Final Report

Citrus postharvest program

Project leader:

John Golding

Delivery partner:

NSW Department of Primary Industries

Project code:

CT19003

Project:

Citrus postharvest program (CT19003)

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the citrus research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Contents

Public summary

The Australian citrus industry is increasing production and to ensure grower profitability there is a need to grow current markets and develop new ones. A key part of the market growth will require the supply of high-quality fruit. Australia receives a premium for its citrus fruit due to its excellent quality. It is critical to maintain and build upon this high-quality status with practical and cost-effective technologies to improve fruit quality. However, maintaining fruit quality is challenging with long storage times (due to disrupted shipping) and phytosanitary end-point treatments required for many export markets. In addition, consumers (and markets) are increasing their expectations of lower chemical residues in fruit. These challenges were addressed with this industry focused and science-based postharvest research, development, and extension program. An active program of industry engagement was undertaken to keep the Australian citrus industry informed of postharvest best practices and allow rapid adoption. The project had an active Project Reference Group (PRG), which provided direction and guidance to ensure industry relevance.

To assess alternative postharvest decay (green and blue mould) control, trials examining alternatives to current postharvest fungicides were conducted. A new formulation of *ortho*-phenylphenol (OPP; Ortocil®) showed promise and was subsequently registered with the APMVA. The addition of new postharvest treatments and tools are a great benefit to industry and gives options for growers to control postharvest decay. Trials to minimise anthracnose development in Imperial mandarins also showed the benefit of good postharvest practices.

To improve market access, a long-term trial examining chilling injury to different Navel cultivars and the interactions between orchard management and postharvest storage were undertaken and showed the complexity of predicting and managing chilling injury. Techniques used in the orchard, such as applying plant growth regulators and restricting irrigation, were also assessed. It was found that there were no negative effects on final fruit quality (including the development of chilling injury) from these orchard treatments. In addition, alternatives to postharvest waxes and their effects on the storage and shelf life of lemons were assessed. These trials showed some plant-based coatings have potential to deliver favourable fruit quality outcomes and meet different market requirements.

The continued success of the Australian citrus industry relies on the delivery and adoption of relevant and timely information that is under-pinned by science-based research results. The program delivered up-to-date postharvest resources (e.g. regular articles in Australian Citrus News) and program updates at grower forums and workshops (e.g. Citrus Australia Regional Forums and Citrus Congress). These are essential to ensure the industry is receiving the latest information, is using best practices, and meeting retailers' and consumers' expectations.

Technical summary

The consistent supply of high-quality citrus fruit to domestic and export markets requires a whole-of-chain approach supported by innovative postharvest technologies and information to meet consumer and regulatory requirements. While the industry currently has solid production and exports, there are several significant challenges with consistently and efficiently delivering high-quality fruit to domestic and export markets.

The main postharvest problem for citrus on both the domestic and export markets is decay caused by blue and green mould. This decay is currently controlled with postharvest fungicides. While 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 up to 10 weeks. 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. This program assessed the efficacy and practicalities of alternative postharvest decay control using commercial treatments (Ortocil®, Cerafruta®, DeccoPlus® and Fruit Mag®), essential oils, photo-oxidation, organic salts (sodium bicarbonate, sodium benzoate, and potassium sorbate), and elevated treatment temperatures. In addition, a series of postharvest trials were also conducted to improve how anthracnose is managed in Imperial mandarins, with some current postharvest fungicides showing potential.

The Australian citrus industry needs improved market access outcomes. To achieve this, postharvest trials were conducted to reduce chemical residues from packing lines. 2,4-D is sometimes used to maintain the calyx of fresh citrus, but alternatives are required. Postharvest WetCit® application to remove Fuller's rose weevil eggs and red scale was evaluated. The use of 3,5,6-trichloro-2-pyridiloxyacetic acid, hydrogen sulphide fumigation, and a comparison of 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide treatments were also undertaken, with results showing some promise for non-2,4-D treatments to maintain fruit calyx (button) condition during storage.

Chilling injury continues to be a major storage problem when exporting Australian citrus. Chilling injury is a physiological storage disorder that can sometimes occur on the fruit at phytosanitary treatment temperatures (i.e. <3 C). To understand some of the underlying factors affecting chilling injury, a survey of different Navel cultivars grown on the same location and rootstock was conducted over five seasons. This survey showed large varietal, seasonal and yearly differences in the expression of chilling injury and highlighted the complexity of predicting and managing chilling injury.

In response to disrupted and extended shipping times due to COVID-19, a series of trials were undertaken to improve fruit quality outcomes following extended storage. Many growers use plant growth regulators (PGR) such as gibberellic acid (GA). They are also using restricted irrigation to reduce water use and improve eating quality, but little is known about its effects on fruit quality following extended storage. Trials conducted on Afourer mandarins showed some effects on fruit quality but no significant effects on chilling injury. Postharvest treatments, such as 1-methyl cyclopropene and gibberellic acid, were assessed for their effects on long-term storage but showed little overall benefit. The assessment of alternatives to postharvest waxes on the storage and shelf life of lemons showed some benefits, but more work should be conducted to match postharvest treatments to market and consumer needs.

These results were delivered in a series of 10 refereed scientific papers in international journals. These results were also extended to the industry in a series of 22 postharvest articles published in industry journals and communications. This was supported by 20 postharvest presentations in all growing regions of Australia at various Citrus Australia Regional Forums, general postharvest presentations and at the national Citrus Congress and Technical Forums.

Keywords

Citrus, quality, storage, decay, export, eating quality

Introduction

The Australian citrus industry is a valuable contributor to regional Australia. In 2022–23, 815,750 tonnes, valued at $977.1 million, were produced, with most (72%) sold as fresh produce (Australian Horticulture Statistics Handbook 2022–23). Underpinning the success of the Australian citrus industry, particularly in export markets, is the consistent delivery of high-quality fruit. Australia demands a premium for its citrus fruit due to its excellent eating quality attributes.

While the Australian citrus industry is currently facing challenges such as increasing production volumes, shipping disruptions, supply chain issues and soft export markets, the need to maintain fruit quality through the supply chain from the orchard to the consumer is critical for grower returns and profitability. Postharvest is the key link between the orchard and the consumer and can either enhance or detract from the investment. However, maintaining fruit quality is particularly challenging with the long storage times (due to disrupted shipping) and the application of phytosanitary end-point treatments required for many export markets. In addition, growing consumer (and market) expectations of lower chemical residues put further demands on consistently delivering high-quality fruit to consumers. These challenges were addressed with this science-based targeted postharvest research, development, and extension program. The overall objectives of this program were to:

- Keep the Australian citrus industry informed on postharvest best practices with clear pathways for adoption identified.
- Ensure that the Australian citrus industry has access to new technologies to maintain postharvest quality.
- Develop resources to enable growers to meet export maximum residue levels (MRLs) while ensuring postharvest quality.

Underpinning these key program objectives were a series of research trials conducted to:

1. Assess the efficacy and practicalities of alternative postharvest decay control. To examine alternatives to current postharvest fungicides for controlling postharvest decay (green and blue mould), commercial treatments (Ortocil®, Cerafruta®, DeccoPlus® and Fruit Mag®), essential oils, photo-oxidation, organic salts (sodium bicarbonate, sodium benzoate, and potassium sorbate), and elevated treatment temperatures were assessed. Postharvest trials with non-chemical (physical) and low-pressure treatments were also included and a series of trials on improving the postharvest management of anthracnose in Imperial mandarins.

2. Improve market access outcomes. Trials were conducted to reduce chemical residues from packinglines and evaluate

postharvest WetCit® application to remove Fuller's rose weevil eggs and red scale, as well as the effects on postharvest decay. 2,4-D is sometimes used to maintain the calyx of fresh citrus, but alternatives are required. A series of postharvest trials were performed to evaluate the use of 3,5,6-trichloro-2-pyridiloxyacetic acid, hydrogen sulphide fumigation, a comparison of 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide treatments, and the effects of 'low' dicamba levels on the shelf life of Navel oranges.

3. Reduce chilling injury. Chilling injury continues to be a major storage problem when exporting Australian citrus. Chilling injury is a physiological storage disorder that can sometimes occur on the fruit at phytosanitary treatment temperatures (i.e. <3 °C). To understand some of the underlying factors affecting chilling injury, a survey of different Navel cultivars grown on the same location and rootstock was conducted over five seasons. In addition, several postharvest trials were conducted to assess the chilling injury susceptibility of new mandarin cultivars.

4. Improve fruit quality outcomes. In response to disrupted and extended shipping times due to COVID-19, a series of trials were undertaken to improve fruit quality outcomes after long-term storage. Plant growth regulators such as gibberellic acid are often used by growers, who are also now using restricted irrigation to reduce water use and improve eating quality, but little is known about the effects on fruit quality following extended storage. Trials were conducted on Afourer mandarins to examine the effects of these pre-harvest treatments on chilling injury and eating quality following long-term storage. In addition, postharvest treatments such as 1-methyl cyclopropene (MCP) and gibberellic acid (GA) were assessed for their effects on long-term storage. Alternatives to postharvest waxes were assessed on the storage and shelf life of lemons. The preliminary development of new citrus firmness meters was assessed.

Extension was a key component of the program to ensure the outcomes were met. Regular presentations at industry and postharvest workshops and regular articles in *Australian Citrus News* were highlights of the program.

This Program fulfilled the following Australian Citrus Industry Outcomes (Citrus Strategic Investment Plan (SIP) 2017–2021):

> *Outcome 1. Market opportunities in both domestic and especially export markets.*
>
> *Outcome 2. Reduced agrichemical-related risks.*
>
> *Outcome 3. Improved product quality from the application of innovation.*
>
> *Outcome 4. Increased skills, capacity, and knowledge*.

Methodology

With guidance and direction from the Program Reference Group, several postharvest storage trials were conducted to find alternative decay control technologies, improve market access outcomes, manage chilling injury, and improve the quality of Australian citrus.

1. **Alternative decay control**
   - Ortocil® (*ortho*-phenylphenol, OPP)
   - Cerafruta® (formulation of natamycin)
   - DeccoPlus® (potassium sorbate compatibility with current postharvest fungicides)
   - Fruit Mag® (magnesium oxide)
   - Essential oils (e.g. lemon myrtle (*Backhousia citriodora*) and lemon-scented tea tree (*Leptospermum petersonii*))
   - Optimising organic salts (sodium bicarbonate (SB), sodium benzoate (SBen), and potassium sorbate (PS)).

   Non-chemical (physical) low-pressure treatments were also assessed on their effects on postharvest decay. In addition, a series of postharvest trials were conducted to improve how anthracnose is managed in Imperial mandarins.

2. **Improving market access outcomes**
   - Meeting MRLs – removing dimethoate residues from packing lines.
   - Evaluating postharvest WetCit® application to remove Fuller's rose weevil (FRW) eggs and red scale, and its

effects on postharvest decay.

- Maintaining calyx condition – alternatives to 2,4-D
    - Evaluating 3,5,6-trichloro-2-pyridiloxyacetic acid dipping
    - Evaluating hydrogen sulphide fumigation
    - Comparing 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide treatments
    - Effect of 'low' dicamba levels on the shelf life of Navel oranges.

3. Chilling Injury

- Navel chilling injury survey (NSW DPI Dareton)
- Chilling injury susceptibility of new mandarin cultivars

4. Improving fruit quality outcomes

- Effects of irrigation frequency on Afourer fruit quality following long-term storage
- Effects of orchard PGR applications on the shelf life and quality of Afourer mandarins
- Effects of postharvest GA applications of on shelf life and quality of Navel oranges
- Improving the storage performance and eating quality of Afourer mandarins during extended shipping
- Evaluating alternative coatings
- Evaluating new citrus firmness meters.

The general methods for measuring fruit quality and decay work used in all trials are in Appendix 1.

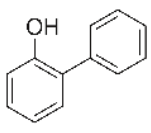
The specific materials and methods for each of the trials are described and summarised in the description of each trial in the Results section of the Technical Report (Appendix 2).

Results and discussion

The Program RD&E was guided by the PRG but had four fruit quality outcomes: (1) alternative postharvest decay control, (2) improving market access outcomes, (3) understanding and managing chilling injury, and (4) improving fruit quality outcomes. The methodology for all experiments is described in Appendix 1 and the full technical report of the results and discussion of each trial is in Appendix 2.

Alternative postharvest decay control

While synthetic fungicides are currently essential for marketing citrus, particularly for long-distance and export markets, there is growing consumer demand for lower chemical residues and alternative decay control measures. This section describes the trials of some new and semi-commercial treatments (Ortocil®, Cerafruta®, DeccoPlus®, Fruit Mag®, essential oils, and optimising organic salts) for postharvest control of green and blue mould and their effects on fruit quality. In addition, a series of trials were conducted to improve how anthracnose is managed in Imperial mandarins. In addition, non-chemical (physical) treatments were also assessed.



Ortocil® *ortho*-phenylphenol (OPP) or 2-phenylphenol is a preservative with E number E231, which is allowed as a postharvest treatment in some countries. It is sold as Ortocil®. A related formulation, sodium *ortho*-phenylphenol (SOPP), is currently registered as Preventol® ON Fungicide for the control of blue mould (APVMA, 2023). However, it is recommended that the SOPP is used in solutions with pH >12, which can cause some phytotoxic issues in some situations. Ortocil® is new and has neutral pH which has broader applications. Several laboratory trials showed good efficacy against *in vivo* green and blue mould in Navel oranges (Figure 1) and on the storage life of Navel oranges stored at either 3 °C or 20 °C for up to 4 weeks. Colin Campbell (Chemicals) Pty Ltd, an Australian local chemical company, has since registered this product for postharvest use against green and blue mould and sour rot (Figure 2) (APVMA, 2024), which is now available for use by the Australian industry. A major benefit of this product is its unique mode of action (FRAC Group M), which is different from other

postharvest fungicides, making it a very useful addition to the postharvest fungicide toolbox and in managing postharvest fungicide resistance.

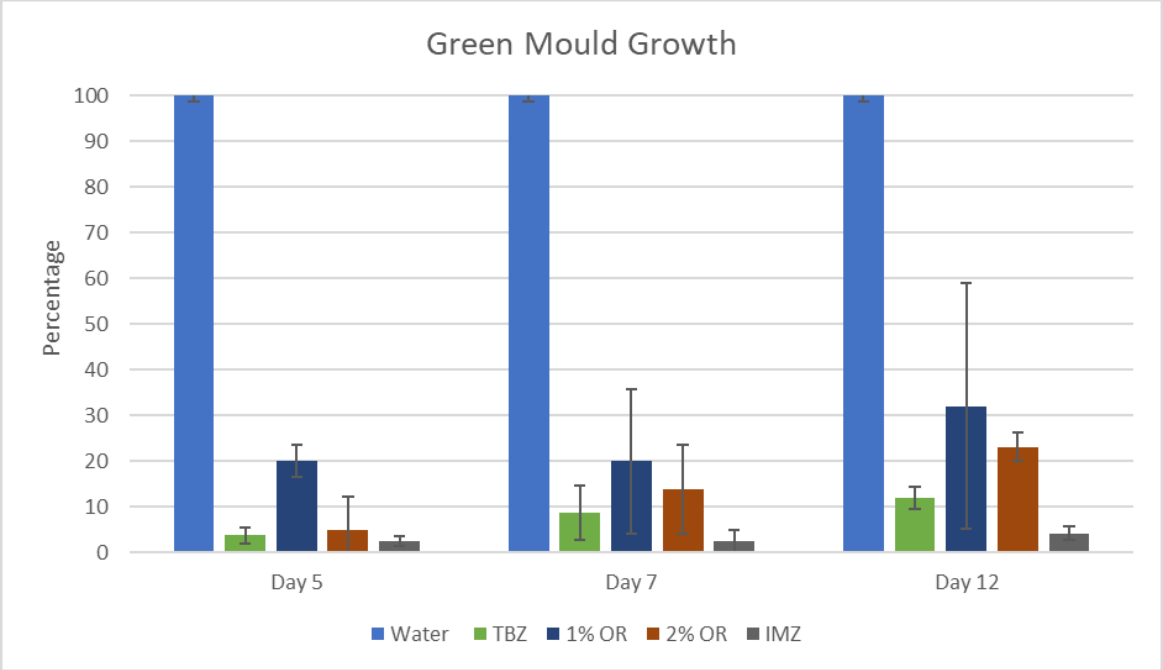
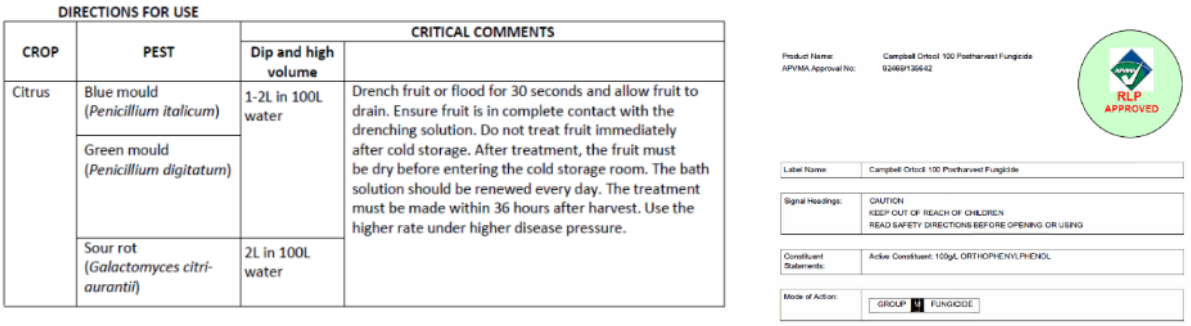


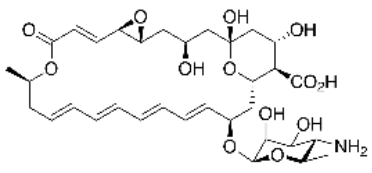
Figure 1. Percentage of green mould following treatment with 1%, 2% Ortocil® (OR), untreated control (water), thiabendazole (TBZ) or imazalil (IMZ) over 12 days at 20 °C. There were 4 replicates assessed per treatment. Bars are standard deviations around the mean.



DIRECTIONS FOR USE

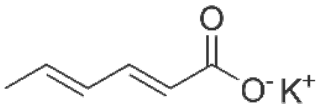
CROP	PEST	CRITICAL COMMENTS	
		Dip and high volume	
Citrus	Blue mould (*Penicillium italicum*)	1-2L in 100L water	Drench fruit or flood for 30 seconds and allow fruit to drain. Ensure fruit is in complete contact with the drenching solution. Do not treat fruit immediately after cold storage. After treatment, the fruit must be dry before entering the cold storage room. The bath solution should be renewed every day. The treatment must be made within 36 hours after harvest. Use the higher rate under higher disease pressure.
	Green mould (*Penicillium digitatum*)		
	Sour rot (*Galactomyces citri-aurantii*)	2L in 100L water	

Figure 2. The label for Campbell Ortocil® 100 Postharvest Fungicide (APVMA Approval Number: 92468/135642).



Cerafruta® Natamycin is a natural antimicrobial peptide produced by the strains of *Streptomyces natalensis*. It acts as an antifungal preservative and is used in a range of food products such as dairy. It is 'generally recognised as a safe' ingredient for various food applications (Meena et al. 2021). Natamycin is a food additive (E235) and is used in the European Union as a surface preservative for certain cheese products. Natamycin is approved in different applications at different levels in over 150 countries. Research on citrus in Egypt and China has shown that natamycin can inhibit green and blue mould, and sour rot (*Geotrichum citri-aurantii*) (Yİğİter et al. 2014; Du et al. 2022). A formulation of natamycin is Cerafruta® (Ceradis Crop Protection), which was trialled in this study against green and blue mould on Navel oranges. The results showed that while the standard postharvest fungicides (thiabendazole (TBZ) and imazalil) worked well (i.e. <10% infection), the different concentrations of Cerafruta® treatments did not affect the levels of green and blue mould. The effects of Cerafruta® (1,000 ppm) on fruit quality were compared to a water dip and a commercial imazalil treatment in Navel oranges stored for 4 weeks at either 5 °C or 20 C. Both the Cerafruta® and imazalil treatments maintained fruit

quality during storage at 20 °C, and the level of natural rots/decay that developed during storage was lower in Cerafruta® and imazalil treated fruit.



DeccoPlus® Potassium sorbate (E-202) is a wide-spectrum antimicrobial food additive with efficacy against mould and yeast, mostly within the pH range of 3.0–6.5. Potassium sorbate is classified by the United States Environmental Protection Agency as a minimal-risk active ingredient and is exempt from residue tolerances. There have been numerous studies on the effectiveness of potassium sorbate on citrus fruit decay. For example, Smilanick et al. (2008) found that potassium sorbate was compatible with some commonly used postharvest fungicides and it also improved their performance against *P. digitatum* and *Geotrichum citri-aurantii*, the causal pathogen of sour rot. A commercial formulation of potassium sorbate is DeccoPlus®, which was evaluated to examine (1) its efficacy against green mould and (2) its compatibility with a range of standard postharvest sanitisers and fungicides. The results showed that 2% DeccoPlus® had better decay control than 1% DeccoPlus®. This result was enhanced when there was a 24-hour delay between infection and treatment compared with a 4-hour delay. This observation requires further investigation.

Additional storage trials examined the compatibility of DeccoPlus® with commercial fungicides and sanitisers. The recommended concentration of DeccoPlus® (1% solution) was applied to green mould-infected Navel oranges with and without the following sanitisers (PAA, hypochlorite) and fungicides (TBZ, imazalil, fludioxonil, propiconazole and fludioxonil, pyrimethanil, imazalil and pyrimethanil, guazatine, and a grand mix containing guazatine + TBZ + PAA, OPP). The results showed the 1% DeccoPlus® treatment reduced the incidence and growth of postharvest decay in Navel oranges (Figure 3) and there were no negative compatibility issues. In all cases, there was no negative effect of DeccoPlus® treatment on the incidence or growth of *Penicillium* decay. While there were no negative effects from mixing DeccoPlus® with other sanitisers (PAA and chlorine), there were also no statistical differences between fruit treated with fungicide alone and fungicide plus DeccoPlus®.

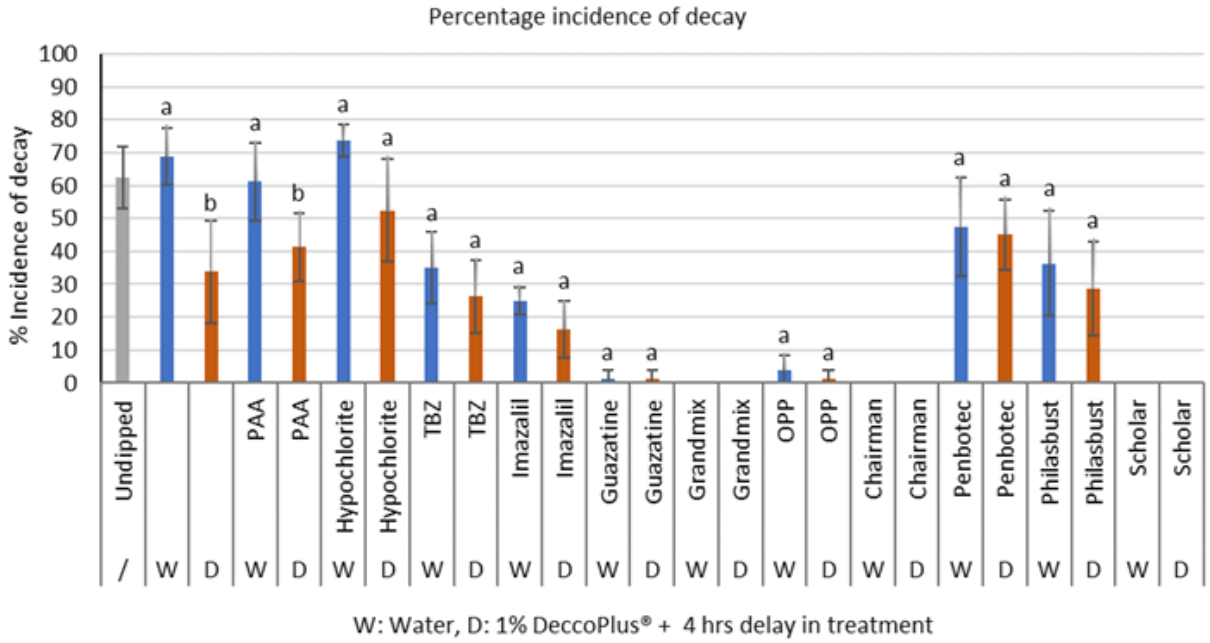


Figure 3. Percentage incidence of decay (%) of fruits treated with DeccoPlus®, commercial fungicides and sanitisers after 1 week at 25 °C with 95% RH. Bars are standard deviations around the means, n=4.
Different letters above the bars on 2 columns of the same fungicides/sanitisers without and with 1% of DeccoPlus® show significant differences (p <0.05, t-test).

Fruit Mag® FruitMag® is a new product with magnesium oxide (MgO) as the active ingredient. MgO is a 'generally regarded as safe' (GRAS) compound by the US FDA (§ 184.1431 Magnesium oxide–CFR). It is also a US FDA-approved food additive with the technical function of a firming agent (§ 184.1431) and has an E number of E530. MgO is used as an anti-caking and firming agent. These trials examined the effect of FruitMag® on decay development in Navel oranges. Two experiments were conducted: (1) Efficacy of FruitMag® on green mould, and (2) different timing of FruitMag® treatment

after infection. The results showed the FruitMag® treatments controlled green mould development. However, its use requires the fruit to dry before processing and the white residue needs to be washed off during processing. More work is required with the commercial application of this product.

Essential oils Plant-based antimicrobial agents such as essential oils offer a safer and more eco-friendly alternative to synthetic fungicides. Their production also makes use of a waste stream from citrus processing. The chemical constituents of essential oils are broadly classified as terpenes and phenylpropanoids, although most of these mainly consist of monoterpenes. The unique chemical structure and exposure to light and oxygen mostly lead to the stability and chemical reactions of essential oils. A series of studies were conducted by Mohammad M Rahman and the citrus postharvest research team at NSW Department of Primary Industries and the University of Newcastle, which contributed to the outcomes of this project. These studies have been published in international peer-reviewed journals and have acknowledged the contributions of this project.

A review was published on the control of postharvest decay in citrus fruits using some of the most common essential oils (EO), the efficacy of EOs in combating fungal infection in both *in vitro* and *in vivo* models, and the mode of action of EOs along with the potency of photochemical by-products were investigated as antifungal agents. For EO use to gain commercial acceptance, it is important to fully understand the efficacy of the bioactive constituents, treatment requirements for different produce and potential effect on the physical and organoleptic parameters of the treated produce. *In vivo* testing typically requires higher treatment concentrations because of differences in the character of food surface properties (e.g. hydrophobicity), which influence the behaviour of both the bioactive and the microbial agent. While many EOs have been demonstrated to possess antifungal activity, the commercial use of these is limited because of issues such as phytotoxicity, intense sensory attributes or technological problems associated with wide-scale production and application. The limitations associated with sensory attributes and phytotoxic effects depend on concentration, application method, treatment duration and the nature of the produce treated. [Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2023) Potential control of postharvest fungal decay of citrus fruits by crude or photochemically changed essential oils–a review. *Food Reviews International*. pp. 1-18]

A comprehensive study examined the efficacy of orange essential oil and citral after exposure to UV-C irradiation to inhibit *Penicillium digitatum* in Navel oranges. The results showed that UV-C irradiated orange essential oil was a potential alternative to synthetic fungicides to inhibit green mould. The source of orange essential oil could be waste flavedo generated by the orange juice processing industry. This research was published in *Horticulturae*. [Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2020) Efficacy of orange essential oil and citral after exposure to UV-C irradiation to inhibit *Penicillium digitatum* in navel oranges. *Horticulturae* 6, 102]

The application of the essential oils of the Australian native plants, lemon myrtle (*Backhousia citriodora*) and lemon-scented tea tree (*Leptospermum petersonii*), were examined as inhibitors of green mould on citrus fruits. The results showed that these essential oils from lemon myrtle and lemon-scented tea tree inhibited the development of green mould in several citrus types. This suggests they could be an alternative to chemical fungicides, especially for fruit marketed as organic. Lemon myrtle essential oil had higher inhibition efficacy, presumably due to having a higher citral content than lemon-scented tea tree essential oil. However, lemon myrtle essential oil was more effective than citral, which suggests that some as yet unknown minor components of lemon myrtle have high antifungal activity. [Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Kirkman, T. and Pristijono, P., 2022. Lemon myrtle and lemon-scented tea tree essential oils as potential inhibitors of green mould on citrus fruits. *The Journal of Horticultural Science and Biotechnology*, 97(4), pp. 524-533.]

A further study of the effectiveness of lemon myrtle essential oil investigated its effects on green mould control using an *in vitro* agar diffusion assay and a vapour assay in artificially infected oranges. The main constituent of lemon myrtle essential oil was shown to be citral. Lemon myrtle essential oil suppressed green mould. While longer dipping times led to some rind injuries, fruit treated with a 5 or 10-second dip were free from any injury. Evaluation after dipping and storage confirmed that the fruits maintained the sensory attributes and were not compromised by the incorporation of the essential oil. The results of this study indicate that lemon myrtle essential oil can be a promising alternative to synthetic fungicides for maintaining the quality of citrus fruit during storage. [Rahman M.M., Wills R.B.H., Bowyer M.C., Vuong V.Q., Golding J.B., Kirkman T. and Pristijono P. (2023) Efficacy of lemon myrtle essential oil as a bio-fungicide in inhibiting citrus green mould. *Plants* 12(21):3742]

The results show the potential of essential oils, particularly following photochemical treatment, to enhance their efficacy against green and blue mould in citrus fruit. Copies of these papers are available from the author.

Postharvest management of anthracnose: Anthracnose is a rind blemish that can develop in the orchard but is more commonly seen in the retail market. The symptoms of anthracnose are often not seen on the fruit at harvest but are

often expressed as the typical ‘gas burn’ symptoms in mandarins after degreening. Anthracnose is generally not a major issue in many drier growing areas but can be in wet growing years. Anthracnose spores infect the fruit during the growing season, where they germinate, invade the rind and then remain dormant. During this time, there are no symptoms of infection and the fruit looks good. However, rind blemish typically develops when the rind is stressed. The fungus can also grow and show the classic gas burn symptoms after the fruit is harvested and stressed, e.g. during degreening. Anthracnose is sometimes referred to as ‘gas burn’ because symptoms can develop after degreening with ethylene, especially in early-season green fruit with no signs of colour break. Ethylene triggers the growth of the dormant fungus and increases the susceptibility of the rind to damage.

While there are many orchard management practices to reduce field infection, there are limited postharvest management options. A series of postharvest trials were conducted during 2019–2021 to identify practices that minimise anthracnose and screen some common postharvest fungicides that can reduce its expression. These common citrus postharvest fungicides are already registered for controlling green and blue mould and sour rot, so these were assessed to potentially reduce anthracnose expression.

Postharvest fungicide screening against anthracnose (2019) Several different fungicides were assessed against anthracnose in Imperial mandarins that were harvested from a commercial orchard in Mundubbera, Queensland, with a history of anthracnose. While TBZ did not affect anthracnose development, Chairman® fungicide (containing fludioxonil and propiconazole), which is registered as a postharvest treatment against green and blue mould in citrus, had some effect against anthracnose in Imperial mandarins.

Postharvest fungicide screening against anthracnose (2020) A similar fungicide screening experiment was conducted in 2020 and showed that Chairman®, fludioxonil, Graduate A®, Sportak® and Cabrio® postharvest dips reduced anthracnose development. Increasing the time in degreening conditions (4 ppm ethylene, 25 °C and 90% relative humidity) did not affect anthracnose development.

Postharvest fungicide screening against anthracnose (2021) The trial design from previous seasons (2019 and 2020) was repeated with Imperial mandarins from Mundubbera, Queensland, with 12 different postharvest fungicide treatments: label rate thiabendazole, imazalil, Chairman® fungicide [fludioxonil and propiconazole], fludioxonil, Philabuster® fungicide [imazalil and pyrimethanil], guazatine, Graduate A+® fungicide [azoxystrobin + fludioxonil], Sportak® fungicide [prochloraz], pyrimethanil, a combination of thiabendazole + fludioxonil active ingredients (which has been shown to have some efficacy on Imperial mandarins by QDAF researchers) and a heated treatment of the previous combination treatment at 50 °C.

Unfortunately (for the experiment), the background level of anthracnose from the same orchards in Mundubbera was very low. The untreated control mandarins only had <10% natural infection, even after 4 weeks in storage. This highlights the inherent seemingly random nature of the preharvest infection and expression of anthracnose between seasons. It was not possible to assess the different fungicides and treatments in this season.

Optimising organic salts to control postharvest decay The development of alternative chemical control treatments has focused on food additives and natural compounds with minimal effects on human health and the environment. For example, there has been a focus on the application of natural organic acid salts such as sodium benzoate and potassium sorbate applied in aqueous solutions. Many research studies have shown the positive effects of food additives (sodium bicarbonate, sodium benzoate, and potassium sorbate), as well as elevated treatment temperatures, on reducing postharvest decay in citrus. However, each salt solution, heated or not, is normally applied as a stand-alone treatment and little information is available on the effectiveness of mixtures of these different GRAS salts to control postharvest decay in citrus. Response surface methodology is a useful procedure to show the interactive effects between different independent and response variables, which can reduce time and cost by simultaneously assessing numerous experimental parameters. Therefore, response surface methodology can be a valuable resource for systematically investigating the interactions and additive effects of these food additives and high treatment temperatures. This study investigated the optimal dipping solutions of the food additives sodium bicarbonate, sodium benzoate, and potassium sorbate (at concentrations of 0.5 to 6.0%) combined with elevated but non-phytotoxic treatment temperatures (20–50 °C) using response surface methodology for the control of green and blue mould in artificially inoculated Valencia oranges. The most suitable food additive concentrations were 4.7% sodium bicarbonate, 1.0% sodium benzoate and 0.7% potassium sorbate with a dipping solution temperature of 50 °C.[Archer, J., Pristijono, P., Vuong, Q.V., Palou, L. and Golding, J.B. (2024) Utilising response surface methodology to optimise food additives and treatments reduces disease caused by *Penicillium digitatum* and *Penicillium italicum* in Valencia oranges. *Horticulturae*, 10(5), 453]

Physical treatments to reduce postharvest decay In addition to chemical treatments to control postharvest decay, physical treatments were assessed for their efficacy against green and blue mould. Physical control measures such as heat treatments (curing and hot water) and irradiation treatments (UV-C and ionising irradiation) have the advantage of leaving no chemical residues but have limited efficacy and a lack of persistence or preventative activity. The effectiveness of low-pressure treatments was assessed against green and blue mould in oranges. Low-pressure storage of 6.6 kPa and low oxygen treatments of 1% O_2 (for 4 and 8 days at 20 °C) decreased both green and blue mould in infected oranges. The reduction in blue mould severity with these physical treatments might also potentially be an alternative to chemical fungicides and could contribute to organic or chemical-free citrus production. It was also noted that low-pressure and low-oxygen treatments reduced weight loss compared to the untreated control fruit. However, these physical treatments also resulted in increased ethanol levels within the fruit, presumably through anaerobic metabolism, but these increased levels were below levels that the consumer can perceive. [Archer J., Pristijono P., Vuong Q.V., Palou L. and Golding J.B. (2021) Effect of low pressure and low oxygen treatments on fruit quality and the *in vivo* growth of *Penicillium digitatum* and *Penicillium italicum* in oranges. *Horticulturae* 7, 582].

Improving market access outcomes

Meeting MRLs – removing dimethoate residues from packinglines Dimethoate is an organophosphate insecticide that can be used as a postharvest end-point treatment to facilitate the interstate trade for some citrus. However, it is thought that dimethoate residues can remain impregnated within the packingline following long treatment times. The presence of potential dimethoate residues in the packingline could then contaminate non-dimethoate-treated fruit processed on the same line. This potential contamination of non-treated fruit could lead to the detection of MRL residues of dimethoate/omethoate in some sensitive export markets. This pilot project assessed different postharvest cleaning products to potentially remove/decontaminate packinglines with embedded dimethoate residues. The trial cleaning products used were: (1) Decco EcoCleaner Line® (active ingredients – sodium dodecylbenzene sulphonate and citric acid), (2) Decco Equipment Cleaner 510® (sodium metasilicate), (3) Vacate wax remover® (sodium hydroxide and potassium hydroxide), (4) Pace Acidex Duo® (phosphoric acid), (5) Chemtech Shock treatment® (sodium hydroxide), (6) sodium bicarbonate, (7) Kitchen Maid® (from packinghouse – Kitchen Maid Multi Purpose Hard Surface Cleaner, butoxyethanol and alkaline salts). All cleaners reduced dimethoate (i.e. combination of dimethoate and omethoate) residues following wash treatment by 84–95% compared to the un-washed rollers. These results were from washing the packing house cups and not in fruit samples, but parallel samples showed no detectable residues in the fruit. While it is ideal not to use organophosphate insecticide treatments on packinglines, these results show that all commercial washing treatments (EcoCleaner®, Vacate®, Acidex Duo®, Equipment Cleaner 510®, Bicarb, Kitchen Maid® and Shock Treatment®) were effective at removing residues from the packingline.

Evaluation of postharvest WetCit® application to remove Fuller's rose weevil (FRW) eggs, red scale, and the effects on postharvest decay

Innovations and new products to improve market access and reduce the risk of residues are crucial for the Australian citrus industry. WetCit® is a new organic adjuvant product that has been trialled in orchards around the world to reduce red scale; however, its effectiveness in postharvest is unknown.

Fuller's rose weevil (FRW) is a quarantine pest for Australian citrus exports. The adult weevils lay their eggs under the orange calyx and are 'cemented' in rafts under the calyx, which makes them very difficult to remove. There is no current postharvest treatment to consistently remove these eggs from under the calyx, which increases export costs as expensive preharvest approaches are required to allow exports. Red scale is another critical quarantine pest that can disrupt exports. It would be ideal to have a postharvest treatment such as WetCit® that could remove FRW eggs and red scale in export consignments. A series of trials were conducted to assess the effects of postharvest applications of WetCit® on (1) *in vivo* survival of FRW eggs in Navel oranges, (2) the effect of different concentrations of WetCit® with dipping and high-pressure washing on red scale removal, and (3) effect on *Penicillium* decay control.

FRW – due to the inconsistent nature of FRW infestation, it was not possible to obtain consistent infestation between fruit and treatments. The experimental unit of each treatment was 40 fruit and each treatment was replicated 4 times. However, there was large fruit-to-fruit variability in FRW infestation and therefore, the results were inconsistent.

Red scale (*Aonidiella aurantia*) is an important quarantine pest in some markets such as South Korea. It is critical to ensure the absence of this pest for this important export market. This trial examined the effect of different concentrations of WetCit® with dipping and high-pressure washing to remove red scale from oranges. One pallet of red

scale-infested Salustiana oranges was obtained from Griffith. Fruit were sorted into 3 categories of red scale infestation: (1) severe/heavy infestation, (2) moderate infestation and (3) low/light infestation. Each fruit was given a unique number and a 4.6 cm diameter circle (area 16.6 cm^2) was drawn with a permanent marker on the surface of each fruit (Figure 4). The number of scale insects inside the marked circle was recorded before and at different times after treatment. The fruit were treated with one of 4 treatments: (1) water dip control, (2) 0.5% WetCit®, (3) 1% WetCit® and (4) no washing. In addition, there were 2 application methods of the dipping treatments: (1) a 30-second dip in a bucket then brushes only and (2) a 30-second dip in a bucket then high-pressure wash with brushes for 30 seconds. Each treatment had 100 fruit and red scale infestation categories.

Figure 4. A sample area around the fruit equator was marked with the permanent pen on the surface of each fruit to allow for the repeated counting of the number of red scale within the circle after treatment.

The results (i.e. combining all the different infestation levels) showed that the 30-second dip in water and WetCit® had little effect on scale removal. Adding a high-pressure wash treatment after the dip resulted in higher levels of scale removal. This reduction in scale following high-pressure washing was observed in both water and WetCit® treatments, and it might be due to the high mechanical impact of the wash treatments on dislodging the scale. This was evident in the wash water from the high-pressure washer, which had high levels of scale in the wastewater. There appeared to be some effect of WetCit® on removing higher levels of scale than the water, but the high-pressure wash is recommended for improving scale removal. While this treatment did not remove all the red scale from the fruit, it could be examined in a systems approach to manage red scale for market access into sensitive markets.

Postharvest decay – applying WetCit® (either dipping or with the addition of high-pressure washing) had little effect on green and blue mould infection in Navel oranges. There appeared to be some benefit with the higher levels (1%) WetCit®, but this was marginal with high green and blue mould development following treatment and storage.

Maintaining calyx condition – alternatives to 2,4-D

Calyx (button) abscission is a major factor contributing to citrus loss during storage, as consumers consider it a negative quality attribute. Calyx loss can also facilitate fungal attack at the abscission zone, leading to an increased incidence of visible decay. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin plant growth regulator that has been widely employed as a preharvest treatment by citrus growers to improve fruit quality attributes such as fruit size and juice acid levels and sugar content. 2,4-D is also sometimes used as a postharvest treatment to delay calyx browning and calyx abscission. Treatment also favourably affects physical characteristics (e.g. colour and firmness) that are important to consumers. While 2,4-D use is currently permitted in Australia and the United States, its use in other countries has diminished due to increased health and environmental concerns (Ma et al. 2015). There is a need for the citrus industry to be proactive and find more acceptable postharvest treatments to replace 2,4-D as a senescence inhibitor.

A series of trials were conducted to examine alternative postharvest treatments to maintain calyx condition, including (1) 3,5,6-trichloro-2-pyridiloxyacetic acid dipping, (2) hydrogen sulphide fumigation, (3) comparison of 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide treatments, and (4) effect of 'low' dicamba levels on shelf life of Navel oranges.

Effects of TPA – the effects of postharvest treatment with 3,5,6-trichloro-2-pyridyloxyacetic acid (TPA) on the deterioration of calyx quality, decay incidence and internal quality parameters in long-term storage on 3 types of citrus were investigated. TPA treatment exhibited a concentration-dependent effect on fruit quality, with higher concentrations reducing calyx deterioration and decay, lowering the respiration rate, ethylene production and ethanol accumulation, and inhibiting change in TSS and TA levels and hence maintaining the TSS:TA ratio. The results show that postharvest TPA treatment can be used to alleviate calyx senescence and maintain postharvest quality in citrus fruits. [Alhassan, N., Bowyer, M.C., Wills, R.B.H., Golding, J.B., Pristijono, P., 2020. Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays calyx senescence and loss of other postharvest quality factors of Afourer mandarins, Navel, and Valencia oranges. *Scientia*

Horticulturae. 272, 109572].

Effects of hydrogen sulphide – short, pre-storage fumigation with hydrogen sulphide (H_2S) gas at 0, 100, 250 and 500 µL L^{-1} affected the development of a range of senescence characteristics of Navel and Valencia oranges and 'Afourer' mandarins during storage at 20 °C for 5 weeks. Fumigation at 100 µL L^{-1} H_2S reduced the incidence of calyx drop, calyx browning, fungal decay and the production of ethylene and ethanol for all 3 citrus species. For Valencia oranges, a lower TSS:TA ratio was observed, arising from both a lower TSS and higher TA than in control fruit. H_2S treatment had no significant effect on the respiration rate in any fruit species. In general, higher concentrations of H_2S were less effective than 100 µL L^{-1} and often resulted in an accelerated loss of quality. The results suggest that H_2S fumigation of citrus before storage might be an alternative treatment for delaying the emergence of senescence characteristics such as calyx browning without the use of synthetic auxins. [Alhassan, N., Wills, R.B.H., Bowyer, M.C., Golding J.B., Pristijono P., 2020. Pre-storage fumigation with hydrogen sulphide inhibits postharvest senescence of Valencia and Navel oranges and 'Afourer' mandarins. *The Journal of Horticultural Science and Biotechnology* 95, 757–762].

Comparative study – The effectiveness of pre-storage dips of different auxin formulations, 2-(4-amino-3,5-dichloro-6-fluoropyridin-2yl) oxyacetic acid (fluroxypyr), 3,6-dichloro-2-methoxybenzoic acid (dicamba) and 2-methyl-4-chlorophenoxyacetic acid (MCPA), were assessed against the standard 2,4-D treatment using Valencia oranges at concentrations of 0.2 and 1 mM. During 4 weeks of storage at 20 °C, fluroxypyr produced the greatest reduction in calyx abscission, calyx browning, fruit decay, and down-regulation in endogenous ethylene production, respiration rate and ethanol formation relative to other treatments. Fluroxypyr dip at 1 mM was most effective, with the 0.2 mM concentration still superior to 2,4-D. MCPA showed only modest activity, while dicamba was ineffective. H_2S fumigation significantly reduced calyx deterioration and delayed the loss of internal quality factors. Therefore, fluroxypyr or H_2S, as a non-auxin treatment, have the potential to replace 2,4-D for commercial use. [Alhassan, N., Wills, R.B.H., Bowyer, M.C., Pristijono, P., Golding J.B., 2022. Comparative study of the auxins 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide to inhibit postharvest calyx senescence and maintain internal quality of Valencia oranges, *New Zealand Journal of Crop and Horticultural Science*, 50(2-3), pp. 131–142].

Chilling injury

Chilling injury can be a devastating postharvest disorder that can occur after low-temperature storage, which can result in a significant downgrade or rejection of fruit in the market. Chilling injury is a disorder (not a disease) that is caused by exposure to cold, but not freezing, temperatures during storage. The major challenge with chilling injury is that the biochemical and physiological basis for its development is unknown.

Navel chilling injury survey To investigate the seasonal and varietal differences in the development of chilling injury, a series of observations examining the expression of chilling injury were made on fruit from the same trees on the same rootstock under the same orchard management over successive seasons (2019–2023). Mature Navel oranges were sourced from trees from the NSW Department of Primary Industries Dareton Navel Trial. This block was planted on *C. citrange* rootstock in 1992. The trial block contained 6 replicates of different early, mid and late-season Navel oranges. Each Navel variety was in a pair with one tree inoculated with the 3532 mild strain and the neighbouring paired tree without the inoculation (control). Each season from 2019–2023, the following fruit varieties were harvested from the NSW DPI Dareton trial block: (1) early season–Leng, Navelina (2020–2023) (Lloyd A and no Navelina in 2019 only), (2) mid-season–Atwood, Houghton, and (3) late season–Chislett, Lanes Late. Fruit from the same labelled trees were harvested at commercial maturity and sent to NSW Department of Primary Industries at Ourimbah and stored at 3 °C for 8 weeks. After storage, the fruit were stored at 20 °C for another week to allow the chilling symptoms to express at room temperature. The results of all data collected (incidence and severity of chilling injury, fruit TSS, TA and vitamin C levels) from the trial over the five years (2019–2023) are presented in the Technical Report (Appendix 2).

The results in Figure 5 show the levels of chilling injury, highlighting the considerable variation between varieties, harvest maturities, and seasons. For example, in the 2019 season, over 90% of all Leng Navels had chilling symptoms, but in the 2022 season, less than 20% of Leng Navels had chilling symptoms. Similarly in 2019, Leng Navel oranges had an average score of 3 (i.e. definite pits up to 10% of the fruit surface), while in 2022, the average chilling score for Leng Navels was less than 0.5 score. In general, there were higher percentages of fruit with chilling injury in 2019 and 2020 than in the following years (2021, 2022 and 2023). Leng consistently had a higher rate and more severe cold-damaged fruit than Navelina from 2020 to 2023. Similar variations in the expression of chilling injury were observed with the mid and late-season fruit. In general, this survey showed large varietal, seasonal and yearly differences in the expression of chilling and

highlighted the complexity of managing chilling injury.

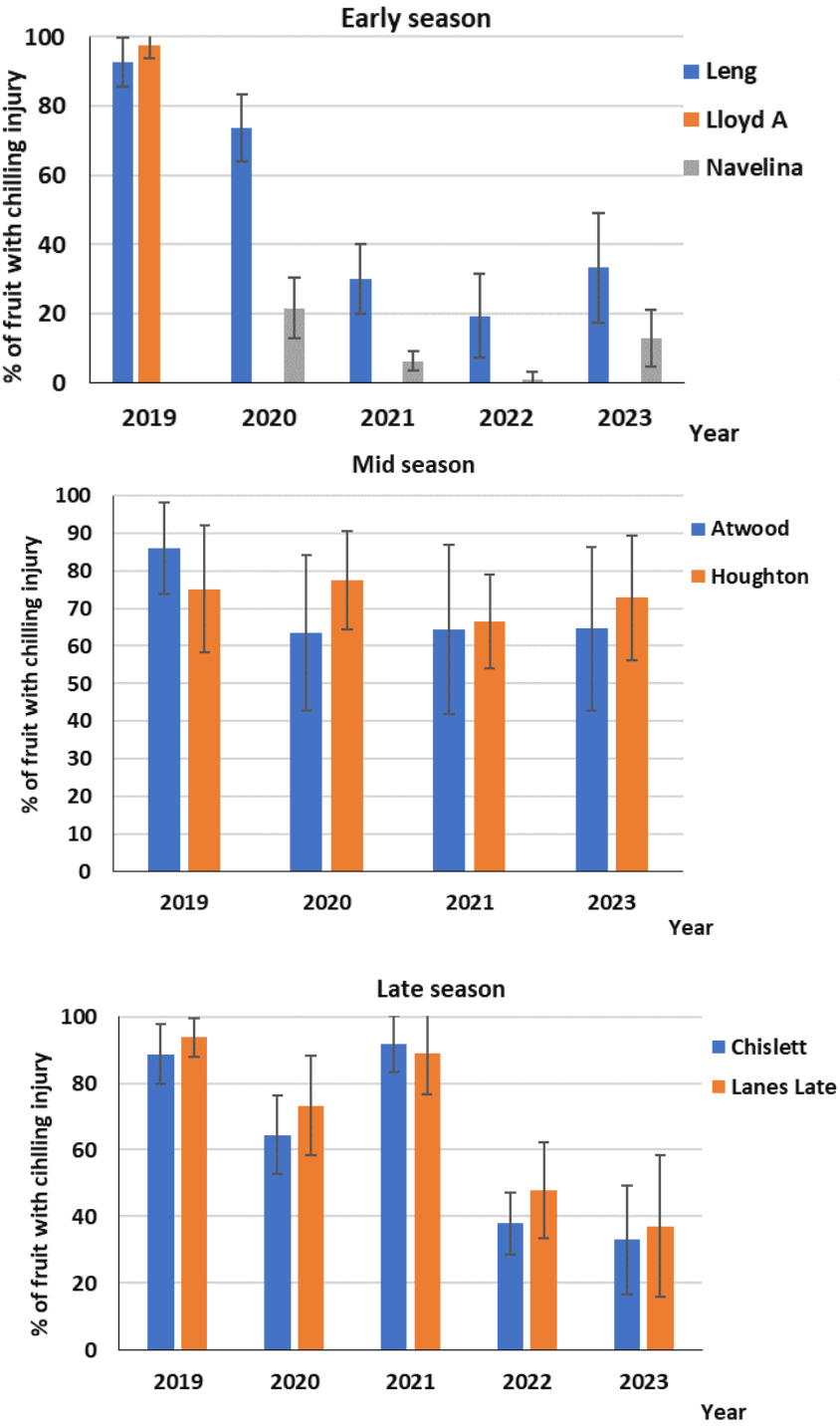


FIgure 5. Percentage of fruit with chilling injury fruit chilling injury score at assessment 2 (8 weeks at 3 °C + 1 week at 20 °C) of:

- early-season (top),
- mid-season (middle) and
- late-season (lower)

Navel varieties during 2019–2023.

Bars are standard deviations around the means, *n*=12.

Chilling injury susceptibility of new mandarin cultivars The citrus industry is continually introducing new citrus types and cultivars to improve consumer choice and profitability. While the production and fruit quality data at harvest of new cultivars are well established, there is no clear local data on postharvest storage behaviour (e.g. susceptibility to chilling injury) to support large-scale production and marketing of new cultivars. These storage trials assessed the effects of long-term storage on Satsuma mandarins (at 2 harvest dates) and Clementine mandarins. In these trials, both the Satsuma and Clementine mandarins showed minimal chilling injury, with any chilling injury symptoms being <5% of all fruit (chilling injury score 2). This is a good result, but more work is required to assess the effects of different harvest times, growers'

management practices, and seasons on these cultivars. In relation to internal fruit quality, as expected, larger fruits contained lower TSS and TA than smaller fruits, and no trends were observed for vitamin C content between the fruits.

Improving fruit quality outcomes

This program was driven by the active Project Reference Group (PRG), which guided the research and extension outcomes. In response to disrupted and extended supply chains with COVID-19 and its aftermath, the PRG highlighted the need to maintain quality with extended shipping times, particularly for mandarins. Subsequent long-term storage trials of Afourer mandarins were conducted to quantify the preharvest growing treatments (e.g. effects of orchard PGRs and irrigation) on postharvest quality and storage life. The results illustrated the effects of pre-harvest orchard management and showed the practicalities of long-term storage to maintain high-quality fruit for extended storage periods.

Effects of irrigation frequency on Afourer fruit quality following long-term storage This trial examined different irrigation strategies on the quality of Afourer fruit following up to 10 weeks of storage at 3 °C. Three irrigation scheduling strategies were compared: (1) control (regular irrigation), (2) reduced irrigation (70% of normal irrigation scheduling), and (3) increased irrigation (120% of normal irrigation scheduling). Afourer mandarin fruit were harvested from each of the replicated orchard blocks in the Riverland and transported to NSW DPI at Ourimbah. Fruit were stored for up to 10 weeks at 3 °C and fruit quality was assessed. A parallel set of fruit was stored at 20 °C for up to 4 weeks. For the fruit kept continuously at 20 °C for up to 4 weeks, the major differences between the treatments were the higher levels of sugars (TSS) and acids (TA) in fruit with reduced irrigation scheduling.

Similarly, in fruit stored for up to 10 weeks at 3 °C and an additional 1 week at 20 °C, the fruit from the reduced irrigation treatment had higher TSS and TA levels. There were minor differences in other quality parameters, and no effect of irrigation treatment on chilling injury or button (calyx) browning was observed in this trial. These results showed that reduced irrigation resulted in fruit with higher TSS and TA levels throughout storage and shelf life with no increase in chilling injury or other storage issues.

Effects of orchard PGR applications on the shelf life and quality of Afourer mandarins The aim of this trial was to examine the effect of commercial PGRs sprayed during growth on the shelf life and quality of Afourer mandarins. The orchard component and PGR applications were conducted in a commercial orchard in South Australia in a block of mature Afourer mandarins. The fruit storage and quality assessment were conducted at NSW DPI, where the mandarins were stored for up to 10 weeks at 3 °C before fruit quality assessments. The results showed no consistent effects of the pre-harvest PGR sprays on the long-term storage of Afourer mandarins.

Effects of postharvest applications of GA on shelf life and quality of Navel oranges Some packers have asked if GA has any postharvest application benefits, but there is insufficient data to support its use. In the previous Hort Innovation project, we used 100 ppm GA (ProGibb®) on lemons and Navel oranges, but this resulted in severe phytotoxicity on the peel (CT15010). As GA regulates growth, very low concentrations can have a profound effect, while too much will have the opposite effect. This trial examined the effects of lower rates of GA (1, 10 and 50 ppm GA) as a postharvest dip on the storage life of Navel oranges, finding that GA aqueous dips did not have any consistent benefits.

Improving the storage performance and eating quality of Afourer mandarins during extended shipping This trial assessed 2 postharvest techniques for managing ethylene in storage:

1. Physically removing the ethylene from the storage environment with potassium permanganate scrubbers: this is a passive system where the potassium permanganate in a sachet oxidises ethylene (and all volatile organics).
2. Preventing the fruit from reacting to ethylene by using 1-methylcyclopropene (1-MCP), which works by stopping the action of ethylene in the fruit.

The results showed that Afourer mandarins could be stored for 15 weeks at 3 °C. There were no consistent differences between the different postharvest treatments and the untreated control fruit. No chilling injury was detected in the experiment. There was no consistent benefit of applying 1-MCP or the potassium permanganate sachet to overall quality; however, there were some individual quality components that benefited from postharvest treatment. Upon tasting the fruit during the storage trial and at the end after 15 weeks, there was some variability between the fruit of the same batch of the same treatment. Informal observations from tasting tests involving 2-fruit from each treatment to consumer panellists, it was a common occurrence to hear that one fruit tasted good and the other fruit was poor. This observation occurred in all different postharvest treatments, and it was difficult to determine if there were any eating differences

between the treatments. A small survey of different fruit sizes and apparent skin colour in mandarins showed no correlation between eating quality. More work is required to improve eating quality consistency.

Literature review of albedo breakdown In response to albedo breakdown in fruit in eastern Australia in the 2022 season, the PGR encouraged the program to review and update the literature on this disorder. This was conducted and presented to the industry (Appendix 3) 'Albedo breakdown research update' and is now a funded Hort Innovation levy-funded R&D project.

Evaluating alternative coatings The natural waxes on the surface of citrus fruit are removed during picking and processing, and food-grade waxes are commercially applied to the fruit before packing. Waxes are essential to maintain the quality of fresh citrus fruit during storage by reducing weight (water) loss.

While the current commercially available waxes are widely used and accepted, the development of alternative waxes/coatings is required to improve the out-turn of citrus. This trial compared the effectiveness of a trial coating (corn-based starch and other natural plant-based ingredients) with a commercial wax on the storage life of lemons. In this trial, weight loss from both the commercial wax and the trial coating was lower than the untreated control (Figure 6). There were some differences in the results such as fruit glossiness, which are discussed in more detail in the Technical Report (Appendix 2), but in general, the trial wax showed some benefits and may be useful in some markets.

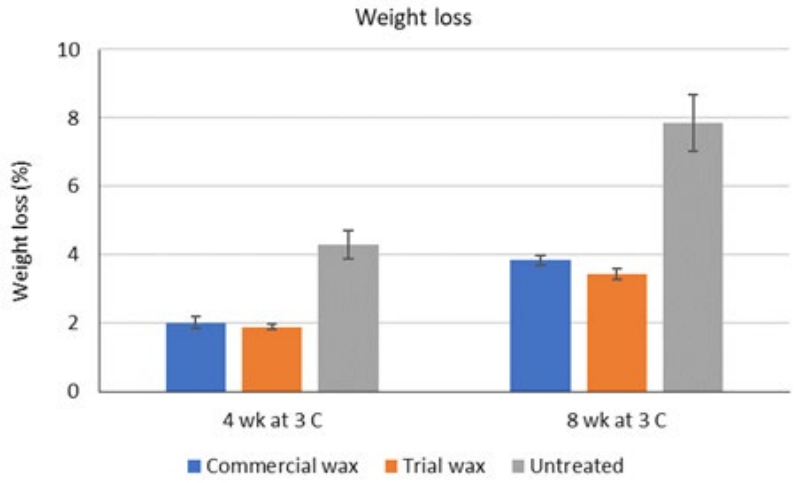


Figure 6. Weight loss (%) of lemons treated with commercial wax, trial wax and untreated after stored for 4 and 8 weeks at 3 °C.

Evaluating new citrus firmness meters Fruit firmness is an important market and consumer quality attribute for all citrus types, yet there are no widespread commercial methods to measure and report fruit firmness (softness). This preliminary study examined a range of cheap and practical methods to objectively assess Navel fruit firmness. A range of sleeves to fit over the standard handheld 'Effigi' penetrometer were trialled and compared. The results showed the most consistent firmness measurement was the hand penetrometer of 13 kg/11 mm tip with sleeve size 42 mm. More work is required to overcome potential user differences.

Outputs

Table 1. Output summary

Output	Description	Detail
A monitoring and evaluation plan developed with the Program Reference Group at MS 102	A monitoring and evaluation plan was delivered to the PRG at MS 102	The monitoring and evaluation plan was delivered and approved by the PRG and Hort Innovation at MS 102. This was used to keep the program on track.
Six monthly milestone reports and an annual work plan developed	Six monthly milestone reports and an annual work plan developed	Six monthly teleconference PRG meetings were conducted, and minutes were reported to PRG and Hort Innovation. Program milestone reports and an annual work plan were

with the PRG	with the PRG	discussed with PRG and reported to Hort Innovation.
Delivery of a *Best Practice Postharvest Manual*	*Best Practice Postharvest Manual*	Not delivered in full. The draft *Best Practice Postharvest Manual* is being prepared and industry stakeholders (including packers and postharvest consultants) are being consulted about the content.
Delivery of regional postharvest workshops	Regional workshops to support the *Best Practice Postharvest Manual*	Regional workshops/postharvest presentations have been delivered in all growing regions (WA, SA, Vic, NSW, FNQ, Central Qld) to support the program. A total of 6 Program postharvest workshops/presentations were conducted.
Presentations at national (and regional) Citrus Australia forums	Presentations at national (and regional) Citrus Australia forums	9 Postharvest presentations were conducted at Citrus Australia Regional Forums around Australia, with 3 general postharvest presentations. In addition, a further 8 postharvest presentations were conducted at the 2022 Citrus Technical Forum, Sunshine Coast, 2 presentations at the Citrus Australia Market Outlook Forum (Mildura), and 7 presentations from the Program were made at the 2024 Citrus Technical Forum, Sunshine Coast.
Regular contributions to industry communications platforms, such as *Australian Citrus News*	Regular contributions to industry communications platforms	A total of 22 postharvest articles were made to industry journals and communications. A regular postharvest article/contribution from the Program was made to *Australian Citrus News* (4 issues per year). In addition, the outcomes of the program were discussed in the Citrus Australia podcast, The Full Bottle.
Contributions to the NSW DPI extension activities	Contributions to the NSW DPI extension activities, such as *Citrus Connect* and future *NSW DPI Citrus Plant Protection Guides*	Regular contributions to NSW DPI extension activities were made during the Program. In addition, a postharvest contribution to the *NSW DPI Citrus Plant Protection Guide* was made in the 2023–24 edition. This addition added postharvest management into the NSW DPI production guide for the first time.
Research papers in international peer-reviewed scientific journals from Year 2	Research papers in international peer-reviewed scientific journals from Year 2	Ten international peer-reviewed scientific papers were produced with assistance from the program. One book chapter on the use of 'green' technology for reducing postharvest losses in fresh horticultural produce was published. In addition, 2 international peer-reviewed papers and a book chapter from the previous Hort Innovation Project (CT15010) were also published–not reported in CT15010.
Final Program report with measurement and evaluation at the end of the Program	Final Program report with measurement and evaluation at the end of the Program	Final Report delivered.

Outcomes

Table 2. Outcome summary

All outcomes have alignment with the Citrus Strategic Investment Plan (2022–2026).

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence

A better working knowledge of the postharvest best practice and adoption of best practice guidelines	Outcome 1 – Protect the production. Strategy 7 – Develop postharvest practices and guidelines to ensure quality product reaches consumers. KPI – Development of postharvest best practice guides to enable consistent delivery of high-quality citrus	The overall level of postharvest knowledge is improving throughout Australia. Improved knowledge and postharvest practices will result in improved fruit quality outcomes and reduced risk of food safety and MRL breaches.	Feedback through grower/packer visits and follow-up from postharvest articles in *Australian Citrus News* and postharvest presentations/workshops highlights the current (and future) needs of postharvest research and extension. Postharvest knowledge and application are improving, as shown by improved sanitation outcomes, leading to lower levels of decay-causing spores in packinghouses (data from 'Sanitation and Fungicide Resistance Service' and presented at Australian Citrus Congress, Sunshine Coast, March 2024).
More effective and sustainable management of citrus postharvest decay and quality management where MRLs and food safety issues are effectively managed	Outcome 1 – Protect the production. Strategy 7 – Develop postharvest practices and guidelines to ensure quality product reaches consumers. KPI – Development of postharvest best practices for the harvest, handling, processing, and storage of Australian citrus	The assessment of alternative decay control measures was an active theme in the program, as well as the assessment of numerous commercial and experimental chemistries and technologies to control postharvest decay. Some treatments show commercial promise, with one treatment being registered for use.	A range of new decay control technologies and products were assessed. One new product, Ortocil®, was subsequently registered with the APMVA by the chemical company for use in the industry (2024). The commercial application of new chemistries/technologies is a great result for the industry in diversifying its decay management tools to meet different market needs.
Increased fruit quality in domestic and export markets	Outcome 1 – Protect the production. Strategy 7 – Develop postharvest practices and guidelines to ensure quality product reaches consumers. KPI – Development of postharvest best practices for the harvest, handling, processing, and storage of Australian citrus	The program has been actively assessing technologies (pre- and postharvest) to improve fruit quality. The assessment of pre-harvest orchard sprays was shown not to affect chilling injury during long-term storage.	Fruit quality continues to be a major competitive advantage of the Australian citrus industry. This Program supports growers in making informed decisions on pre- and postharvest management to improve fruit quality. Where fruit quality issues do occur, such as the development of albedo breakdown in the 2022 season, the program was quick in responding with an update of the literature and extension article in *Australian Citrus News* to minimise albedo breakdown. Surveys and trials with chilling injury supported enhanced fruit quality outcomes.
Reduced risk of fungicide resistance development	Outcome 1 – Protect the production. Strategy 7 – Develop postharvest practices and guidelines to ensure quality product reaches consumers. KPI – Development of postharvest best practices for the	In addition to the assessment of alternative decay control measures (above), the Program also supported the '*Sanitation and Fungicide Resistance Service*' to promote good postharvest practices to reduce risks of	Due to the increased postharvest knowledge and application, the levels of resistance to postharvest fungicides in Australian packing houses have been declining during this Program. This has been due to increased education, monitoring and management of postharvest fungicide resistance. For example, the levels of technical resistance to TBZ declined from 50% in 2020 in all

	harvest, handling, processing, and storage of Australian citrus	postharvest fungicide resistance and improve fruit quality outcomes. This includes a holistic approach to decay management (e.g. fruit quality, sanitation, fungicide use, storage).	sheds throughout Australia to less than 30% in 2023. (Data from 'Sanitation and Fungicide Resistance Service' and presented at Australian Citrus Congress, Sunshine Coast, March 2024).
Reduced risk of chemical and microbiological contamination of Australian citrus	Outcome 1 – Protect the production. Strategy 6 – Monitor and manage food safety risks to maintain consumer confidence in Australian citrus, and Outcome 2 – Market optimisation. Strategy 1. Maintain and improve technical market access for high-value export markets	The presence of chemical residues in some domestic and export markets can affect market access. It is essential that all Australian citrus is clean and meets all market access requirements, including MRLs.	Research in the program showed that current commercial postharvest cleaning products were effective at removing specific chemical residues from packing line equipment, therefore reducing the risks of MRL breaches and loss of market access.
Higher skilled industry in postharvest knowledge and applications	Outcome 1 – Protect the production. Strategy 7 – Develop postharvest practices and guidelines to ensure quality product reaches consumers. KPI – Development of postharvest best practices for the harvest, handling, processing, and storage of Australian citrus	Postharvest is the key link between the grower and the consumer. Maintaining fruit quality from the orchard through the supply chain is critical to grower profitability and industry outcomes. Ensuring the industry (particularly packinghouse staff) are engaged and skilled is essential to add value to the Australian citrus industry.	The Program visited growers and packinghouses in all growing areas around Australia. Specific postharvest workshops have been undertaken to educate, train and improve the competency of growers and packers. On-going postharvest presentations at Regional Forums and regular postharvest sessions/workshops at the Citrus Congress (and Market Outlook Forum) are delivering improved postharvest knowledge and applications to industry. Regular articles in *Australian Citrus News* deliver relevant and on-time seasonal postharvest information.
Maintenance and growth of export market access	Outcome 1 – Protect the production. Strategy 7 – Develop postharvest practices and guidelines to ensure quality product reaches consumers. Outcome 2 – Market optimisation. Strategy 1. Maintain and improve technical market access for high-value export markets	Market access relies on overcoming technical quarantine barriers, such as cold treatment, and meeting and exceeding customers' expectations of Australian citrus. The program has supported both technical and commercial requirements for market access.	The program has conducted R&D with recommendations for longer-term export storage, particularly for mandarins following disrupted export supply chains. In addition, R&D in the chilling susceptibility of new mandarin varieties has supported the industry in export development. Further R&D in improved efficacy of red scale removal in the postharvest processing line from Navel oranges will contribute to increased confidence in exports to sensitive markets.

Increased exports with higher confidence in meeting regulators' and consumers' expectations of Australian citrus	Outcome 1 – Protect the production. Strategy 7 – Develop postharvest practices and guidelines to ensure quality product reaches consumers. Outcome 2 – Market optimisation. Strategy 1. Maintain and improve technical market access for high-value export markets	Fruit quality is a major competitive advantage of the Australian citrus industry. High-quality eating quality fruit which fulfils regulators' requirements and consumer expectations is critical to the profitability of the industry. This Program supported the industry in improving market access outcomes with strategic R&D and extension activities.	Fundamental to fruit quality is decay control. This Program developed tools for growers and packers to successfully control postharvest decay by reducing potential postharvest fungicide resistance and ensuring the fruit meets all market MRLs. New developments in decay control will give industry options for increased exports in changing overseas consumer markets. Increasing consumer and supermarket requirements are necessitating reductions in postharvest fungicide residues. The program has assessed and developed tools to reduce postharvest fungicide use.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project achieved its expected outcomes?	What new knowledge has the project delivered to the citrus industry with regard to postharvest best practices? *Yes. New knowledge and new products (e.g. Ortocil®) are available to the industry to improve decay control and fruit quality.* To what extent has the project increased the adoption of the industry Best Practice Guidelines? *The Program is in the final stages of developing and implementing Postharvest Best Practice Guidelines.*	Continue to respond to industry needs with active and engaged PRG. Continue to engage with international R&D and best practices to ensure new and emerging technologies and products are commercially and practically evaluated in Australian commercial situations.
How relevant was the project to the needs of the intended beneficiaries?	To what extent has the project met the needs of industry levy payers? *The Program has met the needs of the target beneficiaries and provided postharvest tools and knowledge for the industry to continue to improve fruit quality and market outcomes.*	Continue industry engagement, including new players in citrus growing and packing, e.g. in the Shepparton region, where there is a growing citrus industry emerging from the summerfruit industry. These new growers and packers need to be supported with current postharvest best practices to ensure there are no unforeseen issues with novice growers/packers in local and export markets.
How well have intended beneficiaries been engaged in the project?	To what extent were the target engagement levels of industry levy payers achieved? *The Program has exceeded the level of engagement with industry. Postharvest presentations at specific postharvest*	Continue the publication of extension articles supported by face-to-face extension activities, but also explore online opportunities.

	workshops, Regional Forums, and the annual Citrus Congress (and Market Outlook Forum) with specific postharvest workshops for growers/packers were very well attended and received by industry. Off-line contact with growers/packers around Australia demonstrates ongoing engagement and support by industry. Have regular project updates been provided through linkage with the industry communication project? *Yes. This program worked closely with the PRG, industry communication project, Citrus Australia, Hort Innovation, industry stakeholders, chemical companies, and consultants to ensure the program's outcomes were met.*	Increase the distribution of extension materials within Hort Innovation, Citrus Australia, and NSW Department of Primary Industries networks. There are also opportunities within the chemical supply company's networks to supply independent and timely information to packers.
To what extent were engagement processes appropriate to the target audience of the project?	How accessible were extension events to industry levy payers? *Extension activities were delivered around Australia at different times of the year to ensure attendance by growers and packers. 23 extension articles were delivered and 35 presentations/workshops were delivered in different forums (some general regional forums and other specific postharvest workshops). The range of extension activities (face-to-face, telephone support, articles, presentations, and recorded presentations/podcasts) ensured all information was actively available for the industry.*	Continue publishing extension articles that are supported by face-to-face extension activities (e.g. workshops), but also explore online opportunities. Consider the development of local packing house support networks, particularly in new growing areas where new citrus packers can learn from each other to ensure best practices and fruit quality issues such as degreening and oleocellosis are managed in the best possible way. Many packers in new areas are new to citrus packing and some techniques and information they bring from other horticultural industries are not ideal for citrus.
What efforts did the project make to improve efficiency?	What efforts did the project make to improve postharvest performance and efficiency? *Improving performance and efficiency was the basis of the Program. Improving fruit quality out-turns, maintaining and increasing market access outcomes, reducing postharvest decay with reduced inputs, reducing postharvest fungicide resistance, and increasing grower/packer knowledge and skills were delivered.*	There is a continual need to ensure citrus production, including postharvest processing, packing, phytosanitary treatment, storage, and transport, is not only more sustainable but also more cost-efficient. There is not only a need to reduce waste (e.g. decay, rejection with chilling injury, albedo breakdown) but also to reduce greenhouse gas emissions, inputs and costs. Postharvest is a critical step in supplying citrus where its value can be significantly added or removed. Postharvest technology and its application to industry must be continually enhanced, not only to improve quality but also to ensure citrus production and supply are more sustainable.

Recommendations

A significant advantage of this current program was the continual guidance and support of the PRG in meeting industry needs. Refining research and extension directions to meeting changing industry needs was crucial to ensure the Program was current, relevant, and delivered practical outcomes for the industry. It is recommended to continue an active and engaged PRG in any future RD&E.

Review current postharvest best practices and identify gaps that require further R&D, including local and international trends and emerging knowledge or challenges. Examples of key areas of postharvest practice identified which need improvement with further R&D include:

Alternate decay control

Evaluate chemical and non-chemical treatments to control postharvest decay and reduce waste (for example, the assessment of novel treatments to control postharvest decay and reduce waste). This will also include semi and full commercial trials for their applicability to Australian commercial conditions.

Improve market access options for domestic and export markets

Cold treatment–explore the factors that contribute to the development of chilling injury.

Cold treatment is the standard phytosanitary treatment for market access. While it is the basis for most exports, it can also result in superficial damage to the peel following extended cold treatment and storage. This damage is poorly understood and managed. It is recommended that a series of pre and postharvest trials be conducted to develop best practice guidelines for minimising chilling injury.

Phytosanitary irradiation – explore the potential of phytosanitary irradiation for different citrus types to improve fruit quality outcomes following treatment.

While phytosanitary irradiation is successfully being used in some domestic and export markets, there are occasional issues with fruit quality following treatment. These issues include cosmetic phytotoxic damage to the peel and the development of internal pitting. While most fruit treated with phytosanitary irradiation are not affected, some batches of fruit are negatively affected. It is recommended that some of the pre and postharvest factors that contribute to poor fruit quality outcomes be surveyed to develop best practice recommendations to minimise this potential damage. This will give marketers and exporters confidence in the use of phytosanitary irradiation as a market access treatment.

Explore opportunities for improvements in high-pressure washing

The presence of quarantine surface insects such as scale are a major problem in some markets. Improvements in washing and brushing technologies need to be evaluated to remove these pests in Australia.

Improve fruit quality in the supply chain

There are numerous opportunities to optimise pre- and postharvest factors for fruit quality. It is recommended to work with growers and packers to investigate the interaction of pre-harvest and postharvest quality through the supply chain. Additional work to examine fruit variability will increase eating quality and improve overall quality.

Improve sustainability of postharvest practices to reduce greenhouse gas emissions

Sustainability has not been a prominent concept for many Australian citrus growers. While the concepts of sustainability are already used and valued by the industry, they have not been formally documented within the industry, including postharvest management. Sustainability must not become a market access barrier for Australian citrus. Already, the EU has developed regulations for sustainability and industry are preparing Product Environmental Footprint protocols for fresh fruits and vegetables and a common framework for measuring the effects. It will be critical that the Australian industry, particularly the citrus industry, is proactive to ensure sustainability will not become a technical market access issue.

Postharvest management is a high-value producer of greenhouse gas emissions (e.g. refrigeration, processing, chemicals, storage, transport). In some studies, it has been shown that 60% of all greenhouse gas emissions for citrus occur after harvest. It is necessary to quantify and improve the sustainability of postharvest processes within the citrus production system. Alternative abatement processes also need to be identified and applied in postharvest

systems. New challenges in waste management (decay control) and energy use will be crucial in the future of postharvest management of Australian citrus.

Develop new management strategies for the industry and review the effects of achieving MRLs

MRLs are a potential market access issue that need to be continually managed. While it is ideal that Australian citrus has no chemical residues, the need for decay control with postharvest fungicides necessitates residues of approved fungicides in the peel. It is recommended that new management strategies are developed and applied to achieve current and future MRLs.

Increase postharvest education and training

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

Increase RD&E capacity

The future of the Australian citrus industry is to remain flexible and adopt new technologies and strategies to maintain and improve fruit quality, market access and sustainability. This is not only for growers and packers, but also for R&D capacity within Australia. It is recommended to build postharvest R&D capacity for the industry to meet future challenges. Increased capacity in citrus postharvest science will ensure the future of development and application of new technologies to improve fruit quality. It will also bring new and fresh ideas to the industry.

Refereed scientific publications

All articles, posters and presentations are available from the author.

Journal articles

1. Alhassan, N., Bowyer, M.C., Wills, R.B.H., Golding, J.B., Pristijono, P., 2020. Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays calyx senescence and loss of other postharvest quality factors of 'Afourer' mandarins, Navel and Valencia oranges. *Scientia Horticulturae*. 272, 109572. doi.org/10.1016/j.scienta.2020.109572.
2. Alhassan, N., Wills, R.B.H., Bowyer, M.C., Golding J.B., Pristijono P., 2020. Pre-storage fumigation with hydrogen sulphide inhibits postharvest senescence of Valencia and Navel oranges and 'Afourer' mandarins. *The Journal of Horticultural Science and Biotechnology* 95, 757-762. DOI: 10.1080/14620316.2020.17491
3. Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Kirkman, T., Pristijono P., 2020. Efficacy of orange essential oil and citral after exposure to UV-C irradiation to inhibit *Penicillium digitatum* in navel oranges. *Horticulturae* 6, 102. doi:10.3390/horticulturae6040102
4. Archer, J., Pristijono, P., Vuong, Q.V., Palou, L., Golding J.B., 2021. Effect of low pressure and low oxygen treatments on fruit quality and the in vivo growth of *Penicillium digitatum* and *Penicillium italicum* in oranges. *Horticulturae* 7, 582. doi.org/10.3390/horticulturae7120582
5. Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. 2022. Efficacy of citral vapour in controlling citrus green mould. *Acta Horticulturae.* 1336, 235–242. DOI:10.17660/ActaHortic.2022.1336.31
6. Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Kirkman, T., Pristijono P., 2022. Lemon myrtle and lemon scented tea tree essential oils as potential inhibitors of green mould on citrus fruits. *The Journal of Horticultural Science and Biotechnology*, DOI: 10.1080/14620316.2021.2011433
7. Alhassan, N., Wills, R.B.H., Bowyer, M.C., Pristijono, P., Golding J.B., 2022. Comparative study of the auxins 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide to inhibit postharvest calyx senescence and maintain internal quality of Valencia oranges, *New Zealand Journal of Crop and Horticultural Science*, DOI: 10.1080/01140671.2021.2017984
8. Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Kirkman, T., Pristijono P., 2023. Potential control of

postharvest fungal decay of citrus fruits by crude or photochemically changed essential oils–a review. *Food Reviews International*. 18 pages. DOI: 10.1080/87559129.2023.2204157

9. Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Vuong, V.Q., Golding, J.B., Kirkman, T., Pristijono P., 2023. Efficacy of lemon myrtle essential oil as a bio-fungicide in inhibiting citrus green mould. *Plants* 12(21), 3742. doi.org/10.3390/plants12213742
10. Archer, J., Pristijono, P., Vuong, Q.V., Palou, L. and Golding, J.B. 2024. Utilising response surface methodology to optimise food additives and treatments reduces disease caused by *Penicillium digitatum* and *Penicillium italicum* in 'Valencia' oranges. *Horticulturae*, 10(5), 453. doi.org/10.3390/horticulturae10050453

Book chapter

1. Uthairatanakij, A., Laohakunjit, N., Jitareerat, P., Cholmaitri, C., Golding J.B., 2023. Green technology for reducing postharvest losses and improving the nutritional quality of fresh horticultural produce. In Kahramanoglu I. (Ed), New Advances in Postharvest Technology. IntechOpen (on-line) DOI: 10.5772/intechopen.109938 (23 pages).

Previous journal articles/book chapters

Journal articles and book chapters subsequently published from previous Hort Innovation project (Australian Citrus Postharvest Science Program – CT15010) but not reported in CT15010 Final Report (2019)

1. Golding, J.B., Singh S.P., 2020. Advances in the use of irradiation for the market access of fresh horticultural produce. In. Watkins, C. (Ed.), Advances in postharvest management of horticultural produce. pages 251-272. Burleigh Dodds Science Publishing, Cambridge, UK (ISBN: 978 1 78676 288 7).
2. Archer, J., Pristijono, P., Gallien, Q., Houizot, L., Bullot, M., Palou, L., Golding J.B., 2021. Preliminary investigations on the effect of low-pressure treatment on *in vitro* and *in vivo* growth of *Penicillium* sp. in oranges. *Acta Horticulturae* 1325, 55-58. DOI: 10.17660/ActaHortic.2021.1325.9.
3. Papoutsis, K., Stathopoulos, C.E., Bowyer, M.C., Golding J.B., 2021. *In vitro* antifungal activity of lemon (*Citrus limo* L.) waste extracts against *Alternaria alternate* and *Alternaria citri. Acta Horticulturae* 1325, 239-244. DOI: 10.17660/ActaHortic.2021.1325.34

Extension articles

1. Golding J. (2020) One chapter closes, another opens in citrus postharvest research. *Australian Citrus News.* Autumn 2020, p 38-39.
2. Golding J. (2020) One chapter closes, another opens in citrus postharvest research. Citrus e-news. 26 June 2020. https://citrusaustralia.com.au/news/latest-news/one-chapter-closes-another-opens-in-citrus-postharvest-research
3. Golding J. and Archer J. (2020) Postharvest degreening–A refresher. *Australian Citrus News.* Autumn 2020, p 40-41.
4. Golding, J. (2020) Postharvest degreening–A refresher. Citrus e-news. 26 June 2020. https://citrusaustralia.com.au/news/latest-news/a-refresher-on-postharvest-degreening
5. Golding J. and Arche, J. (2020) High levels of technical resistance to some fungicides a concern. *Australian Citrus News*. Winter 2020, p 35-37.
6. Golding J. (2020) Minimising the development of off-flavours in mandarin exports. *Australian Citrus News*. Spring 2020, p 35-37.
7. Golding J., Archer J., Gkountina S. and Singh S.P. (2021) Postharvest evaluation of a new electrolysed water sanitiser system. *Australian Citrus News*. Summer 2020/21, p 31-33.
8. Golding J. and Archer J. (2021) Safe food additives for the control of postharvest decay (2021) *Australian Citrus News*. Autumn 2021, p 36-37.

