess the sanitation and presence of technical resistance were conducted in packinghouses around Australia in 2017 and 2018 seasons. Packinghouses were sent test kits which were put out into the packingline and returned to NSW DPI for assessment and reporting back to the grower. Reports were returned to the grower with recommendations to improve outcomes. This information was confidential to the grower but anonymised data was collated and presented in this Report.

Agar petri dishes with different fungicides were exposed in packinghouses during the main packing season, to estimate the levels of fungicide resistance present. The plates were placed at the start of the packing line, the end of the packing line, and in the cool room. The postharvest fungicides used were; thiabendazole (TBZ) ('Vorlon' or 'Tecto'), imazalil ('Magnate' or 'Fungaflor') and fludioxonil ('Scholar'). If decay spores in the air of the packinghouse landed on the plates with fungicide and then grew, then it was considered there was some technical resistance to that fungicide.

Results

Packinghouse by packinghouse hygiene comparison

Results show that there are large differences between packinghouses. An example is presented in Figure 24 and illustrates that the plates that were sampled from Packinghouse 1 had very few fungi spores, even untreated plates had few spores indicating this packinghouse had good hygiene practices (Table 5). In addition, there was no evidence of any fungicide resistance, with no fungi growing on the plates with fungicide. This is a very good result for this grower and demonstrates the effects of good sanitation and fungicide use. In contrast, the results of Packinghouse 2 showed relatively high levels of decay-causing fungi spores in the untreated plates indicating relatively poor packinghouse hygiene (Figure 25 and Table 5). In addition, some technical resistance to TBZ and imazalil fungicides was detected, particularly against TBZ. The detection of technical resistance is a concern and management of packinghouse hygiene and resistance was required.

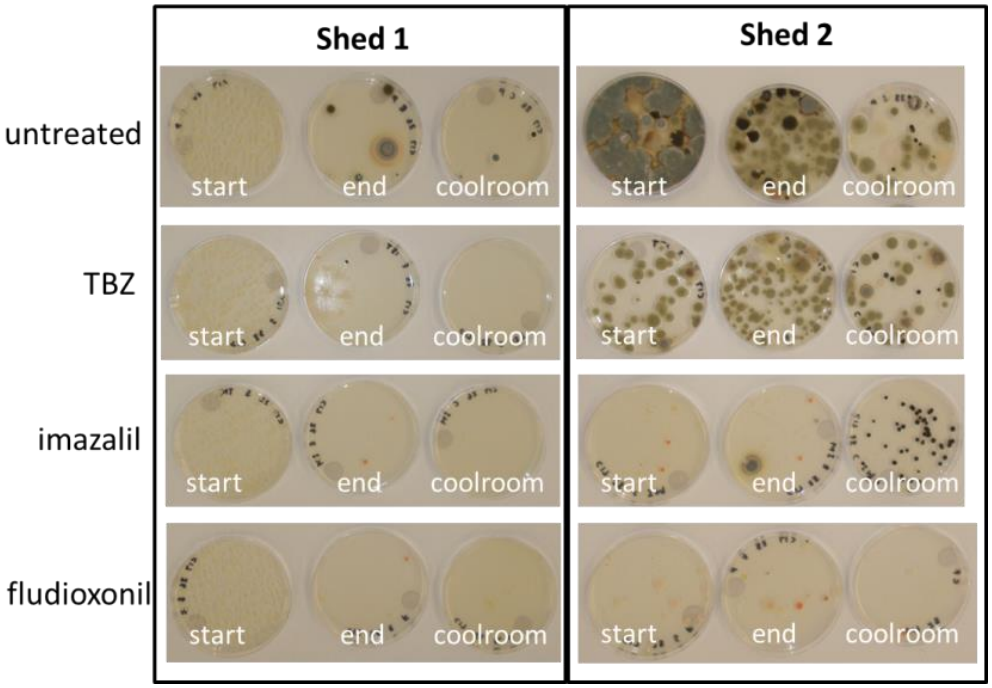


Figure 24. Examples of fungicide resistance survey results from two different packinghouses. Agar plates which were amended with different fungicides (TBZ, imazalil and fludioxonil) were put out into packinghouses at the start of the line (left), end of the line (middle) and in the coolroom (right).

	Packinghouse 1			Packinghouse 2		
	Start	End	Coolroom	Start	End	Coolroom
Untreated (#colonies)	0	4	2	35	+50	17
TBZ resistance	very low	very low	very low	high	high	high
Imazalil resistance	very low	very low	very low	very low	low	very low
Fludioxonil resistance	very low	very low	very low	very low	very low	very low

Table 5. The number of decay causing fungi colonies on the untreated plates is a measure of the hygiene in the packinghouses. The levels of technical resistance are estimated by the number of fungi colonies that grow on plates with added fungicide.

2.1 2017 Packinghouse survey

The results of all the different packing houses around Australia in 2017 showed there was a high level of variability in the management of hygiene and levels of technical resistance between the different packinghouses (Table 6). The results showed very little evidence of resistance to fludioxonil, as this fungicide is not widely used by industry. However there were some packinghouses which had significant technical resistance to TBZ. The management to minimise fungicide resistance requires a whole-of-system approach, starting from harvest through to packing and storage.

Table 6. Summary table of the 2017 sanitation and fungicide resistance packinghouse survey.

		Untreated				TBZ				Fludioxonil			
	Shed	S	E	C	T	S	E	C	T	S	E	C	T
NSW	A	4	4	4	12	4	2	2	8	1	1	1	3
NSW	B	4	3	1	8	2	1	1	4	1	1	1	3
NSW	C	3	4	2	9	1	2	1	4	1	1	1	3
NSW	D	4	4	2	10	2	2	2	6	1	1	1	3
NSW	E	4	4	2	10	1	2	1	4	1	1	1	3
Qld	A	4	4	3	11	2	1	1	4	1	1	1	3
Qld	B	4	4	3	11	2	2	1	5	1	1	1	3
Qld	C	4	4	3	11	2	2	1	5	1	1	1	3
Qld	D	4	4	2	10	1	1	1	3	1	1	1	3
SA	A	4	4	4	12	2	2	2	6	1	1	1	3
SA	B	2	4	2	8	1	1	1	3	1	1	1	3
SA	C	4	3	2	9	2	2	1	5	1	1	1	3
SA	D	4	4	3	11	4	4	4	12	1	1	1	3
SA	E	4	4	2	10	2	4	2	8	1	1	1	3
SA	F	4	4	2	10	1	2	1	4	1	1	1	3
Vic	A	4	4	2	10	1	2	1	4	1	1	1	3
Vic	B	4	4	3	11	3	2	1	6	1	1	1	3
Vic	C	4	3	2	9	2	1	1	4	1	1	1	3
Vic	D	2	2	2	6	1	2	1	4	1	1	1	3
Vic	E	4	4	3	11	2	2	2	6	1	1	1	3
WA	A	4	4	2	10	1	1	1	3	1	1	1	3
WA	B	4	4	2	10	2	1	1	4	1	1	1	3
WA	C	4	4	4	12	1	2	1	4	1	1	1	3
WA	D	4	4	4	12	4	4	1	9	1	1	1	3

S = Start of the line, E = End of the line, C = Coolroom, T = Total score for all different packinghouse locations (S + E + C). Untreated (control) plates measured hygiene where a score of 1 =very low spore levels, 2 = low spore levels, 3 = moderate spore levels, 4 = high spore levels. For TBZ and fludioxonil amended plates a score of 1 = very low resistance detected, 2 = low levels of resistance detected, 3 = moderate levels of resistance detected, and 4 = high levels of resistance detected.

2.2 2018 Packinghouse survey

A summary of the results of the 2018 survey is presented in Table 7. The results show there were large differences in sanitation and technical resistance between the different packinghouses around Australia. Some packinghouses had excellent hygiene and sanitation with very few decay causing fungi detected, while other packinghouses had very high levels of decay causing fungi both in the packinghouse and in the coolroom. In general the highest levels of decay causing fungi were detected at the start of the line. This is not unexpected as this is where the fruit is dumped from the orchard. However it is important to improve hygiene and reduce the numbers of decay causing fungi in all areas of the packinghouse and particular attention should be made in this area, as these spores can remain in the packinghouse and coolroom and be a risk for decay and resistance development.

A comparison of the 2018 results from the 2017 season showed there had been some reductions in the levels of total decay causing spores in the packinghouses and coolrooms in 2018. This is an excellent result and shows that packers have been active in their sanitation programs, particularly in the coolrooms, where total decay causing fungi detections were significantly lower. This lower pressure in the number of decay causing spores reduces the risk of decay and development of resistance. Technical resistance to the postharvest fungicide, TBZ was detected in most packinghouses around Australia. Over half of the samples assessed had technical resistance to TBZ, whilst over 20% of samples at the start of the packingline, had very high levels of technical resistance to TBZ (Table 7). These levels of technical resistance to TBZ are a concern and improvements in packinghouse hygiene and rotation of fungicides is recommended.

Table 7. Summary table of the 2018 sanitation and fungicide resistance packinghouse survey.

		Untreated				TBZ				Imazalil				Fludioxonil			
	Shed	S	E	C	T	S	E	C	T	S	E	C	T	S	E	C	T
NSW	A	1	2	2	5	4	1	1	6	1	1	1	3	1	1	1	3
NSW	B	4	1	1	6	1	2	1	4	1	1	1	3	1	1	1	3
NSW	C	4	4	1	9	2	2	1	5	1	1	1	3	1	1	1	3
NSW	D	4	4	4	12	1	1	1	3	1	1	1	3	1	1	1	3
NSW	E	4	4	1	9	2	1	1	4	1	1	1	3	1	1	1	3
NSW	F	1	1		3	1	1		3	1	1		3	1	1		3
NSW	G	4	4	2	10	1	2	2	5	1	1	1	3	1	1	1	3
NSW	H	2	3	1	6	1	3	4	8	1	1	1	3	1	1	1	3
NSW	I	4	4	1	9	1	3	1	5	1	1	1	3	1	1	1	3
Average		3	3	2	8	2	2	2	6	1	1	1	3	1	1	1	3
Qld	A	4	2	2	8	2	1	1	4	1	1	1	3	1	1	1	3
Qld	B	1	1	1	3	1	1	1	3	1	1	1	3	1	1	1	3
Qld	C	4	3	2	9	4	4	4	12	1	1	1	3	1	1	1	3
Qld	D	4	4	4	12	4	2	2	8	1	1	1	3	1	1	1	3
Qld	E	3	3	2	8	2	2	1	5	1	1	1	3	1	1	1	3
Qld	F	1	1		3	1	1	1	3	1	1	1	3	1	1	1	3
Qld	G		1	1	3	1	1	1	3	1	1	1	3	1	1	1	3
Qld	H	1	1	1	3	1	1	1	3	1	1	1	3	1	1	1	3
Qld	I	1	1	1	3	2	2	3	7	1	1	1	3	1	1	1	3
Average		2	2	2	6	2	2	2	6	1	1	1	3	1	1	1	3
WA	A	4	2		7	1	1		3	1	1		3	1	1		3
WA	B	4	4	3	11	1	1	2	4	1	1	1	3	1	1	1	3
WA	C	4	3	1	8	4	3	1	8	1	1	1	3	1	1	1	3
WA	D	4	4	2	10	4	4	3	11	1	1	1	3	1	1	1	3
WA	E	4	3	1	8	4	4	1	9	1	1	1	3	1	1	1	3
Average		4	3	2	9	3	3	2	8	1	1	1	3	1	1	1	3
SA	A	3	3	4	10	1	4	3	8	1	1	1	3	1	1	1	3
SA	B	1	1	1	3	1	2	1	4	1	1	1	3	1	1	1	3
SA	C	4	3	1	8	2	2	1	5	1	1	1	3	1	1	1	3
SA	D	4	4	1	9	2	3	1	6	1	1	1	3	1	1	1	3
SA	E	4	3	2	9	4	4	4	12	1	1	1	3	1	1	1	3
SA	F	2	1	1	4	1	1	1	3	1	1	1	3	1	1	1	3
SA	G	4	1	1	6	3	3	1	7	1	1	1	3	1	1	1	3
SA	H	4	4	4	12	4	4	4	12	1	2	1	4	1	1	1	3
SA	I	3	1	1	5	1	1	1	3	1	1	1	3	1	1	1	3
SA	J	2	1	1	4	1	1	1	3	1	1	1	3	1	1	1	3
SA	K	3	3	1	7	2	2	2	6	1	2	1	4	1	1	1	3
SA	L	2	2	1	5	3	4	3	10	1	1	1	3	1	1	1	3
SA	M	3	4	3	10	4	4	4	12	2	1	1	4	1	1	1	3
SA	N	4	3	3	10	1	1	1	3	1	1	1	3	1	1	1	3
SA	O	4	3	1	8	1	3	1	5	1	1	1	3	1	1	1	3
SA	P	4	4	1	9	3	4	1	8	1	1	1	3	1	1	1	3
Average		3	3	2	8	2	3	2	7	1	1	1	3	1	1	1	3
Vic	A	3	3	1	7	2	4	1	7	2	1	1	4	1	1	1	3
Vic	B	3	1	1	5	2	2	1	5	1	1	1	3	1	1	1	3
Vic	C	1	3	2	6	2	3	2	7	2	2	1	5	1	1	1	3
Vic	D	3	3	2	8	3	3	2	8	1	2	2	5	1	1	1	3
Average		3	3	2	8	2	3	2	7	2	2	1	4	1	1	1	3

S = Start of the line, E = End of the line, C = Coolroom, T = Total score for all different packinghouse locations (S + E + C). The maximum (worst) total score is 12. (The lower the number = the better the result). Untreated (control) plates measured hygiene, where a score of; 1 =very low spore levels, 2 = low spore levels, 3 = moderate spore levels, 4 = high spore levels. For TBZ, imazalil and fludioxonil amended plates, a score of; 1 = very low resistance detected, 2 = low levels of resistance detected, 3 = moderate levels of resistance detected, 4 = high levels of resistance detected.

The uses of other postharvest fungicides (such as imazalil and fludioxonil) with other modes of action against green and blue mould are widely used and essential to help manage postharvest decay. However this survey also showed some packinghouses had low levels of technical resistance to imazalil in some packinglines and coolrooms. Although this was an un-common observation (<8 % samples), this was a big concern and needs to be eliminated. It is crucial to maintain the effectiveness of postharvest fungicides such as imazalil which is the mainstay of postharvest fungicides. Fortunately no technical resistance to fludioxonil was detected in this Australia-wide survey, but this was probably because this fungicide is not widely used by industry.

The management of resistance to postharvest fungicides requires a whole-of-system approach, starting from harvest through to packing and storage. Some of key management factors have been described in previous '*Australian Citrus News'* articles (Summer 2017) and in the '*Packer Newsletters'*.

An example of the efficacy of good cleaning and sanitation activities is presented in Table 8, which shows the results of different packinghouses at different sampling times. The packinghouses were sampled during the packing season (Time 1) and also after cleaning and improvements in hygiene (Time 2). The results showed a decrease in the total number of decay causing fungi and levels of technical resistance to TBZ in all packinghouses, particularly in Packinghouses A and C. This shows that with both monitoring and targeted cleaning and sanitation, the risk of the failure of decay management can be minimised.

Table 8. Comparison of the results of untreated, TBZ, imazalil and fludioxonil amended plates both before (Time 1) and after intensive sanitation (Time 2) from the same packinghouses. There were three different packinghouses (A, B and C) assessed at two different sampling times (Time 1 and 2). The scores are the same as described in Table 1.

		Untreated				TBZ				Imazalil				Fludioxonil			
Shed	Time	S	E	C	T	S	E	C	T	S	E	C	T	S	E	C	T
A	1	3	3	4	10	1	4	3	8	1	1	1	3	1	1	1	3
A	2	3	1	1	5	1	1	1	3	1	1	1	3	1	1	1	3
B	1	4	4	2	10	4	4	3	11	1	1	1	3	1	1	1	3
B	2	4	3	1	8	4	4	1	9	1	1	1	3	1	1	1	3
C	1	4	3	2	9	4	4	4	12	1	1	1	3	1	1	1	3
C	2	1	1	1	3	2	2	3	7	1	1	1	3	1	1	1	3

Conclusion The results of the two seasons of packinghouse surveys showed there were significant issues with poor postharvest sanitation and the development of technical resistance to common postharvest fungicides. The surveys identified similar issues in all states and growing regions. An active extension program to address these issues was conducted with presentations at regional and national citrus forums, articles in major grower / packer magazines (*Australian Citrus News, Citrus e-News,* NSW DPI *Citrus Connect, Australian Tree Crop* etc.). These are listed in the Extension activities outputs (*Section 4* – below).

Recommendations The Project Reference Committee recommended that this sanitation and fungicide resistance survey become more accessible and available for growers on demand and recommended this become a fee for service. Outside of this Program, this was then developed by NSW Department of Primary Industries and Citrus Australia as a not-for-profit service to industry. This is now available for industry through the Citrus Australia postharvest web page: https://citrusaustralia.com.au/growers-industry/citrus-postharvest-sanitation-and-fungicide-resistance-service.

This service is not supported and has no association with Hort Innovation. No Program time or funding was used to support the development or running of this service.

3. Improved use of sanitising treatments

Sanitation is essential to reduce the number of decay-causing spores and reduce the risk of postharvest decay and development of fungicide resistance to postharvest fungicides. Sanitisers have a non-specific mode of action and controls fungi, bacteria, yeasts, where fungicides are specific to control fungus (e.g. blue and green mould) and can control fungus during storage, even if fungus is present. Each fungicide controls specific decay fungi while sanitisers control a range of microbial pathogens in the water. Pretreatment or washing of fruit with water containing a sanitiser is essential to minimise the risk of bacterial pathogens and reduce the fungal spores.

A number of sanitisers with different chemistries are currently available in the market and registered like fungicides with APVMA. Each sanitiser has its own merits and demerits of adoption. The selection of sanitiser is influenced by a number of factors such as price, water source (channel, ground or council) and quality (pH, hardness), method of application and monitoring (manual vs. automatic), packing line machinery (corrosiveness) and buyer's requirement etc. Following the label guidelines and manufacturers' instructions can ensure correct and safe use of sanitisers at recommended concentrations and conditions.

Chlorine has been a very popular sanitiser for years. Chlorine-based sanitisers include sodium hypochlorite, calcium hypochlorite, bromo-chloro-dimethylhydantoins and chlorine dioxide.

For the effective use of sodium and calcium hypochlorites, an understanding of the basic chemistry of chlorine is the key to manage its active concentration in wash water. When chlorine is dissolved / added to water, it dissociates into hypochlorous acid and hypochlorite ions, depending upon pH. The hypochlorous acid is the most active form and its higher proportion over hypochlorite ions is required for proper sanitation. A pH range of 6.5 to 7.0 is favourable for achieving higher concentration of active chlorine (hypochlorous) and with the increase in pH (>7.5) much less active form (hypocholorite) predominates.

If wash water is alkaline (pH >7.0), the addition of chlorine will further increase the pH, leading to poor antimicrobial efficacy of the chlorine present in water. This will require injection/addition of hydrochloric acid to decrease the pH (6.5- 7.5) for chlorine to be effective. A low pH (<5.0) combined with high water temperature will lead to release of poisonous chlorine gas from chlorinated water which is an occupational health hazard for workers. In nutshell, the pH of wash water after addition of chlorine should be maintained between 6.5 and 7.5.

The use of chlorine in wash water requires more efforts and management in monitoring the pH, chlorine concentration and sanitation potential of water. In addition to the pH, organic load also impacts the efficacy of chlorine because active chlorine gets neutralised due to its reaction with debris and dirt present in water.

Effective monitoring of sanitiser concentration and efficacy is utmost important. Chlorine concentration and pH can be monitored using paper test strips which are easily available from chemical suppliers, but digital equipment is more accurate and objective in measurement. Another indirect measure of sanitising capacity of water is oxidation-reduction potential (ORP) which can be recorded by using an ORP probe. An ORP reading of >700mV suggests the acceptable levels of sanitation potential. However, the ORP reading should be interpreted along with wash water pH and proper chlorine concentration. Some companies supply a digital meter with two or more probes to measure pH and ORP simultaneously. These monitoring tools are not expensive and are highly recommended for packinghouses to measure and record data accurately.

Bromo-chloro-dimethyl hydantoins (BCDH) are another option as a sanitiser registered with APVMA. These sanitisers also provide broad spectrum control of microbial pathogens. They represent a unique blend of chlorine and bromine chemistries which provide sanitising activity in a broad pH range especially towards alkaline conditions (pH 7.0 to 8.5). The basic chemistry of bromine in water is quite similar to chlorine, except the active form of bromine (hypobromous acid) predominates until pH 8.5 opposed to pH 7.5 for chlorine. It is also less affected by organic load compared to chlorine and relatively lower concentrations are required.

Chlorine dioxide is another form of chlorine sanitiser which is currently available in different formulations with stabilized active ingredient. Because of its very high reactivity, a low concentration (up to 10 ppm) and less contact time is required for wash water treatment and is effective against bacteria, moulds and yeast over a pH range up to 8.5. Organic matter in water does not affect the efficacy of chlorine dioxide.

There is a shift towards peracetic acid (PAA) based sanitisers which is driven by environmental impacts of chlorine byproducts and management issues associated with chlorine. Peracetic acid (PAA) is very reactive and quickly decomposes to oxygen, water and acetic acid (acid in vinegar). The use of PAA at recommended concentration (up to 80 parts per million) is effective in reducing microbial load and achieving product sanitation. Unlike chlorine, it is

effective over a wide range of pH and requires less management. However, the effective monitoring and recording of PAA concentrations in wash water is critical as for any other sanitiser and can be done using test strips. The corrosiveness of PAA should be considered for machinery and appropriate protection of workers.

Iodine is an effective sanitiser for water treatment over a wide range of pH with minimal effect of organic load. But the options to adoption are limited due to its availability as a dosing system only. It is corrosive to metals and expensive. To our knowledge, a large citrus packinghouse in Riverina, NSW is still using iodine as a sanitiser.

Ozone is a gaseous sanitiser which can be dissolved in wash water by generating it on-site using an ozone generator. Undoubtedly, ozone is very effective in killing microbial pathogens over a wide range of pH, but it also reacts with organic matter. Its highly corrosive nature and high risks to workers health and safety are among the limitations which have though not hindered its use in some packing houses.

In this section a range of different sanitisers and different sanitation systems were investigated:

1. Effect of common sanitisers on postharvest decay
2. Comparative efficacy of new electrolysed water sanitiser system
3. Evaluation of alternative sanitation treatments – 'ChillSafe'

3.1 Effect of common sanitisers on postharvest decay

Sanitisers are very reactive where they kill pathogens in water on contact but once they are inactivated there is no residual protection. In this trial, the effect of different sanitisers were compared for their effects on the development of postharvest decay in the fruit. Sanitisers are not used for this purpose, as they only clean the water, but this trial examined with effectiveness on green and blue mould in oranges.

Part A. Effect of 'Tsunami' sanitiser of Penicillium infection

Methods Navel oranges were inoculated with and blue green mould spores (as per *General Methods*). After 24 hours after inoculation, the fruit were dipped in 'Tsunami' (peroxyacetic acid and hydrogen peroxide) at the recommended label rates. This was compared to imazalil fungicide at the recommended rate and also compared to water-dip treated fruit (control fruit). Each treatment was replicated three times where 20 fruit used for each replicate. The growth of the green and blue mould infection was determined after 7 days at 20 ^{o}C.

Results The level of infection in the control fruit dipped only in water was >95% infection for both green and blue mould. Imazalil fungicide controlled both fungi (Figure 25). The incidence of fruit with green mould infection following 'Tsunami' treatment was <50% and blue mould was 90%. This shows that treatment with 'Tsunami' 24 hours post-infection reduced green mould infection, but was not effective for blue mould (Figure 25).

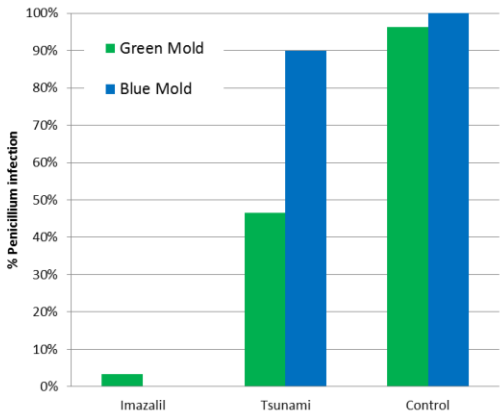


Figure 25. Effect of 'Tsunami' and imazalil treatment 24 hours after infecting oranges with green and blue mould.

Part B. Effect of timing on efficacy of sanitation treatment

Methods Navel oranges were inoculated with green mould spores (as per *General Methods*). Fruit were then dipped in sanitisers at 20 °C at the following times: (1) dip at pre-inoculation (16 hours before inoculation), (2) 6 hours after inoculation, (3) 12 hours after inoculation, (4) 24 hours after inoculation and (5) 48 hours after inoculation. The sanitisers assessed were; chlorine and 'Tsunami' (peroxyacetic acid and hydrogen peroxide) at the recommended label rates. These sanitizing treatments were compared to TBZ fungicide at the recommended rate and also compared to water-dip treated fruit (control fruit). Each treatment was replicated three times where 15 fruit used for each replicate. The growth of the green mould from each of the inoculation point were assessed after 7 days at 20 °C.

Results This trial examined the effect of different timings of the sanitation treatments after infection with green mould. The effect of postharvest treatment with chlorine and 'Tsunami' are presented Figure 26. The results show that chlorine treatment had no effect on the level of postharvest decay, while postharvest 'Tsunami' treatment reduced the level of green mould infection. As expected the fungicide TBZ controlled the green mould.

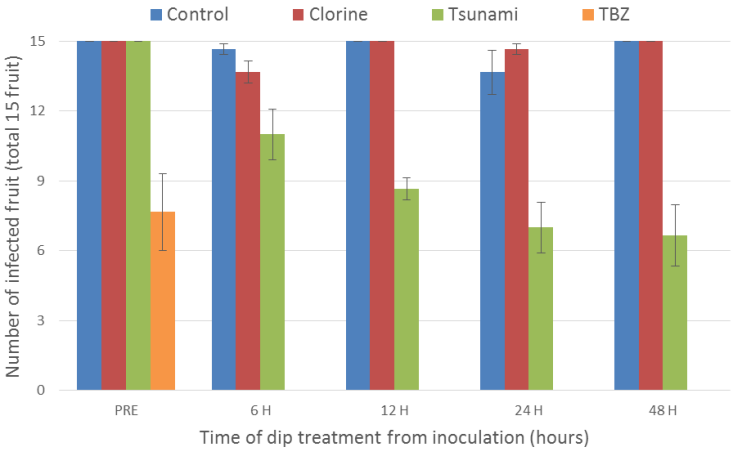


Figure 26. Effect of sanitisers and their timing on subsequent level of green mould infection.

Conclusions While the purpose of postharvest sanitisers is to sanitise the wash water, this experiment showed that 'Tsunami' (peroxyacetic acid and hydrogen peroxide) had some ability to reduce the growth of green mould in oranges post-infection.

3.2. Comparative efficacy of new electrolysed water sanitiser system

Electrolysed water (EW) is a new sanitation system which is used by other industries, such as waste water treatment. EW is produced by the electrolysis water containing dissolved sodium chloride. The electrolysis of such solutions produces a range of chemical species such as hypochlorous acid and highly reactive species. This trial examined the effect of different measurable chlorine levels in Unipolar® EW system against the standard sanitation treatments on development of green and blue mould. Unipolar Water Technologies Pty Ltd supplied and ran the EW system for this trial (Figure 27). This trial was conducted in two parts: (1) treatments in commercial packingline, and (2) treatments in controlled conditions.

Methods In addition to regular infection where 18 Navel oranges per treatment unit were artificially inoculated with green and blue mould spores (as per *General Methods*), water quality and fruit surface testing were also assessed. Water quality testing was conducted by taking samples from each treatment before and after treating the fruit. 100 µl of the samples were spread in duplicate on PCA plates for total plate count and on PDA plates for yeast and mould count. The PCA plates were kept in the incubator at 37 °C for 24 hours and then

counted using the plate counter. The PDA plates were kept in the incubator at 25°C for 72 hours and then counted using the plate counter. Fruit surface testing was conducted by collecting fruit from the drop lines at the end of the packingline. 20ml of neutralizing buffer were poured in plastic bags containing the sample fruit and the fruit were massaged for 30 seconds to dislodge the bacteria and moulds on the fruit surface. 100µl of the buffer from the bags were spread in duplicate on PCA plates for total plate count and on ¼ PDA plates for yeast and mould count. The PCA plates were kept in the incubator at 37 °C for 24 hours and then counted using the plate counter. The PDA plates were kept in the incubator at 25 °C for 72 hours and then counted using the plate counter. The treatments in the commercial packingline were repeated four times to fit into commercial operations, whilst the controlled treatments were independently replicated in separate dipping tubs (4 replicates). The water quality and fruit surface testing were sampled for each replication.

Figure 27. The trial Unipolar® EW system in the commercial packinghouse (left) and treatment of oranges with Unipolar® sanitiser over brushes (right)

Results and Discussion

Part 1. Sanitiser comparison in commercial packingline

This trial examined six treatments in a commercial packingline: (1) Unipolar® EW system set at 5 ppm chlorine, (2) Unipolar® EW system set at 10 ppm chlorine, (3) Unipolar® EW system set at 15 ppm chlorine, (4) 'Tsunami' at the recommended rate (60 ppm), (5) hot water treatment (52 °C) and (6) 'Tsunami' followed by hot water wash (the current in-line commercial treatment). All treatments were conducted on a commercial packingline.

Water Quality

All different levels of the Unipolar® EW system (5, 10 and 15ppm chlorine) resulted in lower total colony forming units and yeast and mould counts (Figure 28). Water samples were measured before and after treatment of the fruit and the results showed few lower total colony forming units and yeast and mould counts before or after dipping the fruit. The 'Tsunami' treatment (peroxyacetic acid and hydrogen peroxide) had lower total colony counts but significantly higher levels of yeast and mould, showing that the 'Tsunami' has more difficulty in controlling yeast and moulds than bacteria. The hot water treatments had high total colony forming units especially after adding the oranges, but lower yeast and mould levels (Figure 28).

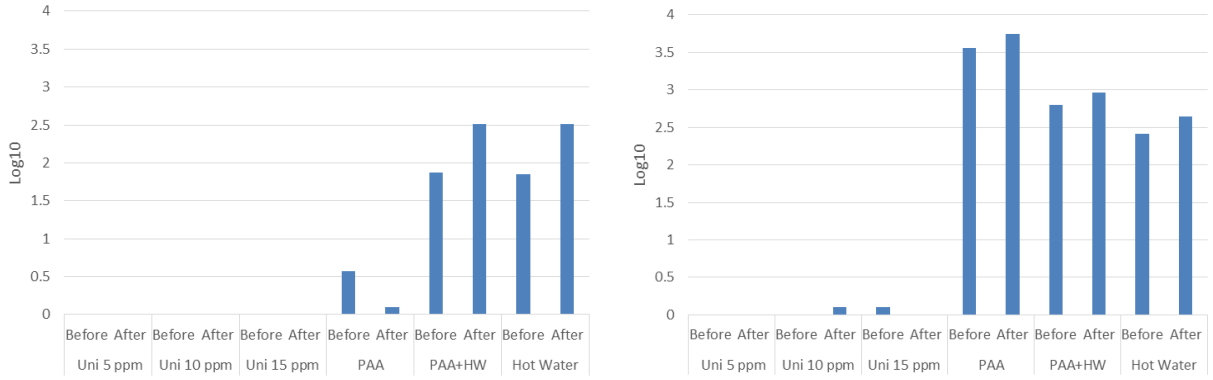


Figure 28. Effect of sanitation treatment on total plate count in water (left) and yeast and mould count in water (right) in commercial packingline. Water samples were taken before and after fruit treatment.

Fruit surface measurements

There was no difference in the number of total colony forming units and yeast and mould count between the treatments, except the yeast and mould count of 'Tsunami' treated fruit which were higher than the other treatments (Figure 29).

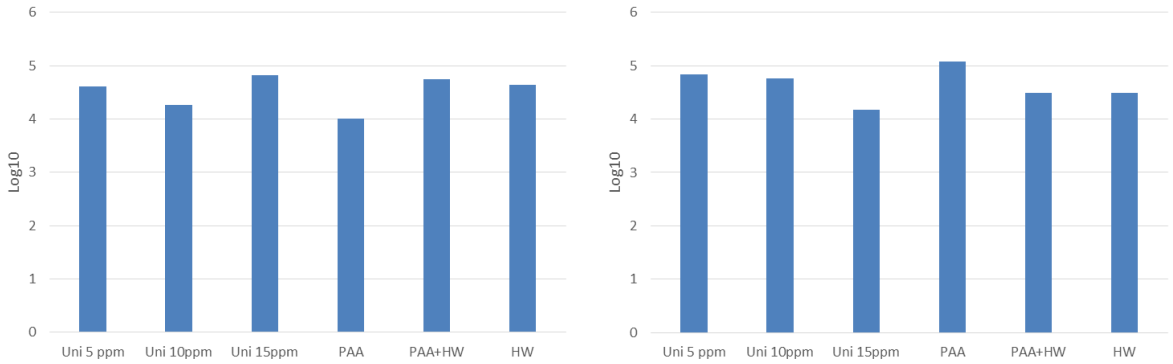


Figure 29. Effect of sanitation treatment on total plate count on the surface of the fruit (left) and yeast and mould count on the surface of the fruit (right) in commercial packingline.

Green and blue mould

There was no difference between the different sanitiser treatments on the incidence and growth of blue and green mould (Figure 30). While there was some reduction of the incidence and growth of green mould due to hot water treatment in some treatment comparisons, this was not always the case. Hot water treatment had no effect on blue mould infection or growth.

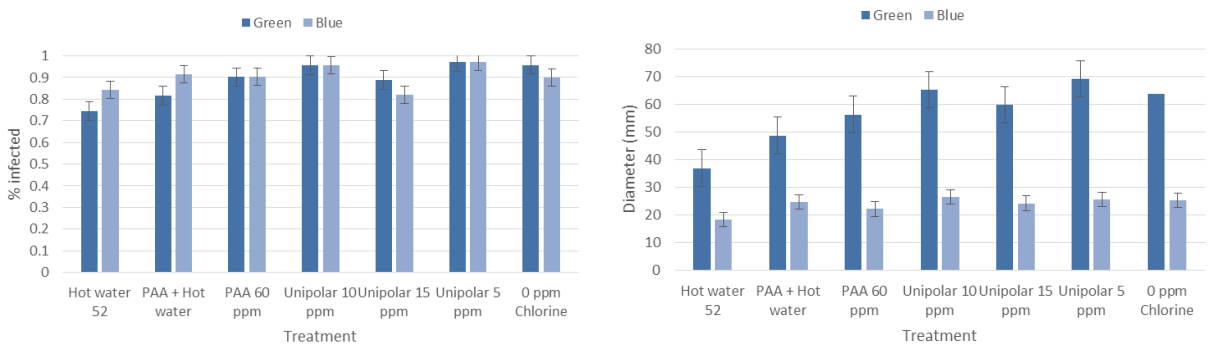


Figure 30. Effect of sanitation treatment on infection rate (%) of green and blue mould (left) and growth (mm diameter) of green and blue mould after 7 days (right) from fruit treated in the commercial packingline. *Bars are standard errors around the mean (n=4).*

Conclusions The Unipolar® EW system at the different levels (5, 10 and 15ppm chlorine) resulted in lower total colony forming units and yeast and mould counts in the wash water. However there were generally no differences in any of treatments on the number of total colony forming units and yeast and mould count on the fruit surface. The samples taken for the fruit surface measurements were taken at the end of the packingline in the drop points at the end of the line. There may have been potential for contamination along the packingline, but this is where fruit are collected for the retailer and consumer. There were no differences in infection and growth rate of green and blue mould following any sanitising treatment in the commercial packingline. However hot water treatment showed some decay control abilities.