9. Golding J. (2021) Review your postharvest fungicides. *Australian Citrus News*. Winter 2021, p 32-33.
10. Golding J. (2021) Review your postharvest fungicides. *CitrusConnect* (NSW DPI) November 2021. https://www.dpi.nsw.gov.au/agriculture/horticulture/citrus/content/post-harvest/articles/review-your-postharvest-fungicides
11. Golding J. (2021) Which postharvest sanitisers are best for you? *Australian Citrus News*. Spring 2021, p 40-41.
12. Golding J. and Donovan N. (2022) Managing anthracnose for successful degreening. *Australian Citrus News*. Issue 1 2022, p 44-45.
13. Golding J.B. (2022) Prevention the best cure for fungicide resistance. *Australian Citrus News*. Issue 2 2022. Pages 32-33.
14. Golding J.B., Kavanagh M., Freriechs J., Pristijono P. and Wang B. (2022) Market access options for export lemons. *Australian Citrus News*. Issue 2 2022. Pages 30-33. (Agricultural Trade and Market Access Cooperation)
15. Chavarria J. and Golding J.B. (2022) Practical steps to minimise albedo breakdown. *Australian Citrus News*. Issue 3 2022. Pages 19-20.
16. Golding J.B. and Rahman M. (2022) New research investigates more natural controls of decay. *Australian Citrus News*. Issue 3. 2022. page 37
17. Golding J.B. (2023) How to control sour rot *Australian Citrus News*. Issue 1. 2023. page 40-41
18. Golding J.B. (2023) Benefits of heating postharvest fungicides. *Australian Citrus News*. Issue 2 2023. Pages 20-21.
19. Golding J.B. et al. (2023) Postharvest section and key article 'Managing potential resistance to postharvest fungicides' – Contributions to NSW Department of Primary Industries Citrus Plant Protection Guide 5th edition. Pages 92-97.
20. Golding J.B. (2023) The benefits of waxing citrus. *Australian Citrus News*. Issue 2 2023. Pages 21-22.
21. Golding J.B. (2023) Avoiding off-flavours. *Australian Citrus News*. Issue 4 2023. Pages 30-31.
22. Golding J.B. and Mark Bullôt M. (2024) Reducing the risk of postharvest fungicide resistance. *Australian Citrus News. In press*

Industry presentations

1. Golding J. (2020) 'Hort Innovation Citrus Postharvest Program (CT19003) update and degreening tips'. Teleconference 5 May 2020. Western Australia citrus packers facilitated by Bronwyn Walsh.
2. Golding J. (2020) 'Postharvest sanitation'. Citrus Australia Far North Queensland Regional Forum. Widem Farming, Dimbulah. 10 March 2021.
3. Golding J. (2020) 'Postharvest sanitation'. Citrus Australia Queensland Regional Forum. Mundubbera. 24 March 2021.
4. Golding J. (2021) 'Update on the Hort Innovation Citrus Postharvest Program (CT19003)' presentation at Citrus Australia and NSW Department of Primary Industries Research and Development Roadshow. Three presentations: Dareton (Sunraysia), Renmark (Riverland) and Yanco (Riverina). 19-22 April 2021.
5. Golding J. (2021) 'Postharvest Refresher' presentation and Postharvest Workshop at Lake Wyangan (Griffith) for Griffith and District Citrus Growers Inc. 3 May 2021.
6. Golding J. (2021) 'Fresh citrus decay control and management of resistance'. Teleconference Campbells Chemicals/Citrosol (Spain). 29 July 2021.
7. Golding J. (2021) 'Degreening'. Citrus Australia Southern Queensland Regional Forum. 26 October 2021. Teleconference.
8. Golding J. (2021) 'Postharvest update' presentation and postharvest packer workshop at Tharbogang (Griffith) for Griffith and District Citrus Growers Inc. 9 December 2021

9. Golding J. (2021) 'Improving and maintaining fruit quality' presentation and discussion at Griffith and District Citrus Growers Association Annual General Meeting. Griffith NSW. 9 December 2021.
10. Golding J.B. (2022) Citrus decay control and management of resistance. Citrosol Postharvest Citrus Webinar series. 10 February 2022.
11. Golding J.B. (2022) Horticulture Innovation Citrus Postharvest Program Update. Presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
12. Golding J.B. (2022) Effects of market access treatments on export lemon quality. Presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
13. Mohammad Rahman, Ron Wills, Michael Bowyer, John Golding, Timothy Kirkman and Penta Pristijono (2022) Photo-oxidation of orange essential oil–A simple photochemistry to fight against green mould in citrus fruit as a biofungicide. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
14. John Archer, Penta Pristijono, Quan Vuong, Lluís Palou and John Golding (2022) Using food additives to control postharvest decay. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
15. John Archer, Penta Pristijono, Quentin Gallien, Laure Houizot, Mark Bullot, Lluís Palou and John Golding (2022) Preliminary investigations on the effect of low-pressure treatment on *in vitro* and *in vivo* growth of *Penicillium* sp. in oranges. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
16. Kostas Papoutsis, Costas Stathopoulos, Michael Bowyer and John Golding (2022) *In vitro* antifungal activity of lemon waste extracts against Alternaria. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
17. Nasiru Alhassan, Michael Bowyer, Ron Wills, Penta Pristijono and John Golding (2022) Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays senescence of mandarins and oranges. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
18. Nasiru Alhassan, Ron Wills, Michael Bowyer, Penta Pristijono and John Golding (2022) Pre-storage fumigation with hydrogen sulphide inhibits postharvest senescence of Valencia and Navel oranges and 'Afourer' mandarins. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
19. John Golding and Mark Bullot (2022) Sanitation and Postharvest Fungicide Resistance Service. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
20. John Golding, Madeline Kavanagh, James Freriechs, Baogang Wang and Penta Pristijono (2022) Effects of market access treatments on export lemon quality. Poster presentation at 2022 Citrus Technical Forum. Citrus Australia. Sunshine Coast, Queensland. 9 March 2022.
21. John Golding (2022) Horticulture Innovation. Citrus Postharvest Program Update presentation at Citrus Australia Tristate Forum. Three presentations: Mildura (Sunraysia), Loxton (Riverland) and Leeton (Riverina). 17-20 October 2022.
22. John Golding (2023) Session organiser and convener of the Citrus Australia Market Outlook Forum. Session 2–Avoiding poor outturns and claims in export markets–who holds the key in your business? Session Introduction and Summary. 9 March 2023.
23. John Golding (2023) What can we do about chilling injury? Citrus Australia Market Outlook Forum. 9 March 2023.
24. John Golding (2023) Hort Innovation postharvest project: how it improves outturns. Teleconference to NSW DPI Dareton Citrus Field Day 2023. 13 September 2023.
25. John Golding (2023) 'Postharvest and fruit quality updates' presentation at Citrus Australia Tristate Forum. Mildura (Sunraysia) 16 October 2023.
26. John Golding (2023) 'Postharvest and fruit quality updates' presentation at Citrus Australia Tristate Forum. Loxton (Riverland) 17 October 2023.

27. John Golding (2023) 'Postharvest and fruit quality updates' presentation at Citrus Australia Tristate Forum. Griffith (Riverina). 19 October 2023.
28. John Golding (2023) 'Review and update of Hort Innovation Citrus Postharvest Program (CT19003)' to Citrus SIAP. Hort Innovation North Sydney 22 November 2023.
29. John Golding (2024) Session organiser and convener of the Postharvest and Packaging Workshop at the Citrus Australia Citrus Congress. Sunshine Coast. 6 March 2024.
30. John Golding (2024) Update Horticulture Innovation Postharvest Project. Citrus Australia Citrus Congress. Sunshine Coast. 6 March 2024.
31. John Golding and Mark Bullot (2024) What has been happening with fungicide resistance in Australian citrus packinghouses? Citrus Australia Citrus Congress. Sunshine Coast. 6 March 2024.
32. Mohammad M. Rahman, Ronald B. H. Wills, Michael C. Bowyer, Van Q. Vuong, John B. Golding, Timothy Kirkman and Penta Pristijono (2024) Lemon myrtle essential oils as a potential inhibitor of green mould in Navel oranges. Poster presentation at Australian Citrus Congress. Sunshine Coast. 6-7 March 2024.
33. Mohammad M. Rahman, Ronald B. H. Wills, Michael C. Bowyer, Van Q. Vuong, John B. Golding, Timothy Kirkman and Penta Pristijono (2024) Effect of Lemon myrtle essential oils on the quality of Valencia oranges. Poster presentation at Australian Citrus Congress. Sunshine Coast. 6-7 March 2024.
34. Nasiru Alhassan, Ron B. H. Wills, Michael C. Bowyer and Penta Pristijono and John B. Golding (2024) The effect of auxins 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide on postharvest calyx senescence of Valencia oranges. Poster presentation at Australian Citrus Congress. Sunshine Coast. 6-7 March 2024.
35. John Golding, Enora Grandjean, Steven Falivene, Thuy Pham and Penta Pristijono (2024) Finding reliable and consistent methods to measure citrus fruit firmness. Poster presentation at Australian Citrus Congress. Sunshine Coast. 6-7 March 2024.

Citrus Australia '*The Full Bottle'* podcast. 'Episode 6: Our regional forums' Monday 31 October 2022. Interview on postharvest research update.https://citrusaustraliathefullbottle.podbean.com/e/episode-6-our-regional-forums/

Collaboration with Euphresco project '*Basics—Basic substances as an environmentally friendly alternative to synthetic pesticides for plant protection*'.

Euphresco is a network of organisations that coordinate national research in the phytosanitary area. The overall goal of Euphresco is to support coordination and collaboration in the area of phytosanitary research and maintain itself as a strong, long-term network of research stakeholders. The project examined the use of basic substances (such as sodium bicarbonate) as phytosanitary measures and concluded in August 2023, but on-going academic networking is continuing.

References

Selected references

Alhassan, N., Bowyer, M.C., Wills, R.B.H., Golding, J.B., Pristijono, P., 2020. Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays calyx senescence and loss of other postharvest quality factors of 'Afourer' mandarins, Navel and Valencia oranges. *Scientia Horticulturae.* 272, 109572. doi.org/10.1016/j.scienta.2020.109572.

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).

Alhassan, N., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Pristijono, P., 2020. Pre-storage fumigation with hydrogen sulphide inhibits postharvest senescence of Valencia and Navel oranges and 'Afourer' mandarins. *J Hortic Sci Biotechnol.* 1-6. doi:10.1080/14620316.2020.1749138

Alhassan, N., Wills, R.B.H., Bowyer, M.C., Pristijono, P., Golding J.B., 2022. Comparative study of the auxins 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide to inhibit postharvest calyx senescence and maintain internal quality of Valencia oranges. *New Zealand Journal of Crop and Horticultural Science*, DOI: 10.1080/01140671.2021.2017984

Alhassan, N., Wills, R.B.H., Bowyer, M.C., Golding J.B., Pristijono P., 2020. Pre-storage fumigation with hydrogen sulphide

inhibits postharvest senescence of Valencia and Navel oranges and 'Afourer' mandarins. *The Journal of Horticultural Science and Biotechnology* 95, 757-762. DOI: 10.1080/14620316.2020.17491

Archer J., Pristijono P., Vuong Q.V., Palou L. and Golding J.B. 2021. Effect of low pressure and low oxygen treatments on fruit quality and the *in vivo* growth of *Penicillium digitatum* and *Penicillium italicum* in oranges. *Horticulturae* 7, 582. doi.org/10.3390/horticulturae7120582

Archer, J., Pristijono, P., Vuong, Q.V., Palou, L. and Golding, J.B. 2024. Utilising response surface methodology to optimise food additives and treatments reduces disease caused by *Penicillium digitatum* and *Penicillium italicum* in 'Valencia' oranges. *Horticulturae*, 10(5), 453. doi.org/10.3390/horticulturae10050453

Australian Horticulture Statistics Handbook 2022–23. Horticulture Innovation Australia Limited, 2024 (MT21006).

Burg S.P. 2004. Postharvest physiology and hypobaric storage of fresh produce. E-Book. Cambridge (MA): CABI Publisher.

Carvalho, P.C., Salvador, A., Navarro, P., Monterde, A., Martinez-Javega, M.J., 2008. Effect of Auxin Treatments on Calyx Senescence in the Degreening of Four Mandarin Cultivars. *HortScience,* 43, 747–752. https://doi.org/10.21273/HORTSCI.43.3.747

Cronjé, P.J.R., Crouch, E.M., Huysamer, M., 2005. Postharvest calyx retention of citrus fruit. Acta Horticulturae 628, 369-376. https://doi.org/10.17660/ActaHortic.2005.682.43

Du, Y., Li, Y., Tian, Z., Cheng, Y. and Long, C.A., 2022. Natamycin as a safe food additive to control postharvest green mould and sour rot in citrus. *Journal of Applied Microbiology*, *133*(6), pp. 3438-3450.

Golding J.B. and Archer J. 2019. Advances in postharvest handling of citrus fruit. In: *Achieving sustainable cultivation of tropical fruits*. Ed. Elhadi M. Yahia. pp 65-90. Burleigh Dodds Science Publishing, Cambridge. UK. ISBN: 978 1 78676 284

Golding J. and Palou L. 2018. Review – Exploring the potential of low dose irradiation phytosanitary treatments for the Australian citrus industry. Contribution to Horticulture Innovation Citrus Postharvest Science Program (CT15010). 24 pages.

Lindhout, K. (2007) Physiology of chilling-related postharvest rind breakdown of navel oranges (*Citrus Sinensis* (L.) Osbeck). PhD thesis. La Trobe University. Australia.

Ma, Q., Zhu, L., Feng, S., Xu, R., Kong, D., Deng, X., Cheng, Y., 2015. Fluroxypyr – a potential surrogate of 2,4-dichlorophenoxyacetic acid for retarding calyx senescence in postharvest citrus fruit. *Postharvest Biol Technol.* 105, 17-25. https://doi.org/10.1016/j.postharvbio.2015.03.006

Marini, R.P., Byers, R.E., Sowers, D.L., Young, R.W., 1990. Fruit Abscission and Fruit Quality of Apples Following Use of Dicamba to Control Preharvest Drop. *J. Amer. Soc. Hort. Sci.* 115, 390-394.

Meena, M., Prajapati, P., Ravichandran, C. and Sehrawat, R., 2021. Natamycin: a natural preservative for food applications—a review. *Food Science and Biotechnology*, pp. 1-16.

Miles A. 2016. Increasing market access, profitability and sustainability through integrated approaches to fungal disease control. Horticulture Innovation Australia Final Report Project C13020. 64 pages.

Montesinos-Herrero, C., del Río, M.Á., Pastor, C., Brunetti, O. and Palou, L., 2009. Evaluation of brief potassium sorbate dips to control postharvest Penicillium decay on major citrus species and cultivars. *Postharvest Biology and Technology*, *52*(1), pp. 117-125.

NSW DPI Citrus Plant Protection guide (2023–24). Creek A, Donovan N, Falivene S, Golding J, Mahmud K, Khurshid T, Mo J and Monks D *Citrus plant protection guide*, NSW Department of Primary Industries, Orange. https://www.dpi.nsw.gov.au/agriculture/horticulture/citrus/content/manuals-guides/citrus-plant-protection-and-management-guide

Obenland, D. and Arpaia M.L. 2019. Effect of harvest date on off-flavor development in mandarins following postharvest wax application. *Postharvest Biology and Technology* 149, 1-8.

Onay F., Akgul N.B. and Akocak P.B. 2014. Natamycin treatment to control postharvest mold development and improve storability of citrus fruits. *Journal of Food, Agriculture and Environment*. 12, 188– 192.

Palou L. 2013. Mini review: Heat treatments for the control of citrus postharvest green mold caused by Penicillium

digitatum. In: Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education. Vol. 1 Editors: Méndez-Vilas. Publisher: Formatex Research Center, Badajoz, Spain

Palou L., Ali A., Fallik E., and Romanazzi G. 2016. GRAS, plant- and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. *Postharvest Biology and Technology.* 122, 41-52.

Palou L., Smilanick J.L., Usall J. and Vinas I. 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. *Plant Disease* 85, 371–376.

Porat R., Feng X., Huberman M., Galili D., Goren R. and Goldschmidt E. E. (2001). Gibberellic acid slows postharvest degreening of Oroblanco citrus fruits. *HortScience* 36, 937-940.

Pristijono P., Bowyer M., Scarlett C., Vuong Q., Stathopoulos C. and Golding J.B. 2018. Combined postharvest UV-C and 1-methylcyclopropene (1-MCP) treatment, followed by storage continuously in low level of ethylene atmosphere improves the quality of Tahitian limes. *Journal of Food Science and Technology* 55, 2467-2475.

Pristijono P., Bowyer M.C., Scarlett C.J., Vuong Q.V., Stathopoulos C.E. and Golding J.B. 2017. The effect of postharvest UV-C treatment and associated with different storage conditions on the quality of Tahitian limes (*Citrus latifolia*). *Journal of Food and Nutritional Disorders* 6:4. doi:10.4172/2324-9323.1000230

Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Kirkman, T., Pristijono P., 2020. Efficacy of orange essential oil and citral after exposure to UV-C irradiation to inhibit *Penicillium digitatum* in navel oranges. *Horticulturae* 6, 102. doi:10.3390/horticulturae6040102

Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Kirkman, T., Pristijono P., 2022. Lemon myrtle and lemon scented tea tree essential oils as potential inhibitors of green mould on citrus fruits. *The Journal of Horticultural Science and Biotechnology*, DOI: 10.1080/14620316.2021.2011433

Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Golding, J.B., Kirkman, T., Pristijono P., 2023. Potential control of postharvest fungal decay of citrus fruits by crude or photochemically changed essential oils–a review. *Food Reviews International.* 18 pages. DOI: 10.1080/87559129.2023.2204157

Rahman, M.M., Wills, R.B.H., Bowyer, M.C., Vuong, V.Q., Golding, J.B., Kirkman, T., Pristijono P., 2023. Efficacy of lemon myrtle essential oil as a bio-fungicide in inhibiting citrus green mould. *Plants* 12(21), 3742. doi.org/10.3390/plants12213742

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.

Schirra M., D'Aquino S., Cabras P. and Angioni A. (2011) Control of postharvest diseases of fruit by heat and fungicides: efficacy, residue levels, and residue persistence. A review. *Journal of Agricultural and Food Chemistry*, 59, 8531-8542.

Sdiri, S., Navarro, P., Salvador, A., 2013. Postharvest application of a new growth regulator reduces calyx alterations of citrus fruit induced by degreening treatment. *Postharvest Biol Technol.* 75, 68-74. doi: 10.1016/j.postharvbio.2012.08.004

Smilanick, J.L., Mansour, M.F., Gabler, F.M. and Sorenson, D., 2008. Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. *Postharvest Biology and Technology*, *47*(2), pp. 226-238.

Wills R.B.H. and Golding J.B. 2016. Postharvest: An Introduction to the Physiology and Handling of Fruit and Vegetables. NewSouth Publishing. Sydney Australia.

Yİğİter, B., Onay, F., Akgul, N.B. and Akocak, P.B., 2014. Natamycin treatment to control postharvest mold development and improve storability of citrus fruits. *Journal of Food, Agriculture & Environment*, *12*(2), pp. 188-192.

Youssef K., Abd-Elsalam K., Hussien A., Sanzani S. and Ippolito A. 2017. Organic and inorganic salts as postharvest alternative control means of citrus. Chapter 8. Citrus Postharvest Pathology. IntechOpen. http://dx.doi.org/10.5772/67228 (19 pages).

Intellectual property

No project IP or commercialisation to report.

Acknowledgements

The Program thanks Hort Innovation and Australian citrus growers and packers for their assistance and co-operation.

Special thanks and appreciation to the Project Reference Group: Nathan Hancock (Citrus Australia), Allen Jenkin (Ironbark Citrus), Michael Kay (Northern Valley Packers), Craig Chappell (Mildura Fruit Company), Kevin Quinlan (NSW DPI) and Ashley Zamek/Jacqui Simpson (Horticulture Innovation). All members of the PRG were active and supportive in guiding the Program both at the official PRG meetings and outside of the meetings with additional meetings and visits. The success of the Program was in-part due to the proactive and engaging role of the PRG.

NSW Department of Primary Industries co-invested in this Program with in-kind salaries to support project management and scientific and technical inputs throughout the Program. NSW DPI also supported the maintenance of research laboratories, electricity, project support, biometric support, and ongoing quality assurance.

The Program thanks all NSW Department of Primary Industries staff, including Thuy Pham, John Archer, Mark Bullot, James Freriechs, Shashi Satyan and Maddy Kavanagh, who all helped conduct many of these postharvest trials and data management. David Cruickshank and Christine Cruickshank for technical assistance during busy times. Steven Falivene and Andrew Creek for collaboration with all experiments, sourcing and harvesting the fruit for experiments and coordinating workshops. Amanda Warren Smith for assisting the Final Report. Kevin Quinlan for management support. We also thank the staff at NSW Department of Primary Industries at Ourimbah for volunteering to assess the fruit for storage trials.

The Program thanks and acknowledges the invaluable assistance and support of:

- The University of Newcastle. Staff–Dr Penta Pristijono, Dr Michael Bowyer, the late Dr. Ron Wills, Dr Quan Vuong. Students–John Archer, Mohammad Rahman and Nasiru Alhassan.
- Institut Valencià d'Investigacions Agràries (IVIA) (Spain) – Prof. Lluis Palou
- Support from Citrus Australia, E.E. Muir & Sons Pty Ltd, Colin Campbell (Chemicals) Pty. Ltd., Landmark
- Visiting students from France: Marine Lardier, Enora Grandjean, Matthieu Morel, Marine Tremblay, Camille Prouff, Chloé Jaquen, Dominique Versini, Tifenn Allard, Marek Falco, Emma LeGal and Aude Sol.

The Program would finally like to thank the growers and packers who have supported this project with packinghouse visits, collaboration with trials and supply of trial fruit.

Appendices

Appendix 1. General Methods

Appendix 2. Technical Report. Results and Discussion

Appendix 3. Literature update–Review of albedo breakdown

Appendix 1. General Methods

Postharvest decay infection and 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 packing houses in Griffith, NSW. Conidia were then harvested from both species by adding 5 mL of sterile, de-ionised water containing 0.05% Triton X-100 to the petri dish. Conidia were then rubbed with a sterile glass rod, and conidia suspension was passed through 2 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 25 °C and 95% relative humidity, and then the number of decayed fruit was counted and the diameter of the infection measured (mm).

Fruit quality assessment

Weight loss

- Weight loss of the fruit was assessed using an electronic balance, where the fruit weight of each treatment unit was recorded each assessment day. Weight change was expressed as a percentage determined by deducting the initial weights (W1) from the final weights (W2), divided by the initial weights and multiplied by a hundred per cent (%).

Fruit glossiness

- Objective measure. A BYK spectrophotometer was used to measure the brightness of the fruit's surface. This measuring device uses beams of light to assess the reflection and scattering of light by the surface of the fruit. Measurements were repeated at 10 points on each fruit to ensure that the sample was representative.
- Subjective assessment. The severity of fruit skin glossiness was assessed visually according to a 4-point scale, as follows: 1 = not shiny/glossy; 2 = little shiny/glossy; 3 = shiny/glossy; and 4 = very shiny/glossy.

Chilling Injury

- The severity of chilling injury symptoms was assessed visually according to a five-stage scale, as follows: 1 = no pitting; 2 = a few scattered pits–just one or 2 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.

Albedo breakdown

- The severity of albedo breakdown symptoms was assessed visually according to a five-stage scale, as follows: 1 = no symptoms; 2 = slight/maybe some; 3 = definite visible and obvious symptoms; 4 = moderate breakdown; and 5 = severe breakdown.

Calyx condition

- Calyx changes were assessed using the method of Alhassan et al. (2019) with calyx colour scored on a 5-point scale where 1 = no browning; 2 = <25% brown; 3 = 25–50% browning; 4 = 50–75% browning; and 5 = >75% browning. A browning score was only assigned to fruit with an intact calyx.

  In general, for each assessment period, the mean browning score of fruit in each treatment unit was calculated. The number of fruit experiencing calyx detachment and the number of fruit showing visible mould were also recorded as a percentage of the fruit in a unit.

Ethylene production and respiration rate

For each assessment, 3 fruits were randomly selected from each treatment unit, weighed and placed in a 2 L airtight glass jar fitted with a septum in the lid. The jar was then sealed for 2 hours at 20 °C, after which a 1 mL sample of the headspace was withdrawn for analysis.

- The concentration of ethylene in the headspace was determined using a flame ionisation gas chromatograph (Gow-Mac 580, Bridgewater NJ) fitted with a stainless-steel column (2 m × 3.2 mm OD × 2.2 mm ID) packed with Porapak Q (80–100 mesh) (Altech, Sydney), with 70, 90 and 110 °C as the operating temperatures of the injector, column and detector, respectively. Hydrogen, nitrogen and air were used as carrier and combustion gases set at flow rates of 30, 60 and 300 mL min^{-1}, respectively. Ethylene production was expressed as µL C_2H_4 kg^{-1} h^{-1} (Huque et al. 2013).
- The fruit respiration rate was measured using the method described by Pristijono et al. (2018) with little modification. Briefly, a 5 mL gas sample was withdrawn from the headspace of the fruit, weighed and sealed in an airtight 2 L glass jar at 20 °C for 2 hours. Carbon dioxide levels were determined using an ICA40 series low-volume gas analysis system (International Controlled Atmosphere Ltd., Kent, UK) with respiration rate expressed as mL CO_2 kg^{-1} h^{-1}.

Measurement of fruit firmness

- Fruit firmness was determined on 10 randomly selected fruit from each treatment unit for each treatment at the end of the storage period using a texture analyser (Lloyd Instrument Ltd., Fareham, UK). The maximum force (N) was measured by compressing the fruit at the equatorial zone between 2 flat surfaces closing together at the rate of 1 mm min^{-1} to a depth of 2 mm. Two readings were taken with the average value recorded as the firmness level.

Determination of internal quality

Juice ethanol concentration

- Ethanol concentration (mg 100 mL^{-1}) in orange juice was measured according to the method of Alhassan et al. (2019). Briefly, aliquots (10 mL) of orange juice were manually squeezed from 5 fruit in each unit into a 20 mL vial. The vials were then sealed with crimp top fitted with a 2 mm rubber septum and incubated for 10 minutes at 30 °C in a water bath. A 1 mL sample of the headspace was withdrawn from the vial and injected in a gas chromatograph (Series 580, GOW MAC, Bethlehem, PA, USA) fitted with a stainless steel (1.2 m × 3 mm) Porapak® QS 80/100 column and equipped with a flame ionisation detector. The gas settings were nitrogen (carrier gas) (30 mL min^{-1}), hydrogen (19 mL min^{-1}) and air (300 mL min^{-1}). The detector, injector and column temperatures were set at 163, 164 and 142 °C, respectively. A 10 mL solution containing ethanol (5 µL L^{-1}) in a 20 mL sealed vial was incubated at the same temperature and used as an internal standard.

Total soluble solids (TSS)

- Total soluble solids (TSS) were determined from the juice of at least 5 fruit per treatment unit, which was filtered through 2 layers of muslin gauze. Measurements were recorded at 20 °C as the refractive index using a portable digital refractometer (Atago, Tokyo) and expressed as a percentage %Brix.

Fruit juice titratable acidity (TA)

- Titratable acidity (TA) was measured by titrating 5 mL of expressed juice with 0.1 M NaOH to pH 8.2 by an automatic titrator (Mettler Toledo, Switzerland). Data were expressed as % citric acid equivalents.

Vitamin C content

- The vitamin C content of the juice was estimated using an iodometric Hanna Ascorbic Acid Test Kit, which required 10 mL of freshly prepared juice. These results were expressed as parts per million ascorbic acid.

Juice content (%)

- Ten fruit per treatment unit were hand-juiced and the juice was weighed after filtering through a stainless-steel strainer (1 mm mesh). The content of the juice was calculated from the fresh weight of the fruit sample.

Appendix 2. Technical Report. Results and Discussion

Alternative decay control

While synthetic fungicides are currently essential for the marketing of citrus, particularly for long-distance and exports, there is growing consumer demand for lower chemical residues and alternative decay control measures.

This section describes studies on a range of new and semi-commercial treatments on the postharvest control of green and blue mould and their effects on fruit quality:

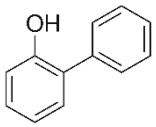
- Ortocil®
- Cerafruta®
- DeccoPlus®
- Fruit Mag®
- Essential oils
- Optimising organic salts

In addition, a series of postharvest trials were conducted to improve how anthracnose is managed in Imperial mandarins. Non-chemical (physical) treatments were also assessed.

Ortocil®

Ortho-phenylphenol (OPP) or 2-phenylphenol is one of the monohydroxylated isomers of biphenyl. It is a biocide used as a preservative with E number E231, which is allowed as a postharvest treatment in some countries. It is sold as Ortocil®.

A related product, sodium *ortho*-Phenylphenol (SOPP), is currently registered as Preventol® ON Fungicide for the control of blue mould (APVMA, 2024). However, a limitation of the use of SOPP is that it is recommended to use the SOPP solution over pH 12. This caustic high pH can cause phytotoxic issues in some situations. This formulation of OPP is at neutral pH and has broader applications.



o-phenylphenol

a. Effect of Ortocil® on blue and green mould

Methods

Organic Navel oranges from NSW DPI Somersby Research farm were infected with green and blue mould (as per General Methods–Appendix 1) (Figure 1) and treated with 1% Ortocil® or 2% Ortocil® and compared to untreated control and an imazalil and thiabendazole dip (as per label recommendations). The fruit were dipped for 30 seconds. There were 40 fruit per treatment unit with 4 independent replicates (i.e. each dip solution was prepared for each replicate).

Figure 1. Infecting oranges with blue and green mould (left), treating infected fruit with Ortocil® fungicide (middle) and assessing the development of decay (right) in storage at 25 °C at NSW Department of Primary Industries.

Results

The results show that a 30-second dip in Ortocil® reduced the incidence of green and blue mould (Figures 2–4). The pH of the dip solutions is presented in Table 1, and it shows that these dip solutions did not significantly change after dipping and remained around pH 7–8 (tap water). This is in comparison to the current formulation of SOPP, which is caustic (very high pH) and can cause phytotoxic fruit damage.

Figure 2. Effect of water dip (control) (top left), imazalil (top right), 1% Ortocil® (bottom left) and 2% Ortocil® (bottom right) treatment on the development of green and blue mould after 7 days of storage at 25 °C.

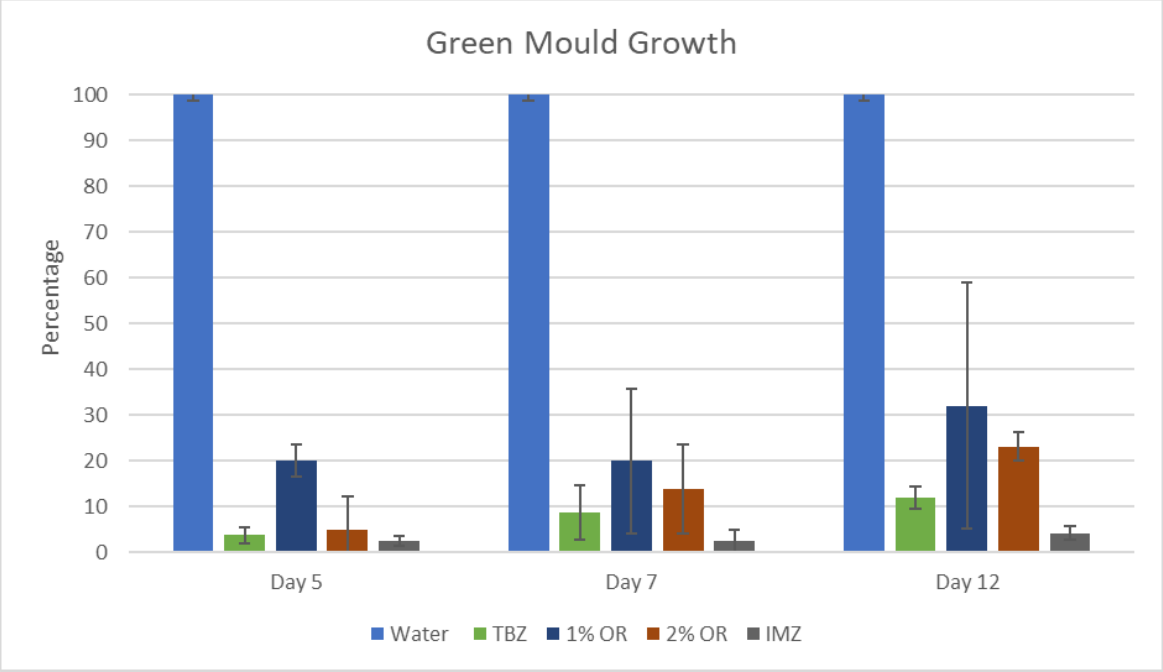


Figure 3. Percentage of green mould (%) following treatment with 1%, 2% Ortocil® (OR), untreated control (water), thiabendazole (TBZ) or imazalil (IMZ) over 12-days at 20 °C. There were 4 replicates assessed per treatment.

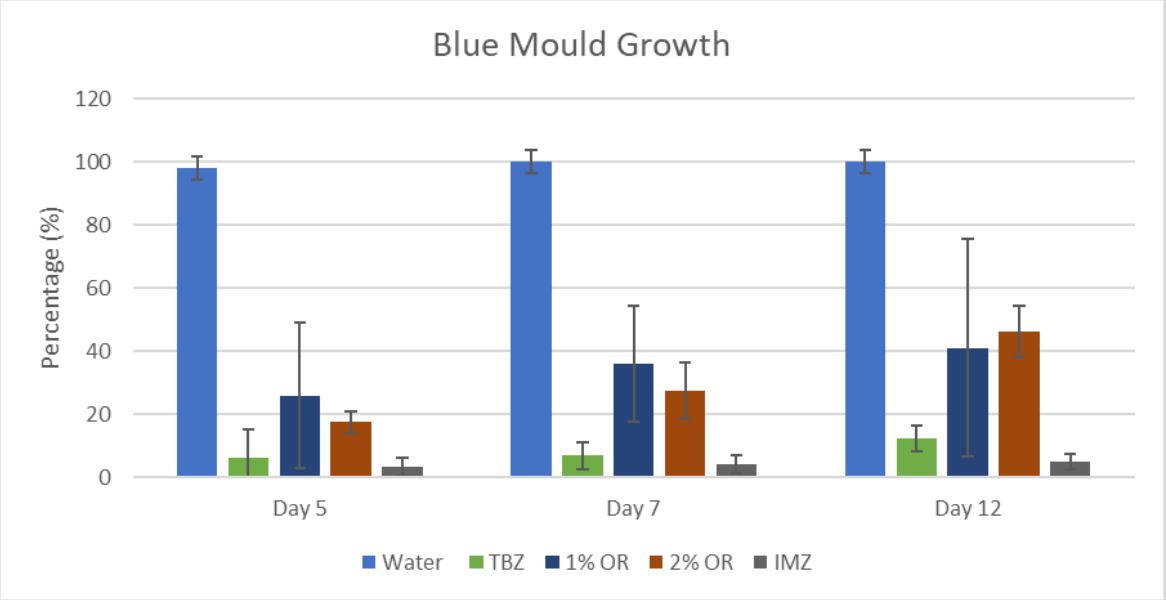


Figure 4. Percentage of blue mould (%) following treatment with 1%, 2% Ortocil® (OR), untreated control (water), or imazalil (IMZ) over 12 days at 20 °C. There were 4 replicates assessed per treatment.

Table 1. Solution pH of the dips following a 30-second dipping treatment

Before treatment	Water	pH 7.83 (at 15.2 °C)
After treatment	1% Ortocil®	pH 7.20 (at 16.7 °C)
	2% Ortocil®	pH 7.25 (at 16.4 °C)
	Imazalil	pH 7.56 (at 16.4 °C)

b. Effect of Ortocil® on Navel fruit quality

Methods

Navel oranges were harvested from a commercial orchard in Leeton (NSW) and transported to NSW DPI at Ourimbah. Fruit were then treated with 1% Ortocil®and 2% Ortocil® and compared to untreated control (water dip) and imazalil (label rate). Fruit were dipped for 30 seconds (Figure 5). There were 10 fruit per treatment unit with 4 replicates (i.e. each dip solution was prepared for each replicate).

After treatment, one set of fruit was stored continuously at 20 °C for 4 weeks (Part A Shelf-life trial). Another set of fruit was stored at 3 °C for 4 weeks (Part B Storage trial). Fruit quality was assessed at weekly intervals according to the General Methods–overall acceptability, weight loss, respiration rate, fruit firmness, chilling injury (3 °C fruit only), TSS, TA, vitamin C content, juice ethanol content and natural decay infection rates.

Figure 5. Dipping Navel oranges with trial dip solutions for fruit quality storage and shelf life assessments at NSW Department of Primary Industries.

Results

Part A. Shelf life trial

The effects of Ortocil® (and imazalil treatment) on the shelf life of Navel oranges stored at 20 °C for 4 weeks are presented in Figures 6–11. There were no negative effects of Ortocil® treatment on the overall quality of the fruit. The results of the average overall acceptability score presented in Figure 6 show that the water treatment (control) had the lowest acceptability over the 4-week shelf life. This was reflected in the fruit firmness (Figure 7). As expected, the untreated water treatment had higher levels of naturally occurring rots than the Ortocil® and imazalil-treated fruit (Figure 8). The treatment did not affect other fruit quality parameters (e.g. TSS).

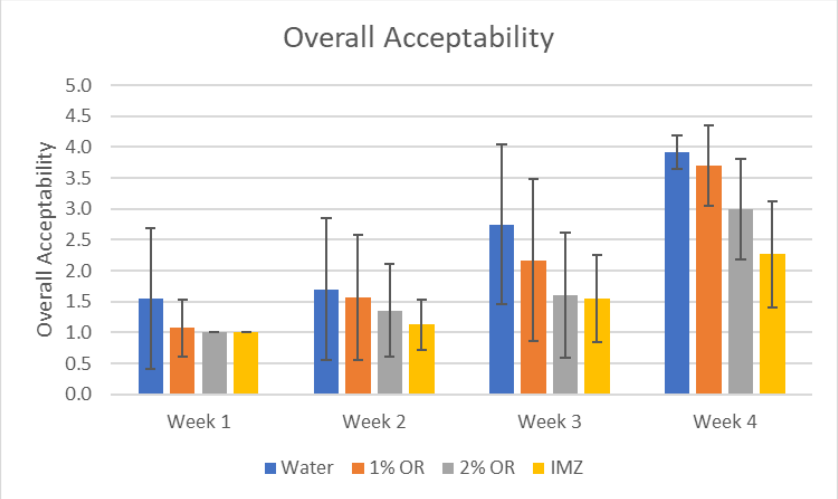


Figure 6. Effect of 1% and 2% Ortocil® and imazalil on the overall acceptability score of Navel oranges during storage at 20 ˚C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

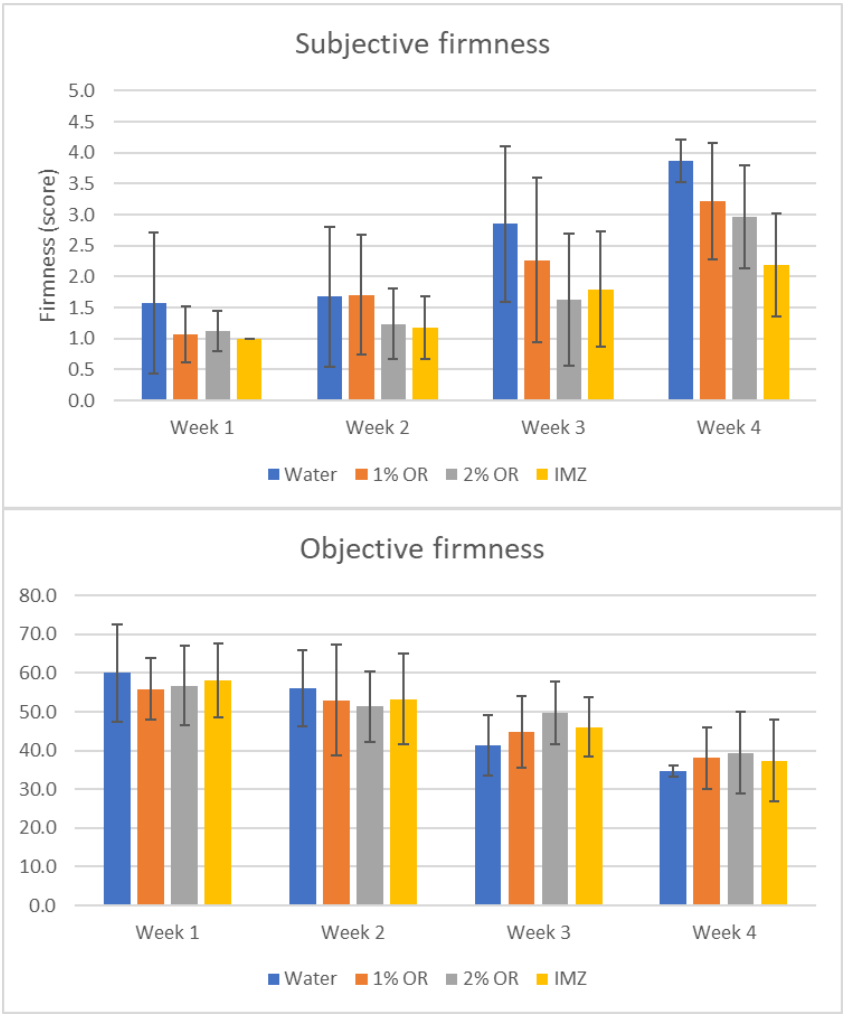


Figure 7. Effect of 1% and 2% Ortocil® and imazalil on the fruit firmness subjective score (top) and objective firmness (lower) of Navel oranges during storage at 20 °C for 4 weeks.
The values are means of 4 replicates with 10 fruit per replicate.

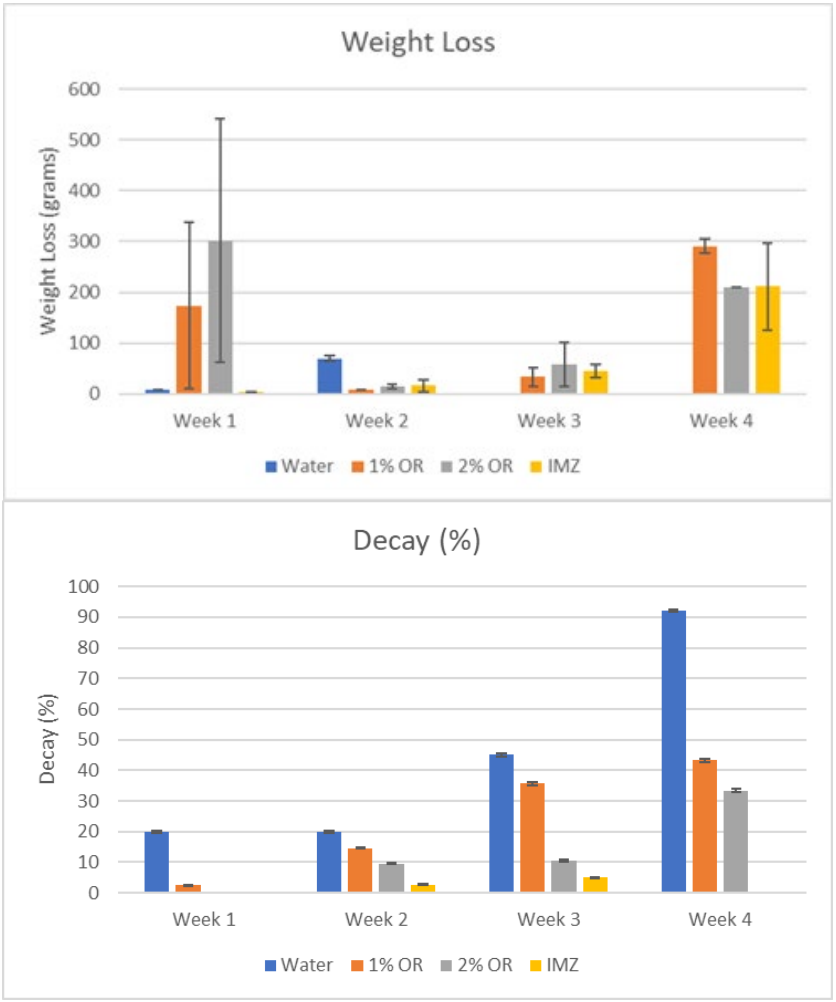


Figure 8. Effect of 1% and 2% Ortocil® and imazalil on average fruit weight loss (top) and percentage of rots in Navel oranges (lower) during storage at 20 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

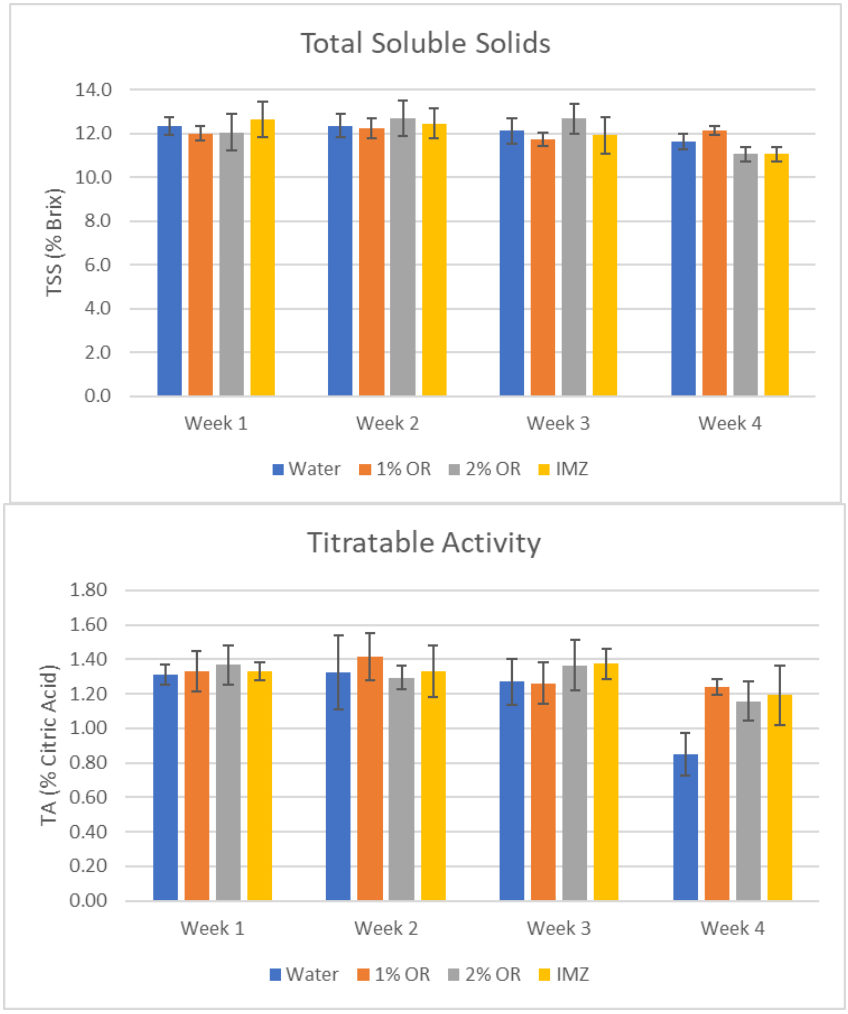


Figure 9. Effect of 1% and 2% Ortocil® and imazalil on the fruit total soluble solids (TSS, % Brix) (top) and titratable acidity (TA, % citric acid) (lower) in Navel oranges during storage at 20 °C for 4 weeks.
The values are means of 4 replicates with 10 fruit per replicate.

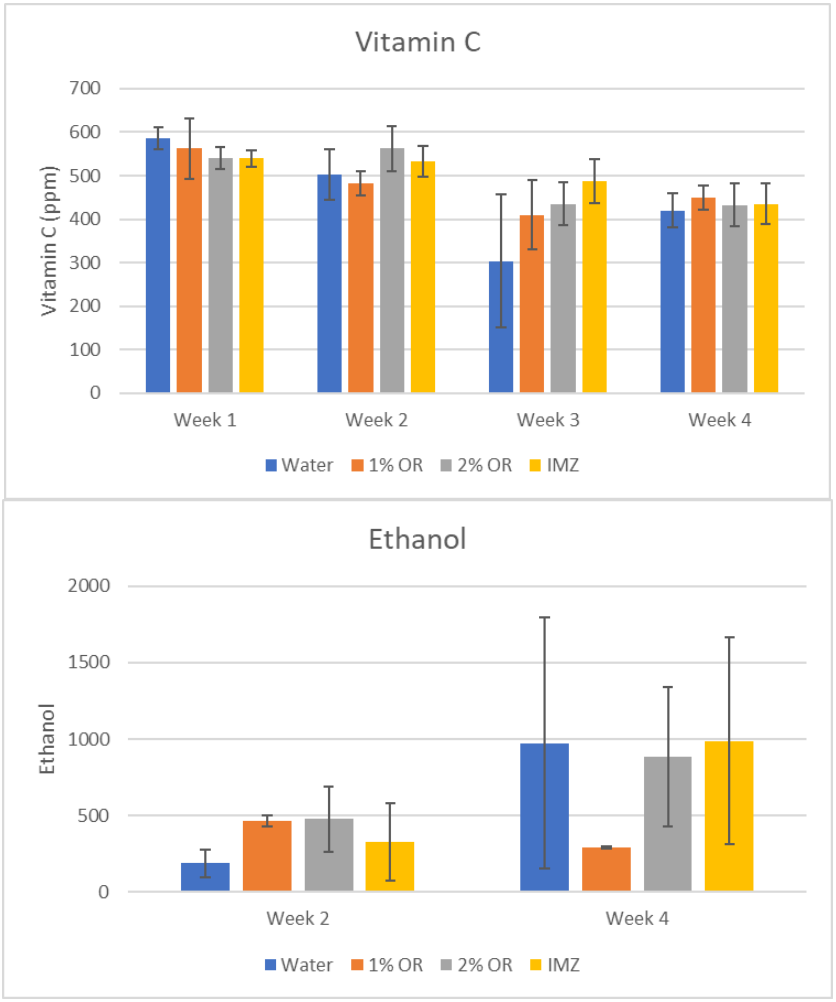


Figure 10. Effect of 1% and 2% Ortocil® and imazalil on the fruit vitamin C content (ppm) (top) and juice ethanol content (mg 100 mL^{-1}) (lower) in Navel oranges during storage at 20 °C for 4 weeks.
The values are means of 4 replicates with 10 fruit per replicate.

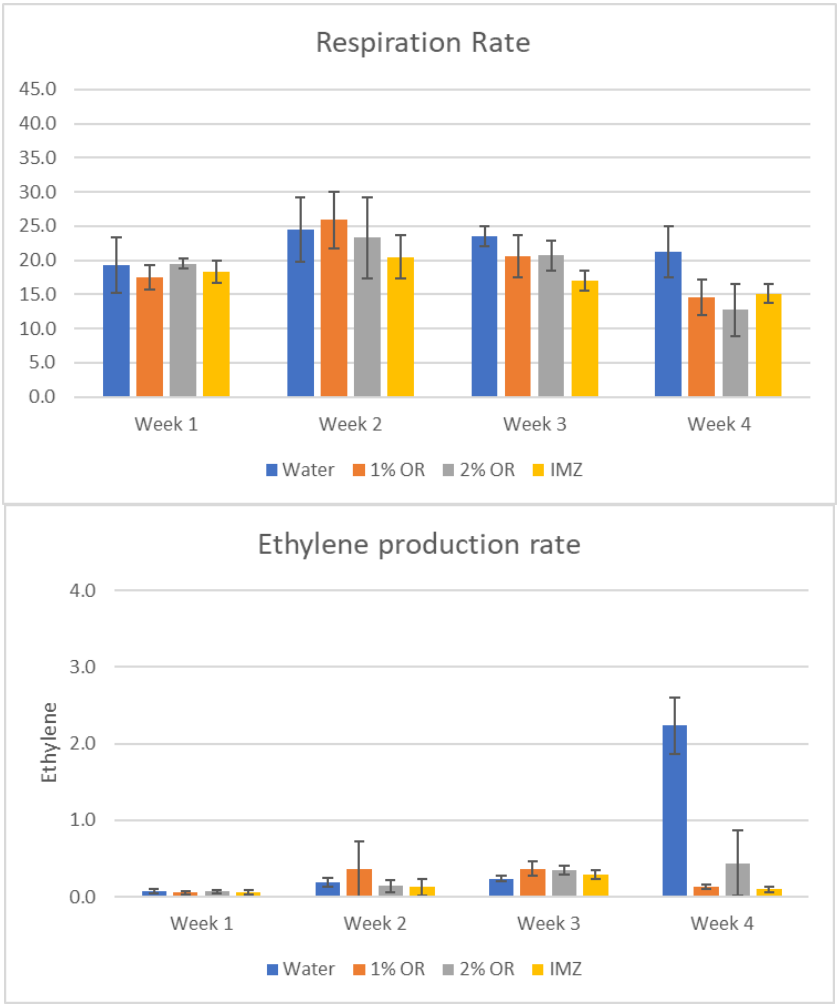


Figure 11. Effect of 1% and 2% Ortocil® and imazalil on the fruit respiration rate (mL CO_2 kg^{-1} h^{-1}) (top) and ethylene production rates (µL C_2H_4 kg^{-1} h^{-1}) (lower) in Navel oranges during storage at 20 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

Results

Part B. Storage trial

The effects of Ortocil® (and imazalil treatment) on the storage life of Navel oranges stored at 3 °C for 4 weeks are presented in Figures 12–18. The results showed that there were no negative effects of Ortocil® treatment on the overall quality and all other fruit quality parameters. The water loss data were confounded with the levels of decay with affected fruit weights in the treatment bags. The treatment did not affect other fruit quality parameters (e.g. TSS).

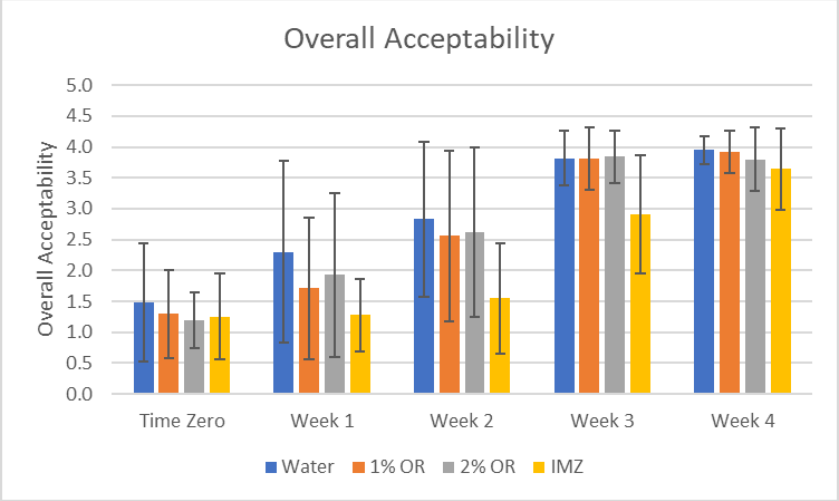


Figure 12. Effect of 1% and 2% Ortocil® and imazalil on the average overall fruit acceptability score in Navel oranges during storage at 3 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

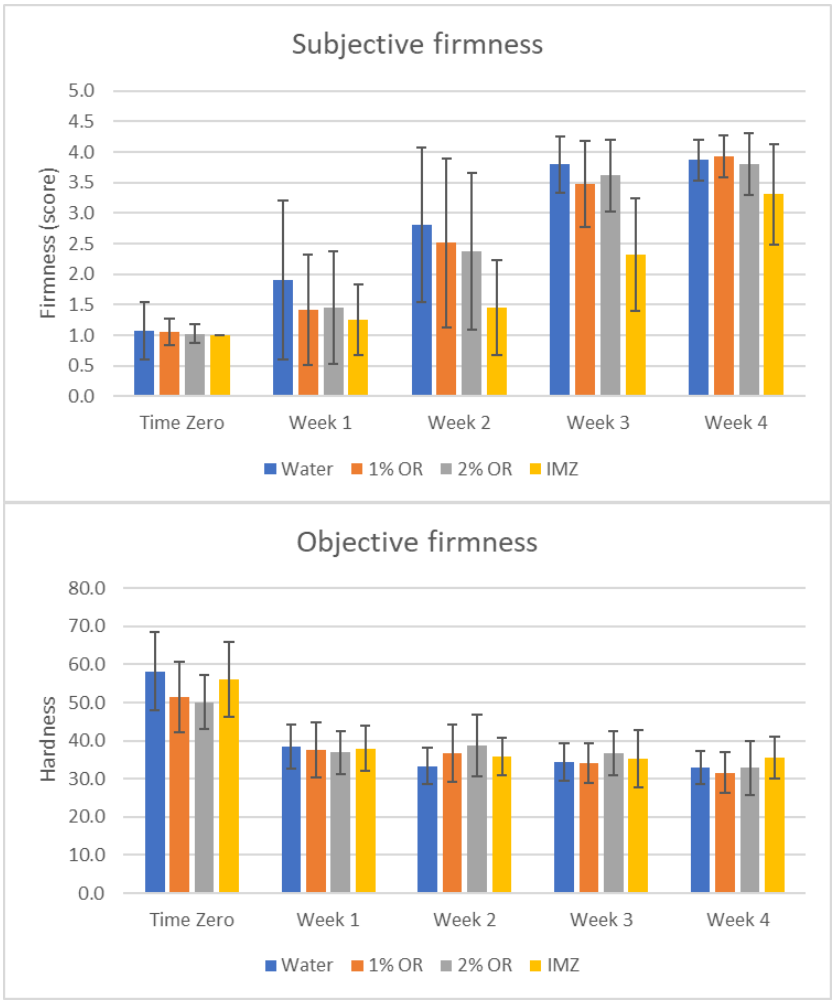


Figure 13. Effect of 1% and 2% Ortocil® and imazalil on the subjective firmness score (top) and objective fruit firmness (lower) in Navel oranges during storage at 3 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

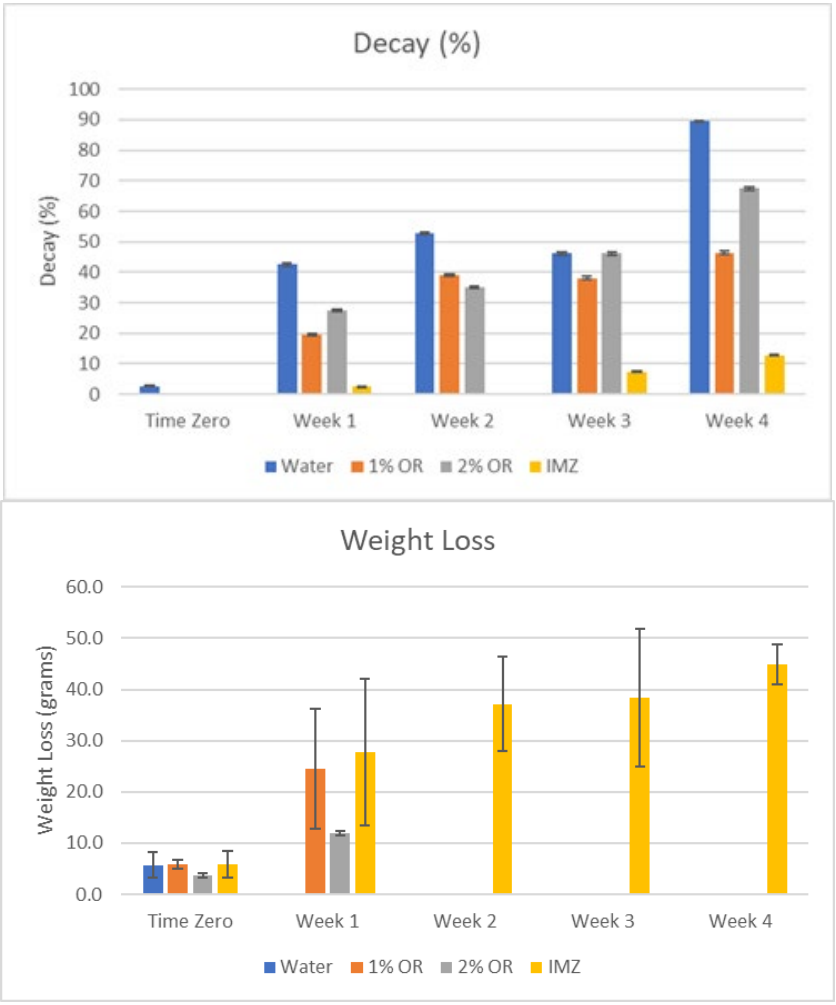


Figure 14. Effect of 1% and 2% Ortocil® and imazalil on the decay (%) (top) and weight loss (g) (lower) in Navel oranges during storage at 3 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

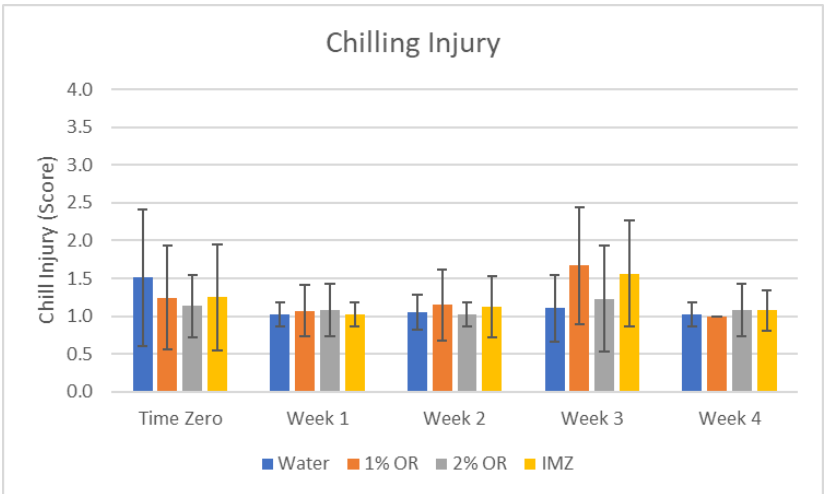


Figure 15. Effect of 1% and 2% Ortocil® and imazalil on the subjective levels of chilling injury (score) in Navel oranges during storage at 3 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

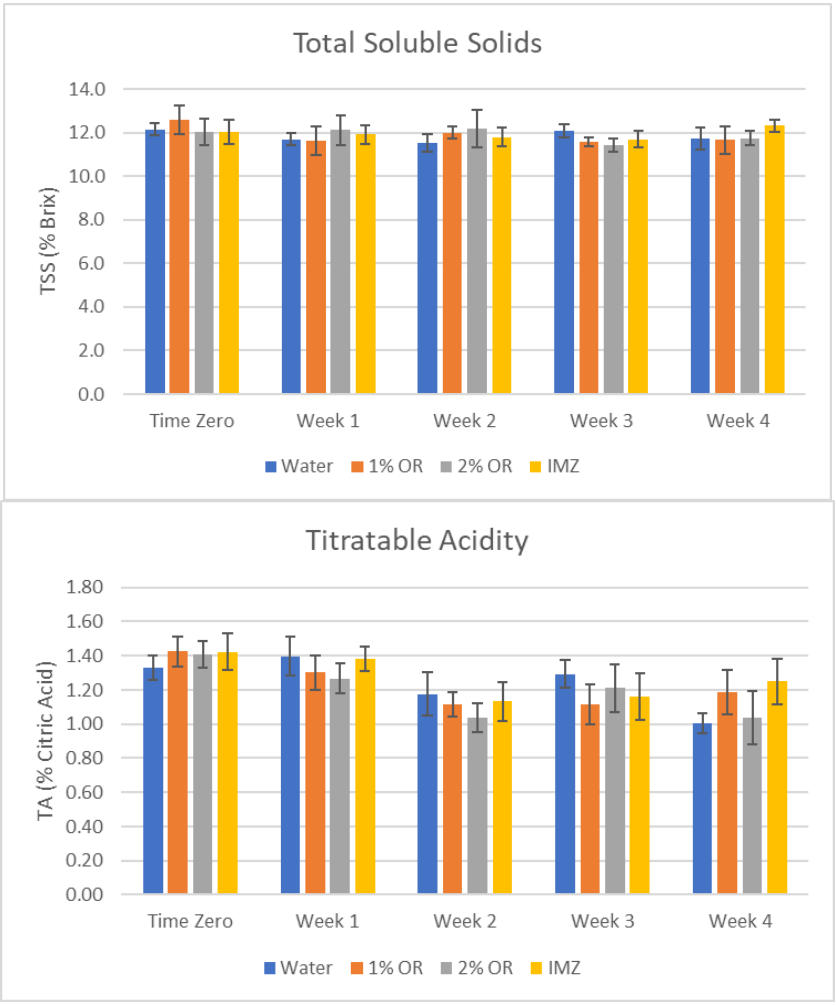


Figure 16. Effect of 1% and 2% Ortocil® and imazalil on the levels of total soluble solids (TSS, % Brix) (top) and titratable acidity (TA, % citric acid) (lower) in Navel oranges during storage at 3 °C for 4 weeks.
The values are means of 4 replicates with 10 fruit per replicate.

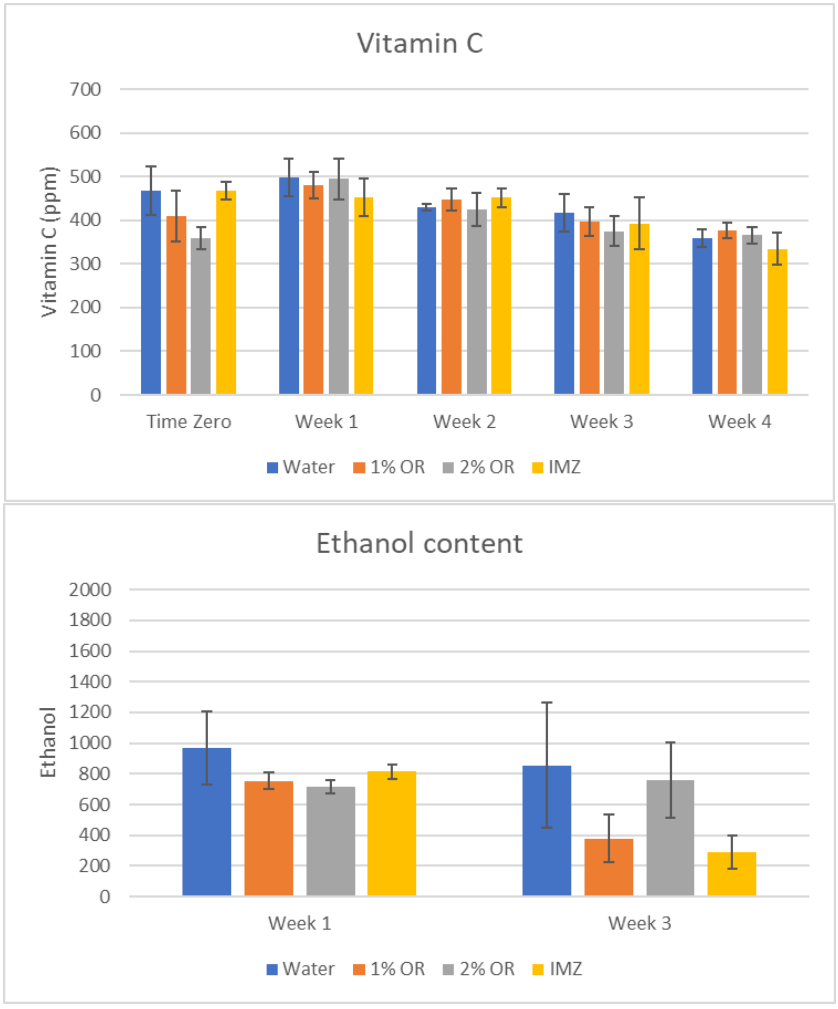


Figure 17. Effect of 1% and 2% Ortocil® and imazalil on the levels of vitamin C (ppm) (top) and juice ethanol content (mg 100 mL^{-1}) (lower) in Navel oranges during storage at 3 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

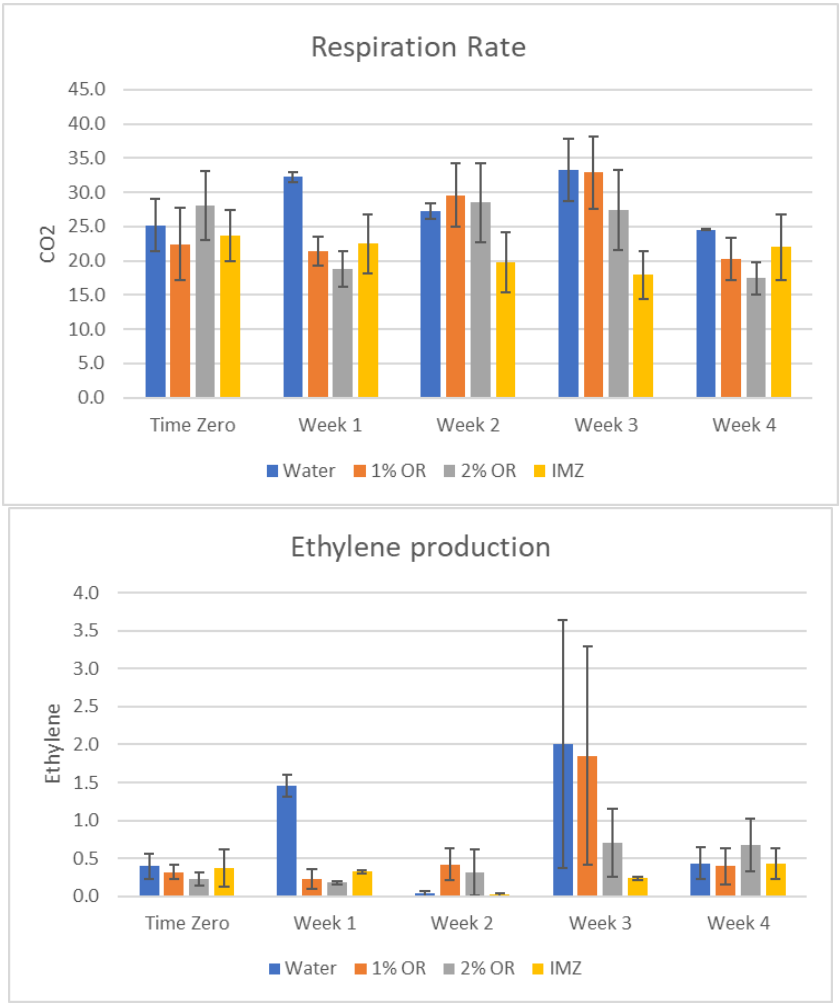


Figure 18. Effect of 1% and 2% Ortocil® and imazalil on the fruit respiration rate (mL CO_2 kg^{-1} h^{-1}) (top) and fruit ethylene production rates (µL C_2H_4 kg^{-1} h^{-1}) (lower) in Navel oranges during storage at 3 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

c. Effect of extended treatment times on the effectiveness of Ortocil®

The dipping time in previous experiments was 30 seconds. This is a good dipping time for commercial situations, but it is important that longer dipping times do not adversely affect final fruit quality. A small trial examined the effect of dipping times up to 10 minutes on fruit quality.

Methods

Navel oranges were harvested from a commercial orchard in Leeton (NSW) and transported to NSW DPI at Ourimbah. Fruit were then treated with 1% Ortocil® and 2% Ortocil® for 1, 2 and 5 minutes. There were 10 fruit per treatment unit with 4 replicates (i.e. each dip solution was prepared for each replicate). A single replicate of fruit was treated with 1% and 2% Ortocil® for 10 minutes. The fruit was stored continuously at 20 °C for 3 weeks with weekly assessments according to the General Methods–overall acceptability, weight loss, respiration rate, fruit firmness, TSS, TA, vitamin C content, juice ethanol content and natural decay infection rates.

Results

The visual quality of the Navel oranges dipped in 1% Ortocil® or 2% Ortocil® for 10 minutes is shown in Figure 19 and shows no adverse effects of the longer treatment times. The data from the different fruit quality parameters on fruit treated with different dip times are presented in Figures 20–23. The longer dip treatment times did not have a negative effect on overall fruit quality in the early stages of shelf life at 20 °C, but after the longest shelf life (3 weeks), the long treatment times were of lower quality than the water control (Figure 20), particularly at the higher treatment concentration (2% Ortocil®). This was reflected in the less firm fruit in the longer dip time treatments (Figure 21). There were few consistent effects of dipping time on fruit vitamin C content, TSS, TA, respiration rate and ethylene production rates (Figures 20, 22–23). These results show that long dip times (>5 minutes) should be avoided and it is important to follow the label recommendations for a 30-second dip treatment.

Figure 19. Visual appearance of Navel oranges dipped in 1% Ortocil® (left) and 2% Ortocil® (right) for 10 minutes after 3 weeks of storage at 20 °C.

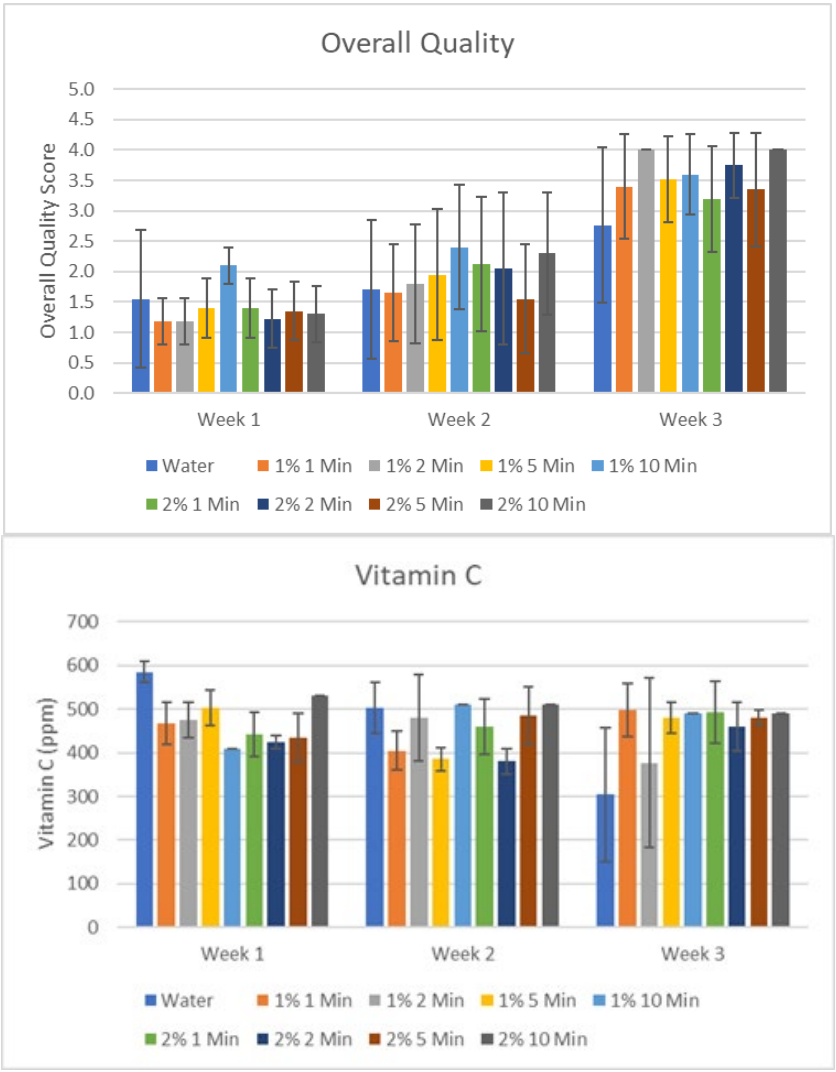


Figure 20. Effect of different treatment times (1, 2, 5 and 10 mins) of 1% and 2% Ortocil® dips on overall fruit quality score (top) and average vitamin C content (lower) over 3 weeks storage at 20 °C. The values are means of 4 replicates with 10 fruit per replicate, except 10 min treatment where there was only one replicate.

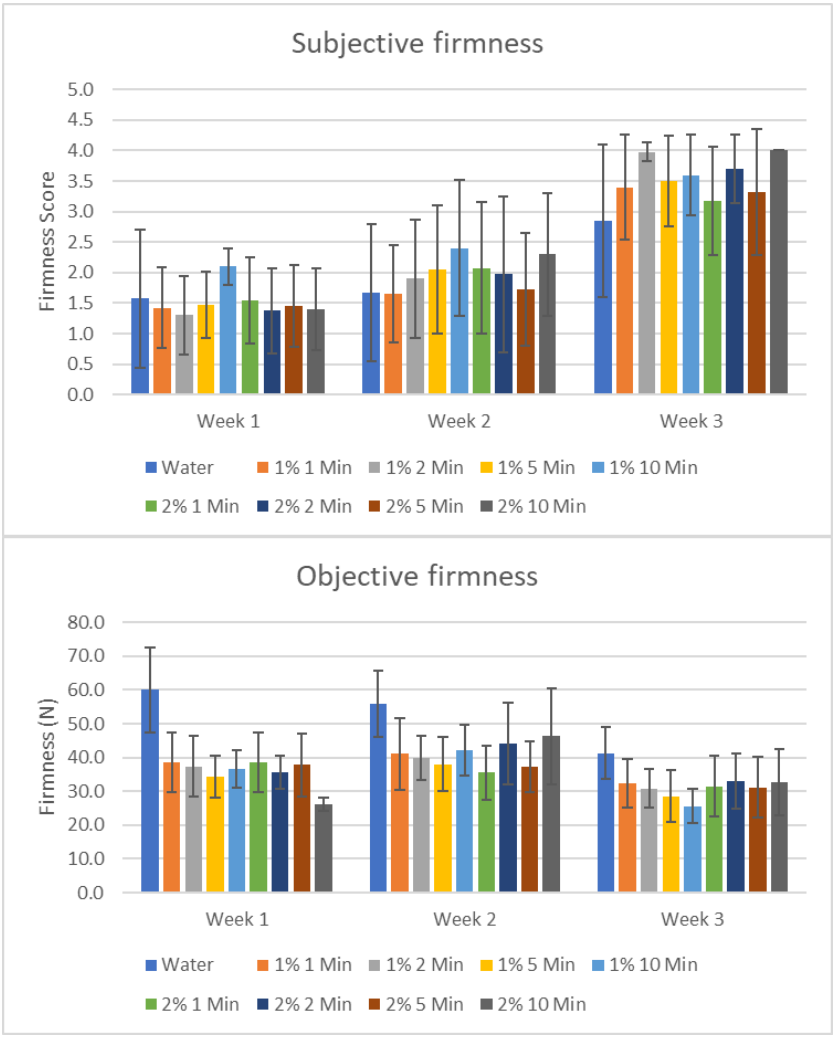


Figure 21. Effect of different treatment times (1, 2, 5 and 10 mins) of 1% and 2% Ortocil® dips on subjective fruit firmness (score) (top) and objective firmness (N) (lower) over 3 weeks storage at 20 °C. The values are means of 4 replicates with 10 fruit per replicate, except 10 min treatment where there was only one replicate.

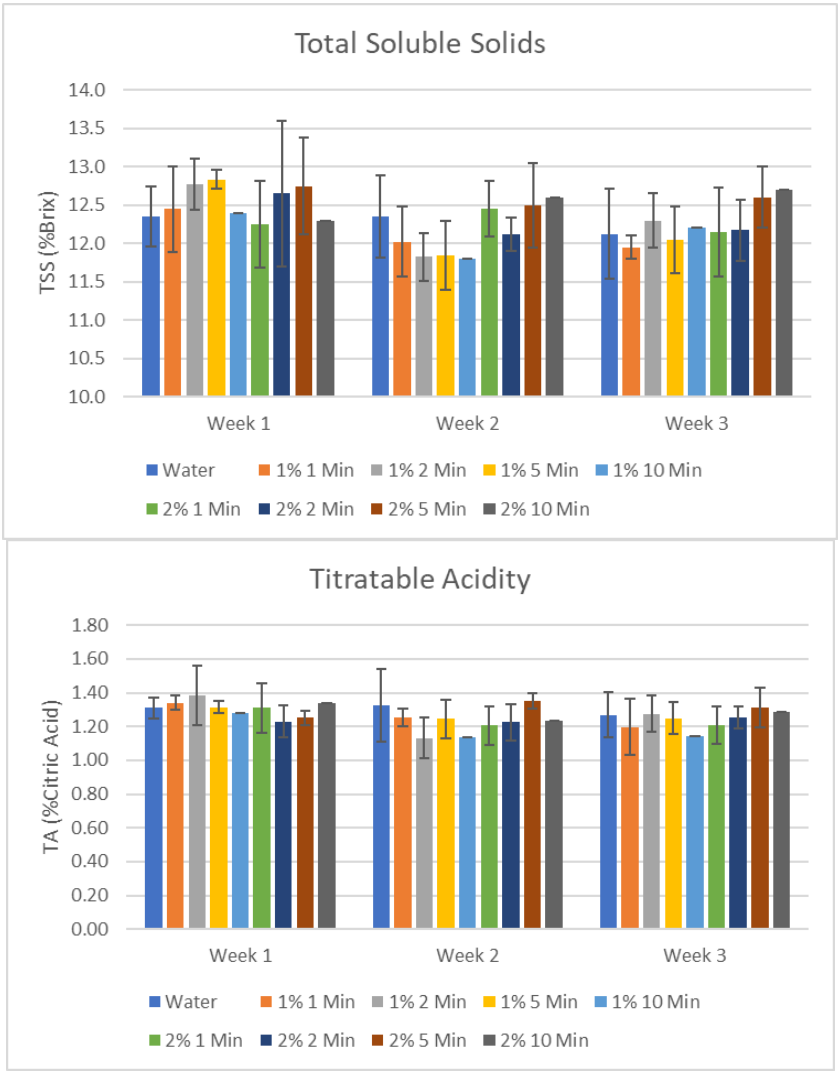


Figure 22. Effect of different treatment times (1, 2, 5 and 10 mins) of 1% and 2% Ortocil® dips on total soluble solids (TSS, % Brix) (top) and titratable acidity (TA, % citric acid) (lower) over 3 weeks storage at 20 °C. The values are means of 4 replicates with 10 fruit per replicate, except 10 min treatment where there was only one replicate.

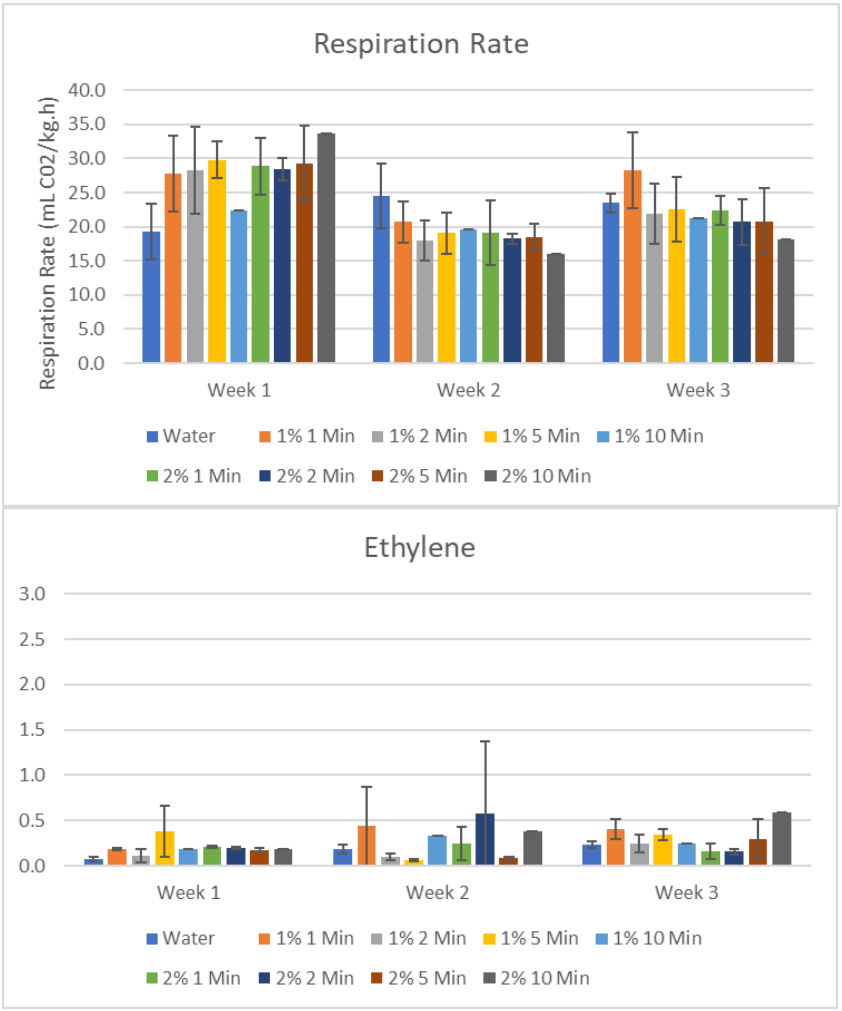
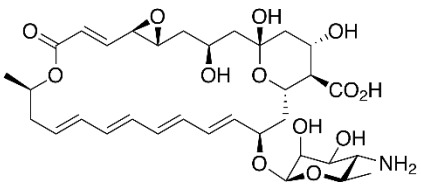


Figure 23. Effect of different treatment times (1, 2, 5 and 10 mins) of 1% and 2% Ortocil® dips on the fruit respiration rate (mL CO_2 kg^{-1} h^{-1}) (top) and fruit ethylene production rates (µL C_2H_4 kg^{-1} h^{-1}) (lower) over 3 weeks storage at 20 °C. The values are means of 4 replicates with 10 fruit per replicate, except 10 min treatment where there was only one replicate.

Cerafruta®

Natamycin is a natural antimicrobial peptide produced by the strains of *Streptomyces natalensis*. It acts as an antifungal preservative and is used in a range of food products such as dairy. It is listed as a 'generally recognised as a safe' (GRAS) ingredient for various food applications (Meena et al. 2021).



Natamycin, also known as pimaricin.

Natamycin is a food additive (E235) and is used in the European Union as a surface preservative for certain cheese and dried sausage products. Natamycin is approved for different applications at different levels in over 150 countries. Research on citrus in Egypt and China has shown that natamycin is capable of inhibiting green and blue mould, and also sour rot (*Geotrichum citri-aurantii*) (Yİğİter et al. 2014; Du et al. 2022). A formulation of natamycin is Cerafruta® (Ceradis Crop Protection), which was trialled in this study against green and blue mould on Navel oranges.

a. Effects of Cerafruta® on green and blue mould

Methods

Organic Navel oranges from NSW DPI Somersby Research farm were infected with green and blue mould (as per General Methods) and treated with 250 ppm (CF1), 500 ppm (CF2), 1000 ppm (CF3) and 2000 ppm (CF4) Cerafruta® and compared to a water dip control and imazalil dip (as per label recommendations). The fruit were dipped for 30 seconds. There were 40 fruit per treatment unit with 4 independent replicates (i.e. each dip solution was prepared for each replicate).

Results

The effect of different concentrations of Cerafruta® (and standard fungicides) on the development of green and blue mould are presented in Figures 24 and 25, respectively. The results show that while the standard postharvest fungicides (TBZ and imazalil) worked well (i.e. <10% infection), the Cerafruta® treatments did not greatly affect the levels of green and blue mould, even after 5 days of incubation. There was also no effect of the different concentrations of Cerafruta® on the incidence of green and blue mould.

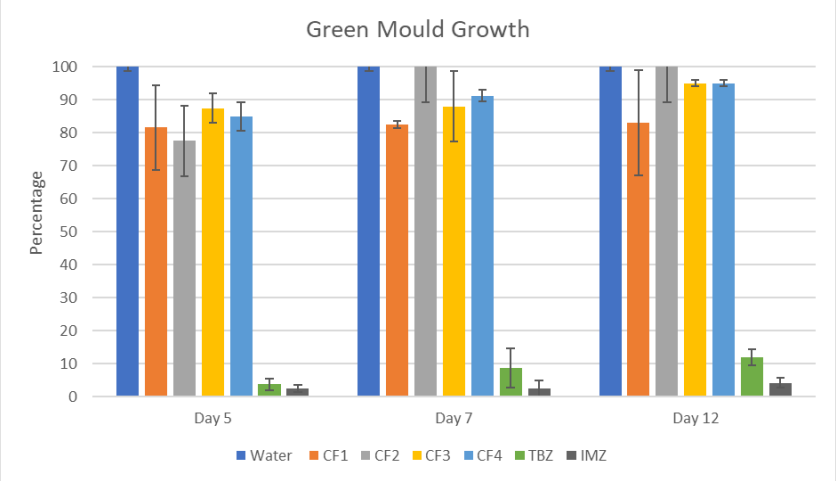


Figure 24. Percentage incidence of green mould (%) in infected Navel oranges treated with 250 ppm (CF1), 500 ppm (CF2), 1000 ppm (CF3) and 2000 ppm (CF4) Cerafruta® and compared to a water dip control and imazalil and TBZ dip (as per label recommendations.

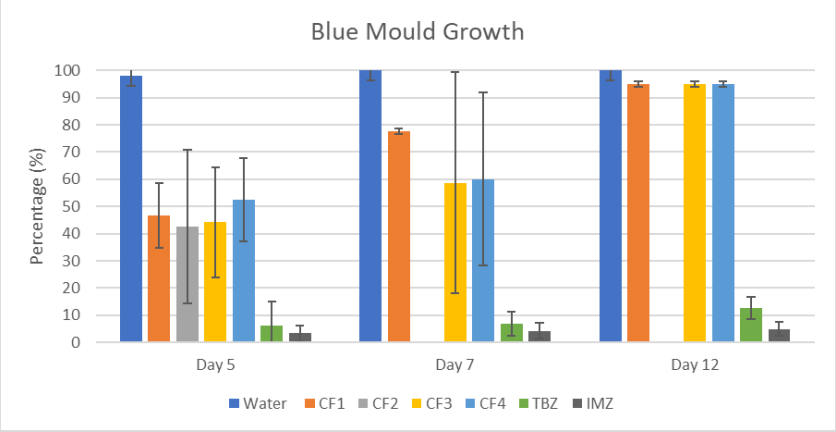


Figure 25. Percentage incidence of blue mould (%) in infected Navel oranges treated with 250 ppm (CF1), 500 ppm (CF2), 1000 ppm (CF3) and 2000 ppm (CF4) Cerafruta® and compared to a water dip control and imazalil and TBZ dip (as per label recommendations. Note there are no data for CF2 after day 6 due to high levels of decay.

b. Effects of Cerafruta® on Navel fruit quality

Methods

Navel oranges were harvested from a commercial orchard in Leeton (NSW) and transported to NSW DPI at Ourimbah. Fruit were then treated with 1000 ppm Cerafruta® and compared to untreated control (water dip) and imazalil (label rate). The fruit were dipped for 30 seconds. There were 10 fruit per treatment unit with 4 replicates (i.e. each dip solution was prepared for each replicate).

After treatment, one set of fruit was stored continuously at 20 °C for 4 weeks (Part A Shelf life trial), and another set of fruit was stored at 3 °C for 4 weeks (Part B Storage trial). Fruit quality was assessed at weekly intervals according to the General Methods–overall acceptability, weight loss, respiration rate, fruit firmness, chilling injury (3 °C fruit only), TSS, TA, vitamin C content, juice ethanol content and natural decay infection rates.

Results

Part A–Shelf life trial

The effects of the application of Cerafruta® (1000 ppm) on fruit quality were compared to a water dip and a commercial imazalil treatment in Navel oranges stored for 4 weeks at 20 °C. These results are presented in Figures 26–30. The results show both the Cerafruta® and imazalil treatments maintained fruit quality during storage at 20 °C (Figure 26). The level of natural rots that developed during shelf life was lower in Cerafruta® and imazalil-treated fruit. Although there were some inconsistencies between the assessment time, in general, there were few other differences in fruit quality between the treatments (Figures 27–30).

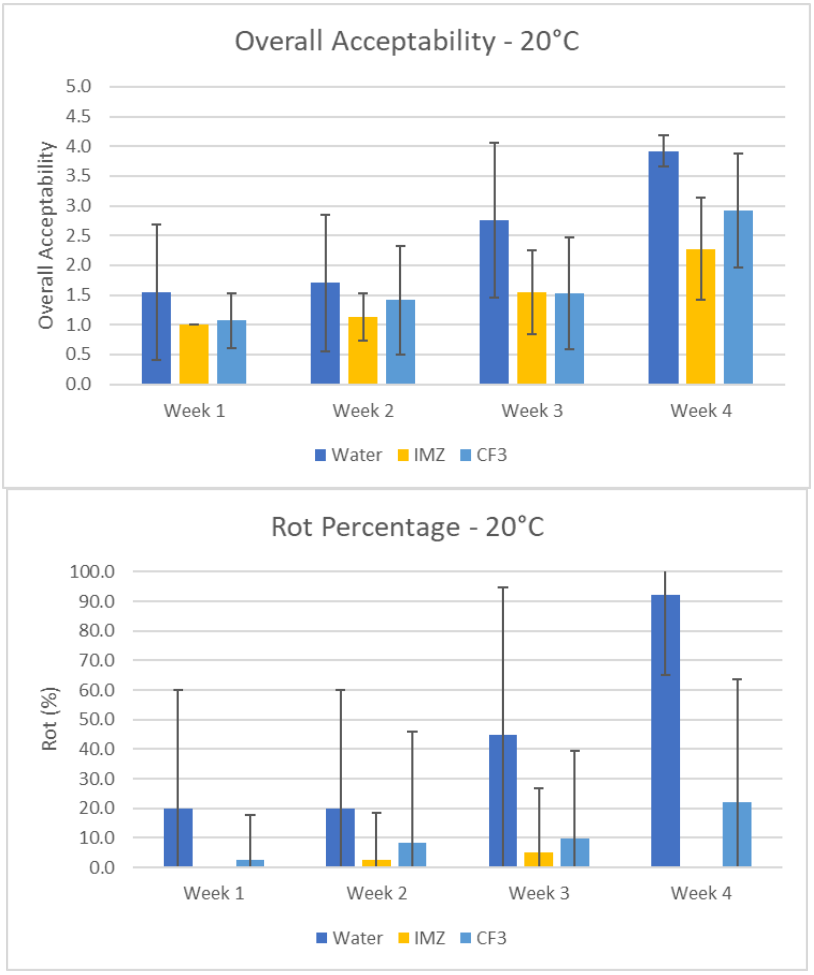


Figure 26. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on the average overall fruit acceptability score (top) and natural fruit rots (lower) in Navel oranges during storage at 20 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

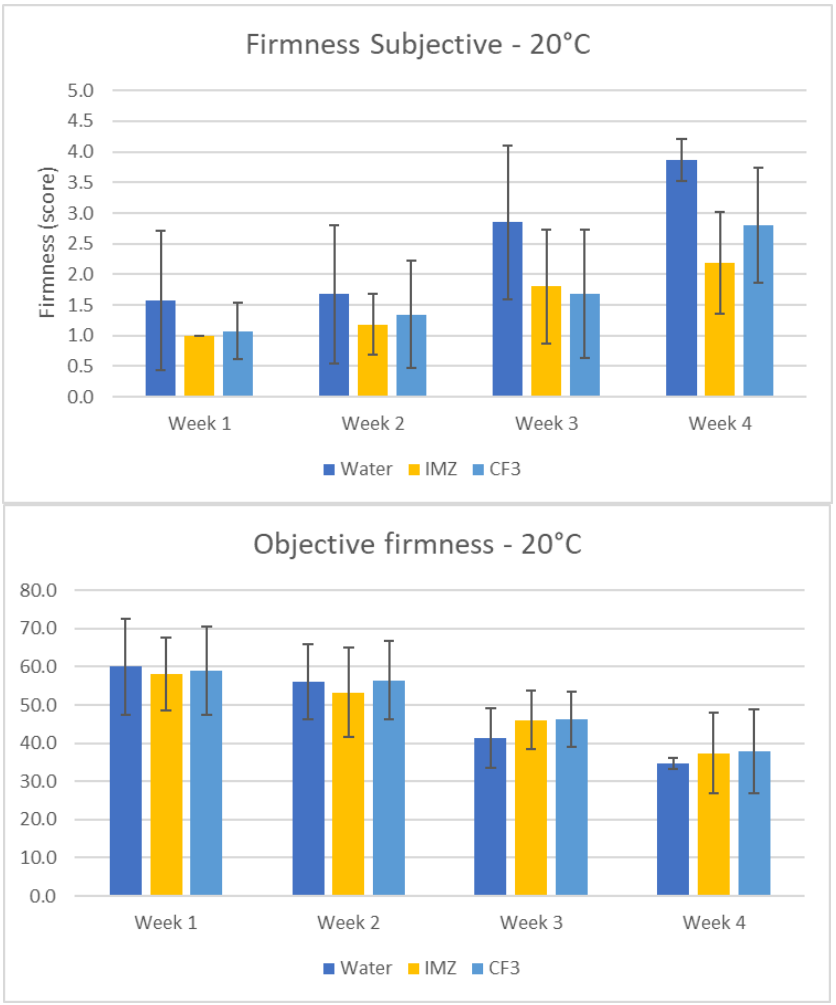


Figure 27. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on subjective fruit firmness score (top) and objective fruit firmness (N) (lower) in Navel oranges during storage at 20 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

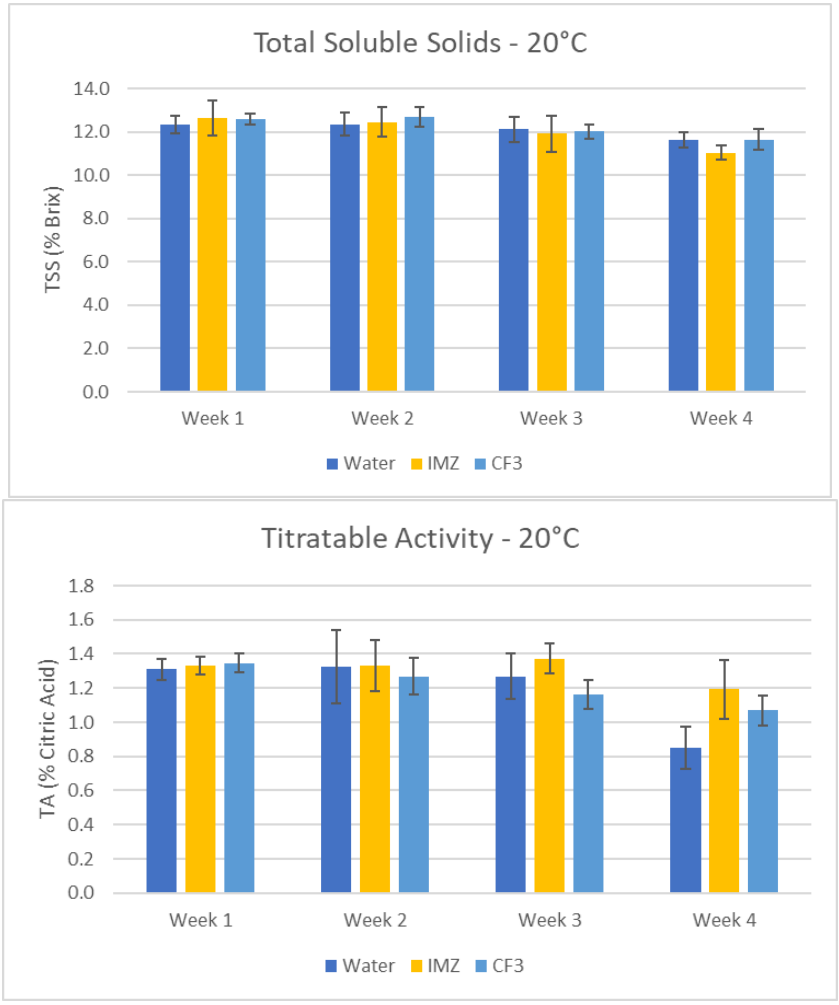


Figure 28. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on fruit TSS (% Brix) (top) and TA (% citric acid) (lower) in Navel oranges during storage at 20 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

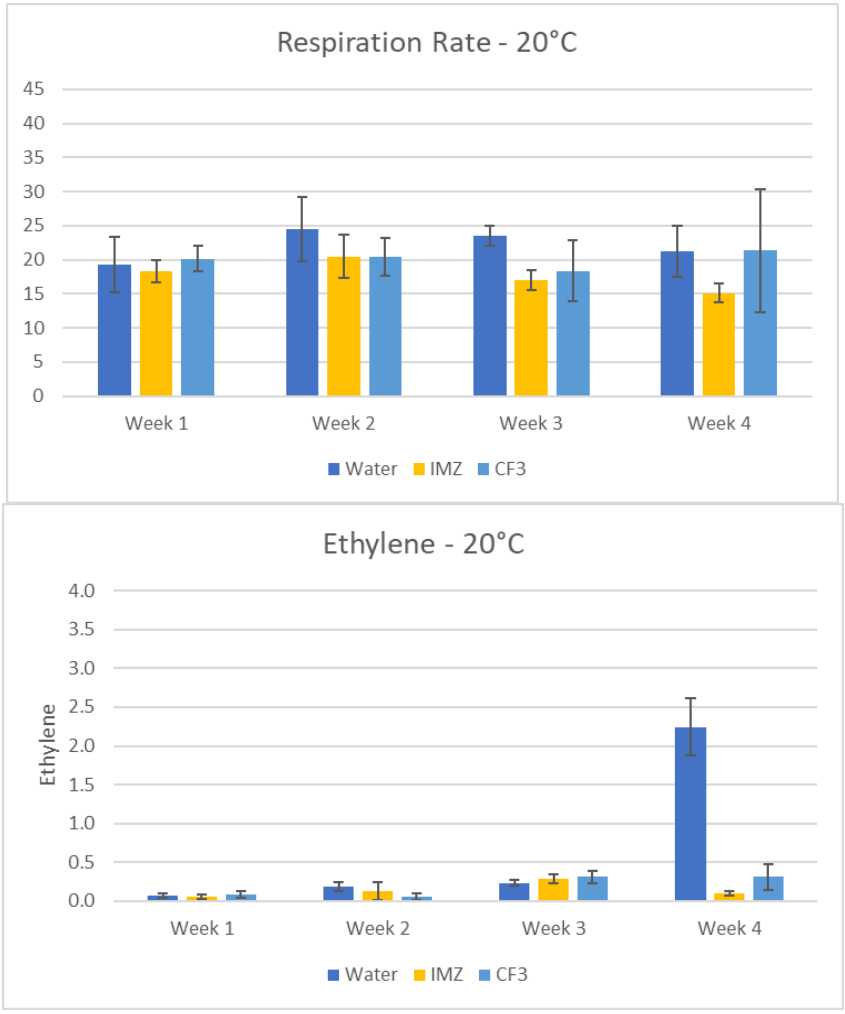


Figure 29. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on the fruit respiration rate (mL CO_2 kg^{-1} h^{-1}) (top) and fruit ethylene production rates (µL C_2H_4 kg^{-1} h^{-1}) (lower) in Navel oranges during storage at 20 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

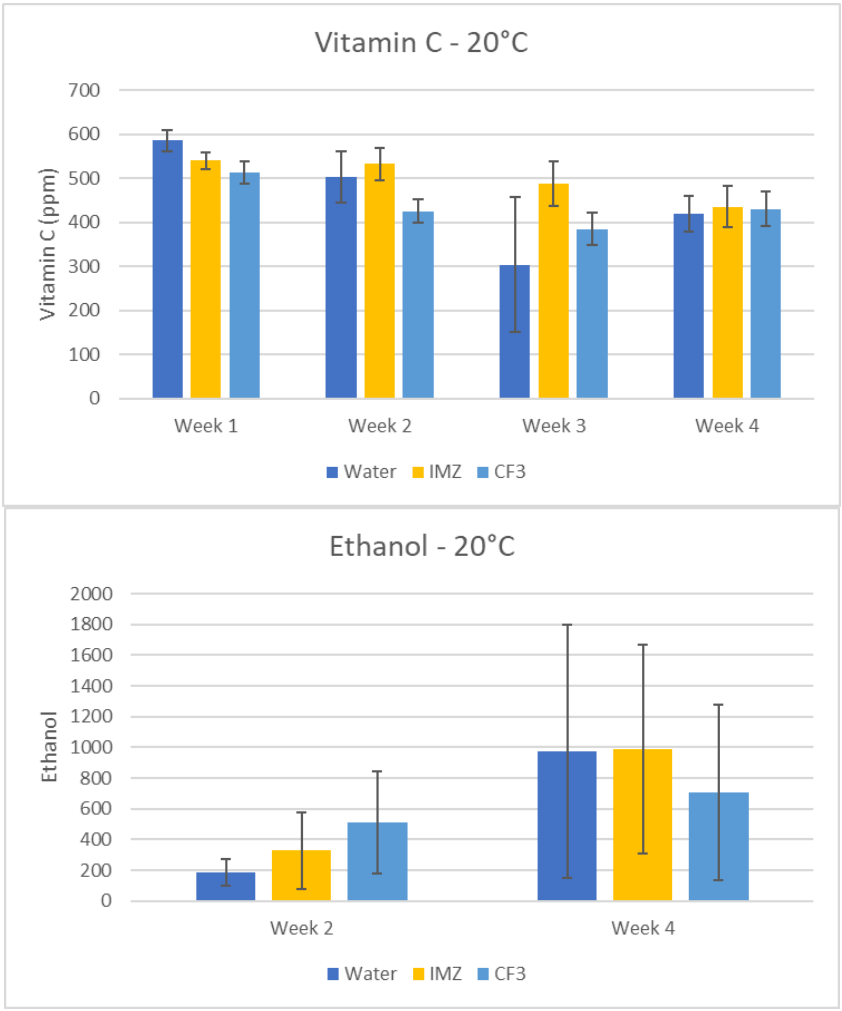


Figure 30. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on vitamin C content (ppm) (top) and fruit juice ethanol content (mg 100 mL^{-1}) (lower) in Navel oranges during storage at 20 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

Part B–Storage trial

In general, treatment with Cerafruta® or imazalil maintained quality, at least in the initial stages of storage at 5 °C, but as storage time continued, there were fewer positive effects of these treatments on overall quality (Figure 31). This was reflected in the subjective assessment of fruit firmness (Figure 32). This was similarly observed with the development of rots, where both Cerafruta® or imazalil treatment suppressed natural decay, but in the later stages of storage, these treatment differences became less obvious. There were few other differences in fruit quality between the treatments (Figures 33–36).

These results show that 1000 ppm Cerafruta® had no negative effects on fruit quality during storage at 5 °C but had the benefit of suppressing natural decay.

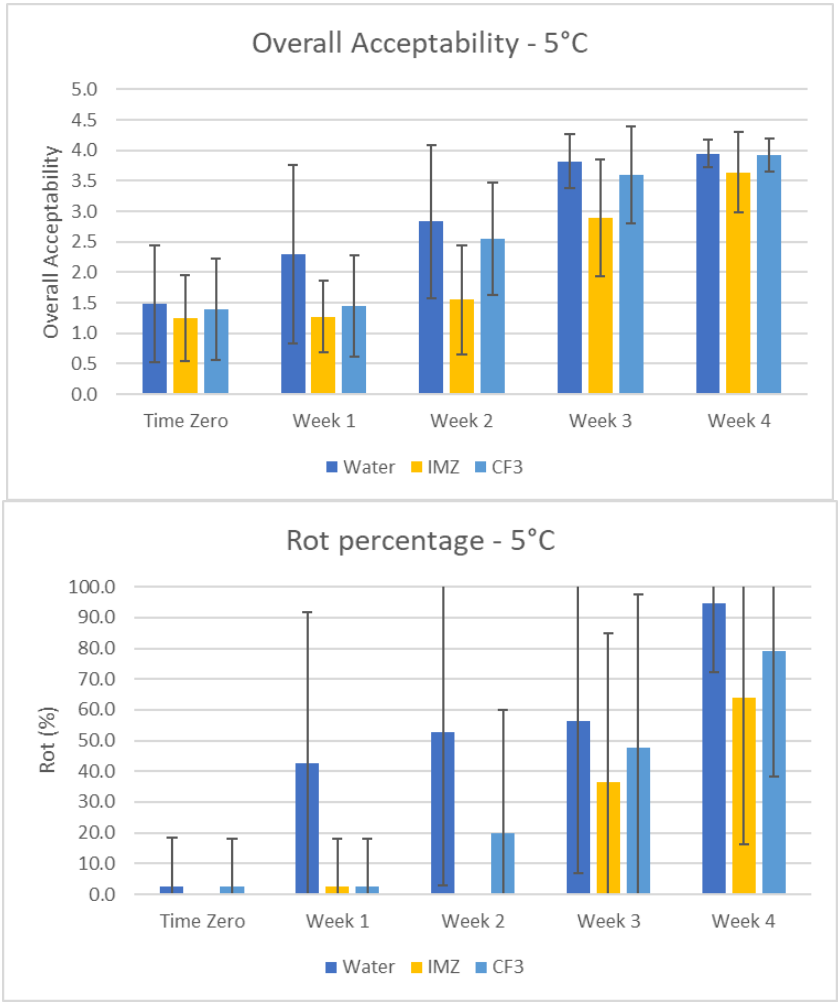


Figure 31. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on the average overall fruit acceptability score (top) and natural fruit rots (lower) in Navel oranges during storage at 5 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

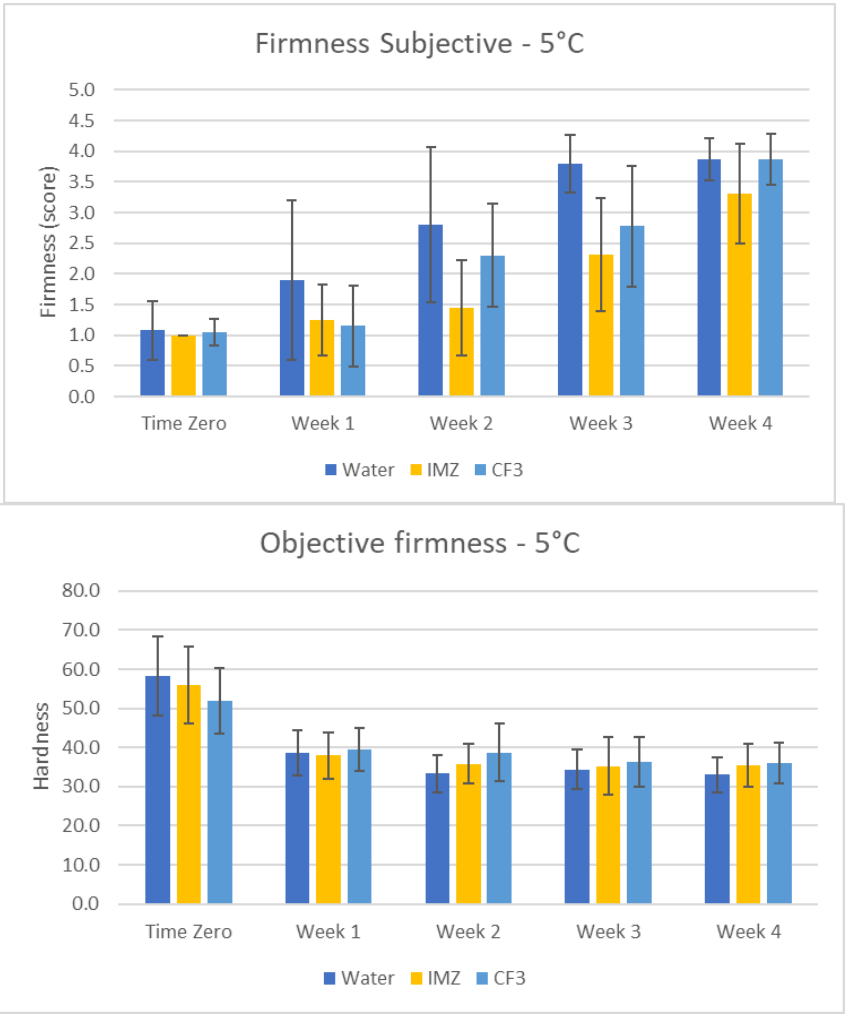


Figure 32. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on subjective fruit firmness score (top) and objective fruit firmness (N) (liower) in Navel oranges during storage at 5 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

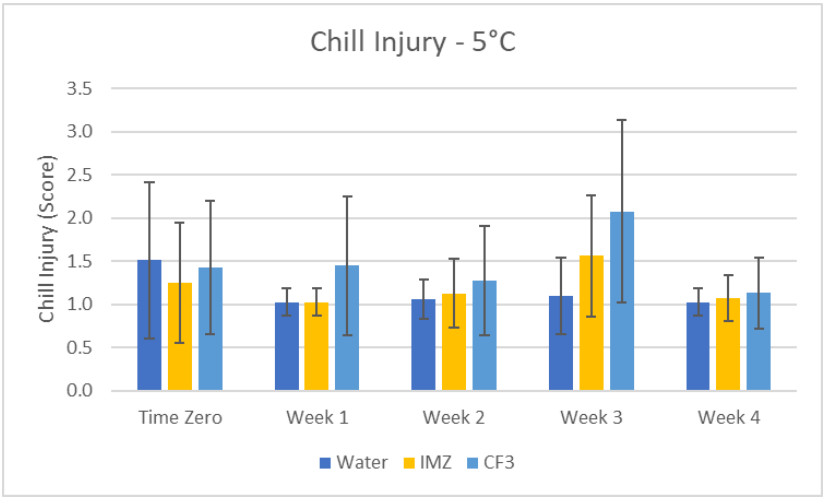


Figure 33. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on the levels of chilling injury in Navel oranges during storage at 5 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

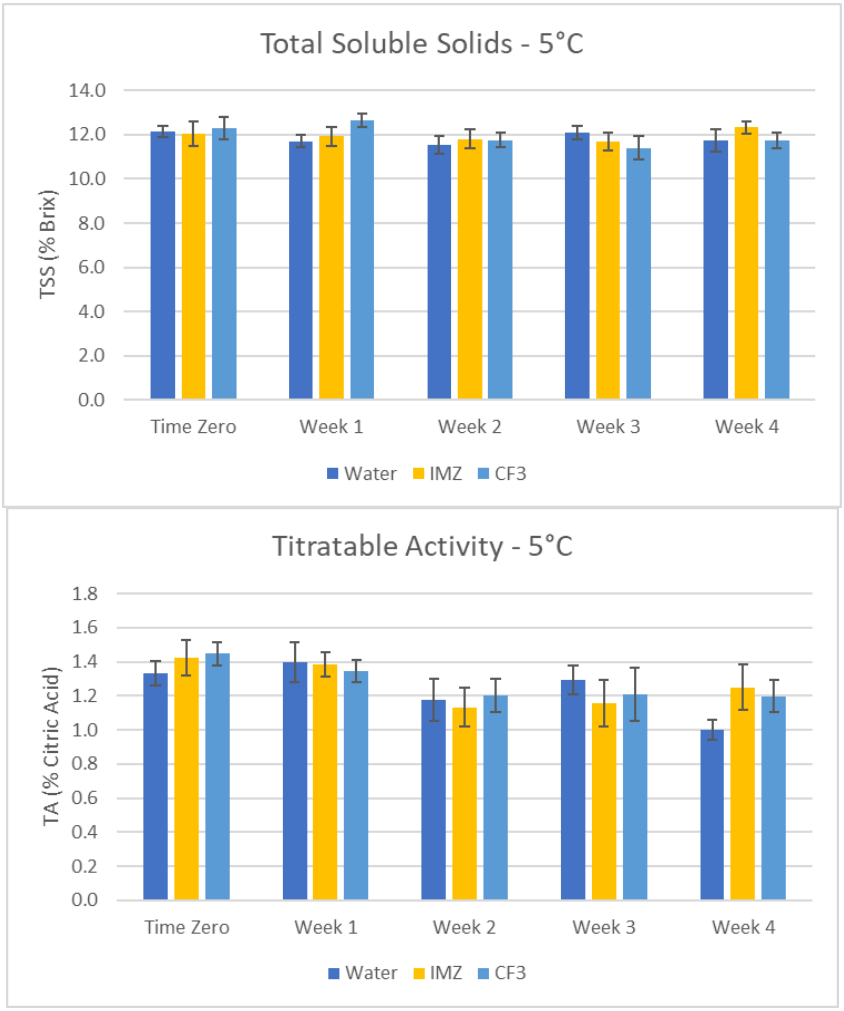


Figure 34. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on fruit TSS (% Brix) (top) and TA (% citric acid) (lower) in Navel oranges during storage at 5 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

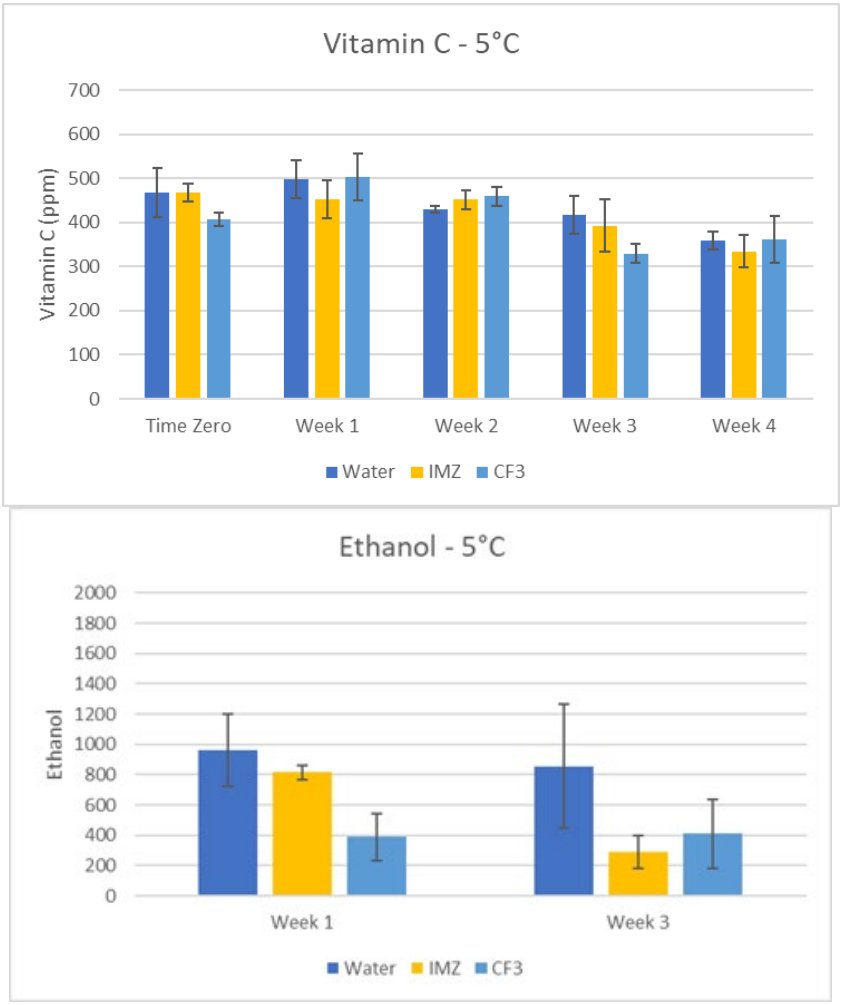


Figure 35. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on vitamin C content (ppm) (top) and fruit juice ethanol content (mg 100 mL^{-1}) (lower) in Navel oranges during storage at 5 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

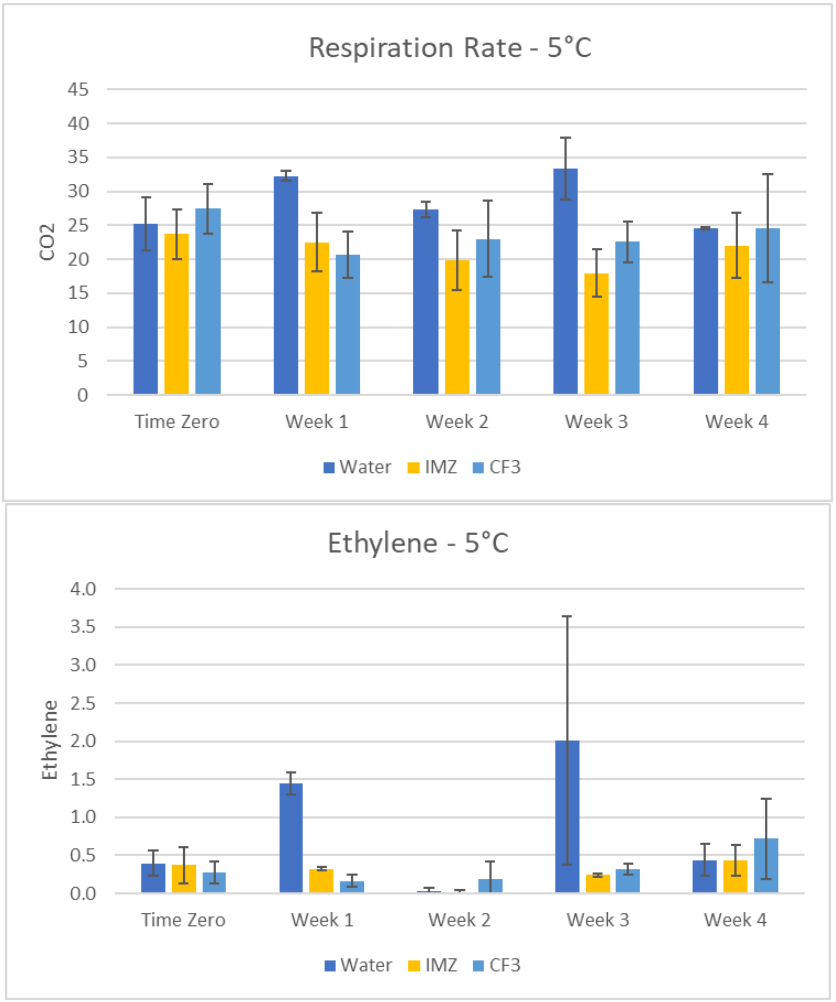
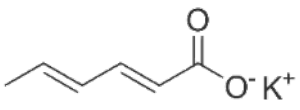


Figure 36. Effect of 1000 ppm Cerafruta® (CF3), imazalil and water dip on the fruit respiration rate (mL CO_2 kg^{-1} h^{-1}) (top) and fruit ethylene production rates (µL C_2H_4 kg^{-1} h^{-1}) (lower) in Navel oranges during storage at 5 °C for 4 weeks. The values are means of 4 replicates with 10 fruit per replicate.

DeccoPlus®

Sorbic acid and some sorbic acid salts have been widely used for years as preservatives in processed food. Potassium sorbate (E-202) is a wide-spectrum antimicrobial food additive effective against moulds and yeasts, mostly within the pH range of 3.0–6.5. Potassium sorbate is classified by the United States Environmental Protection Agency as a minimal-risk active ingredient and is exempt from residue tolerances. There have been numerous studies on the effectiveness of potassium sorbate on citrus fruit decay. For example, Montesinos-Herrero et al. (2009) showed good control of *Penicillium* decay in a range of citrus types. Smilanick et al. (2008) also found that potassium sorbate was compatible with some commonly used postharvest fungicides, but potassium sorbate also improved their performance against *P. digitatum* and *Geotrichum citri-aurantii*, the causal pathogen of sour rot.

A commercial formulation of potassium sorbate is DeccoPlus®, which was evaluated to examine (1) its efficacy against green mould and (2) its compatibility with a range of standard postharvest sanitisers and fungicides.



Potassium sorbate

a. Efficacy DeccoPlus® against green mould with different timings of treatment

Methods

Navel oranges were inoculated with green mould, according to the General Methods. Fruit were then left to incubate at 25 °C for either 4 hours or 24 hours before dip treatments were applied for 30 seconds. Fruit were treated in 1% DeccoPlus®, 2% DeccoPlus®, and Chairman® fungicide (propiconazole and fludioxonil) at the label rate and compared to a water dip control. Each treatment unit was replicated 4 times and there were 20 fruit per treatment unit. After treatment, fruit were stored at 25 °C with +90% relative humidity and both decay incidence and diameter of the decay infections were measured.

Results

The levels of decay infection are presented in Figure 37 and show that 2% DeccoPlus® treatment had better decay control than 1% DeccoPlus® treatment. It was interesting that there were significant differences in the results of the 1% DeccoPlus® treatment between the 4-hour delay and 24-hour delay in dip treatments. The 24-hour delay between infection and treatment provided a superior result than the 4-hour delay in the 1% DeccoPlus® treatment. This observation requires further investigation.

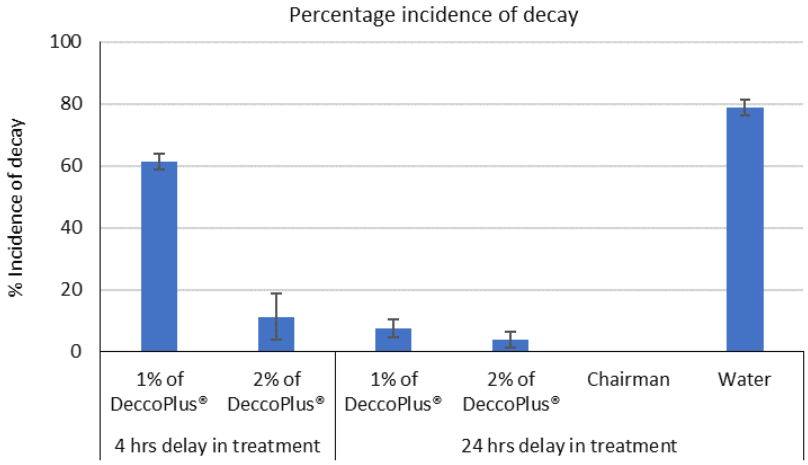


Figure 37. Percentage incidence of decay (%) of fruit treated with DeccoPlus® (1% and 2%), Chairman® and control (water) at 4 and 24 hours delay in treatment. Bars are standard deviations around the means, *n*=4.

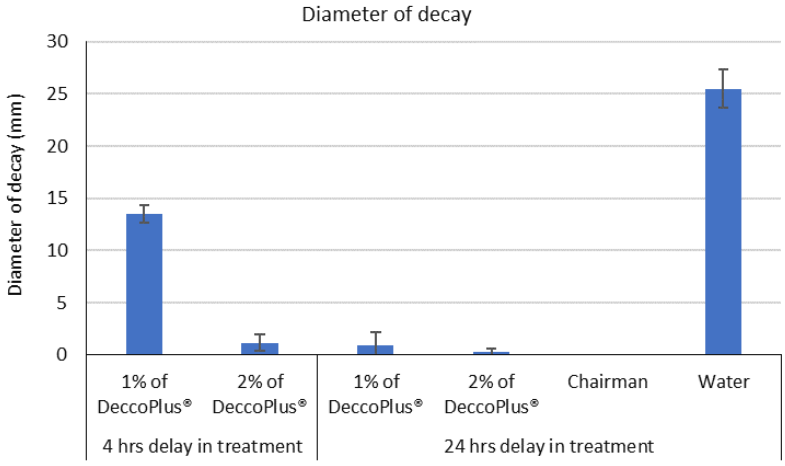


Figure 38. Diameter of decay (mm) of fruit treated with DeccoPlus® (1% and 2%), Chairman® and control (water) at 4 and 24 hours delay in treatment. Bars are standard deviations around the means, *n*=4.

b. Compatibility of DeccoPlus® with commercial fungicides and sanitisers

Methods

Non-treated Navel oranges were inoculated with green and blue mould and left at 20 °C for 4 hours. The recommended concentration of DeccoPlus® (1% solution) was then applied to the infected Navel oranges with and without the following sanitisers (PAA, hypochlorite) and fungicides (TBZ, imazalil, fludioxonil, propiconazole and fludioxonil, pyrimethanil, imazalil and pyrimethanil, guazatine, a grand mix containing guazatine + TBZ + PAA, OPP). All sanitisers and fungicides were prepared at their label rates. Fruit were all treated for 30 seconds. There were 20 fruit per treatment combination and each combination was replicated 4 times. There were both untreated (washed controls) and water dip controls. Treated fruit were stored at 25 °C with +90% relative humidity and both decay incidence and diameter of the decay infections were measured.

Results

The results presented in Figures 39 and 40 showed that Deccoplus® treatment reduced the incidence and growth of postharvest decay in Navel oranges. No negative compatibility issues were observed in this trial. In all cases, there was no negative effect of Deccoplus® treatment on the incidence or growth of *Penicillium* decay. Mixing Deccoplus® with the sanitisers (PAA and chlorine) increased the efficacy of the sanitiser in providing some protection against decay. However, there were no statistical differences between fruit treated with fungicide alone and fungicide plus Deccoplus® mixture, indicating no negative effects of the mixing. However, the results suggested there was a positive effect of adding Deccoplus® to the fungicide, but this benefit in reducing the incidence and growth of decay was not statistically significant (at p<0.05).

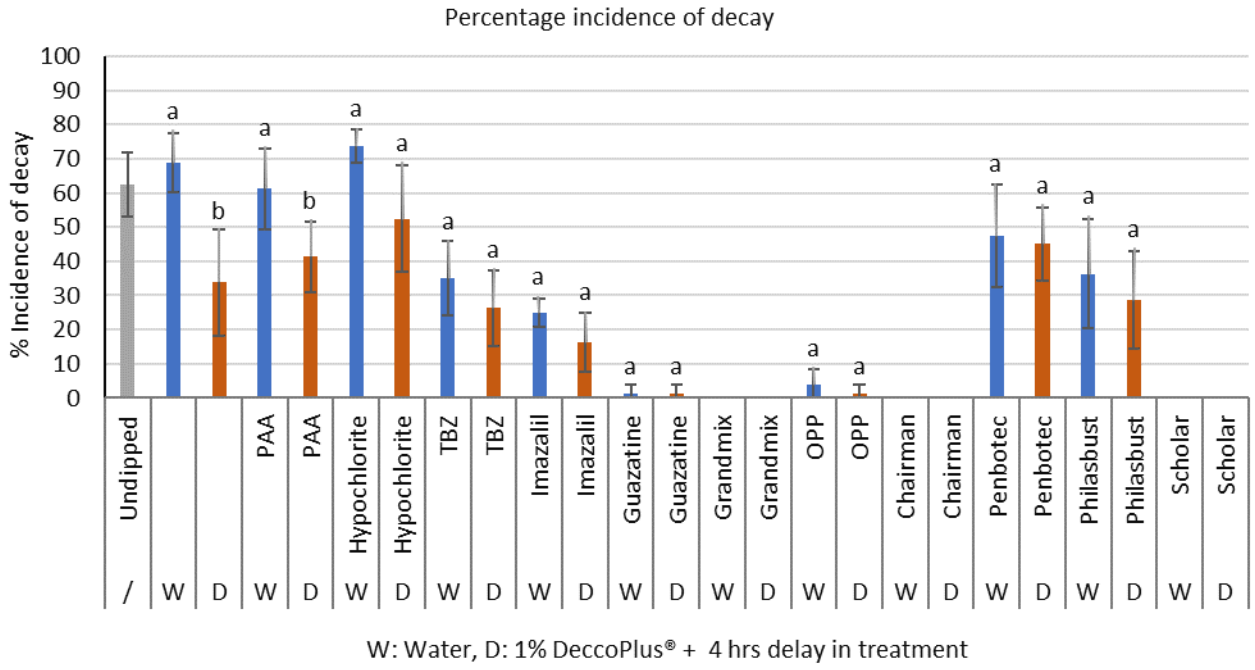


Figure 39. Percentage incidence of decay (%) of fruits treated with DeccoPlus®, commercial fungicides and sanitisers after 1 week at 25 °C with 95% RH. Bars are standard deviations around the means, n=4. Different letters above the bars on 2 columns of the same fungicides/sanitisers without and with 1% of DeccoPlus® show significant differences (p <0.05, t-test).

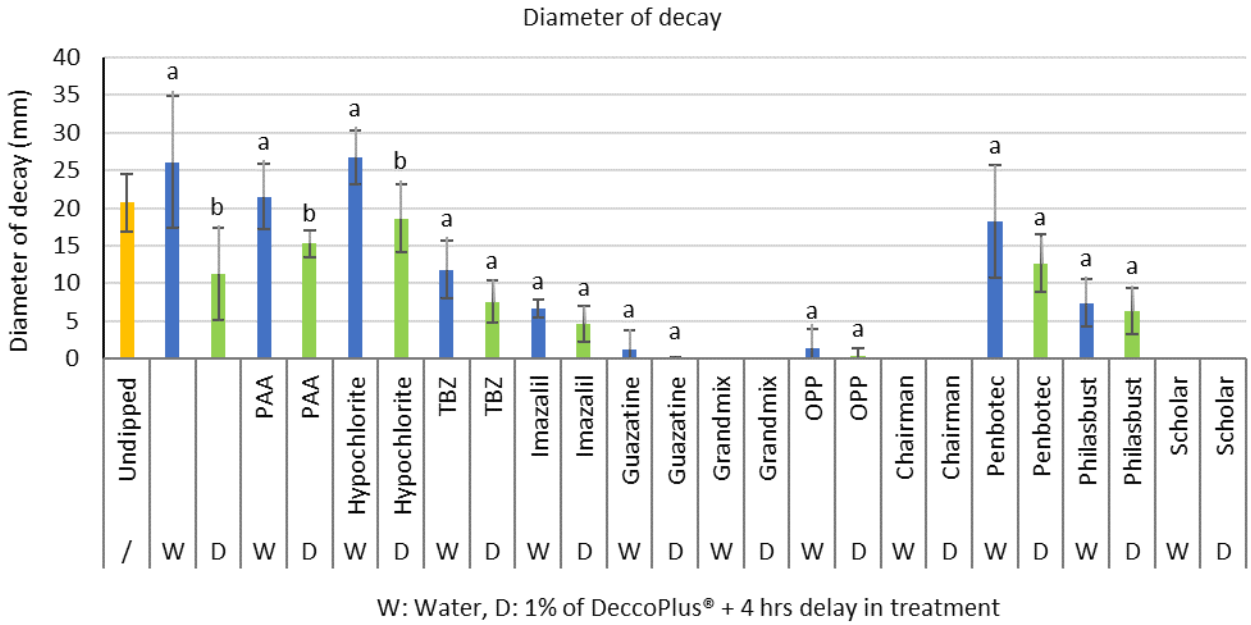


Figure 40. Diameter of decay (mm) of fruits treated with DeccoPlus®, commercial fungicides and sanitisers after 1 week at 25 °C with 95% RH. Bars are standard deviations around the means, *n*=4. Different letters above the bars on 2 columns of the same fungicides/sanitisers without and with 1% of DeccoPlus® show significant differences (p <0.05, *t*-test).

Fruit Mag®

FruitMag® is a new product with magnesium oxide (MgO) as the active ingredient. MgO is classified as a 'generally regarded as safe' (GRAS) compound by the US FDA (§ 184.1431 Magnesium oxide–CFR). It is also a US FDA-approved food additive with the technical function of a firming agent (§ 184.1431) and has an E number of E530.

MgO is used as an anti-caking and firming agent. In citrus, the addition of FruitMag® to pectin (isolated from citrus) promotes rapid gel formation (ICL Group unpublished report). There have also been unpublished reports of FruitMag® reducing postharvest decay in citrus (ICL Group).

This trial examined the effect of FruitMag® on decay development in Navel oranges. Two experiments were conducted: (1) efficacy of FruitMag® on green mould, and (2) different timing of FruitMag® treatment after infection.

Methods

Experiment 1. Efficacy of FruitMag® on green mould

Navel oranges were inoculated with green mould, according to the General Methods. Fruit were then left to incubate at 25 °C for 24 hours before dip treatments were applied for 30 seconds. Fruit were treated in 5% FruitMag® (Figure 41). After 15 minutes, half of the FruitMag® fruit were washed by hand in tap water and the other half remained unwashed. These were compared to the results of fruit dipped in Chairman® fungicide (propiconazole and fludioxonil) at the label rate and to fruit dipped in water (control). Each treatment unit was replicated 4 times and there were 30 fruit per treatment unit. After treatment, fruit were stored at 25 °C with +90% relative humidity and both decay incidence and diameter of the decay infections were measured.

Experiment 2. Different timing of FruitMag® treatment after infection

The same experimental design and procedures were conducted for Experiment 2, except there was a set of fruit that were treated with FruitMag® 4 hours after infection. All fruit were hand-washed after 15 minutes.

Figure 41. Dipping Navel oranges in 5% FruitMag®. The white residue can be removed with post-dip washing.

Results

Experiment 1. Efficacy of FruitMag® on green mould

The effect of treating green mould-infected Navel oranges with 5% FruitMag® treatment (with and without post-dip washing) is presented in Figures 42-44, and shows the FruitMag® treatments controlled green mould development. The hand washing treatment, where the treated fruit were gently washed in tap water 15 minutes after dipping, showed a slight increase in the level of decay, but it was still less than 10% infection.

Figure 42. Photos of green mould-infected Navel oranges treated with water (control, top left), Chairman® fungicide (top right), 5% FruitMag® with no washing (bottom left) and 5% FruitMag® with gentle hand washing 15 minutes after dip treatment. Fruit were infected with green mould and stored at 25 °C for 1 week.

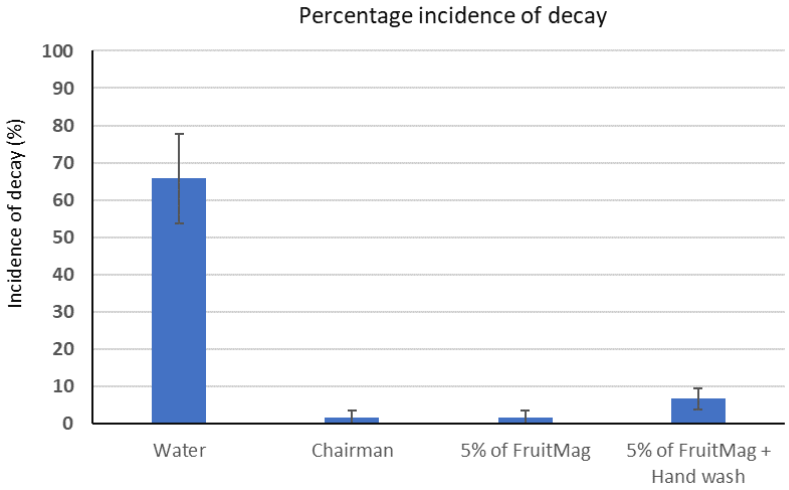


Figure 43. Effect of 5% FruitMag® treatment (with and without post-dip washing), Chairman® fungicide and water dip on the incidence of green mould on infected Navel oranges 24 hours before dip treatment.
Bars are standard deviations around the means, *n*=4.

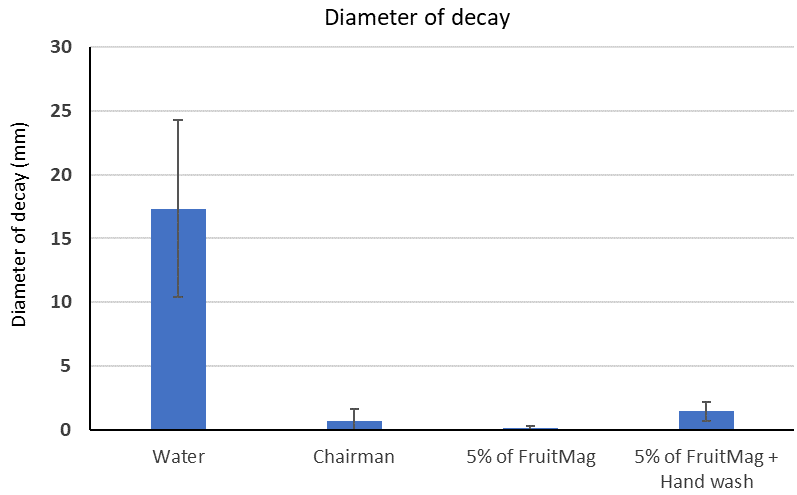


Figure 44. Effect of 5% FruitMag® treatment (with and without post-dip washing), Chairman® fungicide and water dip on the growth (decay diameter, mm) of green mould in Navel oranges 24 hours before dip treatment. Bars are standard deviations around the means, *n*=4.

Experiment 2. Different timing of FruitMag® treatment after infection

The effect of different timings of FruitMag® treatment (4 hours and 24 hours after infection) is presented in Figure 45–47 and shows there were no differences in decay control between the different treatment times. These results are promising, but more work will be required to examine the retention times of the FruitMag® on the fruit and potential washing issues.

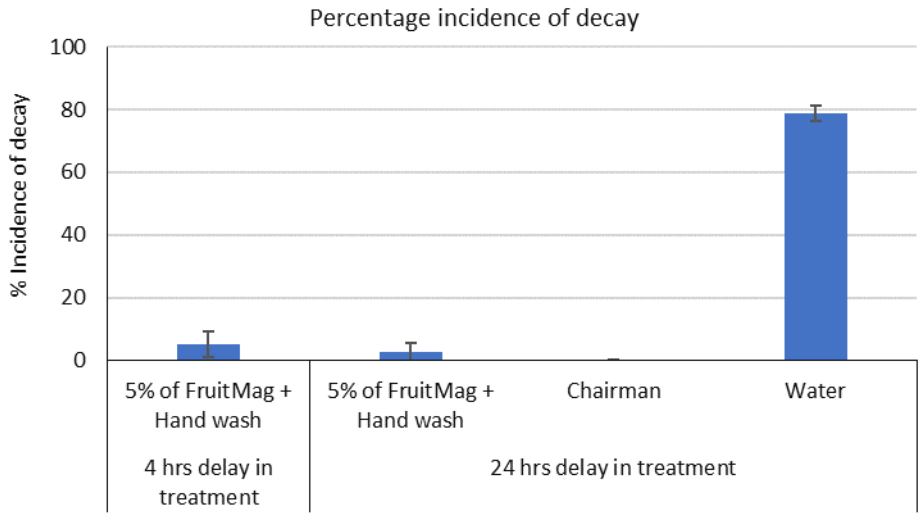


Figure 45. Effect of 5% FruitMag® treatment (with post-dip washing), Chairman® fungicide and water dip on the percentage incidence of green mould in Navel oranges 4 and 24 hours before dip treatment. Bars are standard deviations around the means, *n*=4.

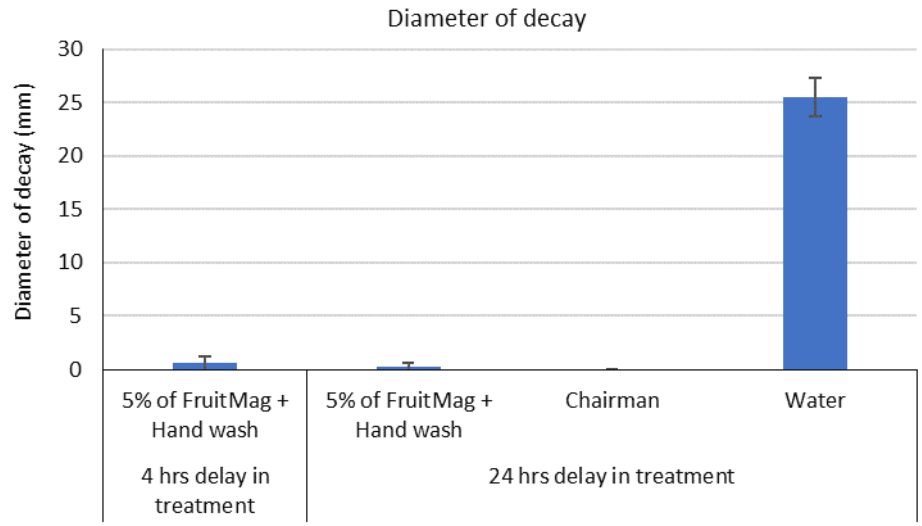


Figure 46. Effect of 5% FruitMag® treatment (with post-dip washing), Chairman® fungicide and water dip on the growth (decay diameter, mm) of green mould in Navel oranges 4 and 24 hours before dip treatment. Bars are standard deviations around the means, *n*=4.

Figure 47. Photos of green mould-infected Navel oranges treated with water (control, top left), Chairman® fungicide (top right), 5% FruitMag® with 4 hours infection then washing (bottom left) and 5% FruitMag® with 24 hours then washing (bottom right).

Essential oils

Plant-based antimicrobial agents such as essential oils offer a safer and more eco-friendly alternative to synthetic fungicides. Their production also makes use of a waste stream from citrus processing. The chemical constituents of essential oils are broadly classified as terpenes and phenylpropanoids, although most of these mainly consist of monoterpenes. The unique chemical structure and exposure to light and oxygen mostly lead to the stability and chemical reactions of essential oils.

A series of studies were conducted by Mohammad M. Rahman and the citrus postharvest research teams at NSW Department of Primary Industries and the University of Newcastle, which contributed to the outcomes of this Project. These studies have been published in international peer-reviewed journals and have acknowledged the contributions of this Project.

1. Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2020) Efficacy of orange essential oil and citral after exposure to UV-C irradiation to inhibit *Penicillium digitatum* in navel oranges. *Horticulturae* 6, 102. doi:10.3390/horticulturae6040102
2. Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2022) Lemon myrtle and lemon-scented tea tree essential oils as potential inhibitors of green mould on citrus fruits. *The Journal of Horticultural Science and Biotechnology*, DOI: 10.1080/14620316.2021.2011433
3. Rahman M.M., Wills R.B.H., Bowyer M.C., Vuong V.Q., Golding J.B., Kirkman T. and Pristijono P. (2023) Efficacy of lemon myrtle essential oil as a bio-fungicide in inhibiting citrus green mould. *Plants* 12(21):3742. https://doi.org/10.3390/plants12213742
4. Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2023) Potential control of postharvest fungal decay of citrus fruits by crude or photochemically changed essential oils–a review. *Food Reviews International.* pp. 1-18. DOI: 10.1080/87559129.2023.2204157

1. Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2020) Efficacy of orange essential oil and citral after exposure to UV-C irradiation to inhibit *Penicillium digitatum* in navel oranges. *Horticulturae* 6, 102. doi:10.3390/horticulturae6040102

horticulturae

MDPI

Article

Efficacy of Orange Essential Oil and Citral after Exposure to UV-C Irradiation to Inhibit *Penicillium digitatum* in Navel Oranges

M. M. Rahman [1], R.B.H. Wills [1], Michael C. Bowyer [1], John. B. Golding [1,2], Timothy Kirkman [1] and Penta Pristijono [1,*]

[1] School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia; mohammad.m.rahman@uon.edu.au (M.M.R.); ron.wills@newcastle.edu.au (R.B.H.W.); michael.bowyer@newcastle.edu.au (M.C.B.); john.golding@dpi.nsw.gov.au (J.B.G.); timothy.kirkman@newcastle.edu.au (T.K.)

[2] NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia

* Correspondence: penta.pristijono@newcastle.edu.au

Received: 2 December 2020; Accepted: 11 December 2020; Published: 14 December 2020

check for updates

Abstract

The effect of UV-C irradiation on the antifungal properties of orange essential oil (EO) against Penicillium digitatum in inoculated Navel oranges was examined. The UV-C irradiation of orange EO resulted in a 20% loss of the major constituent, limonene, and the generation of 3 hydroperoxide oxidation products, (2S,4R)-p-mentha-6,8-diene-2-hydroperoxide,(1S,4R)-p-mentha-2,8-diene-1-hydroperoxide, and (1R, 4R)-p-mentha-2,8-diene-1-hydroperoxide. The *P. digitatum* growth in oranges dipped in non-irradiated orange EO at 1000–4000 µL L−1 was not significantly different to control the fruit. Dipping in UV-C treated orange EO inhibited the growth of *P. digitatum* with 4000 µL L^{-1} having the greatest effect. No phytotoxic injury to the rind was observed at any concentration. Citral, as a known antifungal chemical, was included for comparison. The non-irradiated citral (1000 µL L^{-1}) was more effective than irradiated orange EO, but elicited rind phytotoxicity. The irradiated citral was less effective in inhibiting *P. digitatum* growth with the loss of citral, but not hydroperoxide formation. These results suggest UV-C irradiated orange EO as a potential alternative to synthetic fungicides to inhibit *P. digitatum* decay. The source of orange EO could be waste flavedo generated by the orange juice processing industry.

Conclusions

This study showed that UV-C irradiation of orange EO significantly inhibited the development of *P. digitatum* decay on Navel oranges. While the level of inhibition was not as high as that achieved with the non-irradiated citral, orange EO had a significant commercial advantage of not generating any visible skin damage without the need for an ethanolic dip solution. Given the ready availability of orange skin tissue as a processing waste product and the ability to use the EO in an aqueous solution, it would seem worthwhile to conduct further studies to determine if other methods or combinations of methods, such as radiation, heat, oxygen, and water, could enhance the antimicrobial activity of orange EO to a level where it could be a commercially profitable product for the orange processing industry.

All references are listed in the manuscript. A copy of this manuscript is available from the author.

2. Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2022) Lemon myrtle and lemon scented tea tree essential oils as potential inhibitors of green mould on citrus fruits. *The Journal of Horticultural Science and Biotechnology*, DOI: 10.1080/14620316.2021.2011433

THE JOURNAL OF HORTICULTURAL SCIENCE AND BIOTECHNOLOGY
https://doi.org/10.1080/14620316.2021.2011433

Taylor & Francis
Taylor & Francis Group

Check for updates

Lemon myrtle and lemon scented tea tree essential oils as potential inhibitors of green mould on citrus fruits

M. M. Rahman[a], R.B.H. Wills[a], Michael C. Bowyer[a], John B. Golding[a,b], Timothy Kirkman[a] and Penta Pristijono[a]

[a]School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia; [b]NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia

Abstract

This study examined the ability of essential oils (EOs) obtained from the Australian native plants, lemon myrtle (*Backhousia citriodora*) (LM) and lemon scented tea tree (*Leptospermum petersonii*) (LSTT) to inhibit the in vivo growth of Penicillium digitatum in citrus fruits. The main constituent of LM and LSTT EOs was citral at 85 and 64%, respectively. Citral was included for comparison. Oranges dipped for 120 sec in EO solutions showed less fungal wastage compared to control with efficacy being LM > pure citral > LSTT, with 1000 µL L^{-1} LM EO the optimal treatment. The greater response of LM over LSTT EO was attributed to its higher concentration of citral but the greater effect over citral suggested some minor constituent(s) of LM EO also had antifungal activity. While the 120 sec dips caused severe rind injury, a 30 sec dip in 1000 µL L^{-1} LM EO generated only slight injury and inhibited fungal wastage in Valencia and Navel oranges, mandarins and lemons. A 10 sec dip did not cause rind injury but was less effective for mould inhibition. The findings suggest LM EO as an alternative to synthetic fungicides to inhibit wastage in citrus during storage, particularly for organic produce.

General Discussion

These results show that the EOs from the Australian native LM and LSTT inhibited development of *P. digitatum* wastage on a range of citrus fruits, indicating they could be an alternative to the use of chemical fungicides, especially for fruit marketed as organic. LM EO had higher inhibition efficacy, presumably due to having a higher citral content than LSTT EO. However, LM EO was found to be more effective than citral, which suggests that some as yet unknown minor component of LM has high antifungal activity. A major barrier to the use of LM EO for fresh fruit marketing is the generation of rind injury. However, it might be more acceptable for organic citrus fruit storage that are to be processed for juice or other products. The rind injury could be due to the action of citral, which has been shown to disrupt fruit membrane structure and is assumed to be the mechanism whereby it disrupts the growth of *P. digitatum* (Ben-Yehoshua et al. 1992; Leite et al. 2014). The ability of citral to damage rind cells is supported by the finding that rind injury was more severe on fruit dipped in 100% citral than in LM EO, which comprises only 85% citral.

The reduction in rind injury achieved by dipping fruits for shorter times is probably due to a reduced level of citral accumulation on the rind. For commercial dipping operations, immersion of fruit in the dip solution for 30 sec would seem to be the lowest possible throughput time. On this basis, the above findings indicate that while LM EO was able to inhibit the development of green mould, this would be accompanied by a low level of rind injury on some fruit. However, shorter contact times of the LM EO solution are possible by spraying fruit on a conveyor belt. Dipping fruit in LM EO for 10 sec did not generate rind injury, and while not as effective in inhibiting mould growth as a 30-second dip, it still gave a substantially longer storage life than control fruit. To overcome rind injury but retain longer exposure times, it would be worthwhile investigating the different modes of applying LM EO and the incorporation of the EO into edible films, coatings and/or nano encapsulation. The potential benefit of the formulation was shown by Rodov et al. (2011), who found citral dissolved in 25 % v/v ethanol suppressed *P. digitatum* decay in 'Eureka' lemons without visible rind damage. An alternate strategy would be to identify the minor constituent(s) in LM EO with antifungal activity and seek plant EOs that have higher concentrations of these constituents.

All references are listed in the manuscript. A copy of this manuscript is available from the author.

3. Rahman M.M., Wills R.B.H., Bowyer M.C., Vuong V.Q., Golding J.B., Kirkman T. and Pristijono P. (2023) Efficacy of lemon myrtle essential oil as a bio-fungicide in inhibiting citrus green mould. *Plants* 12(21):3742. https://doi.org/10.3390/plants12213742

plants

MDPI

Article

Efficacy of Lemon Myrtle Essential Oil as a Bio-Fungicide in Inhibiting Citrus Green Mould

Mohammad M. Rahman [1,*], Ronald B. H. Wills [1], Michael C. Bowyer [1], Van Q. Vuong [1], John B. Golding [1,2], Timothy Kirkman [1] and Penta Pristijono [1]

Abstract

The effectiveness of lemon myrtle (LM) (*Backhousia citriodora*) essential oil (EO) was investigated to combat Penicillium digitatum by in vitro agar diffusion and vapour assay and in artificially infected oranges. The main constituent of LM EO was revealed as citral when analysed in gas chromatography–mass spectrometry. Pure citral was also included in the experiment for comparison. The in vitro fungal growth was significantly inhibited by LM EO at 1, 2, 3, 4 and 5 µL per disc while complete growth inhibition by both the pure citral and LM EO occurred at 4 and 5 µL per disc. Inoculated fruits treated by dipping in 1000 µL L^{-1} LM EO solutions for 5, 10, 15, 30 and 120 s showed significantly lower fungal wounds compared to the control. While longer dipping times led to some rind injuries, fruits with a 5 and 10 s dip were found free from any injury. The evaluation after dipping and storage confirmed that the fruits maintained the sensory attributes and were not compromised by the incorporation of the essential oil. The results of this study indicate that LM EO can be a promising alternative to synthetic fungicides for preserving the quality of citrus fruits during storage.

Conclusions

Both extracted and commercial LM EOs contain high levels of citral with a content of approximately 88%. Other constituents were also found in these LM EOs, though their extent was very low, within a range of 0.5 to 2% of total constituents. LM EO was effective in inhibiting mould growth in the in vitro tests and in oranges. In the in vitro tests, the crude oil was used without any dilution in the present investigation. So, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by microdilution were not determined. The level of mould inhibition of LM EO was even greater than that of citral, the major and well-known antimicrobial compound. This indicates that LM EO might have some as yet unknown minor components that might exhibit potent antimicrobial activity. Therefore, future studies are suggested to examine the MIC and MBC of LM EO and the individual and synergistic effects of other minor components in LM EO on the prevention and treatment of bacterial or other fungal growth in oranges and other fresh produce.

All references are listed in the manuscript. A copy of this manuscript is available from the author.

4. Rahman M.M., Wills R.B.H., Bowyer M.C., Golding J.B., Kirkman T. and Pristijono P. (2023) Potential control of postharvest fungal decay of citrus fruits by crude or photochemically changed essential oils–a review. *Food Reviews International.* pp. 1-18. DOI: 10.1080/87559129.2023.2204157

FOOD REVIEWS INTERNATIONAL
2024, VOL. 40, NO. 4, 1029–1046
https://doi.org/10.1080/87559129.2023.2204157

Potential Control of Postharvest Fungal Decay of Citrus Fruits by Crude or Photochemically Changed Essential Oils – a Review

Mohammad M. Rahman[a], Ron B.H. Wills[a], Michael C. Bowyer[a], John B. Golding[a,b], Timothy Kirkman[a], and Penta Pristijono[a]

[a]School of Environmental and Life Sciences, University of Newcastle, Ourimbah, Australia; [b]NSW Department of Primary Industries, Ourimbah, Australia

Abstract

Limiting postharvest losses of fruit from fungal attack is a major priority. While synthetic chemical fungicides are efficacious, the development of chemical resistance and consumer concerns are driving reinvestigation of natural antimicrobial agents. This review emphasizes the control of postharvest decay in citrus fruits with the use of some most common essential oils (EO), the efficacy of EOs in combating fungal infection in both in vitro and in vivo models, and the mode of action of EOs along with the potency of photochemical by-products that have also been investigated as antifungal agents.

Conclusions

The most common and serious diseases affecting postharvest handling of citrus fruits are green and blue moulds caused by *P. digitatum* and *P. italicum*, respectively with sour rot caused by *Geotrichum citri-aurantii*, can be an important disease. Synthetic fungicides are often the current primary means of controlling these postharvest diseases, however public concern over the use of these synthetic agents in food processing requires the development of alternative strategies. Plant-based antimicrobial agents such as EOs and their photochemically modified derivatives offer a safer and more eco-friendly alternative to synthetic fungicides. Their production also makes use of a waste stream from citrus-processing.

For EO use to gain commercial acceptance, it is important to fully understand the efficacy of the bioactive constituents, treatment requirements for different produce and potential impact on the physical and organoleptic parameters of the treated produce. In vivo testing typically requires higher treatment concentrations because of differences in the character of food surface properties (e.g. hydrophobicity) which impact on both the behaviour of the bioactive and the microbial agent. While many EOs have been demonstrated to possess antifungal activity, the commercial use of these agents is limited because of issues such as phytotoxicity, intense sensory attributes or technological problems associated with wide-scale production and application. The limitations associated with sensory attributes and phytotoxic effects depend on concentration, application method, treatment duration and the nature of the produce treated.

All references are listed in the manuscript. A copy of this manuscript is available from the author.

Postharvest management of anthracnose

Anthracnose is a rind blemish that can develop in the orchard but is more commonly seen after harvest in the retail market. The symptoms of anthracnose are often not seen on the fruit at harvest but are often expressed as the typical 'gas burn' symptoms in mandarins after degreening. Anthracnose is generally not a major issue in many drier growing areas but can be a big problem in wet growing years.

Anthracnose is caused by *Colletotrichum* species of fungi, which are commonly found in orchards. Fungal spores are produced on dead twigs and foliage within the tree and are spread by water (e.g. rain) (Figure 48). Therefore, anthracnose can be a problem when conditions are wet. Spores appear as pink- or salmon-coloured masses in humid conditions, or in drier conditions, they appear brown to black. The spores infect the fruit during the growing season where they germinate, invade the rind and then remain dormant. During this time, there are no symptoms of infection, and the fruit looks good. However, rind blemish typically develops when the rind is stressed. The fungus can also grow and show the classic 'gas burn' symptoms after the fruit is harvested and stressed, e.g. during degreening.

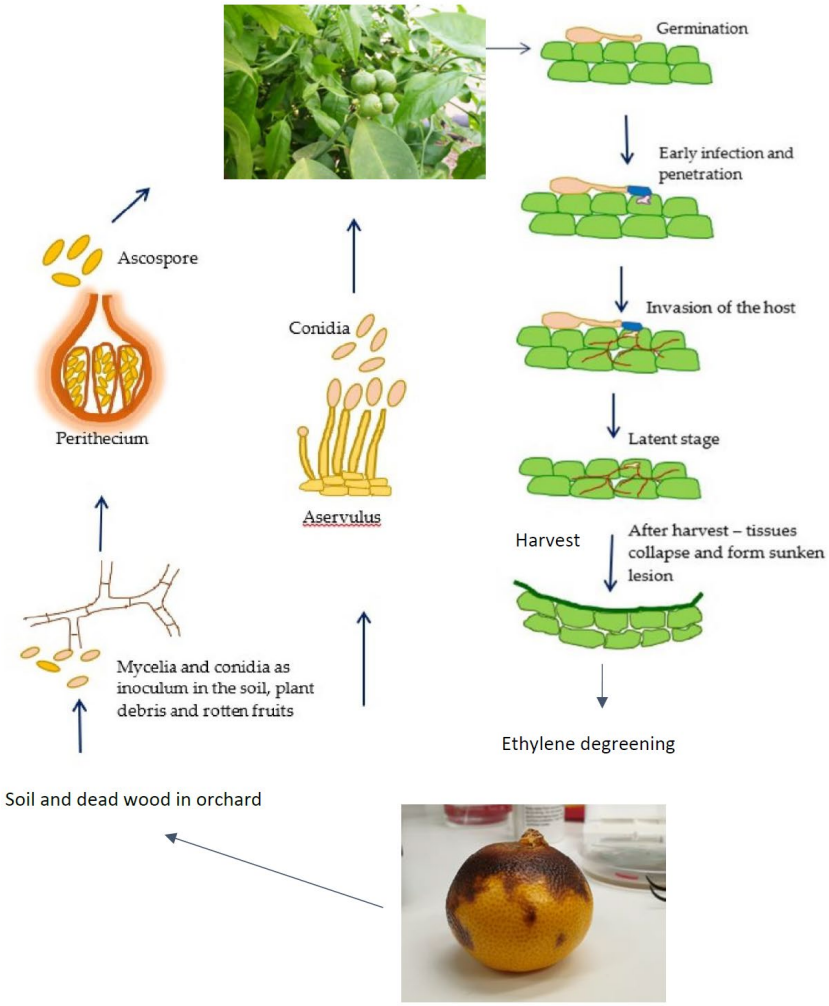


Figure 48. Life cycle and infection of anthracnose in citrus. (Adapted from Zakaria. *Agriculture* 2021, 11(4), 297)

The classic anthracnose symptoms are shown in Figure 49. After ethylene degreening, the peel develops silver-grey legions, which are initially flat. These quickly develop into either sunken black lesions or a superficial reddish-brown discolouration on the fruit skin. Initially only the skin is affected, but in the advanced stages, the fungus can penetrate deep into the flesh, causing fruit to rot.

Anthracnose is sometimes referred to as 'gas burn' because symptoms can develop after degreening with ethylene,

especially in early season green fruit with no signs of colour break. Ethylene triggers the growth of the dormant fungus and increases the susceptibility of the rind to damage.

Figure 49. Classic anthracnose symptoms on Imperial mandarins in the Southern retail market.

There is much that can be done to prevent fruit infection in the field, especially during wet seasons. It is essential to minimise the risk of anthracnose infection. This can be done with annual pruning and keeping the trees free of dead wood to reduce spore production. It is also important to use field sprays of copper-based fungicides to inhibit spore germination. The application of a protectant copper spray before autumn will reduce the development of anthracnose in the orchard, but more sprays are needed in wet seasons. Note that copper sprays are only effective when the copper is on the fruit skin. Copper can easily be washed off with rain and coverage is reduced as the fruit grows. Anthracnose can also develop in fruit that are harvested when over-mature or held too long in storage.

After harvest, ethylene degreening encourages the expression of anthracnose symptoms in the fruit. Therefore, minimising the time spent on degreening and reducing the severity of the degreening treatments helps to reduce anthracnose. For example, the use of ethylene trickle system or ethylene generators, ensuring degreening ethylene levels do not exceed 5 ppm and reducing the time in degreening all help to reduce the risk of anthracnose developing in the supply chain.

A series of postharvest trials were conducted in 2019–2021 to identify practices that minimise the expression of anthracnose and screen a range of common postharvest fungicides that can reduce its expression. These fungicides are not registered for controlling anthracnose but can potentially reduce its expression.

Postharvest fungicide screening against anthracnose (2019)

Imperial mandarins were harvested from a commercial orchard in Mundubbera, Queensland. The orchard block from which the fruit were harvested had previously been known to have a high level of high level of anthracnose. Fruit were transported to NSW DPI, assigned to the treatment groups and dipped.

Commercial postharvest fungicides were prepared according to the existing label (Table 2).

Table 2. Fungicides assessed against anthracnose in Imperial mandarin trial (2019).

Tradename	Formulation	Active ingredients	Registrant
Graduate A+®	suspension concentrate	fludioxonil (239 g/L) and azoxystrobin (239 g/L)	Syngenta
Chairman®	suspoemulsion	fludioxonil (240 g/L) and propiconazole (102.5 g/L)	Syngenta
Cabrio®	emulsifiable concentrate	pyraclostrobin (250 g/L)	BASF
Sportak®	emulsifiable concentrate	prochloraz (450 g/L)	FMC
Tecto®	suspension concentrate	thiabendazole (500 g/L)	Syngenta

Results

Figure 50 shows the effect of the amount of degreening on the expression of anthracnose development and shows fruit degreened for 4 days had significantly more anthracnose than fruit degreened for only 1 day.

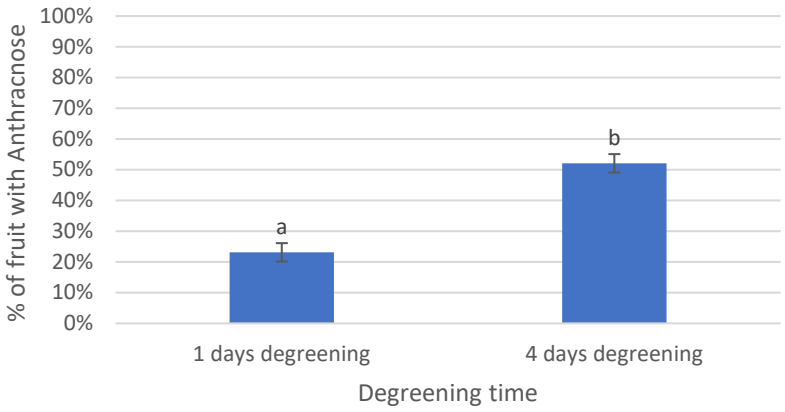


Figure 50. Effect of degreening time on the development of anthracnose in Imperial mandarins. Values are the means of 100 fruit from 4 replicates. Connecting letters report using a Tukey-Kramer (HSD 0.08494 q* 2.00763 α = 0.05).

The results of the different fungicides on the rate of fruit with anthracnose symptoms are presented in Figure 51 and show that TBZ had no effect on controlling anthracnose.

The fungicides Graduate A+®, Chairman® and Cabrio® were found to have statistically reduced anthracnose expression on fruit which had been degreened for one day (Figure 51). Graduate A+® and Chairman® both contain the active ingredient fludioxonil, which is a broad-spectrum fungicide. For fruit that had been degreened for 4 days only, the fungicide Cabrio® was found to statistically reduce the rate of anthracnose infection. Cabrio® is an advanced strobilurin fungicide that is not registered for use in citrus. It is a registered preharvest treatment against husk spot in macadamia nuts and controls downy mildew in grapevines and rust in almonds.

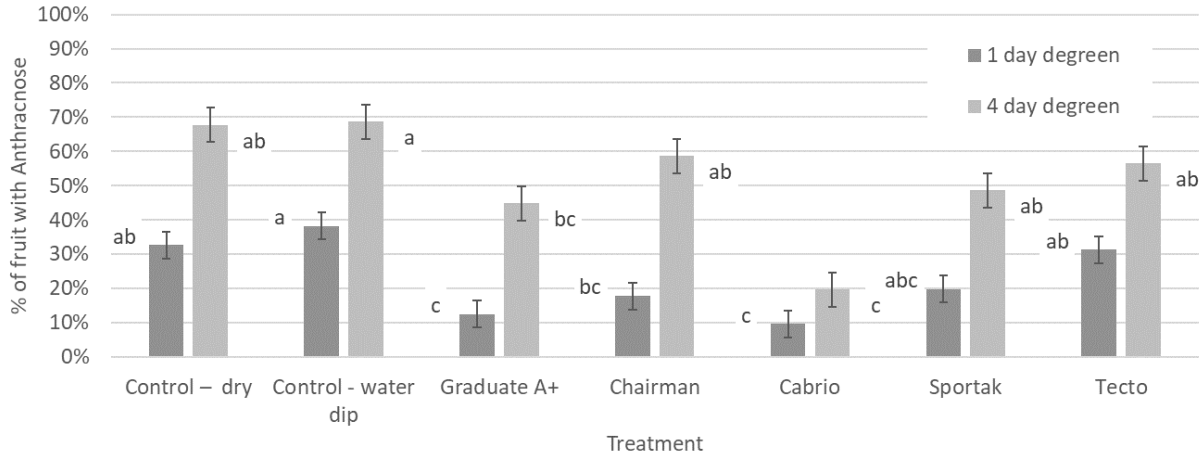


Figure 51. The effect of different fungicides on mandarins following degreening with ethylene for either one day or 4 days. Values are the means of the disease infection rate (%) from 100 fruit with 4 independent replicates. Connecting letters report using a Tukey-Kramer technique (one-day degreening HSD 0.18368 q*3.3044 α = 0.05 and 4-day degreening HSD 0.23307 q*3.3044 α = 0.05). The one-day and 4-day degreening treatments were analysed separately.

The results show that in this research trial, Chairman® fungicide (containing fludioxonil and propiconazole), which is registered as a postharvest treatment against green and blue mould in citrus, also has some additional effect against anthracnose in Imperial mandarins. This trial was repeated with registered postharvest fungicides (not only citrus fungicides) in 2020.

Postharvest fungicide screening against anthracnose (2020)

Imperial mandarins from Mundubbera were harvested, transported to NSW DPI, and treated with the following fungicides (Table 3).

Table 3. Commercial postharvest fungicides (and their codes) used for the storage trial of Imperial mandarins for the expression of anthracnose (2020).

Code	Fungicide	Active ingredient	Fungicide group
1. UTC	Control	water dip	
2. TBZ	Tecto	500 g/L thiabendazole	Group 1
3. IMZ	Fungaflor	500 g/L imazalil	Group 3
4. CHR	Chairman	240 g/L fludioxonil and 102.5 g/L propiconozole	Group 3 and 12
5. FLU	Scholar	230 g/L fludioxonil	Group 12
6. PHL	Philabuster	200 g/L imazalil and 200 g/L pyrimethanil	Group 3 and 9
7. GUZ	Panoctine	400 g/L guazatine	Group M7
8. GRD	Graduate A+	239 g/L azoxystrobin + 239 g/L fludioxonil	Group 11 and 12
9. PRO	Sportak	450 g/L prochloraz	Group 3
10. CAB	Cabrio	250 g/L pyraclostrobin	Group 11

Results

The effect of different postharvest fungicides on the expression of anthracnose in Imperial mandarins is presented in Figure 52 and showed that Chairman ®, Fludioxonil, Graduate A ®, Sportak ®, and Cabrio ® postharvest dips resulted in lower anthracnose development. In this season (2020), increasing the time in degreening conditions (4 ppm ethylene, 25 °C and 90% relative humidity) did not affect the development of anthracnose (Figure 53).

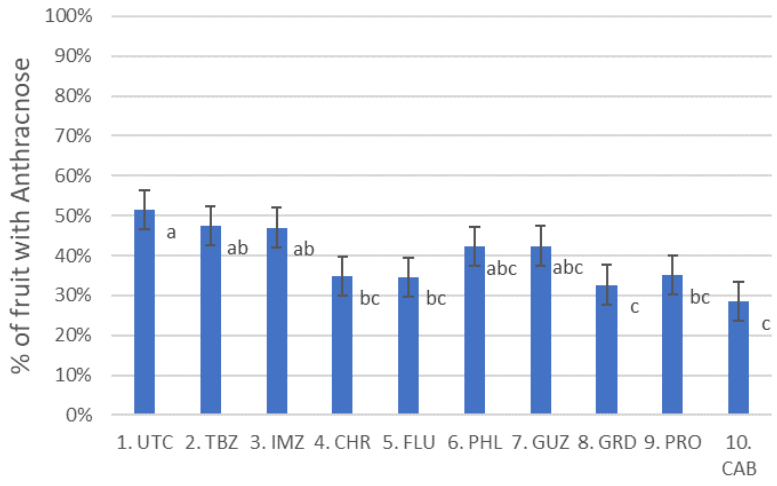


Figure 52. Effect of trial fungicides on the percentage of fruit with anthracnose (%). Bars are standard deviations around the means, *n*=4.

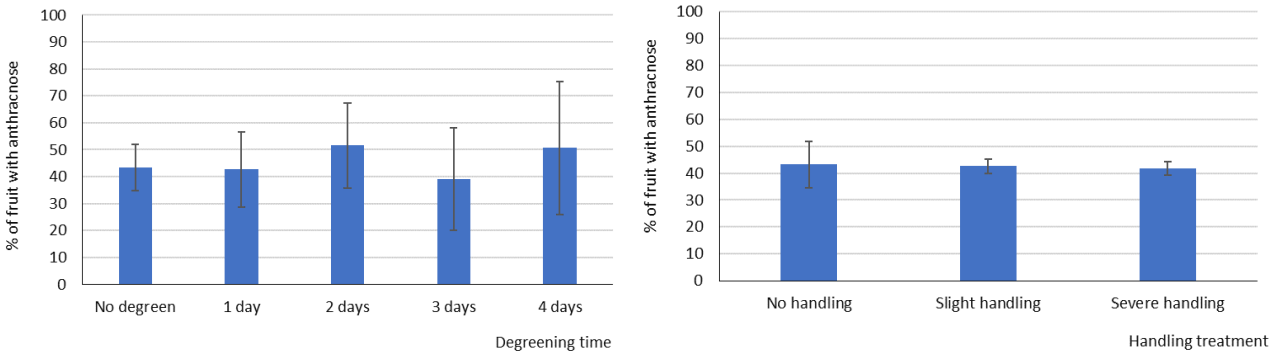


Figure 53. Effect of degreening time (left) and handling treatment (right) on the percentage of fruit with anthracnose (%). No handling: water dip only (no additional handling), slight handling: brushes only for 15 sec, severe handling: brushes and high-pressure wash for 60 sec. Bars are standard deviations around the means, *n*=4.