Part 2. Sanitiser comparison in controlled conditions

Methods In parallel to the commercial assessment (part 1), this trial examined ten treatments in controlled conditions (dipping in tubs) (Figure 31): (1) control (packinghouse water no added chlorine), (2) Unipolar® EW system set at 5 ppm chlorine, (3) Unipolar® EW system set at 10 ppm chlorine, (4) Unipolar® EW system set at 15 ppm chlorine, (5) chlorine as hypochlorite (50 ppm), (6) 'Tsunami' at the recommended rate, (7) hot water treatment (52 °C), (8) imazalil, (9) control (pre-treatment sodium bicarbonate with post-treatment water wash),and (10) no treatment control from orchard. In addition to artificial green and blue mould decay assessment, water quality and fruit surface measurements, the levels of natural postharvest decay infection was recorded after four weeks at room temperature with 100 fruit assessed per treatment unit, where each treatment was replicated four times.

Figure 31. Treating oranges in different sanitisers in controlled conditions (80L tubs) (left), and green and blue mould infected fruit following treatment (right)

Results and Discussion - Controlled conditions

Water quality

Unipolar® EW system (set at 5, 10 and 15 ppm chlorine), chlorine as hypochlorite (50 ppm) and 'Tsunami' had few detectable colony forming units (Figure 32.a) and yeast and mould counts (Figure 32.b), as compared to the control (packinghouse water) and hot water. All sanitation treatments resulted in significantly lower detectable colony forming units and yeast and mould counts. Water quality measurements were taken before and after the fruit were dipped and the results showed there was no increase in detectable colony forming units and yeast and mould counts after the fruit were treated with the sanitisers.

The levels of detectable colony forming units in the control (packinghouse water) samples did not increase after the fruit had been dipped, but the numbers of yeasts and moulds in the dip water did significantly increase after dipping the fruit. The levels of yeasts and moulds in the dip water of the hot water treatment did not increase following dipping the fruit, but the levels of detectable colony forming units were significantly higher after dipping the fruit in hot water. These results demonstrate the presence of detectable colony forming units and yeast and mould counts in both the packinghouse water and hot water. The hot water in the packingline was sourced from the packinghouse, with no treatment. Although this hot water was heated to 52 °C, it was not enough to sanitise the hot water.

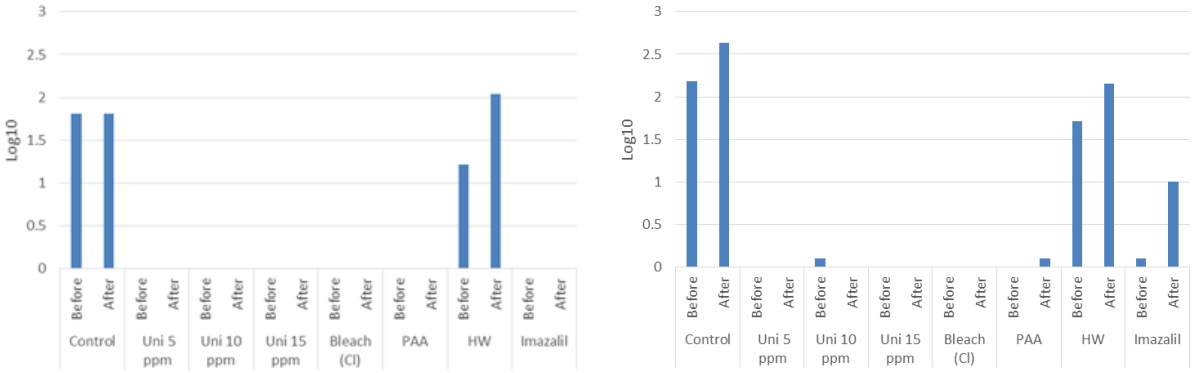


Figure 32. Effect of sanitation treatment on (a) total plate count in water (left) and yeast and mould count in water (right) in controlled conditions. Water samples were taken before and after fruit treatment.

Fruit surface measurements

The levels of total colony units and yeast and mould counts from the surface of the fruit were similar between all treatments except the hot water treatment, where the levels were significantly higher. The treatments in this component were applied as a dip treatment (with no brushes) (Figure 33). The lack of brushing or removal of the surface contamination from the fruit surface may be responsible for the consistent high levels of total colony units and yeast and mould counts on the fruit surface.

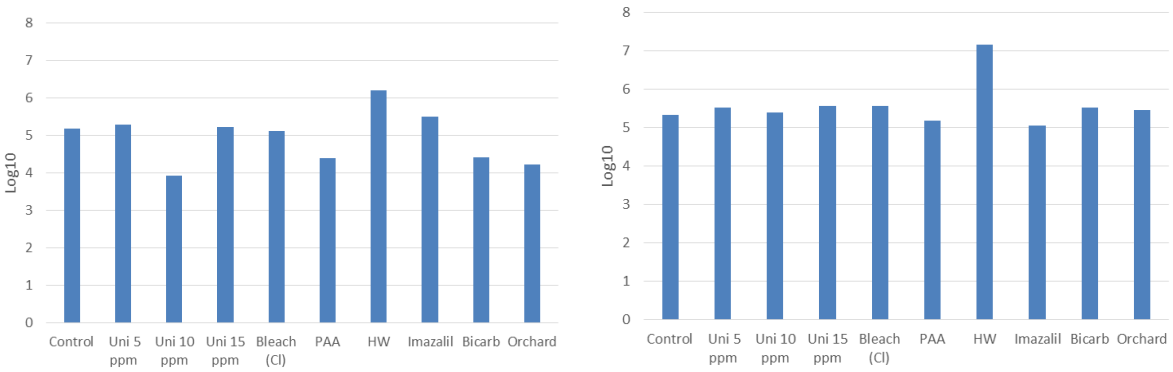


Figure 33. Effect of sanitation treatment on total plate count on the surface of the fruit (left) and yeast and mould count on the surface of the fruit (right) in controlled conditions.

Green and blue mould

As expected, there was no difference between Unipolar® EW system (set at 5, 10 and 15 ppm chlorine), chlorine as hypochlorite and 'Tsunami' in the rate of green and blue mould infection and size of infection following artificial inoculation of the fruit (Figure 34). However hot water significantly reduced the incidence and growth of green and blue mould infection. As expected, the imazalil fungicide controlled green and blue mould.

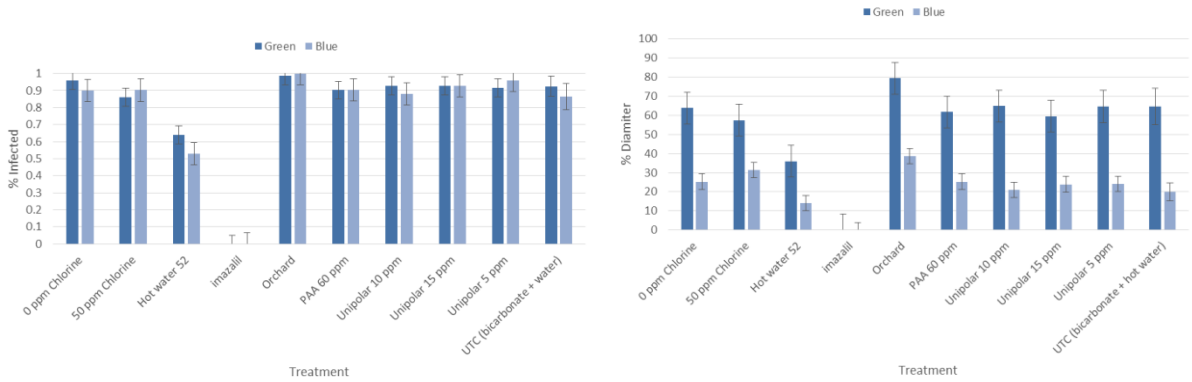


Figure 34. Effect of sanitation treatment on infection rate (%) of green and blue mould (left) and growth (mm diameter) of green and blue mould in controlled conditions after 7 days storage (right).
Bars are standard errors around the mean (n=4).

Natural postharvest decay

The overall levels of natural postharvest decay infection in this experiment were very low. But it must be noted that all fruit (except orchard treatment) were drenched with a 1.5% sodium bicarbonate treatment after harvest which was then washed off with packinghouse water. After treatment and storage of the fruit for one month at room temperature, the different sanitisers / dip treatments did not have any effect on the rates of natural infection of postharvest decay (green and blue mould) (Figure 35).

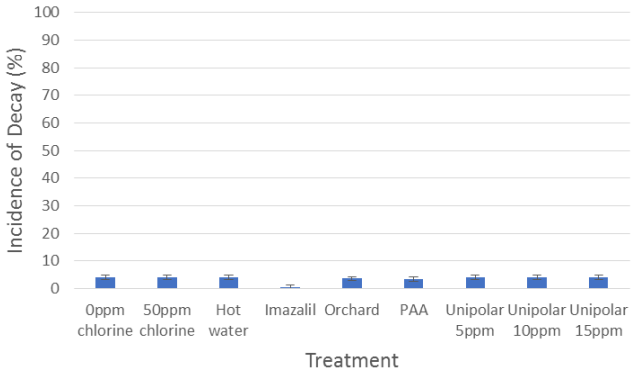


Figure 35. Effect of sanitation treatment on natural postharvest infection rate (%) of treated oranges (*n*=100 fruit per replicate). *Bars are standard errors around the mean (n=4).*

Discussion All sanitisers, i.e. Unipolar® EW system (at 5, 10 and 15 ppm chlorine), chlorine as hypochlorite and 'Tsunami' were effective in reducing detectable colony forming units and yeast and mould counts in the wash water. This is the main function of sanitisers and why they are used in postharvest horticulture. However there were no generally differences in the effectiveness of the different sanitisers reducing detectable colony forming units and yeast and mould counts on the fruit surface. In addition, the sanitisers had no effect on green and blue mould decay. These results were expected as sanitisers only clean up the wash water and no not control postharvest decay. The hot water treatment was the only postharvest treatment (except the commercial fungicide, imazalil) to reduce the development of postharvest decay (green and blue mould).

3.3. Evaluation of alternative sanitation treatments

'ChillSafe'

'ChillSafe' is a relatively new sanitation system developed in Australia and produced by Coolsan® Australia (https://coolsan.com.au/). The active ingredient of 'ChillSafe' is sodium percarbonate which with the addition of water produces hydrogen peroxide (H_2O_2) which is a powerful oxidant.

$$2Na_2CO_3.3H_2O \rightarrow 3H_2O_2 + 4Na^+ + 2CO_3^{2-}$$

As an oxidizing agent, sodium percarbonate is an ingredient in a number of home and laundry cleaning products, including non-chlorine bleach products. But it has been patented by Coolsan® for use as a slow release sanitation system for use in hospitality and other uses, including postharvest sanitation. The slow release of hydrogen peroxide from the 'ChillSafe' sachets is thought to kill postharvest decay spores in coolrooms and reduce ethylene levels (https://coolsan.com.au/). 'ChillSafe' is registered organic by Biological Farmers of Australia and is safe to use.

This evaluation of 'ChillSafe' as an aid for postharvest sanitation was in two parts:

a. *In vitro* effect of high levels of sodium percarbonate on the viability of decay spores following treatment,

b. *In vitro* effect of 'ChillSafe' sachets on the viability of decay spores following treatment.

***a. In vitro* effect of high levels of sodium percarbonate on the viability of decay spores following treatment**

Green and blue mould spores (6 x 10^4 spores) were sealed in a glass jars with 0.1 g of sodium percarbonate and excess water. After 4 or 14 days treatment, the *Penicillium* spores on the glass slides were transferred to agar plates to determine if the spores were still viable after treatment. The results showed that the sodium percarbonate treated spores for 4 and 14 days had significantly lower mould growth following treatment (Figure 36 and 37).

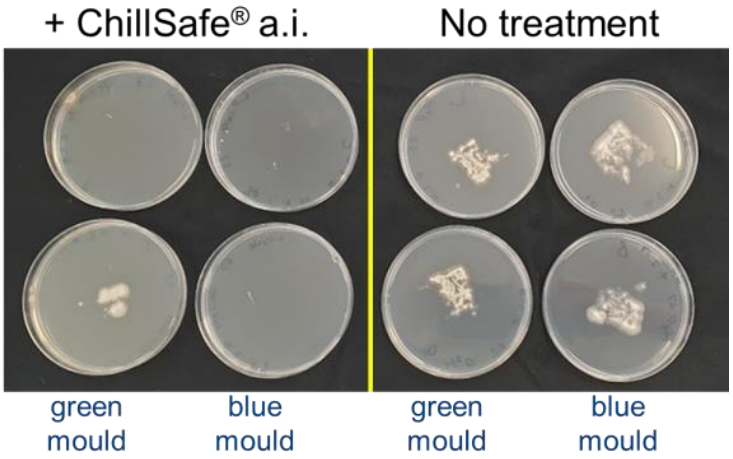


Figure 36. Effect of sodium percarbonate treatment on the growth of green and blue mould spores on PDA agar plates after 4 days treatment.

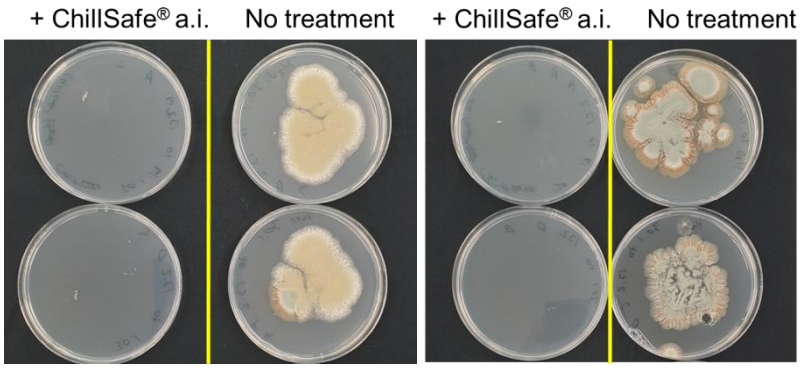


Figure 37. Effect of sodium percarbonate treatment on the growth of green (left) and blue mould (right) on PDA agar plates after 14 days treatment.

Conclusion Treatment with high levels of sodium percarbonate in a sealed glass container reduced the viability of green and blue mould growth.

b. In vitro effect of 'ChillSafe' sachets on the viability of decay spores following treatment.

Methods To assess the effectiveness of 'ChillSafe' sachets in a coolroom, 'ChillSafe' sachets were placed in previously cleaned coolrooms. One coolroom had one 'ChillSafe' sachet, a second coolroom had two 'ChillSafe' sachets and the final coolroom had no sachet (control). Each coolroom had a tub of water to maintain high humidity within the coolrooms. Agar plates were then placed into the rooms to see how 'clean' each coolroom was before the addition of 20 sporulating green and blue mould infected oranges. Agar plates with PDA were placed in the coolroom and the number of decay causing spores on the plates recorded.

Results This experiment was repeated several times in different coolrooms, but the results were inconsistent. This is because of the inherent contamination within the coolroom refrigeration and fans etc. Whilst the rooms were thoroughly cleaned with bleach and ethanol, there was continued contamination. It is proposed to conduct longer-term trials in both experimental and commercial situations to more fully assess this technology.

Conclusions The principle of sodium percarbonate (hydrogen peroxide) controlling green and blue mould spores was illustrated in *Part a*, but its assessment in coolrooms and commercial situations need to be demonstrated.

4. Physical treatments to manage postharvest decay

Sanitation of fruit and wash water is critical to the successful control of postharvest decay. Chemical sanitisers such as chlorine are routinely used to kill fungi spores in wash water and on the fruit. However there are limited non-chemical options for growers and packers to manage postharvest decay. The use of physical treatments such as postharvest heat treatments have been shown to have beneficial effects on reducing pathogens and improving fruit quality, especially in providing tolerance to chilling injury. This section explores different physical and non-chemical treatments to control postharvest decay. Treatments evaluated were:

4.1. Hot water treatment

4.2. Low pressure treatment

4.3. UV-C treatment

4.1 Effect of hot water treatment on postharvest decay

Heat treatments have long been known to control postharvest decay, but have not been used due to their lack of applicability. These experiments assessed the use of hot water treatments on:

i. *In vitro Penicillium* (and sour rot) spore viability and

ii. *In vivo* postharvest decay

i. Effect of hot water on spore mortality

This experiment examined the effects of water temperature (40 to 80 °C) for up to one minute treatment time on the *in vitro* survival of decay-causing spores.

Methods The fungi species used in this trial were *P. digitatum* sensitive to fungicides (wild type – fungicide sensitive), *P. italicum*, *G. Candidum* and *P. digitatum* resistant strain to thiabendazole (TBZ). Spore suspensions of each fungus were treated in glass test tubes at either 40, 50, 60, 70 and 80°C for either 10, 20, 30, 45 and 60 seconds. The control samples were not heat treated. Each combination of time and temperature were tested five times (i.e. 5 independent replicates plated onto 5 agar plates) and the number of colony-forming units on the 1/4 PDA with novobiocin agar plates were measured after 4 days incubation.

Results and discussion Two strains of green mould were tested, a wild type (sensitive to postharvest fungicides) and the other strain that is resistant the fungicide thiabendazole (TBZ). The results show that increasing treatment temperatures resulted in reduced survival of the spores (Figure 38). At 40 ˚C treatment there was a linear increase in the morality of the spores with longer treatment times (r^2 = 0.959), where there was 18% mortality of the spores after 10 seconds treatment, but increased to 46% mortality after 60 seconds treatment. A similar trend was also observed at 50 °C. However at and above 60 °C, there was complete mortality (100%) of the wild strain spores even with the shortest treatment time (10 seconds). A comparison of the green mould sensitive and TBZ resistant showed that resistant strain was more sensitive to the temperature than the wild type. It seemed in this case, the development of resistance to the TBZ fungicide resulted in a decrease in thermal tolerance. This observation is a benefit as the TBZ resistant strain is less 'fit' and would be easier to kill with hot water treatments.

The effect of different treatment temperatures and treatment times on the mortality of blue mould spores is presented in Figure 38 and showed that blue mould had increased tolerance to higher treatment temperatures, as compared to green mould. However at 60 °C, all treatment times had at least 98% mortality of the blue mould spores. Sour rot, *Geotrichum citri-aurantii,* spores were also treated at different temperatures for different times and the results show that treatment above 60 °C resulted in high mortality (>99%).

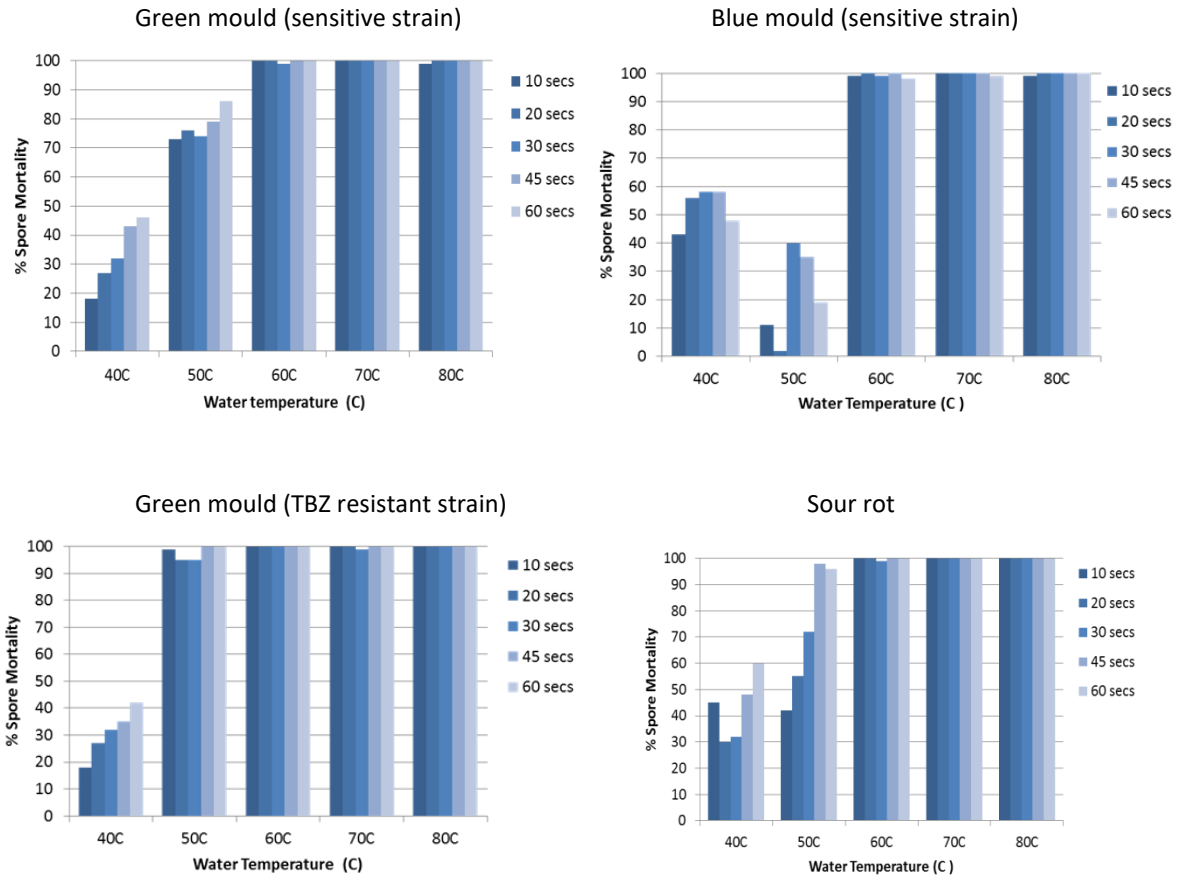


Figure 38. Effect of different temperature (40, 50, 60, 70 and 80 °C) and treatment times (10, 20, 30, 45 and 60 seconds) on the survival of wild-type (fungicide sensitive) green mould spores (top left), blue mould (sensitive strain) (top right), green mould (TBZ resistant strain) (lower left) and sour rot (lower right).

Conclusion. The results of this study showed that at 60 °C approximately 98% to 100% of the spores were killed compared to the control with any treatment time, even as little as 10 seconds which could be achieved in a hot water washing system. This high treatment temperature is just for the wash water to sanitise the water, and the fruit does not have to be at this temperature.

ii. Effect of hot water on postharvest decay

Heat treatments are an effective control measure for the control of postharvest decay. The results of the previous *in vitro* experiment showed that heat treatments above 50 °C were effective at killing fungicide sensitive and resistance green mould, blue mould and sour rot spores. However it was conducted *in vitro* on viable decay spores in water. The results of that experiment are a good approximation of sanitation of wash water, but if the decay had already infected the fruit, what effects does hot water treatment have on decay development? This experiment examined the effect of different hot water treatment times and temperatures on green and blue mould growth on infected oranges.

Methods Navel oranges which had been inoculated 24 hours previously with green (*P. italicum*) and blue (*P. digitatum*) mould spores were dipped in water at different temperatures (20, 45, 50, 55 and 60 °C) for either 30, 60 or 90 seconds. Each treatment was replicated 4 times and there were 20 fruit per replicate. After treatment, the levels of decay were recorded after 7 days storage at 25 °C.

Results and discussion The results presented in Figure 39 show that increasing treatment temperature of the hot water dip reduced the growth of both green and blue mould, whereby at dipping already infected Valencia oranges in hot water at 60 °C for 30 seconds reduced infection to 65 % for green mould and 82 % for blue mould. Previous research and reviews have also shown that the that the application of hot water at 55-65 °C for relatively short periods 10 - 30 seconds can significantly reduce decay by 45-55%. (Palou et al., 2001; Palou, 2013). Hot water / fungicide dips are already regularly used in many packinghouses, where hot fungicide solutions are thought to increase the fungicide deposited residue in the wound (Schirra et al., 2011).

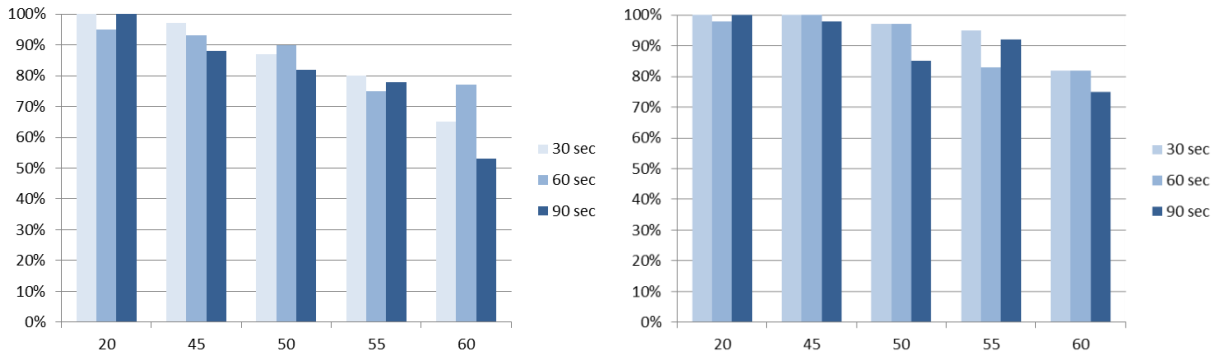


Figure 39. Effect of hot water treatment temperature (40, 45, 50, 55 and 60 °C) and time (30, 60 and 90 seconds) in water on the development of green mould (left) and blue mould (right) in Valencia oranges.

Conclusion These results show the heat treatment itself is helping control postharvest decay and could be used in combination treatments as an alternative to fungicides. However more work is required to understand and quantify potential fruit quality issues with heat treatments.

4.2 Low pressure treatments

Low pressure storage technology has been used in the past and it has recently returned as a technique which can rapidly remove heat, reduce oxygen levels and rapidly remove and manage the storage atmosphere and has the advantage that pressure treatment is homogenous. Modern low pressure systems maintain high humidity levels >95% to lower water loss and wilting, this also lowers respiration and ethylene production to delay fruit ripening during storage (Burg, 2004). Low pressure storage at sub-atmospheric pressures has been shown to extend the storage and shelf-life of many horticultural crops such as tomatoes (Pristijono et al., 2017b). Low pressure can also have direct effect on the growth of postharvest pathogens. We have shown that low pressure treatments of 4 kPa was able to reduce stem decay in green capsicums compared to that of controls at 101 kPa. Green capsicum also had no symptoms of flesh rots for up to 11 days after sub-atmospheric pressure storage compared to atmospheric storage where the green capsicums had 6% flesh rots (Pristijono et al., 2017). This study examined the effectiveness of low pressure on the growth rate of green mould *in vitro* and in citrus fruit at 5°C compared to that of atmospheric pressure (parts 4.2a and b).

In addition, it is important to recognise that with a low pressure treatment, the partial pressure of all gases is reduced, including oxygen. In atmospheric air the partial pressure of oxygen is 21 kPa while in the low-pressure treatment with 0 % humidity partial pressure of oxygen is 1.3 kPa but the treatment use contains 100% humidity meeting at the oxygen partial pressure is even lower at 0.9 kPa. This study examined the effectiveness of low pressure 6 kPa and low oxygen 1% on the growth rate of green mould on citrus at 20°C. The level of oxygen selected for this experiment (1% O_2) was equivalent to that generated within the low pressure treatment (6.6 kPa). At lower pressure (6.6 kPa) the relative partial pressure of oxygen is 1%. An experiment was conducted to compare the effects of low oxygen with low pressure (part 4.2c).

Methods A 'VivaFresh' low-pressure system consisting of six aluminium chambers (0.61 L × 0.43 W × 0.58 H m total volume of 0.152m^3) all identical was used in these experiments (Figure 40). The vacuum was produced and maintained with a two-stage rotary vacuum pump (Model 2005I, Alcatel Adixen, USA) which used a solenoid valve controlled by a proportional/integral/derivative computer control system using 'VivaFresh' software and control parameters. The low-pressure system was able to alter an air flow controller to adjust the air exchange rate to ensure that the accumulation of metabolic gasses did not occur. An air humidifier was used before entering the chamber and the internal air humidity level was monitored with wet-bulb and dry-bulb temperatures using calibrated YSI 55,000 Series GEM thermistors. The data created by the different sensors was sent to the control box and then accessed via ethernet by computers on the network.

Figure 40. 'VivaFresh' low pressure chambers used for these storage trials at NSW Department of Primary Industries, Ourimbah.

a. *In vitro* experiment. A 4mm disc of actively growing green mould was aseptically transferred to a fresh PDA plate and incubated for 24 hours at 25 °C before transferring to the low pressure treatment at 10 °C inside the low pressure chambers at 4 kPa. Similar treatment plates were prepared and placed atmospheric pressure (101 kPa) at 10 °C. After low pressure treatment for either 3 or 6 days, all plates were transferred to 25 °C and the growth of the fungus was measured each day. The treatment unit for each treatment was 10 agar plates which were replicated in three low pressure chambers. The control treatment plates were kept at atmosphere pressure.

b. *In vivo* experiment. Green mould inoculated Navel oranges were either treated with low pressure (4 kPa) or remained at atmospheric pressure (101 kPa). The fruit were stored at 5 °C for 22 days before removal and assessment of growth. There were three separate chambers (independent replicates) for the low pressure treatment and atmospheric pressure. There were 10 fruit per treatment unit.

c. Low oxygen. Green mould inoculated fruit were treated with either the low oxygen or low pressure atmosphere treatments and compared to the untreated fruit kept at regular atmosphere at 20°C. The experimental unit was 15 fruit and each treatment was replicated three times in different low pressure chambers, low oxygen drums or bags. There were removals from each of the treatments at 4 and 8 days of treatment. The relative partial pressures and storage atmospheres of the three treatments are presented in Table 9. The low oxygen treatment (1% O_2) was conducted in 60L steel drums in a flow-through system was maintained at 200 mL.min^{-1} and the non-treated control fruit were stored under high RH (> 95% RH) in an unsealed LDPE bag. The growth of the green mould was assessed up to 3 times; at 24 hours, 4 days and 7 days after removal from treatments by measuring the diameter of the disease tissue. There were removals from the different treatments at 4 and 8 days.

Table 9. Gas pressures and gas percentages used in this experiment.

	Control 101.325 kPa + 100% RH		Low pressure 6.6 kPa + 100% RH		Low oxygen 101.325 kPa + low O_2 + 100% RH	
	%	kPa	%	kPa	%	kPa
		101		6.6		101
water	2.30%	2.3	35.00%	2.3	2.30%	2.3
remainder		*98.9*		*4.3*		*98.9*
nitrogen	76.3%	77.3	50.8%	3.4	96.76%	98.0
oxygen	20.5%	20.7	13.6%	0.9	0.90%	0.9
argon	0.9%	0.9	0.6%	0.0	0.04%	0.0

Results and discussion

a. *In vitro* results

The effects of low pressure on the growth of green mould on PDA petri dishes are presented in Figure A. The low pressure treatment was applied for either three or six days at 10 °C and then returned to regular atmospheric pressure at 25 °C for measurement of fungi growth. The results show that immediately upon removal of the plates after three days treatment, the growth of the fungus was <1 mm, while the fungus growth at atmospheric pressure was 8 mm. After the plates were removed from the low pressure and returned to atmospheric pressure at 25 °C, the growth rates of both treatments significantly increased. There was an increase in growth rate of the fungi which had the initial low pressure treatment (Figure 41). The growth of green mould after six days low pressure treatment was 2.5 mm, while the growth at atmospheric pressure was 14 mm in diameter (Figure 41). After the plates were removed from the low pressure treatment, the growth rate significantly increased where with the growth of the fungi more than doubled on the first day at atmospheric treatment after removal.

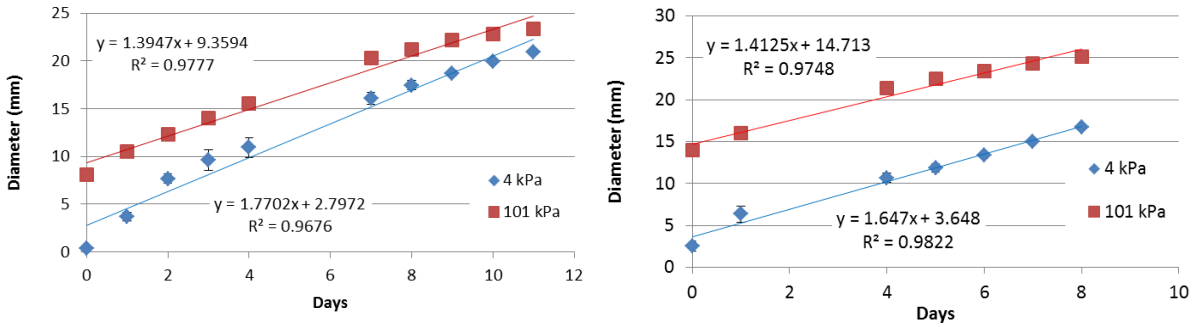


Figure 41. The diameter (mm) of green mould at 25 °C on PDA petri plates following treatment at 4 kPa (or101 kPa) at 10°C for 3 days (left) and 6 days (right).

b. *In vivo* results

Effect of low pressure treatment of 4 kPa on the growth of green mould in Navel oranges after 22 days storage at 5 °C is presented in Figure 42. The results showed that the low pressure treatment significantly reduced the growth of green mould in the fruit. After 22 days at 5 °C, the low pressure treatment had only 4.6 mm growth compared to 16 mm of growth at atmospheric pressure (101 kPa). This difference may be due to reduced oxygen availability during low pressure storage, where the oxygen level at 4 kPa is equivalent to approximately 1% O_2 (v/v). Burg (2004) reported that low oxygen atmospheric storage of 0.1 – 0.25% O_2 have significantly inhibitory effects on pathogens and spore germination.

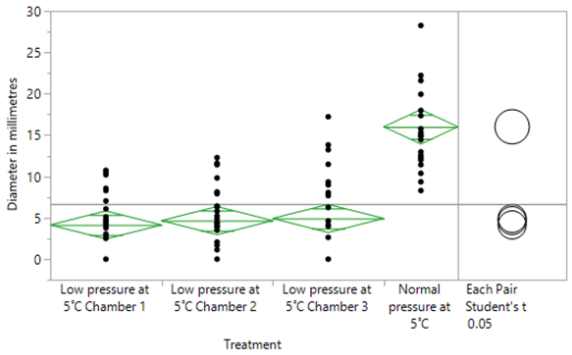


Figure 42. Effect of 3 days low pressure treatment (4 kPa) on growth of green mould growth in Navel oranges in the three low pressure chambers after 22 days storage at 5 °C.
The green diamonds represent the 95% confidence interval for the data.

c. Low oxygen and low pressure comparison

The results show there was a significant difference between the low oxygen and low pressure treatments and the non-treated infected fruit at 20°C (Table 10). This difference was maintained up to 7 days after treatment with the untreated fruit having the greatest average growth of 98 mm compared to that of low oxygen treated fruit which had the lowest average growth of 72 mm. After four days treatment, the fruit with low pressure treatment had reduced growth by 280% compared to the non-treated fruit, while the low oxygen treated fruit reduced growth by 770% compared to the non-treated fruit.

Table 10. Average diameter of green mould growth (mm) in Navel oranges following treatment and assessment after removal.

Treatment	**+24 hours**	**+ 4 days**	**+7 days**
untreated for 4 days	30.1 *c*	63.9 *c*	97.5 *b*
low oxygen for 4 days	4.9 *a*	28.1 a	71.6 *a*
low pressure for 4 days	10.7 *b*	43.3 *b*	80.9 *c*
untreated for 8 days	77.4 *d*	104.9 *d*	
low oxygen for 8 days	3.9 *a*	34.1 *ab*	
low pressure for 8 days	35.0 *c*	64.8 *c*	

The values are the mean of 3 replicates of 15 oranges. *Different letter at each assessment time show significant different at p <0.05 each assessment time was analysed independently*.

Conclusion While the growth of green mould at these low treatment temperatures (5 and 10 °C) is relatively slow, there was a significant difference in the growth between the low pressure treatments and the control treatments at atmospheric pressure. Both *in vitro* and *in vivo* experiments, the growth of green mould was lower when under the low pressure treatment. A subsequent experiment examining the role of low oxygen in low pressure treatment showed that exposure to both low oxygen and low pressure treatments reduced fungal growth compared to that of the non-treated fruit at room temperature and atmosphere. The low oxygen treatment was the most effective in slowing the rate of decay growth compared to the other treatment and suggests that low oxygen and pressure treatments have the potential as an alternative, non-chemical postharvest treatment to reduce mould during storage. More work to look at modified atmospheres and pressures is recommended as a potential addition for a combination treatment to control postharvest decay.