Postharvest fungicide screening against anthracnose (2021)

The trial design from previous seasons (2019 and 2020) was repeated with Imperial mandarins from Mundubbera, Queensland with 12 postharvest fungicide treatments: label rate thiabendazole, imazalil, Chairman ® fungicide [fludioxonil and propiconazole], fludioxonil, Philabuster ® fungicide [imazalil and pyrimethanil], guazatine, Graduate A+ ® fungicide [azoxystrobin + fludioxonil], Sportak ® fungicide [prochloraz], pyrimethanil, a combination of thiabendazole + fludioxonil active ingredients, which previous QDAF researchers have shown to have some efficacy on Imperial mandarins and a heated treatment of the previous combination treatment at 50 °C. The fungicides assessed in 2021 are presented in Table 4.

Table 4. Postharvest fungicides (and their codes) assessed against anthracnose in 2021. All fungicides were used at the table rate and recommendations.

Code	Fungicide
UTC	Control–Water dip
TBZ	Tecto ®
IMZ	Fungaflor ®
CHR	Chairman ®
FLU	Scholar ®
PHL	Philabuster ®
GUZ	Panoctine ®
GRD	Graduate A+ ®
PRO	Sportak ®
PYR	Scala ®
COM	Combination TBZ + FLU
HOT	50 °C hot combination

Each of these treatment combinations was prepared independently in 4 separate batches, and each was replicated in random order to ensure the independence of the fungicide treatment. There were 100 fruit per treatment unit and 4 independent replicates of each treatment. Additional experiments with the same batch of fruit examined (1) the effect of degreening time (0, 1, 2, 3 and 4 days) with or without 5 ppm ethylene at 25 °C and 90% RH, and (2) the effect of storage temperature (3, 10, 20 and 25 °C) on the expression of anthracnose. Over 10,000 Imperial mandarins were treated, stored, and assessed every week for 4 weeks.

Results

Part A. Effect of trial fungicides on postharvest expression of anthracnose

The number and severity of anthracnose infections in the stored fruit following treatment are presented in Figure 55. The untreated control mandarins only had <10% natural infection, even after 4 weeks of storage (Figure 54). This highlights the inherent random nature of the preharvest infection and expression of anthracnose between seasons. The orchard from which the fruit were sourced in Queensland had a history of anthracnose; however, in the 2021 season, these fruits did not develop any anthracnose symptoms and it was not possible to assess the different fungicides and treatments. This was disappointing but illustrates the seasonal nature of agronomic/postharvest trials.

Figure 54. Imperial mandarins, after 4 weeks of storage, showed no anthracnose symptoms.

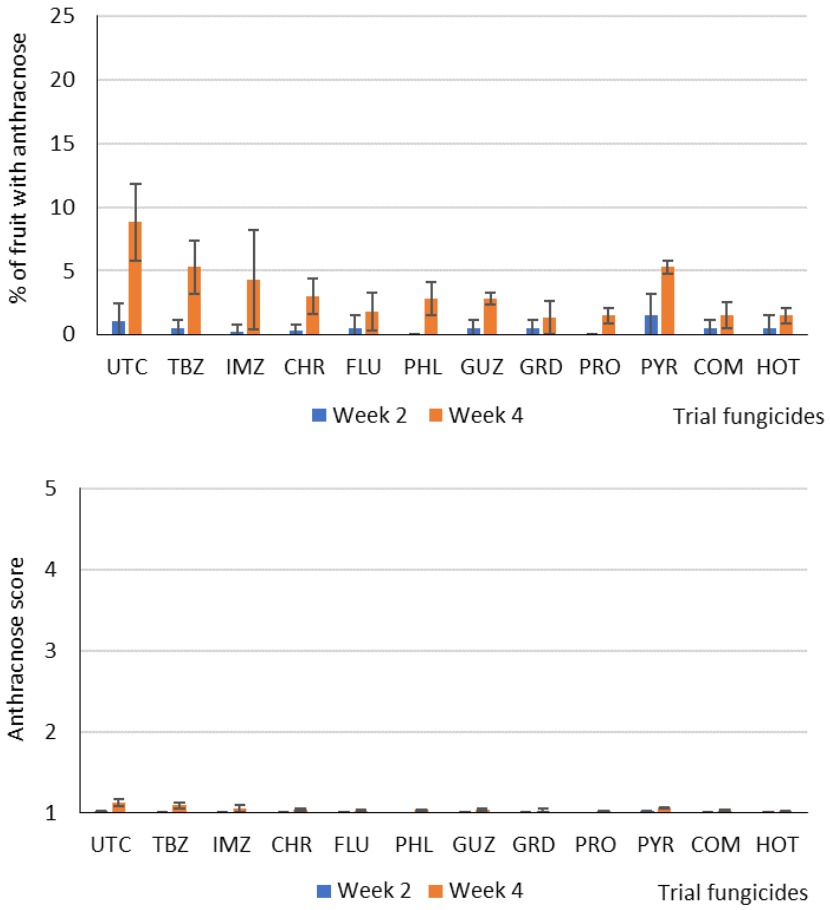


Figure 55. Effect of trial fungicides on the percentage of fruit with anthracnose (%) (top) and anthracnose score (lower) of fruit after 2 and 4 weeks. Anthracnose score: 1 = no damage, 2 = some detected–trace (<2 mm damage), 3 = <10% of fruit detected (<10 mm damage), 4 = 10–25% of fruit affected (10–40 mm damage) and 5 = >25% of fruit affected (>40 mm damage). Bars are standard deviations around the means, *n*=4.

The levels of natural postharvest decay (blue and green mould) following treatment and storage are presented in Figure 56. All postharvest fungicides have efficacy against postharvest decay, with the hot combination treatment showing no decay after 4 weeks of storage.

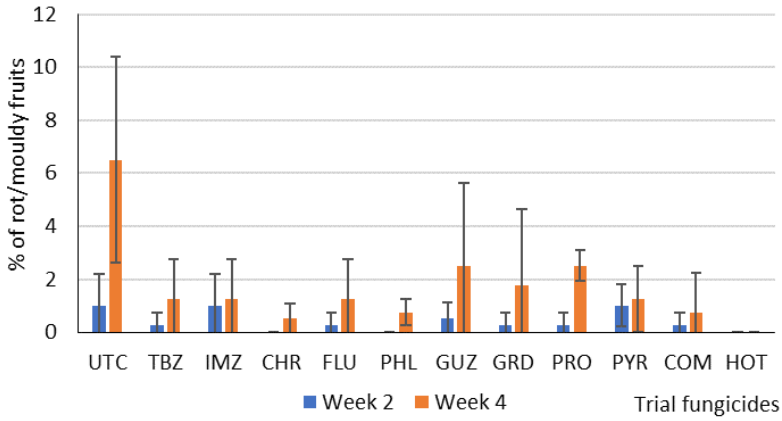


Figure 56. Effect of trial fungicides on the percentage postharvest decay (%) after 2 and 4 weeks. Bars are standard deviations around the means, *n*=4.

Part B. Effect of ethylene and time at 25 °C and postharvest temperature on the expression of anthracnose

B1. Effect of ethylene and time at 25 °C on the expression of anthracnose

The effect of degreening time (i.e., time in ethylene) on the incidence and severity of anthracnose expression was investigated. The fruit in this trial had no postharvest treatments. The results show that longer storage times (4 weeks) and fruit degreened for longer times (3 and 4 days) had a higher incidence of anthracnose (Figure 57).

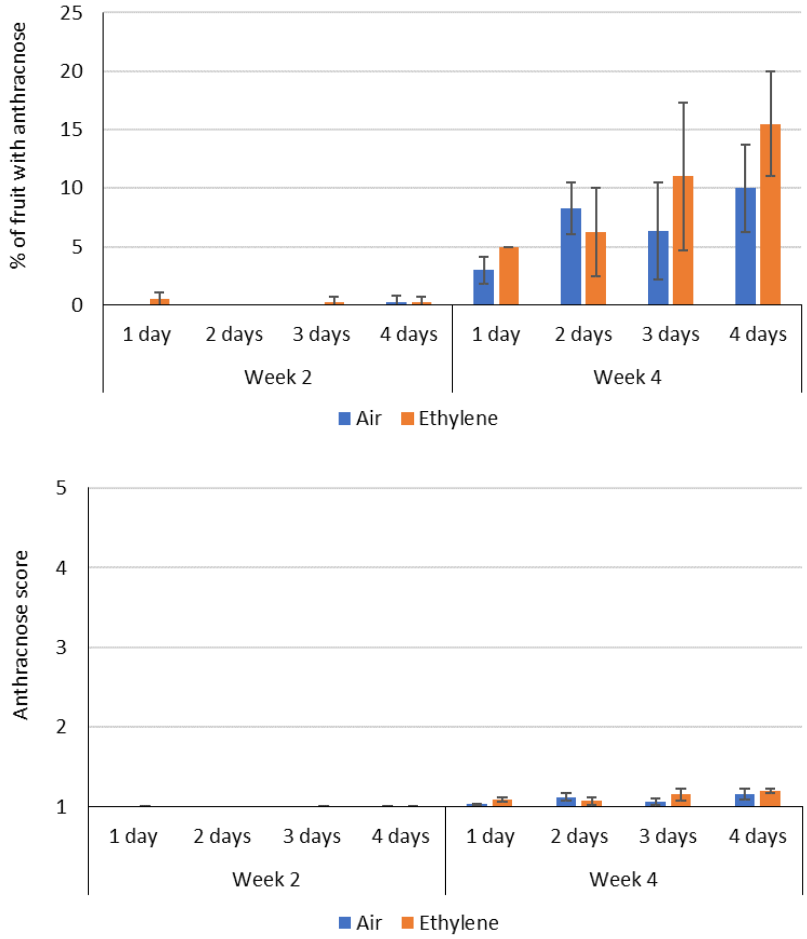


Figure 57. Effect of ethylene and time at 25 ℃ on the percentage of fruit with anthracnose (%) and anthracnose score of fruits. Anthracnose score: 1 = no damage, 2 = some detected–trace (<2 mm damage), 3 = <10% of fruit detected (<10 mm damage), 4 = 10–25% of fruit affected (10–40 mm damage) and 5 = >25% of fruit affected (> 40 mm damage). Bars are standard deviations around the means, *n*=4.

B2. Effect of postharvest temperature on the expression of anthracnose

The effect of different storage temperatures post-degreening (4 days at 25 ℃ in the air) is shown in Figure 58. Lower storage temperatures suppress the development of anthracnose.

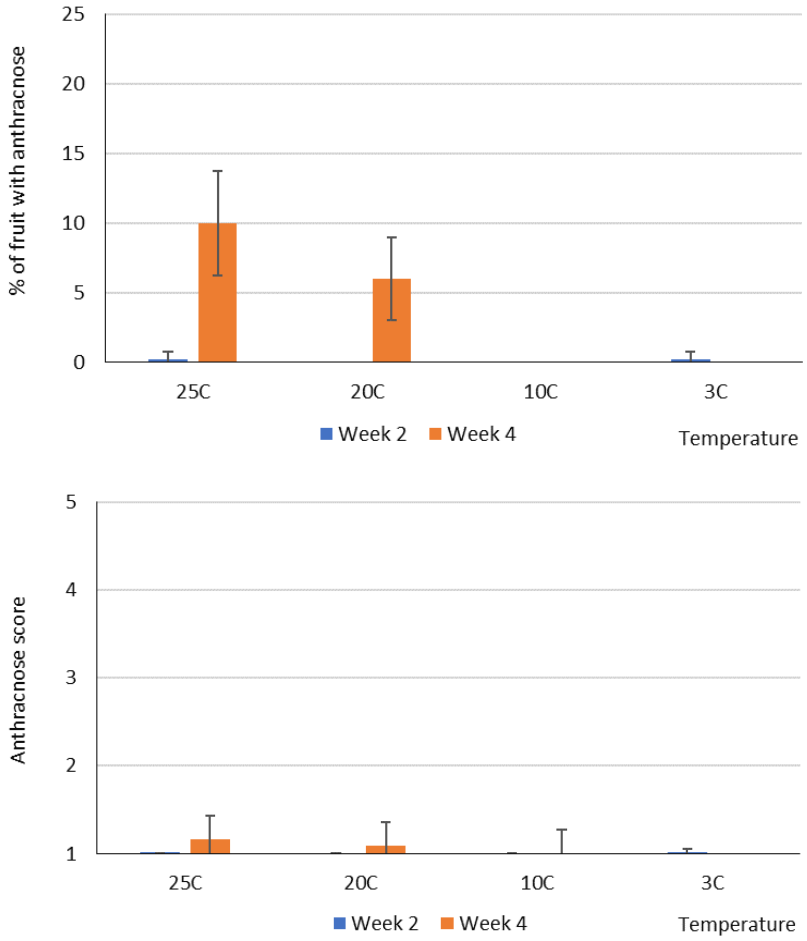


Figure 58. Effect of different postharvest temperatures on the percentage of fruit with anthracnose (%) (top) and on anthracnose score of fruits (lower). Anthracnose score: 1 = no damage, 2 = some detected–trace (<2 mm damage), 3 = <10% of fruit detected (<10 mm damage), 4 = 10–25% of fruit affected (10–40 mm damage) and 5 = >25% of fruit affected (> 40 mm damage). Bars are standard deviations around the means, *n*=4.

Optimising organic salts to control postharvest decay

The development of alternative chemical control treatments has focused on food additives and natural compounds with minimal effects on human health and the environment. There has also been a focus on the application of natural organic acid salts such as sodium benzoate and potassium sorbate in aqueous solutions. Many research studies have shown the positive effects of food additives [sodium bicarbonate (SB), sodium benzoate (SBen), and potassium sorbate (PS)] and elevated treatment temperatures on reducing postharvest decay in citrus. However, each salt solution, heated or not, is normally applied as a stand-alone treatment and little information is available on the effectiveness of mixtures of these different GRAS salts to control postharvest decay in citrus. Response surface methodology (RSM) is a useful way to show the interactive effects between different independent and response variables, which can reduce time and cost by simultaneously assessing numerous experimental parameters. Therefore, RSM can be a valuable resource for systematically investigating the interactions and additive effects of these food additives and high treatment temperatures. This study seeks to fill this knowledge gap and determine the optimal dipping solutions of the food additives SB, SBen and PS (at concentrations of 0.5 to 6.0%) combined with elevated but non-phytotoxic treatment temperatures (20–50 °C) using RSM for the control of GM and BM in artificially inoculated Valencia oranges. This study was published in a refereed scientific journal with acknowledgements of the contribution of this Project:

Archer, J., Pristijono, P., Vuong, Q.V., Palou, L. and Golding, J.B. (2024) Utilising response surface methodology to optimise food additives and treatments reduces disease caused by *Penicillium digitatum* and *Penicillium italicum* in 'Valencia' oranges. *Horticulturae*, 10(5), 453. doi.org/10.3390/horticulturae10050453

horticulturae

MDPI

Article

Utilising Response Surface Methodology to Optimise Food Additives and Treatments Reduces Disease Caused by *Penicillium digitatum* and *Penicillium italicum* in 'Valencia' Oranges

John Archer [1,2,*], Penta Pristijono [1], Quan V. Vuong [1], Lluís Palou [3] and John B. Golding [1,2,*]

Abstract

Penicillium digitatum and *Penicillium italicum* are responsible for citrus green and blue moulds (GM and BM), respectively, which are major citrus postharvest diseases. The aim of this study was to develop an optimal dipping mixture of an aqueous solution of different food additives: sodium bicarbonate (SB), sodium benzoate (SBen), and potassium sorbate (PS), in combination with heat, to control GM and BM using response surface methodology. The ranges of SB (0.0%, 3.0%, 6.0%), SBen (0.0%, 0.5%, 1.0%), PS (0.0%, 0.5%, 1.0%) and temperature (20 °C, 35 °C, 50 °C) with a dipping time of 60s were tested for their impact on GM and BM on artificially inoculated oranges. Within these tested ranges, SB reduced GM severity and incidences of both GM and BM. PS affected BM severity and incidence, but not GM. SBen and temperature did not have impact on GM and BM. The most suitable food additive concentrations were identified to be 4.7% SB, 1.0% SBen and 0.7% PS, with a dipping solution temperature of 50 °C. This treatment was shown to reduce GM and BM incidence from 85 and 86% on control fruit dipped in tap water at 20 °C to 3 and 10%, respectively. Additionally, the severity of GM and BM was reduced from 64 and 26 mm on control fruit to <1 and 2.8 mm, respectively.

Conclusions

This study successfully applied RSM to identify effective combinations of SB, SBen, PS, and temperature for controlling GM and BM, caused by *P. digitatum* and *P. italicum*, respectively, in Valencia oranges. SB notably reduced both GM severity and incidence, along with BM incidence, while it did not significantly affect BM severity. In contrast, SBen and treatment temperature showed no substantial influence on GM and BM at the tested concentrations, likely due to the low concentrations of SBen used in these trials (<1%). By comparison, other research showed significant effectiveness of SBen at higher concentrations, suggesting a threshold-dependent action. PS, although ineffective against GM at the tested concentrations, significantly influenced BM severity and incidence.

Using RSM models, this study identified an optimal postharvest treatment using a mixture of SB at 4.7%, SBen at 1.0%, and PS at 0.7% at a dip temperature of 50 °C, which decreased the incidence and severity of both GM and BM. The reduction in GM incidence was from 85% in the control group treated with water at 20 °C to less than 5% in oranges treated with this combination. In the case of BM incidence, this reduction was from 87% to 10%.

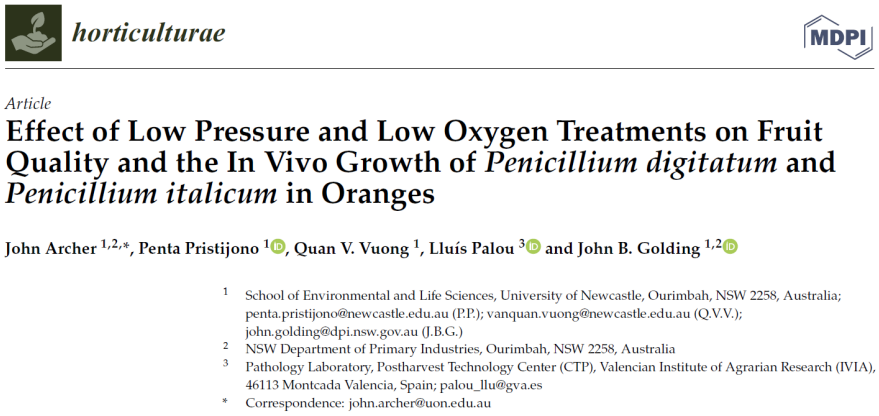
Future experimentation should scale up these experimental results for broader commercial application with the aim to control GM and BM on 'Valencia' oranges. Furthermore, similar research could reduce postharvest decay of other commercially important orange cultivars, such as Navels, mandarins, and lemons. The broader implications of this study are significant where the recommended treatment could potentially improve postharvest disease control in 'Valencia' oranges, reducing food wastage and increasing shipping tolerances, all achieved without the use of synthetic fungicides. Therefore, this approach offers an environmentally friendly and efficient way of preserving the quality and extending the shelf life of citrus fruit.

A copy of this manuscript is available from the author.

Physical treatments to reduce postharvest decay

In addition to chemical treatments to control postharvest decay in citrus, physical treatments were assessed for their efficacy against green and blue mould. Physical control measures such as heat treatments (curing and hot water) and irradiation treatments (UV-C and ionising irradiation) have the advantages of leaving no chemical residues but have limited efficacy and any lack of persistence or preventative activity. The effectiveness of low-pressure treatments were assessed against green and blue mould in oranges. The results of some these were published in a refereed scientific journal acknowledging the contribution of this Project:

Archer J., Pristijono P., Vuong Q.V., Palou L. and Golding J.B. (2021) Effect of low pressure and low oxygen treatments on fruit quality and the in vivo growth of *Penicillium digitatum* and *Penicillium italicum* in oranges. *Horticulturae* 7, 582. doi.org/10.3390/horticulturae7120582



horticulturae

MDPI

Article

Effect of Low Pressure and Low Oxygen Treatments on Fruit Quality and the In Vivo Growth of *Penicillium digitatum* and *Penicillium italicum* in Oranges

John Archer [1,2,*], Penta Pristijono [1], Quan V. Vuong [1], Lluís Palou [3] and John B. Golding [1,2]

[1] School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia; penta.pristijono@newcastle.edu.au (P.P.); vanquan.vuong@newcastle.edu.au (Q.V.V.); john.golding@dpi.nsw.gov.au (J.B.G.)
[2] NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia
[3] Pathology Laboratory, Postharvest Technology Center (CTP), Valencian Institute of Agrarian Research (IVIA), 46113 Montcada Valencia, Spain; palou_llu@gva.es
* Correspondence: john.archer@uon.edu.au

Abstract

Penicillium digitatum and *P. italicum* are the major postharvest pathogens in citrus. To reduce postharvest decay, the use of low-oxygen (0.9 kPa O_2) (LO) or low-pressure (6.6 kPa) (LP) treatments were evaluated during the storage of navel oranges for four or eight days. The results showed that exposure to both LO and LP treatments reduced in vivo pathogen growth compared to the untreated (UTC) oranges, with LO being the most effective. The effects of LO and LP on fruit metabolism and quality were further assessed, and it was found that there was no effect on fruit ethylene production, respiration rate, TSS (total soluble solids), TA (titratable acidity) or fruit firmness. However, both LO and LP treatments did have an effect on juice ethanol concentration and fruit weight-loss. The effect of adding exogenous ethylene at either LP (1 µL/L) or atmospheric pressure (AP) (at either 0.1, 1 µL/L) was also evaluated, and results showed that the addition of ethylene at these concentrations had no effect on mould diameter at LP or AP. Therefore, both LO of 0.9 kPa O_2 and LP of 6.6 kPa at 20 °C are potential non-chemical postharvest treatments to reduce mould development during storage with minimal effects on fruit quality.

Conclusions

The results showed that LP storage of 6.6 kPa and LO treatments of 1% O2 (for 4 and 8 days at 20 °C) decreased *P. digitatum* and *P. italicum* growth in infected oranges. The re-duction in blue mould severity observed with the application of these two physical treatments might also potentially be an alternative to chemical fungicides and could contribute to organic or chemical-free citrus production. It was also noted that LP and LO treatments reduced weight loss compared to the UTC. However, these physical treatments resulted in an increase in ethanol levels within the fruit, presumably through anaerobic metabolism, but these increased levels were below levels that the consumer can perceive. The addition of exogenous ethylene at low concentrations was also assessed at atmospheric pressure and low pressure and was found to have no effect on mould development. These experiments support the potential of LP and LO storage for decay reduction in citrus; however, further experiments need to be conducted to test viability to ensure that commercial treatments can be developed. Whilst the introduction of LP may increase storage duration and quality, the concerns in developing treatments on a commercial level include scaling for the LP vessel.

A copy of this manuscript is available from the author.

Improving market access outcomes

Meeting MRLs – removing dimethoate residues from packing lines

Dimethoate is an organophosphate insecticide that can be used as a postharvest end-point treatment to facilitate the interstate trade for some citrus. However, it is thought that dimethoate residues can remain impregnated within the packing line following long treatment times. The presence of potential dimethoate residues in the packingline may then contaminate non-dimethoate-treated fruit processed on the same line. This potential contamination of non-treated fruit can lead to the detection of MRL residues of dimethoate/omethoate in some sensitive export markets. This pilot project assessed different postharvest cleaning products to potentially remove/decontaminate packinglines with embedded dimethoate residues.

The trial cleaning products used were:

(1) Muirs/Decco EcoCleaner Line® (active ingredients – sodium dodecylbenzene sulphonate and citric acid)

(2) Muirs/Decco Equipment Cleaner 510® (active ingredient – sodium metasilicate)

(3) Campbells Vacate wax remover® (active ingredients – sodium hydroxide and potassium hydroxide)

(4) Campbells/Pace Acidex Duo (active ingredient – phosphoric acid)

(5) Chemtech Shock treatment® (active ingredient – sodium hydroxide)

(6) Sodium bicarbonate

(7) Kitchen Maid® (from packinghouse – Kitchen Maid Multi-Purpose Hard Surface Cleaner®)
(active ingredients – 2 butoxyethanol and alkaline salts)

This trial was planned in the changeover between local and export in southern Queensland, where the packers were changing from using dimethoate. However, Covid travel restrictions at the time limited the planned trial. Fortunately, the packinghouse was cooperative and used different cleaning products on different cups of the packingline that would have been contaminated with dimethoate residues. The washed cups were sent to NSW DPI and then re-washed with a solvent to remove the dimethoate solvent in the packingline cups (Figure 59). This wash solvent was sent to Symbio Laboratories for dimethoate analysis.

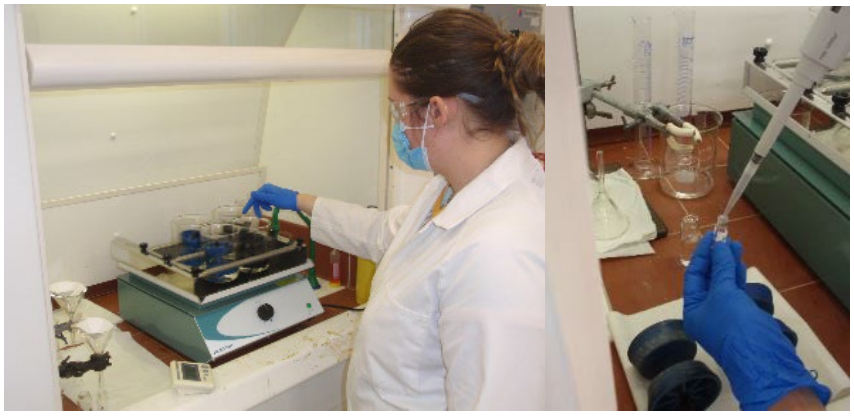


Figure 59. Washing contaminated packingline cups in solvent to assess dimethoate residues.

Results

The results of the % reduction in dimethoate (i.e. combination of dimethoate and omethoate) residues following wash treatments are presented in Table 5. All postharvest wash treatments significantly reduced the levels of dimethoate residues within the packingline and improved the physical appearance of the rollers (Figure 60). The levels of reduction of dimethoate ranged from 84–95 % reduction, as compared to the un-washed rollers. This is a good result as it showed that all commercial washes were effective at reducing the levels of dimethoate in the packinglines.

Table 5. Effect of different commercial postharvest wash treatments on the % reduction in dimethoate residues following wash treatment

Wash treatment *(n = 1 roller)*	**Roller/cup type**		**Average reduction (%)**
	Blue	*Black*	
EcoCleaner®	78.1	88.8	83.5
Vacate®	94.2	95.5	94.9
Acidex Duo®	89.8	93.3	91.6
Equipment Cleaner 510®	94.2	93.3	93.8
Bicarb	85.4	84.3	84.9
Kitchen Maid®	94.2	88.8	91.5
Shock Treatment®	88.3	95.5	91.9

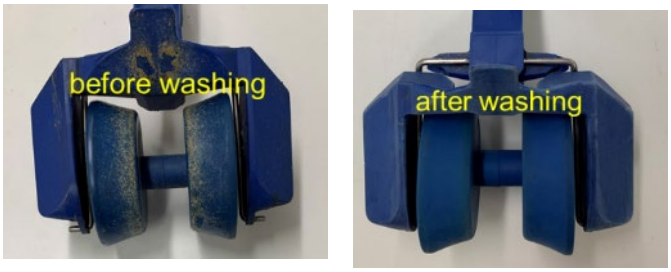


Figure 60. Effect of washing in commercial cleaners on the appearance of rollers/cups.

The results in Table 5 were from one roller wash and solvent residue analysis. This also assumes a constant level of dimethoate residues in the packingline samples and constant removal of residues from the rollers/cups with the standard solvent wash (100% HPLC-grade methanol for 10 minutes at room temperature). This trial was repeated with 2 further replicates of washed packingline cups. After washing, the levels of total dimethoate residues (i.e., a combination of dimethoate and omethoate) were lower than the limit of reporting (LOR). The LOR is the lowest concentration that can be detected with acceptable precision and accuracy. However, the untreated (unwashed) packingline cups had detectable dimethoate residues. As the treated had undetectable residues (i.e. <LOR), then the % reduction in residues could not be determined in the final 2 replicate samples. However, these observations showed the wash treatments reduced the levels of dimethoate residues.

These results are from washing the packinghouse cups and not fruit samples. Before the washing treatments were initiated, the levels of total dimethoate residues (i.e. combination of dimethoate and omethoate) in fruit at the end of the packingline after having been treated with dimethoate in-line within the processing line were, on average, 0.69 mg total dimethoate per kg mandarin fruit. After washing the packingline with their regular commercial treatments, the packer ran Murcott mandarins over the packingline and sent fruit samples for residue analysis. The results showed there were no detectable dimethoate (dimethoate and omethoate) residues in the fruit after this commercial washing. Again, while this was just one sample, no residues were detected in the commercial lot of fruit sample, which shows that, in this case, the commercial wash treatments were successful in reducing any potential residue issues.

Conclusion. While it is ideal not to use organophosphate insecticide treatments on packinglines, these results show that all commercial washing treatments (EcoCleaner®, Vacate®, Acidex Duo®, Equipment Cleaner 510®, Bicarb, Kitchen Maid® and Shock Treatment®) were effective at removing dimethoate residues from the packingline.

Evaluation of postharvest WetCit® application for the removal of Fuller's rose weevil (FRW) eggs, red scale, and effects on postharvest decay

Innovations and new products to improve market access and reduce the risk of residues are crucial for the Australian citrus industry. WetCit® is a new organic adjuvant product that has been trialled in orchards around the world to reduce red scald, however, there is little data on its effectiveness postharvest.

Fuller's rose weevil (FRW) is a quarantine pest for Australian citrus exports. The adult weevils lay their eggs under the orange calyx and are 'cemented' in rafts under the calyx, which makes them very difficult to remove. There is no current postharvest treatment to consistently remove these eggs from under the calyx, which increases export costs as expensive preharvest systems are required to allow exports. Red scale is another critical quarantine pest that can disrupt exports. It would be ideal to have a postharvest treatment such as WetCit® that could remove FRW eggs and red scale in export consignments. The effects of WetCit® on postharvest decay are unknown.

A series of trials were conducted to assess the effects of postharvest applications of WetCit® on (1) *in vivo* survival of FRW eggs in Navel oranges, (2) the effect of different concentrations of WetCit® with dipping and high-pressure washing on red scale removal, and (3) the effect on *Penicillium* decay control.

1. Assess a range of different concentrations of WetCit® with dipping and high-pressure washing on the survival of FRW eggs

A pallet of Washington Navel oranges with a natural infestation of FRW eggs was obtained from a commercial grower in Leeton on 11 August 2021. Fruit were treated with either water, 0.5% or 1% WetCit® by either dipping for 30 seconds or 30 seconds and then high-pressure washing with the sample wash treatment (Figure 61). Each treatment and application method were replicated 4 times and the treatment unit for each treatment was 40 fruit. After treatment, fruit were stored at 20 °C for up to 4 weeks, with the number of FRW larvae assessed every week.

Figure 61. Dipping Washington Navel oranges in WetCit® (left), high-pressure washing treatment after dipping (middle) and assessment of live FRW larvae after treatment and storage with a dissection microscope (right) at NSW Department of Primary Industries.

Results

The results of the effects of dipping in WetCit® at 0.5% and 1.0% and then applying a high-pressure wash on the survival of FRW larvae are presented in Figure 62. Due to the nature of FRW infestations within the orchard, it is not possible to obtain consistent FRW infestation between fruit and treatments. The experimental unit of each treatment was 40 fruit and each treatment was independently replicated 4 times. However, there was large fruit-to-fruit variability in FRW infestation. This was observed in the assessment of the FRW egg rafts and larvae under the fruit calyx, and the results were not consistent or conclusive. High levels of FRW were observed in the 0.5% WetCit® treatment with the high-pressure wash. These levels of FRW were much higher than the water dip (control) and indicate high levels of inconsistency in the results.

These results are inconsistent, and more work should be conducted to assess this treatment.

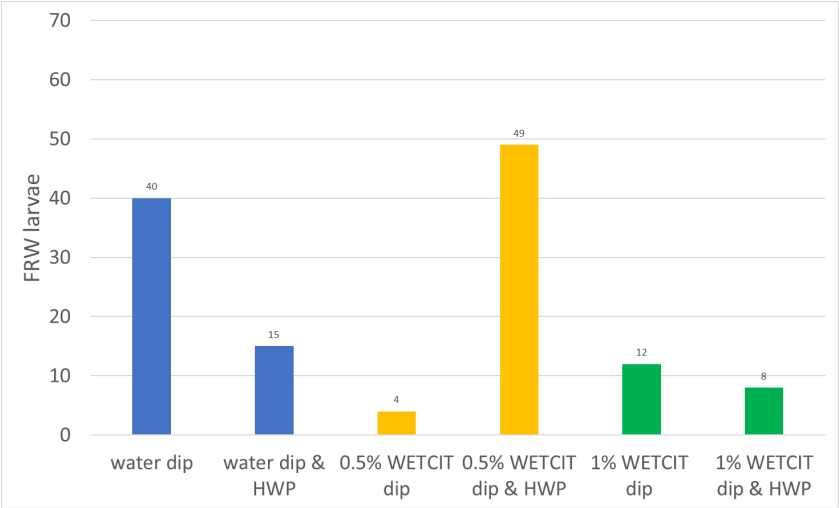


Figure 62. Effect of WetCit® (0, 0.5% and 1.0%) by either dipping alone or with dipping then high-pressure wash on the total numbers of FRW larvae in treated Washington Navel oranges.

2. Assess a range of different concentrations of WetCit® with dipping and high-pressure washing to remove red scale on oranges

Red scale (*Aonidiella aurantia*) is an important quarantine pest in some markets such as South Korea. It is critical to ensure the absence of this pest for exports. This trial examined the effect of different concentrations of WetCit® with dipping and high-pressure washing to remove red scale from oranges.

One pallet of red scale-infested Salustiana oranges was obtained from Griffith. Fruit were sorted into 3 categories of red scale infestation: (1) severe/heavy infestation, (2) moderate infestation and (3) low/light infestation (Figure 63). Each fruit was given a unique number and a 4.6 cm diameter circle (area 16.6 cm^2) was drawn with a permanent marker on the surface of each fruit (Figure 64). The number of red scale inside the marked circle was recorded before and at different times after treatment. Fruit were treated with 4 different treatments: (1) water dip control, (2) 0.5% WetCit®, (3) 1% WetCit® and (4) no washing at all. In addition, there were 2 application methods of the dipping treatments: (1) a 30-second dip in a bucket then brushes only and (2) a 30-second dip in a bucket then high-pressure wash with brushes for 30 seconds. Each treatment had 100 fruit in each red scald infestation category.

Figure 63. Severely infested red scale on oranges used for trial.

Figure 64. A sample area around the fruit equator was marked with the permanent pen on the surface of each fruit to allow for the repeated counting of the number of red scale within the circle after treatment.

Results

The average number and percentage of red scale remaining on the fruit after treatment on each of the infestation severity categories are presented in Figures 65 and 66. The untreated and water only control had a natural decline in the scale of around 20%. The patterns of removal of the red scale were similar to the different infestation levels of the fruit, whereby scale in the low, medium, and high/severe infested fruit were similar, indicating no interaction of treatment and infestation levels. This shows that treatment differences were common between different levels of infestation.

The overall results (i.e. combining all the different infestation levels) are presented in Figures 67 and 68 and show that the 30-second dip of the infested fruit in water and WetCit® had little effect on increasing scale removal. The addition of a high-pressure wash treatment after the dip resulted in higher levels of scale removal. This reduction in scale following high-pressure washing was observed in both water and WetCit® treatments, and it may be due to the high mechanical impact of the wash treatments on dislodging the scale. This was evident in the wash water from the high-pressure washer, which had high levels of scale in the wastewater. There appeared to be some effect of WetCit® on removing higher levels of scale than the water, but the high-pressure wash is recommended for improving scale removal. While this treatment did not remove all red scale from the fruit, it could be examined in a systems approach to manage red scale for market access into sensitive markets.

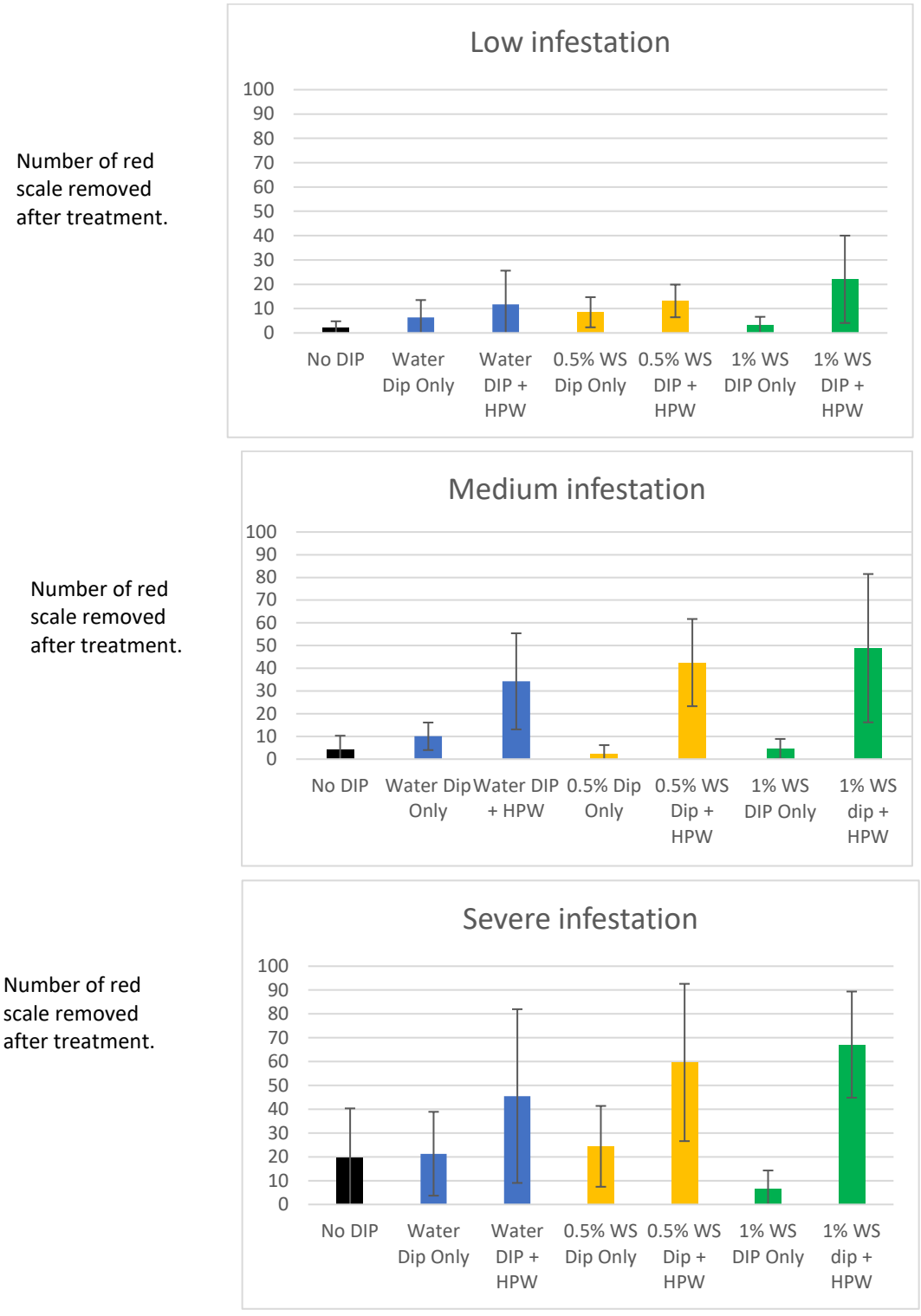


Figure 65. Effect of WetCit® (0, 0.5% and 1.0%) by either dipping alone or with dipping then high-pressure washing on the total numbers of red scale remaining on Salustiana oranges in each of the 3 infestation categories (low infestation–top, medium infestation–middle, and severe infestation–lower).

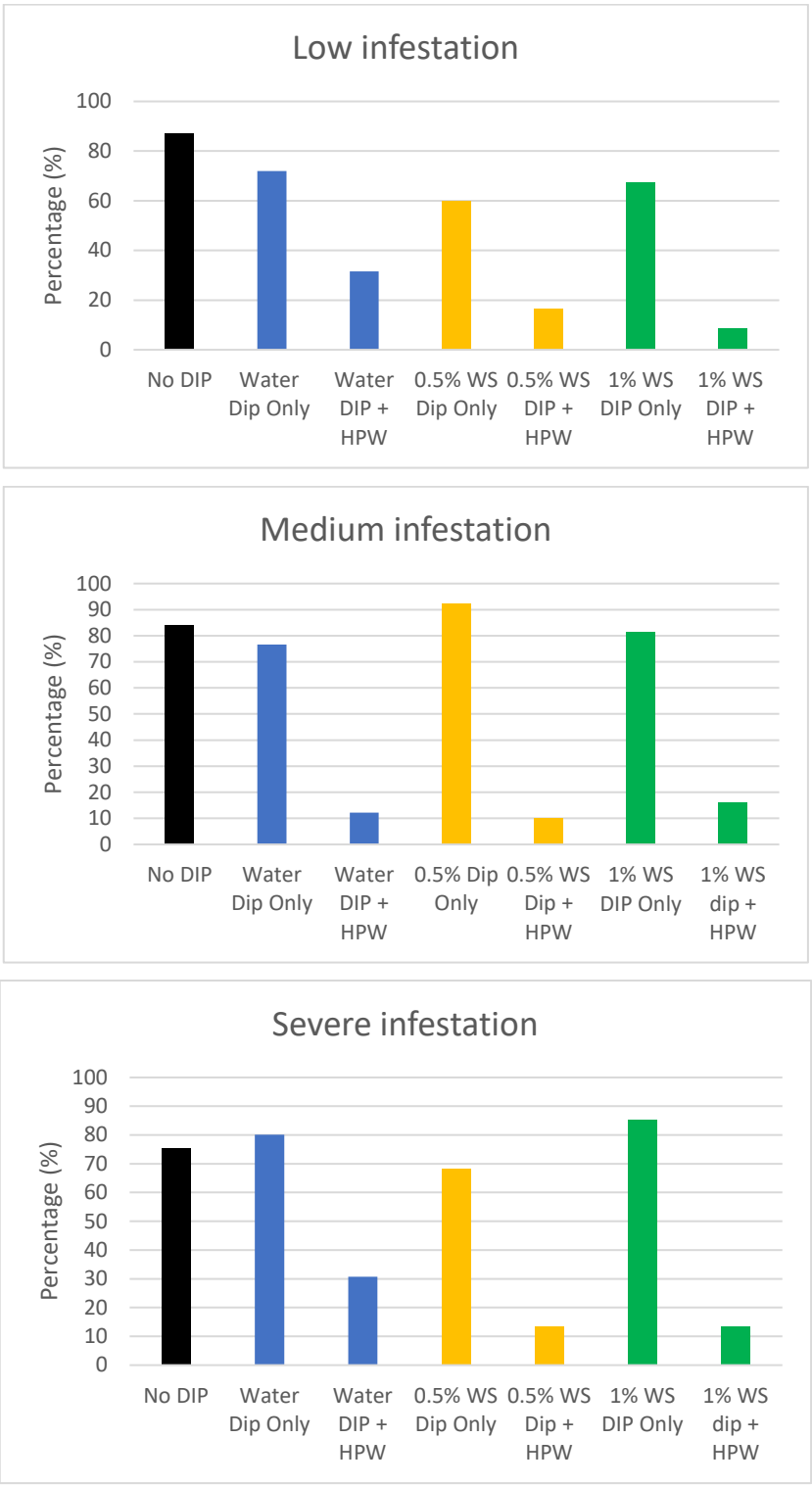


Figure 66. Effect of WetCit® (0, 0.5% and 1.0%) by either dipping alone or with dipping then high-pressure washing on the percentage of red scale remaining on Salustiana oranges following treatment in each of the 3 infestation categories (low infestation–top, medium infestation–middle, and severe infestation–lower).

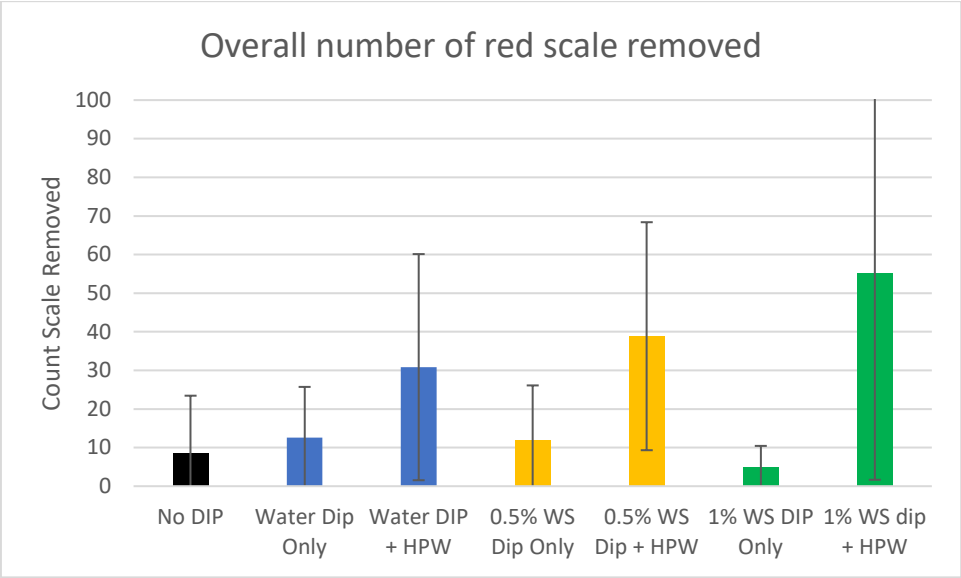


Figure 67. Effect of WetCit® (0, 0.5% and 1.0%) by either dipping alone or with dipping then high-pressure washing on the total number of red scale removed from Salustiana oranges following treatment averaged in all infestation categories.

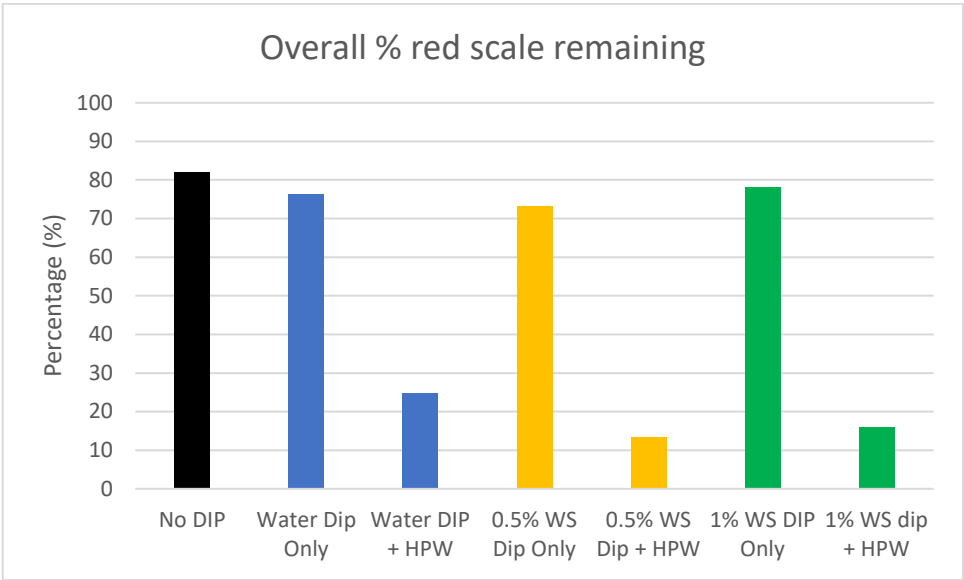


Figure 68. Effect of WetCit® (0, 0.5% and 1.0%) by either dipping alone or with dipping then high-pressure wash on the percentage of red scale remaining on Salustiana oranges following treatment averaged across all infestation categories.

3. Effect of WetCit® on postharvest decay (green and blue mould)

WetCit® has been reported to improve decay control. Consequently, we assessed the effects of WetCit® on green and blue mould infection in Navel oranges.

Organic Navel oranges were inoculated with wild-type green and blue mould spores (*P. digitatum* and *P. italium*) and allowed to germinate for 24 hours at 25 °C. Inoculated fruit were treated with treatments (1) water dip control, (2) 0.5% WetCit®, (3) 1% WetCit® and (4) no washing at all. In addition, there were 2 application methods of the dipping treatments: (1) a 30-second dip in a bucket then brushes only or (2) a 30-second dip in a bucket then high-pressure wash with brushes for 30 seconds. Each treatment had 40 fruit and was replicated 4 times. After treatment, fruit were stored at 25 °C with 90% relative humidity and the percentage of green and blue mould was assessed at regular intervals.

Results

The results presented in Figures 69 and 70 show that WetCit® treatment (either dipping or with the addition of high-pressure washing) had little effect on green and blue mould infection in Navel oranges. There appeared to be some benefit with the higher levels of WetCit® (1%), but this was marginal with significant green and blue mould development following treatment and storage.

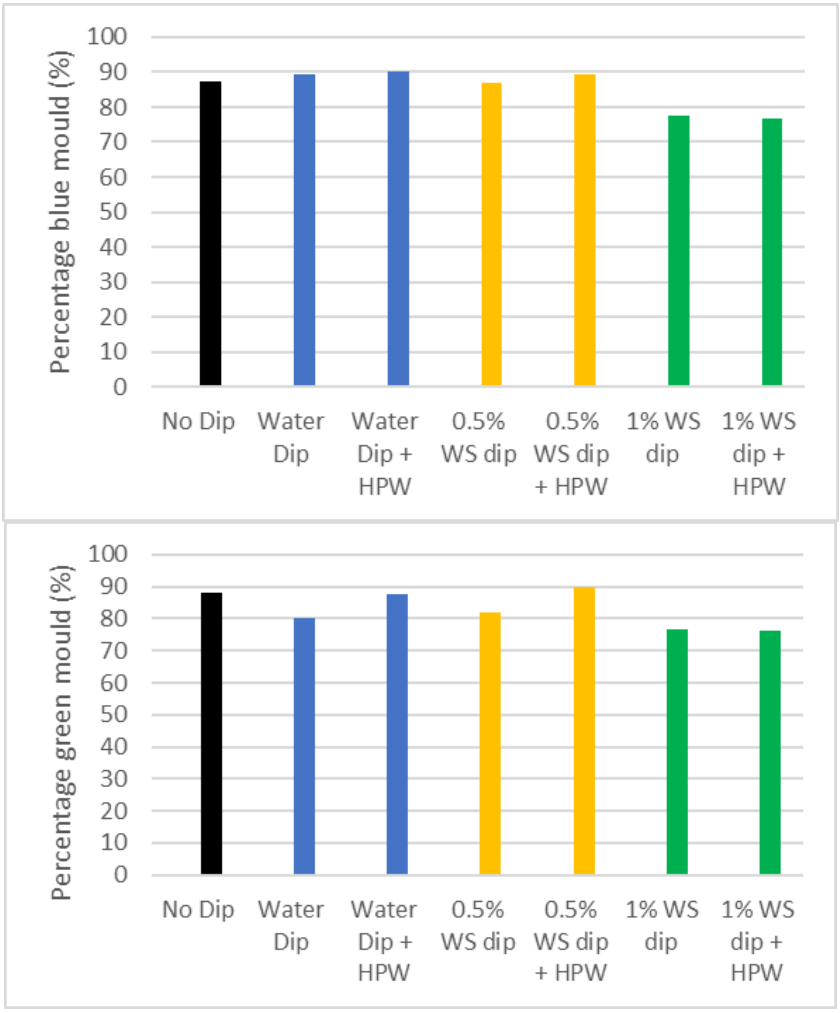


Figure 69. Effect of WetCit® (0, 0.5% and 1.0%) by either dipping alone or with dipping then high-pressure washing on the percentage of blue (top) and green (lower) mould infection in inoculated Navel oranges.

Figure 70. Comparison of the effect of water dip (left) and 1% WetCit® with high-pressure washing (right) on green and blue mould development. Note green and blue mould were inoculated on the same fruit.

Maintaining calyx condition – alternatives to 2,4-D

Calyx (button) abscission is a major factor contributing to citrus loss during storage as it is considered a negative quality attribute by consumers. Calyx loss can also facilitate fungal attack at the abscission zone, leading to an increased incidence of visible decay (Cronjé et al. 2005).

2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin plant growth regulator that has been widely employed as a preharvest treatment by citrus growers to improve fruit quality attributes such as fruit size, juice acid levels and sugar content. 2,4-D is also used as a postharvest treatment to delay calyx browning and calyx abscission. Treatment also favourably influences physical characteristics (e.g. colour and firmness) that are important to consumers. While 2,4-D use is currently permitted in Australia and the United States, use in other countries has diminished due to increased health and environmental concerns (Ma et al. 2015). The citrus industry needs to be proactive and find safer and more acceptable postharvest treatments to replace 2,4-D as a senescence inhibitor.

A series of trials were conducted to examine a range of alternative postharvest treatments to maintain calyx condition:

1. Evaluation of 3,5,6-trichloro-2-pyridiloxyacetic acid dipping
2. Evaluation of hydrogen sulphide fumigation
3. Comparison of 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide treatments
4. Effect of 'low' dicamba levels on the shelf life of Navel oranges. Not published but presented in this report.

The results of some of these trials were published in referred scientific journals with acknowledgement of the contribution of this project:

1. Alhassan, N., Bowyer, M.C., Wills, R.B.H., Golding, J.B., Pristijono, P., 2020. Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays calyx senescence and loss of other postharvest quality factors of 'Afourer' mandarins, Navel and Valencia oranges. *Scientia Horticulturae*. 272, 109572. doi.org/10.1016/j.scienta.2020.109572.
2. Alhassan, N., Wills, R.B.H., Bowyer, M.C., Golding J.B., Pristijono P., 2020. Pre-storage fumigation with hydrogen sulphide inhibits postharvest senescence of Valencia and Navel oranges and 'Afourer' mandarins. *The Journal of Horticultural Science and Biotechnology* 95, 757-762. DOI: 10.1080/14620316.2020.17491
3. Alhassan, N., Wills, R.B.H., Bowyer, M.C., Pristijono, P., Golding J.B., 2022. Comparative study of the auxins 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide to inhibit postharvest calyx senescence and maintain internal quality of Valencia oranges, *New Zealand Journal of Crop and Horticultural Science*, DOI: 10.1080/01140671.2021.2017984

Copies of these papers are available from the author. The abstracts and main findings of these studies are summarised in this report.

Evaluation of postharvest dipping of 3,5,6-trichloro-2-pyridyloxyacetic acid (TPA)

This work was published in *Scientia Horticulturae*:

Alhassan, N., Bowyer, M.C., Wills, R.B.H., Golding, J.B., Pristijono, P., 2020. Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays calyx senescence and loss of other postharvest quality factors of 'Afourer' mandarins, Navel and Valencia oranges. *Scientia Horticulturae*. 272, 109572. doi.org/10.1016/j.scienta.2020.109572

Scientia Horticulturae 272 (2020) 109572

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays calyx senescence and loss of other postharvest quality factors of 'Afourer' mandarins, Navel and Valencia oranges

Nasiru Alhassan[a], Michael C. Bowyer[a], R.B.H. Wills[a], John B. Golding[a,b], Penta Pristijono[a,*]

[a] School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia
[b] NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia

Abstract

The effects of postharvest treatment of three citrus fruit types with 3,5,6-trichloro-2-pyridyloxyacetic acid (TPA) on the deterioration of calyx quality, decay incidence and internal quality parameters in long-term storage were investigated. Navel oranges and 'Afourer' mandarins were treated with TPA concentrations of 0, 2, 4, 8, 16 and 32 µM, while Valencia oranges were treated at concentrations of 0, 15, 30, 60 and 120 µM. Fruit were stored in air at 20˚C for 32 and 28 days, respectively. TPA treatment exhibited a concentration-dependent effect on fruit quality, with higher concentrations resulting in a reduced incidence of calyx deterioration and decay, a lowering of respiration rate, ethylene production and ethanol accumulation, and inhibition of change in TSS and TA levels and hence maintaining the TSS/TA ratio. The results show that postharvest TPA treatment can be used to alleviate calyx senescence and maintain postharvest quality in citrus fruits.

Discussion

TPA treatment led to significant reductions in calyx abscission, calyx browning and fruit decay in all citrus types investigated. The delay observed for each factor increased with increasing TPA concentration which is in broad agreement with previous findings reported by Salvador (2010) and Carvalho (2008). Interestingly, the observed significant effects of the lower concentration TPA treatments conducted on Afourer' mandarins and Navel oranges are an order of magnitude below those reported previously by Carvalho et al. (2008) (2 µM Vs 20 µM), and which if validated through further study, suggests that a considerable reduction in commercial postharvest treatment regimens to preserve citrus quality during long-term storage or export transportation is possible.

There was a general reduction of fruit respiration rate with increasing TPA concentration in all citrus types, but this observation was only statistically significant (P<0.001) in the Valencia oranges. TPA treatment significantly reduced respiration rate in Valencia oranges and exhibited a concentration-dependent relationship, suggesting TPA may lower general metabolic activity in citrus. This proposition is further supported by endogenous ethylene measurements in Valencia fruit, which showed decreased ethylene production with increasing TPA concentration. These results correlate with findings by Ma et al. (2014) who observed that 'Olinda' Valencia treated with 2,4-D (2.3 mM) led to reductions in respiration and endogenous ethylene production. The significantly lower treatment concentrations used in this study suggest TPA be a more favourable treatment for the long-term storage of citrus through its action in lowering general postharvest metabolism.

Flow on effects from a decrease in ethylene production and respiration could explain the reduction in ethanol

accumulation in the fruit that leads to off-flavours during long term storage of citrus (Hagenmaier, 2002; Obenland et al. 2011). The findings on ethanol accumulation in this study due to TPA dipping are in contrast with previous studies that found no significant effect on ethanol accumulation (Carvalho et al. 2008; Salvador et al. 2010). We attribute this difference to the short storage time of just 7 days used in previous studies compared to the 32- and 28-day storage times in these experiments, with ethanol accumulation occurring at a later stage of senescence and hence requiring prolonged storage to manifest.

Retention of internal and external quality parameters in all 3 citrus varieties investigated attests to the potential of TPA as an alternative postharvest treatment to 2,4-D. It would seem that TPA can be applied at much lower concentrations than 2,4-D while still producing comparable outcomes, which, in light of persistent environmental concerns over the widespread use of synthetic agricultural agents at commercial scale, is encouraging. Further study on a wider range of citrus is, however, required to reinforce the findings presented in this study and to determine the commercial applicability to the citrus industry.

All references are listed in the manuscript. A copy of this manuscript is available from the author.

Evaluation of hydrogen sulphide fumigation

This work was published in *The Journal of Horticultural Science and Biotechnology*:

Alhassan, N., Wills, R.B.H., Bowyer, M.C., Golding J.B., Pristijono P., 2020. Pre-storage fumigation with hydrogen sulphide inhibits postharvest senescence of Valencia and Navel oranges and Afourer mandarins. *The Journal of Horticultural Science and Biotechnology* 95, 757-762. DOI: doi.org/10.1080/14620316.2020.1749138

THE JOURNAL OF HORTICULTURAL SCIENCE AND BIOTECHNOLOGY
https://doi.org/10.1080/14620316.2020.1749138

Taylor & Francis
Taylor & Francis Group

Check for updates

Pre-storage fumigation with hydrogen sulphide inhibits postharvest senescence of Valencia and Navel oranges and 'Afourer' mandarins

Nasiru Alhassan[a], Ron B. H. Wills[a], Michael C. Bowyer[a], John B. Golding[b] and Penta Pristijono[a]

[a]School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW, Australia; [b]NSW Department of Primary Industries, Ourimbah, NSW, Australia

Abstract

A short, pre-storage fumigation with hydrogen sulphide (H_2S) gas at 0, 100, 250 and 500 µL L^{-1} affected the development of a range of senescence characteristics of Navel and Valencia oranges and 'Afourer' mandarins during storage at 20°C for five weeks. The greatest beneficial effect was observed with fumigation at 100 µL L^{-1} H_2S which reduced the incidence of calyx drop, calyx browning and fungal decay and production of ethylene and ethanol for all 3 citrus species. For Valencia oranges, a lower total soluble solids (TSS): titratable acidity (TA) ratio was observed, arising from both a lower TSS and higher TA than in control fruit. H_2S treatment had no significant effect on the respiration rate in any fruit species. In general, higher concentrations of H_2S were less effective than 100 µL L^{-1} and often resulted in accelerated loss of quality. The results suggest that H_2S fumigation of citrus before storage might be an alternative treatment for delaying the emergence of senescence characteristics such as calyx browning without the use of synthetic auxins.

Discussion

Maintaining a good external appearance is a critical factor in the successful marketing of any horticultural commodity, as visual cues are often the only guide that consumers use when making purchase decisions. The ability of short-term fumigation with H_2S at a concentration of 100 µL L^{-1} to delay visual degradation of the calyx and delay the appearance of postharvest decay on the 3 citrus fruits investigated in this study suggests its potential use as commercial storage treatment. It is worthy of more intensive study on a wider range of citrus fruits. An added benefit could be if H_2S usage led to reduced reliance on the synthetic auxin 2,4-D to retain calyx condition and synthetic fungicides to inhibit rot

development.

An additional effect of fumigation with 100 µL L^{-1} H_2S on the 3 citrus fruits was to suppress endogenous ethylene production, a finding consistent with previous postharvest studies on other commodities, including green leafy vegetables (Al Ubeed et al. 2018). It is tentatively suggested that suppression of endogenous ethylene production could be a causative link by which H_2S delays calyx senescence and rot development, as ethylene is known to promote maturation, senescence and ripening in horticultural commodities (Wills & Golding, 2016). In our studies, H_2S-related effects occurred in the presence of a low background level of ethylene (0.1 µL L^{-1}) that is commonly found in commercial marketing situations (Wills et al. 2000), suggesting that H_2S treatment remains effective even in the presence of external ethylene sources. Certainly, the suppression of degreening of Valencia oranges by H_2S could be ascribed to the reduction in ethylene synthesis, as exposure to high ethylene levels is used commercially to degreen citrus fruits (Sdiri et al. 2013). Such inhibition of degreening is, of course, undesirable, and H_2S treatment may be inappropriate for green-skinned citrus fruits. Still, it would be a benefit for lime fruit, where green skin is preferred. In addition, an increase in ethylene has been reported to induce the activity of enzymes responsible for calyx abscission (Baird & Webster, 1996).

The reduced incidence of decay observed in the study is consistent with the report by Ali et al. (2019, 2016) that H_2S fumigation suppressed the growth of a range of postharvest pathogens in various fruits and by Hu et al. (2014) in mulberry fruits.

Respiration rate is an essential factor that influences the rate of physiological and biochemical changes in fruits (Wills & Golding, 2016); however, in this study, respiration rates of 3 citrus types were not inhibited by H_2S. This is inconsistent with the previous findings of other non-climacteric produce such as pak choy (Al Ubeed et al. 2019), strawberry (Hu et al. 2012) and water spinach (Hu et al. 2015), where H_2S treatment lowered the respiration rate. This suggests that for citrus fruits, the action of H_2S is not through reducing the rate of general metabolism but is due to a more specific effect of ethylene action.

In regard to the internal fruit quality parameters, many studies have shown that citrus fruit accumulates ethanol during storage, where this increase depends on the type of fruit and treatment conditions, and that the accumulated ethanol leads to perceptions of off-flavour (Hagenmaier 2002; Ke and Kader 1990). In this study, the application of 100 µL L^{-1} H_2S decreased ethanol production by Valencia and Navel oranges, but there was no significant effect on 'Afourer' mandarins. It is noted that the levels of ethanol are not particularly high in the present study, and a more significant effect may occur with a storage period longer than 5 weeks, which would be consistent with the result for the controlled atmosphere storage of Valencia oranges (Ke and Kader, 1990).

The effect of H_2S on TSS and TA also differed between the different citrus fruits. Valencia oranges had a lower TSS, higher TA and hence a lower TSS:TA ratio than control fruit, but the levels in the H_2S-treated fruit were similar to those in freshly harvested fruit. Thus, H2S inhibits change in these quality factors during storage. The results agree with the findings of Ni et al. (2016), who reported that H_2S treatment reduced sugar levels in grapes, and Hu et al. (2014), who found mulberry fruit treated with H_2S showed higher TA levels than control fruits. There was no significant change in TSS, TA, or TSS:TA ratio in Navel oranges or 'Afourer' mandarins.

This is the first report on the application of a pre-storage H_2S treatment on citrus fruit, and more work is required to assess its effect on different citrus types and varieties, as well as on H_2S concentrations and fumigation conditions.

All references are listed in the manuscript. A copy of this manuscript is available from the author.

Comparison of promising alternatives to 2,4-D

This work was published in the *New Zealand Journal of Crop and Horticultural Science*:

Alhassan, N., Wills, R.B.H., Bowyer, M.C., Pristijono, P., Golding J.B., 2022. Comparative study of the auxins 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide to inhibit postharvest calyx senescence and maintain internal quality of Valencia oranges, *New Zealand Journal of Crop and Horticultural Science*, DOI: 10.1080/01140671.2021.2017984

NEW ZEALAND JOURNAL OF CROP AND HORTICULTURAL SCIENCE
https://doi.org/10.1080/01140671.2021.2017984

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Comparative study of the auxins 2,4-D, fluroxypyr, dicamba, MCPA and hydrogen sulphide to inhibit postharvest calyx senescence and maintain internal quality of Valencia oranges

Nasiru Alhassan [a], Ron B. H. Wills [a], Michael C. Bowyer[a], Penta Pristijono[a] and John B. Golding[a,b]

[a]School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW, Australia; [b]NSW Department of Primary Industries, Ourimbah, NSW, Australia

Abstract

The synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) is sometimes applied commercially to delay calyx senescence, decay and maintain citrus fruit internal quality parameters, however, there is a need to find alternative treatments to reduce calyx senescence and maintain fruit quality during storage. In this study, the effectiveness of pre-storage dips of different auxin formulations, 2-(4-amino-3,5-dichloro-6-fluoropyridin-2yl) oxyacetic acid (fluroxypyr), 3,6-dichloro-2-methoxybenzoic acid (dicamba) and 2-methyl-4-chlorophenoxyacetic acid (MCPA), were assessed against the standard 2,4-D treatment using Valencia oranges at concentrations of 0.2 and 1 mM. Hydrogen sulphide (H_2S) a gaseous signalling compound known to delay senescence in postharvest produce was also assessed by fumigating Valencia oranges at 100 µL L−1. During four weeks storage at 20°C, fluroxypyr produced the greatest reduction in calyx abscission, calyx browning and fruit decay and down regulation in endogenous ethylene production, respiration rate and ethanol formation, relative to other treatments. Fluroxypyr dip at 1 mM was most effective, with the 0.2 mM concentration still superior to 2,4-D. MCPA showed only modest activity, while dicamba was ineffective. H2S fumigation significantly reduced calyx deterioration and delayed the loss of internal quality factors. Therefore fluroxypyr or H2S, as a non-auxin treatment, have the potential to replace 2,4-D for commercial use.

Discussion

Treatment of Valencia oranges with 2,4-D, fluroxypyr, MCPA and H_2S resulted in beneficial effects during storage for calyx integrity and a range of internal quality factors. Comparing the effects against the current industry treatment (2,4-D) shows that Fluroxypyr gave superior retention of all factors. It is noteworthy that fluroxypyr was most effective when applied at 1 mM, but was still superior to 2,4-D at the 0.2 mM treatment concentration. MCPA showed only limited effect as a postharvest treatment while Dicamba had little or no impact on postharvest quality. These findings are generally consistent with previous reports by Ma et al. (2015) and Carvalho et al. (2008). Based on toxicological data, fluroxypyr (Category IV toxin, LD_{50} >5000 mg kg^{-1}) (EPA, 2012) appears to be a more environmentally acceptable synthetic auxin treatment than 2,4-D (Category II-III toxin, LD_{50} = 700-900 mg kg^{-1}).

The results for H_2S fumigation in this study confirmed results from our previous investigation showing H_2S exposure beneficially impacts postharvest citrus quality (Alhassan et al. 2020). While less effective than fluroxypyr overall, H_2S treatment in this study produced results generally consistent with 2,4-D across the range of visual and internal senescence parameters assessed. As a low-cost, low-technology treatment capable of being easily scaled, H_2S represents a potential paradigm shift in citrus postharvest management.

The beneficial effects on the calyx and internal quality factors indicate that auxins affect one or more aspects of metabolism, leading to a general reduction in normal senescence rates. The results of this study are consistent with this premise, with fluroxypyr presumably being more effective than the other compounds. Effects on ethylene and respiration rate also correlate with a general reduction in ethanol levels in the fruit for all treatments (except dicamba). This contrasts with previous studies where no significant effect of 2,4-D on ethanol content in Clemenule mandarins and Navelina oranges was observed (Sdiri et al. 2013). Results for H_2S fumigation were consistent with our previous studies conducted on both green produce and citrus, showing that exposure effectively suppresses both ethylene production and respiration (Alhassan et al. 2020). Hydrogen sulphide presence (both endogenous and exogenous) has been linked to natural auxin control in plants, increasing endogenous indole acetic acid (IAA) production in a range of plant species.

Many studies have shown that the rate of senescence is related to the concentration of ethylene around the produce. It

could be speculated that the ability of auxins to inhibit ethylene production is a key factor in their inhibition of calyx and internal quality changes. In this trial, auxin treatments had only a modest effect in lowering TSS levels. Effects on TA were more substantial, showing significant increases in acidity levels recorded in treated fruit from both growing regions. Overall, this led to a reduced TSS:TA ratio (relative to control) for fluroxypyr, 2,4-D and MCPA, with the effect more pronounced in Experiment 1, a result presumably associated with higher natural acidity levels of the fruit. H_2S fumigation also increased TA levels in fruit from both growing regions, leading to a significant decrease in the TSS:TA ratio.

Of the 4 auxins assessed, only dicamba failed to affect the quality parameters. The result is surprising given the close structural relationship to 2,4-D. Previous studies that assessed dicamba as a preharvest treatment found it successfully inhibited fruit drop (Marini et al. 1990).

Fruit softening occurs because of cell wall component degradation, resulting from the coordinated action of cell wall-modifying enzymes. In this study, the effects of the various treatment concentrations on firmness increased with decreased calyx senescence and decay, indicating that cell wall degradation could have affected calyx changes and rot incidence in citrus fruit.

Conclusions

Maintenance of external and internal quality factors in citrus fruit investigated demonstrates the potential of fluroxypyr as an alternative, less toxic synthetic auxin treatment to 2,4-D for controlling calyx senescence in the citrus industry. Fumigation with 100 µL L^{-1} H_2S produced results comparable to 2,4-D, making it a prime candidate for further investigation as a low-cost, natural alternative treatment to synthetic auxins. Recent evidence suggests that H_2S may act in a multifactored manner, downregulating the deterioration of cellular changes at the abscission point and affecting endogenous ethylene production, a key driver of senescence in citrus.

All references are listed in the manuscript. A copy of this manuscript is available from the author.

Effect of low dicamba levels on the shelf life of Navel oranges

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective systemic herbicide and synthetic auxin that functions by increasing senescence and cell death. While the results of the previous experiment showed that dicamba treatment was not effective at those concentrations, this experiment examined the effects of low concentrations of dicamba (0.0025, 0.005 and 0.010 M) on Navel orange quality at 20 °C under continuous low levels of ethylene storage.

Methods

Navel oranges from NSW DPI Somersby Research Station were harvested on 22 July 2019 and briefly sanitised in hypochlorite, rinsed in potable water, and allowed to dry. Fruit were dipped for one minute in the following dicamba concentrations: 0, 2.5, 5.0 and 10.0 mM active ingredient. The fruit were allowed to dry overnight then placed into 60 L steel drums with a constant flow of 0.1 ppm ethylene and stored at 20 °C with 90% relative humidity. Each dip treatment was replicated 3 times and each treatment replicate was placed into a separate drum. The treatment unit was 50 fruit. The initial weights of 15 fruit per treatment unit were recorded and re-weighed at each assessment time.

Fruit were stored for 3 weeks with assessments each week. Non-destructive assessments were conducted each week for button quality, calyx browning, calyx abscission, weight loss, fruit respiration rate and decay development.

After the 3 weeks of storage, destructive assessments were conducted to measure total soluble solids (TSS), titratable acidity (TA) and juice ethanol levels according to the General Methods (Appendix 1).

Results

The effect of different dicamba concentrations on calyx health are presented in Figure 71. The results showed that treatment with dicamba did not improve calyx health, as shown by the levels of acceptable calyxes and calyx health score (Figure 72). The application of dicamba did not affect the level of calyx abscission (Figure 72); indeed, the higher levels of dicamba treatment tended to have higher levels of calyx abscission. Furthermore, dicamba did not affect postharvest decay development (Figure 73) or weight loss (Figure 72). There was also no consistent effect of different concentrations of dicamba on fruit ethylene production rates, fruit TSS and TA (Figures 73 and 74).

In summary, these results show that ‘low’ dicamba concentrations did not affect calyx health or fruit quality.

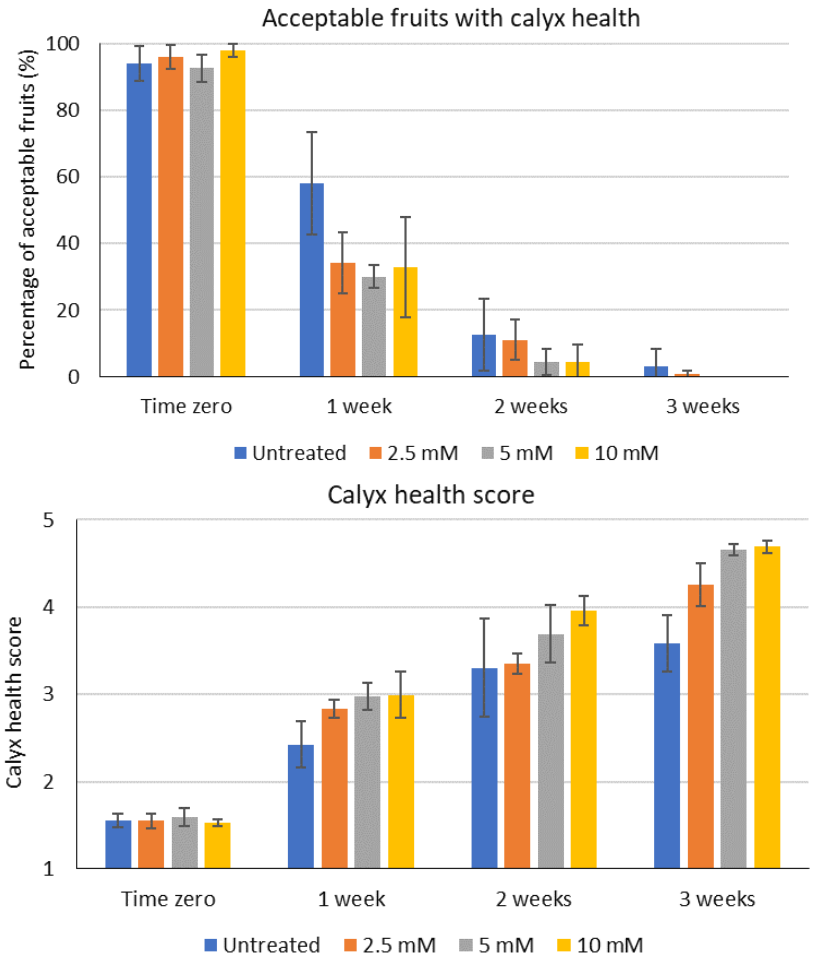
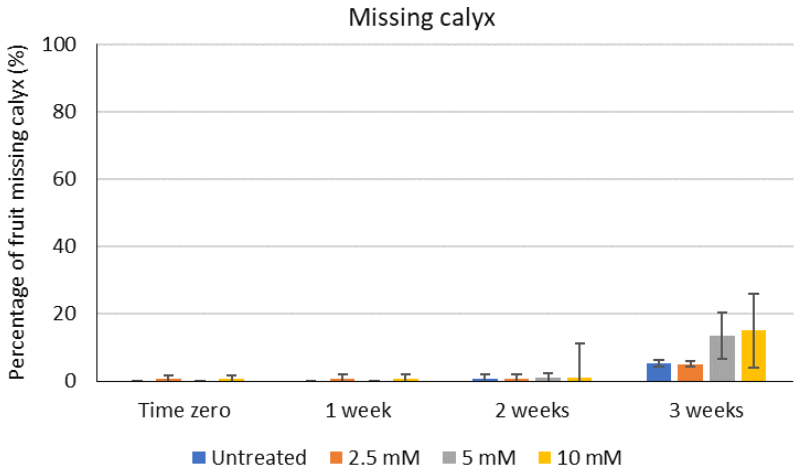


Figure 71. Percentage of acceptable fruits with calyx health (%) (top) and calyx health score of fruit (lower) treated with different concentrations of dicamba at time zero and after 1–3 weeks stored at 20 °C. Calyx health score: 1 = green, 2 = slightly yellow, 3 = mainly yellow, 4 = total yellow, and 5 = brown. Bars are standard deviations around the means, *n*=3.



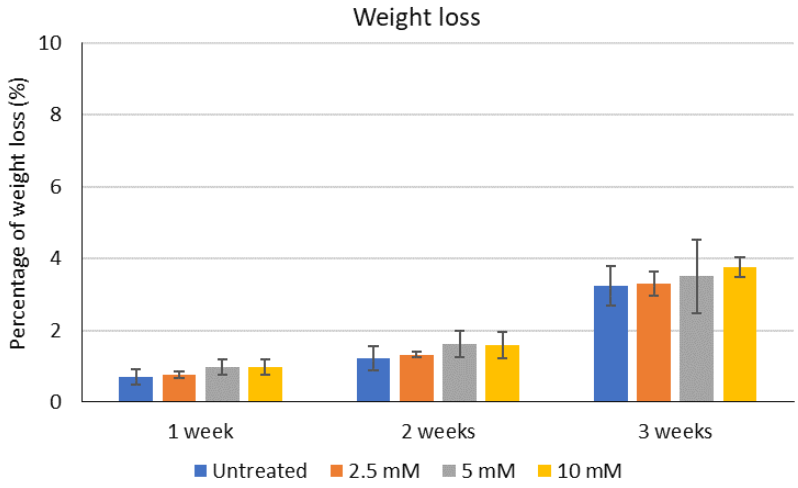


Figure 72. Percentage of fruit missing calyx (%) (top) and weight loss (lower) of individual fruits (%) treated with different concentrations of dicamba and stored at 20 °C for 1–3 weeks. Bars are standard deviations around the means, *n*=3.

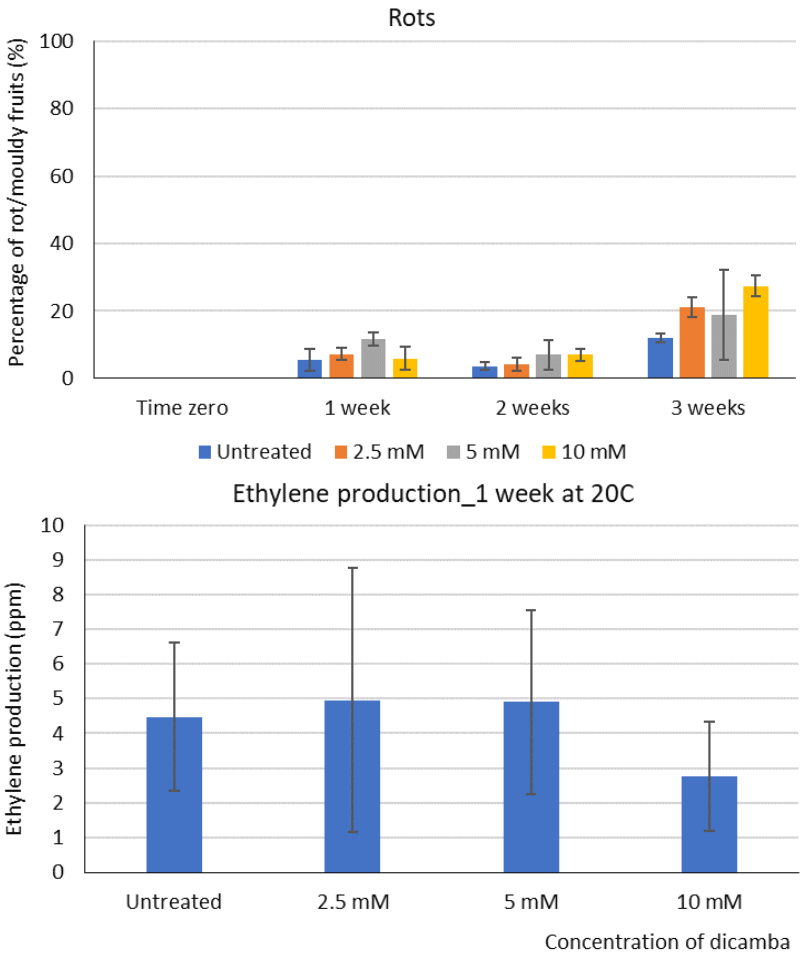


Figure 73. Percentage of rot/mouldy fruits (%) (top) and ethylene production of fruits (ppm) (lower) treated with different concentrations of dicamba and stored at 20 °C for 1–3 weeks. Bars are standard deviations around the means, *n*=3.

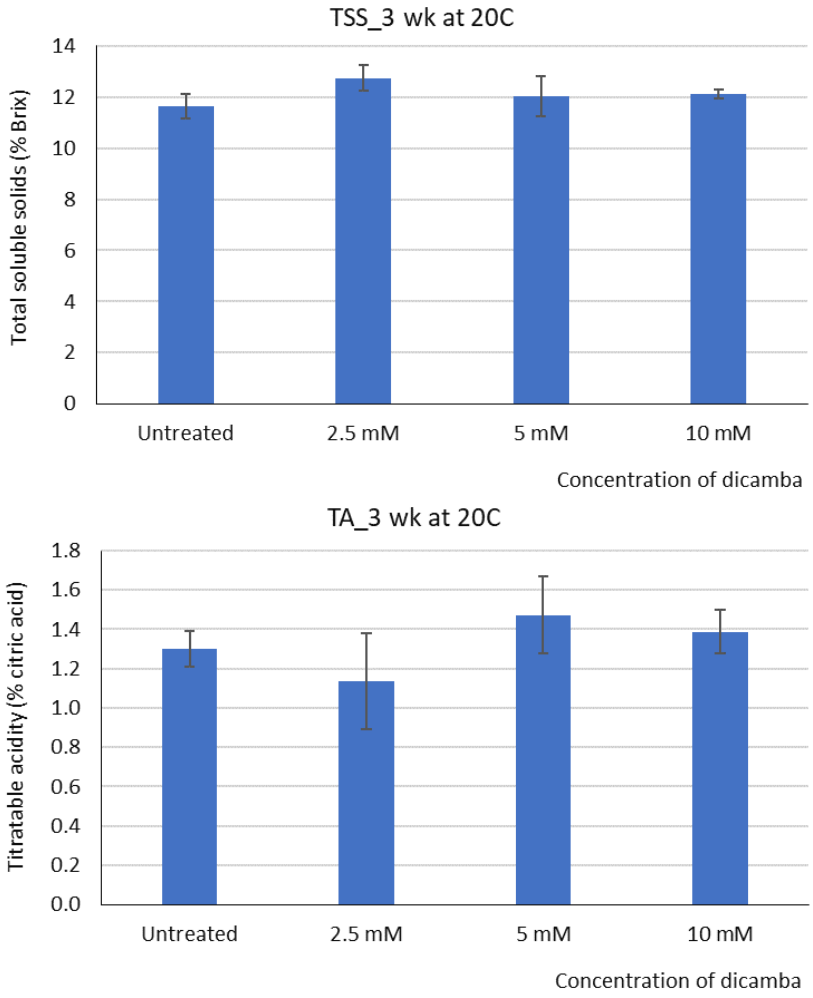


Figure 74. Total soluble solids (% Brix) (top) and titratable acidity (% citric acid) (lower) of fruits treated with different concentrations of dicamba after 3 weeks stored at 20 °C. Bars are standard deviations around the means, *n*=3.

Chilling injury

Chilling injury can be a devastating postharvest disorder that can occur after low temperature storage. It can result in a significant downgrade or rejection of fruit in the market. The classical symptoms of chilling injury are pitting of the peel, superficial scald-like symptoms of the peel and browning of the skin (Figure 75). Chilling injury is a disorder that 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.

Figure 75. Classic chilling injury symptoms on Navel oranges following cold storage.

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 a 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 that operate in the induction of cold tolerance (e.g. lipid metabolism, oxidative stress, dehydrins, osmoprotectants, metallothioneins, defence responses).

Predicting the onset and severity of chilling injury is also difficult due to the unpredictable nature of the time × temperature combinations required to produce the onset of symptoms. A short cold treatment time may not develop any symptoms, but longer cold storage times may express the disorder. The easiest way to avoid chilling injury is to avoid storing citrus at <5 °C; however, many of the phytosanitary cold treatments against fruit flies require cold treatment (1 °C) for up to 3 weeks.

Although the mechanisms of chilling injury are not fully understood, there are several pre and postharvest factors that 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, and growing locations. The susceptibility to chilling injury differs among species and different citrus types. For example, limes are lemons and are generally more susceptible to chilling injury than oranges and mandarins. 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, while Thomson are moderately sensitive and Navelate and Roberts fruit are highly sensitive to chilling temperatures.

Dareton Navel chilling injury survey

To investigate the possible seasonal and varietal differences in the development of chilling injury, a series of observations examining the expression of chilling injury were made on fruit from the same trees on the same rootstock under the same orchard management over successive seasons.

Methods

Mature Navel oranges were sourced from trees from the NSW Department of Primary Industries Dareton Navel Trial. This block was planted in October 1992 on *C. citrange* rootstock. The trial block contained 6 replicates of different early, mid and late-season Navel oranges (Figure 76). Each Navel variety was in a pair with one tree inoculated with the 3532 mild strain and the neighbouring paired tree without the inoculation (control).

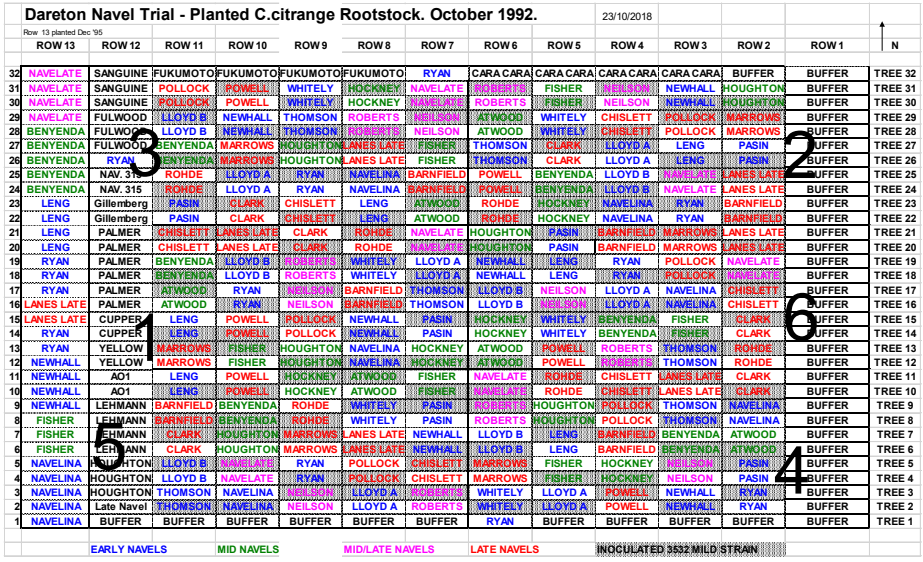


Figure 76. Orchard trial design for the Dareton Navel trial.

Each season for 5 seasons (2019–2023), the following fruit varieties were harvested from the Dareton trial block:

- Early season–Leng, Navelina (2020-2023) (Lloyd A and no Navelina in 2019 only)
- Mid season–Atwood, Houghton
- Late season–Chislett, Lanes Late

Fruit from the same labelled trees were harvested at commercial maturity and sent to NSW Department of Primary Industries at Ourimbah and stored at 3 °C for 8 weeks. After storage, the fruit were transferred to 20 °C for another week's shelf life to allow the chilling symptoms to express at room temperature. Chilling injury was assessed (% fruit with symptoms and severity of symptoms). After selected assessments in some years, a sample of fruit from each box was assessed for TSS and TA (and vitamin C in 2023). All fruit were from the tree with no postharvest fungicide or wax.

Results

The results of all data collected (incidence and severity of chilling injury, fruit TSS, TA and vitamin C levels) from the trial over the 4 years (2019–2023) are presented below and show how different varieties express chilling symptoms differently in each season.

Figure 77 shows the incidence (%) of fruit with chilling injury symptoms and the severity of these symptoms in early-season fruit (Leng, Navelina, and Lloyd A (in 2019 only). There was a large variation in the expression of chilling injury in these early harvest fruit.

In the 2019 season, over 90% of all Leng navels had chilling symptoms, but in the 2022 season, less than 20% had chilling symptoms. Similarly in 2019, Leng navel oranges had an average score of 3 (i.e. pitting covering up to 10% of fruit surface), while in 2022, the average chilling score for Leng navels was less than 1.5. In general, the 2019 and 2020 seasons had higher percentages of fruit with chilling injury than the following years (2021, 2022 and 2023). Leng consistently had a higher rate and more severely cold damaged fruit than Navelina from 2020 to 2023.

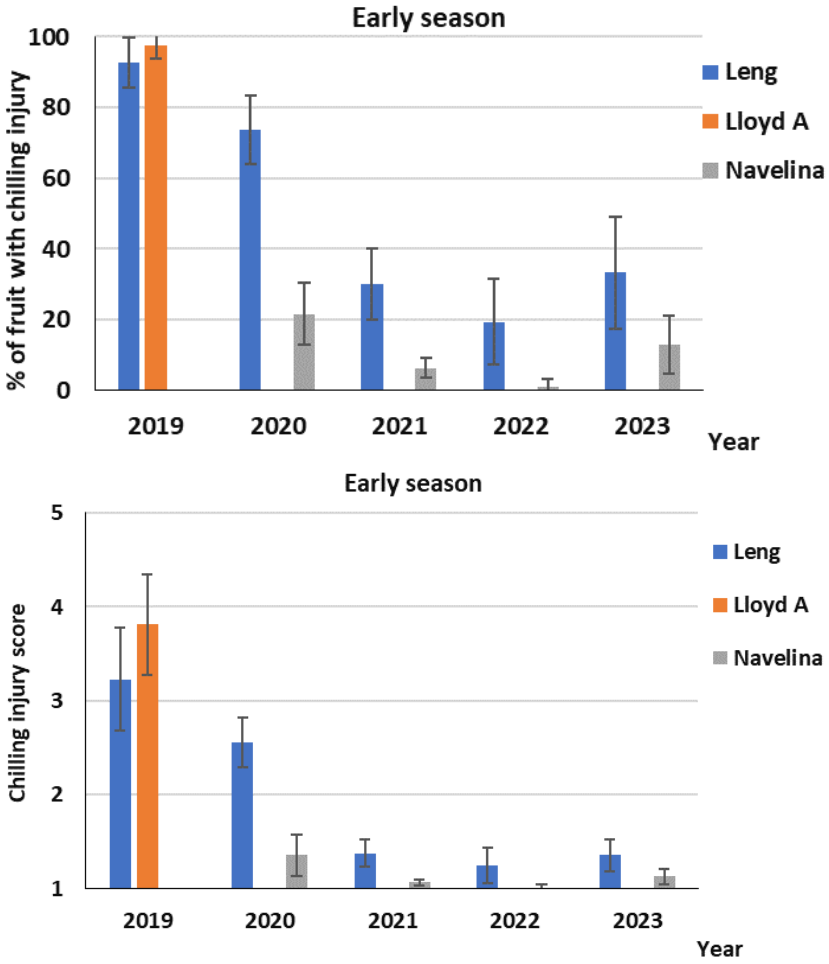


Figure 77. Percentage of fruit with chilling injury (%) (top) and fruit chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of different varieties of early season Dareton oranges 2019–2023.
Bars are standard deviations around the means, *n*=12.

The levels of TA of Leng were consistently higher, while Navelina had higher TSS levels in 2020 and 2022 (Figure 78).

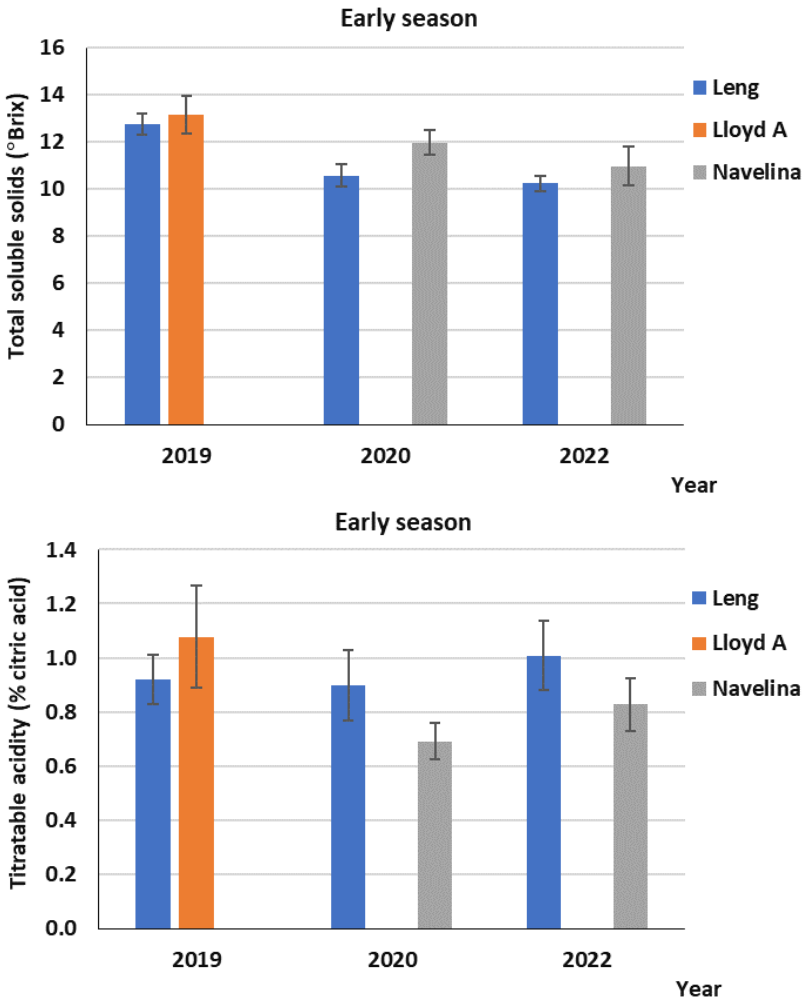


Figure 78. Total soluble solids (°Brix) (top) and titratable acidity (TA, % citric acid) (lower) assessment 2 (upon removal + 1 week at 20 °C) of different varieties of early season Dareton oranges 2019–2023.
Bars are standard deviations around the means, *n*=12.

The results of the chilling expression from the mid-season harvest fruit from Atwood and Houghton oranges are presented in Figure 79. There were relatively high rates of chilling injury (>60–80% of fruit with symptoms) in the 4-year trial. In general, in 2019, Atwood had a higher percentage of fruit with chilling symptoms and higher levels of chilling damage than Houghton in 2019. However, in 2020, this observation was the opposite, and in 2021 and 2023, there was no difference in chilling injurybetween Atwood and Houghton.

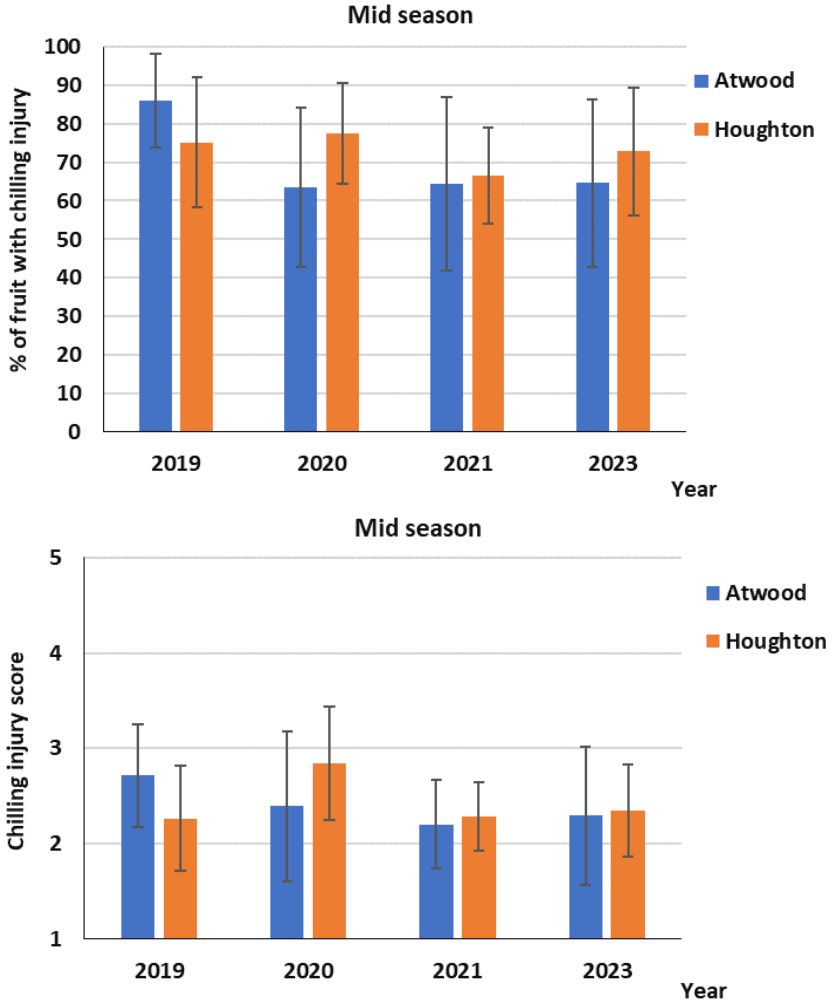


Figure 79. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of different varieties of mid-season Navel oranges 2019–2023.
Bars are standard deviations around the means, *n*=12.

The effects of variety and season on TSS and TA between Atwood and Houghton are presented in Figure 80. There were no differences in TSS and TA between Atwood and Houghton, but there were seasonal differences.

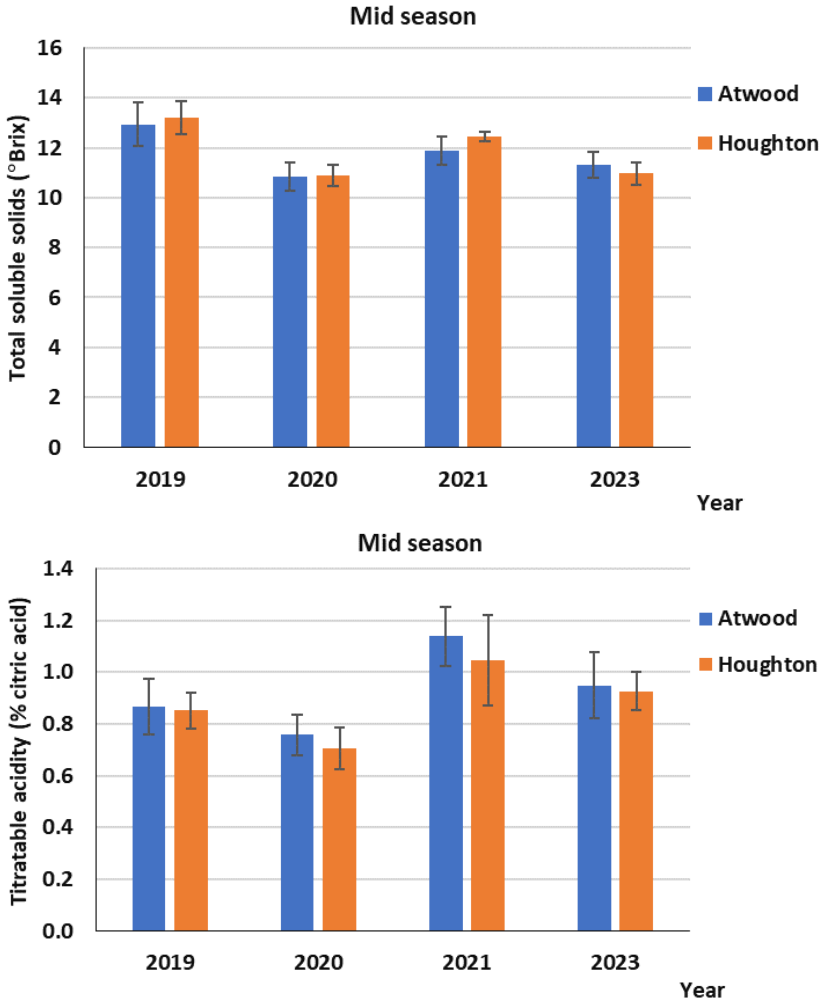


Figure 80. Total soluble solids (°Brix) (top) and titratable acidity (% citric acid) (lower) at assessment 2 (upon removal + 1 week at 20 °C) of different varieties of mid-season navel oranges. Bars are standard deviations around the means, *n* = 12 for years 2019, 2020, 2023 and *n* = 2 for year 2021.\

The results of the late-season chilling injury are presented in Figure 81. In 2019 and 2021, there was a higher percentage of chilling injuries (>80%) and they were more severe than in the other years.

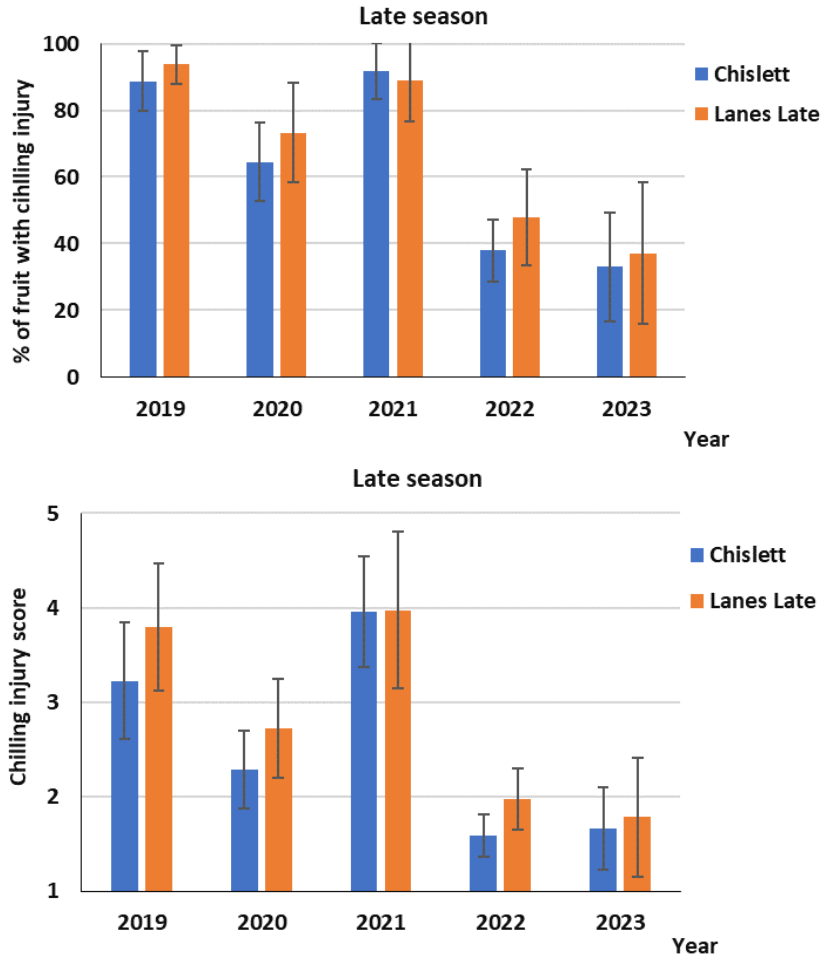


Figure 81. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of different varieties of late-season navel oranges 2019–2023. Bars are standard deviations around the means. Chislett (*n*=12), Lanes Late 2019, 2020, 2021, 2022 (*n* = 12) and Lanes Late 2023 (*n* = 10).

In general, there were no differences in TSS and TA between Chislett and Lanes Late from 2019 to 2023, except for 2021, when Chislett retained higher TSS and TA levels than Lanes Late (Figure 82).

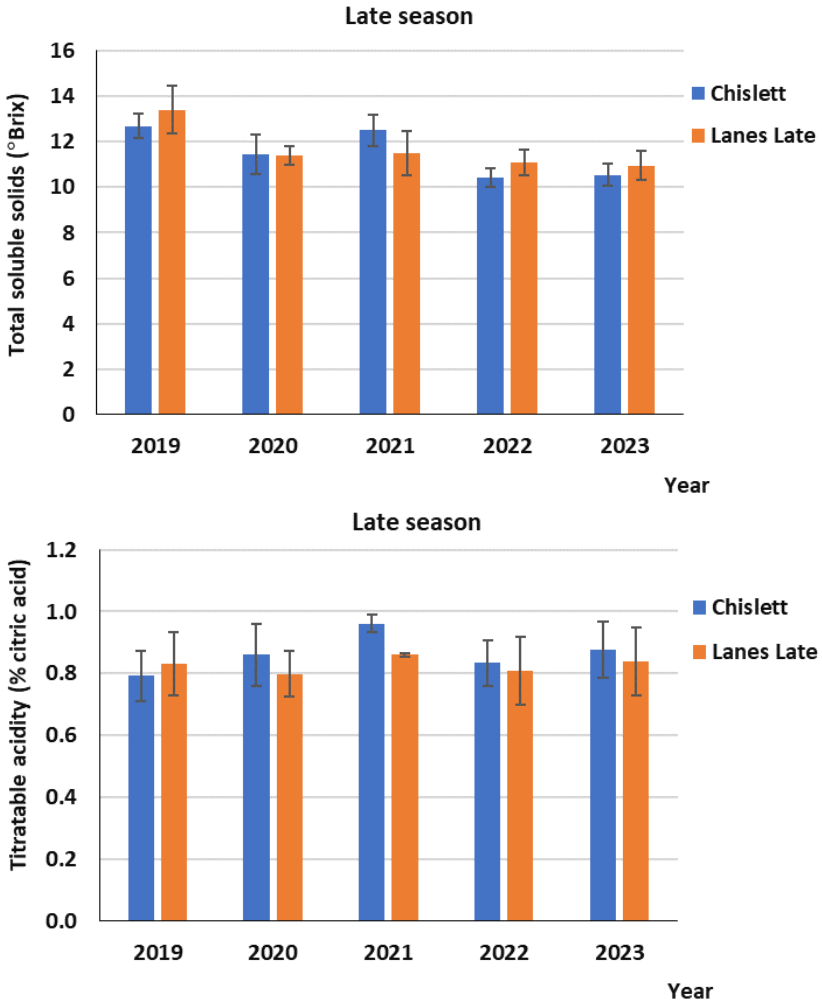


Figure 82. Total soluble solids (°Brix) (top) and titratable acidity (% citric acid) (lower) at assessment 2 (upon removal + 1 week at 20 °C) of different varieties of late-season navel oranges 2019–2023. Bars are standard deviations around the means, *n* = 12 for years 2019, 2020, 2022, 2023 and *n* = 2 for year 2021.

Stress trial

In addition to the variety × season trial, the same block of navel oranges was subject to water (irrigation) stress on certain blocks within the orchard (except for the 2022 season). The results of the water stress on the percentage of fruit with chilling injury and chilling injury score are presented in Figure 83. Water stress may contribute to a greater percentage of fruit with chilling injury and a higher severity of chilling injury in high-incidence seasons (from 2019 to 2021). However, stress had no effect on chilling injury in 2023, where there was a large decrease in the percentage of fruit with chilling injury symptoms in 2023.

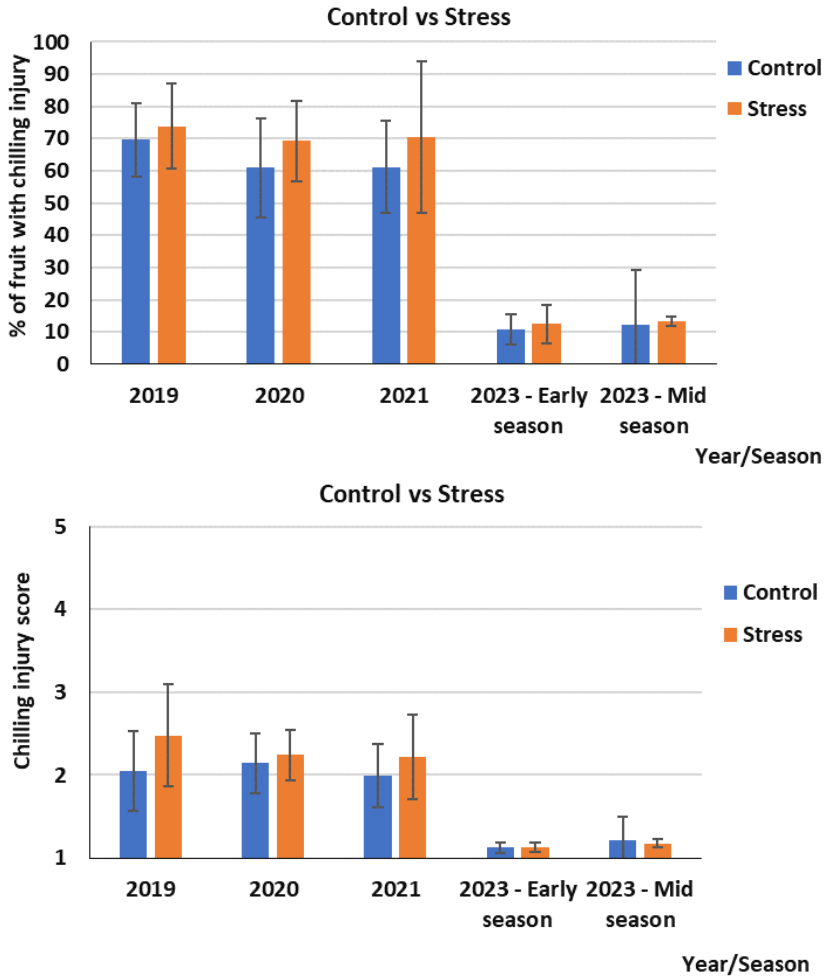


Figure 83. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of the control and stress Dareton oranges 2019–2023. Bars are standard deviations around the means, years 2019, 2020, 2021–mid-season (*n* = 12), 2023–Early season (Atwood, *n* = 6), 2023–mid-season (Hockney, *n* = 3). There are no data for the control and stress in 2022.

There were no consistent effects of water stress on the levels of TSS and TA in different seasons (Figure 84).

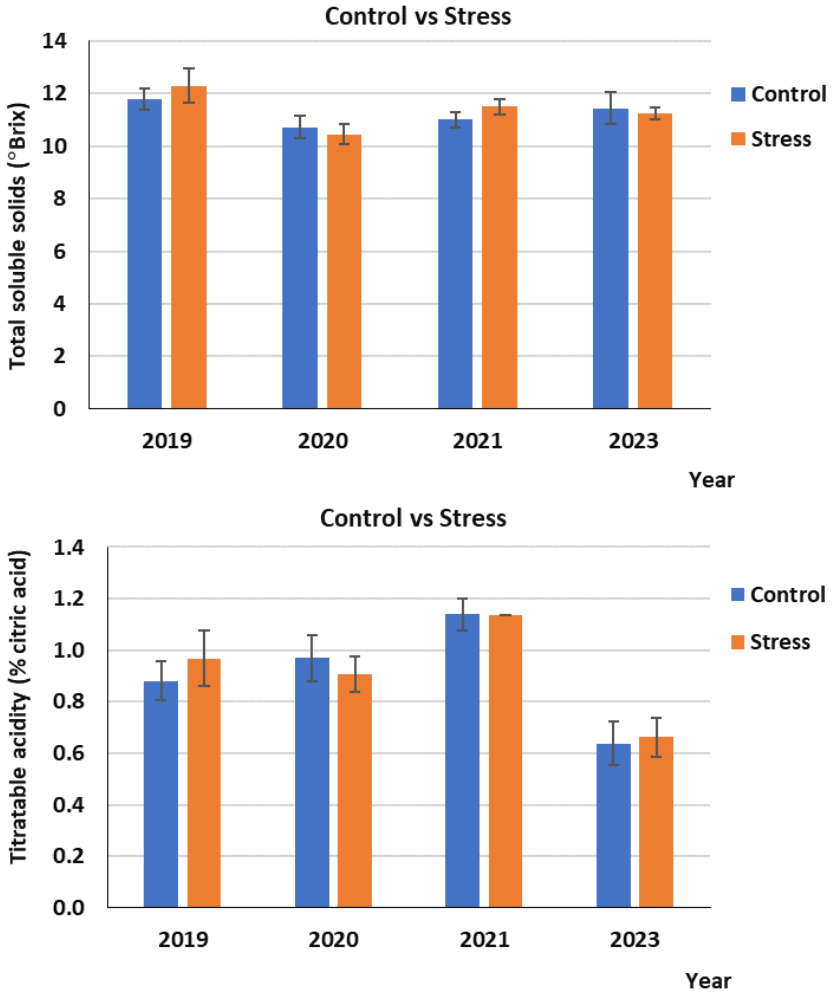


Figure 84. Total soluble solids (°Brix) (top) and titratable acidity (% citric acid) (lower) at assessment 2 (upon removal + 1 week at 20 °C) of the control and stress Dareton oranges 2019–2023 (mid-season). Bars are standard deviations around the means, years 2019, 2020 (*n* = 12), 2021 (*n* = 2) and (*n* = 3). There are no data for the control and stress in 2022.

Effect of mild strain 3532 inoculation on the development of chilling injury in Navel oranges

The effect of mild strain 3532 inoculation on the development of chilling injury in Navel oranges during storage on Navelina (Lloyd A–2019 only), Leng, Atwood, Houghton, Chislett, and Lanes Late navel oranges are presented in Figures 85–90. The results show that inoculation with mild strain 3532 did not affect the chilling injury of Lloyd A and Navelina (early season) (Figure 85).

Navelina (Lloyd A–2019 only)

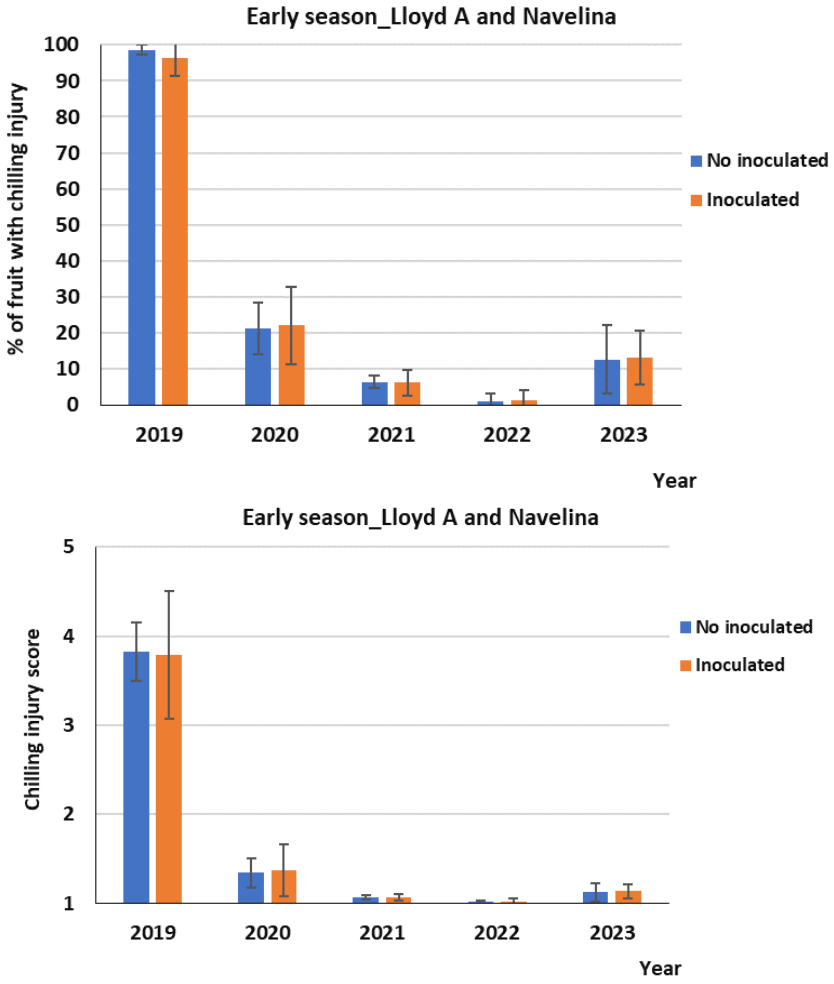


Figure 85. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of early season navel oranges without and with inoculated 3532 mild strain in 2019 (Lloyd A) and 2020–2023 (Navelina). Bars are standard deviations around the means, *n*=6.

Leng

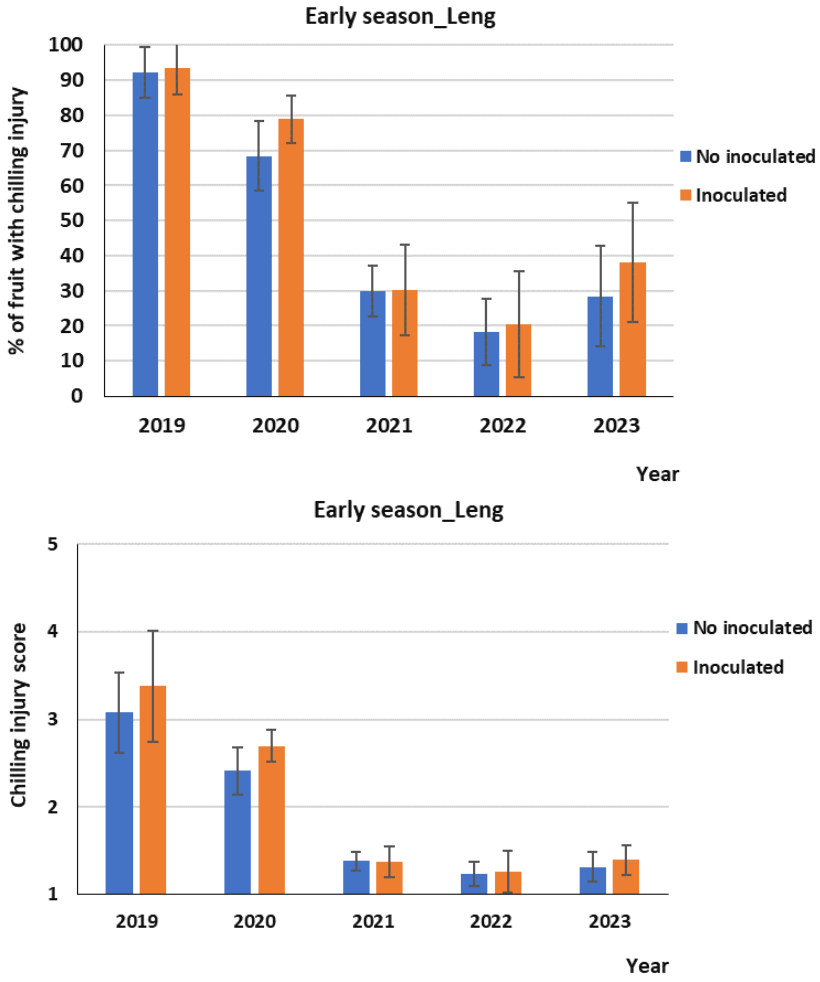


Figure 86. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of early season Dareton oranges without and with inoculated 3532 mild strain from 2019–2023 (Leng). Bars are standard deviations around the means, *n*=6.

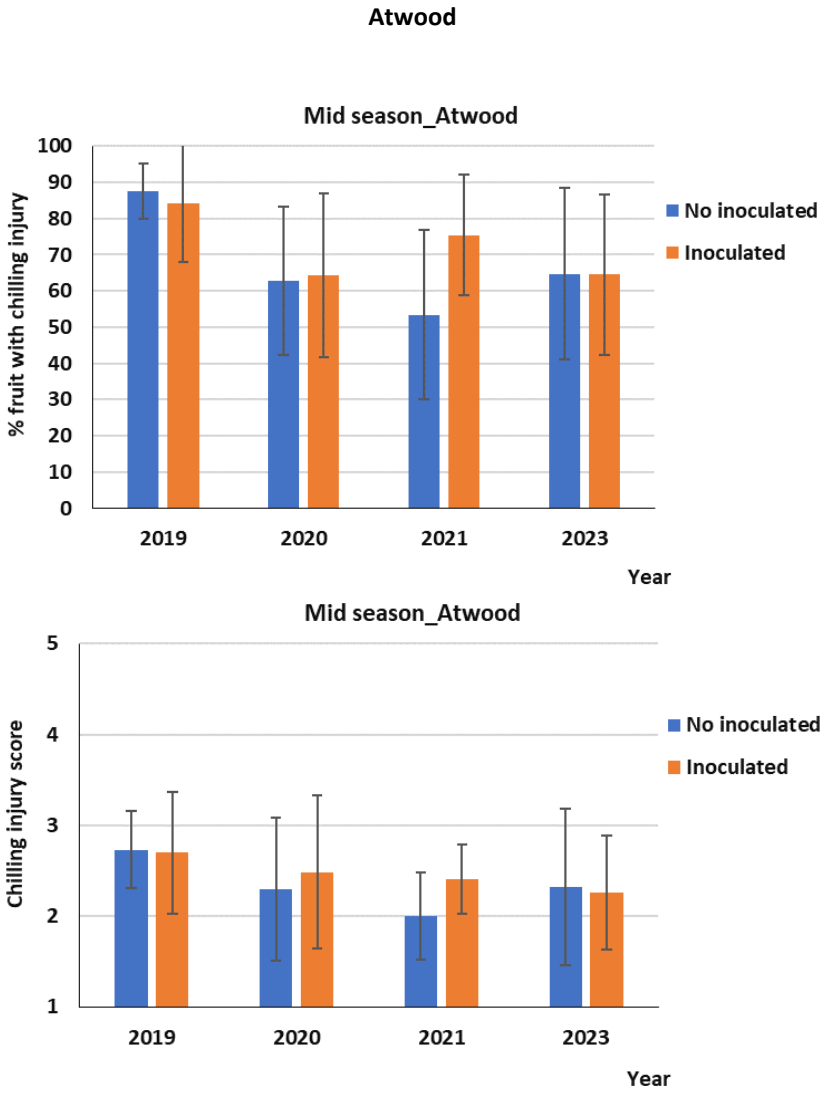


Figure 87. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of mid-season Dareton oranges without and with inoculated 3532 mild strain from 2019–2023 (Atwood). Bars are standard deviations around the means, *n*=6.

Houghton

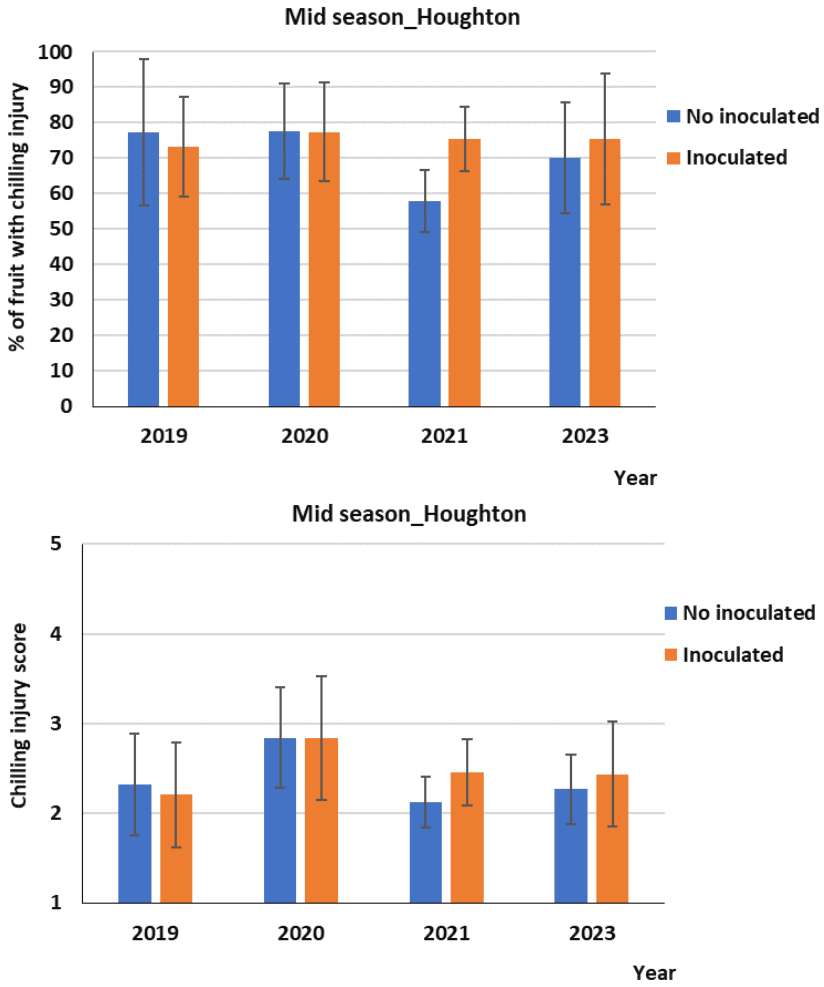


Figure 88. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of mid-season Dareton oranges without and with inoculated 3532 mild strain from 2019–2023 (Houghton). Bars are standard deviations around the means, *n*=6.

Chislett

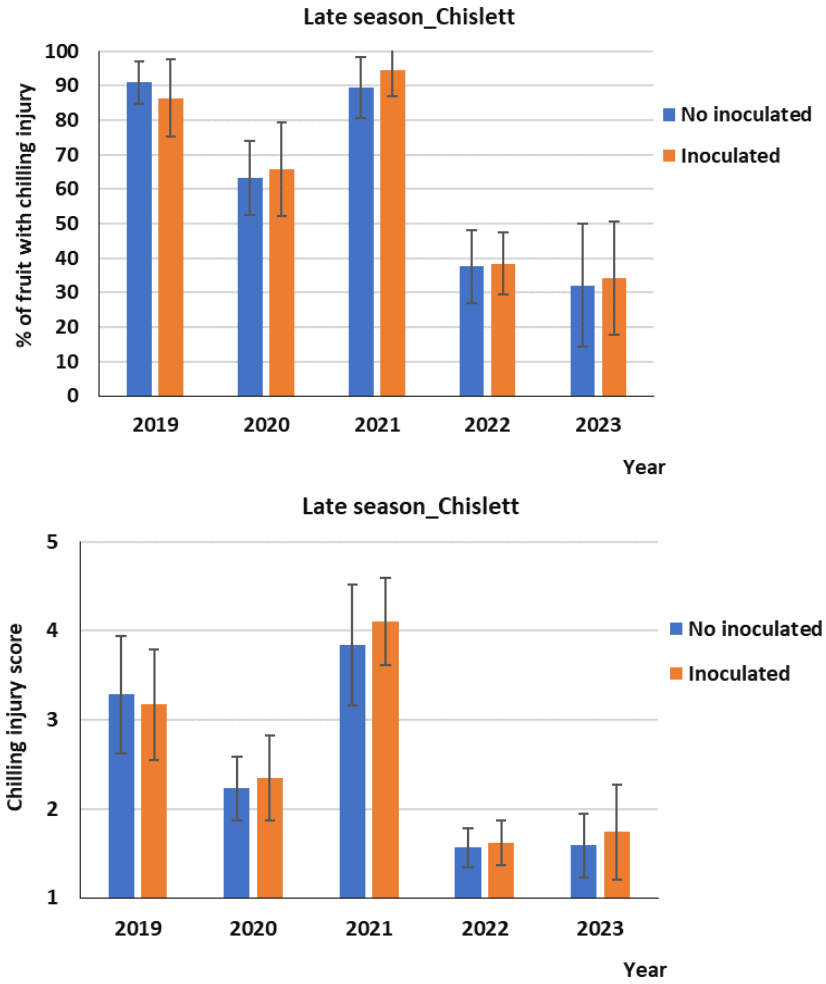


Figure 89. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of late season Dareton oranges without and with inoculated 3532 mild strain from 2019–2023 (Chislett). Bars are standard deviations around the means, *n*=6.

Lanes Late

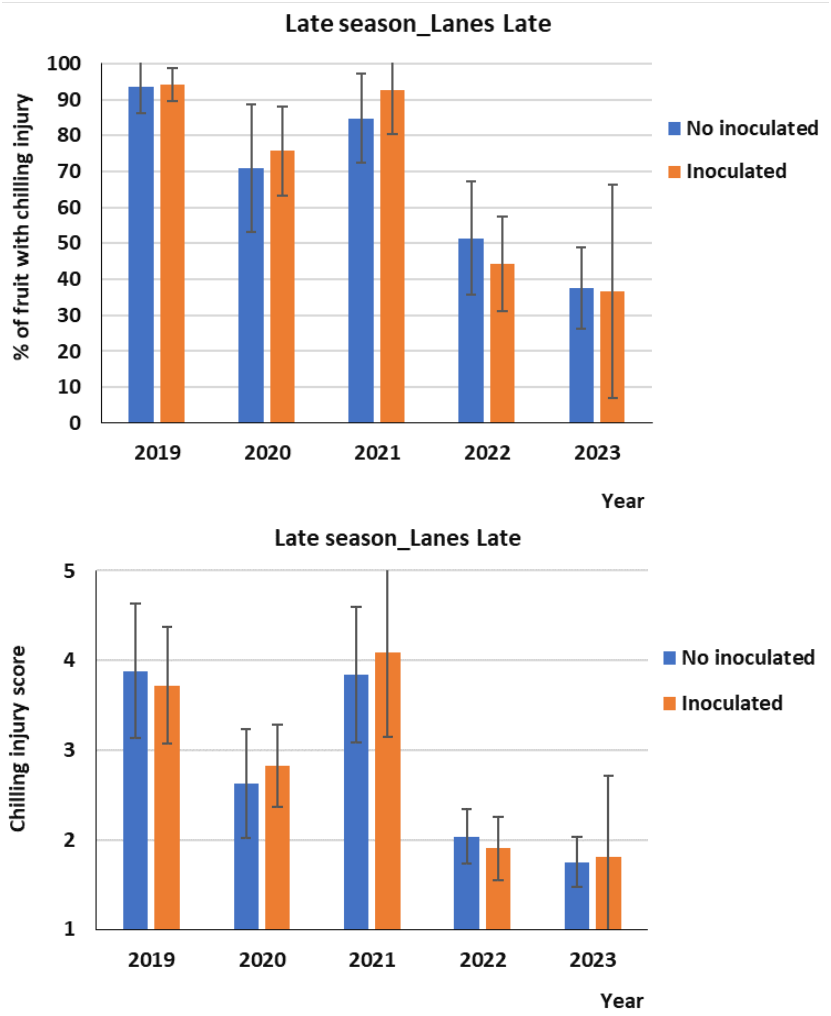


Figure 90. Percentage of fruit with chilling injury (top) and chilling injury score (lower) at assessment 2 (upon removal + 1 week at 20 °C) of late season Dareton oranges without and with inoculated 3532 mild strain from 2019–2023 (Lanes Late). Bars are standard deviations around the means, *n*=5.

Effect of mild strain 3532 inoculation on internal quality in Navel oranges

The main focus of this trial was the effects of variety and season on chilling injury, but the levels of TSS and TA were also irregularly measured in this trial. The effects of the inoculation of the mild strain 3532 on fruit TSS and TA are presented in Figures 91–96, with vitamin C in 2023 data presented in Figure 97. The results show that inoculation of the mild strain 3532 had no consistent effects on TSS, TA or vitamin C content. This is a good result for the industry, who has been using the mild strain 3532 in commercial production for many years.

Early Season

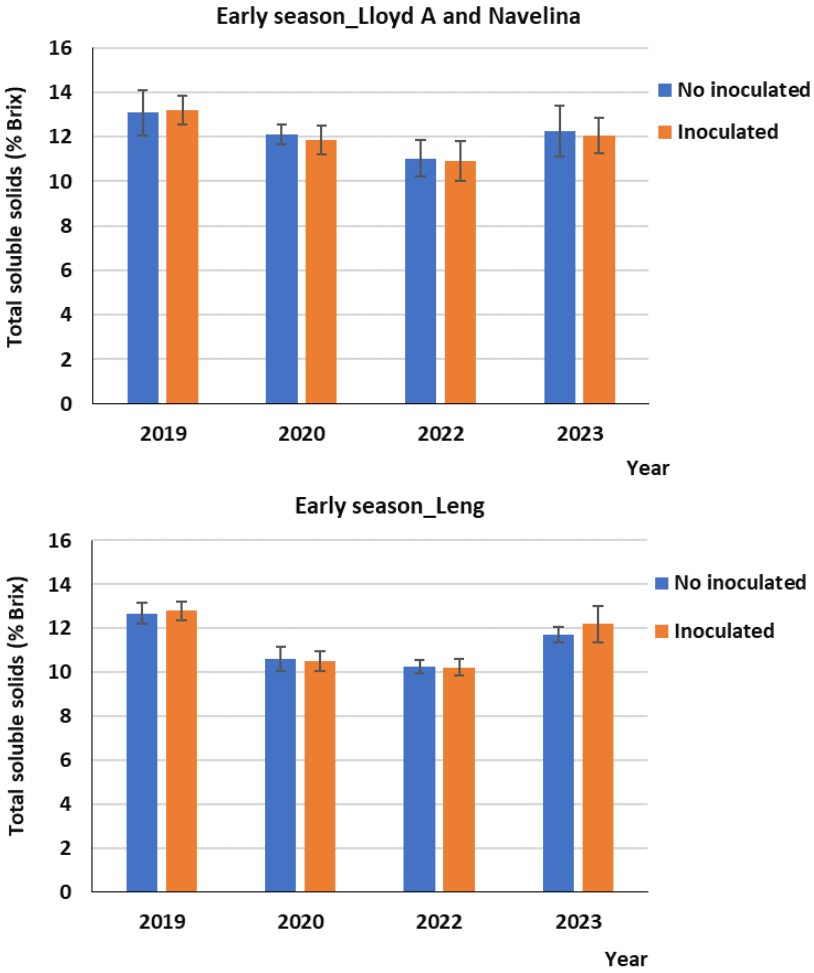


Figure 91. Total soluble solids (% Brix) at assessment 2 (upon removal + 1 week at 20 °C) except for the year 2023 when TSS were measured at time zero of early season Dareton oranges without and with inoculated 3532 mild strain from 2019 (Lloyd A), 2020, 2022 and 2023 (Navelina) (top) and all years Leng navel oranges (lower). Bars are standard deviations around the means, *n*=6. There are no data on TSS of early season oranges in 2021. In 2023, TSS was measured at time zero.

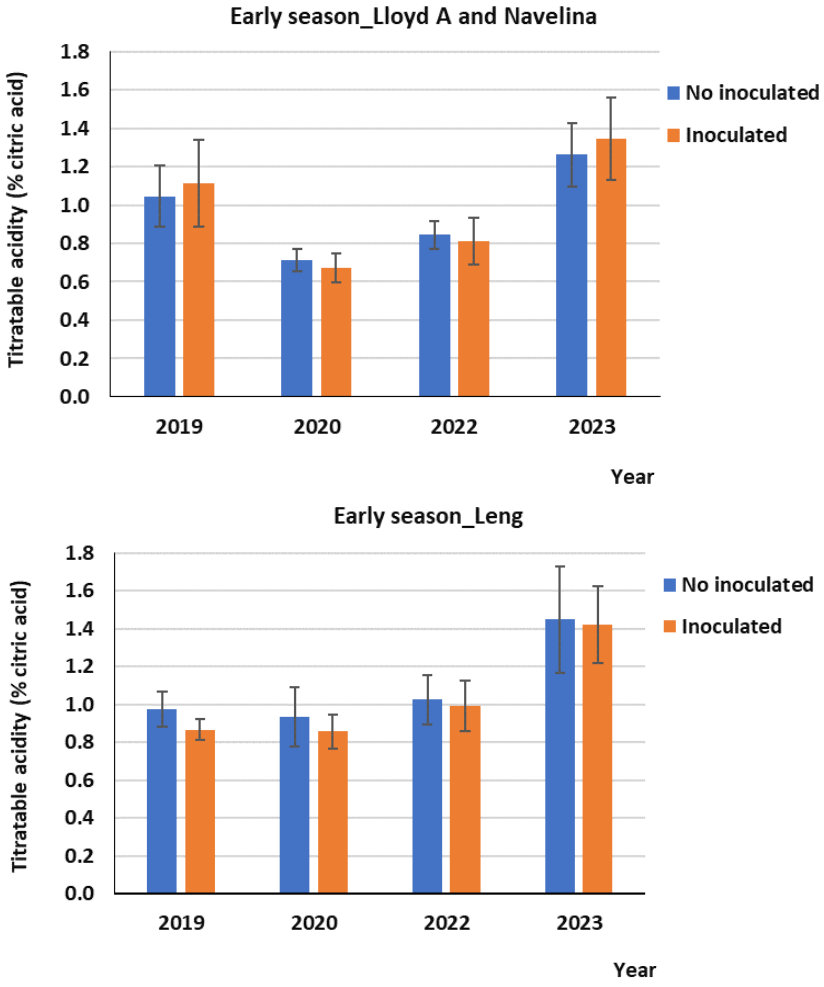


Figure 92. Titratable acidity (% citric acid) at assessment 2 (upon removal + 1 week at 20 °C) except for the year 2023 when TA was measured at time zero of early season Dareton oranges without and with inoculated 3532 mild strain from 2019 (Lloyd A), 2020, 2022 and 2023 (Navelina) (top) and all years Leng navels (lower). Bars are standard deviations around the means, *n*=6. There are no data on TA of early season oranges in 2021. In 2023, TA was measured at time zero.

Mid season

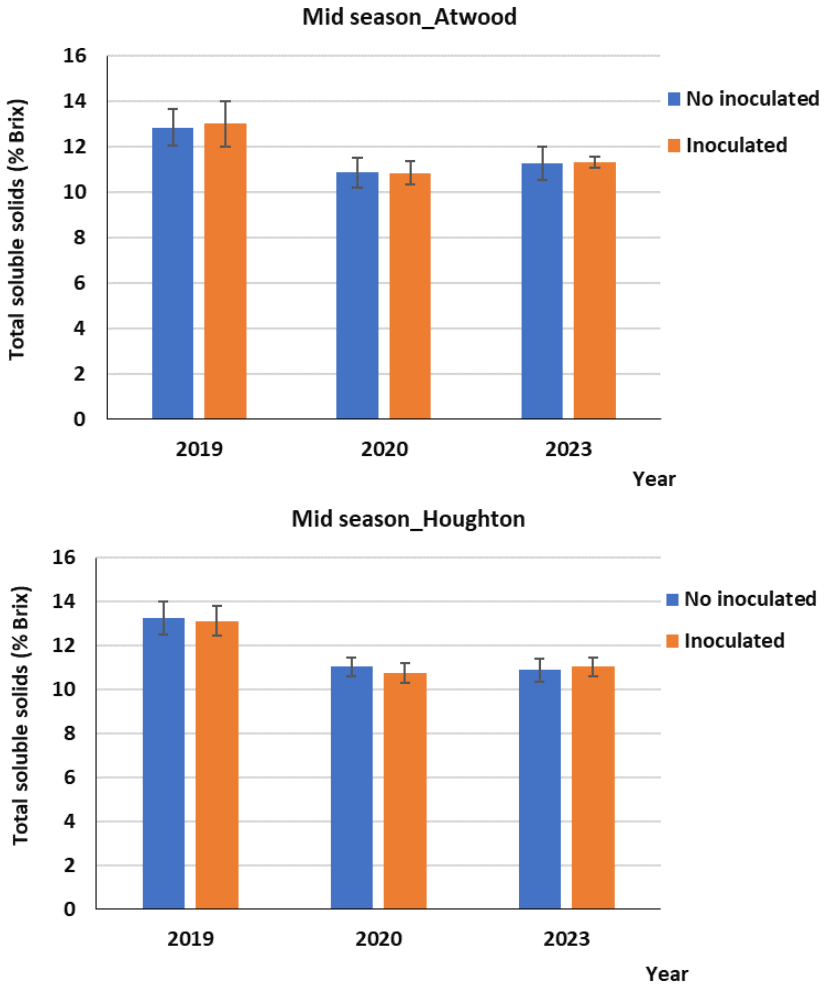


Figure 93. Total soluble solids (% Brix) at assessment 2 (upon removal + 1 week at 20 °C) of mid-season Dareton oranges without and with inoculated 3532 mild strain from 2019, 2020 and 2023 (Atwood) (top) and Houghton fruit from 2019, 2020 and 2023 (lower). Bars are standard deviations around the means, *n*=6. TSS was measured for inoculated and not inoculated mid-season oranges in 2021. There were different box names for mid-season 2022, so TSS could not be used.

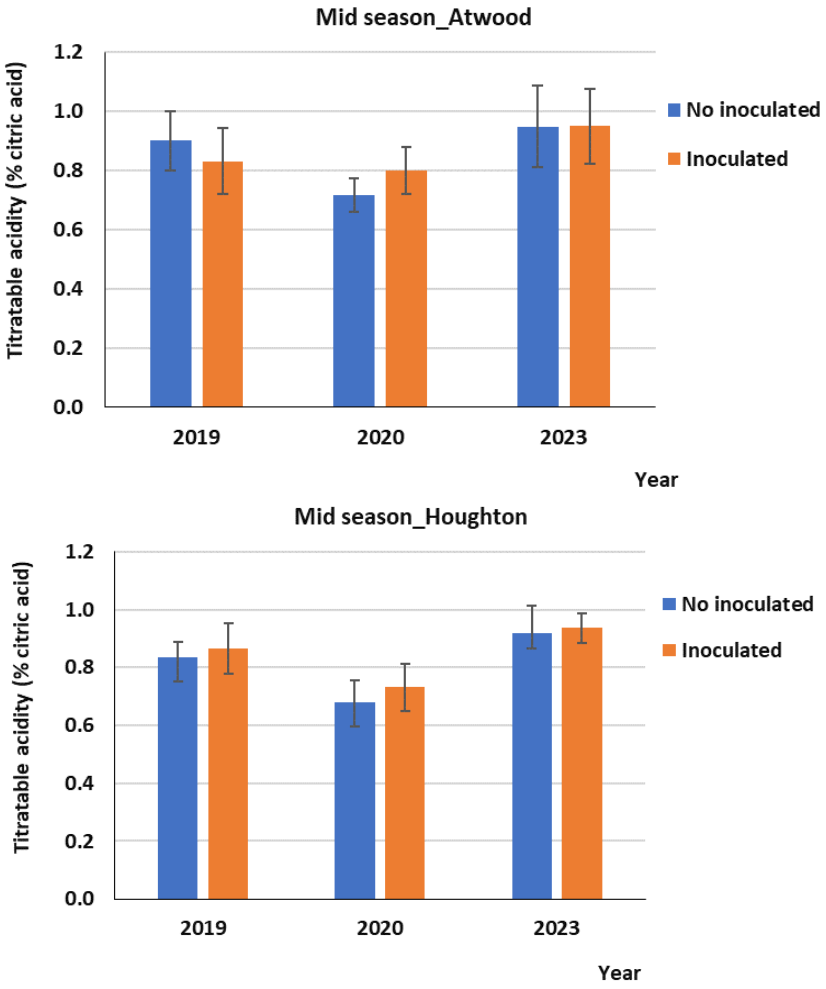


Figure 94. Titratable acidity (% citric acid) at assessment 2 (upon removal + 1 week at 20 °C) of mid-season Dareton oranges with and without inoculated 3532 mild strain from 2019, 2020 and 2023 (Atwood) (top) and Houghton navel oranges in 2019, 2020 and 2023 (lower). Bars are standard deviations around the means, *n*=6. No TA measures were recorded in 2021. There were different box names for mid-season 2022, so TA could not be used.

Late Season

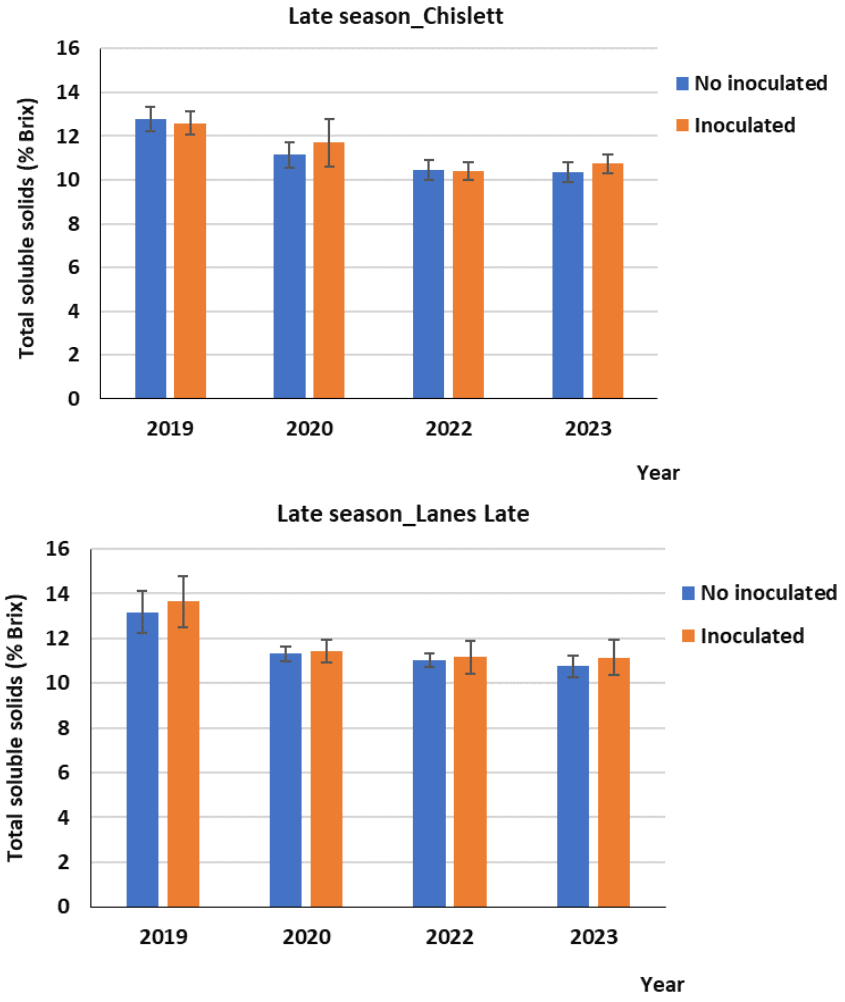


Figure 95. Total soluble solids (% Brix) at assessment 2 (upon removal + 1 week at 20 °C) of late season Dareton oranges without and with inoculated 3532 mild strain from 2019, 2020, 2022 and 2023 (Chislett) (top) and 2019, 2020, 2022 and 2023 Lanes Late (lower). Bars are standard deviations around the means, *n*=6. TSS was not measured in 2021.

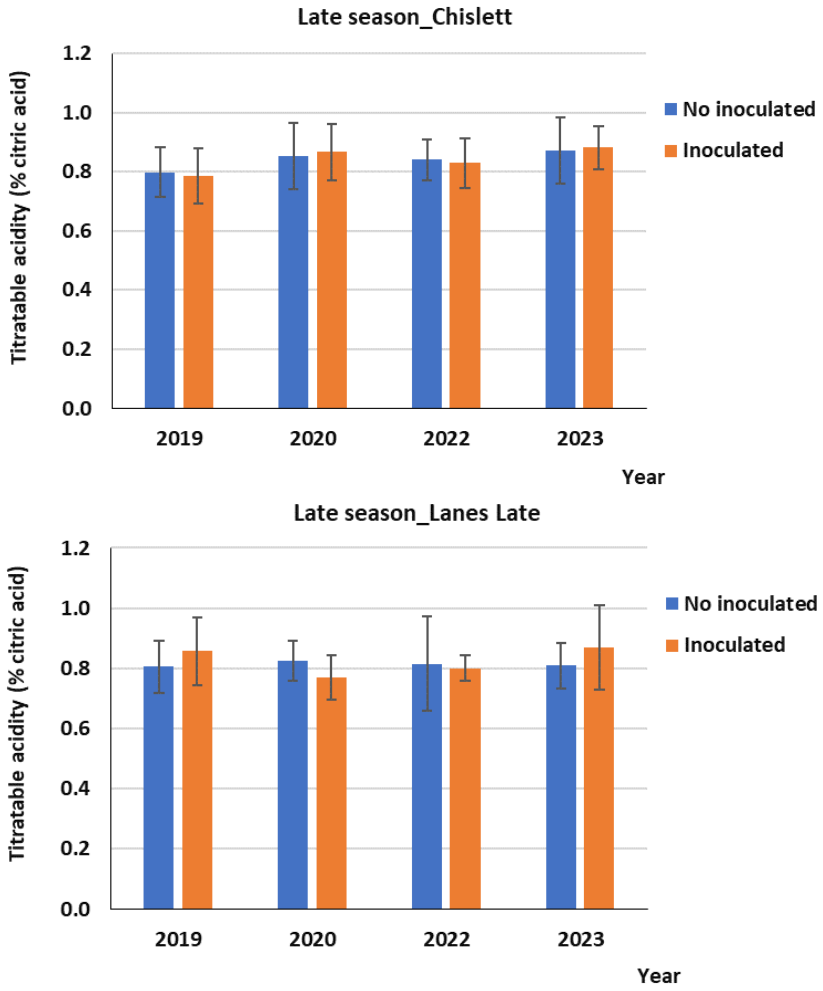


Figure 96. Titratable acidity (% citric acid) at assessment 2 (upon removal + 1 week at 20 °C) of late-season Dareton oranges without and with inoculated 3532 mild strain from 2019, 2020, 2022 and 2023 (Chislett) (top) and from 2019, 2020, 2022 and 2023 Lanes Late (lower). Bars are standard deviations around the means, *n*=6. TA was not measured in 2021.

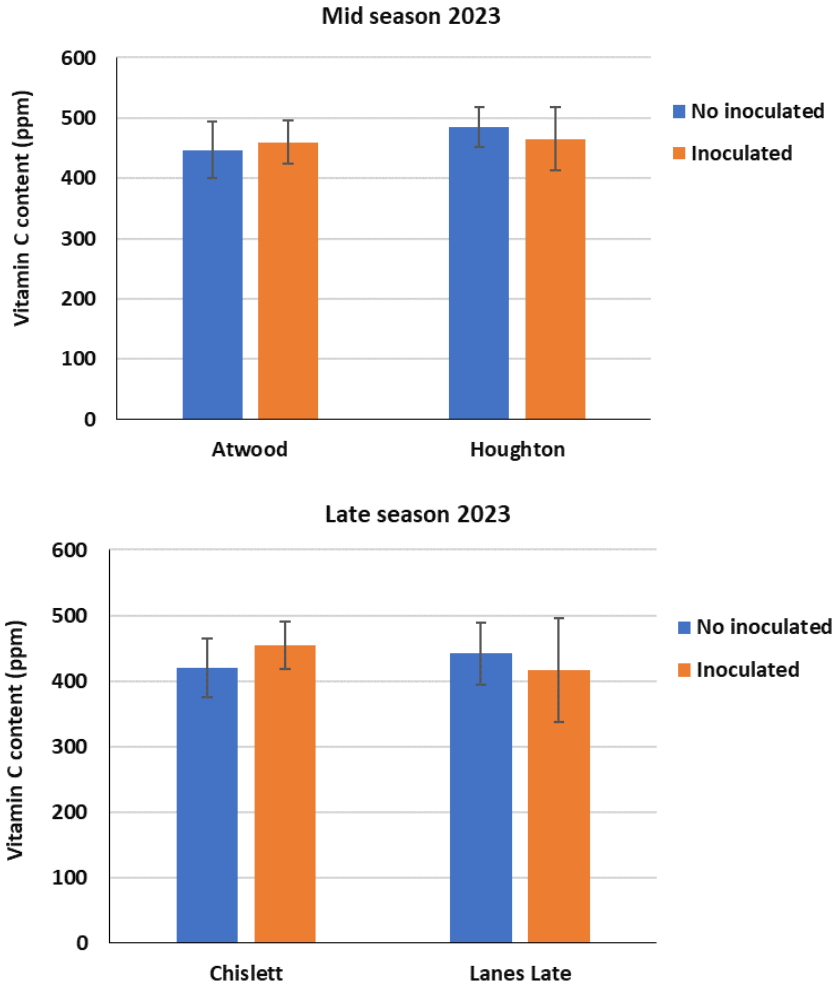


Figure 97. Vitamin C content (ppm) at assessment 2 (upon removal + 1 week at 20 °C) of 2023 mid-season Dareton oranges (Atwood and Houghton) (top) and late season (Chislett and Lanes Late) (lower) without and with inoculated 3532 mild strain. Bars are standard deviations around the means, *n*=6. There were no data for vitamin C in other years.

Chilling injury susceptibility of new mandarin cultivars

The industry is continually introducing new citrus types and cultivars to improve consumer choice and improve profitability. While the production and fruit quality data of new cultivars are well established, there are no local data on postharvest storage behaviour (e.g. susceptibility to chilling injury) to support large-scale production and marketing of new cultivars. This trial assessed the effects of long-term storage on Satsuma mandarins (at 2 different harvest dates) and Clementine mandarins.

Satsuma mandarins

Figure 98. Satsuma mandarins were harvested on 18 March 2021 (Harvest 1) (top) and on 16 April 2021 (Harvest 2) (lower).

Satsuma mandarins were harvested from commercial orchards on 18 March 2021 (harvest time 1) and 16 April 2021 (harvest time 2). Upon arrival, the fruit was separated into small and large sizes. Fruit size was only recorded during Harvest 2, showing the average small fruit size being 74 g weight and 55 mm diameter. In comparison, the large fruit average was recorded as 81 g weight and 57 mm diameter. All fruit were stored at 3 °C for either 4 or 8 weeks and assessed for chilling injury upon removal and again at 7 and 14 days at 20 °C.

Satsuma showed minimal chilling injury with symptoms being <5% of the fruit (chilling injury score 2) (Figures 99 and 100). This is a good result for this cultivar, but more work is required to assess the effects of different harvest times, growers' practices, and seasons. In relation to internal fruit quality, as expected, larger fruit contained a lower TSS and TA when compared to the smaller fruit and no trends were observed between different fruit on vitamin C content.

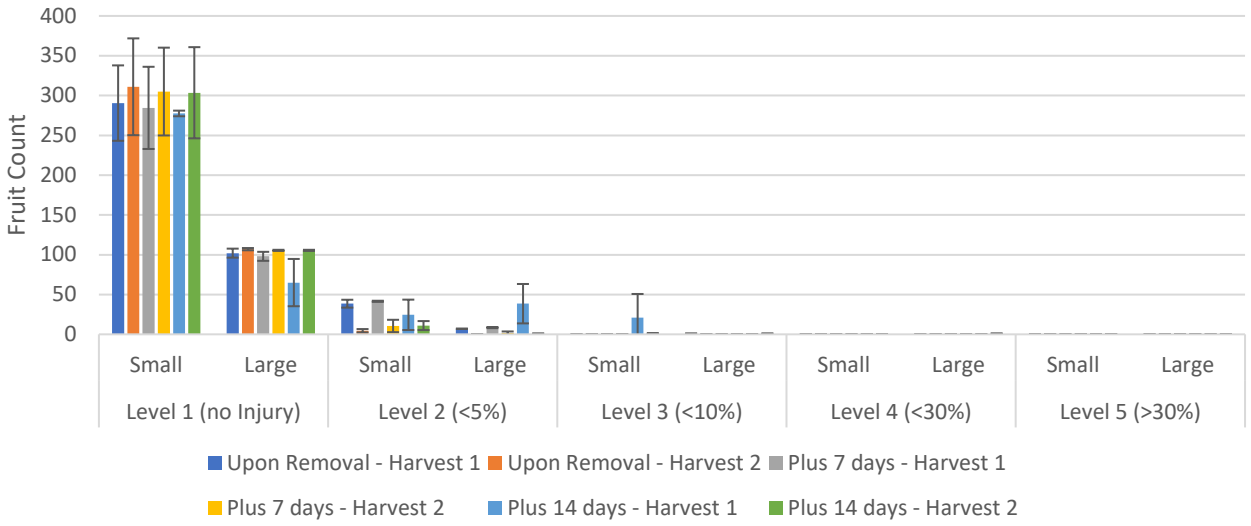


Figure 99. The average number of mandarins within the different classes of chilling injury of small and large Satsumas in 2 harvests. Bars are standard deviations around the mean (*n*=4).

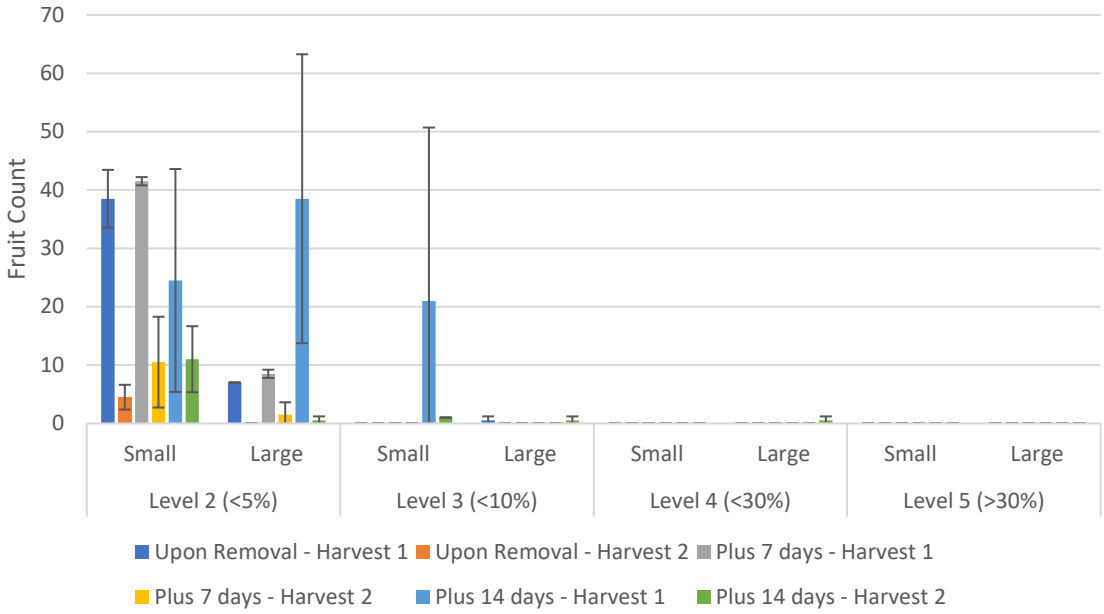


Figure 100. Within the fruit that exhibited chilling injury symptoms, the average number of mandarins within the different classes of chilling injury of small and large satsumas was over 2 different harvests.
Bars are standard deviations around the mean (*n*=4).

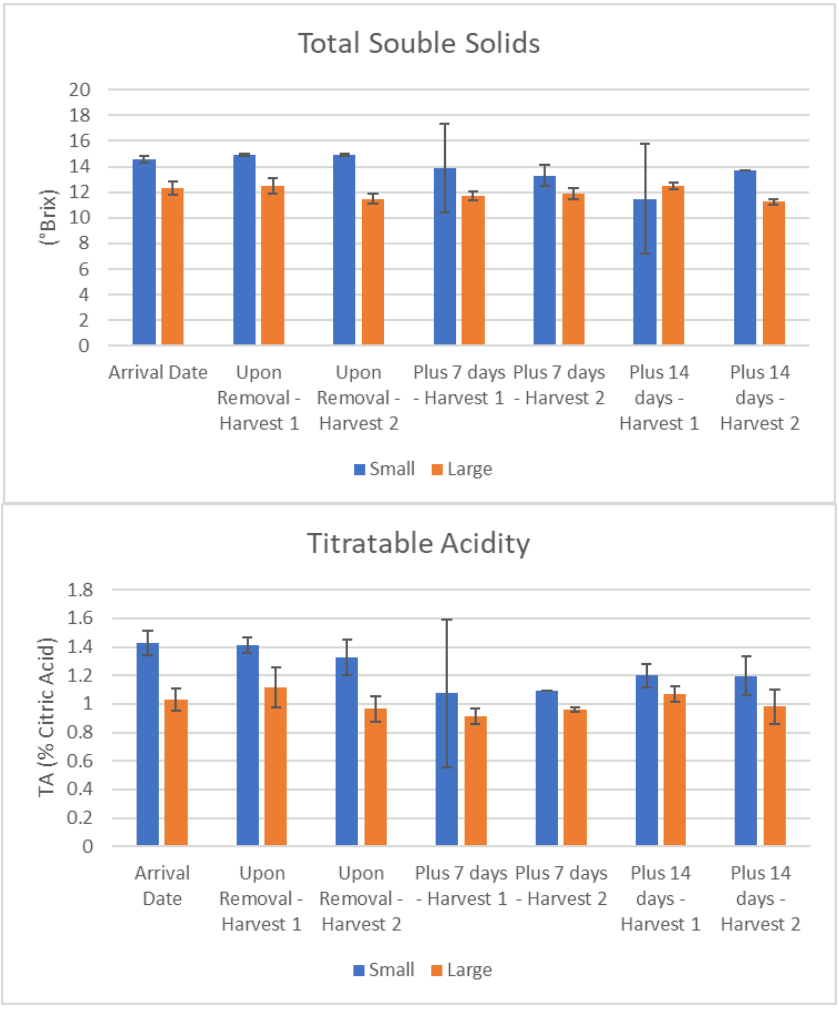


Figure 101. Average TSS (top) and TA (lower) of small and large Satsumas from 2 different harvest times and stored for 8 weeks with additional assessments after 7 and 14 days at 20 °C. Bars are standard deviations around the mean (*n*=4).

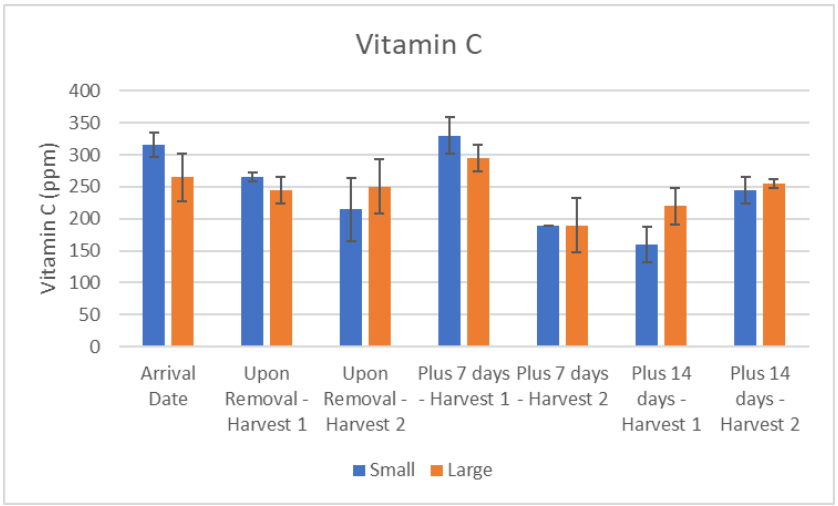


Figure 102. Average vitamin C content (ppm) of small and large Satsumas over 2 different harvests.

Clementine mandarins

Clementine mandarins were harvested on 21 May and 15 June 2021 from a commercial orchard in SA. All fruit were stored at 3 °C for either 4 or 8 weeks and assessed for chilling injury upon removal and again at 7 and 14 days at 20 °C. To investigate whether fruit size had any effects on fruit quality, fruit were segregated into their count sizes C24, 28, 32 and 36 (Figure 103).

Figure 103. Clementine mandarins for storage trial. Count 24 (top left), Count 28 (top right), Count 32 (bottom left), and Count 36 (bottom right).

The results showed there were no differences spotted between sizes or harvest time for chilling injury (Figures 104 and 105). There was minimal chill injury counted in both harvests, with the maximum total chill injury percentages for Clementine of 6.3% chill injury recorded for Week 1 Harvest 1 and 7.2% chill injury recorded for Week 2 Harvest 2 (Figure 104).

Due to only 1 replicate being taken for TSS, TA and vitamin C, determining differences is hard to determine. However, no trend was seen in the size of the fruit with TSS, TA or vitamin C (Figures 106-108).

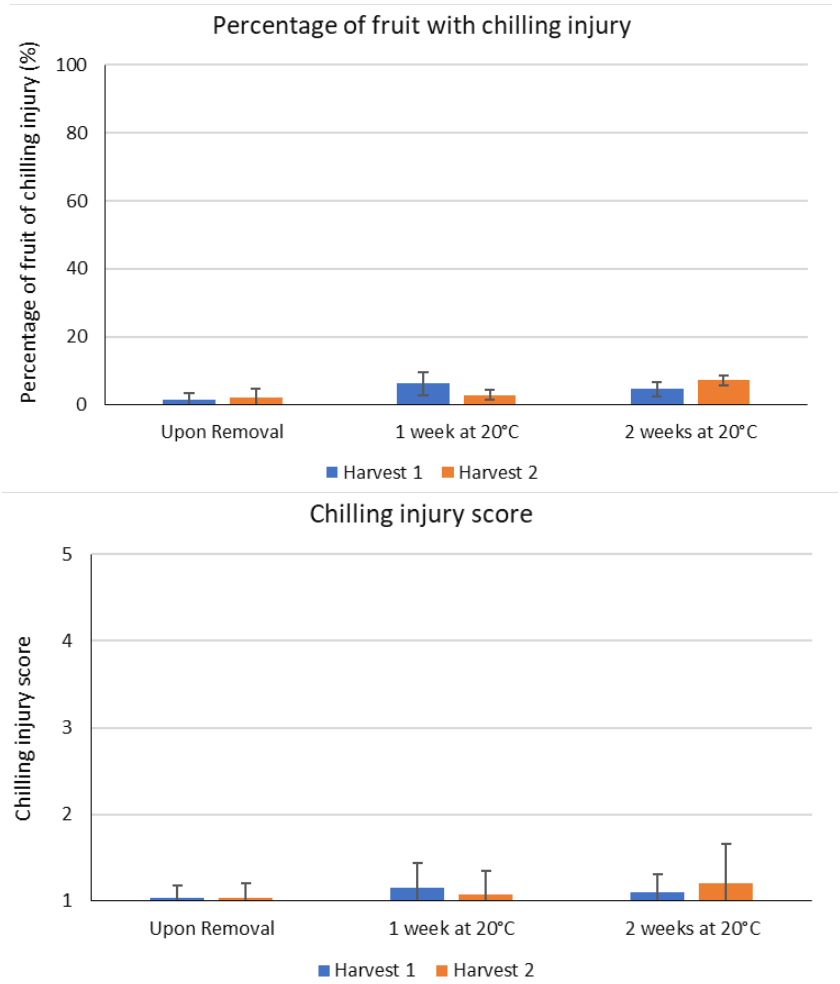


Figure 104. Percentage of fruit with chilling injury (%) (top) and chilling injury score (lower) of Clementine mandarins upon removal and again 1 week and 2 weeks at 20 °C for 2 harvests. Bars are standard deviations around the means, *n* = 4.

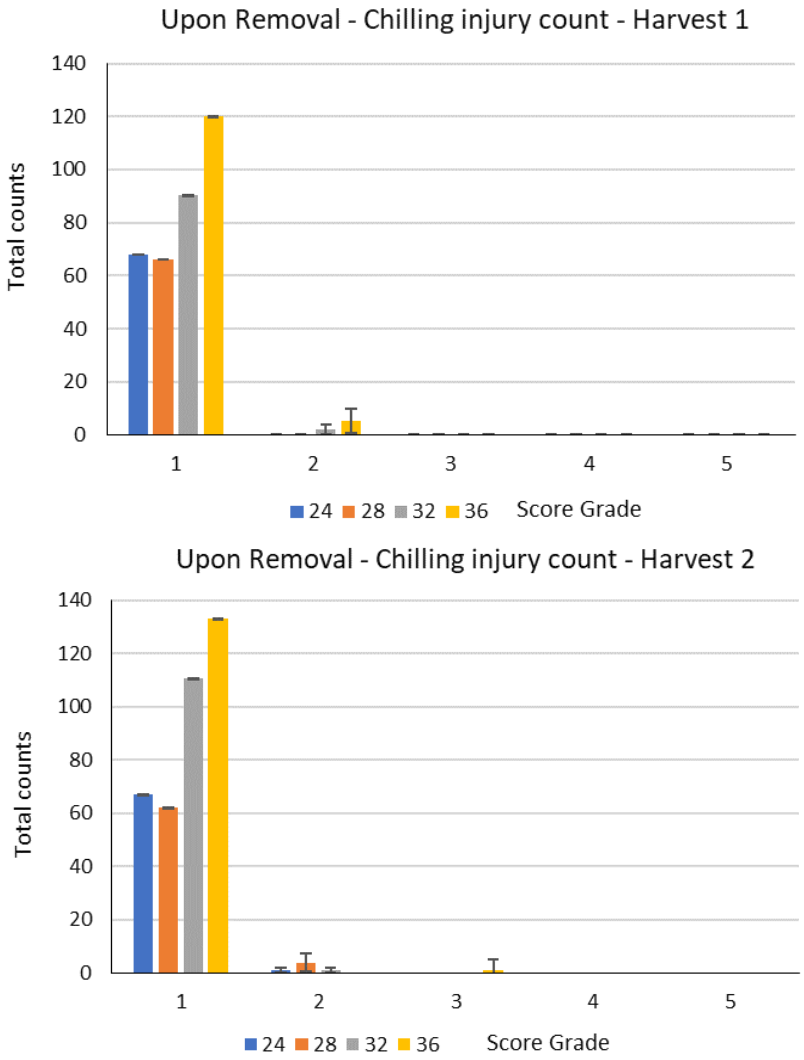


Figure 105. Effect of the fruit size on the chilling injury count of Clementine mandarins upon removal in harvest 1 (top) and harvest 2 (lower). Bars are standard deviations around the means, *n* = 4.