Some of the research in this Section was presented at the 5th International Symposium on Postharvest Pathology in Leige Belgium in May 2019. A paper was submitted for publication in *Acta Horticulturae*.

Presentation by John Archer, University of Newcastle / NSW Department of Primary Industries
at 5th International Symposium on Postharvest Pathology. International Society of Horticultural Science.
(Appendix 1)

Preliminary investigations on the effect of low-pressure treatment on in vitro and in vivo growth of *Penicillium* sp. in oranges

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Abstract

Penicillium digitatum **is the major pathogen causing postharvest decay in citrus fruit and is the organism responsible for green mould. *P. digitatum* decay is currently managed with synthetic fungicides but there is a growing consumer need to reduce the reliance on synthetic fungicides and find alternative non-synthetic treatments. Low-pressure treatments may offer a potential solution for the storage and transport of citrus, as it is a physical treatment and does not leave any chemical residues. To test the effectiveness of low-pressure storage treatments on the growth of *P. digitatum*, small-scale laboratory experiments were conducted with specialist low pressure chambers. *P. digitatum* was grown on PDA agar plates and treated with low pressure (4 kPa) for 3 and 6 days at 10°C before growth assessments in air at regular (101 kPa) atmospheres. The results showed that low pressure treatments slowed the growth of *P. digitatum*. In a further experiment on oranges, *P. digitatum* infected fruit were treated with low pressure (4 kPa) at 5°C for up to 22 days. This experiment also showed a reduced growth of *P. digitatum in vivo*. These results show there is potential to control the growth of *P. digitatum* at low pressure treatments and further experimentation should be conducted.**

Keywords: citrus, storage, decay, non-chemical.

4.3 UV-C treatments

Over the past few decades, ultra violet – C (UV-C) treatment (180 -280 nm) has been evaluated as a postharvest treatment for fresh fruits and vegetables. Postharvest treatment with UV-C light has been reported to have a beneficial effect on maintaining the quality of horticultural produces, such as reducing pathogen growth and delaying the ripening and aging of fruit. This experiment examined the effect of postharvest UV-C light on postharvest decay and Navel orange fruit quality.

Methods Navel oranges were exposed to different intensities of UV-C light and stored up to 21 days at 20 °C where weight loss, peel colour, calyx abscission, ethylene production, respiration rate, soluble solids content (SSC), titratable acidity (TA) and acceptability index were assessed every 7 days. Another set of fruit were inoculated with green mould and treated with UV-C light at either 2 or 24 hours after inoculation at four different UV-C intensities. The treatment unit was 16 fruit per replicate which were replicated 5 times.

UV-C treatments were conducted using a custom made light proof box fitted with two germicidal lamps (Sahkyo Denki Co. Ltd G20T10 20 Watt, low pressure mercury). A SED008/W detector with PIR Irradiance Calibration at 254nm was used to measure UV-C intensity. This was connected to a research radiometer (International Light Technologies 1700 Series) that measured the cumulative exposure over time. The sensor was placed inside the light box at equal height to the produce during irradiation treatment. The UV-C light was switched off manually once exposure time had been reached for each of the required treatment units. The oranges fruit were placed approximately 50 mm from the UV-C light sources and rotated 180°C during irradiation to ensure complete and even coverage. Following treatment, fruit from each treatment unit were stored up to 21 days at 20 °C.

Results Fruit quality (weight loss, firmness and colour) was generally not affected by UV-C treatment, but some changes in the levels of SSC/TA were detected in the highest treatment dose at the final fruit quality assessment time. Navel oranges were also inoculated with green mould and treated with UV-C light at either 2 or 24 hours after inoculation at four different UV-C intensities. The results presented in Figure 43 show there was a different response between the doses and treatment times of UV-C treatment and while these differences were small, there was some reduction in green mould growth with UV-C treatment.

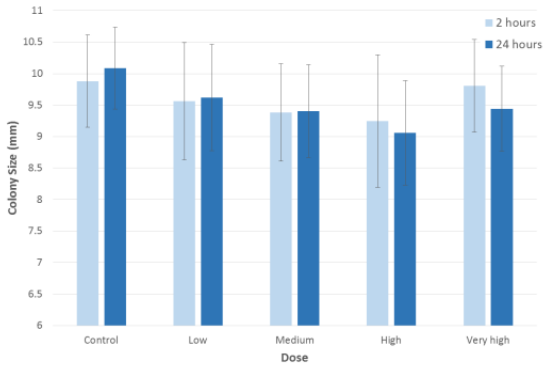

Figure 43. Effect of different doses of UV-C treatment on the growth of green mould in Navel oranges. Fruit were treated for either 2 or 24 hours. *Bars are standard errors around the mean (n=5).*

Conclusion The results in this preliminary experiment suggest that a pre-storage UV-C treatment has limited potential to reduce green mould development. While this treatment could be easily integrated into commercial packinglines, more work should be conducted to investigate and optimise this non-chemical treatment to control postharvest decay.

2. Managing fruit quality through the supply chain

Postharvest is the critical link between the grower and the consumer. Its primary function is to ensure fruit quality is maintained through the supply chain to meet and exceed retailers' and consumers' expectations. While decay is the primary cause of postharvest losses, it can be successfully managed with good postharvest practices and fungicides. However, there are a range of other postharvest issues/challenges which need to be managed to successfully market citrus fruit onto domestic and export markets. These include (1) chilling injury, (2) minimising the development of off-flavours, (3) identifying and implementing new approaches to maintain fruit quality during storage and (4) understanding the potential postharvest quality effects of low dose irradiation as a market access treatment for export.

2.1 Chilling injury

Chilling injury can be a devastating postharvest disorder due to low temperature storage. It can result in significant downgrade or rejection of fruit in the market. The classical symptoms of chilling injury is pitting of the peel and superficial scald-like symptoms of the peel and browning of the skin.

Chilling injury is a disorder that is not caused by a disease and is caused by exposure to cold temperatures, but not freezing temperatures during storage. Chilling injury is distinct from freezing injury as there is no development of ice crystals in the cells in chilling injury. The severity of the chilling injury symptoms is related to both the storage temperature and the length of cold treatment, whereby symptoms are increased with lower storage temperatures for longer treatment times. Some symptoms of chilling injury can occur while the fruit is at low temperature but these symptoms increase when the fruit is removed from the chilling temperature to room temperature.

The major challenge with chilling injury is that the biochemical and physiological basis for its development is not known. While the biochemical and molecular mechanisms involved in chilling tolerance in different citrus types have been extensively studied there seems to be a complex interplay of different metabolic pathways which operate in the induction of cold tolerance (lipid metabolism, oxidative stress, dehydrins, osmoprotectants, metallothioneins, defence responses etc.).

Predicting the onset and severity of chilling injury is also difficult, due to the unpredictable nature of the time x temperature combinations required to produce the onset of symptoms. Short exposure to cold treatment may not cause injury, but longer cold storage times can result in the expression of the disorder. The easiest way to avoid chilling injury is to avoid storing citrus at < 5 ^{o}C, however many of the phytosanitary cold treatment against fruit flies requires cold treatment (1 ^{o}C) for up to three weeks treatment (see *Section 2.4 Market Access Section*).

Although the mechanisms of chilling injury are not fully understood, there are a number of pre- and postharvest factors which interact to affect the development of chilling injury symptoms.

Preharvest factors affecting chilling injury

Preharvest factors contributing to the development of chilling injury include citrus type and cultivar, preharvest orchard temperatures, harvest times, growing locations, fruit maturity at harvest etc. The susceptibility to chilling injury differs among species and different citrus types. For example limes and lemons are generally more susceptible to chilling injury than oranges and mandarins. But even within a citrus type, there are differences in susceptibility to chilling injury. For example comparing the susceptibility of different Navel oranges, 'Navelina' fruit have stronger tolerance to chilling temperatures, 'Thomson' are moderately sensitive and 'Navelate' and 'Roberts' fruit are highly sensitive to chilling temperatures (Lindhout, 2007). Grapefruit are one of the most chilling susceptible citrus types but there are significant differences in susceptibility between red and regular grapefruit types. Lado et al. (2015) showed there is a positive relationship between carotenes content, mainly lycopene, and chilling tolerance during storage of grapefruit at low temperature. The potential protective role of lycopene in chilling injury has been shown to be related to the antioxidant properties of lycopene (Lado et al., 2015).

The incidence of chilling injury also depends on pre-harvest factors, including maturation stage and environmental conditions during fruit growth such as field temperatures prior to cold storage. For example in some situations, fruit harvested earlier and later in the season are more susceptible to chilling injury than mid-season fruit, but this does not always hold in different seasons and growing conditions.

Postharvest management to reduce chilling injury symptoms

Temperature treatments. Short term high temperature treatments in the form of hot water dips, hot water brushing, curing or conditioning with hot humid air have been shown to reduce the incidence of chilling injury in citrus. It is considered that these heat treatments are a form of stress which helps protect the fruit from another stress, i.e. cold stress and chilling injury. The protective nature of heat treatments has been shown to occur through various physiological mechanisms such as the development of heat shock proteins and induction of other defense mechanisms in the citrus albedo. A range of curing and heat treatment conditions have been tested and show a range of results depending on the fruit type and treatment. Conditioning citrus fruits with hot air for three days at 37 ^{o}C has been consistently found to be effective in increasing chilling tolerance without inducing heat damage. This has been demonstrated for different citrus seasons and in fruits harvested at all maturity stages, despite the variable susceptibility of fruit throughout the season. In addition hot water dips treatments, intermittent warming, and rinsing and brushing at temperatures of 60 ^{o}C have been also reported to be effective at reducing the symptoms of chilling injury.

Plant growth regulators. A range of different plant growth regulators such as jasmonic acid, salicylic acid have been implicated in the development of chilling injury. Jasmonic acid and its various metabolites have been shown to regulate plant responses to abiotic and biotic stresses including chilling injury in a range of crops, including citrus. Jasmonic acid and salicylic acid have been shown to reduce the incidence of chilling injury in citrus possibly through enhanced antioxidant activities, increased production of total phenolics and /or suppressed membrane lipid peroxidation and reactive oxygen species production.

Fungicides. Common postharvest fungicides such as thiabendazole are applied to control postharvest decay such as green mould (*see Section 1 - Postharvest Decay*), but it has also been shown to reduce the incidence of chilling injury. Furthermore the application of thiabendazole as a warm dip (40 ^{o}C) has been shown to further decrease the expression of chilling symptoms. It is thought that the increased temperature results in higher deposition of the chemical into the fruit, but it is not known how thiabendazole confers increased chilling tolerance.

Waxes. The application of waxes is commonly used to replace the natural waxes / cuticle which are removed with postharvest brushing, handling and packing. Waxes reduce water loss from the fruit and have also been shown to reduce the incidence of chilling injury but with different outcomes with different wax and fruit types.

Modified atmospheres. The use of modified atmosphere packaging is not widely commercially used, but it has been shown to reduce chilling injury. It is thought that the high relative humidity within the storage carton reduces water loss and chilling injury.

Three postharvest trails were conducted to understand the pre- and postharvest factors that can be managed to minimise the development of chilling injury:

a. Preharvest – Survey of variety effects on chilling injury in Navel oranges,
b. Preharvest – Effect of water stress and location on chilling injury in 'Atwood' Navel oranges, and
c. Postharvest - Effects of postharvest treatments on chilling injury.

2.1.a Preharvest – Survey of variety effects on chilling injury in Navel oranges

A survey of the chilling tolerance / susceptibility of different Navel varieties was conducted on fruit grown from a long term Navel orange varieties trial orchard at the NSW Department of Primary Industries Dareton Research Station. The fruit used for this survey were harvested from a replicated experimental trial plot planted on Citrange rootstock in 1992.

Methods Fruit were harvested at three harvest times during the 2019 season: (1) early harvest – 5 June – 'Leng' and 'Lloyd'; (2) mid harvest 8 July – 'Atwood' and 'Houghton', and (3) late harvest – 28 August – 'Chislett' and 'Lanes Late'. Due to differences in maturity, the fruit were harvested at different times. Fruit were harvested from individual trees and stored separately. For each variety, there were six replicates of two trees per replicate. Within each replicate one tree had been inoculated with the mild strain of viroid which promotes tree dwarfing, whilst the other tree was not infected. After harvest, fruit were transported to NSW Department of Primary Industries at Ourimbah and stored at 2 ^{o}C for 8 weeks before assessing chilling injury symptoms immediately after removal and after one week at 20 ^{o}C (Figure 44).

The severity of CI symptoms were assessed visually according to a five-stage scale: 1 = no pitting, 2 = a few scattered pits – just one or two pits (<5% of the fruit surface), 3 = definite pits up to 10% of the fruit surface, 4 = pitting covering up to 30% of the fruit surface; and 5 = extensive pitting covering >30% of the fruit surface.

Figure 44. Storage of Navel oranges at NSW Department of Primary Industries at Ourimbah where fruit were stored at 2 °C for 8 weeks before assessing chilling injury.

Results and Discussion The average level of chilling injury in the different Navel varieties after storage for 8 weeks storage at 2 °C and another week at 20 °C is presented in Table 11. Each Navel variety had different levels of chilling injury after storage, with the early and late harvested varieties having greater chilling damage. 'Atwood' and 'Houghton' had the lowest levels of chilling injury while 'Lloyd', 'Chislett' and 'Lanes Late' had the highest levels of chilling injury. There was also a significant interaction of variety and storage time, where the levels of chilling injury generally increased with the additional one week at 20 °C. This is where the increased expression of the chilling symptoms developed after removal from cold storage. This is commonly observed and can be a problem in the market where the fruit looks satisfactory upon removal of the fruit from cold storage, but with storage at room temperature (20 °C), the chilling symptoms are expressed (Wills and Golding, 2016).

Table 11. Effect of variety on the severity of average chilling score of different Navel oranges with different harvest times after 8 weeks storage at 2 °C.

Harvest time	**Variety**	**Upon removal**	**+ 1 week at 20 °C**	
early -	Leng	2.9 cA	3.2 cB	*least significant difference comparing removal times = 0.13*
	Lloyd	3.4 eA	3.8 dB	
mid -	Atwood	2.0 bA	2.7 bB	
	Houghton	1.9 aA	2.3 aB	
late -	Chislett	3.2 dA	3.2 cA	
	Lanes Late	3.8 fA	3.8 dA	
		least significant difference comparing different varieties = 0.48		

Chilling injury subjective score: 1 = no pitting, 2 = a few scattered pit symptoms, 3 = up to 10% of the fruit surface with chilling symptoms, 4 = up to 30% of the fruit surface with pit symptoms; and 5 = severe pitting covering >30% of the fruit surface.

Treatments in the same column with the same small letter indicate these treatments are statistically similar ($p<0.05$). Treatments in the same row with the same capital letter indicate these treatments are statistically similar ($p<0.05$)

Instead of looking at the average chilling injury severity score across all the fruit in a sample, another way of examining the chilling data is to look at the percentage of fruit that have no chilling symptoms (Table 12), or those fruit that are commercially acceptable (i.e. fruit with no chilling symptoms and those fruit with just trace amounts of chilling symptoms) (Table 13).

The effect of variety on the percentage (%) of fruit with no chilling symptoms (chilling score = 1) is presented in Table 12 and show that upon removal, 43% of all 'Houghton' fruit had no symptoms, but 'Lanes Late' fruit only had 5% of the fruit with no chilling symptoms. There was a significant interaction of variety and assessment time, where in general the levels of excellent fruit with no chilling symptoms decreased with shelf life assessment at 20 ^{o}C.

Table 12. Effect of variety on the percentage (%) of fruit with no chilling symptoms (chilling score = 1) of different Navel oranges with different harvest times after 8 weeks storage at 2 ^{o}C.
(The estimates of the predicted standard errors are presented in parenthesis)

Harvest time	Variety	Upon removal	+ 1 week at 20 ^{o}C
early -	Leng	16.8 (2.6)	7.2 (1.9)
	Lloyd	11.5 (2.3)	2.2 (1.1)
mid -	Atwood	37.3 (3.8)	14.3 (2.8)
	Houghton	42.7 (3.7)	24.8 (3.4)
late -	Chislett	12.3 (2.7)	11.1 (2.6)
	Lanes Late	4.8 (1.7)	5.8 (1.8)

The results presented in Table 13 show the effect of variety and assessment time on the percentage (%) of fruit with acceptable chilling symptoms, i.e. fruit with no chilling symptoms (chilling score 1) and fruit with just trace amounts of chilling symptoms (chilling score 2). Variety had a significant difference in the proportion of fruit with no commercial chilling damage, where the order of fruit with the highest to lowest percentage of fruit were: 'Houghton', 'Atwood', 'Chislett', 'Leng', 'Lloyd' and 'Lanes Late' which had the lowest numbers of fruit with chilling symptoms. There was no interaction of assessment time and variety.

Table 13. Effect of variety on the percentage (%) of fruit with acceptable chilling symptoms (chilling score = 1 + 2, i.e. combination of fruit with no symptoms and those with trace levels) of different Navel oranges with different harvest times after 8 weeks storage at 2 ^{o}C.
(The estimates of the predicted standard errors are presented in parenthesis)

Harvest time	Variety	Upon removal	+ 1 week at 20 ^{o}C
early -	Leng	39.5 (4.2)	28.8 (4.0)
	Lloyd	25.5 (3.9)	16.8 (3.5)
mid -	Atwood	74.4 (4.2)	56.3 (5.1)
	Houghton	81.1 (3.7)	67.9 (4.5)
late -	Chislett	44.2 (4.9)	43.5 (5.1)
	Lanes Late	24.2 (4.1)	24.0 (4.1)

Total soluble solids (TSS) was measured in all treatments and assessment times and the results showed there was no difference in TSS between the different varieties (Table 14), but fruit assessed after one week storage at 20 °C had lower TSS (13.0% Brix) than when measured upon removal (13.4% Brix) (Table 14).

Table 14. Effect of navel variety on average fruit total soluble solids (TSS).

	Early -		Mid -		Late -	
Variety	Leng	Lloyd	Atwood	Houghton	Chislett	Lanes Late
TSS (% Brix)	12.9	13.4	13.2	13.3	13.0	13.6

Titratable acidity (TA) was also measured in the juice following treatment and shelf-life and a significant interaction between variety and assessment time (Table 15). The results show that the early season fruit ('Leng' and 'Lloyd') had higher TA at harvest and after 1 week at 20 °C. There was a significant decline in TA values of 'Leng' and 'Lloyd' fruit following the one week shelf life at 20 °C, but this decline in TA was not significant for the other varieties.

Table 15. Effect of variety and assessment time on juice titratable acidity (TA, % citric acid) upon removal from storage and after one week at 20 °C.

Harvest time	**Variety**	**Upon removal**	**+ 1 week at 20 °C**	
early -	Leng	1.09 bA	0.92 bB	*least significant difference comparing removal times = 0.10*
	Lloyd	1.29 bA	1.08 cB	
mid -	Atwood	0.91 aA	0.87abA	
	Houghton	0.91 aA	0.85 aA	
late -	Chislett	0.89 aA	0.83 aA	
	Lanes Late	0.90 aA	0.83 aA	
		least significant difference comparing different varieties = 0.06		

Treatments in the same column with the same small letter indicate these treatments are statistically similar (p<0.05). Treatments in the same row with the same capital letter indicate these treatments are statistically similar (p<0.05)

Conclusion This trial was conducted on fruit from the same orchard and grown under the same conditions and rootstock. Due to differences in maturity, the fruit were harvested at different times. The chilling stress conducted in this trial was far more severe than the regular cold disinfestation treatment, but showed significant variety and assessment time effects. The results showed that the mid-season fruit, 'Houghton' and 'Atwood' had both lower numbers of fruit with chilling injury and the overall average severity of chilling injury symptoms than fruit from the earlier and later harvests. As expected the addition of a 20 °C shelf life increased the levels of chilling damage as the symptoms were expressed with shelf life. The varietal and harvest time effects are inter-related, but the results of this experiment from one season (2019) showed that the mid-season fruit had lower susceptibility to chilling that early and late season.

2.1.b Preharvest – Effect of water stress and location on chilling injury in 'Atwood' Navel oranges

Water is a limited commodity in Australian horticulture and citrus production. The current Hort Innovation project '*Improving citrus quality with regulated deficit irrigation*' (CT17000) is looking at the effects of regulated deficit irrigation during citrus maturation and ripening stages to enhance fruit sugar content, while saving irrigation water. This small storage experiment utilised fruit from this trial to examine the effect of a pre-harvest "water stress" treatment on internal fruit quality (TSS/TA) and the development of chilling injury symptoms in 'Atwood' Navel oranges.

Methods 'Atwood' Navel orange trees from the Dareton Agricultural Station at NSW Department of Primary Industries were treated with 50% of the recommended water rate from April to June 2019 and considered 'stressed'. In addition a corresponding row of 'Atwood' Navel orange trees were given their full regular water allocation and considered 'controls'. Fruit were harvested from either the eastern or western half of each tree on 8 July 2019. A total of 24 treatment units (boxes) (i.e. 2 'stress' treatments x 2 tree orientation (East V West) x 6 replicates/trees) of fruit were harvested and were sent to NSW Department of Primary Industries at Ourimbah where the fruit were stored for 8 weeks at 2 ^{o}C then assessed for chilling injury upon removal and after an additional one week shelf life at 20 ^{o}C (as above). TSS and TA were measured at each assessment, as per *General Methods*.

Results Table 16 shows the average chilling scores of the fruit after storage and shelf life, while it appeared like the stressed fruit had higher average chilling scores there was no statistical effect of the water stress treatment, or orientation of the fruit on the tree (east V west). However fruit from water stressed trees assessed after shelf life had significantly more chilling injury (chilling score 2.3) as compared to upon removal from storage (average score 1.8).

Table 16. Effect of water stress and tree position on the severity of average chilling score after 8 weeks storage at 2 ^{o}C.

Treatment	Orientation	Upon removal	+ one week at 20 ^{o}C
Control	East	1.7	2.2
	West	1.7	1.9
Stressed	East	2.0	2.5
	West	1.9	2.4

Chilling injury subjective score: 1 = no pitting, 2 = a few scattered pit symptoms, 3 = up to 10% of the fruit surface with chilling symptoms, 4 = up to 30% of the fruit surface with pit symptoms; and 5 = severe pitting covering >30% of the fruit surface

The effect of stress and tree orientation on the percentage (%) of fruit with no chilling symptoms (chilling score = 1) is presented in Table 17 and show that upon removal, 42% of all fruit had no symptoms, but after one week storage at 20 ^{o}C, 29% of fruit had no chilling symptoms. There was no effect of stress or tree orientation on the percentage (%) of fruit with no chilling symptoms.

Table 17. Effect of assessment time on the percentage (%) of fruit with no chilling symptoms (chilling score = 1) upon removal after 8 weeks storage at 2 ^{o}C or an additional one week at 20 ^{o}C
(The estimates of the predicted standard errors are presented in parenthesis)

Upon removal	+ 1 week at 20 ^{o}C
41.7 (3.0)	28.8 (2.9)

The results presented in Table 18 show the effect of stress treatment and assessment time on the percentage (%) of fruit with acceptable chilling symptoms, i.e. fruit with no chilling symptoms (chilling score 1) and fruit with just trace amounts of chilling symptoms (chilling score 2). There was no interaction of assessment time, treatment time

and any other factor, however there was a significant different in treatment time, where the levels of fruit with acceptable chilling injury were lower after the shelf life assessment (72%) as compared to 85% of fruit that were acceptable upon removal. In addition, the levels of acceptable fruit were lower in stressed fruit (72%) as compared to non-stress fruit (85%), indicating that the stress treatment increased the fruit susceptibility to chilling.

Table18. Effect of stress treatment and assessment time on the percentage (%) of fruit with acceptable chilling symptoms (chilling score = 1 + 2, i.e. combination of fruit with no symptoms and those with trace levels) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C
(The estimates of the predicted standard errors are presented in parenthesis)

Treatment	**Orientation**	**Upon removal**	**+ one week at 20 °C**	*average*
Control	East	88.5 (5.0)	76.4 (7.1)	*85.4 (2.9)*
	West	91.9 (4.6)	84.2 (6.6)	
Stressed	East	78.4 (6.4)	61.0 (7.9)	*72.2 (3.6)*
	West	82.1 (6.3)	66.4 (8.0)	
	average	*85.0 (2.8)*	*71.6 (3.7)*	

Internal quality There were no effects of tree orientation or assessment time on fruit TSS, however there was a significant difference in the levels of TSS between the stressed and non-stressed (control) fruit. The overall TSS of fruit that had been stressed was 12.5% Brix, which was significantly higher than the control (non-stressed) fruit (11.8% Brix). This was expected and is the aim of regulated deficit irrigation management.

There was also a significant interaction between stress treatment and assessment time (Table 19), which shows there was no difference in fruit TA between stressed and control fruit upon removal, but after one week shelf life, the TA in the stressed fruit remained high (0.97% citric acid) while the levels of TA in the control fruit declined (0.88 % citric acid).

Table 19. Effect of water stress and assessment time on fruit titratable acidity (TA, % citric acid) after 8 weeks storage at 2 °C and an additional week at 20 °C.

Treatment	**Upon removal**	**+ one week at 20 °C**
Control	0.97 Aa	0.88 Ba
Stress	0.93 Aa	0.97 Ab

Treatments in the same column with the same small letter indicate these treatments are statistically similar ($p<0.05$). Treatments in the same row with the same capital letter indicate these treatments are statistically similar ($p<0.05$)

Conclusion As expected, the levels of TSS in fruit that had been stressed were higher than in the control fruit. This is a key objective of the Hort Innovation project '*Improving citrus quality with regulated deficit irrigation*' (CT17000). There was no significant effect of the water stress (50% of the recommended water rate from April to June) on the average levels of chilling injury score on Atwood Navel oranges following 8 weeks storage at 2 °C and an additional week at 20 °C. But there was a significant difference in the number of fruit between the stressed and control fruit with chilling symptoms, where control (non-stressed) fruit had higher proportion of acceptable fruit than stressed fruit. But note that this trial was one variety in one season and broader inferences should not be drawn from this one results. Therefore this aspect of water stress management needs to be more thoroughly investigated before wider industry recommendations are made, especially for fruit destined for export which require a cold disinfestation treatment.

There was no effect of the source of the fruit from either the east or western aspect of the tree but as expected, the chilling symptoms increased with the addition of the one week shelf life at 20 °C.

2.1.c Postharvest - Effects of postharvest treatments on chilling injury

As outlined in the brief review in the Introduction, there are several postharvest techniques to reduce chilling injury. This trial assessed some new innovations which have been reported to minimise chilling symptoms:

Postharvest application of salts. 'Fortisol Ca' and 'Fortisol Ca Plus' are considered natural products from Citrosol (Spain). There are some reports that 'Forisol Ca' and 'Fortisol Ca Plus' have some abilities in reducing chilling injury symptoms. For example, Martínez-Hernández et al. (2017) showed postharvest application of 1% 'Fortisol Ca' and 1.5% 'Fortisol CaPlus' reduced chilling injury and red blotch in lemons stored for 33 days at 7°C, which simulated a long storage and transportation period, followed by 5 d at 22°C of retail sale period, simulating then a prolonged shelf life.

'Citrashine Long Life' wax. 'Citrashine Long Life' wax is a new wax formulation based on carnauba and shellac formulation developed by Decco (Spain) which has been reported to reduce chilling injury.

Gibberellic acid (GA3). Preharvest GA3 sprays are used to navel oranges to improve rind firmness and quality at harvest, for increased packouts and market outturns. In addition, GA3 has been shown to reduce albedo breakdown (creasing), and reduce postharvest rind breakdown and extend postharvest shelf-life. GA3 has been shown to reduce chilling injury in other crops such as mature green tomato fruit (Zhu et al. 2016) but some reports have shown no effect of GA3 on chilling injury (Porat et al., 2001). However, as this is already used in the citrus industry, it is worthy treatment for the comparison of other postharvest treatments.

Methods Navel oranges (commercial and mature 'green'), mandarins, grapefruit and lemons were harvested on 18/19 August 2019 from commercial orchards in Leeton, NSW. The focus of this trial was to examine the effects of postharvest treatments on commercial Navel oranges, but some of these treatments were also tested on mature 'green' Navels, mandarins, grapefruit and lemon fruit as outlined below.

Commercial Navel oranges (1) were treated with six postharvest treatments (Figure 45):

1. 1.5% 'Fortisol Ca' (x 30 second dip)
2. 1.5% 'Fortisol Ca Plus' (30 second dip)
3. 100ppm GA3 (ProGibb) (x 30 second dip)
4. 'Natural Shine 960' - carnaubra wax (current packinghouse wax)
5. Decco 'Citrashine Long Life' wax
6. Untreated control (no treatment)

Mature 'green' Navel oranges (with some green colour in peel) (2) and mandarin fruit (3) and grapefruit (4) were treated with:

1. 1.5% 'Fortisol Ca' (x 30 second dip)
2. 1.5% 'Fortisol Ca Plus' (30 second dip)
3. 100ppm GA3 (ProGibb) (x 30 second dip)
4. Untreated control (no treatment)

Lemon fruit (2 replicates only) were treated with:

1. 1.5% 'Fortisol Ca' (x 30 second dip)
2. 100ppm GA3 (ProGibb) (x 30 second dip)
3. Untreated control (no treatment)

The treatment unit was at least 70 fruit per box which were replicated four times, except lemon fruit which only had two replicates. After treatment, the fruit were transported to NSW Department of Primary Industries at Ourimbah, where the fruit were stored for 8 weeks at 2 °C before chilling injury assessment immediately upon removal and also after 1 week at 20 °C. Fruit weights of ten individually labelled fruit from each treatment unit were recorded before and after storage.

Figure 45. Treatment of citrus fruit with postharvest dips (left), preparation of wax in commercial packingline (middle) and storage at NSW Department of Primary Industries (right).

Results

The internal fruit quality (TSS and TA) at the beginning of the storage trial of the different citrus fruit were: commercial Navel orange (TSS 11.9, TA 1.16 % citric acid), mature 'green' Navel orange (TSS 10.0 % Brix, TA 1.24 % citric acid), grapefruit (TSS 13.9% Brix, TA 2.5 % citric acid), mandarin (TSS 15.8% Brix, TA 1.65 % citric acid) and lemon (TSS 8.6% Brix, TA 6.38% citric acid).

A summary table of the chilling injury results in the different citrus types following treatment and cold storage (with an additional shelf life at 20 ^{o}C) is presented in Table 20. This is a comparison of the different results and treatments but the individual comparisons with analysis are presented in each citrus type – below.

Table 20. Summary table of different citrus types and postharvest treatments on average chilling severity score, the proportion (%) of fruit with *'Excellent'* scores (i.e. chilling score 1) and the proportion (%) of fruit with *'Satisfactory'* scores (i.e. chilling score 1 + 2).

		Average Chilling Score *		Proportion of fruit *'Excellent'* score		Proportion of fruit *'Satisfactory'* score	
Citrus type		Upon removal	+ 1 week shelf life	Upon removal	+ 1 week shelf life	Upon removal	+ 1 week shelf life
Commercial Navel	Fortisol Ca	1.7	1.9	60	53	84	79
	Fortisol Ca Plus	1.4	1.5	64	54	99	98
	'Citrashine Long Life' wax	1.4	1.5	59	52	100	98
	ProGibb	1.6	1.7	54	47	91	88
	'Natural Shine 960' wax	1.4	1.5	63	54	100	99
	Untreated control	1.7	2.0	52	38	86	80
Mature 'Green' Navel	Fortisol Ca	1.2	1.3	82	73	100	100
	Fortisol Ca Plus	1.1	1.2	85	77	100	99
	ProGibb	1.2	1.3	82	74	100	98
	Untreated control	1.2	1.2	80	77	100	100
Grapefruit	Fortisol Ca	1.5	1.7	51	39	98	90
	Fortisol Ca Plus	1.8	2.0	28	27	94	88
	ProGibb	1.8	1.9	37	31	90	89
	Untreated control	1.6	1.7	48	39	94	93
Mandarin	Fortisol Ca	1.2	1.3	81	74	100	100
	Fortisol Ca Plus	1.3	1.3	75	73	100	98
	ProGibb	3.9	4.2	0	1	9	10
	Untreated control	1.2	1.3	79	74	100	100
Lemon	Fortisol Ca	2.3	2.9	19	6	70	34
	ProGibb	1.9	2.1	29	25	84	71
	Untreated control	2.3	3.2	17	8	63	31

** Chilling injury subjective score: 1 = no pitting, 2 = a few scattered pit symptoms, 3 = up to 10% of the fruit surface with chilling symptoms, 4 = up to 30% of the fruit surface with pit symptoms; and 5 = severe pitting covering >30% of the fruit surface*

Navel oranges

There was no effect of postharvest treatment on the severity of average chilling score in commercial Navel oranges (Table 21). There was a significant difference in the severity of average chilling score between the different assessment times, where fruit upon removal had an average chilling score of 1.5, while after an additional one week shelf life at 20 °C, the average chilling score was 1.7.

Table 21. Effect of postharvest treatment on the severity of average chilling score in commercial Navel oranges after 8 weeks storage at 2 °C and one week at 20 °C.

Treatment	Upon removal	+ one week at 20 °C
Fortisol Ca	1.7	1.9
Fortisol Ca Plus	1.4	1.5
'Citrashine Long Life' wax	1.4	1.5
ProGibb	1.6	1.7
'Natural Shine 960' wax	1.4	1.5
Untreated control	1.7	2.0

Chilling injury subjective score: 1 = no pitting, 2 = a few scattered pit symptoms, 3 = up to 10% of the fruit surface with chilling symptoms, 4 = up to 30% of the fruit surface with pit symptoms; and 5 = severe pitting covering >30% of the fruit surface

There was no effect of the number of fruit with no chilling symptoms (Table 22), but shelf life had a significant effect on overall fruit with no chilling symptoms. A total of 59% of all fruit (with 2.9% standard error) had no chilling symptoms, and after one week shelf life, the proportion declined to 50% (with 3.0% standard error).

Table 22. Effect of postharvest treatment the percentage (%) of commercial Navel oranges with no chilling symptoms (chilling score = 1) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C (The estimates of the predicted standard errors are presented in parenthesis)

Treatment	Upon removal	+ one week at 20 °C
Fortisol Ca	60 (7.2)	53 (7.5)
Fortisol Ca Plus	64 (6.8)	54 (7.1)
'Citrashine Long Life' wax	59 (7.1)	52 (7.3)
ProGibb	54 (7.2)	47 (7.4)
'Natural Shine 960' wax	63 (6.7)	54 (7.0)
Untreated control	52 (7.4)	38 (7.4)

There was a significant difference between the different treatment when counting the number of fruit with no chilling symptoms or trace / very few chilling symptoms ($p<0.001$). The results presented in Table 23 show the percentage (%) of Navel fruit with acceptable chilling symptoms at each assessment and the average number of fruit across the different assessments. These results show that both wax treatments ('Citrashine Long Life' wax and 'Natural Shine 960' wax) along with the 'Fortisol Ca Plus' pre-storage treatment had more acceptable fruit than the untreated control fruit.

Table 23. Effect of postharvest treatment on the percentage (%) of commercial Navel fruit with acceptable chilling symptoms (chilling score = 1 + 2, i.e. combination of fruit with no symptoms and those with trace levels) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C
(The estimates of the predicted standard errors are presented in parenthesis)

Treatment	**Upon removal**	**+ one week at 20 °C**	***Average***
Fortisol Ca	84 (3.8)	79 (4.3)	*82 (2.9) b*
Fortisol Ca Plus	99 (1.0)	98 (1.4)	*98 (0.8) c*
'Citrashine Long Life' wax	100 (0.6)	98 (1.4)	*99 (0.7) c*
ProGibb	91 (3.0)	88 (3.5)	*90 (2.3) b*
'Natural Shine 960' wax	100 (0.6)	99 (0.8)	*99 (0.5) c*
Untreated control	86 (3.6)	80 (4.2)	*83 (2.7) ab*

Treatments in the same column with the same small letter indicate these treatments are statistically similar (p<0.05).