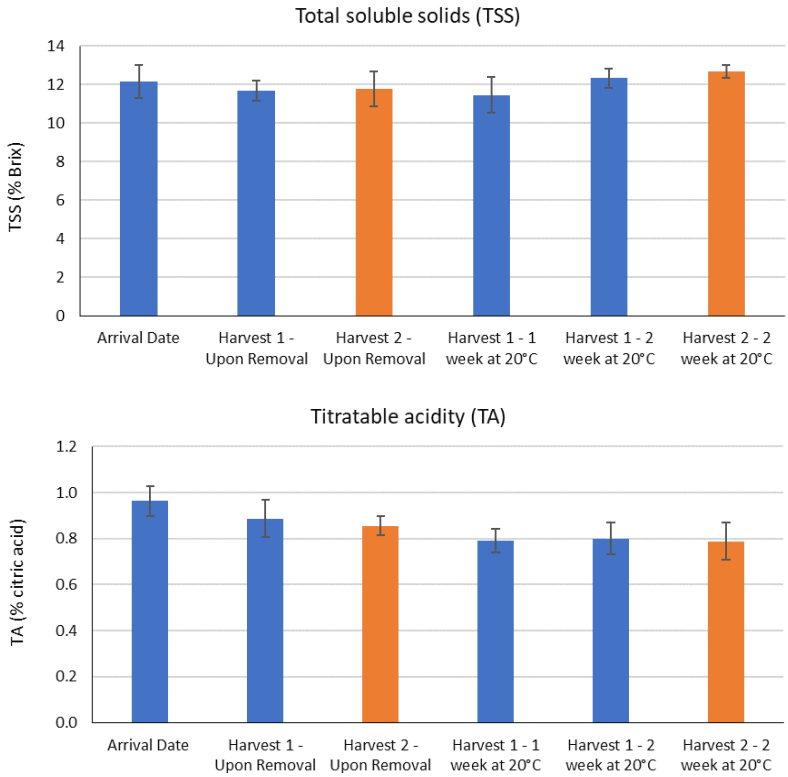


Figure 106. Total soluble solids (% Brix) (top) and titratable acidity (% citric acid) (lower) of Clementine mandarins upon removal plus 1 and 2 weeks at 20 °C for 2 harvests. Bars are standard deviations around the means, *n* = 4.

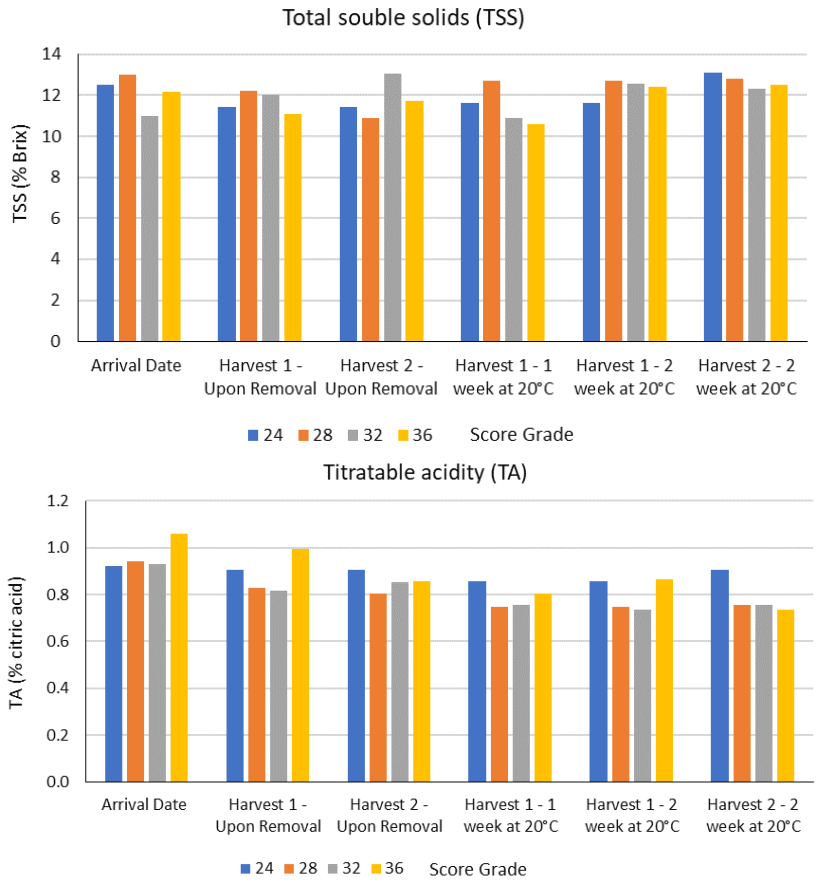


Figure 107. Effect of the fruit size on the total soluble solids (% Brix) (top) and titratable acidity (% citric acid) (lower) of Clementine mandarins upon removal plus 1 and 2 weeks at 20 °C for 2 harvests.

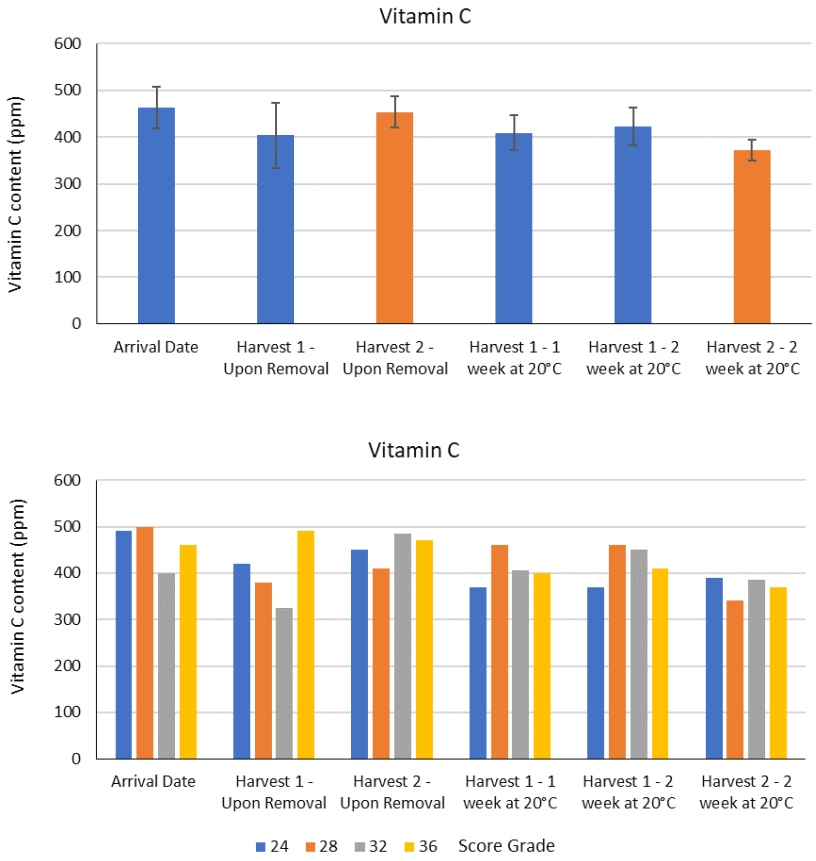


Figure 108. Vitamin C content (ppm) of Clementine mandarins (top) and effect the fruit size on the vitamin C content (lower) upon arrival, upon removal, plus 1 and 2 weeks at 20 °C for 2 harvests. Bars are standard deviations around the means, *n* = 4.

Improving fruit quality outcomes

Effects of irrigation frequency on Afourer fruit quality following long-term storage

Summary

This trial examined different irrigation strategies on the quality of Afourer fruit following up to 10 weeks of storage at 3 °C. Three irrigation scheduling strategies were compared: (1) control (regular irrigation), (2) reduced irrigation (70% of normal irrigation scheduling), and (3) increased irrigation (120% of normal irrigation scheduling). Afourer mandarin fruit were harvested from each of the replicated orchard blocks in the Riverland and transported to NSW DPI at Ourimbah. Fruit were stored for up to 10 weeks at 3 °C and fruit quality was assessed. Upon arrival of fruit at NSW DPI, a parallel set of fruit was stored at 20 °C for up to 4 weeks. For the fruit kept continuously at 20 °C for up to 4 weeks, the major differences between the treatments were the higher levels of sugars (TSS) and acids (TA) in fruit with reduced irrigation scheduling. Similarly, in fruit stored for up to 10 weeks at 3 °C and an additional 1 week at 20 °C, the fruit from the reduced irrigation treatment had higher TSS and TA levels in the storage and shelf life assessments. There were minor differences in other quality parameters and irrigation treatment did not have any effect on chilling injury or button (calyx) browning in this trial. These results showed that reduced irrigation resulted in fruit with higher TSS and TA levels throughout storage and shelf life with no increase in chilling injury or other storage issues.

Background

Management of irrigation is widely used to manipulate Afourer fruit yields and quality. Increasing irrigation is used to increase fruit size, while reduced irrigation is used to conserve limited water and increase fruit sweetness. However, there is no information on the effects of these orchard treatments on fruit quality, particularly after long-term storage.

The aim of this trial was to determine optimum irrigation levels on mature Afourer orchards in the Riverland. This trial was established on dedicated trial blocks of mature Afourer orchards that had established orchard trials examining the effect of 2 irrigation strategies (increased and decreased) to evaluate correlating effects on tree health and stressing and potential effects on fruit sizing and yield. Two strategies were being trialled: reduced irrigation (70% of normal irrigation scheduling) and increased irrigation (120% of normal irrigation scheduling). These treatments were compared to the normal irrigation scheduling. Fruit were harvested and stored for up to 10 weeks at 3 °C before fruit quality assessments.

Methods

The trial was established on a commercial Afourer orchard in South Australia. The irrigation treatments imposed on the orchard were: (1) control (regular management – 100% irrigation), (2) reduced irrigation (70% less irrigation) and (3) increased irrigation (120% of the regular irrigation). Each treatment was replicated 3 times, and each treatment block had 4 rows of trees. Each of the irrigation treatments had true replicates in different treated rows with buffers. The independence of the treatments with the different replicates was maintained throughout the experiment.

Fruit were harvested on 27 July 2022, packed on 28 July, and transported by refrigerated truck to NSW DPI on 31 July 2022. Fruit quality was assessed upon arrival at NSW DPI and on a batch of fruit held at 20 °C for 4 weeks. The main storage trial was conducted on the remainder of fruit stored at 3 °C for up to 10 weeks storage. Each treatment was replicated 3 times, where each replicate was harvested from an independent and separate block in the same orchard (see above). Fruit quality was assessed after 4 weeks of storage (30.8.22) and after 10 weeks (11.11.2022) at 3 °C. An additional fruit quality assessment was conducted after an additional week at 20 °C to simulate retail shelf life. Each treatment unit was a 15 kg loose pack box of fruit.

Fruit quality assessments were conducted according to General Methods (Appendix 1)

Results

Shelf life after harvest

The effects of irrigation scheduling on the initial shelf life of Afourer mandarins that were harvested and stored for up to 4 weeks at 20 °C are presented in the following figures. The results show there were no clear differences in weight loss and fruit firmness between the treatments over the shelf-life period. However, the reduced irrigation fruit was firmer over the shelf life periods. The levels of sugars and acids were higher in reduced irrigation fruit, and this difference was maintained during the 4-week storage at 20 °C.

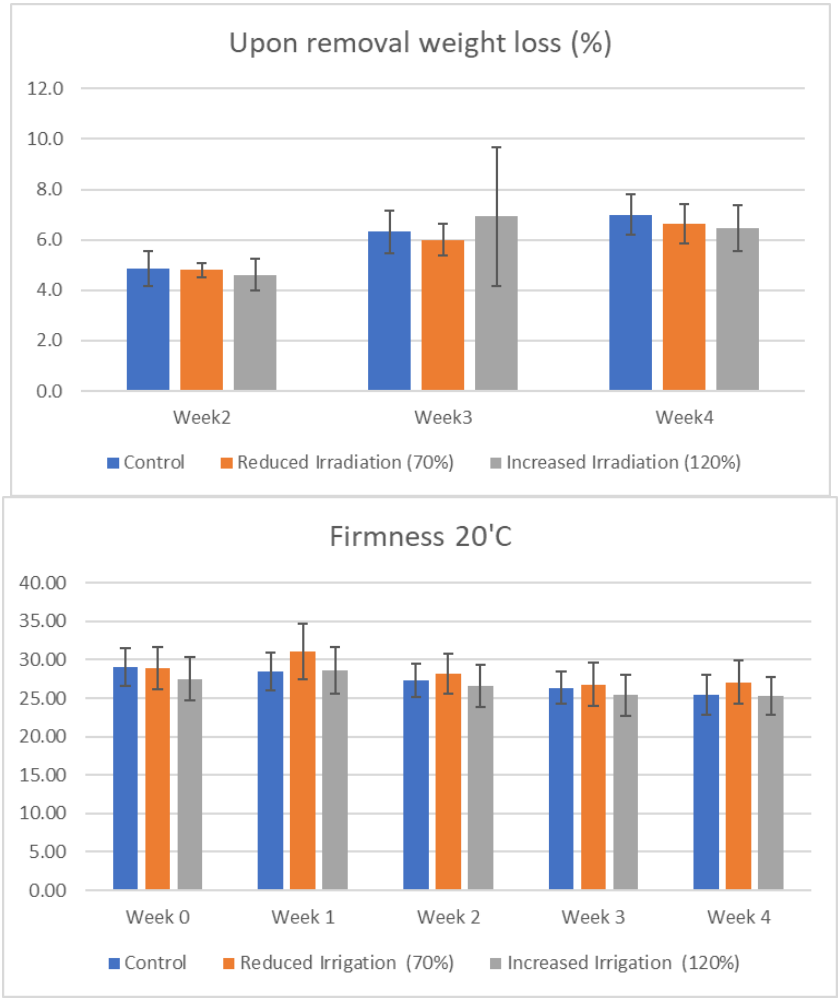


Figure 109. Weight loss (%) from boxes of Afourer mandarins (top) and fruit firmness (N) (lower) during shelf life storage (20 °C) for up to 4 weeks after harvest (bars are standard deviations around the means, *n*=3)

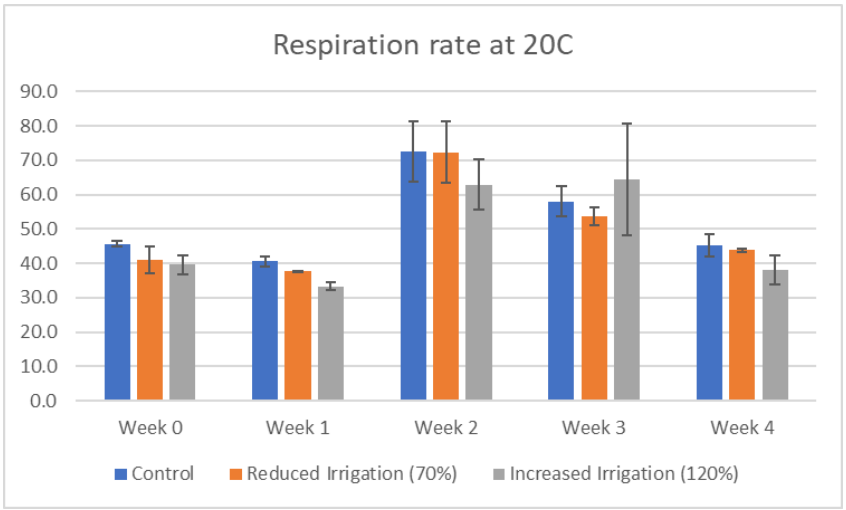


Figure 110. Respiration rate (mL CO_2 kg^{-1} h^{-1}) of Afourer mandarins during shelf life storage (20 °C) for up to 4 weeks after harvest (bars are standard deviations around the means, *n*=3)

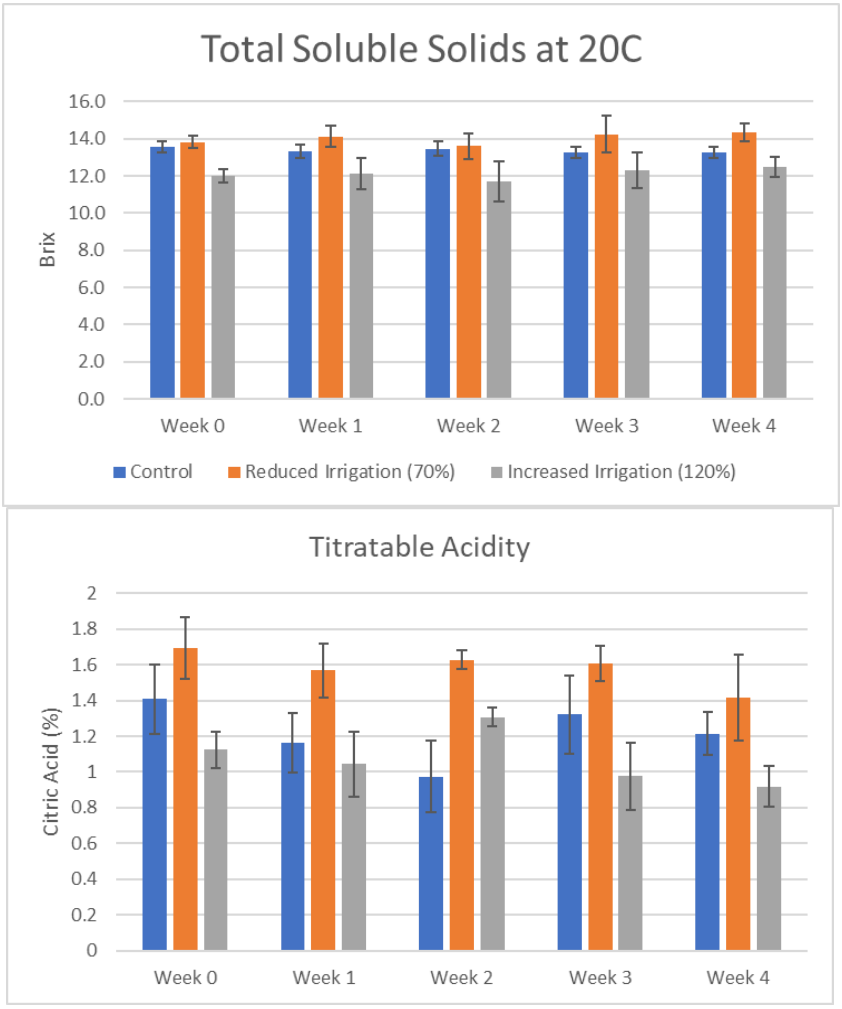


Figure 111. Total soluble solids content (TSS or SSC, % Brix) (top) and titratable acidity (TA, % citric acid) (lower) of Afourer mandarins during shelf life storage (20 °C) for up to 4 weeks after harvest (bars are standard deviations around the means, *n*=3).

Storage life

Afourer fruit quality was assessed after 4 and 10 weeks of storage at 3 °C and an additional one week at 20 °C to simulate retail shelf life.

All fruit were in excellent condition and there were no visual differences between the treatments during the storage trial (Figure 112). In addition, there appeared to be no irrigation treatment effects on either the browning (condition) of the button or on the expression of chilling injury.

In general, there was no treatment effect on weight loss, which increased during storage life at 3 °C but increased when the fruit was kept out at 20 °C for one week. In general, fruit with reduced irrigation scheduling had higher fruit firmness over the storage period and shelf life.

The levels of TSS did not change during the 10-week storage period. However, the reduced irrigation scheduling treatment had higher levels of TSS over the entire storage period, including the additional shelf life. The levels of TA were also higher in the reduced irrigation treatment, which was maintained over the storage life (including shelf life).

Figure 112. The visual appearance of Afourer mandarins after 10 weeks of storage at 3 °C with an additional 1 week at 20 °C. Control fruit (top left), reduced irrigation (70%) (top right) and increased irrigation (120%) (lower).

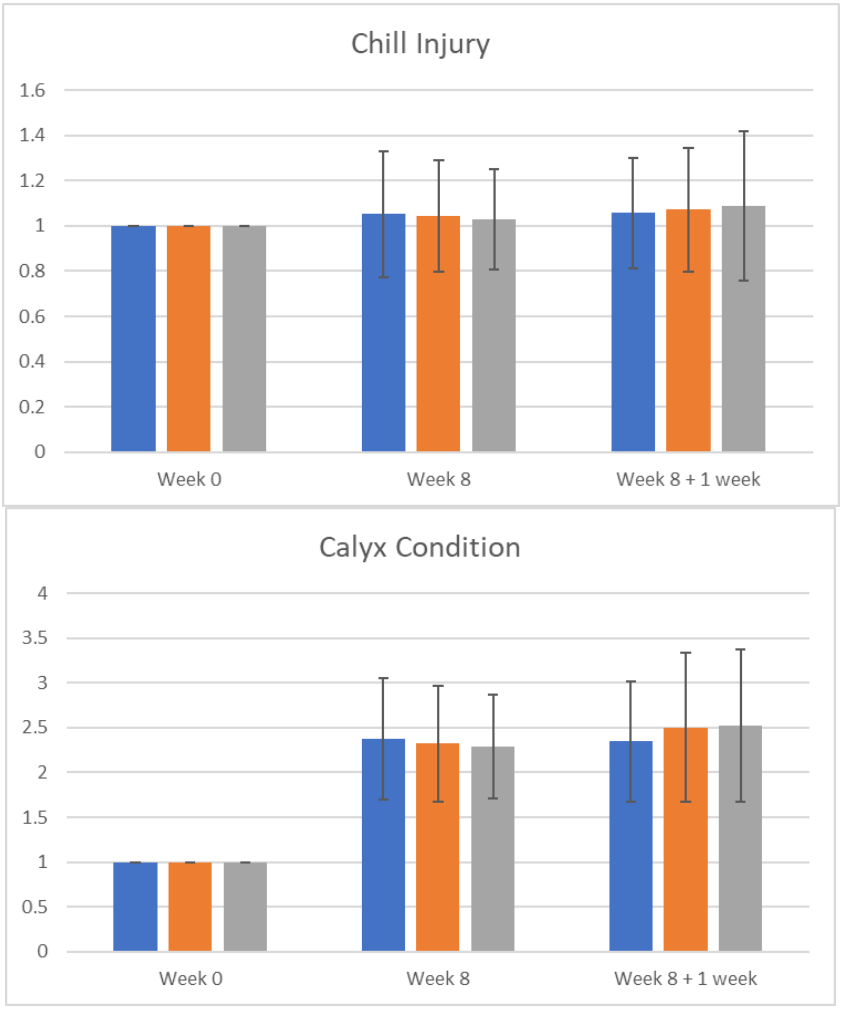


Figure 113. Effect of irrigation scheduling on average chilling injury (top) and average calyx condition/browning (lower) in Afourer mandarins after 10 weeks of storage and an additional one week at 20 °C. Chilling injury score: 1 = normal (no pitting symptoms), 2 = slight pitting (a few scattered pits), 3 = moderate pitting (up to 30% surface covering), 4 = severe pitting (> 30% surface covering). Bars are standard deviations around the means, *n*=3.

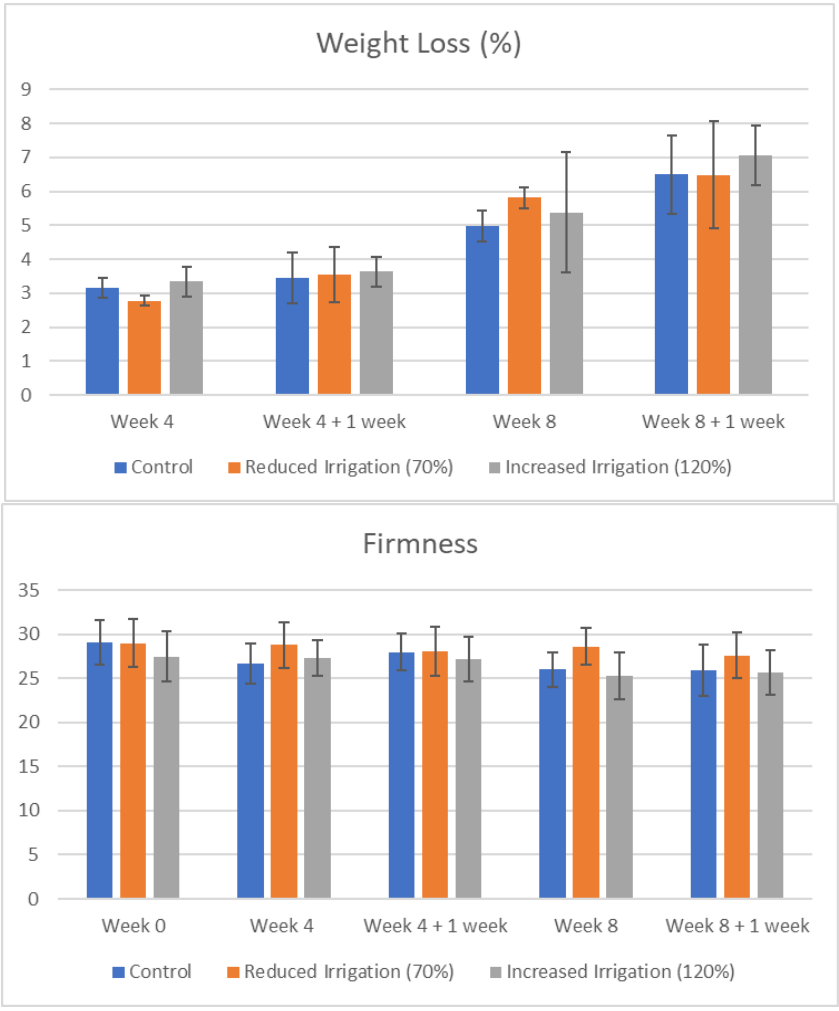


Figure 114. Effect of irrigation scheduling on fruit weight loss (%) (top) and fruit firmness (N) (lower) after 4 weeks and 10 weeks storage and an additional one week at 20 °C. Bars are standard deviations around the means, *n*=3.

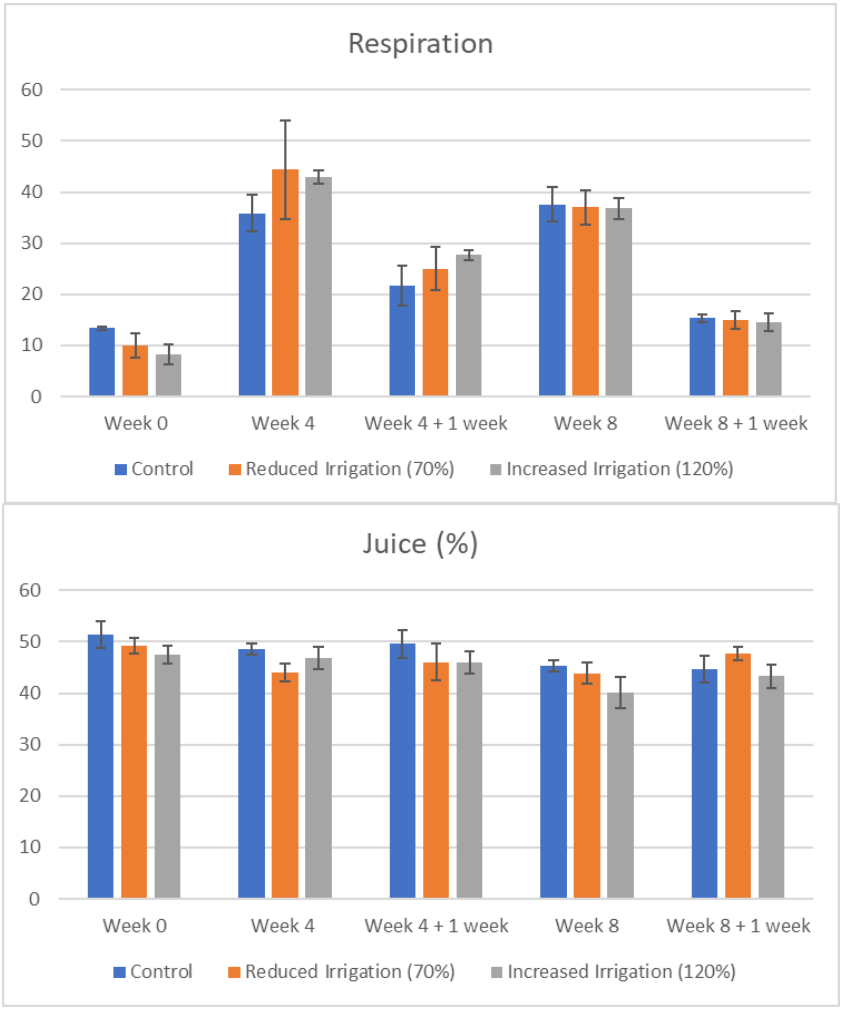


Figure 115. Effect of irrigation scheduling on fruit respiration rates (top) and juice content (lower) after 4 weeks and 10 weeks of storage and an additional one week at 20 °C. Bars are standard deviations around the means, *n*=3.

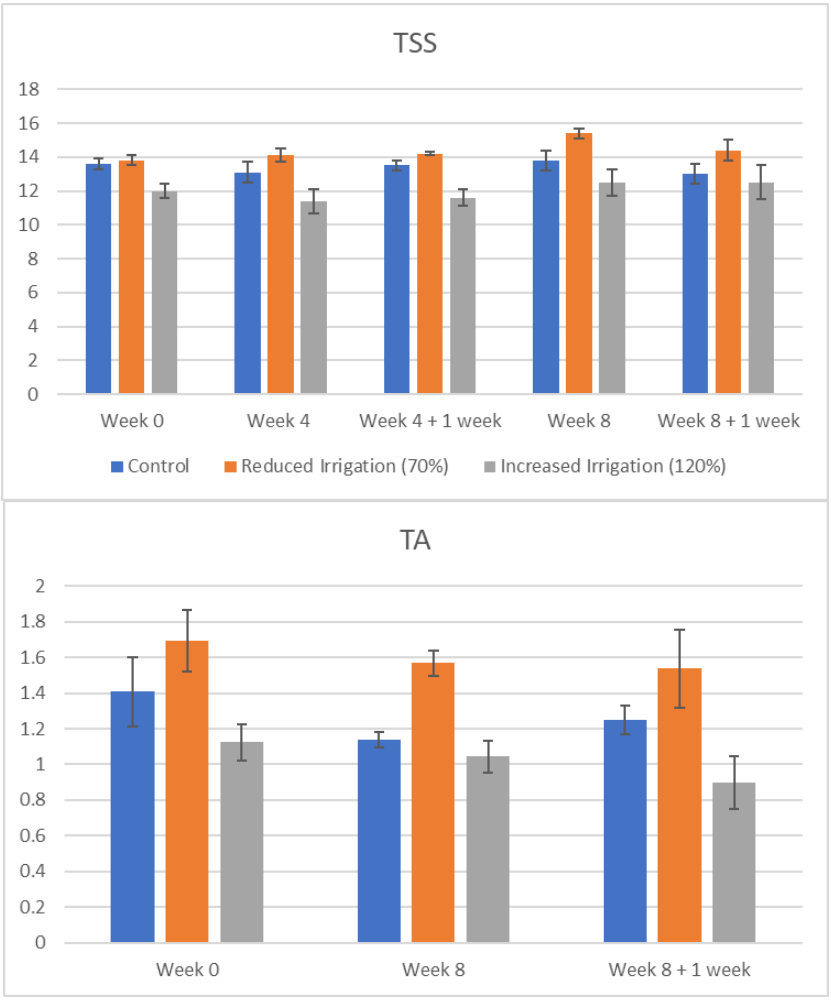


Figure 116. Effect of irrigation scheduling on fruit TSS (top) and TA (lower) after 4 and 10 weeks of storage and an additional one week at 20 °C. Bars are standard deviations around the means, *n*=3.

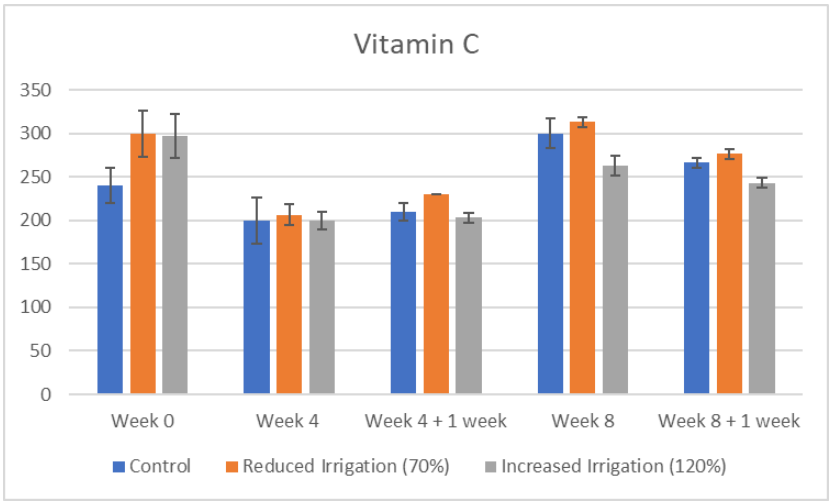


Figure 117. Effect of irrigation scheduling on vitamin C content after 4 and 10 weeks of storage and an additional week at 20 °C. Bars are standard deviations around the means, *n*=3.

Taste testing

To complement the fruit quality assessments for a mandarin storage trial, an informal taste test was conducted to determine if any of the postharvest treatments had any effects on consumer fruit acceptability.

There were 3 pre-harvest treatments (Treatment 1, 2 and 3) and 3 replicates (A, B and C). Volunteers were recruited to taste the mandarins at NSW DPI and fill out a questionnaire on their sensory experiences of the fruit. Fruit from the storage trial were presented to 55 taste testers. The order of presentation to the tasters was randomised according to the table below.

The tasters/assessors were asked to peel and taste the mandarins. The panellists were asked to say how much they dislike or like the fruit on a 1–9 scale with the range from '*Dislike extremely*' (left) to '*Like extremely*' (right). They were asked to assess the following attributes: fruit firmness, appearance, sweetness, sourness, aroma, and overall liking, as well as provide any comments on the fruit. In between samples, the assessors were asked to rest and drink some room-temperature water and plain water crackers to remove the sensory fatigue of the palate.

Taster number	Replicate	First	Second	Third
1	C	1	2	3
2	C	2	3	1
3	C	3	1	2
4	B	3	1	2
5	B	1	2	3
6	B	2	3	1
7	A	2	1	3
8	A	3	2	1
9	A	1	3	2

Figure 118. Tasters assessing mandarins from the irrigation trial at NSW DPI (left). Sample order of tasting order for the presentation of mandarins to tasters (right). This plan was repeated until all panellists were complete.

Results

The results presented in Figure 119 show there were no differences between the consumer liking (or disliking) of any of the fruit quality attributes (fruit firmness, appearance, sweetness, sourness, aroma, and overall liking).

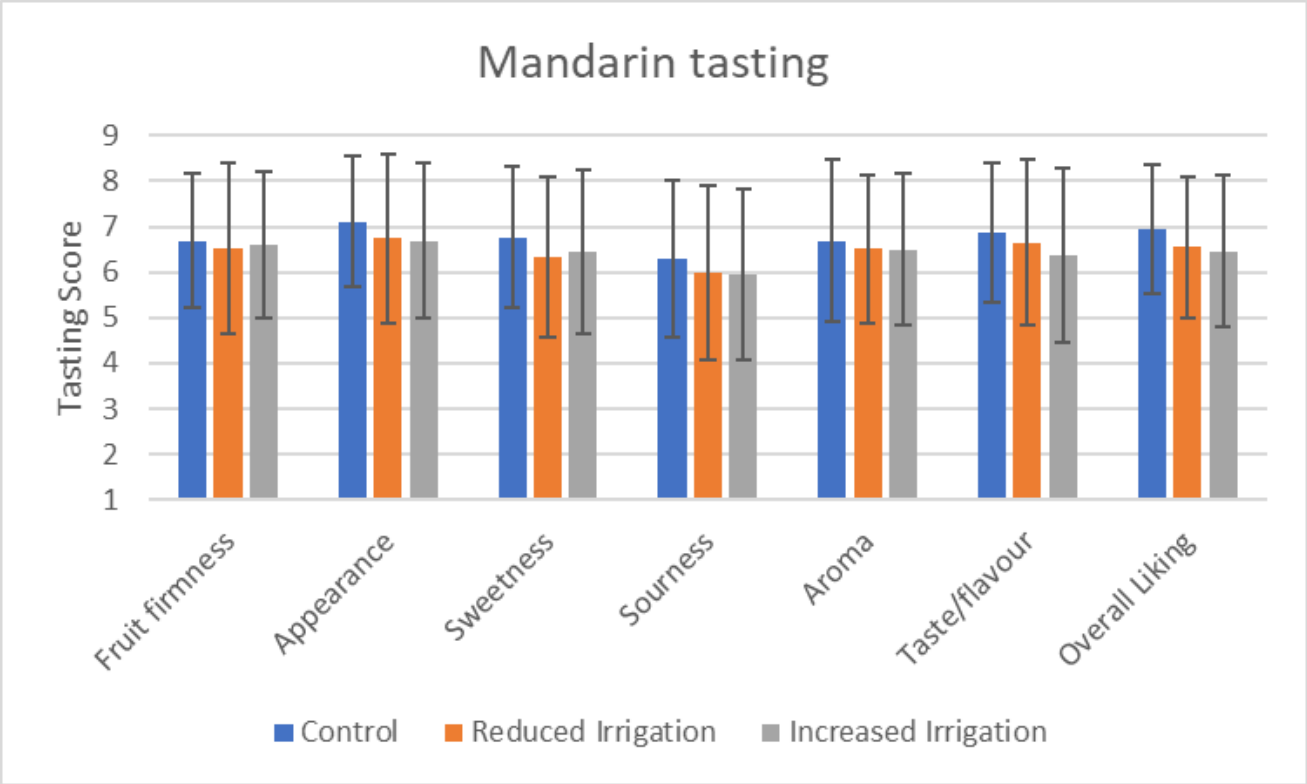


Figure 119. Taste testing scores of fruit firmness, appearance, sweetness, sourness, aroma, and overall liking attributes of Afourer mandarin fruit stored for 10 weeks or 4 weeks at 3 °C plus 1 week at 20 °C.
Bars are standard deviation around the mean, *n*= 55 panellists.

Effects of orchard PGR applications on the shelf life and quality of Afourer mandarins

Plant growth regulators (PGRs) are an important management tool for improving different crop cycles and yield-related processes. Gibberellic acid (GA) is a well-known plant hormone used to delay rind development and reduce albedo breakdown. 2,4-dichlorophenoxy acetic acid (2,4-D) is used to stop fruit drop. While these treatments are routinely used in the orchard, there is little information on the effects of these orchard treatments on fruit quality, particularly after long-term storage.

The aim of this trial was to examine the effect of applications of commercial plant growth regulators sprayed during growth on the shelf life and quality of Afourer mandarins. The orchard component and PGR applications were conducted in a commercial orchard in South Australia in a block of mature Afourer mandarins. The fruit storage and quality assessment were conducted at NSW Department of Primary Industries, where the mandarins were stored for up to 10 weeks at 3 °C before fruit quality assessments.

Methods

Afourer mandarins were treated with different PGRs during the growing season. The treatments were sprayed in a commercial mature block of Afourer mandarins. The treatments were: (1) 10 ppm GA applied mid-February 2022, (2) 20 ppm GA applied mid-February 2022, (3) 10 ppm GA plus 20 ppm 2,4-D, and (4) Control (no spray). All fruit were sourced from the same block and 4 replicates were allocated within the orchard block.

Fruit were harvested and sent to NSW Department of Primary Industries on 05.09.2022. Fruit were assessed upon receipt

at NSW and after one week at 20 °C. Fruit were stored at 3 °C and assessed after 4 and 10 weeks of storage. Fruit quality was assessed immediately after cold storage (when the fruit had warmed to room temperature) and after one week of shelf life simulation at 20 °C (Figure 120). The treatment unit was 1 box of fruit.

Figure 120. Afourer mandarins upon arrival at NSW Department of Primary Industries (left) and assessment of fruit for subjective quality analysis (right).

Results

Figure 121. Appearance of Afourer mandarins at the beginning of the experiment. Fruit sprayed with 10 ppm GA applied mid-February 2022 (top left), fruit sprayed with 20 ppm GA applied mid-February 2022 (top right), fruit sprayed with 10 ppm GA plus 20 ppm 2,4-D (bottom left) and no spray control fruit (bottom right).

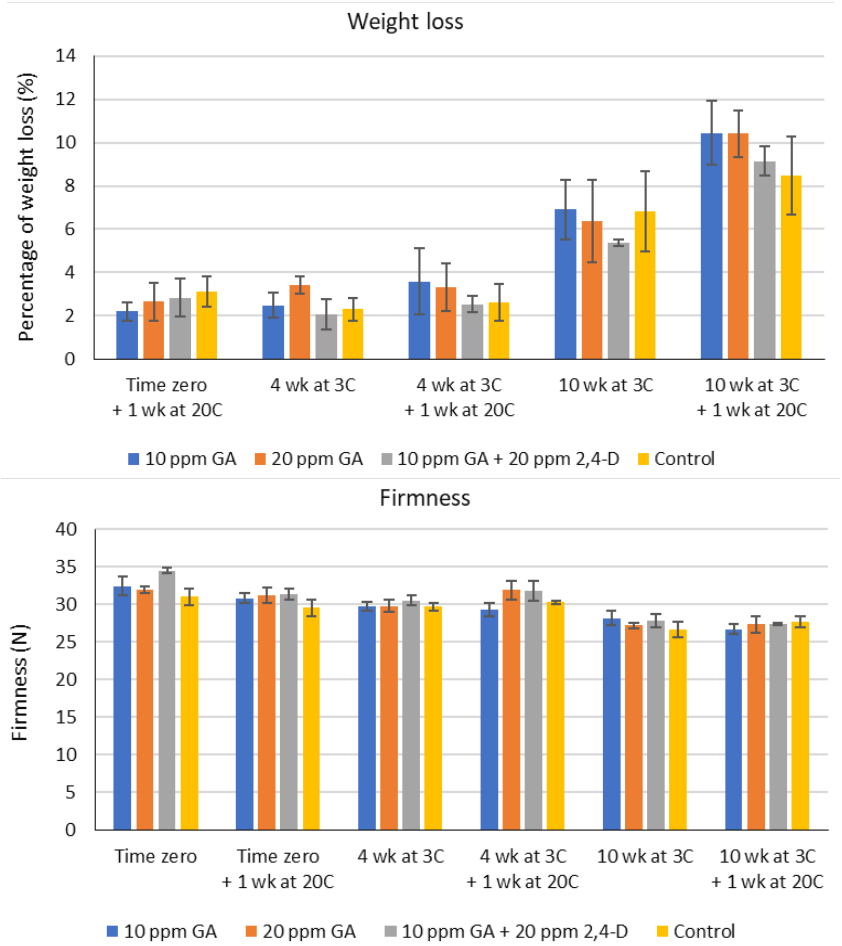


Figure 122. Effect of orchard PGR applications on the percentage of weight loss (%) (top) and the firmness (N) (lower) of Afourer mandarins at the following storage times: time zero, time zero plus 1 week (wk) at 20 °C, 4 weeks at 3 °C, 4 weeks at 3 °C plus 1 week at 20 °C, 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Bars are standard deviations around the means, *n* = 4.

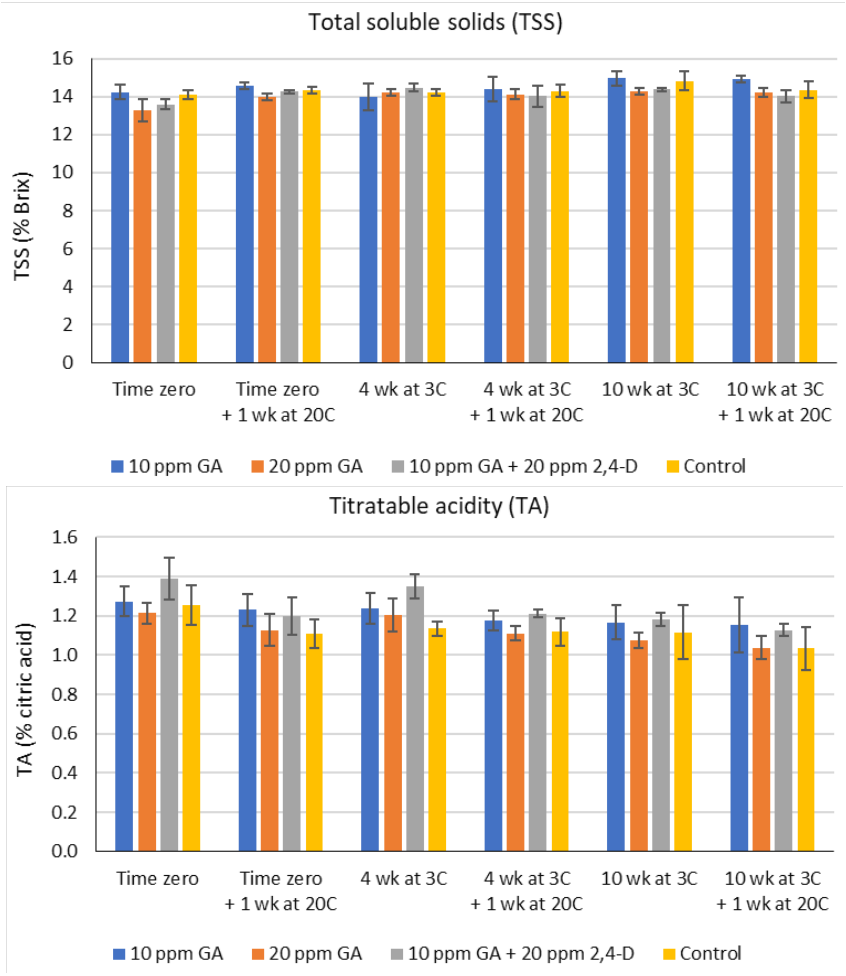


Figure 123. Effect of orchard PGR applications on the total soluble solids (TSS) (% Brix) (top) and the titratable acidity (TA) (% citric acid) (lower) of Afourer mandarins at the following storage times: time zero, time zero plus 1 week (wk) at 20 °C, 4 weeks at 3 °C, 4 weeks at 3 °C plus 1 week at 20 °C, 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Bars are standard deviations around the means, *n* = 4.

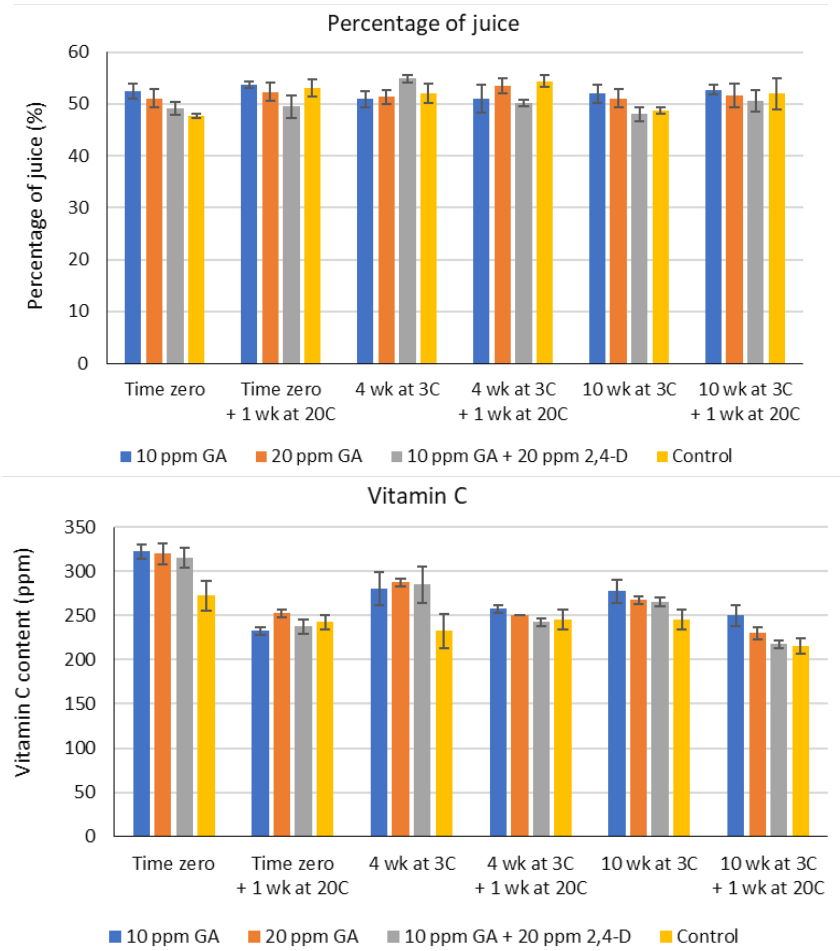


Figure 124. Effect of orchard PGR applications on the percentage of juice (%) (top) and the vitamin C content (ppm) (lower) of Afourer mandarins at the following storage times: time zero, time zero plus 1 week (wk) at 20 °C, 4 weeks at 3 °C, 4 weeks at 3 °C plus 1 week at 20 °C, 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Bars are standard deviations around the means, *n* = 4.

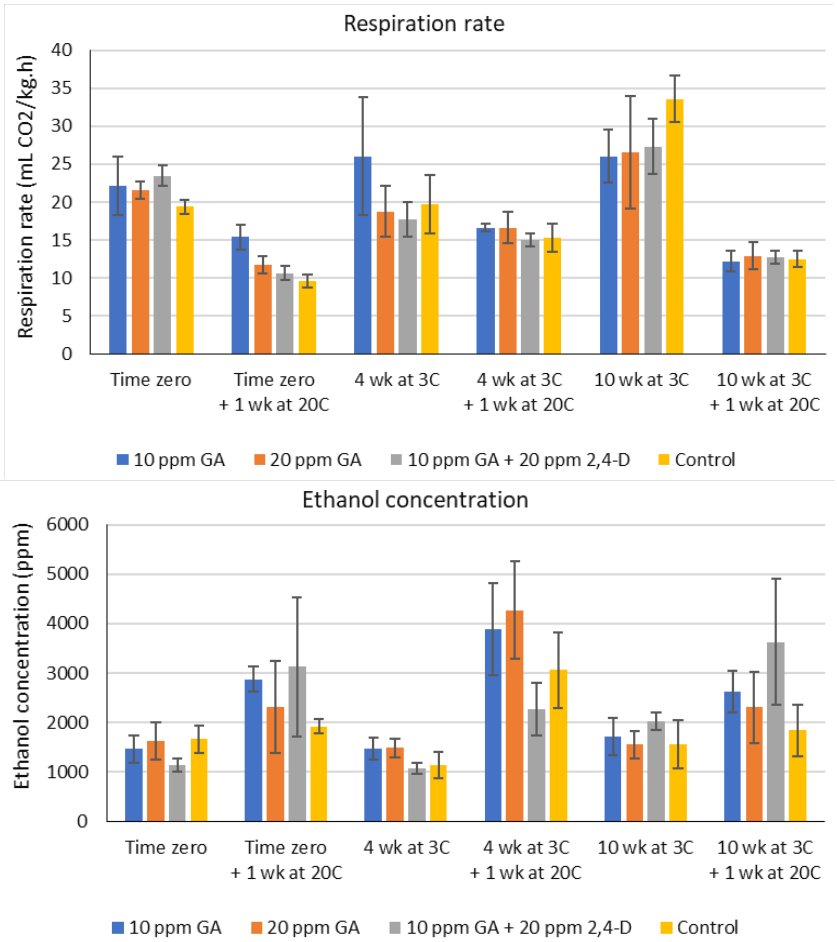


Figure 125. Effect of orchard PGR applications on the respiration rate (mL CO_2/kg.h) (top) and the ethanol concentration (ppm) (lower) of Afourer mandarins at the following storage times: time zero, time zero plus 1 week (wk) at 20 °C, 4 weeks at 3 °C, 4 weeks at 3 °C plus 1 week at 20 °C, 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Bars are standard deviations around the means, *n* = 4.

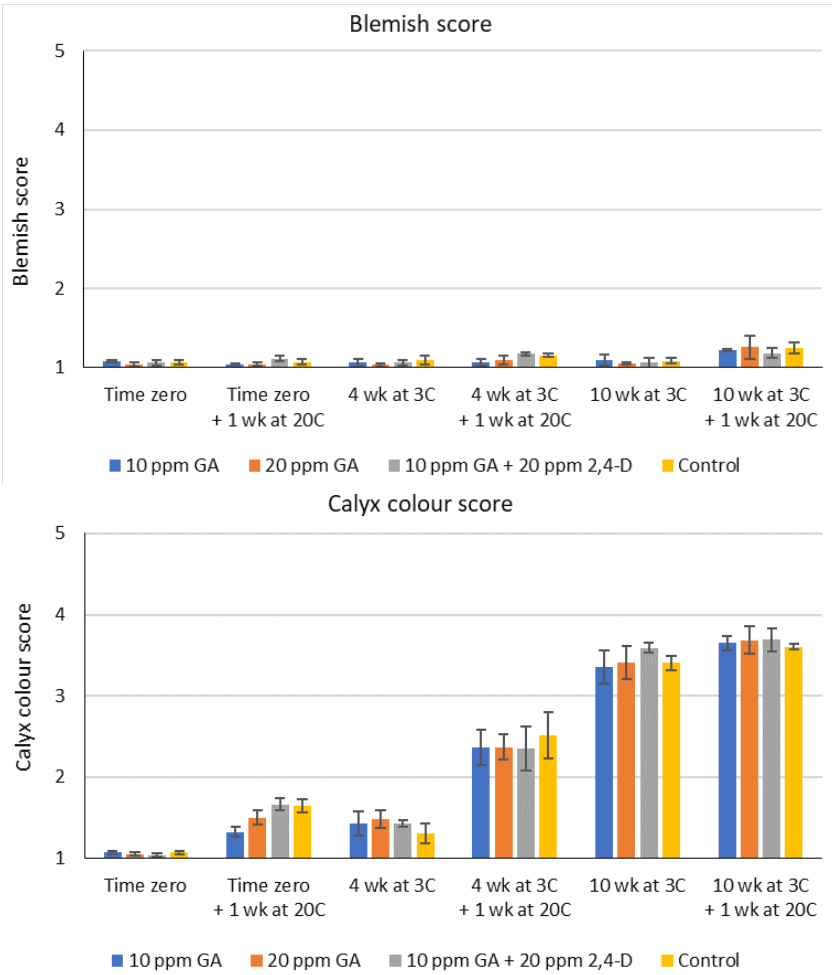


Figure 126. Effect of orchard PGR applications on the blemish score (top) and the calyx colour score (lower) of Afourer mandarins at the following storage times: time zero, time zero plus 1 week (wk) at 20 °C, 4 weeks at 3 °C, 4 weeks at 3 °C plus 1 week at 20 °C, 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Blemish scoring system: 1 = no browning, 2 = trace/some detectable (0–5% of the surface area has browning)–still acceptable, 3 = moderate (5–25% surface area has browning symptoms)–not acceptable, 4 = high levels (35–50% browning) and 5 = very high levels (>50% browning). Calyx colour scoring system: 1 = green–fresh green, 2 = slightly yellow (<25% yellow/brown), 3 = moderately yellow (25–50% brown/yellow), 4 = yellow (50–75% yellow/brown) and 5 = brown (>75% brown). Bars are standard deviations around the means, *n* = 4.

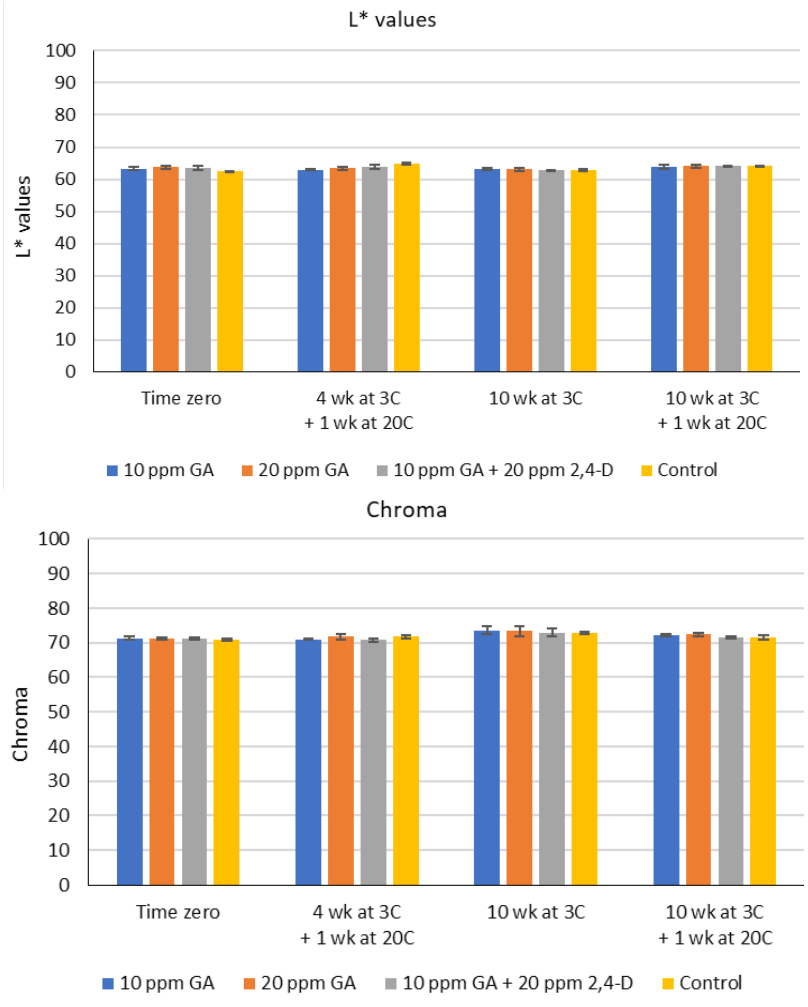


Figure 127. Effect of orchard PGR applications on the L* values (top) and the chroma values (lower) of Afourer mandarins at the following storage times: time zero, 4 weeks at 3 °C plus 1 week at 20 °C, 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Bars are standard deviations around the means, *n* = 4.

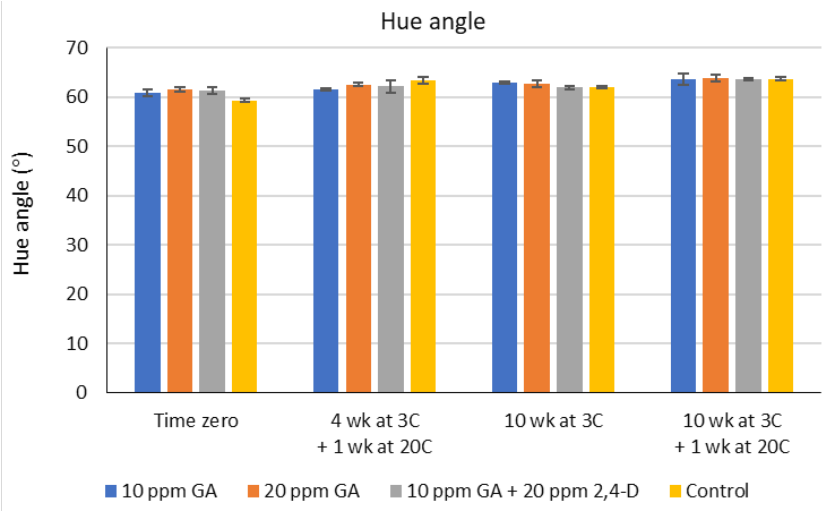


Figure 128. Effect of orchard PGR applications on the Hue angle (°) of Afourer mandarins at the following storage times: time zero, 4 weeks at 3 °C plus 1 week at 20 °C, 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Bars are standard deviations around the means, *n* = 4.

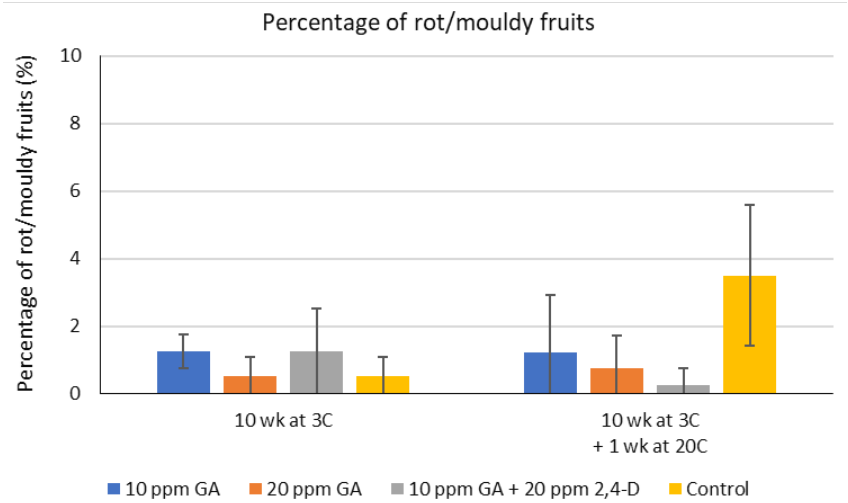


Figure 129. Effect of orchard PGR applications on the percentage of rot/mouldy fruits (%) of Afourer mandarins at 10 weeks at 3 °C and 10 weeks at 3 °C plus 1 week at 20 °C. Bars are standard deviations around the means, *n* = 4.

Effects of postharvest applications of GA on shelf life and quality of Navel oranges

Gibberellic acid (GA) is a naturally occurring plant hormone that promotes the growth and elongation of cells. In the orchard, GA is used to reduce flowering and increase the ratio of leafy to leafless inflorescence. In addition, appropriately timed GA treatments have been shown to reduce rind sensitivity to mechanical damage in citrus (NSW DPI Citrus Plant Protection guide, 2023–24).

Some packers asked if GA has any postharvest application benefits, but there is insufficient data to support its use. In the previous Hort Innovation project, we used 100 ppm GA (ProGibb) on lemons and Navel oranges, but this treatment caused severe phytotoxicity on the peel (CT15010). As GA regulates growth, applications of very low concentrations can have a profound effect, while too much will have the opposite effect. This trial examined the effects of lower rates of GA (1, 10 and 50 ppm GA) as a postharvest dip on the storage life of Navel oranges.

Results

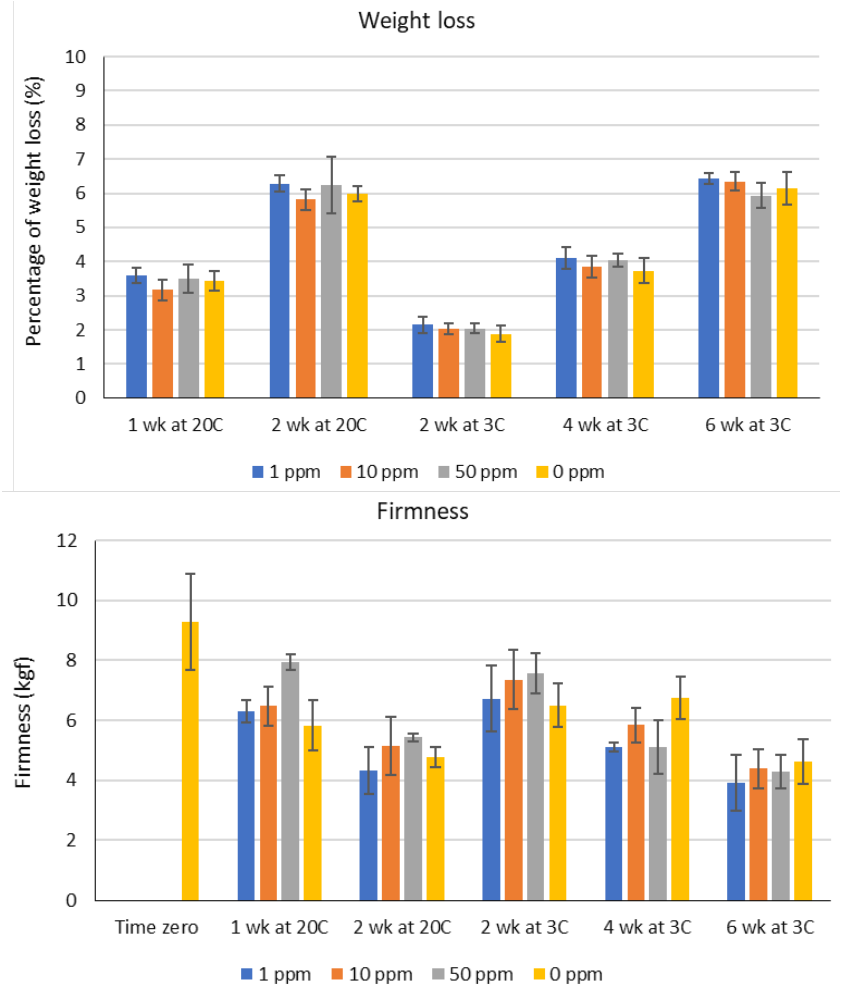


Figure 130. Effect of different concentrations of GA (ppm) on the weight loss (%) (top) and the firmness (kgf) (lower) of Navel oranges at the following storage times: time zero, 1 week at 20 °C, 2 weeks at 20 °C, 2 weeks at 3 °C, 4 weeks at 3 °C and 6 weeks at 3 °C. Bars are standard deviations around the means, *n* = 4.

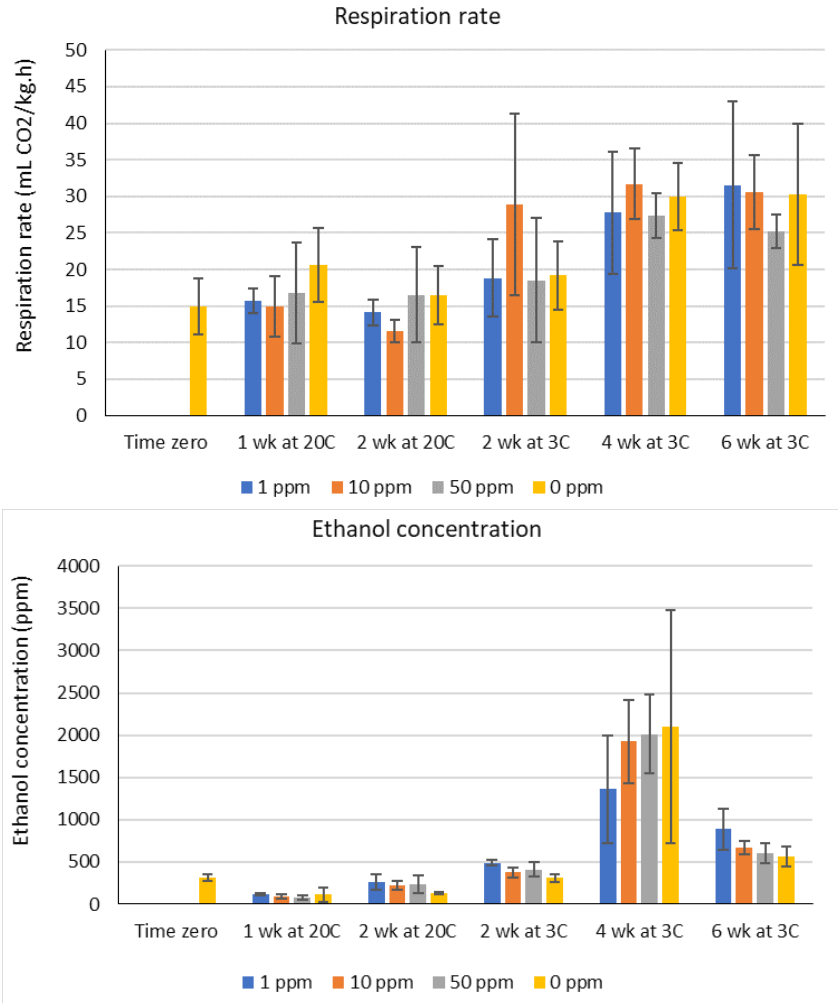


Figure 131. Effect of different concentrations of GA (ppm) on the respiration rate (mL CO_2/kg.h) (top) and the ethanol concentration (ppm) (lower) of Navel oranges at the following storage times: time zero, 1 week at 20 °C, 2 weeks at 20 °C, 2 weeks at 3 °C, 4 weeks at 3 °C and 6 weeks at 3 °C. Bars are standard deviations around the means, *n* = 4.

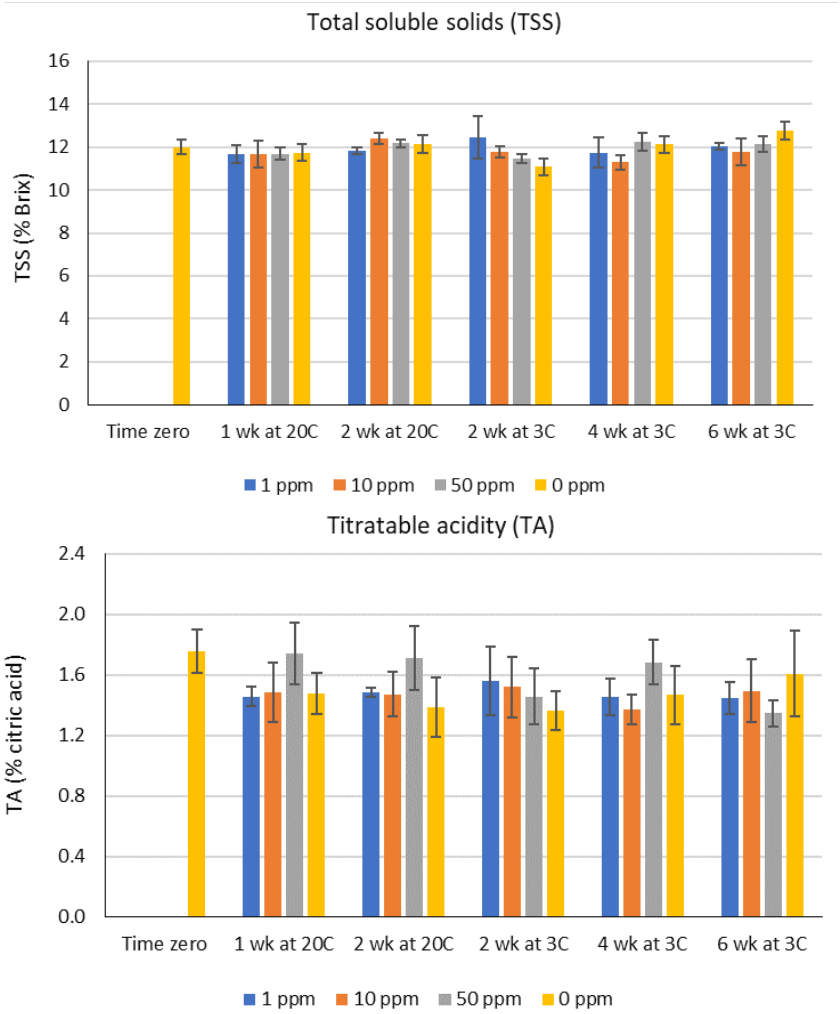


Figure 132. Effect of different concentrations of GA (ppm) on the total soluble solids (% Brix) (top) and the titratable acidity (% citric acid) (lower) of Navel oranges at the following storage times: time zero, 1 week at 20 °C, 2 weeks at 20 °C, 2 weeks at 3 °C, 4 weeks at 3 °C and 6 weeks at 3 °C. Bars are standard deviations around the means, *n* = 4.

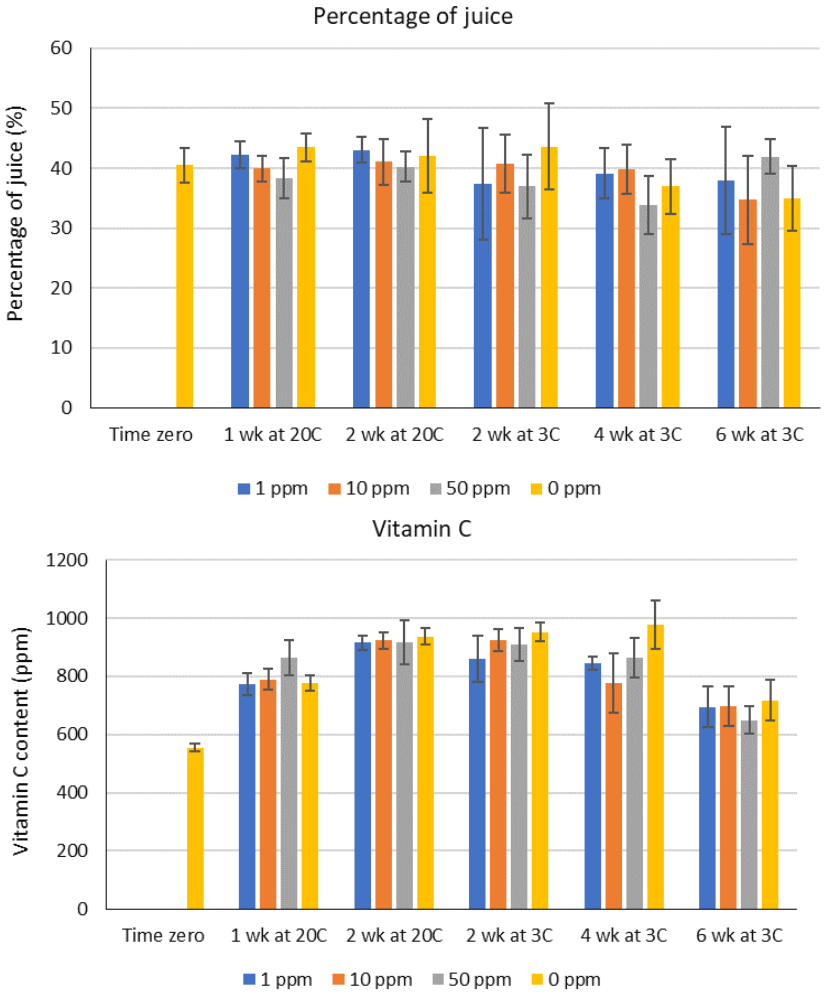


Figure 133. Effect of different concentrations of GA (ppm) on the percentage of juice (%) (top) and the vitamin C content (ppm) (lower) of Navel oranges at the following storage times: time zero, 1 week at 20 °C, 2 weeks at 20 °C, 2 weeks at 3 °C, 4 weeks at 3 °C and 6 weeks at 3 °C. Bars are standard deviations around the means, *n* = 4.

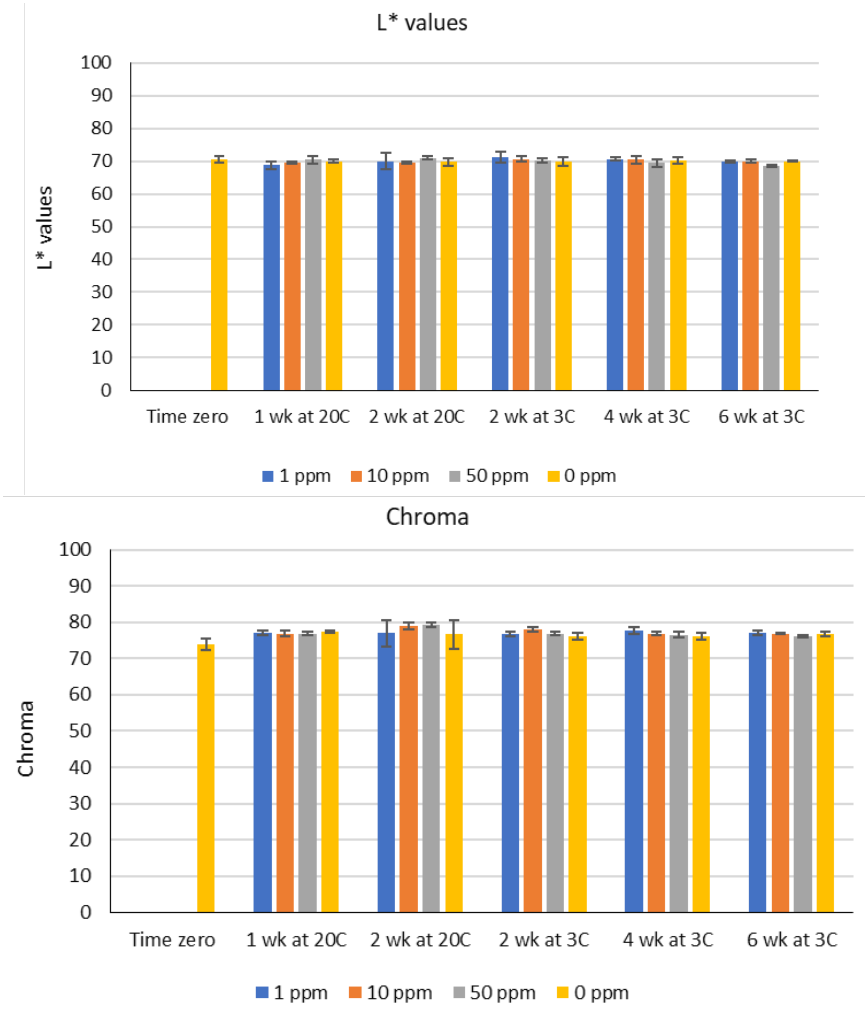


Figure 134. Effect of different concentrations of GA (ppm) on the L* values (top) and the chroma values (lower) of Navel oranges at the following storage times: time zero, 1 week at 20 °C, 2 weeks at 20 °C, 2 weeks at 3 °C, 4 weeks at 3 °C and 6 weeks at 3 °C. Bars are standard deviations around the means, *n* = 4.

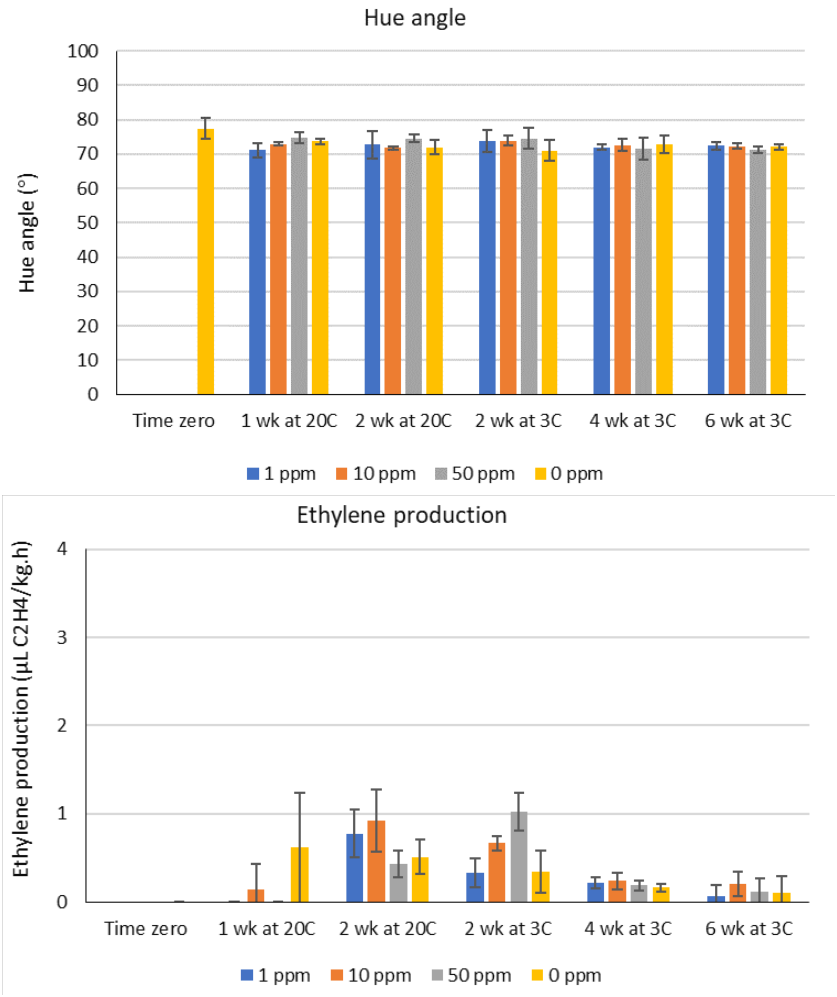


Figure 135. Effect of different concentrations of GA (ppm) on the Hue angle (°) (top) and the ethylene production (µL C_2H_4/kg.h) (lower) of Navel oranges at the following storage times: time zero, 1 week at 20 °C, 2 weeks at 20 °C, 2 weeks at 3 °C, 4 weeks at 3 °C and 6 weeks at 3 °C. Bars are standard deviations around the means, *n* = 4.

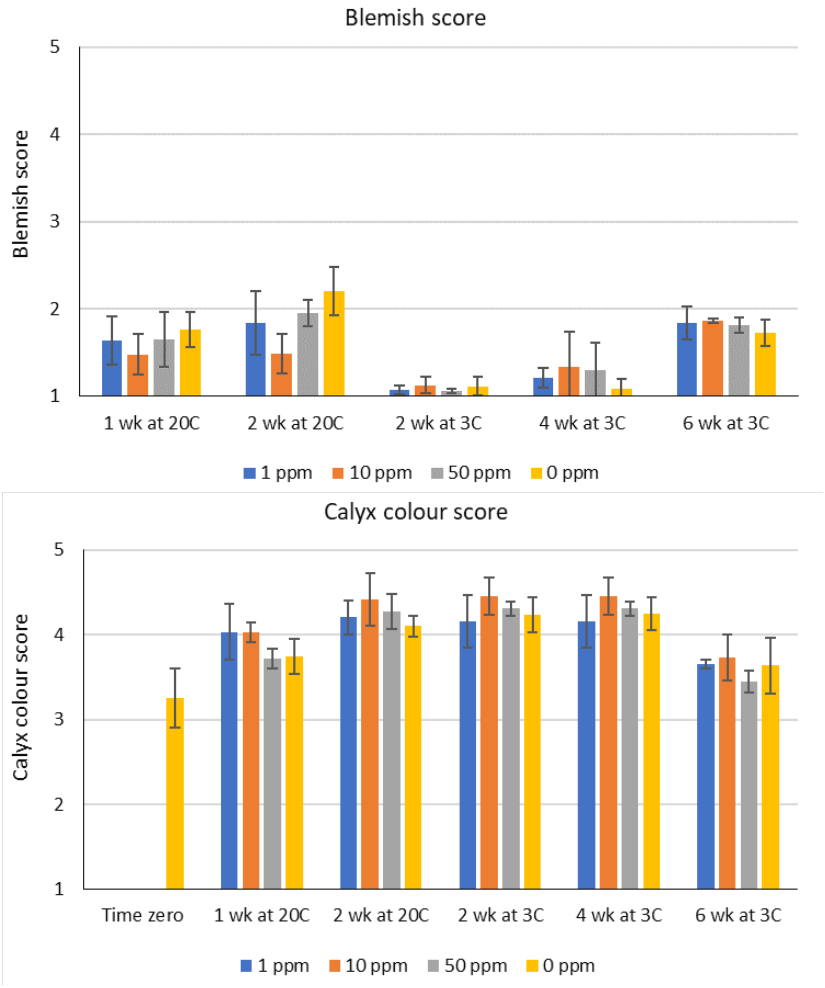


Figure 136. Effect of different concentrations of GA (ppm) on the blemish score (top) and the calyx colour score (lower) of Navel oranges at the following storage times: time zero, 1 week at 20 °C, 2 weeks at 20 °C, 2 weeks at 3 °C, 4 weeks at 3 °C and 6 weeks at 3 °C. Blemish scoring system: 1 = no browning, 2 = trace/some detectable (0–5% of the surface area has browning)–still acceptable, 3 = moderate (5–25% surface area has browning symptoms)–not acceptable, 4 = high levels (35–50% browning) and 5 = very high levels (>50% browning). Calyx colour scoring system: 1 = green–fresh green, 2 = slightly yellow (<25% yellow/brown), 3 = moderately yellow (25–50% brown/yellow), 4 = yellow (50–75% yellow/brown) and 5 = brown (>75% brown). Bars are standard deviations around the means, *n* = 4.

Improving the storage performance and eating quality of Afourer mandarins during extended shipping

Current delays in shipping times, extended storage, and breaks in the cold chain are challenging the final eating fruit quality of Australian mandarins in distant export markets. We have previously shown that lowering atmospheric ethylene levels at reduced storage temperatures maintains fruit quality during long-term storage of Afourer mandarins. Ethylene is commonly used to degreen mandarins and its effects on the long-term storage of citrus are minimal. However, we have shown the continuous application of very low levels of ethylene was detrimental to the storage life of mandarins (Li et al. 2018), where the storage of fruit at the lowest ethylene levels possible resulted in the best quality fruit, although chilling injury may be increased. This trial will assess 2 different techniques for managing ethylene in storage:

1. Physically removing the ethylene from the storage environment with potassium permanganate scrubbers. This is a passive system where the potassium permanganate in a sachet oxidises ethylene (and all volatile organics).
2. Preventing the fruit from reacting to ethylene with the use of 1-methylcyclopropene (MCP). MCP works by stopping the action of ethylene in the fruit.

Methods

Afourer mandarins (export grade, waxed and export grade and export × 10 kg boxes, count 113) were harvested and packed on 29.9.21. These were sent to NSW Department of Primary Industries via the Sydney markets.