There was no effect of treatment or assessment time on TSS. In addition, there was no effect of treatment on TA, but TA was significantly lower after shelf life assessment, where upon removal the average TA was 1.0 % citric acid and after 1 week at 20 °C, the average TA was 0.9 % citric acid (Table 24).

Table 24. Effect of postharvest treatment on the TSS and TA in commercial Navel oranges upon removal from storage and after one week at 20 °C.

	TSS		**TA**	
Treatment	**Upon removal**	**+ one week at 20 °C**	**Upon removal**	**+ one week at 20 °C**
Fortisol Ca	12.7	12.0	1.05	0.82
Fortisol Ca Plus	12.3	12.2	1.01	0.91
'Citrashine Long Life' wax	12.1	11.9	1.10	0.96
ProGibb	11.9	11.9	1.00	0.91
'Natural Shine 960' wax	12.0	12.3	1.16	0.90
Untreated control	12.0	12.1	0.97	0.86

Mature Green Navels

Mature navel oranges with some green colour in the peel were used for this component. The treatments used for this comparison were: 'Fortisol Ca', 'Fortisol Ca Plus', 'ProGibb' and untreated control. The levels of chilling injury in these fruit were low with most fruit having none or just trace levels of chilling injury (Table 25). There was no difference between the treatments on the severity of average chilling score, but as expected there was a difference between assessment times, where fruit assessed upon removal had lower average chilling scores (1.2), than after one week shelf-life at 20 °C (1.2).

Treatment also did not have any effect on the number of fruit with no chilling symptoms (chilling score = 1), but the shelf life resulted in more fruit with chilling damage (Table 26).

Table 25. Effect of postharvest treatment on the severity of average chilling score in mature green Navel oranges after 8 weeks storage at 2 °C and one week at 20 °C.

Treatment	Upon removal	+ one week at 20 °C
Fortisol Ca	1.2	1.3
Fortisol Ca Plus	1.1	1.2
ProGibb	1.2	1.3
Untreated control	1.2	1.2

Chilling injury subjective score: 1 = no pitting, 2 = a few scattered pit symptoms, 3 = up to 10% of the fruit surface with chilling symptoms, 4 = up to 30% of the fruit surface with pit symptoms; and 5 = severe pitting covering >30% of the fruit surface

Table 26. Effect of postharvest treatment on the percentage (%) of mature green Navel fruit with no chilling symptoms (chilling score = 1) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C (The estimates of the predicted standard errors are presented in parenthesis)

Treatment	Upon removal	+ one week at 20 °C
Fortisol Ca	82 (3.3)	73 (4.0)
Fortisol Ca Plus	85 (3.2)	77 (3.9)
ProGibb	82 (3.4)	74 (4.0)
Untreated control	80 (3.5)	77 (3.8)
Average	*82 (1.7) a*	*75 (1.9) b*

Treatments in the same row with the same small letter indicate these treatments are statistically similar ($p<0.05$).

There was a statistical effect on the interaction of TSS and assessment time, but the magnitude of these differences were small (Table 27) and not considered commercial. There was no difference in the treatments or assessment time on TA.

Table 27. Effect of postharvest treatment on the TSS and TA in mature green Navel oranges upon removal from storage and after one week at 20 °C.

	TSS		TA	
Treatment	**Upon removal**	**+ one week at 20 °C**	**Upon removal**	**+ one week at 20 °C**
Fortisol Ca	9.9	10.3	1.08	1.20
Fortisol Ca Plus	10.1	10.3	1.12	1.10
ProGibb	11.0	10.6	1.22	1.28
Untreated control	10.6	10.3	1.27	1.03

Grapefruit

There was no effect of postharvest treatment on the severity of average chilling score in grapefruit (Table 28). There was a significant difference in the severity of average chilling score between the different assessment times, where fruit upon removal had an average chilling score of 1.7, while after an additional one week shelf life at 20 °C, the average chilling score was 1.8. There was a treatment difference detected in the proportion of grapefruit with no chilling symptoms, where 'Fortisol Ca Plus' had lower acceptable fruit (Table 29). When combined with the total acceptable fruit (i.e. fruit with no symptoms and fruit with trace chilling injury symptoms), there were no differences in treatment or assessment time (Table 30).

Table 28. Effect of postharvest treatment on the severity of average chilling score in grapefruit after 8 weeks storage at 2 °C and one week at 20 °C.

Treatment	Upon removal	+ one week at 20 °C
Fortisol Ca	1.5	1.7
Fortisol Ca Plus	1.8	1.9
ProGibb	1.8	1.9
Untreated control	1.6	1.7

Chilling injury subjective score: 1 = no pitting, 2 = a few scattered pit symptoms, 3 = up to 10% of the fruit surface with chilling symptoms, 4 = up to 30% of the fruit surface with pit symptoms; and 5 = severe pitting covering >30% of the fruit surface

Table 29. Effect of postharvest treatment on the percentage (%) of grapefruit with no chilling symptoms (chilling score = 1) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C (The estimates of the predicted standard errors are presented in parenthesis)

Treatment	Upon removal	+ one week at 20 °C	Average
Fortisol Ca	51 (6.0)	39 (6.2)	46 (4.3) b
Fortisol Ca Plus	28 (5.8)	27 (6.0)	28 (4.2) a
ProGibb	37 (5.7)	31 (5.7)	34 (4.0) ab
Untreated control	48 (5.9)	39 (5.9)	44 (4.1) b

Treatments in the same column with the same small letter indicate these treatments are statistically similar ($p<0.05$).

Table 30. Effect of postharvest treatment on the percentage (%) of grapefruit fruit with acceptable chilling symptoms (chilling score = 1 + 2, i.e. combination of fruit with no symptoms and those with trace levels) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C (The estimates of the predicted standard errors are presented in parenthesis)

Treatment	Upon removal	+ one week at 20 °C
Fortisol Ca	98 (1.4)	91 (2.8)
Fortisol Ca Plus	94 (2.3)	88 (3.4)
ProGibb	90 (2.7)	89 (2.9)
Untreated control	94 (2.1)	93 (2.3)

There was no difference in TSS and TA between the different treatments and assessment times (Table 31).

Table 31. Effect of postharvest treatment on the TSS and TA in grapefruit upon removal from storage and after one week at 20 °C.

	TSS		TA	
Treatment	**Upon removal**	**+ one week at 20 °C**	**Upon removal**	**+ one week at 20 °C**
Fortisol Ca	14.2	14.0	2.2	2.2
Fortisol Ca Plus	14.1	14.0	2.4	2.0
ProGibb	14.3	14.3	2.3	2.2
Untreated control	14.5	14.5	2.2	2.4

Mandarins

Treatment of the mandarin fruit with ProGibb resulted in phyto-toxicity of the peel (Figure 46). These phyto-toxicity symptoms were included in the symptoms of chilling injury as it was not possible to differentiate the different skin damage types. As expected the results showed that there was a treatment difference in the severity of average chilling score of chilling injury (Table 32), where ProGibb treatment was had higher damage, and there was no effect between the other treatments. This was also observed with the number of fruit with no chilling symptoms (Table 33) and fruit with acceptable chilling damage (Table 34).

Figure 46. Phyto-toxicity damage on the peel of mandarin fruit following 100 ppm postharvest ProGibb treatment.

Note severe burning / discolouration of the peel following treatment.

Table 32. Effect of postharvest treatment on the severity of average chilling score in mandarins after 8 weeks storage at 2 °C and one week at 20 °C.

Treatment	**Upon removal**	**+ one week at 20 °C**	*Average*
Fortisol Ca	1.2	1.3	*1.2 A*
Fortisol Ca Plus	1.3	1.3	*1.3 A*
ProGibb	3.9	4.2	*4.0 B*
Untreated control	1.2	1.3	*1.2 A*
Average	*1.9 a*	*2.0 b*	

Chilling injury subjective score: 1 = no pitting, 2 = a few scattered pit symptoms, 3 = up to 10% of the fruit surface with chilling symptoms, 4 = up to 30% of the fruit surface with pit symptoms; and 5 = severe pitting covering >30% of the fruit surface. Treatments in the same column with the same small letter indicate these treatments are statistically similar (p<0.05). Treatments in the same row with the same capital letter indicate these treatments are statistically similar (p<0.05)

Table 33. Effect of postharvest treatment on the percentage (%) of mandarins with no chilling symptoms (chilling score = 1) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C (The estimates of the predicted standard errors are presented in parenthesis)

Treatment	**Upon removal**	**+ one week at 20 °C**	*Average*
Fortisol Ca	81 (3.0)	74 (3.3)	*77 (2.2) a*
Fortisol Ca Plus	75 (3.2)	73 (3.4)	*74 (2.3) a*
ProGibb	0 (0.4)	1 (1.0)	*1 (0.5) b*
Untreated control	79 (3.1)	74 (3.3)	*76 (2.2) a*

Treatments in the same column with the same small letter indicate these treatments are statistically similar (p<0.05).

Table 34. Effect of postharvest treatment on the percentage (%) of mandarins with acceptable chilling symptoms (chilling score = 1 + 2, i.e. combination of fruit with no symptoms and those with trace levels) upon removal after 8 weeks storage at 2 °C or an additional one week at 20 °C (The estimates of the predicted standard errors are presented in parenthesis)

Treatment	**Upon removal**	**+ one week at 20 °C**	*Average*
Fortisol Ca	100 (0.3)	100 (0.3)	*100 (0.3) a*
Fortisol Ca Plus	100 (0.0)	98 (0.9)	*99 (0.4) a*
ProGibb	9 (1.7	10 (1.9)	*9 (1.2) b*
Untreated control	100 (0.3)	100 (0)	*100 (0.1) a*

There was no difference in TSS between the different treatments and assessment times (Table 35). There was no effect of treatment on mandarin fruit TA, but assessment time had a significant effect on TA, where fruit upon removal had an average TA of 1.5 % citric acid, and after one week at 20 °C the average TA was 1.3% citric acid.

Table 35. Effect of postharvest treatment on the TSS and TA in mandarins upon removal from storage and after one week at 20 °C.

	TSS		**TA**	
Treatment	**Upon removal**	**+ one week at 20 °C**	**Upon removal**	**+ one week at 20 °C**
Fortisol Ca	16.4	16.3	1.4	1.3
Fortisol Ca Plus	17.0	16.6	1.5	1.4
ProGibb	16.2	16.2	1.5	1.3
Untreated control	16.1	16.2	1.6	1.3

Lemons

There were only two replicates for the lemons, therefore full statistical analysis was not possible, but a summary of the means and standard errors are presented in Tables 36 and 37.

Table 36. Effect of postharvest treatment on the average chilling injury severity score, the percentage (%) of lemons with no chilling symptoms and acceptable chilling levels upon removal from storage and after one week at 20 °C.

	Severity score		Excellent		Acceptable	
Treatment	**Upon removal**	**+ one week at 20 °C**	**Upon removal**	**+ one week at 20 °C**	**Upon removal**	**+ one week at 20 °C**
Fortisol Ca	2.3 (0.2)	2.9 (0.1)	19 (3.6)	6 (2.1)	70 (4.2)	34 (7.8)
ProGibb	1.9 (0.0)	2.1 (0.1)	29 (0.7)	25 (5.0)	84 (1.4)	71 (2.8)
Untreated control	2.3 (0.1)	3.2 (0.1)	17 (4.2)	8 (0.7)	63 (5.7)	31 (6.4)

Table 37. Effect of postharvest treatment on the TSS and TA in lemons upon removal from storage and after one week at 20 °C.

	TSS		TA	
Treatment	**Upon removal**	**+ one week at 20 °C**	**Upon removal**	**+ one week at 20 °C**
Fortisol Ca	8.0 (0.1)	8.3 (0.3)	5.2 (0.9)	5.3 (0.3)
ProGibb	8.2 (0.3)	8.3 (0.2)	5.0 (1.2)	5.7 (0)
Untreated control	8.3 (0.1)	8.2 (0.2)	5.9 (1.6)	5.5 (0)

Conclusions This trial examined a range of postharvest treatments which have previously shown promise to minimise chilling injury during cold storage of citrus. Overall the level of chilling injury in the trial was low, meaning that treatment differences are generally difficult to detect. The primary aim of the study was to trial all the different treatments on commercial Navel oranges. The results showed when averaging across all fruit in the box, there were no differences in average chilling injury between the treatments, but the numbers of fruit with chilling injury symptoms was significantly reduced with the both wax treatments ('Citrashine Long Life' wax and 'Natural Shine 960' wax) while 'Fortisol Ca Plus' pre-storage treatment also had more acceptable fruit than the untreated control fruit. There were no postharvest treatment differences observed in the stored mature green Navel oranges which had some green tinge to the peel, but these fruit had lower overall chilling symptoms. The postharvest gibberellic acid (ProGibb) had no effect on chilling injury in any treatment, but caused significant phyto-toxicity to the mandarin peel.

Chilling injury is an inconsistent chilling disorder which is difficult to predict. The postharvest application of treatment to minimise the incidence and severity of the disorder would improve confidence in cold treated fruit and reduce the risk of claims. This one season study showed that some postharvest treatments ('Citrashine Long Life' wax and 'Natural Shine 960' wax, and 'Fortisol Ca Plus' pre-storage treatment) had the potential to minimise the number of fruit which develop postharvest chilling symptoms. More work should occur to further examine these treatments in different seasons.

2.2 Minimising the development of off-flavours in mandarins in export markets

There are occasional out-turn issues with the development of off-flavours in some export mandarins to south east Asia. It is generally accepted that off-flavours are the result of anaerobic stresses which produce high levels of ethanol and acetaldehyde in storage. Dr Andrew MacNish (Queensland DPI) and his research team have done extensive work looking at the role of waxes in providing a barrier to the diffusion of gases between the fruit and the air. They showed that different waxes have different permeabilities to oxygen and carbon dioxide, and that carnauba waxes are recommended in order to minimise the likelihood of anaerobic stress. However, while carnauba waxes allow greater gas exchange there still remains the potential issue for the production of off-flavours.

Another potential source of anaerobic stress leading to off-flavours is low ventilation rates in shipping containers. Low ventilation rates potentially cause a lack of enough fresh air into the container leading to a build-up of carbon dioxide (CO_2) and lowering of oxygen levels within the consignment. The current recommendations for the air exchange / ventilation rates for shipping Murcott mandarins are unclear, with different shipping companies recommending different ventilation rates (Table 38). The ideal ventilation rate will be high enough not allow a build-up of CO_2 within the container, but not too high to have minimal impact on water loss from the fruit. Excessive ventilation rates will cause excessive water loss from the fruit, even with wax application.

The aim of this trial was to investigate different container ventilation rates on fruit quality and the development of off-flavours in waxed and unwaxed Murcott mandarin fruit after a simulated export and shelf-life assessment.

Table 38. Current ventilation rate recommendations for 40' reefer shipping containers for citrus exports. (from literature and personal communications)

Shipping container / reference	Citrus type	Storage temperature	Ventilation / air exchange (cbm/hr)	Relative humidity (%)
Hamburg Reefers	Mandarins, tangelos, easy peelers	4-8 °C	15 - 25	90-95
Hamburg Reefers	Oranges	2-10 °C	15 – 25	85-90
Shipping Australia / FSA / AQIS*	Grapefruit	10-15 °C	12	85-90
Shipping Australia / FSA / AQIS*	Lemon	10-14 °C	10 *	85-90
Shipping Australia / FSA / AQIS*	Lime	8-10 °C	4 *	85-90
Shipping Australia / FSA / AQIS*	Mandarin	-	*No recommendation made*	-
Shipping Australia / FSA / AQIS*	Orange	5-7 °C	6 (Valencia = 10, Navel = 8)	85-90
Emirates Shipping	Grapefruit	-	15	90
Emirates Shipping	Lemon	-	15	90
Emirates Shipping	Limes	8-10 °C	15	90
Emirates Shipping	Oranges	4-12 °C	15	90
Costco Shipping	Lemons and limes	11 °C	25	90
Costco Shipping	Oranges	1-9 °C	25	90-95
Exporter – Queensland mandarins	Mandarins	0.8-1.0 °C	10	

**from Shipping Australia / FSA / AQIS draft 'Code of Practice for handling fresh fruit and vegetables in refrigerated shipping containers for Australian exports'. These ventilation rates for 40' (12m) containers assume 20 tonnes of product at its correct carriage temperature. Rates have been calculated to keep carbon dioxide levels below 1%.*

Methods Low-seeded Murcott mandarin fruit were sourced from a commercial exporter in Queensland. All fruit were premium quality and had been treated with imazalil. Half the fruit were taken from the packingline before the waxer (i.e. unwaxed) and the other half the fruit for the experiment were waxed with 'Decco Canauba Premium' wax (0.5L per tonne). Upon arrival of fruit at NSW Department of Primary Industries, fruit within each of the waxed and unwaxed categories were randomised, sorted and allocated four treatments (5, 15, 25 and 35 cbm per hour) (Figure 47). Fruit at the cold temperature (1 ^{o}C) were weighed before placing into 60L steel drums with the same fill capacity as the export container. Similarly the drums had equivalent flow rates as the export container (5, 15, 25 and 35 cbm per hour). Each treatment unit (drum) was replicated three times. Fruit were stored at 1 ^{o}C for a simulated cold treatment export treatment of 24 days. Fruit weights of ten individually labelled fruit from each treatment unit were recorded before and after storage. During storage, the O_2, CO_2 and ethylene levels from the outlet of each drum were assessed twice per week. After cold treatment, fruit were assessed; (1) immediately upon removal from cold storage, (2) after an addition 3 days at 20 ^{o}C and (3) after an additional 7 days at 20 ^{o}C. ANOVA was used to test the effects of wax, ventilation, storage time and their interactions on a range of postharvest variables. A $\log_e$ transformation prior to the ANOVA was necessary for both internal ethanol and juice ethanol to satisfy the ANOVA assumptions. Statistical analysis was conducted by NSW Department of Primary Industries Biometrician, Lorraine Spohr.

On a side batch of fruit from the same batch (waxed and unwaxed), fruit respiration production rates were measured two times per week on fruit stored at 1 ^{o}C and 20 ^{o}C in air.

At each assessment time, fruit weight loss, fruit firmness (15 fruit per replicate), fruit respiration rate (also CO_2 levels inside fruit cavity) (x 2 fruit per sample, x 5 samples per rep), ethanol levels in juice (also ethanol levels inside fruit cavity) (x 5 individual fruit per rep) and fruit juice TSS / TA were measured.

Figure 47. Setting up the mandarin ventilation trial at NSW Department of Primary Industries.

Sensory In addition, consumer taste panels were conducted at NSW Department of Primary Industries on the test fruit, three and seven days after removal from cold treatment. This was conducted by Dr Soumi Paul Mukhopadhyay, Sensory and Consumer Science Researcher at NSW Department of Primary Industries.

The experimental design consisted of multiple 4 x 4 Latin square designs – where each judge assessed all 4 ventilation treatments. A feature of a Latin square design is that the order of tasting is balanced so that each treatment is presented either 1st, 2nd, 3rd or 4th in a specified order. The effects of judge and order were accounted for in the analysis. Four sensory assessments were undertaken at NSW Department of Primary Industries at Ourimbah with varying numbers of untrained judges:

Assessment 1. + 3 days shelf life with waxed fruit (21 judges)
Assessment 2. + 3 days shelf life with unwaxed fruit (19 judges)
Assessment 3. + 7 days shelf life with waxed fruit (16 judges)
Assessment 4. + 7 days shelf life with unwaxed fruit (14 judges)

Since the judges were volunteers, it was not possible to ensure judges were the same people for all 4 assessments and wax treatments. This required 4 separate statistical analyses to be undertaken. The effects of postharvest storage and wax were not tested in the sensory phase of this trial.

Untrained consumers / judges were asked to detect any off-flavours (aroma and taste) in each mandarin and if there were any off-flavours, what was the strength of the off-flavours on a 5-point scale. After smelling the fruit, the consumers were asked to peel and eat each mandarin and then asked to rate the following attributes on a 9-point scale (aroma, sweetness, acidity / sourness, flavour / taste, and overall liking). The sensory evaluation questionnaire is attached as Appendix 2.

Statistical analysis Only Assessment 3 could be analysed as a standard Latin square design since it included 16 judges. (16 / 4 = 4 balanced Latin squares in this design). Unbalanced experimental designs (assessments 1, 2 and 4) were analysed using REML. Statistical analysis was conducted by Lorraine Spohr, Biometrician NSW Department of Primary Industries. The ventilation treatment effect was tested at the p=0.05 significance level. Treatment means were then compared using the Fisher's protected least significant difference (l.s.d.) procedure.

Results

In the side batch of fruit (both waxed and unwaxed), fruit respiration production rates were measured two times per week on fruit stored at 1 °C and 20 °C and the results are presented in Figure 48. The results show the strong effect of storage temperature on fruit respiration, where fruit stored at 20 °C had significantly higher respiration rates than fruit stored at 1 °C (Figure 48). The fruit stored at 1 °C were transferred to 20°C on Day 39, and the increase in respiration rate upon transfer to 20 °C was dramatic.

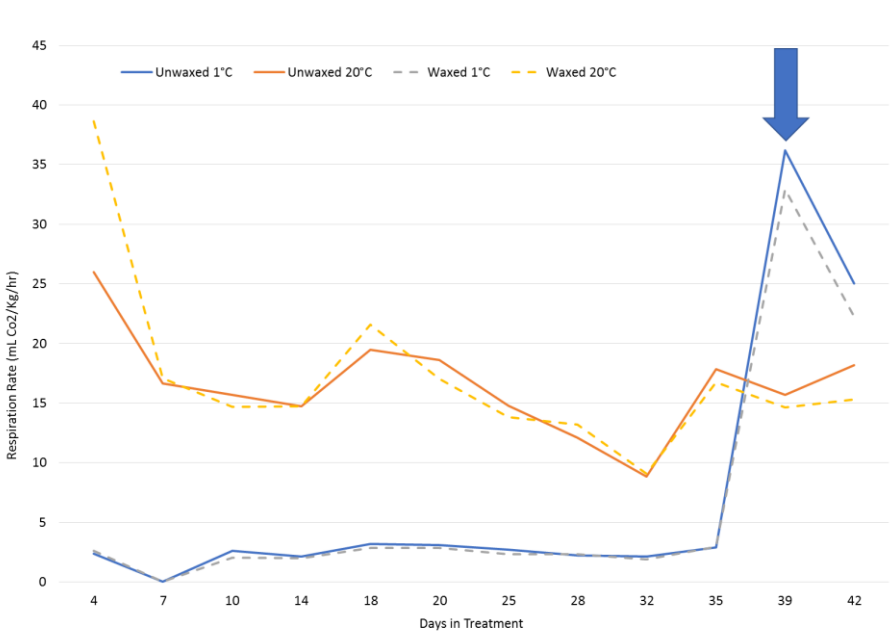

Figure 48. Fruit respiration rate of waxed and unwaxed Murcott mandarins stored at either 1 °C or 20 °C. After 39 days at 1 °C, this fruit was transferred to 20 °C.

The carbon dioxide (CO_2) content of the drums containing the fruit during the main storage experiment at 1 °C are shown in Figures 49 and 50. The effect of ventilation rate (5, 15, 25 and 35 cbm/hr) on the carbon dioxide (%) level of the exiting ventilation stream of waxed Murcortt mandarins is shown in Figure 49 and shows that the lowest ventilation rate (5cbm) had an average % CO_2 in the drums of 0.8 % CO_2. The 15cbm resulted in 0.4% CO_2 in the drum and the highest ventilation rates (25 and 35cbm) were around 0.2% CO_2. Similarly the CO_2 levels in the drums with the unwaxed Murcortt mandarins also showed that the lowest ventilation rate (5cbm) had an average % CO_2 in the drums of 1.0 % CO_2. The 15cbm was 0.4% CO_2 and the highest ventilation rates (25 and 35cbm) were around 0.2% CO_2 (Figure 50). These results show that as expected, the lower ventilation flow rates resulted in high levels of CO_2 in the storage drums.

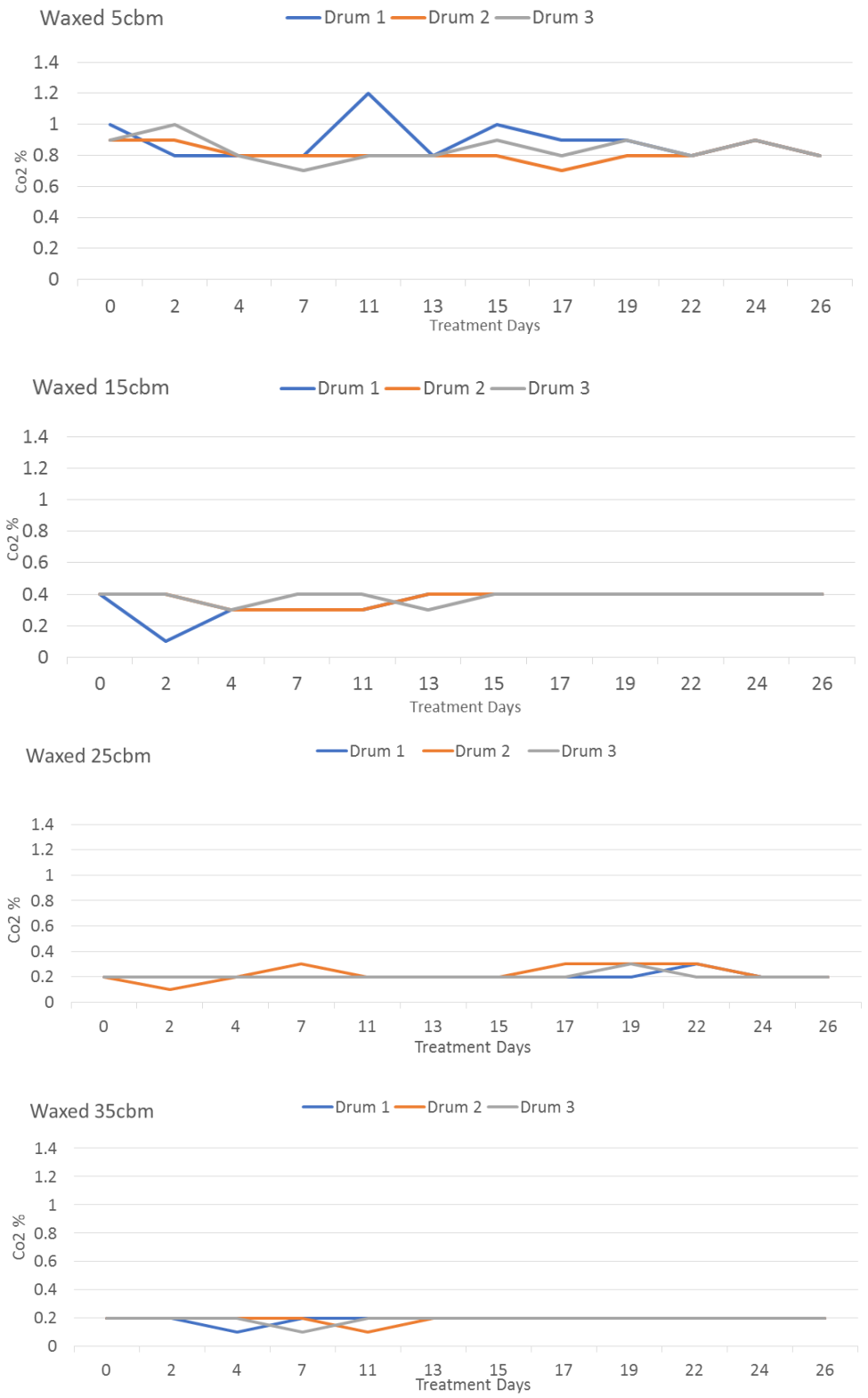

Figure 49. Effect of ventilation rate (5, 15, 25 and 35 cbm/hr) on the carbon dioxide (%) level of the exiting ventilation stream of waxed Murcortt mandarins stored at 1 °C.
There were 3 drums / replicates per treatment

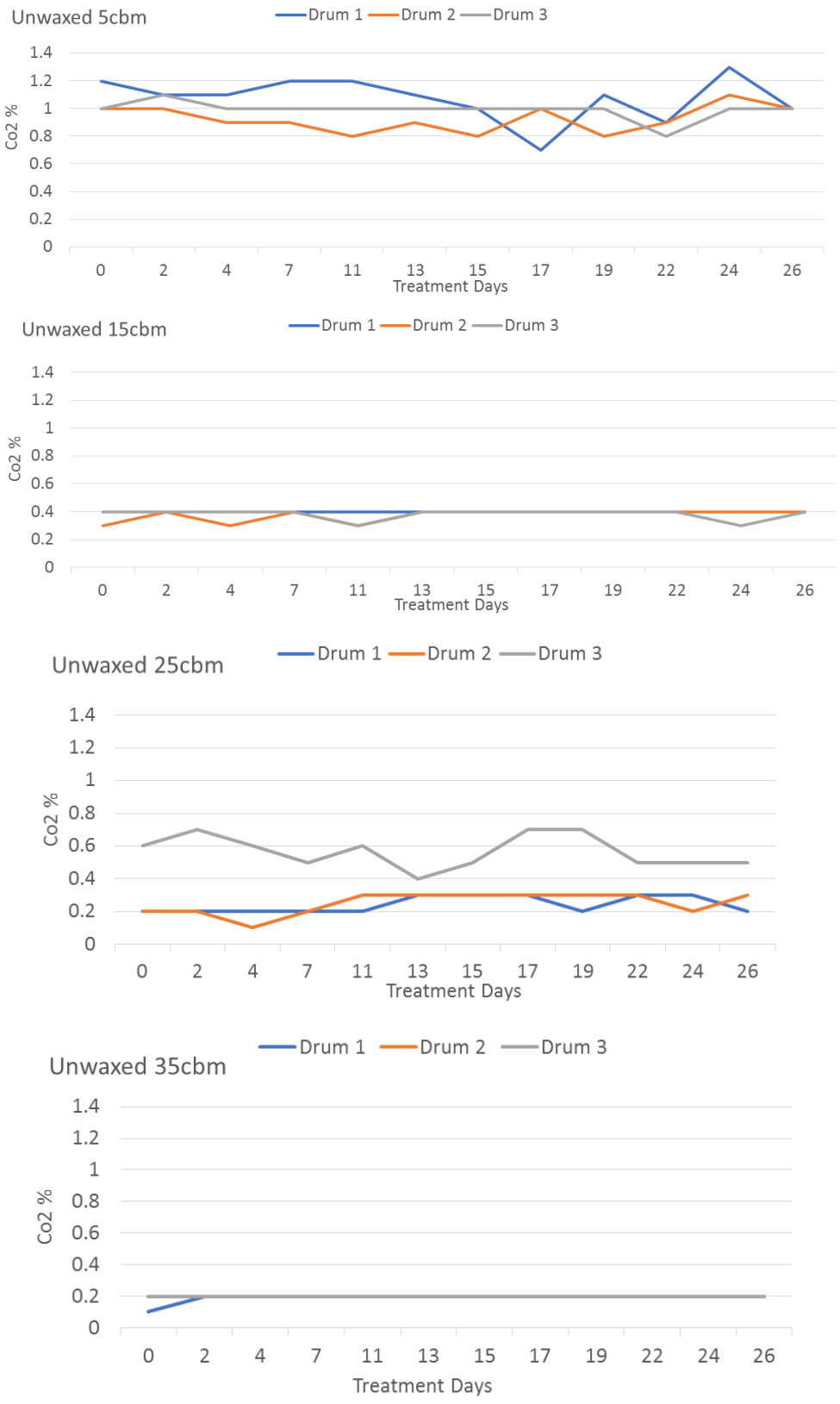

Figure 50. Effect of ventilation rate (5, 15, 25 and 35 cbm/hr) on the carbon dioxide (%) level of the exiting ventilation stream of unwaxed Murcortt mandarins stored at 1 ^{o}C.
There were 3 drums / replicates per treatment

Weight loss Weight loss equates to fruit water loss through transpiration and respiration. As expected, weight loss increased with storage time (Table 39). There was a significant effect of ventilation rate and storage time on weight loss (Table 40), where upon removal there was no effect of the different ventilation rates on water loss, but as time progresses at 20 °C, fruit weight loss increases, with the 15 and 25 cbm treatments having higher losses after 7 days storage at 20 °C (Table 40). While these differences in water loss were statistically significant, these differences would not have large commercial differences. There was also no effect of wax treatment on water loss immediately upon removal from cold storage, but as time progressed, waxed fruit retained more weight than unwaxed fruit (Table 41).

Table 39. Effect of storage time on % weight loss in Murcott mandarins stored for 24 days at 1 °C with 3 and 7 days shelf life at 20 °C.

Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C
0.3 a	1.9 b	4.0 c

Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Table 40. Effect of ventilation rate and storage time on % weight loss in Murcott mandarins stored for 24 days at 1 °C with 3 and 7 days shelf life at 20 °C.

Ventilation (cbm)	Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C
5	0.29 Aa	1.8 Ab	3.8 Ac
15	0.33 Aa	1.8 Ab	4.1 Bc
25	0.32 Aa	1.9 Ab	4.2 Bc
35	0.41 Aa	1.9 Ab	3.7 Ac

Treatments in the same column with the same capital letter indicate these treatments are statistically similar (p<0.05). Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Table 41. Effect of wax and storage time on % weight loss in Murcott mandarins stored for 24 days at 1 °C with 3 and 7 days shelf life at 20 °C.

	Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C
Unwaxed	0.38 Aa	2.3 Ab	4.4 Ac
Waxed	0.30 Aa	1.4 Bb	3.6 Bc

Treatments in the same column with the same capital letter indicate these treatments are statistically similar (p<0.05). Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Fruit respiration rate There was a significant three way interaction between wax, ventilation rate and storage time (Figure 51). These were a series of complex interactions which make interpretation of the results difficult but in general results show fruit respiration increased after removal from cold storage and with shelf life and waxed fruit had lower respiration rates than unwaxed fruit. However there were many interactions between the different factors.

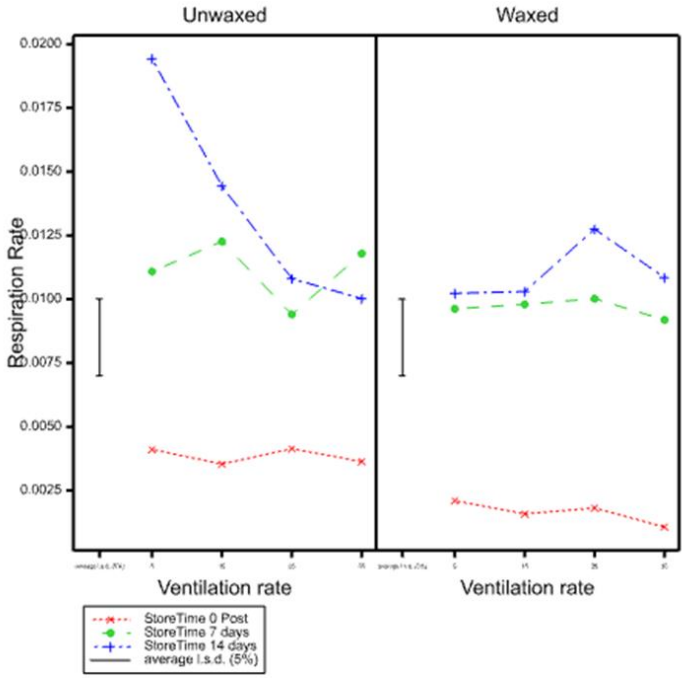

Figure 51. Effect of ventilation rate, storage time and wax on the fruit respiration rate of Murcott mandarins stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

Internal carbon dioxide (CO_2) content The level of CO_2 within the fruit is thought to be an indicator of fruit metabolism and can relate to anaerobic respiration (Oberland and Arpia, 2019). In this experiment, there was no effect of ventilation rate or wax on the levels of internal CO_2 within the Murcott mandarins following treatment and storage (Table 42). There was a significant difference detected with assessment time, where in general, the longer shelf life periods resulted in higher internal CO_2 levels in the fruit.

Table 42. Effect of ventilation rate and wax on the internal CO_2 levels (%) in Murcott mandarins stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

	Ventilation (cbm)	Upon removal	+ 3days at 20 °C	+ 7 days at 20 °C	+ 14 days at 20 °C
Unwaxed	5	1.3	2.7	1.8	2.8
	15	1.0	2.7	2.0	2.8
	25	1.3	2.5	2.1	3.5
	35	1.1	2.6	2.8	3.0
Waxed	5	0.9	2.0	1.9	3.9
	15	1.0	2.6	2.4	2.8
	25	0.7	2.4	2.5	3.2
	35	0.9	3.0	2.2	3.2
	Average	*1.0 a*	*2.6 c*	*2.2 b*	*3.1 d*

Ethanol content in juice There was a significant interaction of wax treatment and assessment time on the level of ethanol in the juice of stored mandarins (Table 43). In general the level of ethanol in the juice increased with assessment time at 20 °C, and the waxed fruit accumulated more ethanol only at the first assessment time (upon removal).

Table 43. Effect of wax on the ethanol levels (ppm) in Murcott mandarin juice stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

	Upon removal	+ 3days at 20 °C	+ 7 days at 20 °C	+ 14 days at 20 °C
Unwaxed	8 Aa	136 Ab	140 Ab	289 Ac
Waxed	25 Ba	164 Ac	89 Ab	283 Ad

Treatments in the same column with the same capital letter indicate these treatments are statistically similar (p<0.05). Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Ethanol content inside fruit A significant three way interaction between wax, ventilation and storage time (Table 44) was detected which over-rides other two way interactions and other effects such as what is presented in Tables 45-48).

Table 44. Effect of ventilation rate, storage time and wax on the internal ethanol levels (ppm) in Murcott mandarins stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

	Ventilation (cbm)	Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C	+ 14 days at 20 °C
Unwaxed	5	0.8	7.1	7.0	7.6
	15	2.7	1.3	7.5	31.2
	25	2.1	1.2	2.2	9.9
	35	1.0	0.4	1.7	10.6
Waxed	5	16	1.6	12.6	24.9
	15	3.4	0.6	1.6	5.2
	25	0.24	1.4	0.7	6.3
	35	0.9	8.3	5.7	5.2

Given the data presented in Table 44 is a summary of the three-way interaction of the overall experiment, there was a significant interaction between ventilation rate and storage time (Table 45) which showed the differing effects of the ventilation rate the internal ethanol levels at the different storage times.

Table 45. Effect of ventilation rate and storage time on the internal ethanol levels (ppm) in Murcott mandarins stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

Ventilation (cbm)	Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C	+ 14 days at 20 °C
5	3.4	3.4	9.4	13.8
15	3.0	0.9	2.2	12.7
25	0.7	1.0	1.3	7.9
35	1.0	1.9	3.1	7.4

In general the lowest flow rate (5cbm) resulted in the highest levels of internal ethanol (Table 46) and longer storage times after 3 days storage at 20 °C resulted in higher internal ethanol levels (Table 47), but these observations were also affected by storage time (Table 45) and wax (Table 44).

Table 46. Effect of ventilation rate on the internal ethanol levels (ppm) in Murcott mandarins stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

Ventilation (cbm)			
5	15	25	35
6.2 b	2.9 a	1.6 a	2.6 a

Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Table 47. Effect of storage time on the internal ethanol levels (ppm) in Murcott mandarins stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C	+ 14 days at 20 °C
1.6 a	1.5 a	3.0 b	10.1 c

Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

There was a significant interaction of wax and ventilation rate, where waxed fruit held at 15cbm had lower internal ethanol levels than unwaxed fruit, but at all other flow rates there was no difference between waxed and unwaxed fruit (Table 48)

Table 48. Effect of ventilation rate and wax on the internal ethanol levels (ppm) in Murcott mandarins stored for 24 days at 1 °C with 3, 7 and 14 days shelf life at 20 °C.

	Ventilation (cbm)			
	5	15	25	35
unwaxed	4.1 Aa	5.3 Ab	2.3 Aa	1.7 Aa
waxed	9.4 Ac	1.6 Bab	1.1 Aa	3.9Ac

Treatments in the same column with the same capital letter indicate these treatments are statistically similar (p<0.05). Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Fruit firmness As expected, fruit stored for longer periods were less firm than fruit which had just been removed from cold storage (Table 49). In addition waxed fruit were firmer than unwaxed fruit (Table 50). There was no effect of ventilation rate on fruit firmness.

Table 49. Effect of storage time on fruit firmness (Newtons) in Murcott mandarins stored for 24 days at 1 °C with 3 and 7 days shelf life at 20 °C.

Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C
26.8 a	22.6 b	19.0 a

Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Table 50. Effect of waxing on fruit firmness (Newtons) in Murcott mandarins stored for 24 days at 1 °C with 3 and 7 days shelf life at 20 °C.

wax	unwaxed
24.7 a	20.9 b

Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Fruit TSS There was no effect of ventilation rate, wax or storage time on fruit TSS.

Fruit TA While there were significant differences in fruit TA during shelf life (Table 51), these differences were not commercially significant. In addition waxed fruit had lower TA than unwaxed fruit (Table 52), but this was due to the different fruit (from the same batch), used for this experiment. There was no effect of ventilation rate on fruit firmness.

Table 51. Effect of storage time on fruit TA (% citric acid) in Murcott mandarins stored for 24 days at 1 °C with 3 and 7 days shelf life at 20 °C.

Upon removal	+ 3 days at 20 °C	+ 7 days at 20 °C
0.87 a	0.95 b	0.85 a

Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Table 52. Effect of waxing on fruit firmness (Newtons) in Murcott mandarins stored for 24 days at 1 °C with 3 and 7 days shelf life at 20 °C.

wax	unwaxed
0.84 a	0.94 b

Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Sensory There were no ventilation treatment effects detected for any of the sensory attributes investigated (off-flavours (aroma and taste), aroma, sweetness, acidity / sourness, flavour / taste, and overall liking) (Table 53). However there was one significant ventilation effect was detected for '*Overall liking'* at the 4th assessment (7 days in unwaxed fruit) ($_{F3,35}$ =2.97 ; *p*=0.045) (Table 54), where the 15 cbm appeared to have the lowest overall score, but this was similar to the 5 cbm. However these differences were < 1 subjective unit score which is not commercially significant in the *'neutral to like'* acceptability range. Therefore overall there was no effect of ventilation on sensory acceptability in Murcott mandarins.

Table 53. Table of P values for the different sensory attributes for each assessment time of Murcott mandarins sensory assessment.

Sensory attribute	*Assessment 1* + 3 days with wax	*Assessment 2.* + 3 days with unwaxed	*Assessment 3* + 7 days with wax	*Assessment 4* + 7 days with unwaxed
	# judges = 21	*# judges = 19*	*# judges = 16*	*# judges = 14*
% Off flavour	0.153	-	-	0.360
Acidity	0.700	0.262	0.336	0.557
Aroma	0.748	0.985	0.744	0.413
Flavour	0.701	0.877	0.297	0.086
Sweetness	0.521	0.505	0.260	0.099
Overall	0.524	0.851	0.480	*0.045*

Table 54 Effect of ventilation rate, storage time and wax on '*Overall liking*' of Murcott mandarins stored for 24 days at 1 °C with 3 or 7 days shelf life at 20 °C.

Shelf life at 20 °C	Treatment	Ventilation (cbm)			
		5	15	25	35
3 days	unwaxed	6.4	6.3	6.4	6.7
	waxed	6.3	6.5	5.9	6.4
7 days	*unwaxed**	*5.9ab*	*5.8a*	*6.8c*	*6.6bc*
	waxed	6.6	6.2	5.9	6.4

** significant treatment effect for 7 days unwaxed fruit. Average least significant difference = 0.81*
Treatments in the same row with the same small letter indicate these treatments are statistically similar (p<0.05)

Conclusions There was no effect of ventilation rate on fruit weight loss, indicating that the high humidity of the flow through system used in this experiment did not differentially remove more water from the fruit. This may be different in the commercial situation, where the air exchange is from the outside of the container on the ship, and it would certainly be below the 95% relative humidity used in this trial.

There were complex interactions between most factors, where ventilation rate, shelf life time and wax had different effects on each other at the different levels and times. This makes the development of clear conclusions difficult. But in most cases, expectations of increasing shelf life resulting in greater water loss, respiration rate with higher internal CO_2 and ethanol levels with softer fruit were all significant. Similarly waxed fruit had lower respiration and the fruit were firmer.

The levels of components of postharvest quality which are through to contribute to the development of off-flavours (such as internal CO_2 and ethanol levels) were variable between the ventilation, wax and shelf life treatments with significant interactions between many factors. The lack of differences or clear differences in the physical postharvest parameters (internal CO_2 and ethanol levels) was also reflected in the lack of sensory differences detected by the untrained consumers. No differences in off-flavours (aroma and taste), aroma, sweetness, acidity / sourness, flavour / taste, and overall liking, were detected between the ventilation treatments at each assessment time for each wax (except 'Overall liking' in the unwaxed fruit at 7 days), indicating that the ventilation treatment did not affect consumer acceptability of the treatments.

There were no clear results which supported the hypothesis that lower container ventilation rates resulted in fruit with more off-flavours, which suggests that the current set points for the container ventilation are adequate. But this work was conducted with a high humidity flow-through and was only conducted in one season with fruit from one harvest time, and more work is required to test this hypothesis more thoroughly.

2.3 New approaches to maintain fruit quality during storage

The development and application of new postharvest innovations to maintain fruit quality are key for the Australian citrus industry to be proactive in delivering high quality, safe and nutritious fruit to domestic and export consumers. New innovations to maintain fruit quality through the supply chain such as the concept of ethylene management and new edible films / coatings have been developed with the support of this Program.

2.3.1 Ethylene management

Citrus fruit are considered non-climacteric, but are responsive to postharvest application of ethylene which has been shown to induce a desirable colour change in citrus fruit, and is widely used to commercially degreen early season fruit. However, ethylene has also been shown to produce undesirable effects related to accelerated fruit senescence including abscission of the calyx, calyx browning and calyx drying. Li et al. (2016) showed that when ethylene levels were decreased from 1 to <0.001 µL L^{-1}, the incidence of calyx senescence in 'Afourer' mandarins diminished. The development of undesirable characteristics in citrus as a consequence of ethylene exposure varies with citrus type and cultivar.

There are few reports on assessing the effect of long-term ethylene exposure on calyx condition and internal quality attributes of mandarins and oranges held in long-term storage (cold or ambient temperature). The few cited studies conducted have not undertaken an extensive evaluation of the impact of ethylene concentrations and storage temperature on fruit quality parameters, including vitamin C content and antioxidant activity. The objective of this study was to assess the impact of four ethylene concentrations and different storage temperatures on calyx senescence and internal quality parameters of 'Afourer' mandarins and Navel oranges during long-term storage.

In this study, Alhassan et al. (2019) stored 'Afourer' mandarin and Navel orange fruit in a continuous ethylene exposure at different storage temperatures was investigated to assess their effect on calyx senescence and internal qualities. 'Afourer' mandarin fruit were stored at ≤0.001 (equivalent to ethylene-free air), 0.01, 0.1 and 1 µL L^{-1} of ethylene at either 5, 10 or 20 °C, whilst in a parallel experiment, Navel oranges were exposed to ≤0.001, 0.1 and 1 µL L^{-1} ethylene at either 1 or 10 °C. Changes in external and internal postharvest quality parameters were assessed for up to 8 weeks for 'Afourer' mandarins and 10 weeks for Navel oranges. At all storage temperatures, high levels of ethylene were found to increase the level of calyx senescence, weight loss, loss of fruit firmness and respiration rates. Also, there were significant effects of ethylene and storage temperatures on total soluble solids (TSS) content, titratable acidity (TA), and ethanol accumulation in both citrus species. Continuous exposure to high ethylene also significantly reduced vitamin C and ferric reducing antioxidant power (FRAP) in 'Afourer' mandarins after 8 weeks of storage. Overall, ethylene treatments had a significant effect on both the external and internal qualities of the fruit during storage. The relationship between ethylene concentrations and storage temperatures demonstrate that lowering atmospheric ethylene levels at reduced storage temperatures maintain fruit quality during long term storage.

This study was supported by this Hort Innovation project and published in in international peer-reviewed journal:

> Alhassan N., Golding J.B., Wills R.B.H., Bowyer M.C., Pristijono P. (2019) Long term exposure to low ethylene and storage temperatures delays calyx senescence and maintains 'Afourer' mandarins and Navel oranges quality. *Foods* 8(1), 19. (13 pages).

In another study, Li et al. (2018) stored Afourer' mandarins in air containing ethylene at 0.001, 0.01, 0.1, and 1 µL L^{-1} at 20, 10, 5 and 0 °C and changes in a range of external and internal quality parameters were examined for up to 10 weeks in storage. At all storage temperatures, reducing ethylene concentration in the storage environment decreased the rate of respiration, visible deterioration of the calyx region, ethanol accumulation in the juice, loss of eating quality, and at chilling temperatures reduced rind pitting. The quality attributes limiting mandarin storage life differed between the different storage temperatures but retention of mandarin quality was always optimised by maintaining the lowest possible ethylene atmosphere around fruit. Thus, the primary target should be to ensure the ethylene levels are ≤0.01 µL L−1, as loss of quality was accelerated above this concentration.

This study was supported by this Hort Innovation project and published in in international peer-reviewed journal:

> Li. X., Golding J.B., Arcot J., Wills R.B.H. (2018) Continuous exposure to ethylene in the storage environment adversely affects 'Afourer' mandarin fruit quality. *Food Chemistry* 242, 585-590.

2.3.2 New edible coatings / films

Edible films and coatings are widely used to maintain the quality and shelf life of citrus which act as semi-permeable membranes to restrict the movement of gases and water vapor to reduce the rate of respiration and water loss from the fruit. Many films/coatings due to their barrier and mechanical properties can reduce the rate of physiological postharvest degradation. Citrus waxes are made of shellac (derived from the lac bug, *Kerria lacca*) or carnauba (derived from the leaves of the carnauba palm, *Copernicia prunifera*). However there is a need to improve the efficiency and sustainability of waxes applied to citrus. Readily sourced and inexpensive coating materials which are effective at maintaining fruit quality during storage and shelf life are required.

Pea (*Pisum sativum*) is widely grown around the world and contains 22–45% starch as the most plentiful carbohydrate in the seed. Pea starch (PS) is comprised of a mixture of two homopolymers; a linear fraction, amylose, and a highly branched fraction, amylopectin. Pea starch has high content of amylose; therefore it is a potential option for the production of starch based edible films. Guar gum (GG), which is derived from the endosperm of the guar bean (*Cyamopsis tetragonoloba*) is a type of linear galactomannan with ratio of mannose to galactose units of 2:1. Owing to the long polymeric chain, high molecular weight and wide availability of pea starch and guar gum, they can be potential alternatives for production of renewable source based biodegradable edible coatings or packaging materials. In our previous studies, it has been shown that pea starch in combination with guar gum can form biocomposite edible films with preferable physical, optical and mechanical properties (Saberi et al., 2016a, 2016b, 2016c). However, edible coatings based on pea starch and guar gum have not been comprehensively explored as fruit coatings.

In this study, we investigated novel edible composite coatings based on pea starch and guar gum (PSGG), pea starch-guar gum-shellac (PSGG-Sh), and PSGG/Sh bilayer composite coating, formed by first applying PSGG and then shellac (Sh) compared with fruit coated with commercial wax and uncoated fruit (control) on maintaining the quality of fresh ‘Valencia’ oranges during four weeks at 20 °C and four weeks of storage at 5 °C followed by one week at 20 °C, simulating marketing shelf life (Figure 52).

Figure 52. Bahar Saberi (University of Newcastle) applying novel edible composite coatings based on pea starch and guar gum (PSGG), pea starch-guar gum-shellac (PSGG-Sh) at NSW Department of Primary Industries.

The incorporation of lipid compounds into the PSGG coatings (PSGG-Sh) generally resulted in the best performance in reducing fruit respiration rate, ethylene production, weight and firmness loss, peel pitting, and fruit decay rate of the coated oranges. Fruit coated with PSGG-Sh and a single layer PSGG coatings generally resulted in higher scores for overall flavor and freshness after four weeks at 5 °C followed by one week at 20 °C than uncoated fruit, as assessed by a sensory panel. Although the LBL coating reduced weight loss and respiration rate with improved firmness retention to a greater extent than the single layer PSGG coating, the bilayer coating also resulted in higher levels of ethanol causing increased perception of off-flavors. Overall results suggested that PSGG-based edible coatings could be a beneficial substitute to common commercial waxes for maintaining quality and storability, as well as extending shelf life of citrus fruit and potentially other fresh horticultural produce.

This study was supported by this Hort Innovation project and published in in international peer-reviewed journal:

> Saberi B., Golding J.B., Marques J.R., Pristijono P., Chockchaisawasdee S., Scarlett C.J., Stathopoulos C.E. (2018) Application of biocomposite edible coatings based on pea starch and guar gum on quality, storability and shelf life of ‘Valencia’ oranges. *Postharvest Biology and Technology* 137, 9-20.

2.4 Review of irradiation as a market access treatment on fruit quality

Market access is the key priority of the Australian citrus industry. While cold treatment protocols have successfully enabled exports of oranges and mandarins to key export markets, the development of chilling injury in response to cold treatment can limit exports in some susceptible citrus types such as lemons. Another market access tool for the Australian citrus industry is low dose irradiation. Food Standards Australia New Zealand (FSANZ) have granted food safety approval for irradiation (at 150 – 1,000 Gy) as a technique for insect pest disinfestation of citrus and it has also been approved in several export markets such as Indonesia and Vietnam. A review of the effects of low dose irradiation (< 1,000 Gray) as a market access treatment on citrus fruit quality was requested by the Program Reference Committee (18 July 2017). This was completed on 30 April 2018. The full literature review (24 pages) and recommendations are presented in Appendix 3.

Hort Innovation Strategic levy investment | CITRUS FUND

Contribution from **Australian Citrus Postharvest Science Program (CT15010)**

Exploring the potential of low dose irradiation phytosanitary treatments for the Australian citrus industry

30 April 2018

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Executive Summary

This review examined the published literature on the effects of low dose irradiation (< 1,000 Gy) as a market access treatment on citrus fruit quality. In summary the results highlighted:

- Postharvest low dose irradiation is an accepted safe phytosanitary treatment for some key export markets.
- Irradiation overcomes any issues of chilling injury with cold treatment for export.
- There are few consistent negative effects of irradiation on final citrus fruit quality following treatment and storage.
- Some surface damage (such as pitting) can occur in some types of different citrus but the contributing factors to this damage are not known but could potentially be managed.

Recommendations

Low dose irradiation provides market access options for the Australian citrus industry, but requires solutions to ensure consistent treatment out-turns which do not affect final fruit quality. Recommendations for future R&D investments include:

- Identify and manage pre- and postharvest factors (such as cultivar, harvest time, postharvest but pre-irradiation treatments) that contribute to potential post-treatment damage.
- Lemons and a lesser extent mandarins should be the focus of future industry R&D investments.

Contents

Glossary

Gy	Gray is a derived unit of ionizing radiation dose which is defined as the absorption of one joule of radiation energy per kilogram of matter. 1,000 Gy = 1 kGy
TA	Titratable acidity (% citric acid)
TSS	Total soluble solids, or soluble solids content (% Brix)
Vitamin C	Also known as ascorbic acid (AA) and is an essential nutrient in the diet

The full review *'Exploring the potential of low dose irradiation phytosanitary treatments for the Australian citrus industry'* is presented in Appendix X and available on the Hort Innovation grower portal (23 November 2018):

https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/exploring-the-potential-of-low-dose-irradiation-phytosanitary-treatments/

3. Postharvest treatments to reduce MRLs

Maintaining market access is critical for the Australian citrus industry, in addition to phytosanitary barriers, chemical maximum residue limits (MRL) are a constant and growing issue. This Program has focused on the management of postharvest fungicides, but pre-harvest chemical residues can be an issue for market access. Postharvest processing and washing can have some effects at reducing surface contamination. This study examined the effect of postharvest removal of dithiocarbamate residues in lemons.

Background Dithiocarbamates are an important group of protective non-systemic fungicides with surface protection against black spot (*Guignardia citricarpa*). These fungicides only work if the fruit / foliage are thoroughly covered before a fungal spore lands on the fruit or leaf and tries to infect it. There are several products of dithiocarbamates including 'Dithane', 'Antracol', 'Zineb' etc. Products which have dithiocarbamates as the active ingredients include mancozeb, propineb, zineb. These are usually applied at 6 and 12 weeks after the copper application/s at petal fall. Subsequent applications for the control of mites (Brown citrus mite and Citrus rust mite) also occur depending on the activity of the pest.

Mancozeb is classified as a dithiocarbamate non-systemic agricultural fungicide with multi-site, protective action. Mancozeb (manganese ethylenebis (dithiocarbamate) polymeric complex with zinc salts) is further classified as an ethylenebisdithiocarbamate (EBDC) fungicide. EBDCs have been regarded as relatively harmless because of their low acute toxicity to mammals, but they are generally unstable in the presence of moisture or oxygen and in biological systems. Under these conditions, several degradation products are formed, including ethylenethiourea (imidazolidine-2-thione, ETU). ETU is the main degradation product by hydrolysis and photolysis of EBDCs. ETU is a relatively stable and very polar (water soluble) metabolite. Because of the report of its carcinogenic, mutagenic, and goitrogenic effects in laboratory animals, ETU has become a major human health concern (López-Fernández et al., 2017). But it is the actual dithiocarbamate residue itself that is measured and considered the MRL issue.

The temporary MRL is 7 mg/kg, but this is only temporary and the previous MRL was 0.2 mg/kg. Different overseas export markets have different tolerances to dithiocarbamates, and reducing the MRL in citrus fruit is a priority for growers and exporters. López-Fernández et al. (2017) showed that the optimum conditions to degrade mancozeb and limit their toxicological impact are pH 2, at 25 °C in the presence of light. A previous Horticulture Innovation project (CT13020) showed some postharvest treatments reduced dithiocarbamate residues but their data were variable and limited postharvest washing treatments were used. Miles (2016) further recommended "*several replicate samples to ensure meaningful results*". Therefore this trial increased the number of replicates to six (with 8 fruit sample size (>1kg fresh weight) – as recommended by Symbio Laboratories, Brisbane).

The trial examined the effect of different common postharvest treatments and sanitisers on dithiocarbamate residues in lemon fruit. Chlorine and peroxyacetic acid (PAA) are widely used as sanitisers in the Australian citrus industry. Chlorine has been a very popular sanitiser for years. Chlorine-based sanitisers include sodium hypochlorite, calcium hypochlorite, bromo-chloro-dimethylhydantoins and chlorine dioxide. In this experiment, sodium hypochlorite was used as a postharvest treatment to reduce the levels of dithiocarbamate in lemons. PAA is a strong oxidizing sanitiser which is widely used as a sanitiser in the citrus industry. PAA is commercially available as 'Tsunami' and is a mixture of acetic acid (CH_3CO_2H), hydrogen peroxide (H_2O_2), PAA (CH_3CO_3H) and water (H_2O).

The use of hot fungicides (40-50 ^oC) are used by some citrus packers to improve the efficacy of the fungicides (Golding and Singh, 2017), and increased temperature has been shown to improve the degradation of mancozeb (Hwang et al., 2001). This trial examined the effect of higher washing temperatures on dithiocarbamate residues in lemon fruit. In addition different combinations of hot washing with different sanitisers were assessed. The overall aim of this trail was to reduce dithiocarbamate residues in lemon fruit with standard postharvest treatments.

Methods. Lemon fruit from Queensland with a record of high in-field mancozeb (dithiocarbamate) usage were harvested and transported to the NSW DPI Centre of Excellence for Horticultural Market Access at Ourimbah, via Sydney markets on 1 May 2018. The fruit were harvested after spraying and not dipped or processed. The lemons were washed with different washing treatments with the custom built high pressure washing system with brushes (Figure 53). The following six postharvest treatments were trialed:

1. No postharvest treatment (fruit just from orchard / bin)
2. Cold water wash at 20 ^oC for 30 seconds
3. Hot water at 40 ^oC for 30 seconds

4. 'Tsunami' (peroxyacetic acid and hydrogen peroxide) (label rate) at 20 °C for 30 seconds
5. 'Tsunami' at 40 °C for 30 seconds
6. Chlorine wash (50ppm, 20 °C for 30 seconds)

The fruit were randomly allocated treatments and each treatment was randomly allocated within each replicate. All treatments were independently replicated six times according to a randomized treatments allocation and the washing machine was thoroughly washed at least twice (and cooled/ heated) between treatments. The pH and temperature of wash water was recorded before and during treatment. In addition, the levels of PAA and chlorine were also measured with test strips from samples taken during the washing process.

A total of 48 residue samples were assessed (8 treatments x 6 replicates). After treatment, fruit were drained / air dried for approximately 60 mins at 20 °C and then frozen at -20 °C. The samples were coded and frozen samples couriered and analysed for dithiocarbamates (mg/kg) in the same random order at Symbio Laboratories Pty. Ltd. in Brisbane. Symbio are NATA accredited ISO/IEC 17025 laboratory and the lemon samples were analysed with the accredited method for dithiocarbamates (ferbam; mancozeb; maneb; metham-sodium; metiram; propineb; thiram; zineb; ziram) by GC, GC/MS using in-house CR007 method.

Figure 53. Lemons were randomly selected for treatment (top left), washed in a custom built experimental washing unit (top right), with brushes and high pressure nozzles (bottom left), before drying for 1 hour at 20 °C (bottom right) then frozen (-20 °C) and sent for dithiocarbamate residue analysis at Symbio Laboratories (Brisbane)

An additional storage trial was conducted to examine the effect of an additional de-greening and storage period on the dithiocarbamate residues following postharvest treatment. There were two additional treatments which were conducted and analysed:

7. Untreated control fruit plus additional postharvest storage
8. 'Tsunami' at 40 °C for 30 seconds fruit plus addition postharvest storage

After the washing treatment, fruit were de-greened in ethylene (5ppm) at 25 °C for 4 days, then stored at 10 °C for 1 week before the additional residue assessment. This was to simulate the actual postharvest storage and handling of lemons destined to the market.

All fruit samples were frozen (-20 °C) and sent by courier with freezer packs to Symbio Laboratories (Brisbane) for determination of dithiocarbamate residues.

Statistical design and analysis was conducted by Lorraine Spohr (Biometrician NSW Department of Primary Industries). The effect of washing treatment on dithiocarbamate residue was tested using analysis of variance. Treatment means were compared using the least significant difference procedure with a significance level of p=0.05.

Results and discussion The results of the different postharvest washing treatments on the levels of dithiocarbamates in lemon fruit is presented as a dot-plot of the dithiocarbamate residue raw data along with the means for each washing treatment (Figure 54). These results illustrate the variability of the residues of each sample replicate for each treatment and show that the mean dithiocarbamate concentration in untreated (control) lemon fruit was 2.9 mg/kg. This average dithiocarbamate residue level was lower than the MRL of 3 mg/kg, but the results presented in Figure 54 show that half of the samples (three samples of the six replicate samples) were higher than 3 mg/kg. This illustrates the high variability of residue data (presumably due to spray and orchard factors), and shows the importance of reducing the MRL in fruit with postharvest washing.

There was a significant effect of postharvest washing treatment on dithiocarbamate residues ($F_{7,35} = 41.02$; p<0.001), where all washing treatments resulted in lower dithiocarbamate residues, as compared to the non-washed fruit. There was no statistically significant difference between any of the postharvest wash treatments, including hot washing.

Indeed further testing within the different washing treatments, i.e. using orthogonal contrasts to test potential differences in hot (40 °C) washing V cold (20 °C) washing and also the water treatments V 'Tsunami' treatments, showed there were no significant effects detected for each contrast. This indicates there was no effect of treatment temperature or addition of PAA to the wash on dithiocarbamate residues in the fruit.

In the side experiment which examined the effect of an additional storage time of de-greening and storage of lemons, the results showed that treating fruit 'Tsunami' at 40 °C for 30 seconds fruit before de-greening and storage had similar residue levels, as compared to fruit where the analysis was conducted immediately after treatment (Table 55). This suggests that the initial postharvest washing was an important factor in reducing the dithiocarbamate levels from the fruit, and all washing treatments were statistically similar. Even the addition of a de-greening and storage simulation did not further reduce dithiocarbamate residues in the fruit.

It is interesting to note that the non-washed stored fruit had significantly higher dithiocarbamate residues (5.4 mg/kg) than the non-washed fruit that were immediately analysed (2.9 mg/kg). This observation is difficult to reconcile. The loss of water from the fruit during de-greening and storage would not be enough to contribute to the increase in dithiocarbamate residues (as expressed as mg/kg) observed in stored non-washed fruit. This observation could simply be due to sampling of these fruit or another unknown occurrence.

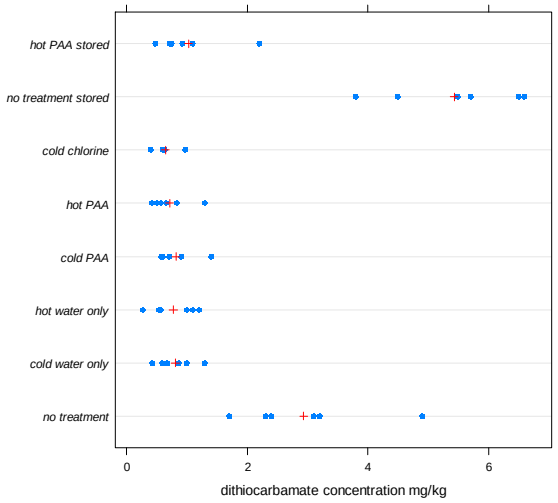


Figure 54. Dithiocarbamate residue raw data and means for each washing treatment (and after de-greening and storage) in lemon fruit.

Table 55. Effect of washing treatment on dithiocarbamate residue concentration in lemon fruit.

Treatment	Concentration (mg/kg)	
cold chlorine	0.643	a
hot 'Tsunami'	0.712	a
hot water only	0.775	a
cold water only	0.810	a
cold 'Tsunami'	0.817	a
hot 'Tsunami' – stored	1.027	a
no treatment	2.933	b
no treatment – stored	5.433	c

Treatments with the same letter indicate these treatments are statistically similar (p<0.05)

The statistical analysis for this experiment is presented in Appendix 4.

Conclusion Any postharvest washing (irrespective of chlorine or 'Tsunami', treatment temperature (20 °C or 40 °C), or additional postharvest storage time) reduced the dithiocarbamate residues in lemon fruit, as compared to non-washed fruit. This reduction in dithiocarbamate residues was significant and contributes to lowering the dithiocarbamate MRL in lemon fruit. While these levels are below the current temporary MRL, many export markets are sensitive to dithiocarbamate and all efforts should be undertaken to reduce the risk of chemical contamination. Holding the fruit for longer periods (e.g. de-greening and storage) did not further reduce dithiocarbamate residues.

This report '*Evaluation of postharvest removal of dithiocarbamate residues in lemons'* is available on the Hort Innovation grower portal (23 November 2018).

https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/evaluation-of-postharvest-removal-of-dithiocarbamate-residues-in-lemons/

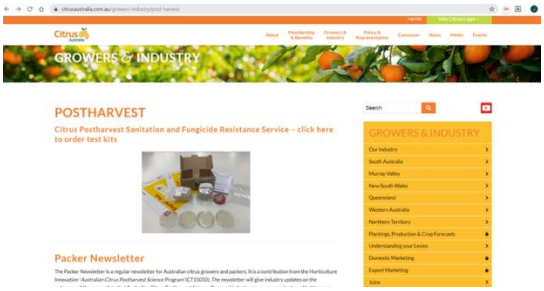
4. Extension resources

A range of extension materials have been developed and extended to industry. Regular postharvest articles to *Australian Citrus News* and the continuation of the *Packer Newsletter* are key extension tools developed from the Program. A postharvest article from this Program has been regularly published in *Australian Citrus News* is the national citrus publication and is the quarterly magazine of Citrus Australia. A major output of the Program has been the archiving, searchability and continuation of the *Packer Newsletter* with two newsletters per year. These are made available on the Citrus Australia website (https://citrusaustralia.com.au/growers-industry/packer-newsletters). The Program works closely with Citrus Australia providing technical information for the postharvest page on Citrus Australia website (https://citrusaustralia.com.au/growers-industry/post-harvest). This webpage is a key resource for citrus growers and was developed by this Program. For example a You tube video was produced and hosted on the Citrus Australia postharvest webpage. The video (3.18 mins) outlines practical steps to successfully de-green mature citrus fruit. In addition, the Program has made contributions to *HortLink*, *Australian Tree Crop* and *Citrus Connect* (NSW DPI).

4.1 Postharvest resources

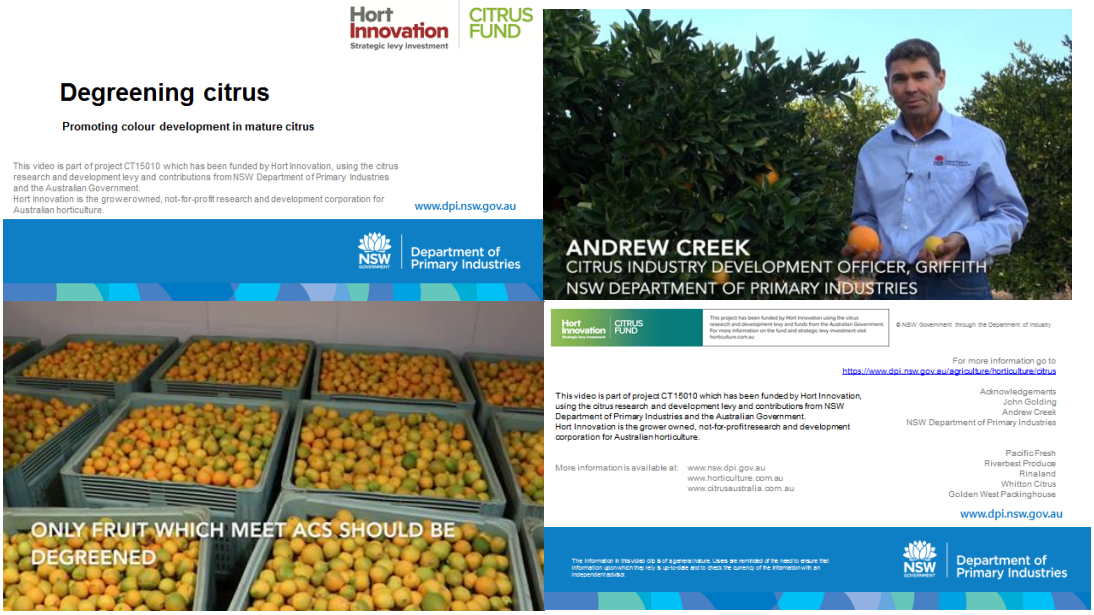
The Program has developed and continues to support the postharvest resource page on Citrus Australia website (https://citrusaustralia.com.au/growers-industry/post-harvest) with articles on:

- Postharvest decay
  - Key postharvest diseases
  - A guide to common postharvest diseases and disorders of Navel oranges, mandarins grown in inland Australia (not a contribution from this Program
- Fungicides and sanitisers
  - Current citrus postharvest fungicides
  - Postharvest decay control – fungicides and sanitisers
- Postharvest fungicide resistance and sanitation
  - Management change can reduce fruit decay
  - Preventing postharvest fungicide resistance
- Food safety
  - Food safety is everyone's responsibility
  - Packing house hygiene - Sanitation and food safety
- Degreening
  - The essentials of degreening. Part 1 and Part 2
  - Degreening You tube video
- Postharvest disorders
  - A review of chilling injury causes and control
  - A guide to the common postharvest diseases and disorders of Navel oranges and mandarins grown in inland Australia (not a contribution from this Program)
- Limes
  - Postharvest research update: Maintaining lime fruit quality during storage
- Horticulture Innovation Project
  - Australian Citrus Postharvest Science Program (CT15010)
- Asian Markets Project (not a contribution from this Program)
  - Hort Frontiers Asian markets project – Queensland Department of Agriculture and Fisheries
- Other postharvest articles
  - Health benefits of oranges and making use of citrus juice waste
  - Collaboration – Top Spanish researcher works with NSW DPI to improve fruit quality
- Postharvest Resources (not contributions from this Program)
  - Citrus Pressure Washer Manual (Plant & Food Research Australia)
  - Product Description Language (PDL) Oranges: reference guide (HIA publication)
  - PDL Oranges General Parameters: colour plates (HIA publication)
  - PDL Oranges Defect Parameters: colour plates (HIA publication)



Degreening You tube video

A practical guide to the degreening of mature citrus fruit was produced and hosted on the Citrus Australia postharvest webpage (https://citrusaustralia.com.au/growers-industry/post-harvest). The video *Degreening – promoting colour development in mature citrus,* was presented by NSW DPI citrus Development Officer and Program team member, Andrew Creek. The video outlines the need and practicalities of degreening processes and runs for 3.18 mins.