Upon arrival, the fruit were allocated to one of 4 treatments (Figure 137):

1. pre-storage MCP treatment–as per commercial standard (MCP)
2. potassium permanganate (PP) sachet (one sachet per box)
3. combination of MCP plus potassium permanganate sachet (MCP PP)
4. untreated control (no MPC or sachet) (UTC).

Fruit were stored at 3 °C and boxes were removed at 3-week intervals, i.e. removals at 3, 6, 9, 12 and 15 weeks (= total 105 days) storage. At each removal time, the fruit were assessed when the fruit had warmed to 20 °C (i.e. upon removal) and after an additional shelf life for 1 week at 20 °C. Each treatment was replicated 4 times and each treatment unit was one box of mandarins. All fruit were stored in the cool room together in their treatment groups to prevent any potential cross-contamination of ethylene between treatments. All removals from the same treatment were stored in the same over-wrapped plastic bag with each treatment replicated and stored separately at 3 °C for up to 15 weeks.

Figure 137. Treating Afourer mandarins in boxes with 1-MCP gas in a sealed tent before long-term storage (left) and with the addition of a potassium permanganate (PP) sachet within the treatment box (right).

At each assessment time and after an additional 7 days at 20 °C, weight loss, fruit respiration rates, ethylene production rates, fruit firmness, the levels of chilling injury, rots, button condition, and rind disorders were assessed (Figure 138). In addition, the fruit were juiced and the TSS, TA, vitamin C content and juice ethanol content were measured (as off-flavours). All methods are described in the General Methods section.

Figure 138. Setting up the storage trial and measuring fruit quality (ethylene, respiration rate and weight loss) during storage at NSW Department of Primary Industries.

Results

The trial was composed of 2 post-treatment conditions:

Part 1. Storage at 3 °C for up to 15 weeks with an additional shelf life of 1 week at 20 °C (long-term storage)

Part 2. Shelf life only at the beginning of the experiment (storage at 20 °C for 6 weeks, with no cold storage)

Part 1. Fruit quality after long-term storage

The effect of the different postharvest treatments and storage time on the quality of Afourer mandarins stored for up to 15 weeks are presented in Figures 139–152. Each of the quality parameters was assessed immediately upon removal from cold storage (when the fruit had warmed up) and again after an additional one week at 20 °C as a simulation of shelf life.

Objective measures of fruit quality

Weight loss. The effect of different postharvest treatments on the percentage weight loss of mandarins during storage is shown in Figure 139. The boxes of fruit were re-weighed every 3 weeks (left), and the results show that the untreated control fruit lost more weight than the other treatments, up to 12 weeks of storage. In addition, 10 fruit samples were stored in netted bags within the treatment boxes and re-weighed every 3 weeks (Figure 139). These results show there were no differences between the treatments (except at the final removal and shelf life at 20 °C in potassium permanganate-treated fruit, which had higher weight loss).

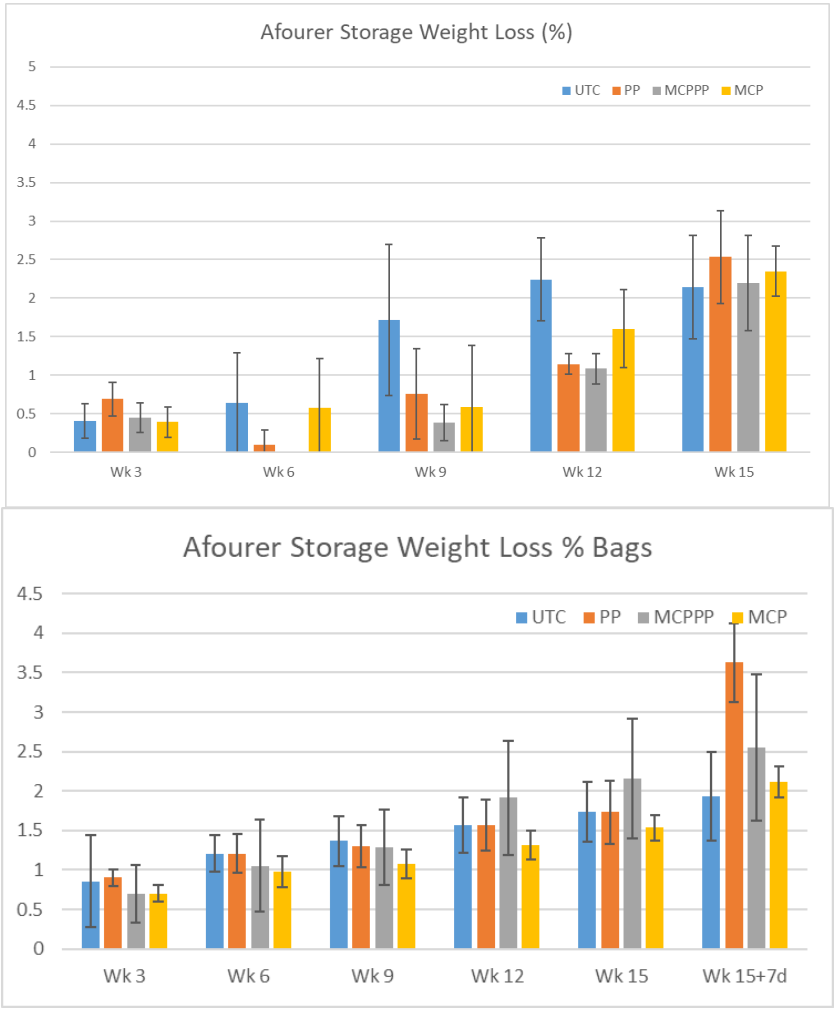


Figure 139. Average weight loss (%) of an entire box of Afourer mandarins (top) and average weight loss of 10 fruit per box (lower) stored at 3 °C for up to 15 weeks and an additional one-week shelf life at 20 °C. *n* = 4 boxes/netted bags (10 fruit per bag).

TSS. The effect of the different postharvest treatments on TSS levels immediately upon removal from cold storage and after 1 week of shelf life is presented in Figure 140. It shows the decline in TSS from upon removal to shelf life. In general, there was not a great decrease in TSS over the 15-week storage time. There were no great differences in the TSS levels between the different treatments over the storage time, however, the combined MCP + PP tended to have lower TSS than the other treatments.

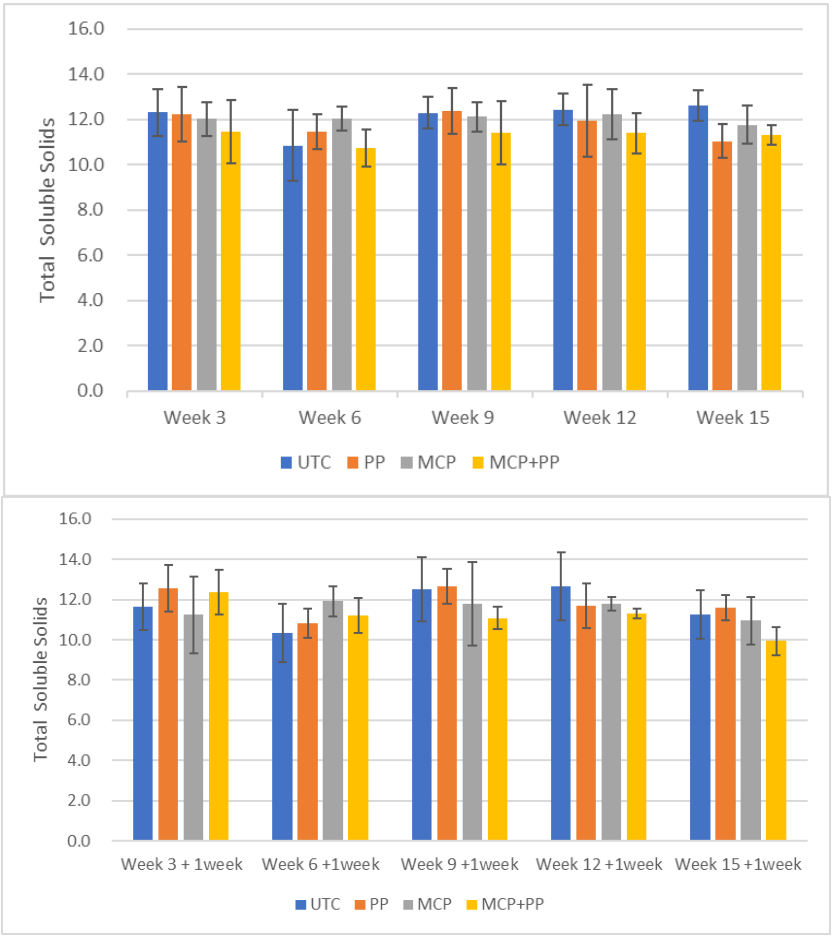


Figure 140. Average total soluble solids (TSS) content in Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower).

TA. The effect of the different storage times and treatments on fruit TA is presented in Figure 141, which shows there was some decline in TA during storage in all treatments. There were no consistent treatment effects on the levels of TA during storage.

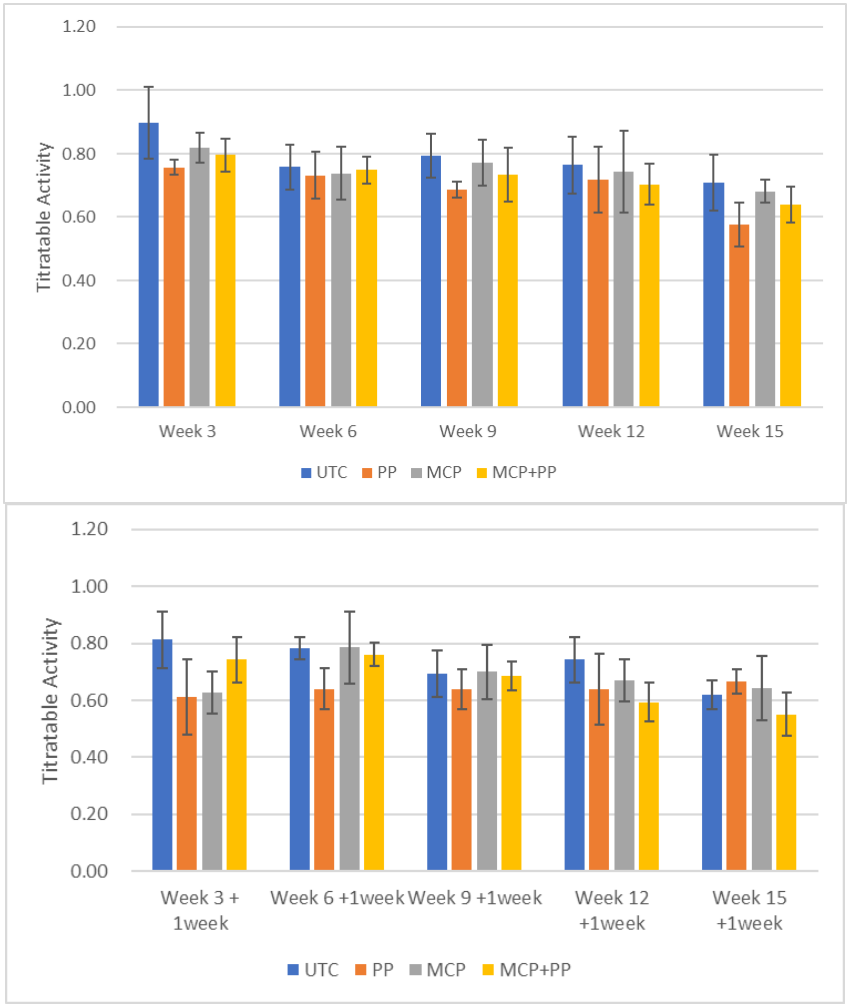


Figure 141. Average titratable acidity (TA, % citric acid) in Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower).

Vitamin C content. The effect of the different treatments on the vitamin C content is presented in Figure 142.

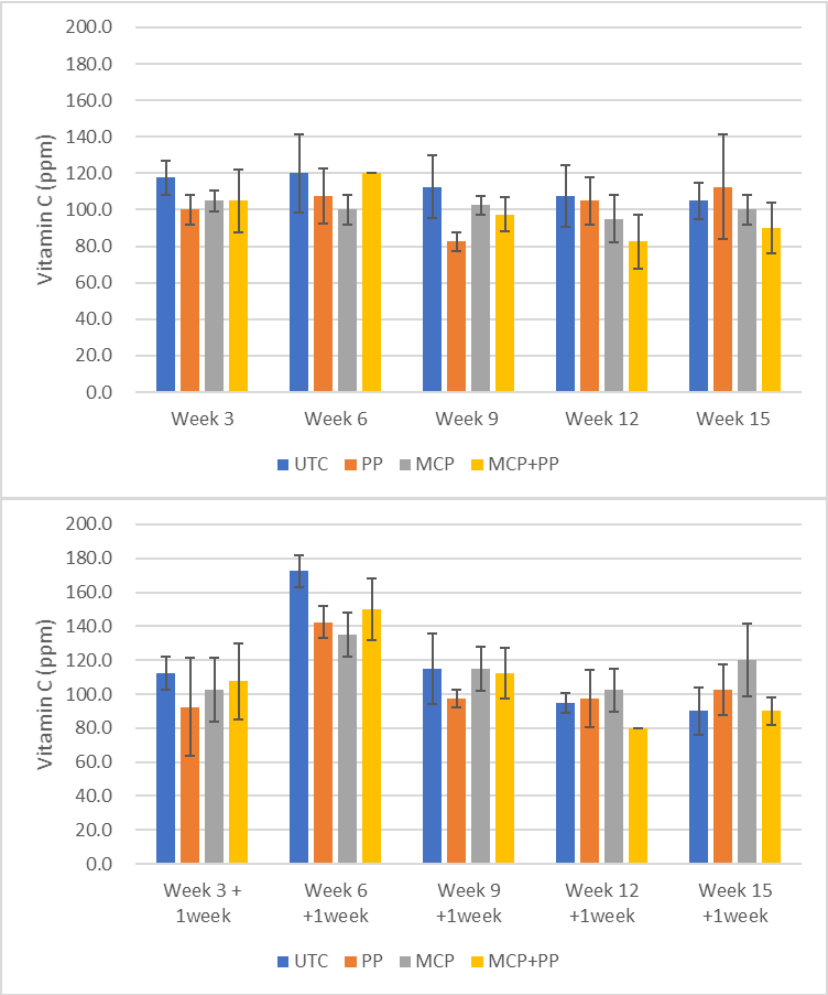


Figure 142. The average level of vitamin C (ppm) in Afourer mandarins when stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower).

Ethanol content. The levels of ethanol in the headspace of the juice of Afourer mandarins during storage are presented in Figure 143. Ethanol is a fermentation product and is associated with off-flavours during storage. The results showed all postharvest treatments tended to have higher levels of ethanol in the juice, but this was not statistically significant.

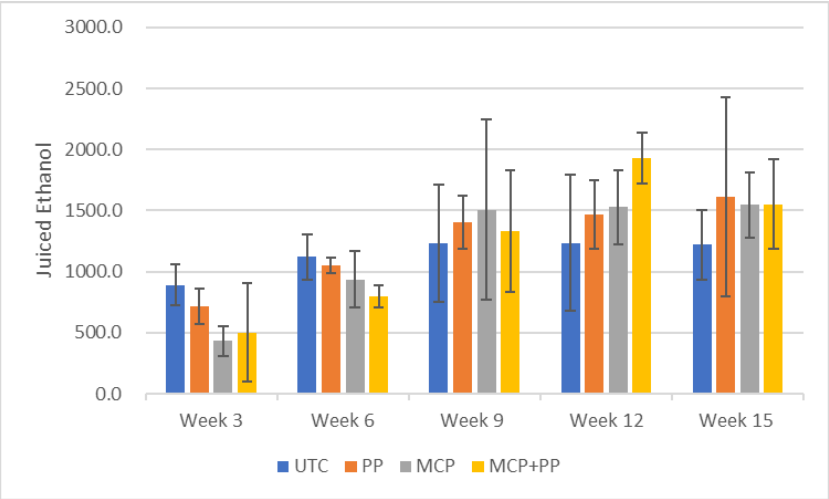


Figure 143. The average concentration of ethanol (ppm) in the headspace of the juice of Afourer mandarins stored at 3 °C for up to 15 weeks.

Atmospheric ethylene and CO_2 concentrations: the levels of ethylene (ppm) and CO_2 (%)in the boxes of Afourer mandarins at 6 and 12 weeks during storage at 3 °C are presented in Table 6. The levels of ethylene were very low. There was no difference between all postharvest treatments in both ethylene concentration and CO_2 levels within the boxes at these sampling times.

Table 6. Average levels of atmospheric ethylene (ppm) and CO_2 content (%) within the box of Afourer mandarins at 3 °C (*n*= 4 boxes).

	Ethylene (ppm)			CO_2 levels (%)	
	6 weeks storage	12 weeks storage		6 weeks storage	12 weeks storage
UTC	0.0025 ± 0.50	0.0075 ± 0.50		0.08 ± 0.10	0.03 ± 0.05
PP	0.0050 ± 1.00	0.0075 ± 0.50		0.70 ± 0.69	0.00 ± 0.08
MCP	0.0000 ± 0.00	0.0100 ± 0.00		0.18 ± 0.17	0.03 ± 0.05
MCP + PP	0.0000 ± 0.00	0.0075 ± 0.50		0.30 ± 0.12	0.08 ± 0.01

Fruit respiration rate. The fruit respiration rate is a measure of the metabolism of the fruit. The results presented in Figure 144 show no consistent effect of the different postharvest treatments upon removal and after 7 days shelf life, but the respiration rate declined with the additional shelf life.

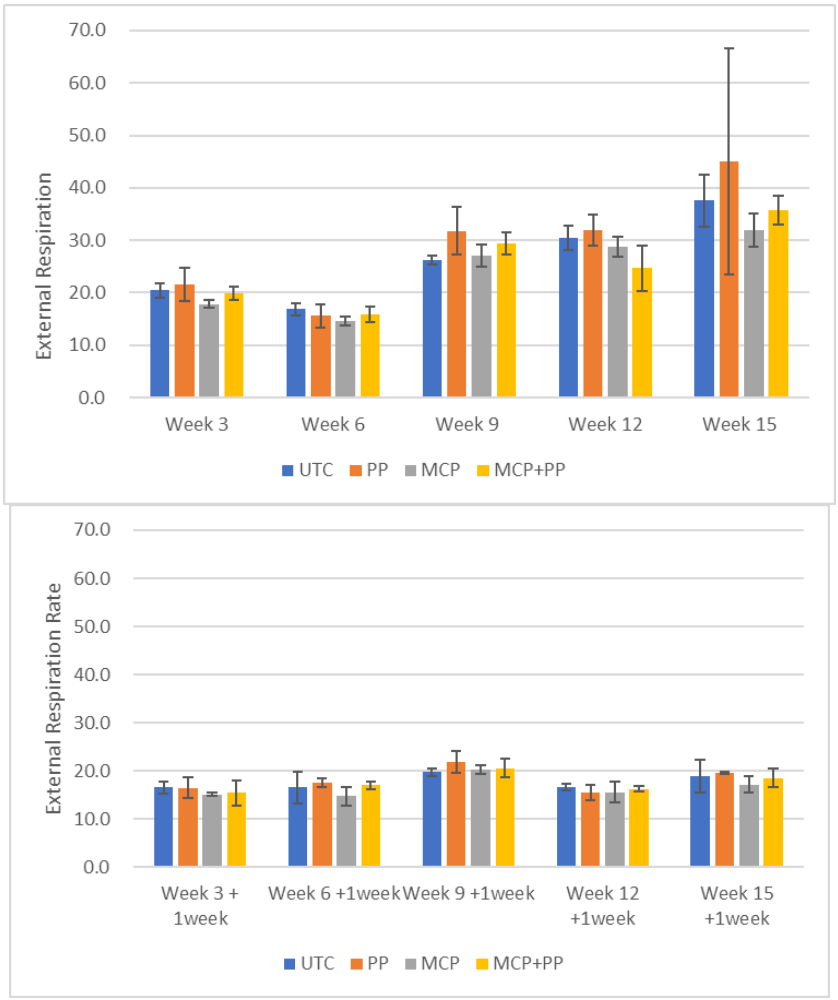


Figure 144. Average respiration rate of Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower).

Fruit ethylene production rate. The rate of ethylene production in the stored mandarins was low and inconsistent.

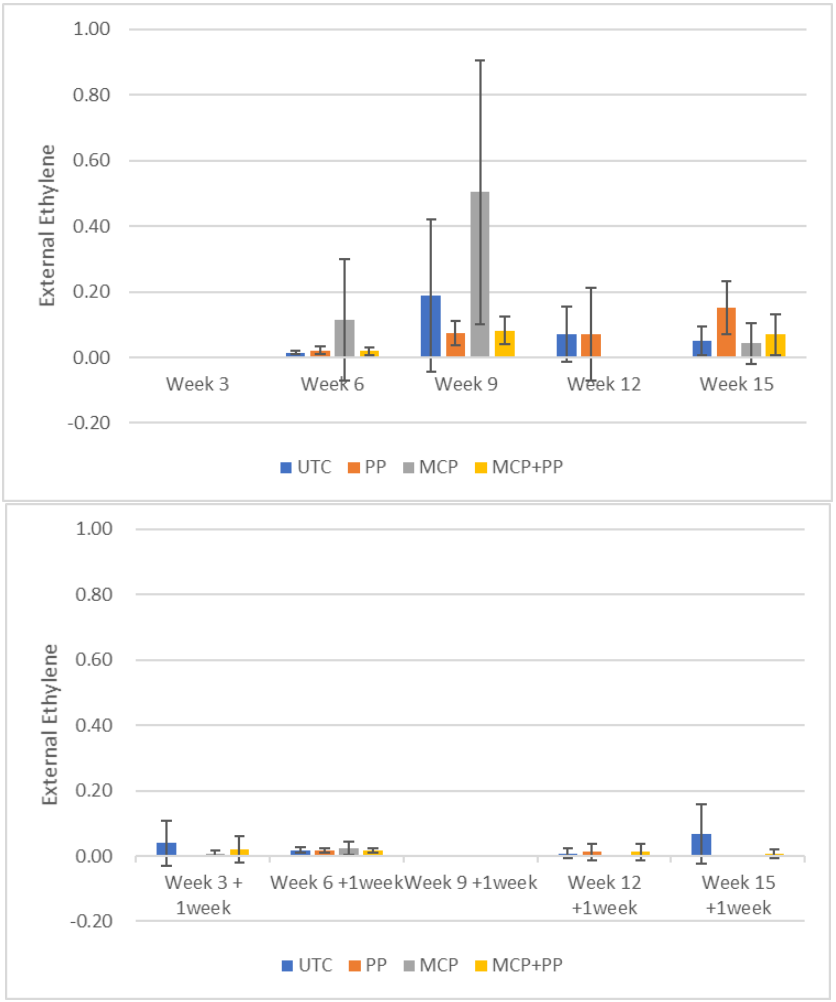


Figure 145. The average ethylene production rate of Afourer mandarins that were stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower).

Subjective measures of fruit quality

Overall subjective fruit quality. The effect of the different postharvest treatments on overall acceptability during the 15-week storage period with the addition of a shelf life assessment 7 days after removal is presented in Figure 146. The results show the Afourer mandarins maintained their quality during long-term storage. Even after 15 weeks at 3 °C and an additional 1-week shelf life, the fruit were still commercially acceptable (above score 3).

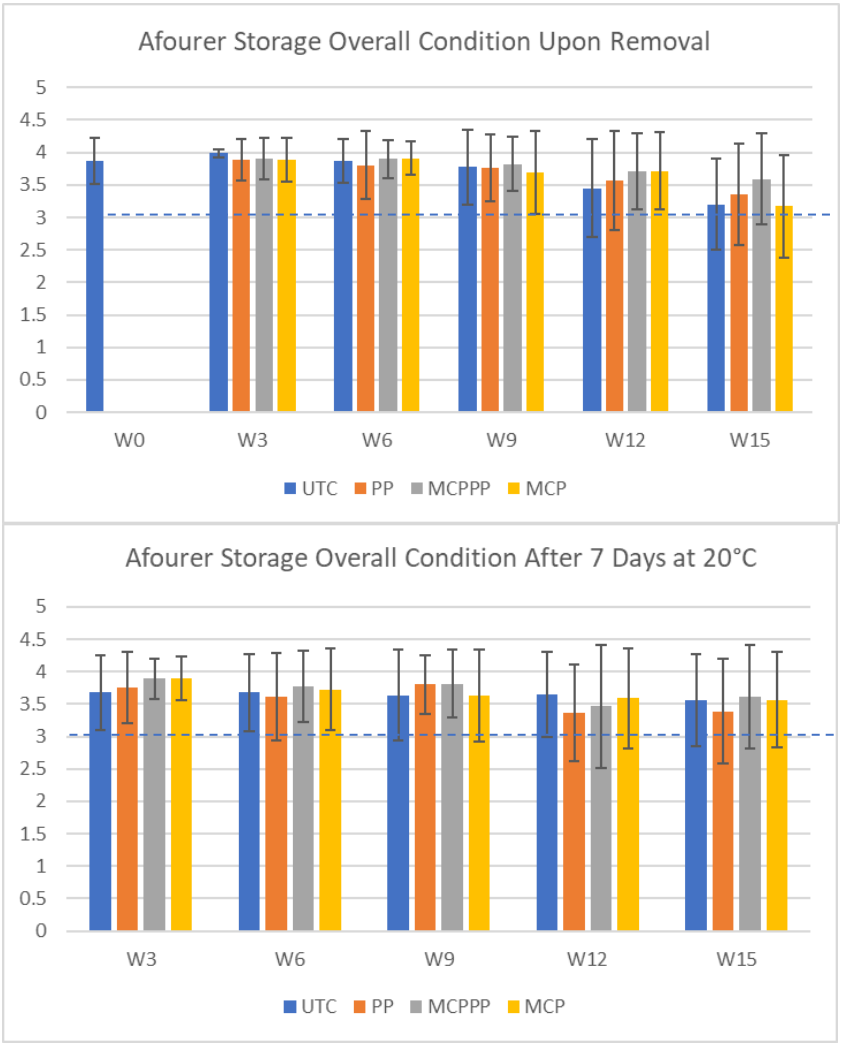


Figure 146. Average overall fruit quality acceptability score of Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower). Subjective fruit scores were 5–excellent condition firm, good colour, and gloss, 4–good quality, 3–acceptable quality–purchase OK, 2–unacceptable, soft, 1 unacceptable, very soft.

Calyx (button) condition. The effect of the different postharvest treatments on the condition of the calyx/button is shown in Figure 147. The results show that, in general, the postharvest treatments maintained the condition of the calyx better than the untreated fruit.

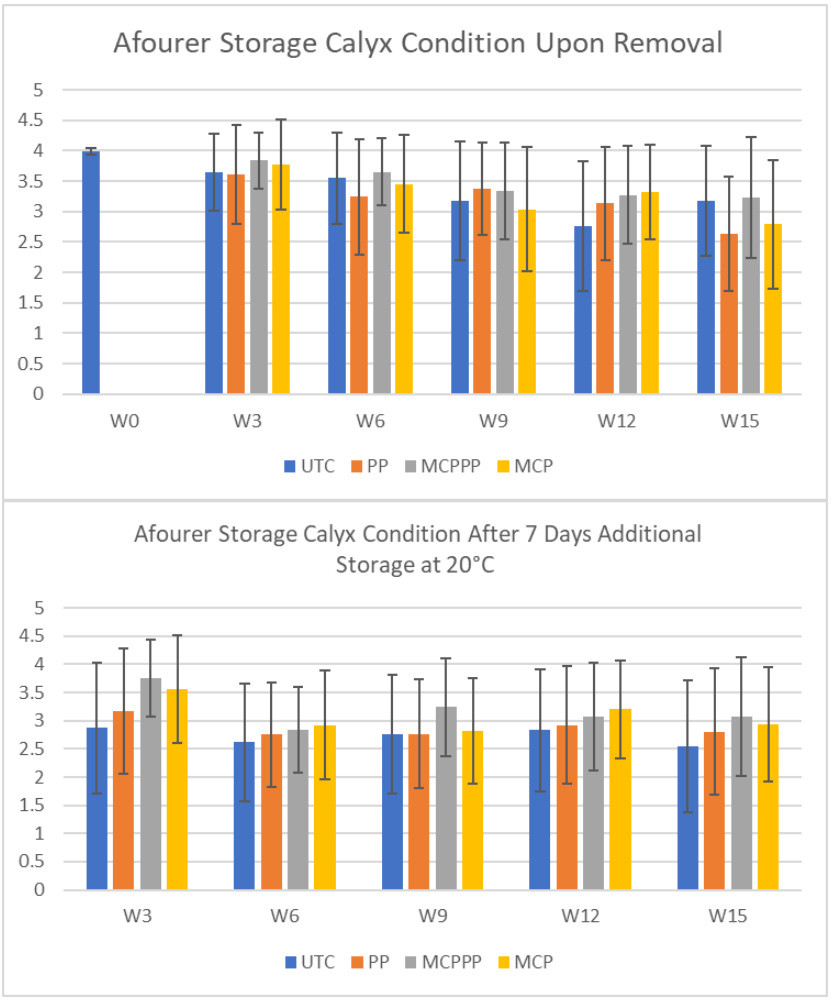


Figure 147. The average calyx condition score of Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower). Subjective fruit scores: 5 = no browning; 4 = <25% brown; 3 = 25–50% browning; 2 = 50–75% browning; and 1 = >75% browning.

In addition to the 3-weekly removals of the assessment of calyx condition during the storage experiment (data presented in Figure 147), the condition and number of all calyxes were counted and assessed in each box at the end of the storage trial (15 weeks at 3 °C and one week at 20 °C). These results (Figure 148) show that at the end of the experiment, fruit treated with MCP had greener calyxes and greater retention of the calyx onto the fruit.

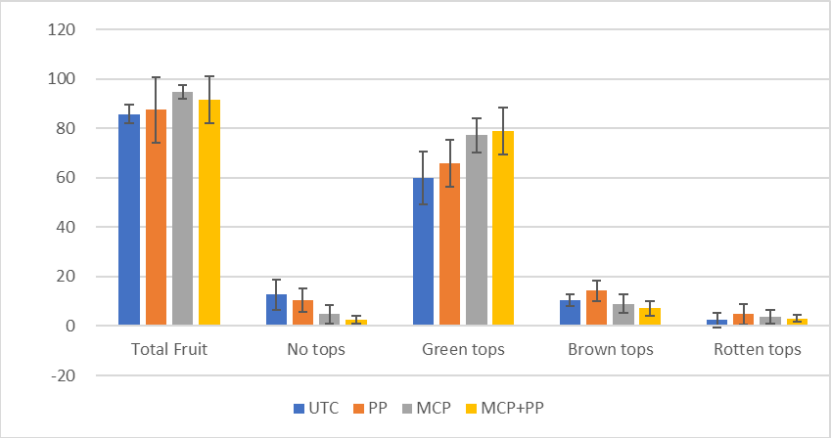


Figure 148. Total number and condition of the fruit calyx (button) on Afourer mandarins in each box stored at 3 °C 15 weeks and after an additional one-week shelf life at 20 °C.

Fruit firmness. Fruit firmness was objectively measured with a texture analyser (Figure 149) and subjectively assessed by hand and scored with a firmness scale (Figure 150). The fruit maintained its firmness during storage. In general, there were no differences between treatments, but MCP-treated fruit tended to retain high firmness levels.

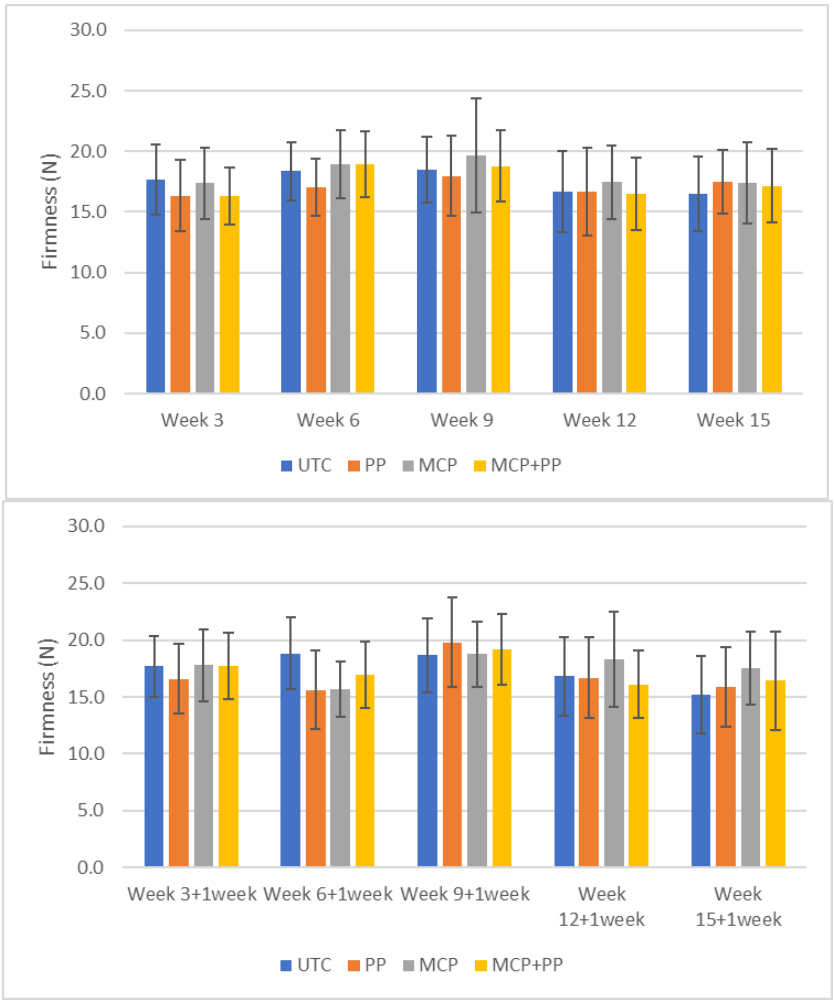


Figure 149. Average fruit firmness (Hardness, Newtons) of Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower).

The subjective assessment of fruit firmness as assessed by hand feel on Afourer mandarins during storage and shelf life is presented in Figure 150. There were relatively minor changes and only small differences were detected between the treatments.

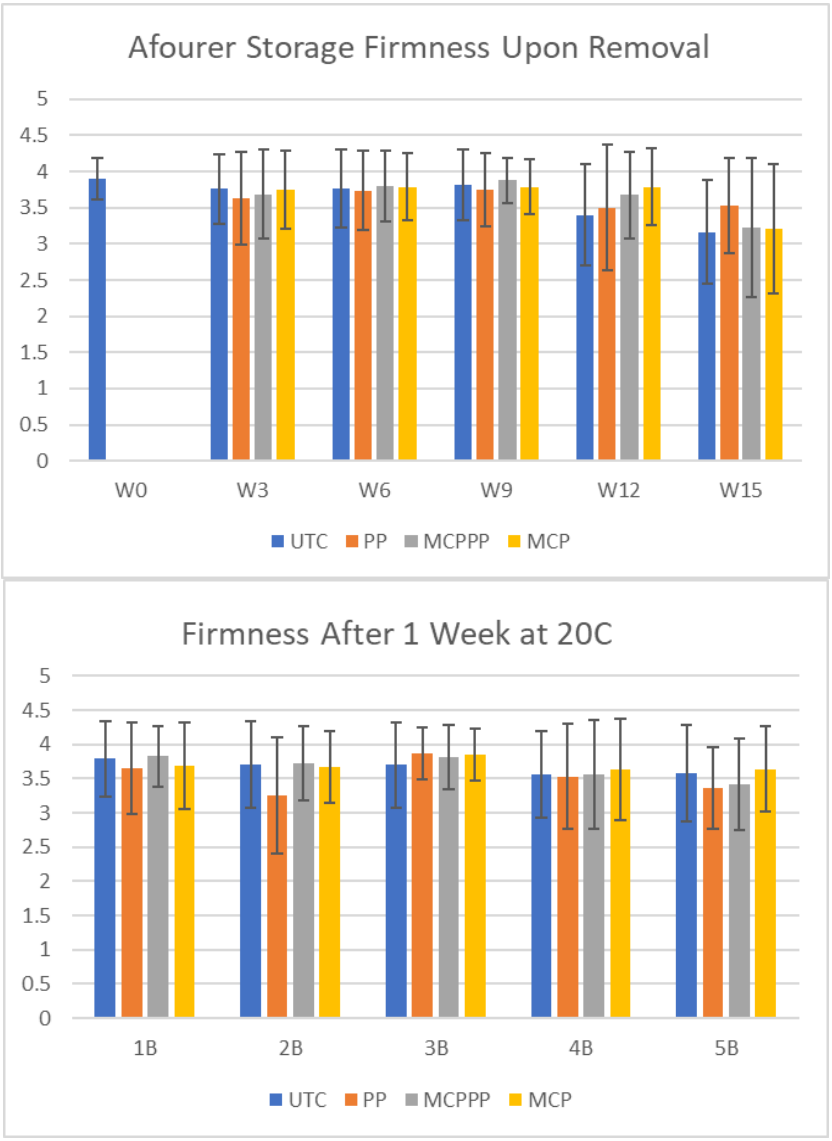


Figure 150. Average subjective fruit firmness score of Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower). Subjective fruit scores: 5 = very firm/hard; 4 = firm; 3 = acceptable to purchase–OK; 2 = soft and unacceptable; 1 = unacceptable–very soft.

Skin blemish. The effects of the different postharvest storage treatments on the levels of skin blemish are presented in Figure 151. There were relatively low levels of superficial skin blemish, which did not increase during storage. This shows no chilling injury symptoms were observed in this storage experiment, even though the fruit were stored for 15 weeks at 3 °C.

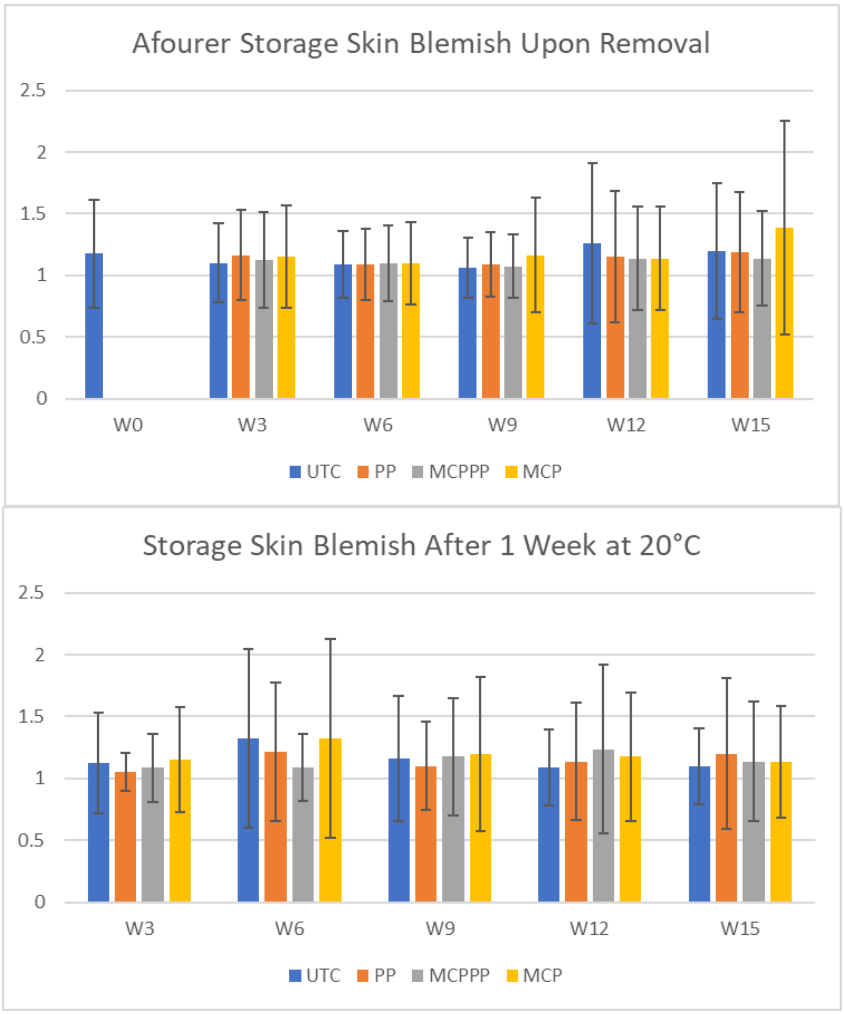


Figure 151. Average subjective level of skin blemish on Afourer mandarins stored at 3 °C for up to 15 weeks immediately upon removal (top) and after an additional one-week shelf life at 20 °C (lower). Subjective fruit scores: 1 = no pitting; 2 = a few scattered pits–just one or 2 pits (<5% of the fruit surface)–still acceptable; 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.

Postharvest rots. The levels of postharvest rots are presented in Figure 152. There were no rots detected for the first 6 weeks of storage. Even after 15 weeks of storage, the rot levels were low, indicating the postharvest fungicide and management were satisfactory.

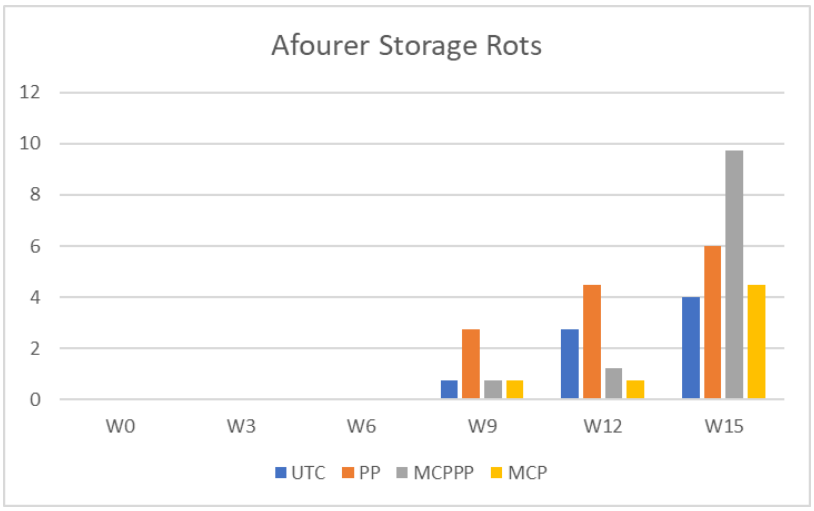


Figure 152. The average number of postharvest rots in each box of Afourer mandarins (count 113) stored at 3 °C for up to 15 weeks immediately upon removal.

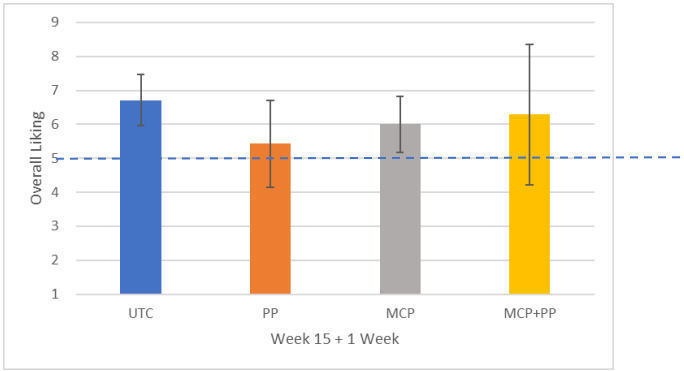
Informal taste tests

After 15 weeks of storage at 3 °C and one-week shelf life at 20 °C, an informal taste testing was conducted with staff at NSW Department of Primary Industries. Due to Covid restrictions at the time, only 7 assessors were available to taste the stored mandarins. All assessments were done in a random order, with the assessors not knowing the treatments. Two mandarins were presented to each assessor. The subjective taste quality parameters assessed were firmness, appearance, sweetness, sourness, aroma, flavour, and overall liking.

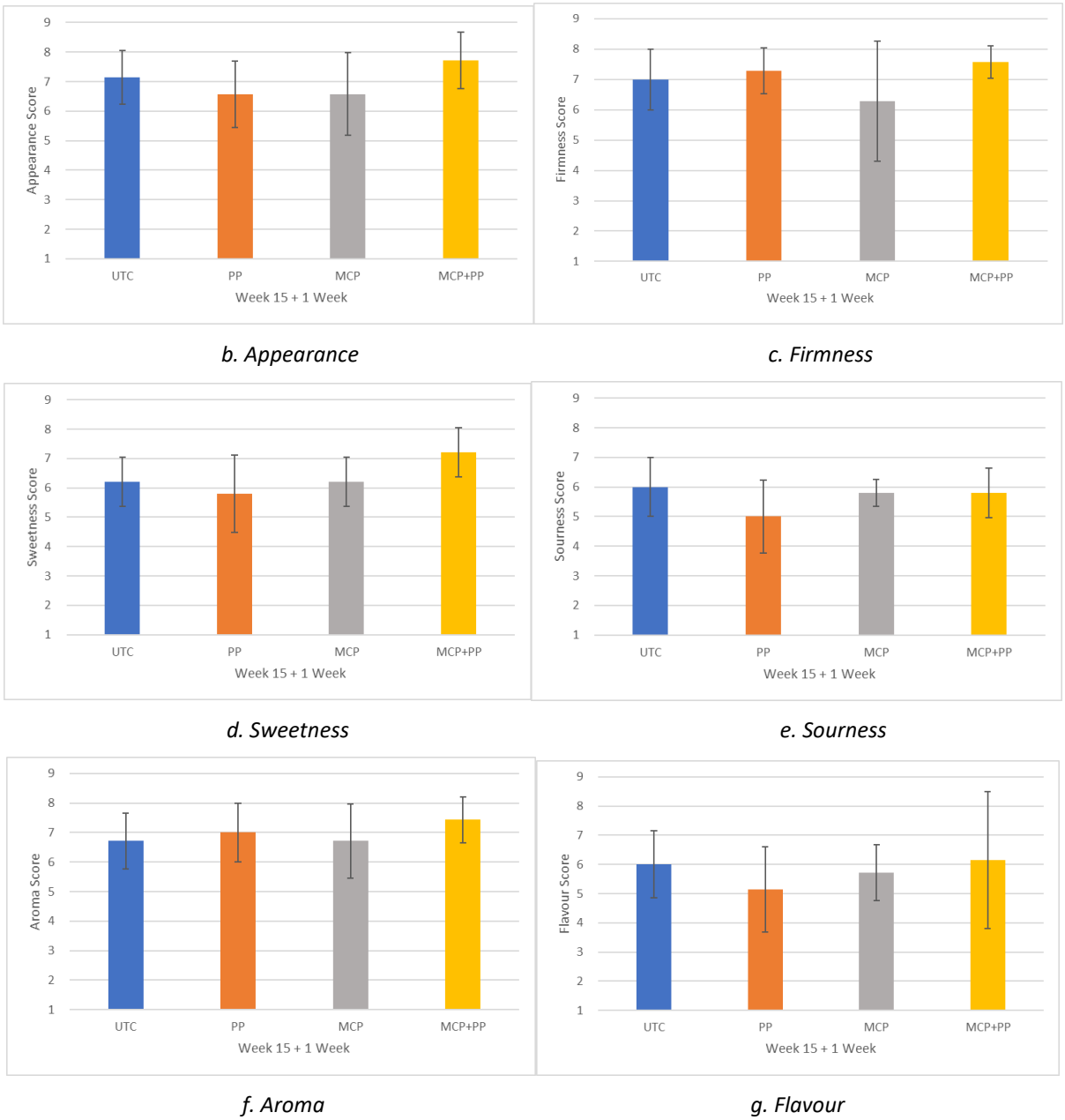
Results

The results are presented in Figure 153 with each of the subjective quality assessments presented. The results show that there were few differences between the treatments after 15 weeks of storage at 3 °C and one week of shelf life at 20 °C. All treatments were acceptable (i.e., above score 5) for overall liking, and there was an overlap in treatment effects. Similar observations were made for the other quality parameters, such as firmness, appearance, sweetness, sourness, aroma, and flavour.

There appeared to be some variability between the different fruit from the same batch / lot. This was examined in a small side study.



a. Overall 'liking'



b. Appearance *c. Firmness*

d. Sweetness *e. Sourness*

f. Aroma *g. Flavour*

Figure 153. Effect of postharvest treatment on the subjective assessment of Afourer mandarins stored for 15 weeks storage at 3 °C and one-week shelf life at 20 °C. The subjective taste quality parameters assessed were: a. overall liking, b. firmness, c. appearance, d. sweetness, e. sourness, f. aroma and g. flavour. The scoring rating system was 9 = excellent, 5 neutral and 1 = horrible. The presented data are means ± standard deviations. **Note: only limited taste testing panellists*.

Conclusions

Fruit could be stored for 15 weeks at 3 °C and there were no consistent differences between the different postharvest treatments and the untreated control fruit. No chilling injury was detected in the experiment. There was no consistent benefit of applying MCP or the potassium permanganate sachet to the overall quality. However, some individual quality components were improved by postharvest treatment. For example, the condition of the calyx (button) was maintained during long-term storage with the application of MCP. The taste testing results after 15 weeks of storage showed that all treatments resulted in acceptable fruit and there was no clear benefit of any postharvest treatment; however, the results

were variable. This apparent variability was followed up in the next section.

Limitations of the inferences that can be drawn from this storage trial include:

- This was a small-scale laboratory trial that tried to simulate commercial conditions.
- The treatment unit for each treatment was a single box that was replicated 4 times.
- All boxes of the same treatment and replicate were stored together in an over-wrapped plastic bag to prevent cross-contamination of ethylene and CO_2. However, these treatments do not simulate the conditions within a full commercial 40-foot shipping container of fruit. This laboratory trial will have different storage atmosphere conditions and shelf life conditions, i.e. potentially lower atmospheric ethylene and CO_2 within the box.

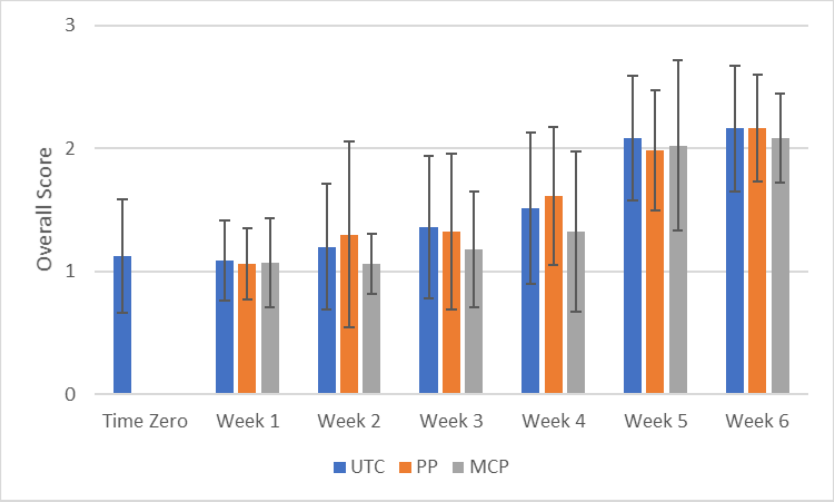
Part 2. Fruit quality after just shelf life at 20 °C (i.e. without any storage at 3 °C)

Immediately after treatment, another sample of fruit was continuously held at 20 °C for 6 weeks to examine the effects of the different postharvest treatments on fruit quality. This component is just a sub-component of the main storage experiment to see what happened during extended shelf life at 20 °C. This was conducted with the remaining 'spare' fruit. Note: there was not enough fruit to assess the potassium permanganate sachet (PP) and MCP treatment combination.

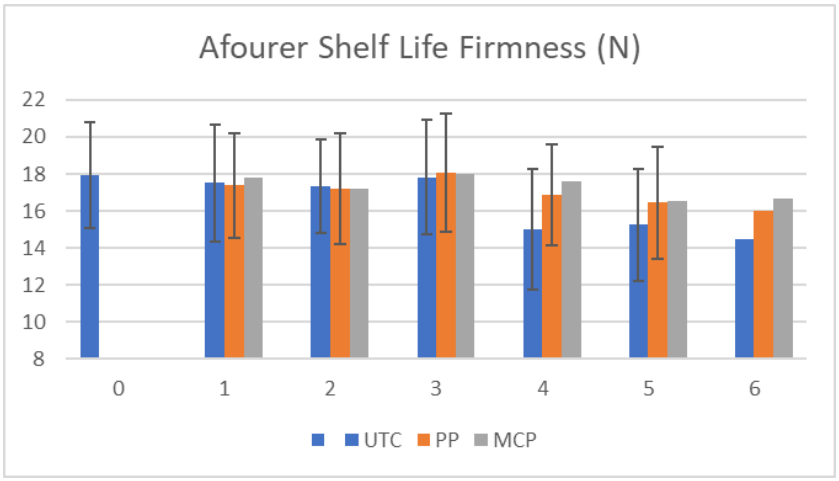
Results

The effect of postharvest treatment on fruit quality attributes on treated Afourer mandarins stored at 20 °C for 6 weeks are presented in the figures below. The fruit remained untreated (UTC), treated with a potassium permanganate sachet (PP), or treated with MCP (MCP). Note: no combination of PP and MCP was assessed in this shelf-life experiment.

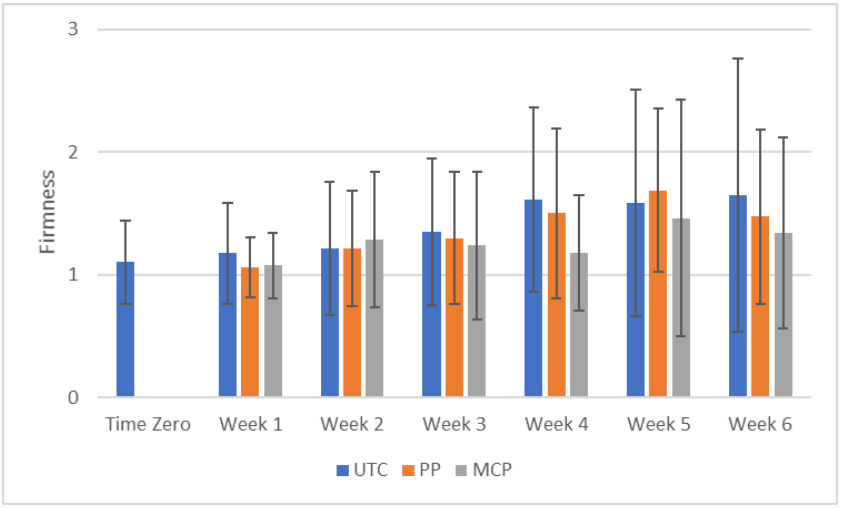
The results presented in Figure 154 show there were no differences in overall fruit quality acceptability score, objective fruit firmness measurement, subjective fruit firmness (hand feel), fruit respiration rate, fruit ethylene production rate, internal CO_2 and ethylene levels within the fruit, headspace ethanol content, vitamin C content, subjective blemish score, subjective calyx condition score (browning), fruit TSS levels (% Brix) and fruit TA levels (% citric acid).



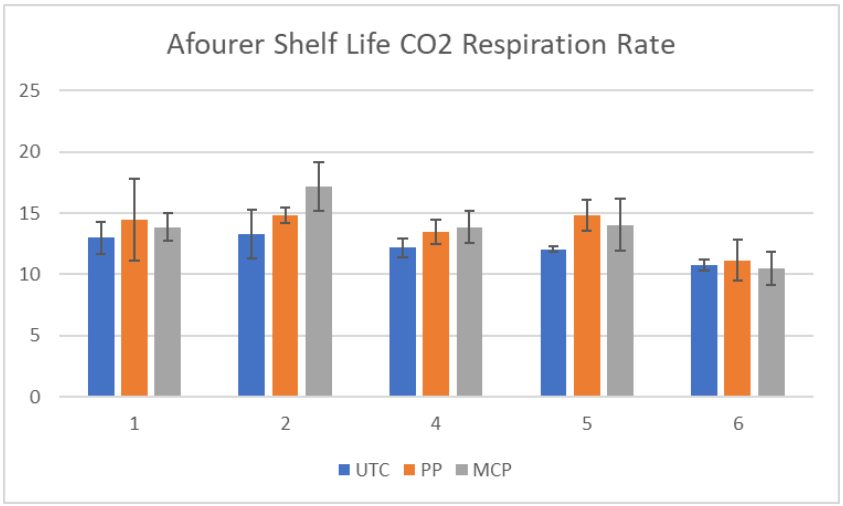
a. Subjective overall acceptability score



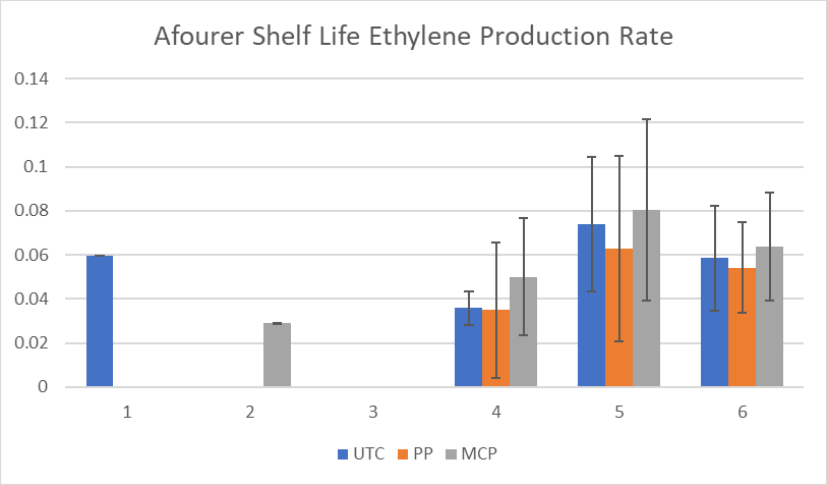
b. Objective fruit firmness measurement (machine)



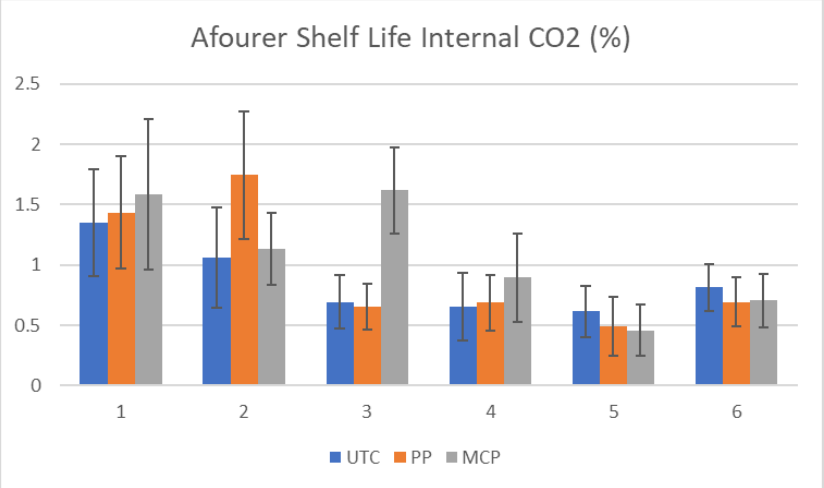
c. Subjective fruit firmness (hand feel)



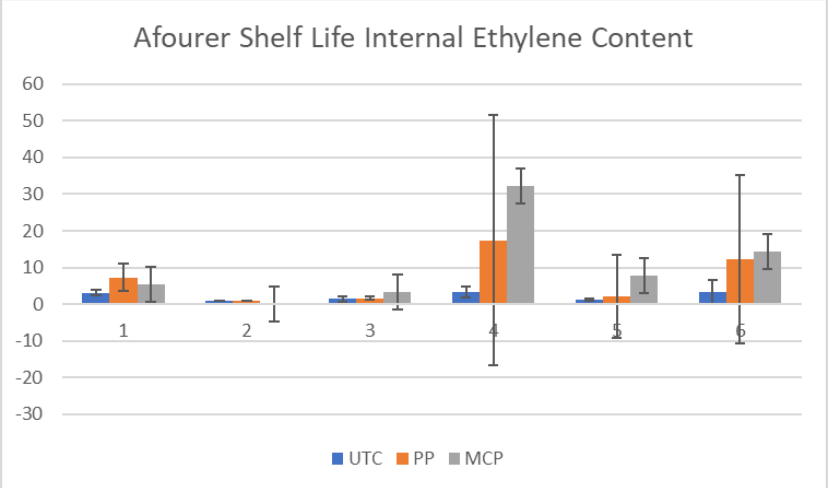
d. Fruit respiration rate



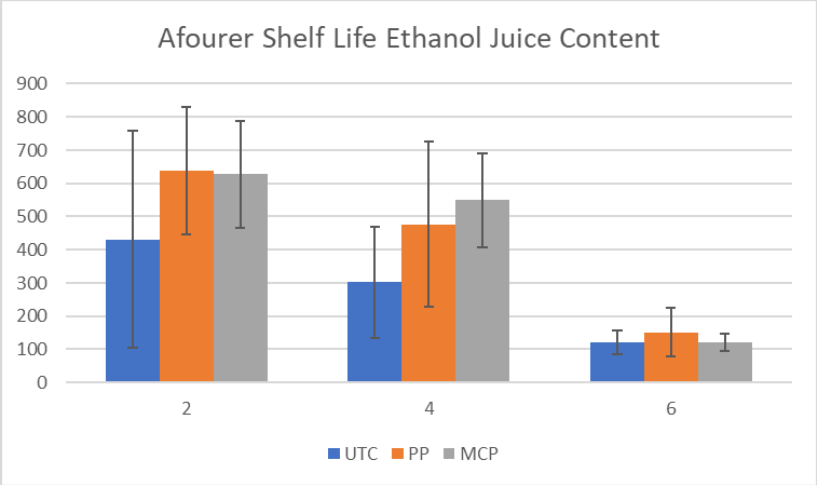
e. Fruit ethylene production rate



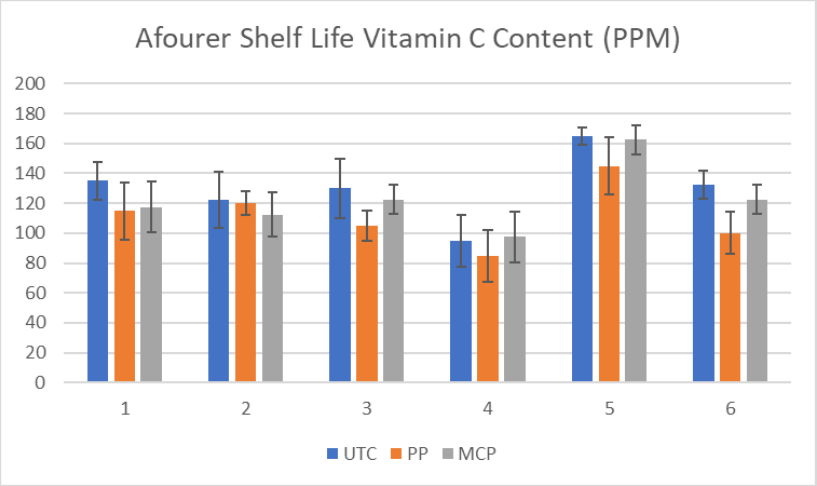
f. Internal CO_2 level within fruit



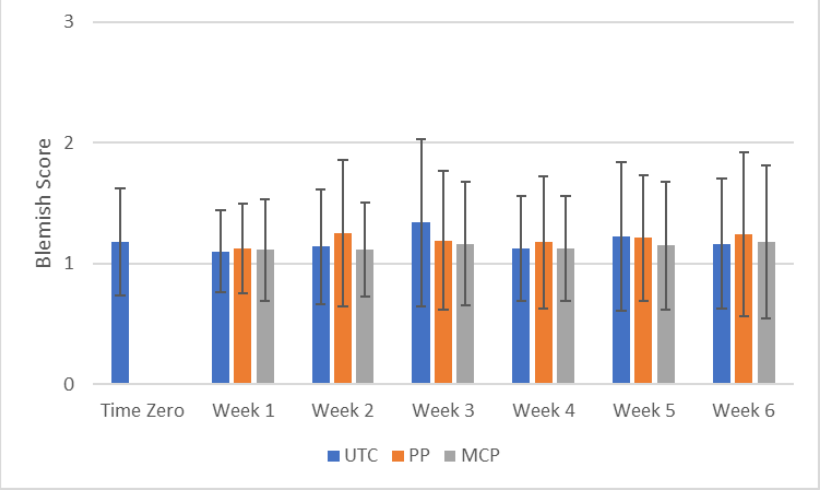
g. Internal ethylene level within fruit



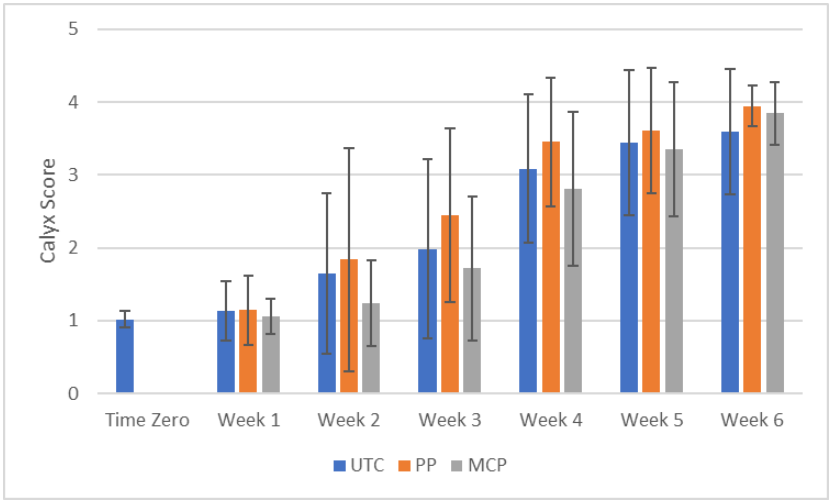
h. Headspace ethanol content



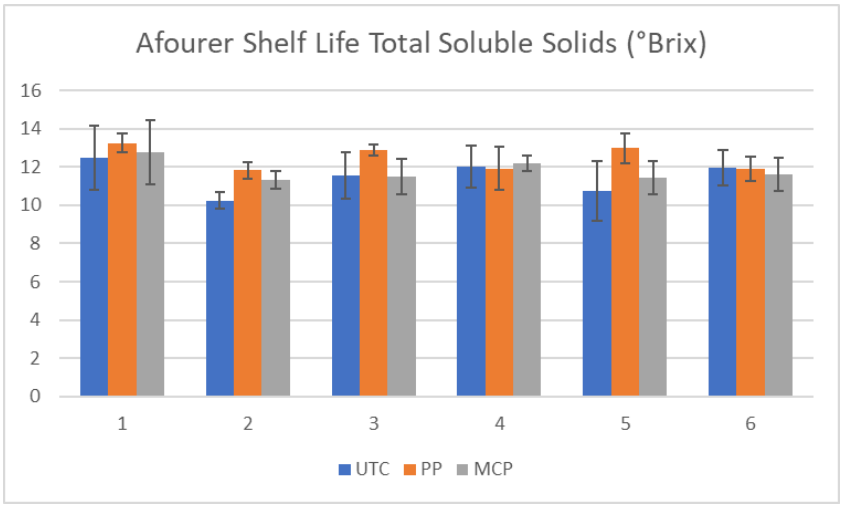
i Vitamin C content



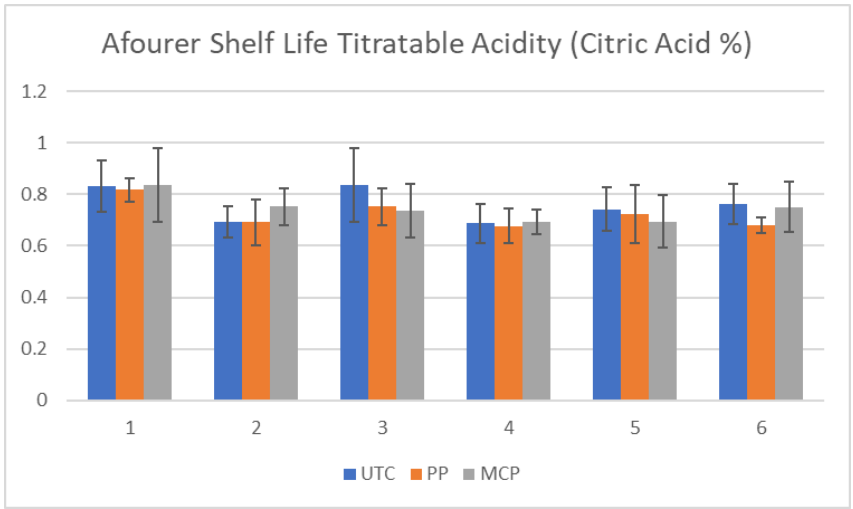
j. Subjective blemish score



k. Subjective calyx condition score (browning)



l. Fruit TSS levels (% Brix)



m. Fruit TA levels (% citric acid)

Figure 154. Effect of postharvest treatment on the subjective assessment of Afourer mandarins stored for up to 6 weeks (shelf life) at 20 °C. (a) overall fruit quality acceptability score, (b) objective fruit firmness measurement, (c) subjective fruit firmness (hand feel), (d) fruit respiration rate, (e) fruit ethylene production rate, (f) internal CO2 and (g) ethylene levels within the fruit, (h) headspace ethanol content, (i) vitamin C content, (j) subjective blemish score, (k) subjective calyx condition score (browning), (l) fruit TSS levels (% Brix) and (m) fruit TA levels (% citric acid).

Part C. Preliminary investigation into fruit variability and its effects on eating quality and acceptability/rejections

Upon tasting the fruit during the storage trial and at the end after 15 weeks, there was some variability between the fruit of the same batch of the same treatment. Informal observations of when presenting 2 fruit from each treatment to consumer panellists, it was a common occurrence to hear that one fruit tasted good and the other fruit was poor. This occurred in all different postharvest treatments, and it was difficult to determine if there were any eating differences between the different postharvest treatments.

In a box of untreated control fruit, we measured the TSS and TA of 20 fruit. The average TSS was 10.86% Brix, with a range from 6.5 to 14.5% Brix. Similarly with TA, the average TA was 0.51% citric acid and the individual fruit ranged from 0.287 to 0.731% citric acid. In addition, the BrimA values ranged from 88 to 192, averaging 145, which is well within the accepted ACS. This large variability in both TSS and TA will result in variable eating quality. This needs to be addressed.

Table 7. Levels of TSS and TA (and their ratio and Brim A) of different Afourer mandarins from the same batch/box.

Fruit number	TSS (% Brix)	TA (% citric acid)	TSS:TA ratio	BrimA
1	12.0	0.709	16.9	151
2	14.5	0.731	19.8	191
3	10.3	0.436	23.6	141
4	6.5	0.287	22.6	88
5	12.8	0.439	29.2	182
6	9.2	0.393	23.4	126
7	12	0.588	20.4	159
8	12.1	0.476	25.4	168
9	12.4	0.667	18.6	161
10	9.2	0.590	15.6	113
11	8.1	0.367	22.1	109
12	10.8	0.494	21.9	146
13	9.6	0.535	17.9	123
14	9.4	0.349	26.9	132
15	13.6	0.493	27.6	192
16	11.7	0.475	24.6	162
17	8.9	0.449	19.8	117
18	13.9	0.680	20.4	184
19	10.7	0.581	18.4	138
20	9.6	0.518	18.5	124
Average of 20 fruit	*10.86*	*0.513*	*21.68*	145
Range within 20 fruit	**6.5 to 14.5**	**0.287 to 0.731**	***15.6 to 29.2***	***88 to 192***

A casual observation of the external colour of the fruit skin showed that even after 15 weeks of storage at 3 °C and 1 week at 20 °C, within the box of Afourer mandarins, there was some variability in fruit skin colour. Some fruit were paler yellow and some were deeper orange (Figure 155). This was common in all treatments.

Figure 155. Fruit appearance at the beginning of the storage trial (left) and after 15 weeks of storage and 1 week at 20 °C (right).

To test the possibility that there was an internal quality difference between the yellow and orange skin colour fruit contributed to the variability in eating quality, we then measured the TSS and TA of 10 fruit with yellow skin and 10 fruit with deeper orange skin. The results are presented in Table 8.

Table 8. Levels of TSS and TA (and their ratio and Brim A) of different Afourer mandarins of different skin colours from the same batch/box*.

Skin colour	Fruit number	TSS	TA	TSS: TA	BrimA
Yellow	1	11.4	0.554	20.6	152
Yellow	2	11.8	0.503	23.5	162
Yellow	3	12.1	0.614	19.7	159
Yellow	4	10.5	0.808	13.0	120
Yellow	5	11.8	0.649	18.2	152
Yellow	6	8.7	0.419	20.8	116
Yellow	7	13.7	0.724	18.9	178
Yellow	8	11.3	0.485	23.3	154
Yellow	9	11.7	0.670	17.5	149
Yellow	10	11.6	0.768	15.1	141
Average	**–**	**11.46**	**0.6194**	**19.1**	**148**
Min and max	**–**	**8.7 to 13.7**	**0.419 to 0.808**	**13.0 to 23.5**	**120 to 178**
Orange	1	9.5	0.646	14.7	114
Orange	2	11.4	0.655	17.4	145
Orange	3	11.3	0.26	43.5	169
Orange	4	11.7	0.655	17.9	150
Orange	5	11.7	0.621	18.8	152
Orange	6	14.1	0.795	17.7	180
Orange	7	9.6	0.434	22.1	130
Orange	8	12.7	0.837	15.2	154
Orange	9	12.2	0.644	18.9	159

Orange	10	12.3	0.744	16.5	154
Average	**–**	**11.65**	**0.629**	**20.3**	**151**
Min and max	**–**	**9.5 to 14.1**	**0.260 to 0.837**	**14.7 to 43.5**	**114 180**

*Note that this was only a sample of 10 fruit with no replication and the selected fruit may not be representative of the population.

It was proposed that the more yellow fruit may have lower TSS and TA, which contribute to poor eating quality, but this was not the case. The results showed that the average TSS for the yellow fruit was 11.46% (range between 8.7 and 13.7%), and the orange fruit was 11.65% (range between 9.5 and 14.1%). Similarly for TA, the average for yellow fruit was 0.619% citric acid (range between 0.419 and 0.808) and for orange fruit, the average TA was 0.6291% citric acid (range between 0.260 and 0.837). The BrimA values for the yellow fruit ranged from 120 to 178 (average 148), and the orange fruit were between 114 and 180 (average 151). The BrimA values are well into the good internal quality of the ACS, but the fruit were variable in eating quality, indicating other measures of internal quality and eating are needed.

The results show there was no difference in the internal objective quality of yellow and orange-coloured mandarins.

It may be possible that if a NIR sorter was available to measure internal TSS, then would it be possible to reject very low TSS fruit (e.g. <9% TSS). All fruit in this trial were the same size (count 113) and the fruit sweetness does not increase in storage. This would be costly in terms of operations, time, and fruit, but just removing a small percentage of fruit that are clearly not going to meet consumer expectations may help the overall acceptability of the batch.

It is important to understand what contributed to the inconsistent eating quality in this batch of fruit. Some aspects of this question are being addressed in the 'Sweeter Citrus' project, but the variability within the tree/box needs to be fixed. Increasing the overall total TSS increases the overall fruit population TSS, but reducing variability and eliminating unacceptable fruit is also important.

Literature review of albedo breakdown

In response to the development of albedo breakdown in eastern Australia in fruit in the 2022 season, the PGR encouraged the program to review and update the literature on albedo breakdown. This was conducted and presented to the industry (Appendix 3) 'Albedo breakdown research update' and is now a funded Hort Innovation levy-funded R&D project. An article from the main findings of this review was extended to industry and published in *Australian Citrus News* [Chavarria J. and Golding J.B. (2022) Practical steps to minimise albedo breakdown. *Australian Citrus News*. Issue 3 2022. Pages 19-20].

Evaluating alternative coatings

Assessment of trial Akorn technology coating on lemons

The natural waxes on the surfaces of citrus fruit are removed during picking and processing, and food-grade waxes are applied to the fruit before packing. Waxes are essential to maintain the quality of fresh citrus fruit during storage. While the current commercially available waxes are widely used and accepted, the development of alternative waxes/coatings is required to improve the out-turn of citrus. Akorn Technology coatings are based on corn-based starch and other natural plant-based ingredients. The coating is seeking organic status. Australian citrus growers have been interested in this product. This trial compared the effectiveness of the Akorn Technology coating with a commercial wax on the storage life of lemons.

Methods

Freshly harvested lemons from Leeton were washed and processed in a commercial packing house. After fungicide treatment, the fruit were treated with the commercial wax (as per packers current handling practices) or treated with the trial coating (Figure 156). An unwaxed sample of fruit (but similarly processed and fungicide-treated) was used as an untreated control (Figure 157). The treatment unit consisted of 20 lemon fruit with 4 replicates.

After treatment, fruit were stored at 20 °C for 4 weeks or stored at 3 °C for up to 8 weeks. Fruit quality assessments were conducted at 2-week intervals. Fruit quality assessments (weight loss, fruit respiration rate, fruit colour (Hue angle, chroma and L value), fruit glossiness (objective and subjective), fruit firmness, TSS, TA, fruit juice ethanol content and consumer visual appeal) were conducted according to the General Methods.

Figure 156. Coating lemon using trial Akorn technology.

Figure 157. Lemon treated with trial Akorn technology coating (middle row) compared to commercial wax (top row) and untreated control (bottom row) at time zero.

Results

The major reason waxes are applied to fresh citrus fruit is to reduce weight (water) loss. In this trial, weight loss from both the commercial wax and the trial coating was lower than the untreated control (Figure 158). In fruit stored constantly at 20 °C, the commercial wax retained more weight, but both were superior to the untreated control. In fruit stored at 3 °C, there was no difference in water loss from the treated fruit, and these treatments reduced water loss by 50% compared to the untreated fruit.

Fruit firmness and water (weight) loss are often related; softer fruit generally have higher water loss. This was also observed in this experiment, where fruit firmness was highest in the waxed/coated fruit (Figure 159). As expected, the fruit became softer during increasing lengths of storage, but the application of wax/coating reduced this fruit softening.

Fruit shine, lustre or glossiness is an important consumer attribute. Many consumers who 'buy with their eyes' expect a glossy lemon. In this experiment, glossiness was assessed by objectively measuring with a specialist instrument (BYK spectrophotometer) and with a subjective scale of fruit glossiness. The results presented in Figures 160 and 161 for both the objective and subjective measures show the commercial wax presented a higher gloss/lustre than the untreated and trial coating. Many consumers seek the highly glossy fruit, but a more natural appearance of the fruit, particularly for the large beverage trade (cocktails and garnishes), may be of some appeal to some buyers. The highly glossy fruit of the commercial wax can appear too bright to some consumers, particularly when the peel is being used as a garnish.

There was no effect of the different coatings on TSS and TA (Figures 162 and 163). The fruit respiration rates were measured over the storage period, and the results showed that at 3 °C after 8 weeks of storage, the untreated fruit had lower respiration rates than the wax/coated fruit (Figure 164). This higher fruit respiration rate may be associated with the higher juice ethanol levels in treated fruit (Figure 165). There were no consistent effects of wax/coating on vitamin C content (Figure 166). Both the conventional wax and the trial wax showed some protection against chilling injury (Figure 167), where both the incidence of chilling and the severity of the symptoms were lower in both treated samples. The positive effects of waxing on suppressing the symptoms of chilling injury have been long recognised, but there were no differences between the different wax/coatings. A visual symptom of green spots/blotches on the skin was observed during storage, but this was low and not affected by coating treatments (Figure 168). There was no effect of treatment on fruit colour (hue angle, L* value and chroma) as measured by the Minolta colour meter (Figures 169-171).

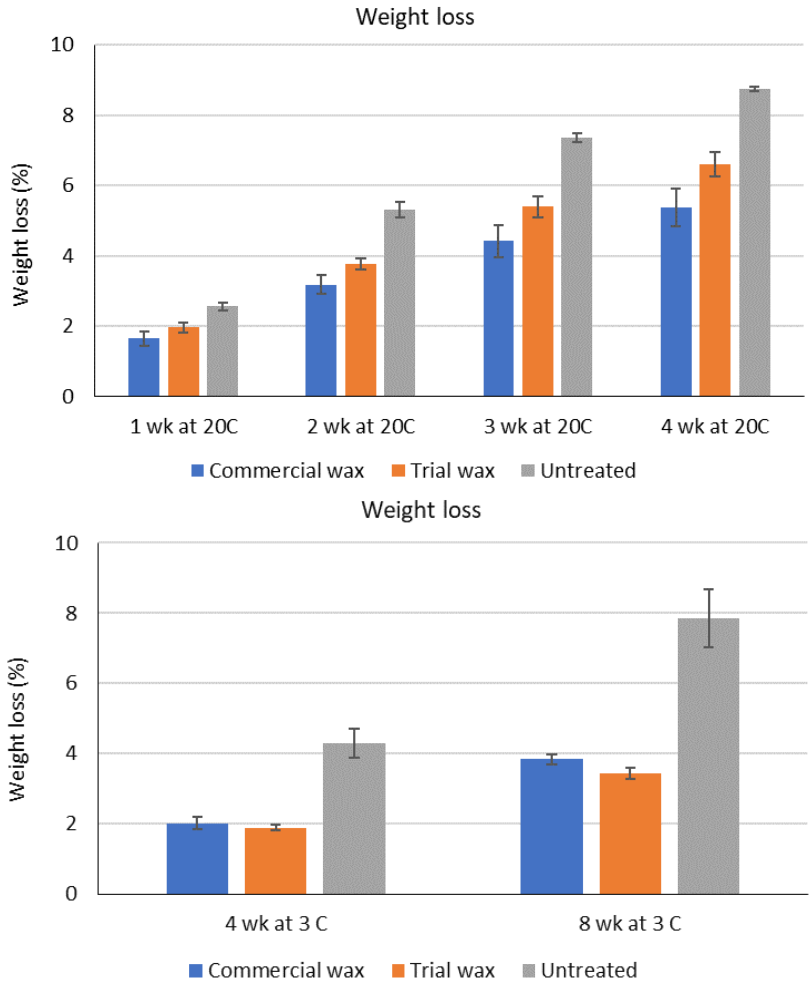


Figure 158. Weight loss (%) of lemons treated with commercial wax, trial wax and untreated after stored at 1, 2, 3 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower). Bars are standard deviations around the means, *n* =4.

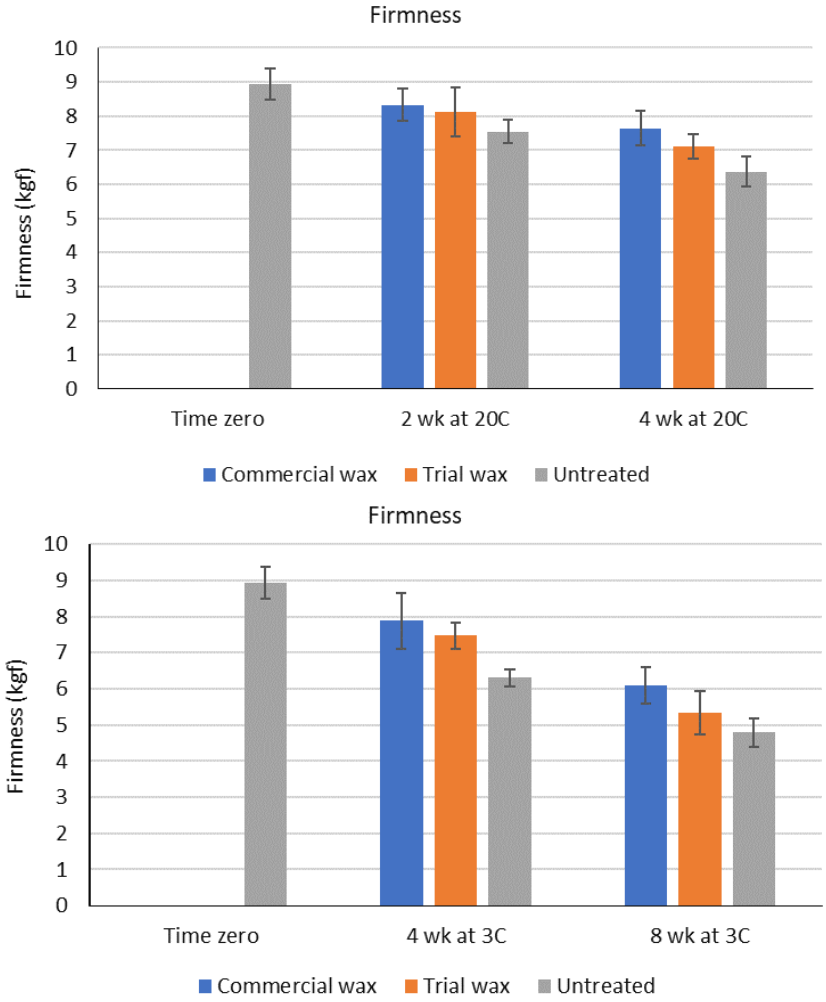


Figure 159. Firmness (kgf) of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top), 4 and 8 weeks at 3 °C (lower). Bars are standard deviations around the means, *n* = 4.