Australian Citrus News

Australian Citrus News is the quarterly magazine of Citrus Australia and is the premier information source for Australian citrus growers. The Program contributed postharvest articles to every issue which are available on the Citrus Australia website (https://citrusaustralia.com.au/media/australian-citrus-news). *Australian Citrus News* provides focused up-to-date information in an easy-to-read style covering technical information, export and domestic markets, production developments, key industry issues, research and development progress and results, profiles and on-farm reports. It is published quarterly with 1,600 copies distributed each issue reaching an estimated 5,000 people across Australia's citrus industry including commercial growers, industry bodies, consultants, state agriculture departments, agronomists, research institutes, economists and the media. The magazine is distributed free of charge to all Australian commercial citrus growers. A total of 12 postharvest articles were published from the Program in *Australian Citrus News* (see below).

2016

1. Golding J. (2016) Postharvest Program to target low residues. *Australian Citrus News*. Summer 2016/17. Page 34.

2017

2. Golding J. (2017) The Essentials of degreening. Part 1. *Australian Citrus News*. Autumn 2017. Page 37.
3. Golding J. (2017) Degreening for optimal results. Degreening Part 2. *Australian Citrus News*. Winter 2017. Pages 20-21.
4. Golding J. and Singh SP. (2017) Postharvest decay control – fungicides and sanitisers. *Australian Citrus News*. Spring 2017. Pages 12-14.
5. Golding J. and Archer J. (2017) Preventing postharvest fungicide resistance. *Australian Citrus News*. Summer 2017. Pages 34-36.

2018

6. Golding J. and Singh S.P. (2018) Packing house hygiene - Sanitation and food safety. *Australian Citrus News*. Autumn 2018. Pages 28-29.

7. Golding J. and Pristijono P. (2018) Postharvest research update: Maintaining lime fruit quality during storage. *Australian Citrus News*. Winter 2018. Pages 26-27.
8. Singh SP Golding J. (2018) Food safety is everyone's responsibility. *Australian Citrus News*. Spring 2018. Pages 26-27.
9. Golding J. and Archer, J. (2018) Management change can reduce fruit decay. *Australian Citrus News*. Summer 2018/19. Pages 36-37.

2019

10. Golding J. (2019) Top Spanish researcher shares knowledge with industry. *Australian Citrus News*. Autumn 2019. Pages 38-39.
11. Golding J. (2019) New developments in decay control. *Australian Citrus News.* Winter 2019. Pages 36-37.
12. Golding J. and Archer J. (2019) A review of chilling injury causes and control. Spring 2019. *Australian Citrus News*. Pages 32-33.

Packer Newsletter

The *Packer Newsletter* is a regular newsletter for Australian citrus growers and packers. The newsletter is run by this current Horticulture Innovation '*Australian Citrus Postharvest Science Program*' (CT15010). The newsletter provides industry with updates on the outcomes of the research from the Program with practical and up-to-date postharvest information for Australian growers and packers. The *Packer Newsletter* was run by Peter Taverner (SARDI) many years and is now hosted by Citrus Australia (https://citrusaustralia.com.au/growers-industry/packer-newsletters). In addition to the continuation of the *Packer Newsletter* with this Program, a major outcome of this Program is the hosting and searchability of the *Packer Newsletter* archives, which contain a wealth of valuable postharvest information.

1. Golding J. (2017) *Packer Newsletter* Edition 115. https://citrusaustralia.com.au/growers-industry/packer-newsletters (3 pages).
2. Golding J. (2018) *Packer Newsletter* Edition 116. https://citrusaustralia.com.au/growers-industry/packer-newsletters (5 pages).
3. Golding J. (2018) *Packer Newsletter* Edition 117. https://citrusaustralia.com.au/growers-industry/packer-newsletters (3 pages).
4. Golding J. (2019) *Packer Newsletter* Edition 118. https://citrusaustralia.com.au/growers-industry/packer-newsletters (12 pages).
5. Golding J. (2019) *Packer Newsletter* Edition 119. https://citrusaustralia.com.au/growers-industry/packer-newsletters (4 pages).

Citrus e-News

Citrus e-News is the regular communication tool for Citrus Australia to communicate directly with growers and provides an up-to-date and timely information. The newsletter is sent to growers with links and archives at the Citrus Australia website (https://citrusaustralia.com.au/news/industry-news). The Program has contributed 12 articles / information to Citrus Australia *Citrus e-News*:

2017

1. Postharvest Program to target low residues. J Golding (25 January 2017) (https://citrusaustralia.com.au/news/latest-news/postharvest-program-to-target-low-residues-25-jan-2017)
2. The Essentials of degreening. J Golding (12 April 2017) (https://citrusaustralia.com.au/news/latest-news/the-essentials-of-degreening)
3. Assessing resistance to postharvest fungicides. J Golding (6 July 2017) (https://www.citrusaustralia.com.au/news/latest-news/assessing-resistance-to-postharvest-fungicides)
4. Postharvest decay control – fungicides and sanitisers. J Golding and SP Singh (23 October 2017) (https://citrusaustralia.com.au/news/latest-news/postharvest-decay-control-fungicides-sanitisers)
5. Post season meeting in Gayndah a success (21 December 2017) (https://citrusaustralia.com.au/news/latest-news/post-season-meeting-gayndah)

2018

6. Preventing postharvest fungicide resistance. J Golding and J Archer (21 February 2018) (https://citrusaustralia.com.au/news/latest-news/preventing-postharvest-fungicide-resistance)
7. Pack house hygiene – sanitation and food safety. J Golding and SP Singh (20 June 2018)

(https://www.citrusaustralia.com.au/news/latest-news/pack-house-hygiene)
8. Food Safety Forum next Wednesday, October 3. SP Singh (21 September 2018) (https://citrusaustralia.com.au/news/latest-news/food-safety-forum-next-wednesday-october-3)

2019

9. New fungicide, foggers, sanitisers feature in 2019 Citrus Tech Forum post-harvest workshops. J Golding (21 January 2019) (https://citrusaustralia.com.au/news/latest-news/new-fungicide-foggers-sanitisers-feature-in-2019-citrus-tech-forum-post-harvest-workshops)
10. 2019 Citrus Tech Forum: Spanish expert to share post-harvest knowledge. J Golding (24 January 2019) (https://citrusaustralia.com.au/news/latest-news/spanish-expert-to-share-post-harvest-knowledge)
11. A review of chilling injury causes and control. J Golding (13 August 2019) (https://citrusaustralia.com.au/news/latest-news/a-review-of-chilling-injury-causes-and-control)
12. Building postharvest research ties with South Korea. J Golding (9 September 2019) (https://citrusaustralia.com.au/news/latest-news/building-postharvest-research-ties-with-south-korea)

Australian Tree Crop

Australian Tree Crop is a bi-monthly national grower magazine to provide information to growers that helps them improve the profitability and productivity of their business. The magazine focusses on new research, practical commercial information and business practices of value to tree crop growers (https://www.treecrop.com.au/).

Golding J. (2018) Preventing postharvest fungicide resistance in citrus. *Australian Tree Crop.* December 2017 / January 2018. Pages 34-35.

HortLink

HortLink provided updates on all Hort Innovation investments to growers (https://www.horticulture.com.au/).

Update of the Australian Citrus Postharvest Science Program (CT15010). HortLink. November 2017. 2017 Edition 4. (http://horticulture.com.au/hortlink-2017-edition-4/citrus/#2)

Citrus Connect

Citrus Connect is a NSW Department of Primary Industries citrus e-newsletter published three times per year. It provides the citrus industry with updates on projects, the latest technical information and events (https://www.dpi.nsw.gov.au/agriculture/horticulture/citrus/citrus-connect).

2017

1. Golding J. (2017) NSW DPI manages Australian citrus postharvest program. *Citrus Connect.* January 2017. (http://www.dpi.nsw.gov.au/agriculture/horticulture/citrus/citrus-connect-articles/citrus-postharvest-program)
2. Golding J. (2017) Assessing resistance to postharvest fungicides. *Citrus Connect.* September 2017.

2018

3. Golding J. and Pristijono P. (2018) Maintaining lime fruit quality during storage. *Citrus Connect.* December 2018. (https://www.dpi.nsw.gov.au/agriculture/horticulture/citrus/content/post-harvest/articles/maintaining-lime-fruit-quality-during-storage-dec-2018)
4. Golding J. (2017) Resistance to postharvest fungicides. *Citrus Connect.* March 2018.

4.2 Industry communication / presentations / visits

A range of visits to citrus growing areas and postharvest presentations to Citrus Australia regional forums and specific postharvest workshops were presented by the Program (Table 56).

Table 56. Postharvest presentations to grower forums / meetings from the Program

	Grower Forum	Location	Date	Title of presentation
1	Citrus Australia Queensland regional forum	Gayndah (Qld)	21 February 2017	Outline of the Australian Citrus Postharvest Science Program
2	Citrus Australia 2017 Citrus Technical Forum	Mildura (Vic)	1 March 2017	Introduction to the Australian Citrus Postharvest Science Program
3	Riverina Postharvest Workshop	Griffith (NSW)	19 May 2017	Update of degreening, citrus postharvest fungicides and sanitisers
4	Citrus Australia Queensland post-season regional forum	Gayndah (Qld)	29 November 2017	Postharvest fungicides and resistance update
5	WA citrus packers workshop	Fremantle (WA)	2 December 2017	Postharvest fungicides and resistance update
6	Citrus Australia Food Safety Forum (SA)	Barmera (SA)	3 October 2018	Microbiological pathogens on fresh food. Understanding the risks – Dr SP Singh (NSW DPI)
7	Citrus Australia pre-conference tour of 2019 Citrus Technical Forum	Renmark (SA)	4 March 2019	Controlling postharvest decay- The Essentials
8	Citrus Australia 2019 Citrus Technical Forum	Adelaide (SA)	6-7 March 2019	Australian fungicide resistance survey
9	Citrus Australia 2019 Citrus Technical Forum	Adelaide (SA)	6-7 March 2019	Hort Innovation postharvest project - Fungicide use, timing, irradiation and chilling review
10	Citrus Australia Far North Queensland citrus workshop	Mutchilba (Qld)	4 June 2019	Use of fungicides and storage of limes
11	Citrus Australia Riverina regional forum	Griffith (NSW)	7 November 2019	Postharvest Program update
12	Citrus Australia Riverland regional forum	Loxton (SA)	11 December 2019	Postharvest Program update

In addition to this these presentations (Table 56), face-to-face visits to packinghouses and discussions with packinghouse managers about postharvest practices and the outcomes of Program were regularly undertaken. The Program Leader visited different growing regions: Riverland (x 5 different visits), Sunraysia (x 5 different visits), Gayndah / Mundubberah (x 3 different visits), Griffith / Leeton (x 4 different visits), Harvey / Moora (WA) (x 1 visit), NSW Central Coast (x 2 different visits), Gunnedah (NSW) (x 1 visit) and Muchilba (Qld) (x 1 visit). In each visit to the growing regions, the Project Leader visited key growers and packinghouses in each region with up to 7 packinghouse visits per region / visit.

4.3 Technical outputs

A range of technical outputs have been produced from the Program. Seven research papers / reviews that have attributed contributions from this Program have been published in a range of international journals. In addition, eleven technical posters from the Program were presented at the 2019 Citrus Technical Forum in Adelaide in March 2019. The posters contributed to the postharvest workshops and presentations at the Citrus Technical Forum. The Program Leader was also invited to present two key note lectures at two leading international postharvest conferences in Korea and Spain. In addition, a presentation of work from the Program was presented by John Archer (talk and poster) at the 5th International Symposium on Postharvest Pathology in Leige Belgium in May 2019. While these international presentations were contributions from the Program, they were funded from external sources.

Peer-reviewed scientific articles

Seven research papers / reviews that have attributed contributions from this Program have been published. These are available on-line or upon request.

2018

1. Li. X., Golding J.B., Arcot J., Wills R.B.H. (2018) Continuous exposure to ethylene in the storage environment adversely affects 'Afourer' mandarin fruit quality. *Food Chemistry* **242**, 585-590. https://doi.org/10.1016/j.foodchem.2017.09.088
2. Papoutsis K., Vuong Q.V., Tesoriero L., Pristijono P., Stathopoulos C.E., Gkountina S., Lidbetter F., Bowyer M.C., Scarlett C.J., Golding J.B. (2018) Microwave irradiation enhances the in vitro antifungal activity of citrus by-product aqueous extracts against *Alternaria alternata*. *International Journal of Food Science and Technology*. **53**, 1510-1517. https://doi:10.1111/ijfs.13732
3. Saberi B., Golding J.B., Marques J.R., Pristijono P., Chockchaisawasdee S., Scarlett C.J., Stathopoulos C.E. (2018) Application of biocomposite edible coatings based on pea starch and guar gum on quality, storability and shelf life of 'Valencia' oranges. *Postharvest Biology and Technology* **137**, 9-20. https://doi.org/10.1016/j.postharvbio.2017.11.003
4. Saberi B., Golding J.B. (2018) Postharvest application of biopolymer-based edible coatings to improve the quality of fresh horticultural produce. In: Gutiérrez T. (ed) Polymers for Food Applications. pages 211-250. Springer, Cham. Switzerland. https://doi.org/10.1007/978-3-319-94625-2_9

2019

5. Alhassan N., Golding J.B., Wills R.B.H., Bowyer M.C., Pristijono P. (2019) Long term exposure to low ethylene and storage temperatures delays calyx senescence and maintains 'Afourer' mandarins and Navel oranges quality. *Foods* **8(1)**, 19. (13 pages). https://www.mdpi.com/2304-8158/8/1/19

2020

6. Archer J., Pristijono P., Gallien Q., Houizot L., Bullot B., Palou L., Golding J.B. (2020) Preliminary investigations on the effect of low-pressure treatment on *in vitro* and *in vivo* growth of *Penicillium* sp. in oranges. *Acta Horticulturae*. In press.
7. Golding J.B., Archer J., 2020. Chapter 10. Advances in postharvest handling of citrus fruit. In: Yahia, E. (Ed), Achieving sustainable cultivation of citrus and other tropical and subtropical fruits - Volume 1 Citrus fruits. Burleigh Dodds Series in Agricultural Science: no. 65. Burleigh Dodds Publishing, London UK. In press (26 pages).

International technical presentations contributed by the Program *(but funded by external sources)*

1. John Golding. *Reducing postharvest losses with ethylene management during storage.* Invited keynote address to 4th ISHS Asia Symposium on Quality Management in Postharvest Systems. Jeonju, Korea (12-14 September 2017)
2. John Golding. *Fresh produce supply chain: Innovations to reduce postharvest losses*. Invited keynote address to 6th International Postharvest Unlimited Conference. Madrid, Spain (17-20 October 2017)
3. John Archer, Penta Pristijono, Quentin Gallien, Laure Houizot, Mark Bullot, Lluís Palou, and John Golding *Preliminary investigations on the effect of low-pressure treatment on in vitro and in vivo growth of Penicillium sp. in oranges*. Oral talk and poster presentation at 5th International Symposium on Postharvest Pathology -

From consumer to laboratory: Sustainable approaches to managing postharvest pathogens. Leige Belgium (19-24 May 2019).

Poster Presentations at the 2019 Citrus Technical Forum

Adelaide. 6-7 March 2019

1. Exposure to low ethylene and storage temperatures delays button (calyx) aging and maintains 'Afourer' mandarins and Navel oranges quality during storage (*Nasiru Alhassan, John Golding, Ron Wills, Michael Bowyer and Penta Pristijono)*
2. Evaluating the effects of plastic film packaging on postharvest quality of Navel oranges (*Nasiru Alhassan, John Golding, Ron Wills, Michael Bowyer and Penta Pristijono*)
3. Alternative decay control strategies using citrus essential oils (*M. M. Rahman, John Golding, Ron Wills, Michael Bowyer and Penta Pristijono*)
4. Effects of temperature and time on postharvest fungicide efficacy (*John Golding, Martin Emonet, Fabien Huteau and John Archer)*
5. Effect of postharvest washing on residues of preharvest sprays in lemons (*John Golding, Laure Houizot, Mark Bullot, Lorraine Spohr and John Archer)*
6. Development and assessment of new citrus fruit coatings (*Bahareh Saberi, John Golding, José Marques, Penta Pristijono, Suwimol Chockchaisawasdee, Chris Scarlett and Costas Stathopoulos*)
7. Antifungal activity of citrus by-product aqueous extracts against *Alternaria* (*Kostas Papoutsis, Quan Vuong, Len Tesoriero, Penta Pristijono, Costas Stathopoulos, Stela Gkountina, Fiona Lidbetter, Michael Bowyer, Chris Scarlett and John Golding*)
8. Assessment of postharvest UV-C treatments to maintain quality of limes (*Penta Pristijono, Michael Bowyer, Chris Scarlett, Quan Vuong, Costas Stathopoulos and John Golding*)
9. Combined postharvest treatments of UV-C and 1-methylcyclopropene (1-MCP) to maintain the quality of limes (*Penta Pristijono, Michael Bowyer, Chris Scarlett, Quan Vuong, Costas Stathopoulos and John Golding*)
10. Effect of postharvest UV-C light on postharvest decay and Navel orange fruit quality (*Penta Pristijono, Julien Thomas, Clémence Lerat, John Archer and John Golding*)
11. Evaluating the effects of low pressure and low oxygen on the postharvest growth of green mould (*John Archer, Typhaine Haurogné, Mark Bullot, Penta Pristijono and John Golding*)

Outcomes

Research Outcomes

The Program delivered a series of postharvest storage trials to support the growth of the Australian citrus industry. The major focus was on postharvest decay control, where a range of alternative chemical decay control measures were assessed. The results reinforced the need for good postharvest practices (e.g. postharvest fungicide applications within 24 hours of harvest), and the potential of new and improved postharvest chemistries to control green and blue mould. In addition the assessment of potential postharvest fungicides to manage anthracnose damage showed potential of new postharvest treatments to reduce anthracnose symptoms in Imperial mandarins. The improved use of current sanitisers and assessment of sanitising systems such as the new electrolysed water sanitiser system were assessed and show promise as commercial sanitation treatments of the industry. Physical treatments for the control of postharvest decay gave some mixed results, but there are opportunities for the incorporation of physical treatments to supplement postharvest decay control.

Packinghouse surveys of sanitation and postharvest fungicide resistance over two seasons identified this issue as a broad industry issue which needs constant management. This was addressed with a range of extension activities (see below).

Managing fruit quality through the supply chain to ensure consumers have a good eating experience is essential to the outcomes of the Program. This work reviewed irradiation as a market access treatment on fruit quality, and also reviewed the opportunities to manage chilling injury. Several pre-harvest and postharvest treatments were assessed and showed some promise to minimise potential chilling injury. The management of off-flavours in mandarins in export markets was assessed and more work is required in this area.

The potential to reduce dithiocarbamate residues in lemon fruit with postharvest washing treatments was highlighted with the use of commonly used sanitation treatments (chlorine or 'Tsunami'). This result will give industry confidence to meet export MRLs.

Extension Outcomes

Improved postharvest practice and knowledge were the aim of this Program. The program delivered: 12 postharvest articles in Australian Citrus News, a YouTube instructional video on practical degreening hosted on the Citrus Australia website, a re-developed postharvest resource portal on the Citrus Australia website, a re-developed 'Packer Newsletter' hosted on the Citrus Australia website with 5 new editions, 12 articles / contributions to Citrus Australia Citrus e-News, articles to Australian Tree Crop, HortLink and NSW DPI Citrus Connect. The Program leader also delivered 12 postharvest presentations to industry with addition face-to-face visits and packinghouse visits to all growing regions.

A survey of the postharvest needs was conducted at the Postharvest Workshops at the 2019 Citrus Technical Forum (7 March 2019) and showed that the Program is generally well received by industry with its research focus and extension activities.

Industry feedback A survey of growers / packers was conducted at the postharvest workshops at the 2019 Citrus Technical Forum in Adelaide on 7 March 2019, in the final session of the workshop. The survey form is presented in Appendix 5. The aim of the survey was to obtain feedback on the postharvest needs of the Citrus industry. A total to 31 people responded to the following questions:

Question 1. Do you use any of these citrus postharvest resources? And how useful are they?

	Do you use?	How useful are these resources?			
	Yes?	Not very	OK	Good	Excellent
Australian Citrus News	24		6	12	3
Packer Newsletter	9		1	3	2
NSW DPI *Citrus Connect*	5			2	1
Chemical re-sellers	19	1	3	12	2
Consultants	17		4	11	
Other resources	0				

These results show that major source of postharvest information for respondents were from *'Australian Citrus News'* and the chemical re-sellers. *'Australian Citrus News'* was rated by over half the respondents who thought this was a good / excellent source of postharvest information. This survey was taken at the postharvest workshop at Citrus Technical Forum (7 March 2019), where there was a strong presence of chemical resellers and other stakeholders.

Question 2. Where would you like to get your future postharvest information?

web-based (ACN, Packer Newsletter etc.)	20
direct emails	20
specialist workshops	22
general CAL regional / technical forums	17
other ways of getting information – list here	0

Question 3. What postharvest issues need more R&D?

fruit quality improvement, fungicides and sanitisers, fungicides, sanitation, degreening, internal quality, fungicides, storage, fruit quality improvement through export chain, fungicide resistance, storage, chilling injury, sanitation and fungicides, storage, residues, quality management, degreening, fungicides, improving quality, market access

Question 4. What areas of postharvest training and education would you like to see in the future?

packinghouse training video in sanitation and cleaning, training videos, packinghouse studies and examples of innovation, packinghouse resources, training resources, fruit quality posters, training

Question 5. Are you aware of the Hort Innovation 'Australian Citrus Postharvest Science Program' (CT15010)? How could this Project be improved?

Yes	No
26	4

No respondents made suggestions to improve the Project.

The results of this survey were presented to the PRG and submitted to MS 106 report (29/3/19).

Results highlights

1. The major source of postharvest information was from '*Australian Citrus News'* and over half the respondents thought this was a good / excellent source of postharvest information.

2. While growers also liked web-based sources of information, there was a perceived need for workshops.

3. The requirements for future R&D were similar to the current Program research themes: decay control and fungicide use, fruit quality and storage (chilling injury), sanitation and quality management through the export chain.

4. Key areas of education and training included: general packinghouse resources including packinghouse training in sanitation and cleaning

5. Over 85% of respondents were aware of the Program.

Conclusion This feedback from industry and stakeholders were encouraging for the Program, as the current research and development themes (decay control and quality management) of the current Program were key priorities for the respondents. In addition the extension needs are also being met through the current extension activities (articles to '*Australian Citrus News'* and other web-based areas, and workshops at regional forums etc.).

Innovation Outcomes

A range of technical outputs were produced from the Program and demonstrated the high level innovation for the Australian citrus industry. Seven international peer-reviewed research papers / reviews that have attributed contributions from this Program have been published. In addition, eleven technical posters from the Program were presented at the 2019 Citrus Technical Forum in Adelaide in March 2019. The papers and posters have been reported in the Program milestones and are listed in *Outputs Section 4*.

Collaboration and Training Outcomes

The Program worked closely with Hort Innovation, Citrus Australia through market programs such as Australian Citrus Industry Innovation and Market Development Program. The Program had good working relationships with growers, packers, and other industry stakeholders, such as chemical re-sellers. Regular and open feedback was given through the PRC which guided the development and outcomes of the Program.

The Program also worked closely with postharvest experts at the Institut Valencià d'Investigacions Agràries (IVIA) in Spain where Prof Lluis Palou (Program collaborator) gave two presentations at the postharvest workshop in Adelaide at the 2019 Citrus Australia Technical Forum. Prof Palou also visited and the Sunraysia and Riverland packinghouses and talked with growers / packers. He also spent time in the NSW Department of Primary Industries postharvest research laboratories (March 2019).

The Program also collaborated with University of Newcastle, where it is supporting new postharvest research. The Program co-funded the postgraduate training of John Archer who is investing better was to control postharvest decay. The University of Newcastle also supported the Program with support from Faculty staff (Dr Penta Pristijono, Prof Ron Wills, Dr Michael Bowyer and Dr Quan Vuong) and postgraduate students working in citrus postharvest science and technology to support the Australian citrus industry.

Monitoring and evaluation

A monitoring and evaluation (M&E) plan was delivered and approved by Hort Innovation (July 2017). This Section reports on the performance and evaluation of the Program as outlined in the M&E plan – below.

Broader Program Goals		
Contribution to Industry Objectives • Citrus Industry Strategic Investment Plan • Horticulture Innovation Australia		
Program details	**Performance measures**	**Evaluation methods**
Australian Citrus Industry – Strategic Investment Plan 2017-2022 (SIP)	Extent to which Best Practice postharvest strategies and practices are used in the citrus industry to minimise postharvest MRLs and improve fruit quality	• Export data and new markets • NRS reports • Industry surveys/reports • Surveys of stakeholders • Value of industry / trade etc
Evaluation	Postharvest is the key link between the orchard and the consumer. This Program worked with industry to ensure postharvest related issues (such as MRL, fruit quality etc.) were not a barrier to continued export growth, where exports in 2019 were over $500 million. The continued development of best practices and the assessment of new technologies and innovative solutions to postharvest issues were assessed in this Program to minimise postharvest MRLs and improve fruit quality. This Program was also active in extending these results by developing and extending postharvest extension resources with the re-development of the Citrus Australia postharvest portal, the re-development of *The Packer Newsletter* and regular articles to *Australian Citrus News*.	

Research and Development **1. Ultra-low residues. Conventional decay control**		
Program details	**Performance measures**	**Evaluation methods**
Benchmark current postharvest industry practices	*Maintain clean MRL record for industry (and response to any MRL issues. Record and document any breaches reported.*	*Program Milestone reports with details of activities undertaken and issues to Horticulture Innovation*
Evaluation	The Program was active in working with exporters and growers to ensure that MRLs were not an issue. Evidence to support this goal is the postharvest trial which showed that any postharvest washing treatment reduced the dithiocarbamate residues in lemon fruit. These results were reported to the PRC, Hort Innovation and industry and is available on the Hort Innovation grower portal: https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/evaluation-of-postharvest-removal-of-dithiocarbamate-residues-in-lemons/ (accessed November 2019)	
	Complete packingshed survey for current industry practices (target >50% of sheds to participate)	*Report on current postharvest practices. Case studies of packer improvements*
Evaluation	Packinghouse surveys were completed over two seasons where over 77 packinghouses were surveyed (2017/2018), and the Program Leader has also visited	

	over 70 packinghouses around Australia and talked with growers and packers. Postharvest fungicide and sanitation use were reported in *Australian Citrus News*, Summer (2017). Case studies of improvements in postharvest sanitation were reported in *Australian Citrus News* Summer (2018).	
Assess new fungicides, application approaches and new treatments	*Report results to stakeholders* *Maintain communication and relations with chemical companies and resellers*	*Application / product evaluation reports in Milestones*
Evaluation	Results of new fungicides, application approaches and new treatments were reported to industry at the Citrus Technical Forum (March 2019), milestone reports and discussed with PRC (PRC minutes). The Program Leader regularly talked with all chemical companies and resellers to ensure the results of the Program are extended and to also to get up-to-date information on current and new chemicals for industry.	
1. Ultra-low residues. Fungicide resistance management		
Conduct fungicide resistance survey to build awareness and management of issue	*Fungicide resistance and management options reported per region and nationally*	*Fungicide resistance reports to individual grower / packer, and regional / national summaries presented to industry.*
Evaluation	A two year packinghouse survey of technical resistance to postharvest fungicides was conducted. Sanitation and fungicide resistance reports to individual grower / packer, and regional / national summaries presented to industry. This was extended with grower presentations (e.g. Citrus Technical Forum in Adelaide, March 2019), and with industry articles, *Australian Citrus News.* Summer (2017), pages 34-36; Autumn (2018), pages 28-29 and Summer (2018/19), pages 36-37).	
Assess and facilitate fee-for-service postharvest (including fungicide resistance service)	*If industry need - facilitate development of service*	*Fee-for-service postharvest available and used by industry*
Evaluation	The PRC recommended that the Program develop a fee-for-service sanitation and fungicide resistance testing service for industry (PRC Meeting #2 on 13 March 2018). This was done independently to this Program (as agreed by Hort Innovation) and is now available to industry with partnership with Citrus Australia.	
1. Ultra-low residues. Non-conventional decay control. Alternate treatments and technologies		
Assess non-synthetic chemicals, physical treatments and combination treatments and technologies for decay control	*Reports on effectiveness of alternative treatments to control postharvest decay, maintain fruit quality and button green-ness. Number of new products evaluated/ recommended for further development and reported to industry.*	*Alternative treatments assessed, reported and adopted (if commercially practicable)* *New markets / market segments opened for 'nil-residue' fruit*
Evaluation	A range of different products and technologies have been evaluated (e.g. Unipolar™ sanitation system (*Outputs Section 1.3.2*), new postharvest fungicides for potential anthracnose control (*Outputs Section 1.1.1.iii*). New postharvest fungicides such as 'Chairman' and 'Propirly' were evaluated (Outputs Section *1.1.1i* and *1.1.2.5*). Alternative postharvest decay control, improved use of sanitising treatments and physical treatments to manage postharvest decay are presented in Section (*Outputs Section 1.1.4*). 'Chairman' fungicide is now being used by industry, while other technologies are being assessed for individual commercial use. Other technologies need more research and commercial assessment (see *Recommendations*).	

Assess alternative treatments to 2,4-D to maintain button green-ness		
Evaluation	Low ethylene technologies have been evaluated and technically successful for maintaining the green button (calyx) quality without the use of 2,4-D (Li et al., 2018 and Alhassan et al., 2019), but more work is required to apply these results for commercial outcomes.	
Research and Development **2. Communication**		
Program details	**Program details**	**Program details**
On-going liaison with industry provided via *• articles in Australian Citrus News, Citrus E-news, NSW DPI Citrus Connect, Hort Innovation newsletter*	*Articles to Australian Citrus News, Citrus E-news, NSW DPI Citrus Connect, Growing Innovation, etc. A minimum of 6 articles per year.*	*Summary of articles, presentations and videos presented in Milestones.*
Evaluation	A summary of all extension outputs were presented to the PRC and reported in the Milestones. The Program re-developed the Citrus Australia postharvest webpage with over 20 links to postharvest articles / pages. A total of 12 postharvest articles were published in '*Australian Citrus News*', x 5 '*Packer Newsletter*' editions (hosted on Citrus Australia website), x 12 articles for Citrus Australia Citrus e-News, articles in Australian Tree Crop, HortLink and NSW DPI '*Citrus Connect*'. These are listed in *Outputs Section 4*.	
• presentations at Citrus Australia forums and appropriate workshops	*Presentations at Citrus Australia forums and appropriate workshops*	
Evaluation	A total of 10 postharvest presentations from the Program have been delivered to growers at Citrus Australia forums and regional workshops. Two additional postharvest workshops were also delivered. These are listed in Table 56 (*Outputs Section 4*).	
• one to one consultation with industry	*• Available to industry*	
Evaluation	The Program Leader has been active in engaging with industry for one to one consultation. At all postharvest presentations / workshops, the Program leader has visited growers and packinghouse managers on regional visits. The Project leader has also been open for consultation with industry through email and telephone.	
• on-line instructional videos	*• On-line instructional videos hosted by Citrus Australia / NSW DPI*	
Evaluation	A practical guide to the degreening of mature citrus fruit was produced and hosted on the Citrus Australia postharvest webpage (https://citrusaustralia.com.au/growers-industry/post-harvest). The video '*Degreening – promoting colour development in mature citrus*', outlines the need and practicalities of degreening processes.	
Re-developing 'Packer Newsletter' as	*'Packer Newsletter' searchable and hosted on Citrus Australia website*	*Packer Newsletter' hosted on Citrus Australia website*

searchable resource		
Evaluation	The *'Packer Newsletter'* has been re-developed with five new newsletters added by the Program. It is now searchable and is hosted on the postharvest portal of the Citrus Australia website (https://citrusaustralia.com.au/growers-industry/packer-newsletters).	
Research and Development **3. Collaboration**		
Program details	**Program details**	**Program details**
On-going collaboration with other industry funded programs	*Regular collaboration with Citrus Australia and key collaborators*	*Good working relationship with Horticulture Innovation, Citrus Australia and industry.*
Evaluation	The Program has regular meetings and collaboration with all relevant Hort Innovation and Citrus Australia market programs such as Australian Citrus Industry Innovation and Market Development Program and Australian Citrus Quality Standards Program. The Program worked closely with Citrus Australia (e.g. updating postharvest portal on the Citrus Australia website) and the Program Leader has given ten postharvest presentations at Citrus Australia forums and regional workshops. The Program also has a good working relationship with Hort Innovation, PRC, industry and other stakeholders.	
On-going and developing new collaborations with overseas researchers and developments	*Regular communication with collaborators such as IVIA (Spain), and fostering new linkages where industry can benefit*	*Visit to Australia by leaders in postharvest research and development*
Evaluation	The Program has worked closely with postharvest experts at the Institut Valencià d'Investigacions Agràries (IVIA) in Spain. The Program funded Prof Lluis Palou to give two presentations at the postharvest workshop in Adelaide at the 2019 Citrus Australia Technical Forum. Prof Palou also visited and the Sunraysia and Riverland packinghouses and talked with growers / packers. He also spent time in the NSW DPI postharvest research laboratories (March 2019).	
Project Management		
Milestones	*Program Milestone reports with details of activities undertaken and issues to Horticulture Innovation*	*Program Milestone reports with details of activities undertaken and issues to Horticulture Innovation*
Evaluation	Milestone reports have been delivered and approved by Hort Innovation. All milestones have reported all activities undertaken by the Program and highlighted any potential issues.	
Project steering committee	*Project steering committee meets every six months*	*Project steering committee ensures the outcomes of the project are met and all issues are resolved*
Evaluation	The PRC was briefed and updated on progress of the Program twice a year (face-to-face and/or teleconference) where issues and future planning were discussed, feedback given and all issues resolved. The PRC approved the Program work plan.	
Budget	*Project leader ensures the budget is met*	*Budget is reported to Horticulture Innovation in Final Report*
Evaluation	The Program budget has been met and final reconciliation report delivered with the Final Report.	

Recommendations

This Program identified major areas of postharvest practice which need improvement with further R&D. These include:

Extension and adoption

1. Develop Postharvest Best Practice Manual for industry dissemination

There are range of postharvest resources have been developed in the Program and extended to industry through presentations, articles to '*Australian Citrus News'*, postharvest portal on the Citrus Australia website, re-development of the '*Packer Newsletter'* and through NSW Department of Primary Industries '*Citrus Connect'*. In addition there are many other valuable resources available from previous Hort Innovation citrus postharvest projects which are not being fully utilized. It is recommended that these resources are collated and developed into a '*Best Practice Postharvest Manual'* for the Australian citrus industry. This guide should cover general principles of fruit harvest and handling, postharvest sanitation, use of fungicides, waxes, packing, storage and transport and compliance of market access treatments.

2. Feasibility and demand for formal regular postharvest workshop updates

Feedback from the stakeholder survey at the postharvest workshops at the 2019 Citrus Technical Forum in Adelaide highlighted that growers appreciate workshops and presentations for the delivery of postharvest information (see *Outcomes*). It is recommended that '*Best Practice Postharvest Manual'* for should be made available to all growers and extended through a nationwide series of postharvest workshops.

In addition, it is recommended to examine the feasibility of regular postharvest workshops to update industry with the latest postharvest and market information. Such regular specific workshops would deliver new information on maintaining fruit quality through the supply chain, improved use of sanitisers and fungicides, meeting MRLs, updates on market requirements etc. If feasible and required by industry, these could be run with Citrus Australia in all growing regions.

Research and development

3. Evaluation of new and improved chemical and non-chemical treatments to control postharvest decay

Postharvest decay continues to be the major problem for the storage and marketing of citrus both on domestic and export markets. While the control of decay after harvest requires an integrated approach (handling, sanitation, storage conditions and fungicides), the correct use of fungicides and sanitisers are essential for the successful storage and export of citrus. In addition it is critical that industry have management strategies to meet regulatory and retail MRLs.

3a. Assessment new and improved fungicide chemistries. New fungicides and formulations are continually being improved by chemical manufacturers for the control of citrus postharvest decay. More R&D is required to assess these formulations for the Australian citrus industry and assess their application within Australian handling practices. The continued assessment of new fungicide chemistries is essential for the Australian citrus industry remain competitive. For example this Program assessed the efficacy of 'Chairman' fungicide (containing the active ingredients fludioxonil and propiconazole), which is now being integrated into many commercial fungicide programs. In addition the Program highlighted the potential of the postharvest fungicide, 'Propirly®' (containing the active ingredients propiconazole and pyrimethanil) to control postharvest decay. This fungicide is recommended for use in other countries, such as South Africa, and this needs to be assessed and applied for Australia. Similarly it is important to assess new improved formulations of old fungicides. For example new and improved formulations of SOPP (sodium ortho-phenylphenate) need to be assessed. The Program showed the success of new OPP formulations, but more work is required in this area.

3b. Improved use of salts to control postharvest decay. Salts such as sodium bicarbonate are already used by many

packers to control postharvest decay, but there is a need to improve its efficacy and find alternative 'generally regarded as safe' fungicide alternatives which are easier to handle. These 'non-fungicide' treatments further development to improve, evaluate and assess their effectiveness in commercial situations.

3c. Novel postharvest treatments to minimise postharvest decay. A range of different chemical and physical approaches to the control of postharvest pathogens are being developed and assessed. In addition, combining these treatments within current handling systems will be beneficial to provide non-chemical additive decay control protection. More R&D in this area is productive area of decay control without the use of fungicides.

4. Improved postharvest sanitation

Sanitisers are an under-estimated component of citrus postharvest practices and their importance and correct use need to be highlighted with more R&D to improve their efficacy and use. A number of sanitisers with different chemistries are currently available in the market and each sanitiser has its own merits and limitations. In addition, there are range of new technologies such as electrolysed water, which are being developed and being applied from other industries. For example this Program completed some preliminary assessments on the electrolysed system by as 'Unipolar Water Technologies' and showed some significant water sanitizing benefits. However these types of systems need to be further evaluated for their efficacy and application to the Australian citrus industry. These technologies are potentially more cost efficient but need to be assessed for their commercial application to the Australian citrus industry.

5. Improvements in fruit quality following market access treatments

Market access is critical for the profitability of the Australian citrus industry. The current market access treatments for Australian citrus are effective and widely used, but improvements in the consistency of fruit quality out-turns following treatment would give the market and exporters confidence in Australian citrus.

5a. Reducing chilling injury. Chilling injury is an important storage problem that can affect many citrus fruit and occurs when fruit is held below recommended storage temperatures, i.e. during phytosanitary cold treatment (3 ^{o}C). While not all fruit develop chilling injury during cold treatment, it is a low-level inconsistent problem but can sometimes cause significant out-turn claims in export markets. More R&D into the pre- and postharvest factors that contribute to chilling injury needs to be conducted to develop management strategies to reduce commercial out-turn losses.

5b. Improving out-turns following irradiation treatment for market access. A review of the use of irradiation as a market access treatment for citrus has conducted (*Output 2.4 Review of irradiation as a market access treatment on fruit quality*; Appendix 3) and for future R&D investments had recommended (1) identify and manage pre- and postharvest factors (such as cultivar, harvest time, postharvest but pre-irradiation treatments) that contribute to potential post-treatment damage, and (2) lemons and a lesser extent mandarins should be the focus of future R&D.

5c. Market access treatments. To assist growers and exporters a direct side by side comparison of different market access treatments (cold V irradiation V methyl bromide) should be conducted. To alleviate the potential of chilling injury and irradiation damage, shorter cold treatments and low dose irradiation should be examined.

6. Improving fruit quality in the supply chain

The consistent supply of high fruit quality to the consumer is paramount to the success of the Australian citrus industry. Fruit quality declines after harvest and it is the role of the supply chain and postharvest technology to maintain quality through to the consumer. There are different postharvest challenges for the different citrus types. Mandarins and other 'soft' citrus types are a growing category in Australia (and around the world) and often require specific handling and postharvest practices. Visits to packing houses, particularly in Queensland in the early part of the season showed significant quality issues associated with de-greening of mandarins. This was primarily due to anthracnose infection being expressed after de-greening and through the supply chain. This was initially addressed with research in this Program which showed considerable promise for new potential postharvest fungicides for the management of this problem. More work to highlight the role of pre-harvest and postharvest

management on brush-burn / anthracnose damage, e.g. use of orchard copper sprays, degreening duration and handling. More R&D in this area is required.

Maintaining the button 'freshness' quality is an important aspect of overall fruit quality, as brown or detached buttons can reduce overall fruit acceptability. This currently managed with postharvest applications of 2,4-dichlorophenoxyacetic acid (2,4-D – 'Citrus Stop Drop™') in the wax however alternatives are required. This Program showed there are some postharvest management options such as ethylene management are available, but more work is required to commercially assess these options.

Although limes are a small component of the citrus industry, it is essential to maintain fruit quality (green skin) through to the consumer. Work from this Program showed the use of pre-storage UV-C treatment and 1-methylcyclopropene (1-MCP) maintained lime fruit peel condition, even in the presence of ethylene (Golding and Pristijono, 2018; Pristijono et al., 2018). More work and commercialisation of this treatment is required to allow its use and cost-effective commercial application methods for its introduction.

Refereed scientific publications

Journal articles

Alhassan N., Golding J.B., Wills R.B.H., Bowyer M.C., Pristijono P. (2019) Long term exposure to low ethylene and storage temperatures delays calyx senescence and maintains 'Afourer' mandarins and Navel oranges quality. *Foods* **8(1)**, 19. (13 pages). https://www.mdpi.com/2304-8158/8/1/19

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Li. X., Golding J.B., Arcot J., Wills R.B.H. (2018) Continuous exposure to ethylene in the storage environment adversely affects 'Afourer' mandarin fruit quality. *Food Chemistry* **242**, 585-590. https://doi.org/10.1016/j.foodchem.2017.09.088

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Intellectual property, commercialisation and confidentiality

The Project Reference Committee requested that a not-for-profit service to industry be developed for a sanitation and fungicide resistance testing service. This was developed independent to this Program and with approval from Hort Innovation.

No other project IP, project outputs, commercialisation or confidentiality issues to report.

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Appendices

List of Appendices:

Appendix 1. Abstract to 5th International Symposium on Postharvest Pathology

Abstract submitted to the 5th International Symposium on Postharvest Pathology in Leige Belgium in May 2019. A paper was submitted for publication in *Acta Horticulturae*.

Preliminary investigations on the effect of low-pressure treatment on *in vitro* and *in vivo* growth of *Penicillium* sp. in oranges

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Abstract

Penicillium digitatum is the major pathogen causing postharvest decay in citrus fruit and is the organism responsible for green mould. *P. digitatum* decay is currently managed with synthetic fungicides but there is a growing consumer need to reduce the reliance on synthetic fungicides and find alternative non-synthetic treatments. Low-pressure treatments may offer a potential solution for the storage and transport of citrus, as it is a physical treatment and does not leave any chemical residues. To test the effectiveness of low pressure storage treatments on the growth of *P. digitatum*, small-scale laboratory experiments were conducted with specialist low pressure chambers. *P. digitatum* was grown on PDA agar plates and treated with low pressure (4 kPa) for 3 and 6 days at 10°C before growth assessments in air at regular (101 kPa) atmospheres. The results showed that low pressure treatments slowed the growth of *P. digitatum*. In a further experiment on oranges, *P. digitatum* infected fruit were treated with low pressure (4 kPa) at 5°C for up to 22 days. This experiment also showed a reduced growth of *P. digitatum in vivo*. These results show there is potential to control the growth of *P. digitatum* at low pressure treatments and further experimentation should be conducted.

Appendix 2. Sensory questionnaire for assessment of Murcott mandarins

This questionnaire was use for assessing sensory qualities of Murcott mandarins in *Output Section 2.2 Minimising the development of off-flavours in mandarins in export markets*

Sample:

Sensory taste tasting of Mandarin

Please open the fruit in front of you, smell the fruit; eat the sample and answer the following questions...

1. **Can you detect any *off-flavours (aroma and taste)* from this particular sample?**

   YES _____ (please go to Q2)

   NO _____ (please go to Q3)

2. **If you can detect any off-flavours, how would you rate the *STRENGTH* of the *OFF-FLAVOURS* of this sample?**

Attributes	Definitely Too Weak	Slightly Too Weak	Just Right	Slightly Too Strong	Definitely Too Strong
Off-Flavour					

3. **How much do you like/dislike the *following attributes* of this sample?**

Attributes	Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
Aroma									
Sweetness									
Acidity/ sourness									
Flavour/ Taste									
Overall liking									

4. **Do you have any *COMMENTS/FEEDBACK* about this sample?**

_____

_____

_____

_____

Appendix 3. Exploring the potential of low dose irradiation phytosanitary treatments for the Australian citrus industry

Contribution from **Australian Citrus Postharvest Science Program (CT15010)**

Exploring the potential of low dose irradiation phytosanitary treatments for the Australian citrus industry

30 April 2018

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Executive Summary

This review examined the published literature on the effects of low dose irradiation (< 1,000 Gy) as a market access treatment on citrus fruit quality. In summary the results highlighted:

- Postharvest low dose irradiation is an accepted safe phytosanitary treatment for some key export markets.
- Irradiation overcomes any issues of chilling injury with cold treatment for export.
- There are few consistent negative effects of irradiation on final citrus fruit quality following treatment and storage.
- Some surface damage (such as pitting) can occur in some types of different citrus but the contributing factors to this damage are not known but could potentially be managed.

Recommendations

Low dose irradiation provides market access options for the Australian citrus industry, but requires solutions to ensure consistent treatment out-turns which do not affect final fruit quality.

Recommendations for future R&D investments include:

- Identify and manage pre- and postharvest factors (such as cultivar, harvest time, postharvest but pre-irradiation treatments) that contribute to potential post-treatment damage.
- Lemons and a lesser extent mandarins should be the focus of future industry R&D investments.

Contents

Glossary

Gy	Gray is a derived unit of ionizing radiation dose which is defined as the absorption of one joule of radiation energy per kilogram of matter. 1,000 Gy = 1 kGy
TA	Titratable acidity (% citric acid)
TSS	Total soluble solids, or soluble solids content (% Brix)
Vitamin C	Also known as ascorbic acid (AA) and is an essential nutrient in the diet

Introduction

Market access is the key priority of the Australian citrus industry. While cold treatment protocols have successfully enabled exports of oranges and mandarins to key export markets, the development of chilling injury in response to cold treatment can limit exports in some susceptible citrus types such as lemons. Another market access tool for the Australian citrus industry is low dose irradiation.

Irradiation is a technologically proven, viable and scientifically sound disinfestation treatment (Follet, 2009). Moreover, irradiation is increasingly becoming an approved and agreed treatment in world trade of food and horticultural products. Generic irradiation treatments have been approved by U.S. Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) at doses of 150 Gy (Gray) for tephritid fruit flies and 400 Gy for all insects except pupal and adult Lepidoptera (USDA-APHIS, 2006). Further APHIS rulings and new rulings by the International Plant Protection Convention (IPPC) have approved new minimum doses for 6 fruit fly pests and 14 other plant insect pests regardless of the host product, at doses between 60 and 300 Gy (USDA-APHIS, 2008). An excellent review of irradiation quarantine treatments has been published by Follet (2009).

Food Standards Australia New Zealand (FSANZ) have granted food safety approval for irradiation (at 150 – 1,000 Gy) as a technique for insect pest disinfestation of citrus and it has also been approved in several export markets such as Indonesia and Vietnam. Irradiation breaks chemical bonds in DNA and other molecules, thereby sterilizing the pest or preventing it from achieving sexual maturity. This review examines the published literature on the effect of low dose irradiation (< 1,000 Gy) as a market access treatment on citrus fruit quality.

Internal quality

Total soluble solids (TSS) also known as soluble solids content (SSC, Brix %) is a relative measure of the sugar content in citrus fruit. Together with titratable acidity (TA) which is a measure of the acidity or sourness of the fruit, these fruit quality components combine to determine overall fruit taste and acceptability. The effects of low dose irradiation on TSS and TA levels in citrus fruit are presented in Table 1 and show that in general irradiation does not consistently affect TSS or TA in citrus fruit.

Table 1. Effects of irradiation on total soluble solids (TSS, % Brix) and titratable acidity (TA, % citric acid) in citrus fruit.

Fruit	Dose (Gy)	TSS	TA	Reference
Grapefruit Rio Red	70, 200, 300, 400, 700	Early season not affected. Later harvest no differences after storage	Early season not affected. Later harvest irradiated higher TA	Patil et al. (2004)
Grapefruit Rio Red	150, 300, 400, 500	No change	No change	Hallman and Martinez (2001)
Orange Navel	200, 400, 600	No difference, (except 400 and 600 Gy lower TSS)	No difference, (except at 600 Gy lower TA)	McDonald et al. (2013)
Orange Ambersweet, Hamlin, Navel, Pineapple, Valencia	250, 300, 450	No effect (except decrease in Ambersweet at 300 Gy)	No effect	Miller et al. (2000)
Mandarin	510, 875	No consistent	No consistent	Palou et al. (2007a)

Fruit	Dose (Gy)	TSS	TA	Reference
Clementine		differences	differences	
Mandarin Clementine	195, 395, 510, and 875	No consistent differences	No consistent differences	Palou et al. (2007b)
Mandarin Fallglo, Minneola, Murcott, Sunburst, Temple	250, 300, 450	No effect (except decrease in Sunburst at 450Gy)	No effect (except decrease in Sunburst and Tangelo at 450Gy, and increase Murcott at 300Gy)	Miller et al. (2000)
Mandarin Seedless Kishu	150, 400, 1,000	No change	No change	Ornelas-Paz et al. (2017)
Mandarin Shatang	200, 300, 400, 500, 600	Up to 30 days storage - no differences	Decrease TA with increasing dose, during storage	Zhang et al. (2014)
Orange Navel	200, 400, 600, 800, 1,000	No change	No change	Noh et al. (2016 a, b)
Orange Marrs	150, 300, 400, 500	No change	No change	Hallman and Martinez (2001)
Lime Tahitian	50, 100, 150 and 200; 250, 750	Response varied with harvest time	Response varied with harvest time	da Silva et al (2016)
Pommelo Sarawak, Chandler	150, 1,000	No consistent effects	No consistent effects	Jain et al. (2017)

Orange

O'Mahony and Goldstein (1987) and McDonald et al. (2013) found a decrease in TSS and TA at irradiation dose levels between 300 and 600 Gy but Miller et al. (2000) did not report changes in TA of oranges treated at irradiation doses of 150 – 450 Gy. McDonald et al. (2013) concluded that the overall changes in combined TSS and TA as shown by changes in BrimA after 4 weeks of storage, were relatively small (0.31 units). This, combined with the results their sensory testing, suggested that the effects of irradiation on TSS and TA may have limited importance in terms of an impact on flavor.

In a study of low dose irradiation (200, 400, 600, 800 and 1,000 Gy) on imported Navel oranges stored for either 20°C for 12 days or 3°C for 45 days reported no significant effect of irradiation in TSS/TA ratio, total sugar content, reducing sugar content (Noh et al., 2016 a, b). While Hallman and Martinez (2001) found no change in TA or TSS in Marrs oranges treated up to 500 Gy and stored for 21 days at ambient temperature.

Mandarin

Miller et al. (2000) showed that irradiation (150, 300 and 450 Gy) did not generally affect TSS or TA of five mandarin hybrids, Fallglo, Minneola, Murcott, Sunburst, and Temple, with the exception of a decrease in of Sunburst mandarin at 450 Gy and a reduction in TA of Sunburst and Temple at 450 Gy, but an increase at 300 Gy in Murcott. While these few differences may have been statistically different, the effects on overall taste and flavour would be low. Indeed with a small seven person untrained panel, Miller et al. (2000) showed the acceptability of juice and pulp flavor was not affected. Sensory and consumer aspects of irradiation on consumer acceptability is discussed in the *Consumer Acceptability Section*. Ornelas-Paz et al. (2017) further showed no consistent effect of low dose irradiation (150, 400, and 1,000 Gy) on TSS or TA in Seedless Kishu mandarins. While Zhang et al. (2014) showed with Shatang mandarin treated at 200, 300, 400, 500 and 600 Gy, TSS and TA had no significant differences compare to those of the control during the first 2 weeks storage, but

after this storage time at 4 °C, increasing dose reduced TA levels. However there were no significant differences in TSS between irradiated and non-treated fruits after 30 day storage (Zhang et al. 2014). Palou et al. (2007a, b) also showed there were no consistent differences in TSS and TA in Clemenules mandarins after different times in storage where in general, irradiation had no effect on TSS or TA.

Other citrus

Grapefruit Patil et al. (2004) showed harvest time had a significant effect on the treatment effects of irradiation on Rio Red grapefruit. In early season grapefruit, TSS and TA were not affected due to irradiation or storage, but late harvest grapefruit exposed to irradiation (70 – 700 Gy) retained acidity better than the fruit not exposed to irradiation. Patil et al. (2004) further showed that the initial TSS was the lowest in the late season fruit exposed to the 700 Gy treatments; however, no differences among treatments were observed after storage. Hallman and Martinez (2001) further found no change in TA or TSS in Rio Red grapefruit treated up to 500 Gy and stored for 21 days at ambient temperature.

Lime The effects of irradiation in the internal quality of Tahitian limes were affected by harvest time ('on'- and 'off'-season fruit) and irradiation dose (da Silva et al. 2016).They showed irradiation reduced TSS in fruit from the 'off'-season, but not fruit harvested in the regular harvest period. They also showed higher TA values in the 'off'-season fruits treated with 50 Gy, as compared with untreated fruits (da Silva et al. 2016). This in this trial, the interaction of harvest time and irradiation was significant and should be taken into account.

Pummelo Jain et al. (2017) showed no consistent effects of 150 and 1,000 Gy irradiation on TSS and TA values in Chandler and Sarawak pummelos stored for 3 weeks at 12 °C and after an additional week at 20 °C. The differences were low and did not affect consumer acceptability of the treatment (Jain et al. 2017).

Injury and disorders

Citrus is relatively sensitive to irradiation and the response to treatment is highly variable and dependent on species, hybrid, and cultivar (Miller et al, 2000). Physical injury can occur at low doses (< 1,000 Gy) and usually occurs in the peel as peel pitting and fruit softening (Wall 2015). For example Miller et al. (2000) treated ten citrus cultivars grown in Florida, including the five orange [*Citrus sinensis* (L.) Osbeck] cultivars, Ambersweet, Hamlin, Navel, Pineapple, and Valencia, and the five mandarin hybrids (*Citrus reticulata* Blanco), Fallglo, Minneola, Murcott, Sunburst, and Temple, with low dose irradiation at 0, 250, 300 and 450 Gy, and stored for 14 days at 1 °C or 5 °C plus 3 days at 20 °C, to determine dose tolerance based on fruit injury. They showed that fruit softening of Valencia, Minneola, Murcott, and Temple was dose-dependent, but that of other cultivars was unaffected. Only Ambersweet, Valencia, Minneola, and Murcott did not develop peel pitting at 150 Gy or higher, whilst all the other cultivars were injured (Miller et al. 2000).

While limited studies have been conducted on the causes of damage, Riov (1975) showed that irradiation induced pitting which may be caused by the accumulation of phenolic compounds in flavedo cells leading to cell death and peel necrosis that is manifest as pitting (Figure 1).

A summary table of the literature on the effect of irradiation on citrus disorders in presented in Table 2, and shows different citrus types develop different types of symptoms at different treatment doses.

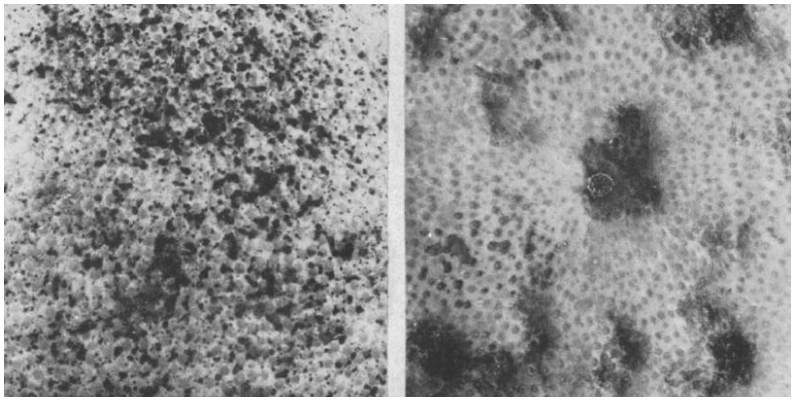


Figure 1. Symptoms of external radiation damage in Marsh Seedless grapefruit peel 7 days after irradiation with 2,400 Gy. Damage symptoms at the stem end (left) and damage symptoms at the stylar end (right).
From Riov (1975) Radiation Botany 15, 257-260.

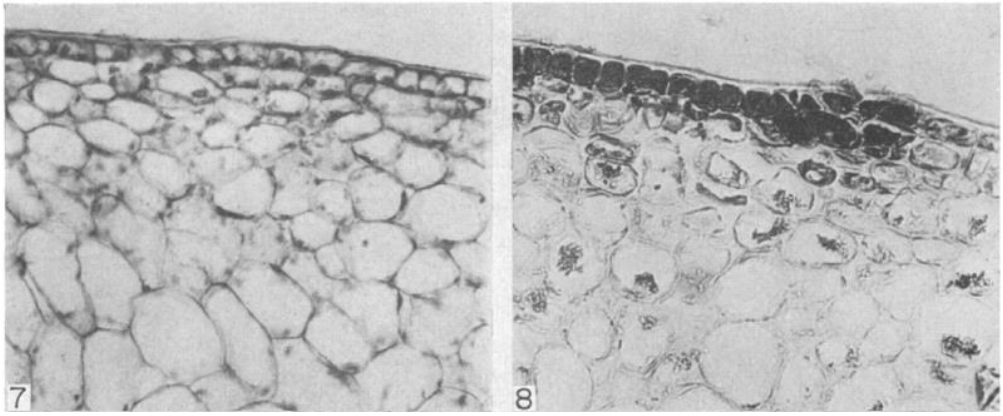


Figure 2. Cross sections in the outer flavedo layers at the stem end of control and irradiated Shamouti orange treated with 2,400 Gy. Untreated control Shamouti orange stained with diazo-safranin (× 290) (left). Irradiated Shamouti orange stained with the GIBBS' reagent (right). Phenolic compounds which accumulate in the outer flavedo layers are seen mainly in the epidermis (× 290).
From Riov (1975) Radiation Botany 15, 257-260.

Table 2. Effects of irradiation on visual quality of citrus fruit.

Fruit	Dose (Gy)	Injury / Disorder	Reference
Orange Mosambi	250, 500, 1,000, 1,500	Peel disorder in the form of brown sunken areas after 90 days and reduced fruit firmness at > 250 Gy	Ladaniya et al. (2003)
Orange Ambersweet, Hamlin, Navel, Pineapple, and Valencia	0, 250, 300, 450	Fruit softening of Valencia was dose-dependent, but that of all other cultivars was unaffected. Pitting - Hamlin, Navel, Pineapple developed peel pitting injury, but Ambersweet and Valencia did not develop any symptoms.	Miller et al. (2000)
Orange Lane Late	200, 400, 600	Surface pitting and visual damage after treatment with 400 and 600 Gy – see Figure 3	McDonald et al. (2013)
Orange Navel	320-370 and 520-600	Increased brown blemishing and pitting	O'Mahony and Goldstein (1987)
Orange Valencia	300, 500, 750, 1,000	No damage at 750 Gy	Nagai and Moy (1985)
Orange Washington Navel, Valencia	75, 150, 300	No damage up to 300 Gy	Macfarlane and Roberts (1968)
Orange Valencia Late	350, 800	No damage	Betancurt et al. (2007)
Mandarin Fallglo, Minneola, Murcott, Sunburst, Temple	0, 250, 300, 450	Fruit softening of Minneola, Murcott, and Temple was dose-dependent, but that of other cultivars was unaffected. Pitting - Minneola, and Murcott did not develop peel pitting at 150 Gy or higher, whilst all the other cultivars were injured	Miller et al. (2000)
Mandarin Nagpur	250, 500, 1,000, 1,500	No rind disorders up to 1,500 Gy	Ladaniya et al. (2003)
Mandarin Clemenules	195, 395	Slight to moderate rind browning immediately after treatment, but no damage after 12 days cold storage	Alonso et al. (2007)
Mandarin Seedless Kishu	150, 400, 1,000	400 and 1000 Gy promoted browning of the calyx end and fungal infection	Ornelas-Paz et al. (2017)
Mandarin Shatang	200, 300, 400, 500, 600	No effect, but increased decay with high levels	Zhang et al. (2014)
Grapefruit Rio Red	150, 300, 400, 500	No effect	Hallman and Martinez (2001)
Grapefruit Rio Red	70, 200, 400, 700	Early season Rio Red grapefruit was more sensitive to later season fruit, particularly at 700 Gy in the early season fruit. But overall appearance of all fruit was still acceptable as judged by consumers	Patil et al. (2014)

Fruit	Dose (Gy)	Injury / Disorder	Reference
Pumello Chandler, Sarawak	150, 1,000	Peel damage was greater and developed more quickly in irradiated fruit and was more severe when the fruit was stored at ambient temperature for a week	Jain et al. (2017)
Lemon Lisbon	75, 150, 300, 600, 1,000	Peel damage increased with dose. Lemons at a green stage were more severely affected than yellow lemons	Jessup et al. (1992)
Lemon Eureka	500 – 3,000	Some minor peel damage and cavitation at 500 Gy	Maxie et al. (1964)
Lime Tahitian	50, 100, 150 and 200; 250, 750	> 100 Gy caused skin yellowing	da Silva et al (2016)

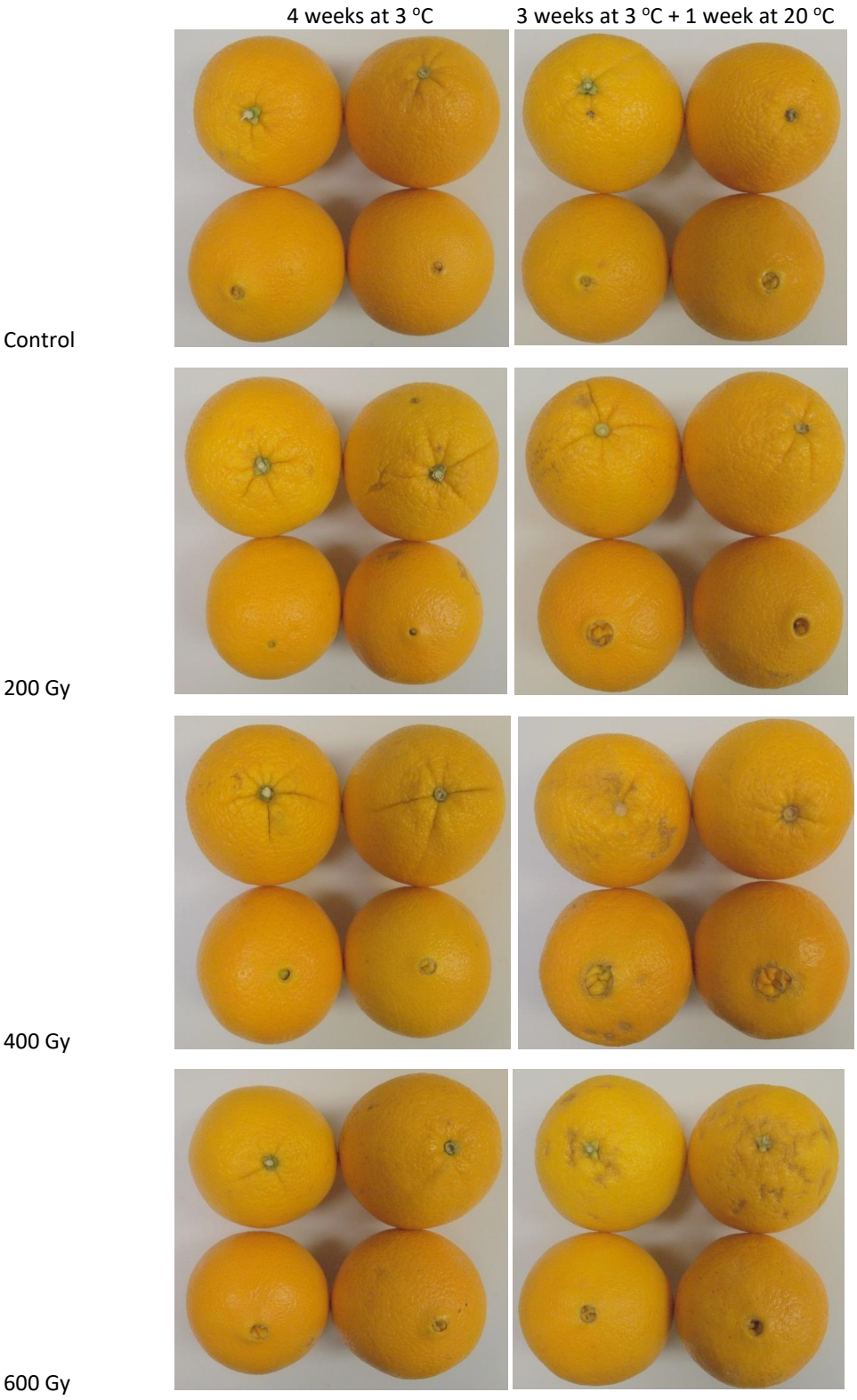
Orange

Lane Late navel oranges had increased surface pitting and visual damage after treatment with 400 and 600 Gy (McDonald et al. 2013). Figure 3 shows the typical symptoms of irradiation damage, with brown blemish and pitting in Lane Late navel oranges following treatment at 400 and 600 Gy and storage. Table 3 quantifies the levels of damage in these fruit following treatment and storage. O'Mahony and Goldstein (1987) also found increased brown blemishing and pitting of whole navel oranges irradiated at 300 and 600 Gy. While in another study, Valencia oranges were tolerant to 750 Gy treatment and storage for 7 weeks at 7 °C (Nagai and Moy 1985). In a comparison of different orange cultivars, Miller et al. (2000) showed that Ambersweet and Valencia oranges tolerated 500 - 600 Gy irradiation, but Hamlin, Navel, and Pineapple cultivars were injured at 150 Gy.

Macfarlane and Roberts (1968) showed treating Washington Navel oranges with less than 300 Gy were commercially acceptable, however injury increased as the dose was increased over the range studied. In addition, the severity of injury depended on the variety and, particularly for Washington Navels, on the part of the season when the fruit was picked and treated (Macfarlane and Roberts, 1968). From a limited data set, early season Washington Navel fruit harvested from coastal NSW was very susceptible to injury, whilst late season fruit was relatively resistant. In a subsequent smaller experiment with Valencia oranges, time of harvesting made little difference (Macfarlane and Roberts 1968). Indeed Betancurt et al. (2007) also showed no effect of 350 and 800 Gy irradiation on Late Valencia appearance and fruit quality.

In addition to external appearance, McDonald et al. (2013) found detected some internal drying and granulation in Lane Late navel oranges with higher irradiation treatments. They showed that after 3 weeks of storage, 25% and 29% of the fruit treated at doses of 400 and600 Gy, respectively, showed some degree of segment drying, but none of the control or fruit treated at 200 Gy showed any symptoms. In addition, they showed granulation was present in an average of 17% of the 600 Gy fruit and in none of the other treatments. However the extensive taste panel assessment did not detect any differences changes due to dose or storage time (McDonald et al., 2013). However in other studies, with Clemenules mandarin fruit (Alonso et al., 2007), Fortune hybrid fruit Alonso et al. (2002) and Nagpur mandarins (Ladaniya et al. 2003) there has been no reports of juice loss due to irradiation.

Figure 3. Effect of low dose irradiation on visual quality of Lane Late navel oranges after 4 weeks storage at 3 °C (left) and 3 weeks at 3 °C and one week at 20 °C shelf-life (right). [*from McDonald et al. 2013*]



(from McDonald et al. (2013). Effect of gamma irradiation treatment at phytosanitary dose levels on the quality of 'Lane Late' navel oranges. Postharvest Biology and Technology 86, 91-99)

Table 3. External damage values for Lane Late navel oranges treated at four different dose levels and evaluated after 1 d, 3 weeks and 4 weeks following irradiation treatment with injury / damage scored using 1–6 scale where 1 = no damage, 4 = moderate damage, 6 = very severe damage. % of fruit rated moderate or above is indicated next to predicted mean score. *(from McDonald et al. 2013).*

	External damage score and % of fruit rated 4 (moderate) and above					
Dose (Gy)	1 day		3 weeks		4 weeks	
0	2.0	6.9%	2.1	6.6%	2.0	3.5%
200	1.9	4.5%	2.3	7.3%	2.4	11.1%
400	2.1	10.8%	3.0	33.0%	3.4	50.3%
600	2.1	9.4%	3.6	55.9%	3.8	68.4%

Mandarin

Similar to oranges, the tolerance of mandarins to irradiation is dependent on cultivar and other pre and postharvest factors. Miller et al. (2000) showed that the mandarin hybrids Minneola and Murcott showed tolerance at 500-600 Gy, but peel pitting occurred for Fallglo, Sunburst and Temple cultivars at 150 Gy. Ladaniya et al. (2003) showed the Nagpur mandarin tolerated doses up to 1,000 Gy. While Shatang mandarin was not affected by treatment up to 600 Gy (Zhang et al. 2014).

Alonso et al. (2007) observed a slight to moderate rind browning was observed in X-ray irradiated Clemenules mandarin fruit at both 195 and 395 Gy after two days at 20 °C. However, this damage was not evident after the 12-day cold storage period and this underscores the random nature of these disorders and could be attributed to interactions with other postharvest factors such as the coating used in this experiment (Alonso et al. 2007).

While some cultivars have tolerance to irradiation, some mandarin types are very sensitive to irradiation. For example, Ornelas-Paz et al. (2017) found Seedless Kishu mandarins (*Citrus kinokuni mukakukishu*) treated with 150 Gy developed peel damage and browning of the calyx. This is not the classic pitting damage, but a general browning and senescence of the peel which becomes highly susceptible to fungal damage (Figure 4). The severity of the browning damage increased with irradiation dose, especially for fruit located on the top layer of the cases but browning severity was not objectively evaluated in this study and further studies are needed in this regard.

Figure 4. Irradiation damage on the peel of kishu mandarins after three weeks of storage.

(from Ornelas-Paz et al. (2017) Effect of phytosanitary irradiation on the postharvest quality of Seedless Kishu mandarins (Citrus kinokuni mukakukishu). Food Chemistry 230, 712-720.)

Other citrus

Grapefruit Treatment of grapefruit with a dose of 300 Gy resulted in minimal injury to the fruit (Spalding and Davis 1985; Miller and McDonald 1996). While Hallman and Martinez (2001) demonstrated that Rio Red grapefruit exposed to irradiation doses of up to 500 Gy did not affect appearance compared to untreated fruit. Type and intensity of injury to grapefruit due to low dose irradiation (300–900 Gy) has been attributed to time of harvest. Early-season grapefruit, harvested from October to December (northern hemisphere), were more susceptible to scald and less susceptible to rind breakdown, while late-season fruit were more susceptible to rind breakdown after irradiation and storage (Hatton et al., 1982). Patil et al. (2014) further showed early season Rio Red grapefruit was more sensitive to later season fruit, particularly at 700 Gy in the early season fruit, overall appearance of all fruit was still acceptable as judged by consumers.

Pummelo There was peel damage in Chandler (red flesh) and Sarawak (white flesh) pummelos treated with 150 Gy and 1000 Gy is described in Figure 5 (Jain et al. 2017). The damage was greater and developed more quickly in irradiated pummelos and became even more severe when the fruit was stored at ambient temperature for a week. However this damage was low and Jain et al. (2017) suggest this could be managed with minimal handling and good temperature control. Indeed the quality of irradiated pummelos stored at refrigerated temperature for 3 weeks was similar to untreated pummelos, however, physical handling and exposure to higher temperature resulted in increased peel pitting of irradiated fruit compared to non-treated fruit. Jain et al (2017) conclude that irradiation could serve as a potential phytosanitary treatment for Chandler and Sarawak pummelos, provided that the fruit is subjected to minimal handling and not temperature abused.

Figure 5. Peel damage in 150 Gy Chandler pummelo after 4 weeks of storage - 3 weeks at 12 °C and fourth week at 20 °C.

(from Jain et al. (2017) Effect of phytosanitary irradiation on the quality of two varieties of pummelos (Citrus maxima (Burm.) Merr.). Scientia Horticulturae 217, 36-47)

Lemon Lisbon lemons harvested at two different maturity stages, green and completely yellow which grown in the NSW Central Coast, were treated with 75, 150, 300, 600 and 1,000 Gy (Jessup et al. 1992). They showed that peel damage increased with increasing irradiation dose. Lemons that were harvested at a green stage, were more severely affected than yellow lemons (Table 4). In addition irradiation caused flesh discolouration and cavitation (Table 4), as well as albedo discolouration and toughness, particularly at the higher dose rates (Jessup et al. 1992).

Maxie et al. (1964) treated Eureka lemons with high irradiation treatments (500 – 3,000 Gy), but even at the lower irradiation doses (500 Gy), there was some minor peel damage and cavitation.

Table 4. Effect of low dose irradiation and subsequent storage at 15 °C on damage to Lisbon lemons harvested at either a green and completely yellow stage. (from Jessup et al. 1992)

Type of damage to fruit and maturity tested						
	Peel Damage		Flesh discolouration		Cavitation	
	Yellow	Green	Yellow	Green	Yellow	Green
5% lsd	*0.42*		*0.45*		*0.23*	
0	1.0	1.0	1.0	1.0	1.0	1.0
75	1.8	2.3	1.1	1.0	1.0	1.0
100	2.4	3.1	2.0	2.1	1.0	1.0
300	2.6	3.6	2.7	2.1	1.2	1.0
600	2.5	3.1	2.6	2.3	1.3	1.2
1000	3.0	4.0	3.5	2.5	1.7	1.2

Lime In a preliminary trial, da Silva et al (2016) showed 250 and 750 Gy doses negatively affected skin quality and pulp of Tahitian limes. They further used lower doses of irradiation (50, 100, 150, and 200 Gy) and showed doses > 100 Gy caused skin yellowing of fruits harvested and concluded doses between 50 Gy and 700 Gy caused damage to lime fruit quality during storage at room temperature (de Silva et al., 2016).

Nutrition

Numerous independent reviews have been published on the effects of irradiation on the nutritional quality of food, including fresh fruit and vegetables (World Health Organization 1981; World Health Organization 1994; World Health Organization 1999; Scientific Committee on Food 2003; Arvanitoyannis 2010; European Food Safety Authority 2011). These reviews have examined the efficacy, safety and nutritional effects of irradiation on a wide range of foods, including citrus. Irradiation can induce changes in nutrient content, depending on a variety of factors including the irradiation dose, composition of the food, packaging material, ambient temperature and atmospheric oxygen concentration (Diehl et al. 1991; Kilcast 1994; World Health Organization 1994). A relatively small proportion of nutrients are sensitive to irradiation, with higher doses of irradiation associated with greater nutritional losses (World Health Organization 1999), however these doses are more than the maximum 800 Gy used for disinfestation of fresh fruit and vegetables. Indeed, there has been no demonstrated effect of irradiation up to 1,000 Gy on the amount and nutritional quality of carbohydrates, proteins or fats and no evidence to suggest that irradiation reduces the mineral content of food (Diehl et al. 1991; World Health Organization 1994). Therefore, macronutrients and minerals have not been given further consideration in this review.

Vitamins have been shown to be susceptible to oxidation and breakdown with high levels of irradiation treatment. There is a general hierarchy of vitamin sensitivity to irradiation, with vitamins A, C, E and thiamin being most sensitive (Figure 6) (Kilcast 1994; Diehl 1995). As fruits and vegetables are the predominant dietary sources of vitamin A (as carotene) and vitamin C, the majority of studies examining the effects of irradiation on fruit or vegetable quality have focused on these nutrients.

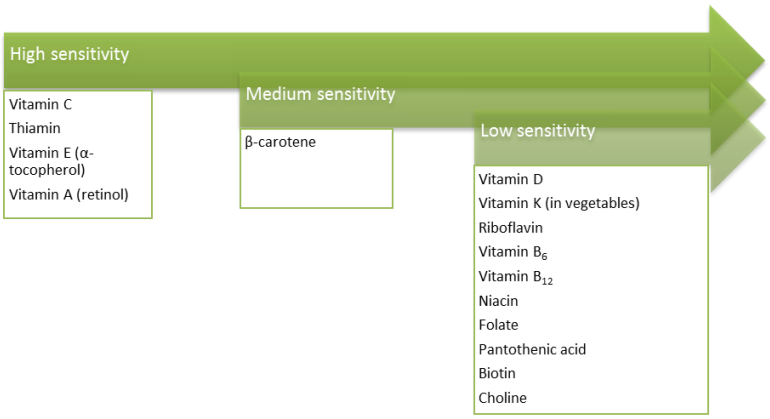


Figure 6. General sensitivity of vitamins in fresh fruit and vegetables to irradiation.

(modified from FSANZ 2014 and Kilcast 1994)

Vitamin C

Citrus is a rich source of ascorbic acid (AA, vitamin C) and is a major dietary contributor to AA in all age and gender groups in Australia and New Zealand, with the exception of 17-18 year old Australian females (FSANZ 2014). Indeed, citrus fruit provides between 5 - 17% of AA intake of Australians (FSANZ 2014). Therefore the potential effects of irradiation of vitamin C content of citrus is important. Citrus are not a major source of dietary carotene, thiamin, riboflavin, niacin, folate or vitamins E and B6 in Australia and New Zealand (FSANZ 2014), therefore this review will focus on the effects of irradiation treatment on AA and carotenes.

Substantial data documents the natural variation in levels of vitamin C in citrus fruit (Lee and Kader 2000; Magwaza et al. 2017). Extensive natural variation occurs in the vitamin C content in of citrus where the main sources of variation are cultivar, season, growing location and orchard management, indeed there is more than four-fold being common between citrus types (Magwaza et al. 2017). In addition, postharvest treatments and storage result in decreased levels of vitamin C during storage (Lee and Kader 2000; Mditshwa et al. 2017).

Vitamin C is widely regarded as a most important water-soluble antioxidant. It includes all compounds exhibiting the biological activity of L-ascorbic acid (AA) plus L-dehydroascorbic acid (DHAA), its oxidation product (Lee and Kader, 2000). However vitamin C is inherently unstable in solution, with its destruction affected by temperature, light and pH (Eitenmiller et al. 2008). As such, vitamin C is one of the most sensitive vitamins to irradiation, with the effects of irradiation influenced by exposure to oxygen, storage and temperature, as well as the pH of the food matrix or storage medium (Kilcast 1994). Irradiation results in some AA being converted to DHAA (Kilcast 1994), however both forms have vitamin C activity (Tsujimura et al. 2008). Therefore, when interpreting findings of irradiation studies it is important to consider that losses due to irradiation may be overestimated if only AA is reported. Hence, total vitamin C (AA plus DHAA) content is a more reliable indicator of post-irradiation vitamin C.

Citrus fruit

There have been a number of studies on the effects of irradiation on nutrient composition of orange, mandarin, lemon, lime and grapefruit. The findings of these studies are summarised in Table 5 (FSANZ 2014) with additional data from the literature.

Orange In Kau oranges, there was no significant effect of X-ray irradiation at 750 Gy on AA levels. Similarly, total carotenoids did not change with irradiation after two days, but were increased 33% after nine days storage in irradiated oranges (Boylston et al. 2002). In blood oranges, irradiation with 250 and 500 Gy slowed the loss of AA during six weeks storage, resulting in higher AA levels in oranges irradiated with 500 Gy (Khalil et al. 2009).

In Mosambi oranges, AA decreased initially at doses of 1,000 Gy (-22%) and 1,500 Gy (-16%). However, this effect was lost throughout the storage period as all groups exhibited AA losses (0 Gy; -31%. 250 Gy; -26%, 500 Gy; -33%, 1,000 Gy; -4%, 1,500 Gy; -16%) (Ladaniya et al. 2003). As only AA was measured, some of the variability in these data may be through transformation to DHAA. Furthermore, the statistical analyses were limited to ANOVA; results of post-hoc testing were not presented thereby limiting interpretation as dose-effects cannot be separated.

De Bortoli et al (2015) report that low dose irradiation (10, 20, 30, 40, 50, 100, 150 and 200 Gy) had no effect on AA levels in Valencia oranges, however no data or methodology were reported and little impact should be taken from this report.

Noh et al. (2016) and Cho et al. (2015) showed that in general treatment with 200, 400, 600, 800 and 1,000 Gy resulted in statistically lower vitamin C levels in Navel oranges imported into Korea during storage at 3°C and at room temperature (20°C) respectively, but these effects were variable at different storage times.

Mandarin Irradiation with 75 and 300 Gy had no significant effect on total vitamin C content in Ellendale mandarins, within a week of irradiation or after three weeks storage (Mitchell et al. 1992). Vitamin C levels decreased 10-12% in Ellendale mandarins, irrespective of irradiation. In contrast, Imperial mandarins showed no early effects of irradiation, but after three weeks storage vitamin C levels decreased 46% in non-irradiated fruit and 69% and 78% in fruit irradiated at 75 and 300 Gy respectively. At this time, total vitamin C levels were significantly lower in irradiated compared to control mandarins.

Another study measured AA levels in Nagpur mandarins irradiated with 250, 500, 1,000 and 1,500 Gy. Irradiation doses of ≥ 500 Gy decreased AA content by approximately 15% (Ladaniya et al. 2003). However, diminution of AA was not dose-dependent, and AA levels fluctuated throughout the storage period. This variability in AA suggests conversion between AA and DHAA may be occurring. As DHAA was not measured, it is not possible to determine the extent of vitamin C loss in this study.

Clementine mandarin A study in Clementine mandarins detected no significant change in total vitamin C levels after X-ray irradiation with 510 and 875 Gy (Rojas-Argudo et al. 2012). Similarly, work from the same group using up to 164 Gy in combination with up to 12 days storage showed no significant change in total vitamin C, except for an early increase in total vitamin C in Clementine mandarins irradiated with 54 Gy (Contreras-Oliva et al. 2011). A third study in Clementine mandarins assessed the impact of irradiation in combination with washing / waxing and storage (Mahrouz et al. 2002). In this study, AA levels fluctuated throughout the seven week experimental period, but decreased in all groups during storage. After seven weeks, there was no significant effect of irradiation on AA content (Mahrouz et al. 2002).

Other citrus

Grapefruit Two studies from the same group indicate no consistent effect of low dose irradiation on AA and β-carotene levels in grapefruit. In the first study, there was no effect of irradiation with 70 – 700 Gy on β-carotene or total carotenoid levels in Rio Red grapefruit in early harvest fruit after 35 days storage at 10C (Patil et al. 2004). However, irradiation doses of 200 Gy or higher, significantly reduced vitamin C content after 4 weeks of storage at 10 °C in late-season Rio Red grapefruit (Patil et al., 2004).

β-carotene levels increased with storage in early harvest fruit irrespective of irradiation dose, but not in late harvest fruit. A similar study used 300 Gy doses and again showed no significant change in either AA or β-carotene levels after 4 and 6 days storage (Vanamala et al. 2005). A third study from the same group indicated significant losses of total vitamin C in two cultivars with very high doses of electron-beam irradiation (Girennavar et al. 2008). In this study, fruit were exposed to 1,000, 2,500, 5,000 and 10,000 Gy. These treatments are extreme but showed no significant change in Rio Red grapefruit with 1,000 Gy, but a statistically significant loss in Marsh White grapefruit at the same dose. However, at higher doses, large losses of vitamin C

occurred in a dose-dependent manner, with losses of >50% at 10,000 Gy. In contrast, β-carotene levels were unaltered by irradiation in Rio Red grapefruit at any dose. But these iradition doses are ten times the levels used for quarantine treatment and are only presented as a guide for extreme high level treatment.

Lemon Irradiation with 75 and 300 Gy had no significant effect on total vitamin C content in lemons up to three weeks after irradiation. Storage had little effect on vitamin C content in irradiated lemons (+1% and -5% change), while vitamin C content decreased 9% in non-irradiated lemons (Mitchell et al. 1992).

Lime In limes, AA levels fluctuated during storage, but were decreased initially by doses of ≥500 Gy. AA levels remained lower in limes irradiated with ≥1,000 Gy during 90 days storage (Ladaniya et al. 2003).

Other non-vitamin bioactive compounds

Whilst citrus is a good source of traditional nutrients such as certain vitamins, minerals and fibre, nutritionists have more recently focused on a range of substances called 'non-vitamin bioactive compounds' or phytochemicals. An orange has over 170 different phytochemicals and more than 60 flavonoids which have been shown to have anti-inflammatory and anti-tumour activity as well as inhibiting blood clots and having strong antioxidant activity (Baghurst, 2000). This section reviews the use of low dose irradiation on these compounds.

Irradiation with 30, 54 and 164 Gy did not decrease total antioxidant capacity and total phenolic content in Clementine mandarins, and flavanone glycoside levels were similar or increased in irradiated fruit after 0 and 6 months storage (Contreras-Oliva et al. 2011). Irradiation of Clementines at a mean dose of 300 Gy followed by storage for 49 days at 3^{o}C resulted in enhanced synthesis of phenolic compounds, primarily hesperidin as the major flavanone glycoside, and nobiletin and heptamethoxyflavone as the major polymethoxylated flavones. Initially, the content of these flavonoids in peel was significantly lower than in controls but biosynthesis increased between days 14 and 21. The irradiation-enhanced content of these flavonoids and of para-coumaric acid, a biosynthetic precursor to the coumarins scopoletin and scopolin, may relate to enhanced resistance to mould decay, while the low irradiation dose and cold storage helped to minimize losses due to pitting of the peel (Oufedjikh et al. 2000). After 12 months storage, small decreases in flavanone glycoside levels occurred in fruit irradiated with 164 Gy (-7% to -12%). However, irradiation of clementine mandarins with 51 and 875 Gy did not alter flavanone glycoside levels after two months storage (Rojas-Argudo et al. 2012).

In grapefruit, effects of irradiation on flavanones were variable; higher doses (400 and 700 Gy) led initially to small reductions in naringin and narirutin in early season fruit, but these changes were lost after 35 days storage, and did not occur in late season fruit (Patil et al. 2004). However, total flavanone levels were increased by irradiation with 70 and 200 Gy after 35 days storage in early season fruit. Lycopene levels were similar between control and ≤1,000 Gy irradiated grapefruit, with the exception of a small decrease (~10%) after 35 days storage in late harvest fruit irradiated with 700 Gy (Patil et al. 2004; Vanamala et al. 2005; Girennavar et al. 2008). Lycopene levels were >25% lower in late compared to early harvest fruit, irrespective of irradiation. Limonin levels in grapefruit were also unaffected by irradiation with <1,000 Gy (Patil et al. 2004).

Table 5. Effects of irradiation on radiation-sensitive nutrients in citrus fruit.
Data primarily complied by FSANZ (2014) with addition of recent literature (2014- 2018).

Fruit	Dose (Gy)	Carotene	Vitamin C	Other components	Analysis method / Reference
Grapefruit	70, 200, 300, 400, 700	No change	No change	Flavonones: variable Lycopene: similar Limonin: no change	AA by HPLC Patil et al (2004) Vanamala 2005
Grapefruit (≥1,000 Gy)	1000, 2,500, 5,000, 10,000	No change	Dose-dependent decrease	Flavonoids and lycopene: No change with 1,000 Gy, variable effects with >1,000 Gy	Total vitamin C by HPLC Girennavar et al (2008)
Lemon	75, 300	n.d.	No change	n.d.	Total vitamin C by derivatization Mitchell et al (1992)
Lime Kagzi	0, 250, 500, 1,000, 1,500	n.d.	Variable. Decreased with 1,500 Gy#	n.d.	AA by titration Ladaniya et al (2003)#
Mandarin Clementine	30, 54, 164, 510, 875	n.d.	No change	Antioxidant capacity, phenolics: no change Flavanone glycosides: no change ≤6 months storage	Total vitamin C by HPLC Rojas-Argudo et al (2012) Contreras-Oliva et al (2011)
Mandarin Ellendale	75, 300	n.d.	No change	n.d.	Total vitamin C by derivatization Mitchell et al (1992)
Mandarin Imperial	75, 300	n.d.	-43%* and -60%* after 3 wk	n.d.	
Mandarin Nagpur	0, 250, 500, 1,000, 1,500	n.d.	Dose-dependent decreases for ≥ 500 Gy#	n.d.	AA by titration Ladaniya et al (2003)#
Orange Lane Late	200, 400 and 600	n.d.	No change	Phenolic content: no change Antioxidant capacity, phenolics: no change	AA by titration McDonald et al (2013)
Orange Kau	750	+33%* after 9 d	No change	n.d.	AA by titration Boylston et al (2002)
Orange Navel	200, 400, 600, 800 and 1,000	n.d.	Dependent on storage time	n.d.	Cho et al. (2015) Noh et al. (2016)
Orange Mosambi	0, 250, 500, 1,000, 1,500	n.d.	Immediate decrease with ≥1,000 Gy, but no difference after storage#	n.d.	Ladaniya et al (2003)#
Orange	10, 20, 30, 40,	n.d.	No change	n.d.	AA methods not

Fruit	Dose (Gy)	Carotene	Vitamin C	Other components	Analysis method / Reference
Valencia	50, 100, 150 and 200				reported De Bortoli et al (2015)
Orange blood	0, 250, 500	n.d.	AA higher in irradiated fruit after 1-6 weeks storage	n.d.	AA by titration Khalil et al (2009)

**Significant difference. n.d.; not determined.*

[#]AA determined, therefore some losses may be due to conversion to DHAA, and statistical analyses limit individual comparisons in this study.

In summary, Food Standards Australia New Zealand (FSANZ 2014) reviewed the published literature demonstrated that phytosanitary doses of irradiation on fresh fruit and vegetables and concluded that low dose irradiation:

- had no effect on carotene levels in fruits and vegetables
- did not decrease vitamin C levels in the majority of fruits and vegetables
- had little effect on other non-vitamin bioactive compounds.

In some cultivars of some fruits vitamin C levels decreased following irradiation. This was also seen in the citrus irradiation treatment literature (Table 5). However, in the majority of these cases the vitamin C content of irradiated fruit remained within the range of natural variation. In addition, when the effects of these changes were compared to dietary consumption patterns it was evident that these changes were unlikely to impact on dietary vitamin C intakes in Australia and New Zealand. FSANZ (2014) concluded that phytosanitary doses of irradiation do not pose a nutritional risk to the Australian and New Zealand populations and recommended that the data requirements for applications to irradiate fruits and vegetables can be streamlined to focus on data for vitamin C, with requirements for other nutrients to be determined on a case-by-case basis. In the case of citrus, the results above show that different citrus fruits and cultivars respond differently to irradiation treatments. This calls for the development of cultivar-specific irradiation treatment protocols. Vitamin C is water-soluble and it is sensitive to irradiative degradation, particularly at higher treatment doses (Kilcast 1994). To reduce vitamin C loss, irradiation treatments could be conducted at low temperatures (Mditshwa et al 2017).

Consumer acceptability

The consumer acceptability of irradiated fruit is the ultimate determinant of commercial acceptability. There have been limited studies on the trained panel and consumer acceptability of citrus following irradiation. Mc Donald et al. (2013) examined the dose tolerance of Lane Late navel oranges to identify the sensory attributes that maybe affected by the treatment, and determine which changes, if any, influence consumer liking. They showed shows that trained panelists were able to detect increased pitting and visual damage in fruit treated by irradiation at 400 and 600 Gy and was corroborated by the consumer panels, which showed lower liking scores in overall appearance for the irradiated oranges compared to the control fruit. The effect was exacerbated by storage for 3 and 4 weeks (McDonald et al., 2013). Similarly, trained panelists in a study done by O'Mahony and Goldstein (1987) found increased brown blemishing and pitting of whole navel oranges irradiated at 300 and 600 Gy. However McDonald et al. (2013) found that overall liking was not affected by irradiation with similar overall liking scores for the control and treated fruit. Although consumers stated that they liked the

appearance of untreated fruit significantly more than irradiated fruit, their overall liking of irradiated fruit was not different than control, although this could be a result of removal of the most heavily pitted fruit from consumer evaluation (McDonald et al., 2013). O'Mahony et al. (1985) also observed that untrained consumers were not able to tell the difference between untreated and irradiated navel oranges (600 – 800 Gy) even though expert judges were able to detect differences in brown blemishing and flavour of irradiated fruit after 5 – 6 weeks in storage. Betancurt et al. (2009) further showed irradiation (350 and 800 Gy) did not affect overall acceptability, appearance or juiciness of Late Valencia oranges after 20 and 40 days of storage as determined by 30 untrained panelists. While Hallman and Martinez (2001) found no differences detected by informal taste panels in Rio Red grapefruit, Marrs oranges, and Dancy tangerines treated up to 500 Gy and stored for 21 days at ambient temperature.

Noh et al. (2006a) evaluated the effects of 200, 400, 600, 800 and 1,000 Gy on Navel oranges and showed that after treatment with continuous storage at 20 °C for 12 days there was no differences in sensory acceptability of treated fruit. But after storage at 3 °C for up to 45 days, consumer perception of sweetness and overall acceptability of irradiated samples greater than 600Gy, were negatively affected by irradiation treatment (Noh et al. 2016b). McDonald et al. (2013) also showed there was a significant interaction between age and irradiation treatment suggesting that irradiation stresses the fruit and makes it more sensitive to handling. The Lane Late navel oranges used by McDonald et al. (2013) were commercially packed for the Pacific Rim (high export pack in which fruit are packed so as to place as much fruit as possible in the box). The fruit packs for visual damage evaluation were kept intact for the entire storage period. For other evaluations, fruit was removed from the original packs; this latter sample did not show as much visual damage as the fruit that was tightly packed. In addition, the fruit used in this study were late season navels, which had suffered stress due to a wet winter. This may also have contributed to the greater impact of irradiation. This observation suggests that packing compression in conjunction with the irradiation may enhance bruising of navel oranges. O'Mahony and Goldstein (1987) also observed greater pitting in navel oranges of damp oranges. The effect of handling, packing density as well as time of harvest should be evaluated in future studies.

While fruit softening has been shown to be not affected to irradiation in Late Valencia fruit (Betancurt et al., 2009), it has also been measured to increase (Miller et al., 2000; O'Mahony et al., 1985; McDonald et al., 2013) but this increased softness has not been able to be detected by trained panelists (O'Mahony et al., 1985; McDonald et al., 2013). Although O'Mahony et al. (1985) rated the resistance to bite of the treated oranges to be slightly higher compared to the control. Similarly McDonald et al. (2013) showed that trained sensory panelists were unable to determine differences in internal dryness or granulation among the control and irradiation treatments, even though analytical testing had indicated that there was a positive association of irradiation with segment drying at 400 and 600 Gy and with granulation at 600 Gy. This difference in results could have been due to the fact that the trained panelists were evaluating fruit sections rather than whole slices or that the drying and granulation, although present in a number of the fruit, was fairly minor on a whole fruit basis. The low prevalence of the drying and granulation was also indicated by results from the consumer panels, which found there to be no differences among treatments for either texture or juiciness.

McDonald et al. (2013) also found that the 400 and 600 Gy doses of irradiation enhanced the concentrations of aroma volatiles present in the Lanes Late but again this did not appear to result in a loss in flavor quality given that both the trained and consumer sensory panels which found there to be no significant differences in the flavor or overall liking between the control and irradiated fruit. The lack of sensory impact of the increases in volatile concentrations was also noted in sensory panel evaluations of aroma of both whole and cut fruit, which found no differences due to treatment (McDonald et al, 2013). Although there was no sensory impact of the increases in aroma volatiles, these changes indicate that irradiation is inducing metabolic alterations in the fruit that have the potential to alter flavor at higher dose levels (McDonald et al., 2013). Similarly McDonald et al. (2013) showed there was decreased TSS at doses 400 and 600 Gy, these small effects had little impact on

flavour. Indeed with other citrus types such as pummelos, while Jain et al. (2017) showed no consistent effects of 150 and 1,000 Gy irradiation on TSS and TA values, these differences were low and did not affect consumer acceptability of the treatments (Jain et al., 2017).

In summary, McDonald et al. (2013) concluded that while there maybe some measurable effects of irradiation on fruit quality (such as aroma volatiles, internal drying/granulation, TA, and TSS) these have not been shown to influence perceived flavour as indicated by the trained sensory panel or consumers.

Observations and opportunities

From a postharvest perspective, the development of physical damage to the peel following treatment is a consequence of localized uncontrolled senescence and necrosis. However there have been very few studies to investigate different pre- and postharvest treatments that could minimise or prevent this damage. All previous irradiation research has been simple empirical research, where the experiment has generally examined different irradiation doses on a single batch of fruit. This type of research is beneficial to compare different irradiation doses, but does not give any information on how irradiation effects quality and how the observed differences can be better managed.

Dr. Marisa Wall from ARS / USDA concluded from her extensive experience and review of irradiation effects on fruit quality that cultivar effects may be related to slight variations in maturity at harvest, and few carefully designed studies have differentiated these factors (Wall, 2015). Hence the differences observed between the different cultivars maybe due to different maturities when treated. While this somewhat problematic for citrus, Jessup et al. (1992) showed green Lisbon lemons were more severely affected by low dose irradiation than yellow lemons. Therefore objective assessments of physiological maturity are needed to evaluate multiple cultivars for sensitivity to irradiation during quarantine treatment. Furthermore there could be other pre- and postharvest factors that could affect the fruit response to irradiation. In other areas of postharvest storage and treatment of citrus, the use of hot fungicides, treatment temperatures and the use of curing have been shown to reduce physical damage to citrus peel following postharvest stresses (such as chilling). These factors have not been assessed in their effects on irradiation damage and are worth exploring to minimise any potential damage.

Conclusions

Low dose irradiation is an important market access tool which can be used for the export of Australian citrus to some markets. This review showed that in general there are few consistent negative effects of low dose irradiation treatment and subsequent cold storage on citrus quality. While some minor quality issues with internal quality were identified, these are generally not consistent and even if these differences are statistically significant they are often not commercially significant. However there are some commercial issues identified with the development of disorders or damage (such as pitting, browning of the calyx and loss of firmness) due to treatment in some types and cultivars of citrus. This damage can be commercially unacceptable and may limit the use of irradiation for some citrus types and cultivars. However this damage is cultivar and even harvest-time specific and more work is required to identify these hybrids / cultivars in local conditions. Better understanding the causes of potential damage offers the potential of relatively simple harvest and postharvest treatments to better manage any potential treatment effects.

Recommendations

Fruit quality issues such as the development of chilling injury during the current cold treatment protocol for chilling sensitive citrus types (particularly lemons) can potentially be overcome with the use of low dose irradiation. However the commercial acceptance of low dose irradiation as a market access treatment will rely

on its routine efficacy to maintain fruit quality through the supply chain. While this review identified some fruit damage in response to low dose irradiation, more work is required to identify and quantify the conditions and citrus types where this damage does not occur.

It is recommended to focus research on citrus types where the current cold treatment can cause chilling injury and limits out-turn on export markets. Lemons and to a lesser extent mandarins should be a focus which could benefit from future research and development investments to improve quality out-turn. Many previous studies have only evaluated a single type and cultivar of citrus harvested from a one geographic area location at one time period. This work should be expanded to include multiple varieties harvested at different times (even years), and from varied locations. This will give growers, exporters, importers and the market confidence in the commercial use of this treatment. Following on from the work of McDonald et al. (2013) who showed some significant skin damage following irradiation in five orange cultivars and five mandarin cultivars, further research should also consider pack configuration on sensitivity of fruit to damage. In addition, it is recommended to explore the pre-treatment effects such as harvest time, postharvest storage and treatment (e.g. hot fungicides and curing), on the fruit response to irradiation. It may be possible to minimise the potential negative effects of irradiation with simple curing or management issues before treatment.

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Appendix 4. Statistical analysis of dithiocarbamate residues in lemon fruit

Part 3 – Postharvest treatments to reduce MRLs

Statistical analysis conducted by Lorraine Spohr. Biometrician, NSW Department of Primary Industries

Results

There was a significant effect of washing treatment on dithiocarbamate residue ($F_{7,35}$=41.02; p<0.001).

Table 1: Effect of washing treatment on dithiocarbamate residue concentration in lemon fruit

Treatment	Concentration mg/kg	
cold chlorine	0.643	a
hot PAA	0.712	a
hot water only	0.775	a
cold water only	0.810	a
cold PAA	0.817	a
hot PAA – stored	1.027	a
no treatment	2.933	b
no treatment – stored	5.433	c

Summary tables of Concentration_mg_kg by Trt_Name

	Nobservd	Mean	s.e.mean
Trt_Name			
cold chlorine	6	0.643	0.0750
cold PAA	6	0.817	0.1256
cold water only	6	0.810	0.1281
hot PAA	6	0.712	0.1311
hot PAA – stored	6	1.027	0.2499
hot water only	6	0.775	0.1527
no treatment	6	2.933	0.4536
no treatment – stored	6	5.433	0.4514

Analysis of variance

Variate: Concentration_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	1.5857	0.3171	0.74	

Rep.*Units* stratum					
Trt_Name	7	122.4498	17.4928	41.02	<.001
Residual	35	14.9240	0.4264		
Total47		138.9595			

Message: the following units have large residuals.

Rep Rep 2 *units* 2	1.663	s.e.	0.558
Rep Rep 4 *units* 7	1.312	s.e.	0.558
Rep Rep 4 *units* 8	-1.495	s.e.	0.558

Tables of means

Variate: Concentration_mg_kg

Grand mean 1.644

Trt_Name	cold chlorine	cold PAA	cold water only
	0.643	0.817	0.810
Trt_Name	hot PAA	hot PAA – stored	hot water only
	0.712	1.027	0.775
Trt_Name	no treatment	no treatment – stored	
	2.933	5.433	

Standard errors of differences of means

Table	Trt_Name
rep.	6
d.f.	35
s.e.d.	0.3770

Least significant differences of means (5% level)

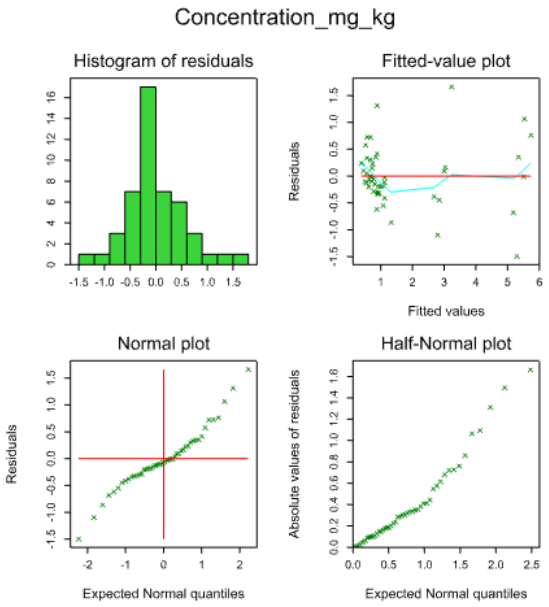
Table	Trt_Name
rep.	6
d.f.	35
l.s.d.	0.7654

```
129  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
130  SCALAR _scode; VALUE=0
131  AKEEP [FACTORIAL=9] Trt_Name; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid; STATUS=_scode
132  IF _scode > 0
133    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
134    AMCOMPARISON [PRINT=letter; METHOD=fplsd; DIRECTION=ascending; PROB=0.05; FACTORIAL=9;\
```

135 STUDENTIZE=no] Trt_Name

Fisher's protected least significant difference test

Trt_Name	Mean	
cold chlorine	0.643	a
hot PAA	0.712	a
hot water only	0.775	a
cold water only	0.810	a
cold PAA	0.817	a
hot PAA – stored	1.027	a
no treatment	2.933	b
no treatment – stored	5.433	c



Additional analysis

Using orthogonal contrasts to test Hot v Cold and Water v PAA.

Result: No significant effect was detected for each contrast.

Contrast 1: Hot v Cold (Trt 2 & 4 v Trt 3 & 5)

Contrast 2: Water v PAA (Trt 2 & 3 v Trt 4 & 5)

Analysis of variance

Variate: Concentration_mg_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	1.5857	0.3171	0.74	
Rep.*Units* stratum					
Tmt	7	122.4498	17.4928	41.02	<.001
Contrast 1	1	0.0294	0.0294	0.07	0.794
Contrast 2	1	0.0048	0.0048	0.01	0.916
Residual	35	14.9240	0.4264		
Total	47	138.9595			

Message: the following units have large residuals.

Rep Rep 2 *units* 2	1.663	s.e.	0.558
Rep Rep 4 *units* 7	1.312	s.e.	0.558
Rep Rep 4 *units* 8	-1.495	s.e.	0.558

Tables of means

Variate: Concentration_mg_kg

Grand mean 1.644

Tmt	1	2	3	4	5	6	7
	2.933	0.810	0.775	0.817	0.712	0.643	5.433

Tmt	8
	1.027

Standard errors of differences of means

Table	Tmt
rep.	6
d.f.	35
s.e.d.	0.3770

Least significant differences of means (5% level)

Table	Tmt
rep.	6
d.f.	35
l.s.d.	0.7654

Appendix 5. Industry survey form citrus postharvest needs

This questionnaire was given as a survey of participants at the postharvest workshop at the Citrus Technical Forum in Adelaide on 7 March 2019 - Postharvest Workshop 2 (pm session)

This survey is to obtain feedback on the postharvest needs of the Citrus industry

1. Do you use any of these citrus postharvest resources? And how useful are they?

		How useful are these sources of information?			
Do you use?		*Not very*	*OK*	*Good*	*Excellent*
☐	Australian Citrus News	☐	☐	☐	☐
☐	Packer Newsletter	☐	☐	☐	☐
☐	NSW DPI Citrus Connect	☐	☐	☐	☐
☐	Chemical re-sellers	☐	☐	☐	☐
☐	Consultants	☐	☐	☐	☐
☐	Other resources – please list: _____				

2. Where would you like to get your future postharvest information?
(Rank your Most Liked preference = 1, and Lowest preference = 5)

☐ web-based (ACN, Packer Newsletter etc) ☐ direct emails

☐ specialist workshops ☐ general CAL regional / technical forums

☐ other ways of getting information – list here:

3. What postharvest issues need more R&D? (eg fungicides, storage etc)

4. What areas of postharvest training and education would you like to see in the future?
(eg packinghouse resources, training courses etc)

5. Are you aware of the Hort Innovation '*Australian Citrus Postharvest Science Program*'

(CT15010)? Yes ☐ No ☐

How could this Project be improved?

Thanks. Your feedback is important to improve the delivery of postharvest R&D and training for industry.
If you would like to give more feedback, please contact John Golding
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