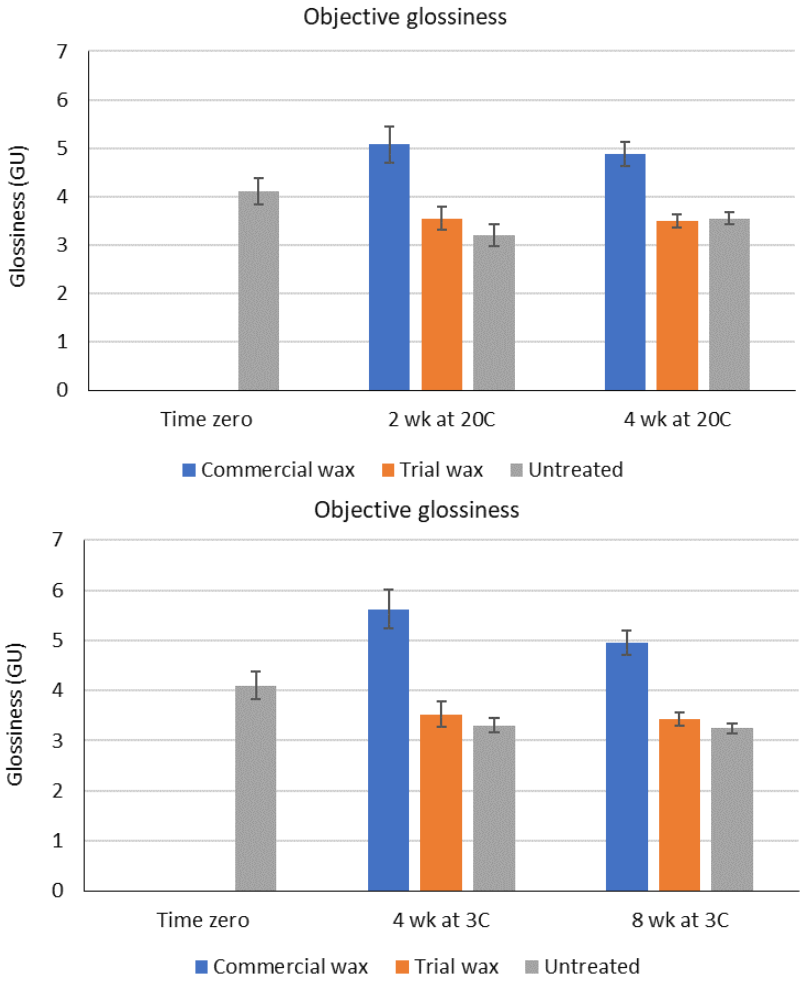


Figure 160. Objective glossiness (GU) measured by BYK spectrophotometer of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top), 4 and 8 weeks at 3 °C (lower). Bars are standard deviations around the means, *n* =4.

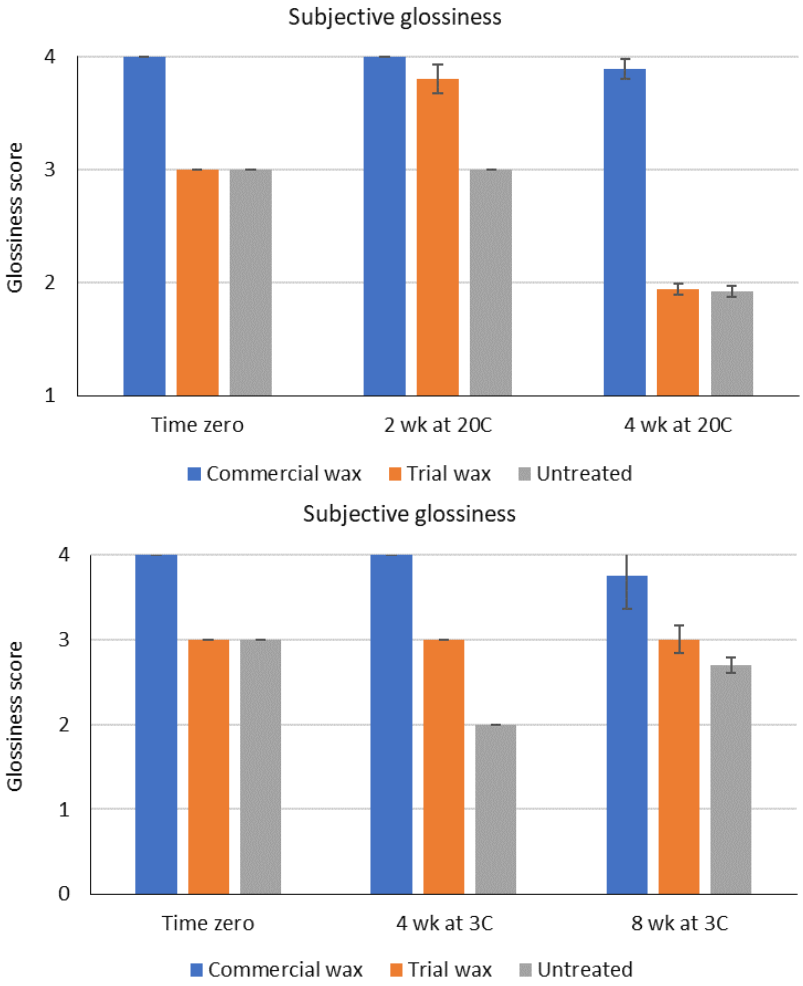


Figure 161. Subjective glossiness score of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower). Subjective glossiness score: 1 = not shiny, 2 = little shiny, 3 = shiny, 4 = very shiny. Bars are standard deviations around the means, *n* = 4.

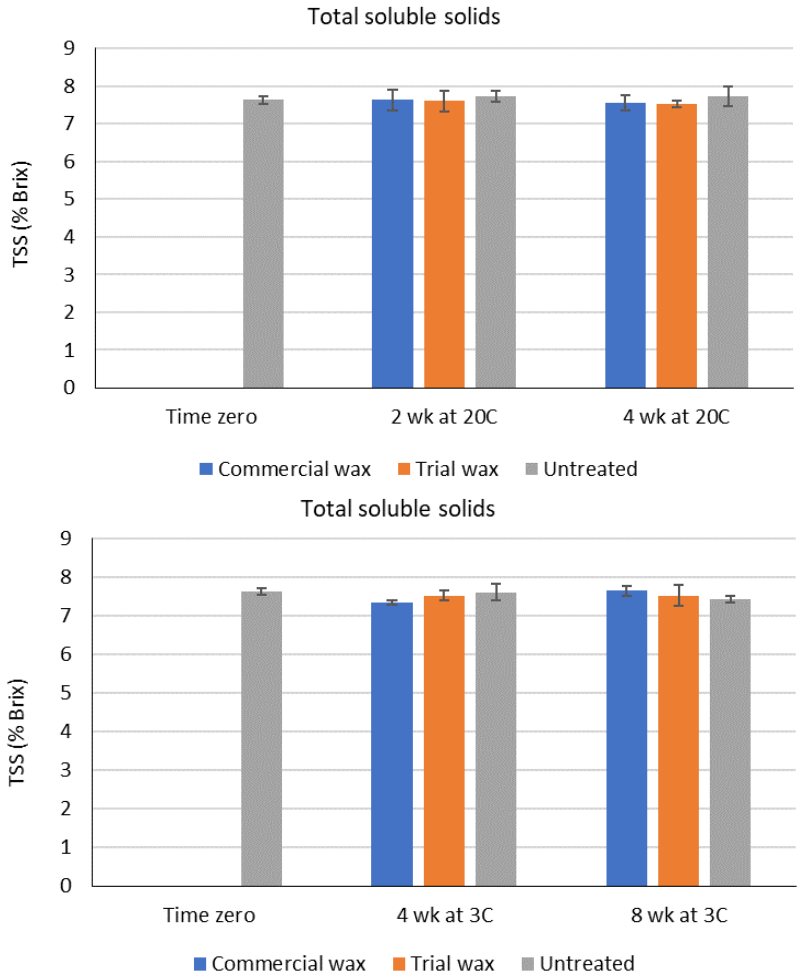


Figure 162. Total soluble solids contents (% Brix) of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower). Bars are standard deviations around the means, *n* = 4.

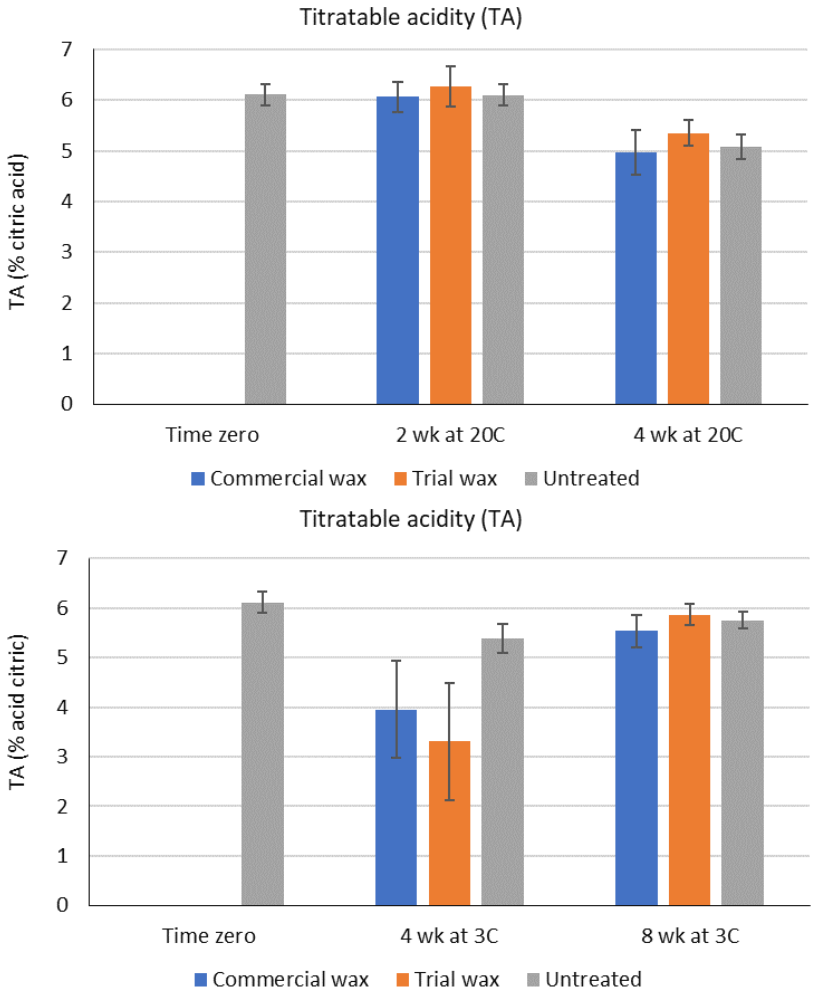


Figure 163. Titratable acidity (% citric acid) of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower). Bars are standard deviations around the means, *n* = 4.

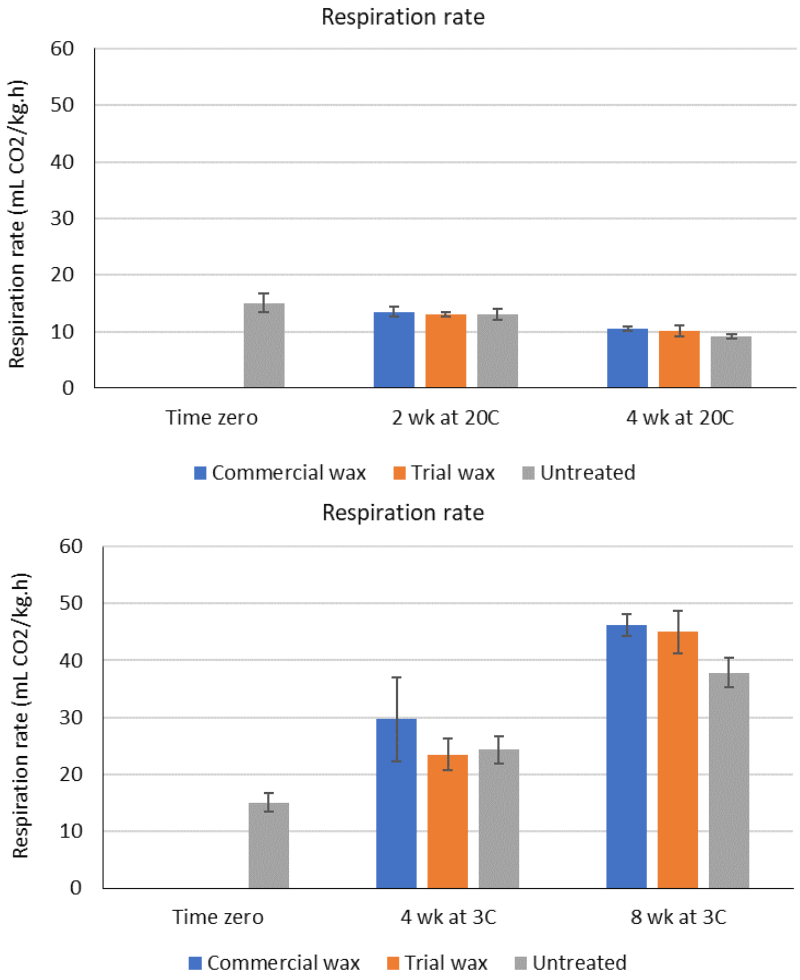


Figure 164. Respiration rate (mL CO_2/kg.h) of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower). Bars are standard deviations around the means, *n* =4.

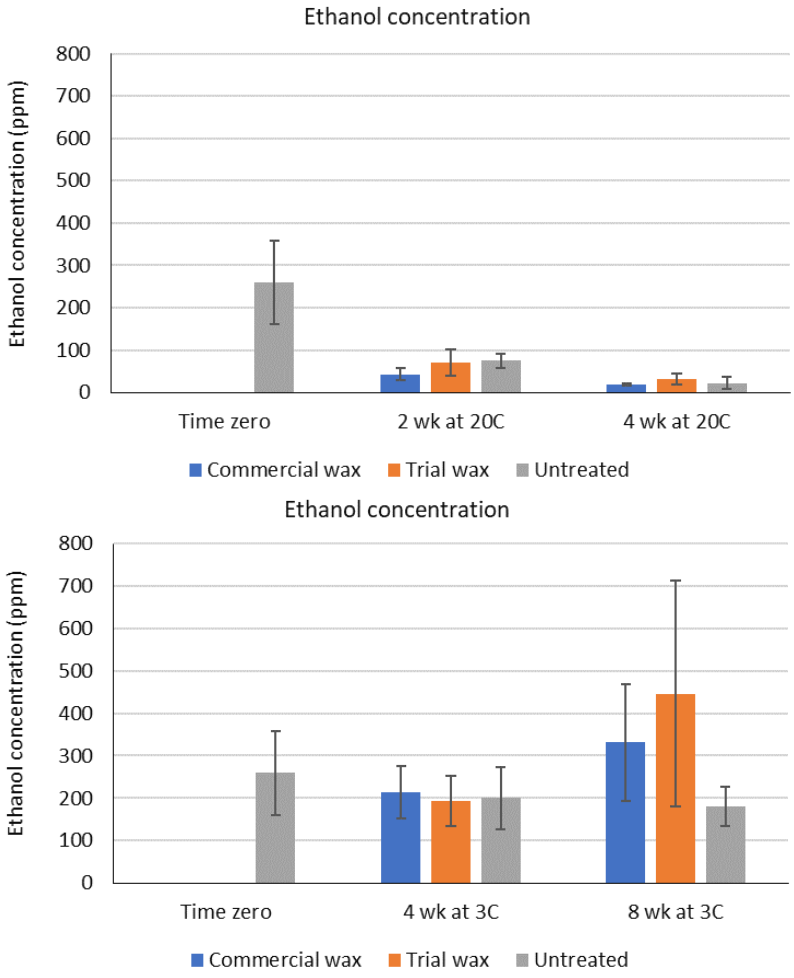


Figure 165. Ethanol concentration (ppm) of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower). Bars are standard deviations around the means, *n* = 4.

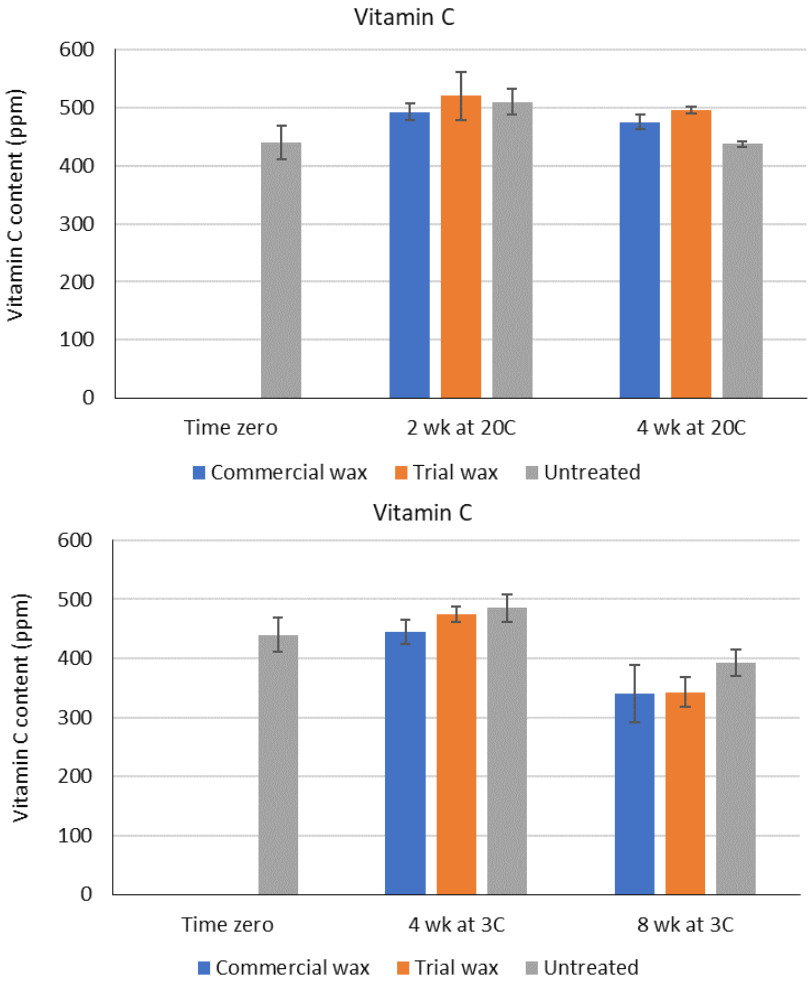


Figure 166. Vitamin C (ppm) of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage times: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower).
Bars are standard deviations around the means, *n* = 4.

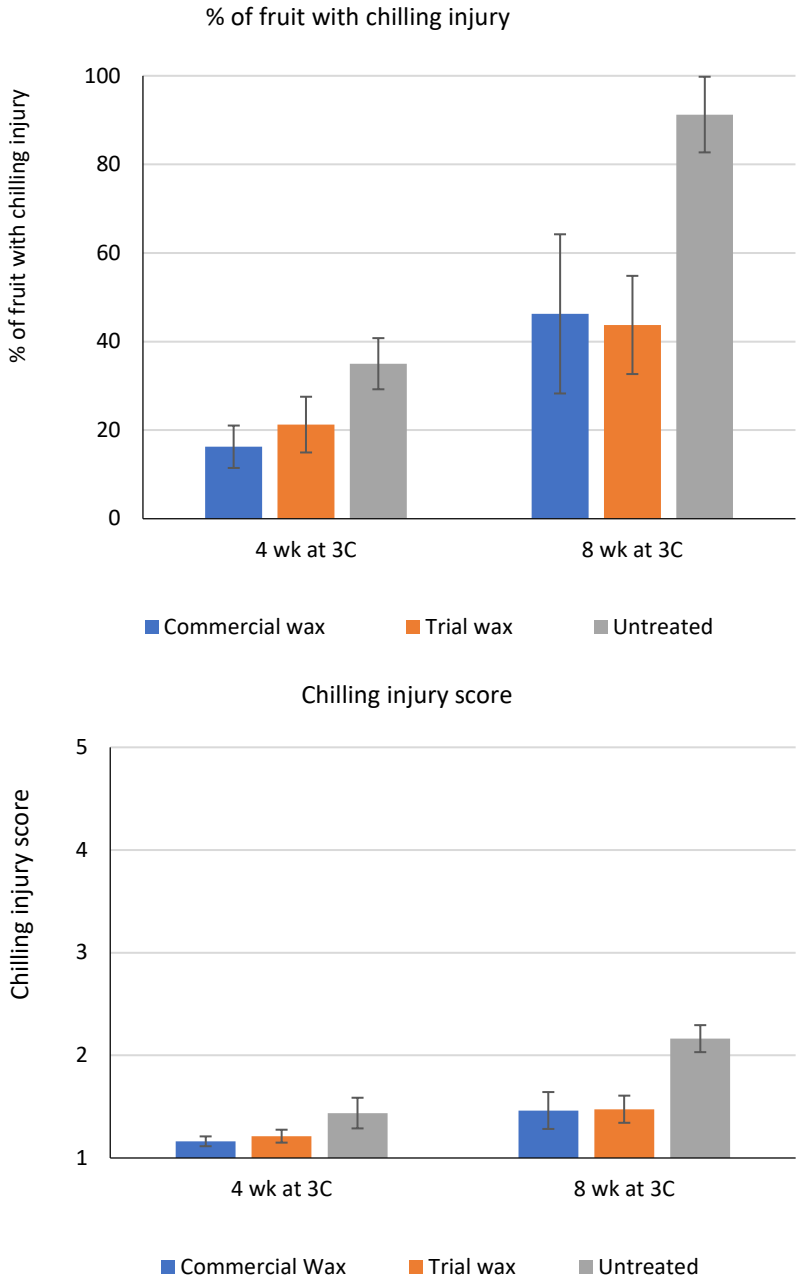


Figure 167. Percentage of fruit with chilling injury (%) (top) and chilling injury score (lower) of lemons treated with commercial wax, trial wax and untreated after stored at 3 °C for 4 and 8 weeks. Subjective chilling injury score: 1 = no pitting, 2 = <5% of fruit surface, 3 = up to 10% of fruit surface, 4 = up to 30% of fruit surface, 5 = >30% of fruit surface. Bars are standard deviations around the means, *n* = 4.

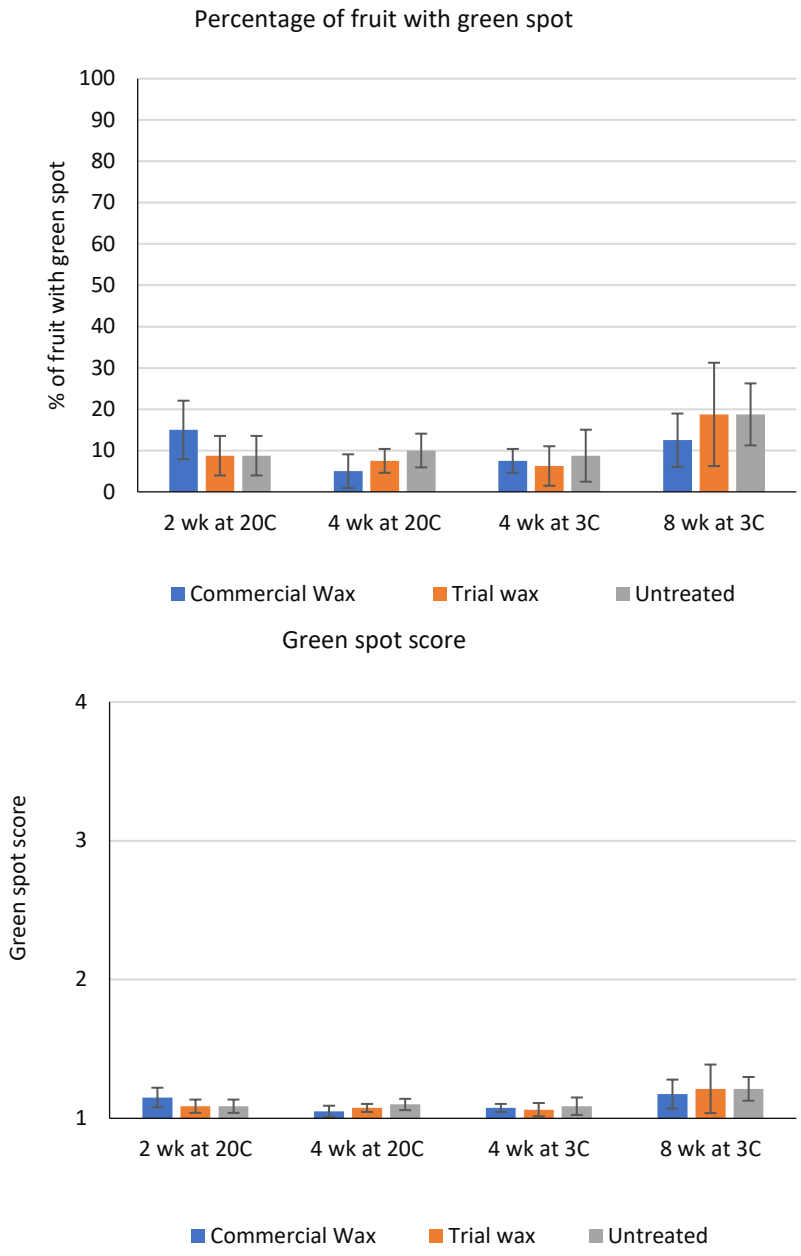


Figure 168. Percentage of green spot (%) (top) and green spot score (lower) of lemons treated with commercial wax, trial wax and untreated after stored at 20 °C for 2 and 4 weeks, and at 3 °C for 4 and 8 weeks. Subjective green spot score: 1 = none, 2 = 10% of fruit surface, 3 = 30% of fruit surface, 4 = >30% of fruit surface.
Bars are standard deviations around the means, *n* = 4.

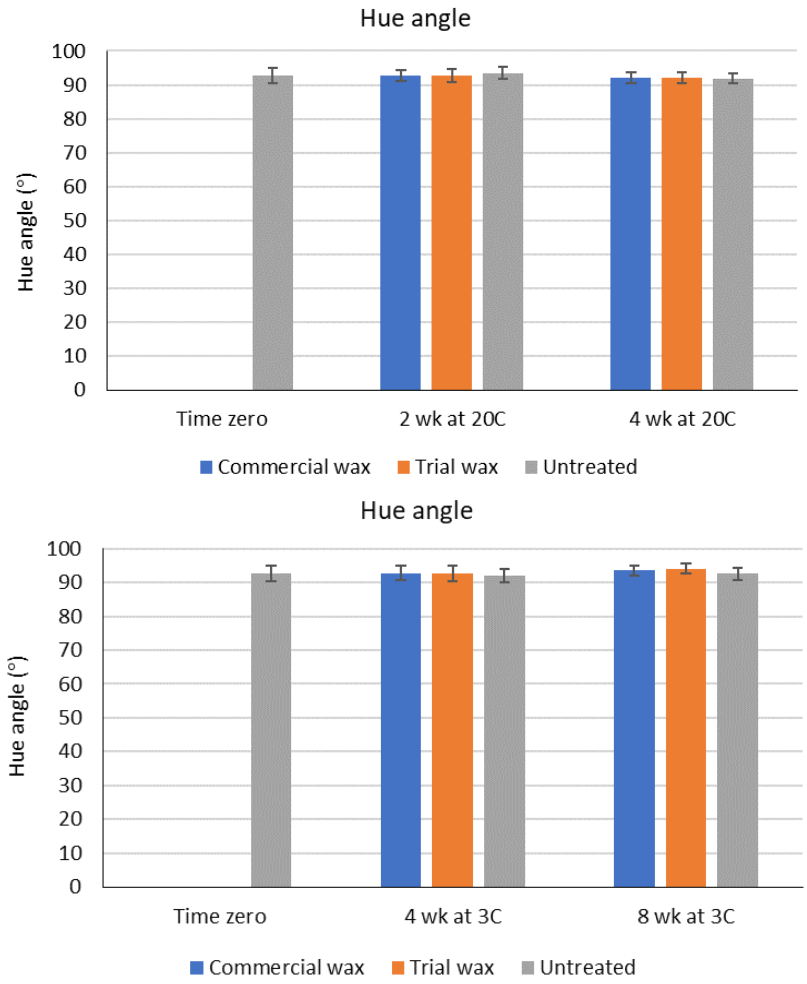


Figure 169. Hue angle (°) of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage time: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower).
Bars are standard deviations around the means, *n* = 4.

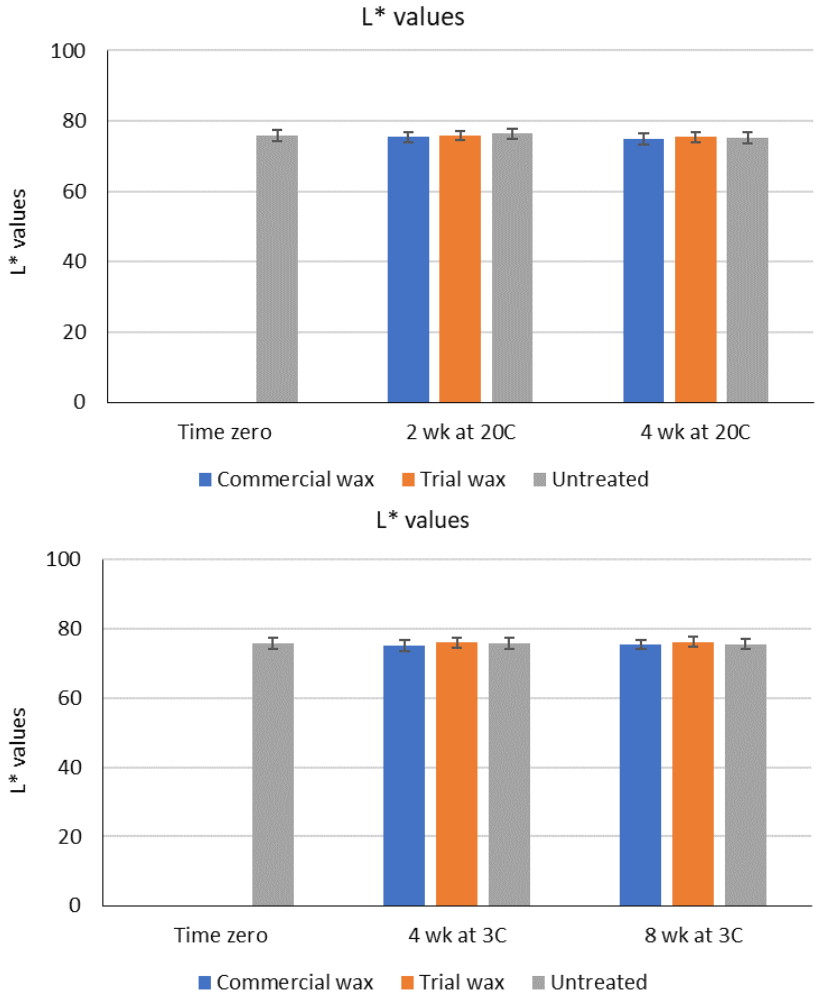


Figure 170. L* values of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage time: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower).
Bars are standard deviations around the means, *n* = 4.

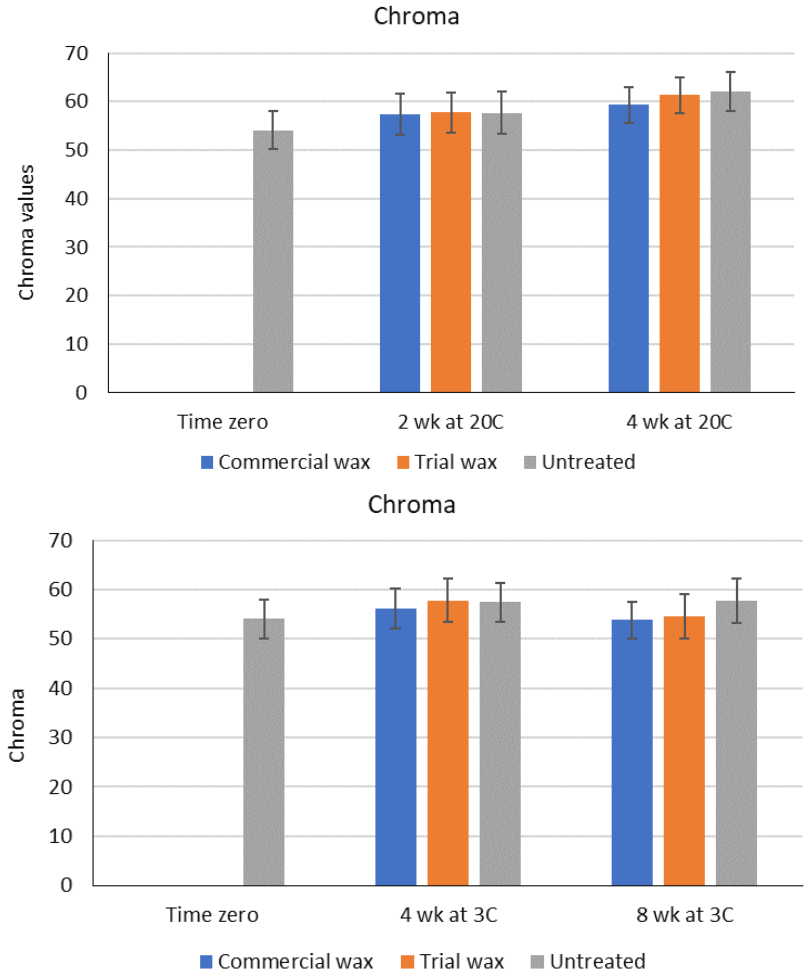


Figure 171. Chroma values of lemons treated with commercial wax, trial wax and untreated at time zero and at the following storage time: 2 and 4 weeks at 20 °C (top) and 4 and 8 weeks at 3 °C (lower).
Bars are standard deviations around the means, *n* = 4.

Evaluating new citrus firmness meters

Fruit firmness is an important market and consumer quality attribute for all citrus types, yet there are no widespread commercial methods to measure and report fruit firmness (softness). Firmness is currently assessed by market agents or quality assurance technicians squeezing the fruit with their hands and reporting the fruit as 'soft' and unmarketable with no actual independent measurement of fruit firmness. In the laboratory we regularly use a texture analyser to measure fruit, and many other industries have standard fruit firmness measures. For example, in the apple industry, fruit firmness is routinely used as a fruit maturity and quality parameter, where fruit firmness is destructively measured with a penetrometer. However, there are no standards in citrus. A cheap, easy-to-use, reliable, and accurate method is required by the industry to measure and report fruit firmness confidently.

Methods

A range of sleeves to fit over the standard handheld Effigi penetrometer, which is used in other industries (such as summer fruit and apples), were trialled and compared. We trialled different sleeve lengths, penetrometer diameters and different instruments on a range of different Navel oranges with different 'hand' firmness classes.

Results

The results showed that the most consistent firmness measurement was the hand penetrometer of 13 kg/11 mm tip with sleeve size 42 mm. While no differences were detected in measuring orange firmness by hand or lever, there were differences in firmness outcomes among different operators. This work needs to be further explored.

Effect of different sleeve lengths A range of different lengths of sleeves were produced to fit over the standard Effigi penetrometer (FT327, Effigi Italy): 39, 39.5, 40, 40.5, 41, 41.5, 41.75, 42, 42.25, 42.5, 42.75, 43, 43.25, 43.5, 43.75, 44, 44.25, 44.5 and 45 mm. The protrusion over the end of the penetrometer is listed in Table 9.

Table 9. Protrusion of penetrometer tip over the different sleeve sizes in both the 8 mm and 13 mm probe diameters in both Effigi FT327 (13 kg capacity) (top) and Effigi FT011 (5 kg capacity) (lower).

FT 327 – 13 kg – Probe diameter 11 mm

Sleeve sizes	Protrusion (mm)			
	Data-1	Data-2	Data-3	Average
39,0	7.72	7.74	7.77	**7.7**
39.5	7.01	7.28	7.22	**7.2**
40.0	6.66	6.69	6.73	**6.7**
40.5	6.19	6.20	6.27	**6.2**
41.0	5.45	5.63	5.60	**5.6**
41.5	5.58	5.49	5.51	**5.5**
41.75	5.3	5.32	5.31	**5.3**
42.0	4.40	4.57	4.60	**4.5**
42.25	4.83	4.91	4.83	**4.9**
42.5	4.30	4.31	4.26	**4.3**
42.75	4.05	4.12	4.16	**4.1**
43.0	3.82	3.74	3.80	**3.8**
43.25	3.57	3.61	3.52	**3.6**
43.5	3.41	3.41	3.48	**3.4**

FT 327 – 13 kg – Probe diameter 8 mm

Sleeve sizes	Protrusion (mm)			
	Data-1	Data-2	Data-3	Average
39.0	9.04	9.11	8.96	**9.0**
39.5	8.90	8.43	8.52	**8.6**
40.0	7.93	7.90	7.73	**7.9**
40.5	7.10	7.22	7.32	**7.2**
41.0	6.77	6.70	6.76	**6.7**
41.5	6.17	6.05	6.11	**6.1**
41.75	6.31	6.35	6.4	**6.4**
42.0	5.97	5.98	5.93	**6.0**
42.25	5.8	5.73	5.82	**5.8**
42.5	5.60	5.51	5.41	**5.5**
42.75	5.29	5.24	5.23	**5.3**
43.0	5.03	4.98	5.02	**5.0**
43.25	4.56	4.58	4.63	**4.6**
43.5	4.57	4.45	4.58	**4.5**

43.75	3.22	3.36	3.18	**3.3**
44.0	2.82	2.55	2.47	**2.6**
44.25	2.61	2.7	2.73	**2.7**
44.5	1.61	2.01	1.99	**1.9**
45.0	1.69	1.86	1.91	**1.8**

Old sleeves (tested)

New sleeves (tested)

43.75	4.18	4.16	4.16	**4.2**
44.0	3.91	3.77	3.87	**3.9**
44.25	3.78	3.59	3.74	**3.7**
44.5	3.59	3.46	3.43	**3.5**
45.0	2.74	2.86	2.88	**2.8**

Old sleeves (tested)

New sleeves(tested)

FT 011 – 5 kg – Probe diameter 11 mm

Sleeve sizes	Protrusion (mm)			
	Data-1	Data-2	Data-3	Average
39.0	7.61	7.73	7.90	**7.7**
39.5	7.21	7.11	7.59	**7.3**
40.0	6.46	6.55	6.56	**6.5**
40.5	6.53	6.51	6.50	**6.5**
41,0	5.81	5.78	5.86	**5.8**
41.5	5.41	5.44	5.47	**5.4**
42.0	4.77	4.70	4.73	**4.7**
42.5	4.42	4.67	4.69	**4.6**
43.0	3.73	3.97	3.85	**3.9**
43.5	3.46	3.47	3.59	**3.5**
44	2.95	2.89	2.91	**2.9**
44.5	2.25	2.10	2.24	**2.2**
45.0	2.04	2.14	2.15	**2.1**

Tested sleeves

FT 011 – 5 kg – Probe diameter 8 mm

Sleeve sizes	Protrusion (mm)			
	Data-1	Data-2	Data-3	Average
39.0	8.94	8.51	8.76	**8.7**
39.5	8.03	8.18	8.11	**8.1**
40.0	7.31	7.49	7.58	**7.5**
40.5	7.07	6.95	7.10	**7.0**
41.0	6.74	6.82	6.75	**6.8**
41.5	6.44	6.49	6.67	**6.5**
42.0	6.08	5,92	6.01	**6.0**
42.5	5.50	5.26	5.42	**5.4**
43.0	5.10	5.12	5.05	**5.1**
43.5	4.60	4.59	4.35	**4.5**
44.0	4.13	4.12	4.02	**4.1**
44.5	3.43	3.29	3.66	**3.5**
45.0	3.19	3.11	3.03	**3.1**

Tested sleeves

The different sleeves were tested on a batch of similarly firm Navel oranges. The results are presented in Figure 172 and showed that the 13 kg capacity penetrometer with the 11 mm tips delivered more consistent results. The results from the 13 kg capacity penetrometer provided higher differences in firmness compared to 5 kg (Figure 173). The 8 mm tip sometimes caused peel damage/injury, and therefore, this tip size was discarded. Therefore, in subsequent experiments, the 13 kg penetrometer was used.

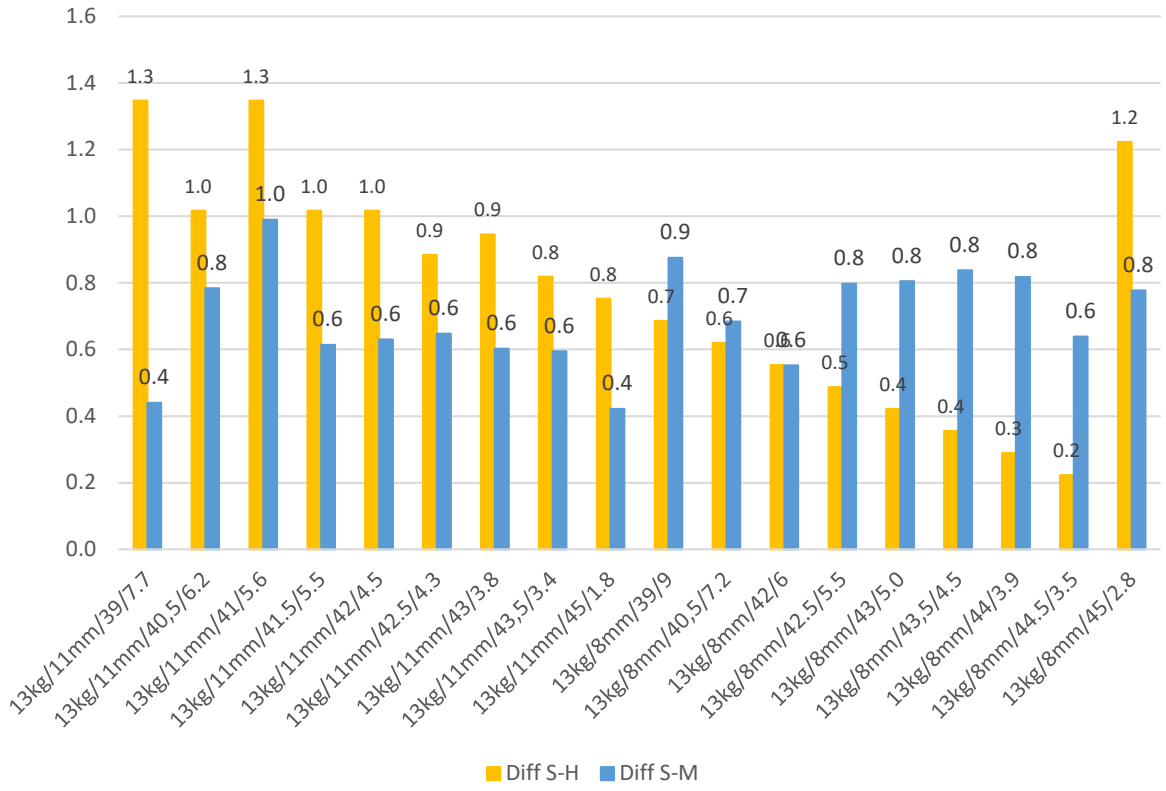


Figure 172. The difference in fruit firmness (kg) using the different trial sleeve sizes with the 13 kg/11 mm penetrometer with an 8 mm tip.

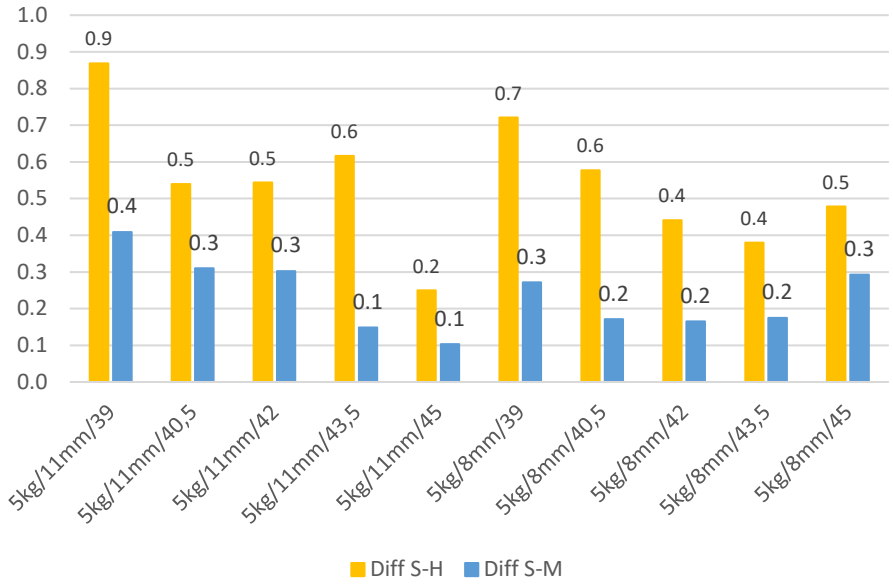


Figure 173. Difference in fruit firmness (kg) using the penetrometer 5 kg/11 mm and 8 mm.

3. Selecting probe diameters (8 mm and 11 mm) using 13 kg (protrusion was selected for this experiment)

- Results: the penetrometer 13 kg with 11 mm showed a more sensitive firmness reading compared to the 8 mm (Figures 174 and 175). So, the probe of 11 mm was selected for subsequence experiments.

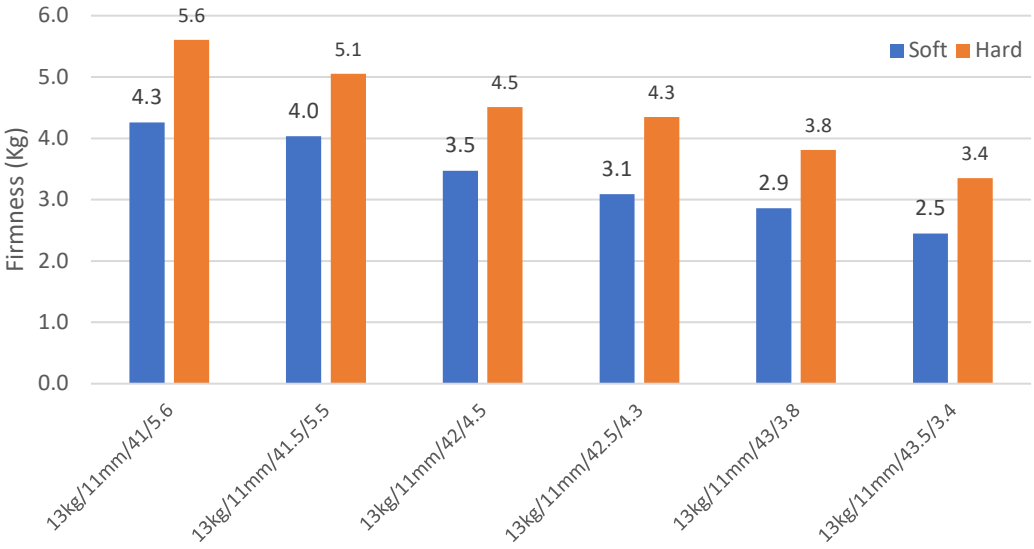


Figure 174. Firmness of Navel oranges using 13 kg/11 mm with different sleeves.

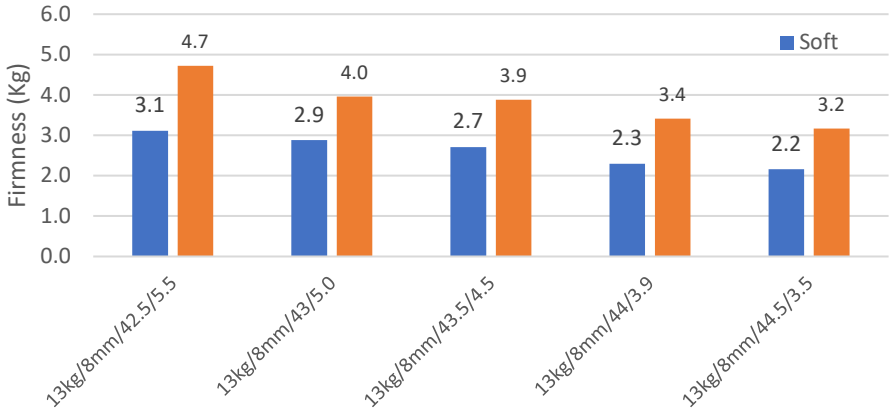


Figure 175. Firmness of oranges using 13 kg/8 mm with different sleeves.

1. Screening of sleeve sizes with 13 kg (8 mm and 11 mm)–new sleeves

The data are combined for all sleeve sizes (old and new size).

Results: The most reliable method was the 13 kg/11 mm and 41.75 sleeves; this was used in subsequent experiments.

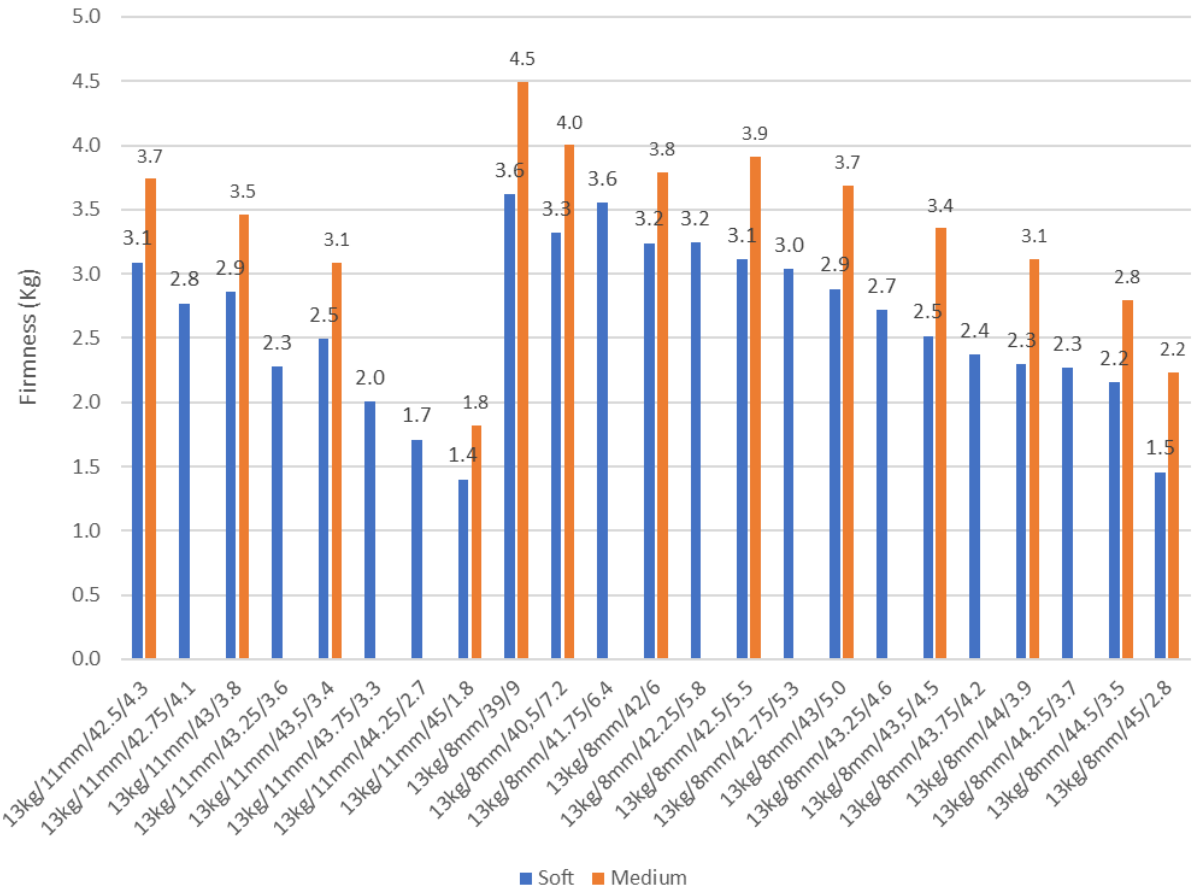


Figure 176. Firmness of oranges using 13 kg/11 and 8 mm with different sleeves.

The second component of this trial was to compare different penetrometers (4 penetrometers)

- In this experiment, there were 30 readings for each penetrometer, one orange for 4 penetrometers.

- Results:
  - ✓ There was no difference between penetrometers for both hard and soft fruits.
  - ✓ All new penetrometers showed similar results, while old penetrometers showed significantly higher results than other penetrometers.

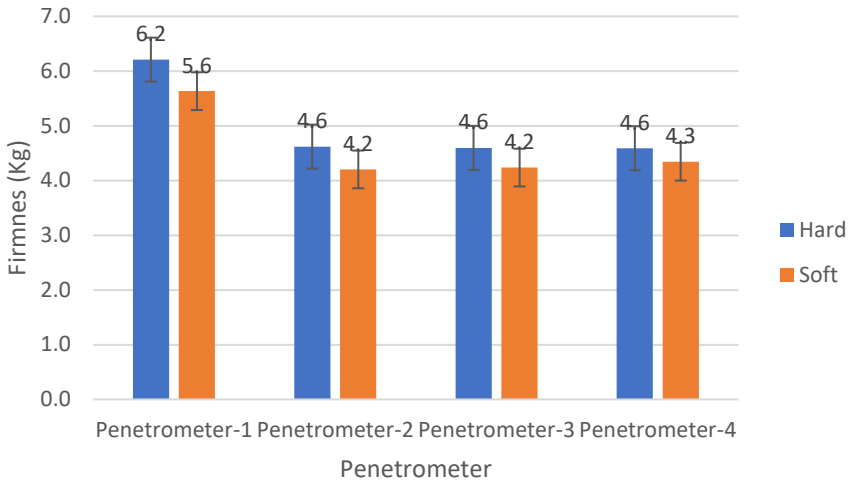


Figure 177. The firmness of oranges was measured using 4 different penetrometers of 143 kg/11 mm and a sleeve of 41.75 (protrusion 5.3).

The third component was comparing 3 penetrometers, by hand and by lever:

- Based on the previous experiment (different penetrometers), this experiment was conducted to test 3 different penetrometers (all Steve's penetrometers) in comparison with a lever.
- 30 readings for each penetrometer by hand on the same fruit
- 30 readings for each penetrometer by lever on the same fruit.

Results: There was no difference between all 3 penetrometers either by hand or by lever (Figure 178).

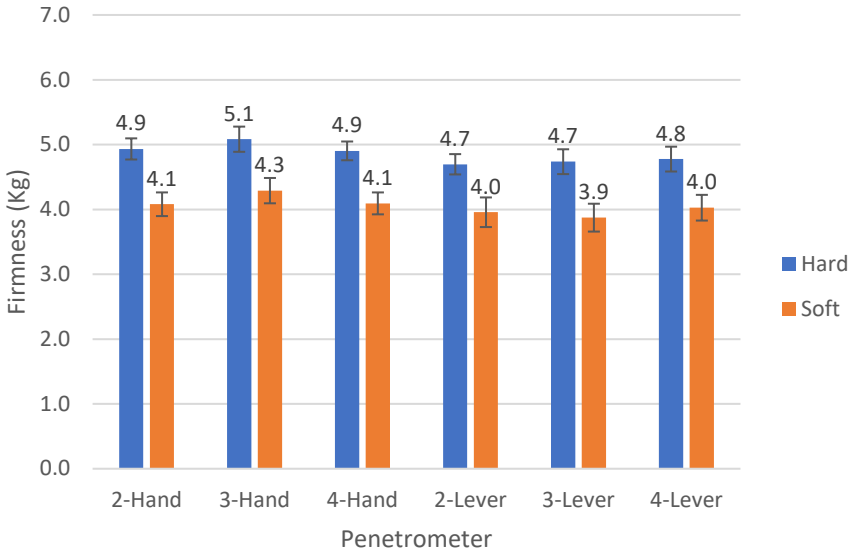


Figure 178. The firmness of oranges was measured using 3 different penetrometers of 13 kg/11 mm and a sleeve of 41.75 (protrusion 5.3).

Comparing hand and lever using the best method 13 kg/11 mm and 41.75 sleeves:

- Oranges from Dareton (soft and medium firmness). The number of oranges was as follows:
  - ✓ Soft = 118 fruits
  - ✓ Medium = 107 fruits
- Firmness measurement: each fruit had 4 readings (2 reading with hand and 2 reading with lever)

Results: There was no difference between hand and lever for both medium and soft oranges (Figure 179).

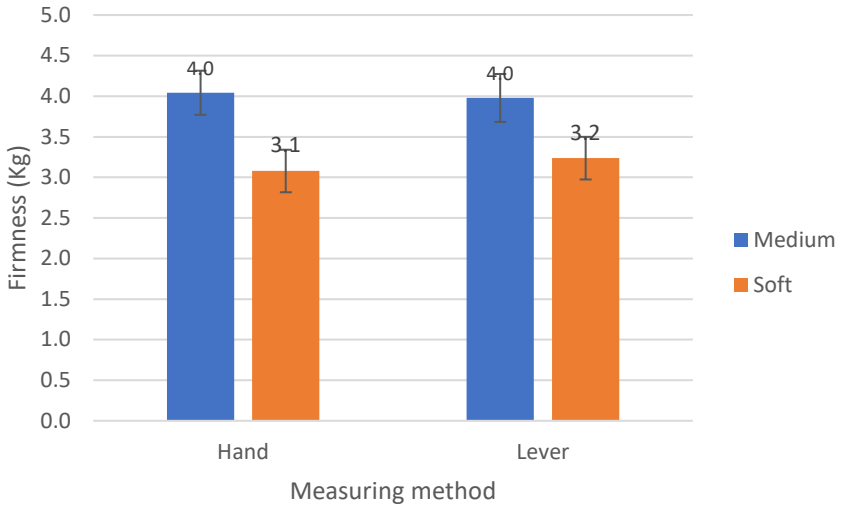


Figure 179. Firmness of oranges (soft and medium) using penetrometers of 13 kg/11 mm with sleeve of 41.75 (protrusion 5.3)

Another trial tested different Navel orange fruits (Dareton and Ourimbah) and between **hand** and **lever** using the best method, 13 kg/11 mm and 41.75 sleeve.

- The number of oranges was as follows:
  - ✓ Soft (27/07) 118 fruits
  - ✓ Medium (27/07) 107 fruits
  - ✓ Dareton soft (03/08) 50 fruits
  - ✓ Ourimbah medium (04/08) 50 fruits

Results showed that there was no difference in orange firmness between measuring by hand and by lever for both soft and medium firmness of oranges (Figure 180).

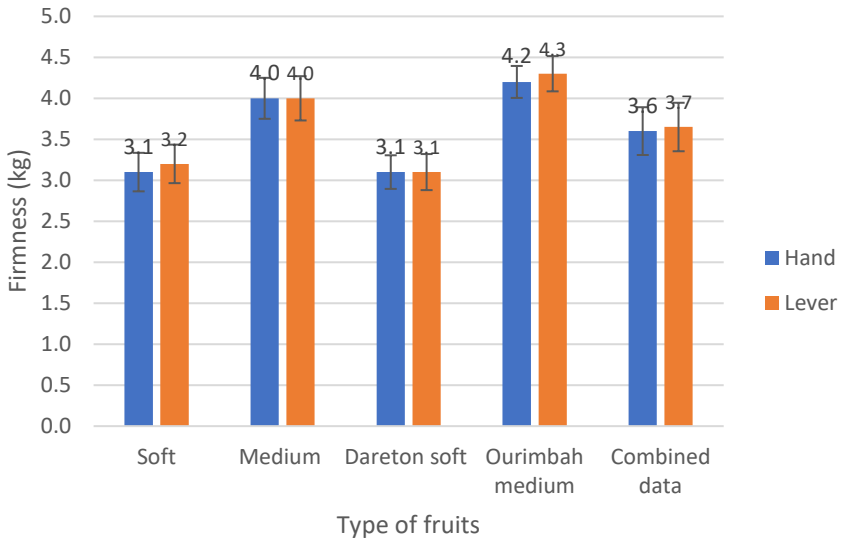


Figure 180. The firmness of oranges using different types of oranges with penetrometers of 13 kg/11 mm using the sleeve of 41.75 (protrusion 5.3)

Comparing user/tester–By hand and by lever (soft and firm oranges).

The data are combined between soft and firm for Figure 181.

Results showed that there was no difference in orange firmness among operators and between measuring by hand and by lever (Figure 181).

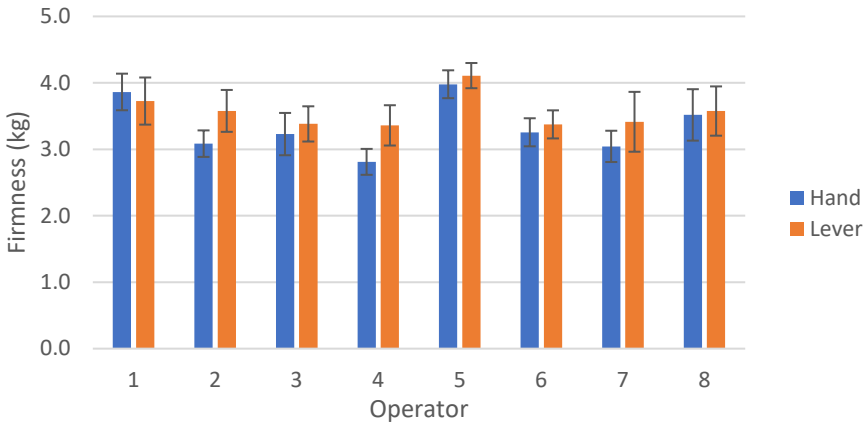


Figure 181. Firmness of oranges by different operators using penetrometers of 13 kg/11 mm with sleeve size of 41.75 (protrusion 5.3)

Data are combined for all operators (for Figure 182). The results showed that there was no difference in orange firmness for measurement by hand and lever for both soft and firm oranges (Figure 182).

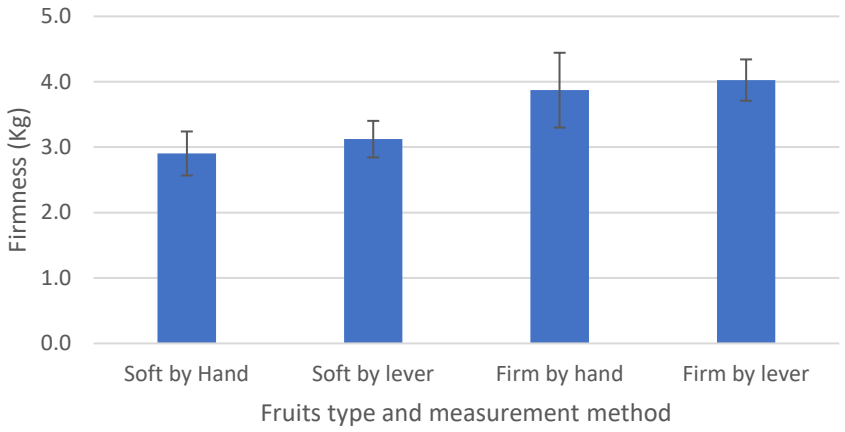


Figure 182. Firmness of oranges was assessed by band and lever for soft and firm oranges using penetrometers of 13 kg/11 mm with sleeve size of 41.75 (protrusion 5.3)

Overall conclusions of the fruit firmness study

- The reliable firmness measurement was penetrometer of 13 kg/11 mm with a sleeve size of 41.75 mm (protrusion 5.3 mm).
- There were differences in firmness outcomes among operators.
- There was no difference in measuring orange firmness between by hand or lever.
- More work is required to test these conclusions.

Appendix 3. Albedo breakdown literature review update

Due to the increase in albedo breakdown in eastern Australia in the 2022 season, the PGR encouraged the program to review and update the literature on albedo breakdown. This was conducted and presented to industry and the research community in a timely manner.

In addition, an article on the management of albedo breakdown was published in *Australian Citrus News* [Chavarria J. and Golding J.B. (2022) Practical steps to minimise albedo breakdown. *Australian Citrus News*. Issue 3 2022. Pages 19-20].

Albedo breakdown research update

Literature collected as part of Hort Innovation '*Citrus Postharvest Program'* (CT19003)

John Golding

NSW Department of Primary Industries

22 July 2022

(Images from NSW DPI Citrus Plant Protection Guide 2021–22. NSW DPI Management Guide. (August 2021). 98 pages. www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/1187654/Citrus-plant-protection-guide-2021.pdf)

Albedo breakdown research update

Contents

Most references are available online or upon request from John Golding.

NSW Department of Primary Industries. john.golding@dpi.nsw.gov.au

Albedo breakdown

Previous Australian research work and resources

Storey R. and Treeby M.T. (1994) The morphology of epicuticular wax and albedo cells of orange fruit in relation to albedo breakdown. *Journal of Horticultural Science*, 69(2), 329-338.

Treeby M.T., Storey R. and Bevington K.B. (1995) Rootstock, seasonal, and fruit size influences on the incidence and severity of albedo breakdown in Bellamy navel oranges. *Australian Journal of Experimental Agriculture*, 35(1), 103-108.

Moulds G., Treeby M., Storey R., Tugwell B. and Bevington K. (1995). Managing albedo breakdown. *Australian Citrus News* 71, 9-10.

Treeby M. (1996) Albedo breakdown in oranges (CT019). Horticultural Research and Development Corporation. 36 pages.

Tugwell B. (1996) Control of albedo rind breakdown with gibberellic acid. (CT119). Horticulture Australia. 18 pages.

Treeby M. and Storey R. (1997) Sprays for albedo breakdown prevention (CT324). Horticultural Research and Development Corporation. 32 pages.

Treeby M.T. and Coote M.J. (1997) Calcium sprays for albedo breakdown prevention in navel oranges. In: 1997 National Citrus Field Day, S. Falivene (ed) (Mildura: November 11, 1997) pp. 61-63.

Treeby M.T., Milne D.J., Storey R., Bevington K.B., Loveys B.R. and Hutton R. (2000) Creasing in Australia: causes and control. In: Proceedings of International Society Citriculture, 9th Congress, 3–7 December 2000, Orlando, Florida. International Society for Citriculture (2003), pp. 1099-1103.

Storey R. and Treeby M.T. (2000) Seasonal changes in nutrient concentrations of navel orange fruit. Scientia Horticulturae 84, 67-82. https://doi.org/10.1016/S0304-4238(99)00093-X.

Treeby M. (2002) Eliminating albedo breakdown in oranges (CT98026). Horticulture Australia. 57 pages.

Storey R., Treeby M.T. and Milne J. (2002) Crease: another Ca deficiency-related fruit disorder? *The Journal of Horticultural Science and Biotechnology*, 77:5, 565-571, DOI: 10.1080/14620316.2002.11511539

Treeby M. T. and Storey R. (2002) Calcium-spray treatments for ameliorating albedo breakdown in navel oranges. *Australian Journal of Experimental Agriculture* 42, 495-502. https://doi.org/10.1071/EA00149

Storey R. and Treeby M.T. (2002) Nutrient uptake into navel oranges during fruit development. *The Journal of Horticultural Science and Biotechnology* 77(1), 91-99. https://www.tandfonline.com/doi/abs/10.1080/14620316.2002.11511463

Morris G. and Foord G. (2006). Improving citrus fruit quality using Gibberellic Acid (GA).WA FarmNote 194. 4 pages. www.doc-developpement-durable.org/file/Culture/Arbres-Fruitiers/FICHES_ARBRES/agrumes/Improving%20fruit%20quality%20using%20Gibberellic%20Acid.pdf

Bevington K., Zeng L., Falivene S., Lindhout K., Treeby M. and Storey R. (2007) Communicating the effects of production conditions on outturn quality (CT01029). Horticulture Australia Limited. 134 pages.

Lindhout K., Treeby M., Hardy S. and Bevington K. (2008) Using gibberellic acid sprays on navel oranges June 2008. NSW Department of Primary Industries. Primefact 800. 5 pages. www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/232197/Using-gibberellic-acid-sprays-on-navel-oranges.pdf (see attached)

Previous and recent research

Jona R., Goren R. and Marmora M. (1989) Effect of gibberellin on cell-wall components of creasing peel in mature Valencia orange. *Scientia Horticulturae* 39(2), 105-115. https://doi.org/10.1016/0304-4238(89)90083-6.

> *Histochemical studies showed that spraying the trees with GA in the summer (July) successfully prevented the appearance of external and internal creasing symptoms in the peel of mature Valencia fruit next April. Microscopic and histochemical analyses made it possible to detect the disorder at a very early stage of fruit development.*

Ali A., Summers L., Klein G. and Lovatt C. (1998) Crease (Albedo Breakdown) can be Predicted by Peel Thickness and Peel Nutrient Status as Early as the End of the Cell Division Stage of Fruit Development. *HortScience* 33(3), p. 498. (Conference abstract only)

> *The 2-year experiment was conducted at seven California 'Valencia' and navel orange (Citrus sinensis L. Osbeck) orchards with known differences in the incidence of crease. Maximum peel thickness, which occurs at the end of cell division during fruit development, was significantly negatively correlated with percent crease at harvest for all sites in both years. There was no significant correlation between leaf N, P, or K concentrations and maximum peel thickness or crease. There was a significant negative correlation between peel K concentration and percent crease for all sites in both years. There was a weak correlation between the incidence of crease and peel nitrogen content in both cultivars. Peel P concentration was not correlated to the incidence of crease in either cultivar. These data identified threshold values for maximum peel thickness and peel K concentration at maximum peel thickness below which the incidence of crease would be greater than 10% for both cultivars.*

González-Altozano P. and Castel J.L. (1999) effects of regulated deficit irrigation on 'Clementina De Nulesâ' citrus trees growth, yield and fruit quality. *Acta Horticulturae* 537, 749-758. DOI: 10.17660/ActaHortic.2000.537.89

> *Lower levels of RDI during September and October produced significant reductions in fruit size and external peel disorders (creasing) in a large proportion of the fruit that decreased their quality and price. Creasing occurred especially in 1998 and exclusively in the 50%-A-O treatment, where minimal psi.lc.gif a values were –0.71 MPa and –1.5 MPa in 1997 and 1998, respectively.*

González-Altozano P and Castel J.R. (1999) Regulated deficit irrigation in `Clementina de Nules' citrus trees. I. Yield and fruit quality effects. *The Journal of Horticultural Science and Biotechnology* 74(6) 706-713. DOI: 10.1080/14620316.1999.11511177

In autumn, for the 25% and 50% RDI levels there was a 25 to 11% reduction of fruit size, respectively, with some external peel disorders (creasing) which reduced fruit quality, even at the lower water stress levels reached in the 50% treatment during this period (ca 20.64 MPa and 20.83 MPa in 1995 and 1996, respectively).

Holtzman S., Greenberg J., Yacov B., Cadmon A. and Felic P. (2000) Effects of NAA and 2, 4-DP on creasing and fruit size of 'Valencia' orange. *Alon Hanotea* 54(8), 313-315.

Sprays with NAA at 300 ppm, combined with 4% potassium nitrate, applied to 22- and 30-mm diameter citrus cv. Valencia fruitlets, decreased creasing to 9% and 7%, respectively, compared to 42% in the unsprayed control. Spray of gibberellic acid at 20 ppm, acidified by 0.1% phosphoric acid, applied on mid-August, decreased creasing to 10% and had no effect on yield nor on fruit size. Sprays with 50 ppm 2,4-D, combined with 4% potassium nitrate, increased yield and fruit size but had no effect on creasing incidence.

Achilea O., Y. Soffer D. Raber and Tamim M.S. (2002) Bonus N, P, K highly concentrated enriched potassium nitrate an optimum booster for yield and quality of citrus fruits. *Acta Horticulturae* 594: 461–466. DOI: 10.17660/ActaHortic.2002.594.59

In Nova tangerine (Citrus reticulate Bla.), a single application of a 10% solution significantly increased total yield by 30% and reduced the incidence of rind creasing by 20%.

Ortuzar F.J.E., Barrales V.L., Peña R.I., Carmona M.P., Martiz M.J., Farias C.A. and Quinteros L.J. (2000) Influence of gibberellic acid spraying date on the development of creasing during ripening of Navel oranges. *Ciencia e Investigación Agraria*, 26(2), 111-118. In Spanish (English summary)

GA3 seemed to delay the evolution of albedo cracking to rind creasing of the flavedo. In Newhall, albedo cracking was reduced in May by the March and April sprays. In June, all GA3 sprays were effective at reducing creasing incidence. As the season progressed, GA3 lost its effectiveness, and by August none of the treatments prevented rind creasing. In Atwood and Frost, creasing incidence was reduced by the April spray, whereas the January spray was less effective.

Bower J.P. (2002) The physiological control of citrus creasing. *Acta Horticulturae* 632, 111-115. 10.17660/ActaHortic.2004.632.14

It is suggested that the solution to creasing is through identification of the critical restriction to pectin synthesis at any particular site, and application of the required element or elements.

Jiezhong C., Xuejuan L., Zixing Y. and Ling W. (2002) Study on the relation between mineral nutrition levels and creasing peel in mature orange. *Plant Nutrition and Fertitizer Science* 8(3), 367-371. https://europepmc.org/article/cba/389670 - abstract only. Chinese

A significant difference in creasing fruit rates was observed between shaded halves of fruits and exposed halves of fruits. Major creasing peel, above 86%, occurred in shaded halves of fruits. There was not a significant correlation between N or P contents and creasing fruits and shaded halves of fruits. Mg contents in cell wall or peel of creasing fruits and shaded halves of fruits were fewer lower than that of normal fruits and exposed halved of fruits, but there was a no significant negative correlation between Mg contents and rates of creasing fruit. Ca contents in cell was or peel of normal fruits and exposed halves of fruits were higher than that of creasing fruit. Ca contents in cell wall or peel of normal fruits and exposed halves of fruits were higher than that of creasing fruit and shaded of fruits. There was a significant negative correlation between Ca contents in peel and creasing fruit rate.

Ritenour M.A., Wardowski W.F. and Tucker D.P. (2003) Effects of water and nutrients on the postharvest quality and shelf life of citrus 1. Document HS942. Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. https://hos.ifas.ufl.edu/media/hosifasufledu/documents/pdf/in-service-training/shared-related-publications/Effects-of-Water-and-Nutrients-on-the-Postharvest-Quality-and-Shelf-Life-of-Citrus.pdf

Non-referenced observations:

- *Effects of N on creasing have been inconsistent.*
- *Creasing can also become more of a problem as P leaf levels rise between 0.10 and 0.14 percent.*
- *increasing K reduces the incidence of creasing.*

Gonzalez C.M. and Lovatt C.J. (2004) Foliar-applied aminoethoxyvinylglycine (AVG) reduces albedo breakdown of late-harvested Navel orange fruit–preliminary results. In *Proc. Int. Soc. Citricult*.

No access but AVG reduces albedo breakdown (Hussain Z. and Singh Z. (2020)–below)

Jiezhong C., Zixing Y., Biyan Z., Chunxiang X. and Juan L. (2005) Effects of pectins and pectinesterase activity on creasing fruit formation in orange (*Citrus sinensis* Osbeck). *Acta Horticulturae Sinica* 32(02), 202. http://ahs.ac.cn/EN/abstract/abstract1520.shtml

> *Examined differences in pectin level, pectinesterase activity and creasing-fruit rates between different oranges and showed the content of TP, WP, PE of the creasing fruits is higher than the normal fruit, and the content of HP is lower than normal fruit.*

Erner Y., Artzi B., Tagari E. and Hamou M. (2005) Potassium affects citrus tree performance. Department of Fruit Trees, Institute of Horticulture, The Volcani Center. Israel. pages 405-414.

> *Report that Potassium decreases the loss of fruit from creasing (Greenberg et al. 1995)*

Table 4. Effect of Potassium and Auxins on 'Valencia' Fruit Quality

Treatment	*Creasing %*	*Roughness %*	*Peel Thick. mm*
Control	42.8 a	4.7 b	5.23 b
NAA 300 ppm 4 June	5.4 c	17.7 ab	5.70 ab
NAA 300 ppm 4 July	14.9 b	10.7 ab	5.55 ab
KNO_3 4% + 2,4-D 18 ppm 7 June	23.6 b	33.4 a	6.15 a

Adapted from Greenberg et al. 1995 (Hebrew).

> *Greenberg, Y., Eshel, G., Gotfrid, A., Rozenberg, O., Katz, T., Zarka, S. and Lindenboim, H. 1995. Effects of auxins spray with NAA, 2,4-D, and 2,4DP on yield, fruit size and creasing in 'Valencia'. Alon Ha'Notea 49: 527536 (Hebrew).*

Greenberg J., Kaplan I., Fainzack M., Egozi Y. and Giladi B. (2006). Effects of auxins sprays on yield, fruit size, fruit splitting and the incidence of creasing of 'Nova' mandarin. *Acta Horticulturae* 727, 249-254. DOI: 10.17660/ActaHortic.2006.727.28

> *Effects of the plant growth regulators (PGRs) 2,4-dichlorophenoxyacetic acid (2,4-D, 40 mg.L^{-1}), naphthaleneacetic acid (NAA, 300 mg.L^{-1}) and 3,5,6-trichloro-2-piridil oxyacetic acid (3,5,6-TPA, 15 mg.L^{-1}) on yield, fruit size, fruit quality, fruit splitting and the incidence of creasing of 'Nova' mandarin [hybrid of Citrus clementina Hort. ex Tanaka × (Citrus paradisi Macf. × Citrus tangerina Hort. ex Tanaka)] were studied. All PGR solutions were tank-mixed with 'Bonus-NPK' fertilizer (5%). Sprays were applied to whole trees twice: early spray –at 13-mm fruitlet diameter and late spray–at 26-mm fruitlet diameter. The early spray of 2,4-D had no effect on the incidence of creasing. The early NAA spray, thinned fruitlets, increased fruit size, decreased splitting to 30%, decreased the incidence of creasing to 28% compared to 36% in the control, and had no effect on the yield. The late NAA spray did not thin fruit and had no effect on fruit size but the number of fruit harvested and yield was increased to 52 kg/tree due to reducing split fruit drop to 21%, and the incidence of creasing was reduced to only 10% of the fruit The late 3,5,6-TPA spray did not thin fruit, had no effect on fruit size, reduced the incidence of creasing to 22% and reduced fruit splitting to 17%, increasing yield to 52 kg/tree. These data suggest that late*

sprays of auxins on fruitlets that do not affect fruit size and do not thin fruitlets anymore, are effective for reducing fruit splitting and the incidence of creasing of 'Nova' mandarin.

Li J. (2006) Creasing fruits and related research of the cell wall metabolism in citrus. Postgraduates Dissertations. South China Agric. Univ. China (in Chinese with English abstract)

Verreynne S. (2006) Evaluation of alternative means of controlling creasing (albedo breakdown). Stellenbosch Uni outputs–South Africa

Gravina A. (2007) Aplicación del ácido giberélico en Citrus. *Agrociencia Uruguay* 11(1), 57-66. http://164.73.52.167/ojs/index.php/agrociencia/article/view/769/800

A reduction of creasing incidence and severity was found with the application of GA3 (10 - 20 mg.L^{-1}) sprayed between 90 and 120 days after flowering in "Washington" navel sweet orange.

Pham T.T.M. (2009) Pre-harvest factors affecting fruit quality in sweet oranges with an emphasis on albedo breakdown. PhD thesis. School of Agriculture and Environment. Curtin University. 192 pages. http://hdl.handle.net/20.500.11937/2300 *(also see page 19)*

The applications of deficit irrigation, exogenous 2% $Ca(NO_3)_2$ containing 'Tween 20' as a surfactant and the exogenous spray application of boron (600 mg.L^{-1}) influenced the incidence and severity of albedo breakdown. The single spray application of boron in early summer at 600 mg.L^{-1} was the most effective in increasing boron concentration in the leaf, rind and pulp of fruit, reducing the incidence of albedo breakdown in 'Washington Navel'.

Greenberg J., Holtzman S., Fainzack M., Egozi Y., Giladi B., Oren Y. and Kaplan I. (2010). Effects of NAA and GA_3 sprays on fruit size and the incidence of creasing of 'Washington' navel orange. *Acta Horticulturae* 884, 273-279. DOI: 10.17660/ActaHortic.2010.884.32

NAA (naphthaleneacetic acid, 300 mg.L^{-1}) sprays on large fruitlets, at a timing that doesn't affect fruit size or thin fruitlets, is effective for creasing reduction. When fruit thinning and enlarging fruit size is also desired, the NAA spray should be applied on fruitlets when they are smaller in size. When a major effect on creasing reduction is desired, two sequential sprays–the first with NAA and the second with GA_3 are recommended.

Li J., Zhang P., Chen J., Yao Q. and Jiang Y. (2009) Cellular wall metabolism in citrus fruit pericarp and its relation to creasing fruit rate. *Scientia Horticulturae* 122, 45-50. https://doi.org/10.1016/j.scienta.2009.03.022

Enhanced loss of pectin and cellulose in the cellular walls of peel tissue of sweet orange could result in fruit creasing.

Phiri Z.P. (2010) Creasing studies in citrus (Masters dissertation, Stellenbosch. University of Stellenbosch). 127 pages.

The position of fruit in the tree and light influenced the development of creasing and the distribution of mineral nutrients in the albedo. Creasing incidence was higher on the south side than on the north side of the tree and fruit from the inside sub-sectors had a greater creasing incidence compared to fruit from the outside sub-sectors. The shady part of outside fruit was more creased compared to the sunny part of the fruit and covering fruit with brown paper bags increased creasing severity. The light manipulation techniques used on the leaves and fruit increased the nitrogen (N), phosphorus (P), potassium (K) and manganese (Mn) concentrations in the albedo and differences in the albedo mineral nutrients amongst the sub-sectors evaluated were observed, but creasing severity or creasing incidence was not significantly correlated with the albedo mineral concentrations at harvest. Albedo mineral concentrations earlier in the season may play a role in creasing development, as creasing severity was significantly correlated with copper (Cu), K, and Mn concentrations in the albedo during stage II of fruit development. Creasing incidence and albedo mineral concentrations were not affected by any of the carbohydrate manipulation techniques used in this study. The incidence and severity of creasing was significantly reduced, with a minor negative effect on fruit rind colour development, by the application of GA3, from mid

November to mid January.

Pham T.T.T., Singh Z. and Behboudian M.H. (2012) Different surfactants improve calcium uptake into leaf and fruit of 'Washington navel' sweet orange and reduce albedo breakdown. *Journal of Plant Nutrition* 35, 889-904. https://doi.org/10.1080/01904167.2012.663442

Five foliar sprays of aqueous solution containing 2% calcium nitrate [$Ca(NO_3)_2$] and 0.05% 'Tween 20' starting from 81 days after full bloom (DAFB) at 10-day intervals improved Ca uptake in leaf, rind and pulp of fruit and reduced the incidence of albedo breakdown in 'Washington Navel' and maintained the other important fruit quality parameters.

Hussain Z. (2014) Role of polyamines and ethylene in creasing of sweet orange fruit. PhD thesis. School of Agriculture and Environment. Curtin University of Technology. 284 pages. http://hdl.handle.net/20.500.11937/1539

Higher levels of endogenous ethylene and lower levels of free polyamines (PAs) initiate the incidence of creasing in sweet orange fruit. The reduction of creasing with exogenous application of ethylene inhibitors such as putrescine, aminoethoxyvinylglycine, cobalt sulphate and acceleration of creasing with inhibitor of PAs biosynthesis and exogenous application of ethrel signifies the involvement of PAs and ethylene in creasing of sweet orange fruit.

Saleem B.A., Hassan I, Singh Z., Malik A.U. and Pervez M.A. (2014) Comparative changes in the rheological properties and cell wall metabolism in rind of healthy and creased fruit of Washington navel and navelina sweet orange ('*Citrus sinensis*' [L.] Osbeck). *Australian Journal of Crop Science* 8, 62-70.

Higher activities of pectinesterase, exo- polygalacturonase, endo- polygalacturonase, and endo-1, 4-ß -D-glucanase in the albedo of creased fruit at commercial harvest seem to be associated with the enhanced loss of pectins and starch in the cell walls of albedo tissue, leading to cell wall loosening and cracks formation consequently reducing hardness, stiffness and tensile force of the rind.

Ibánez A.M., Martinelli F., Reagan R.L., Uratsu S.L., Vo A., Tinoco M.A., Phu M.L., Chen Y., Rocke D.M. and Dandekar A.M. (2014) Transcriptome and metabolome analysis of citrus fruit to elucidate puffing disorder. *Plant Science* 217, 87-98.

"Puffing" disorder, characterized by albedo breakdown and separation between peel and pulp. This leads to disintegration of the albedo tissue, causing the formation of air spaces and albedo with weaker mechanical resistance during peeling in mature fruits. The results showed that transcriptome changes for sucrose and starch metabolism are linked to puffing disorder. Gibberellins and cytokinins probably play key roles in puffing disorder.

Hussain Z. and Singh Z. (2015). Involvement of ethylene in causation of creasing in sweet orange [*Citrus sinensis* (L.) Osbeck] fruit. *Australian Journal of Crop Science* 9(1), 1-8. www.cropj.com/singh_9_1_2015_1_8.pdf

Higher levels of endogenous ethylene in the creased fruit and promotion of the CI with the exogenous application of ethrel and its reduction with the application of ethylene inhibitors suggested the involvement of ethylene in the causation of creasing in sweet orange fruit.

Hussain Z. and Singh Z. (2015) Involvement of polyamines in creasing of sweet orange [*Citrus sinensis* (L.) Osbeck] fruit. *Scientia Horticulturae* 190, 203-210. https://doi.org/10.1016/j.scienta.2015.04.013.

Putrescine application increased endogenous free PUT, SPD, SPM in the albedo and flavedo tissues of the fruit. PUT (500–1000 µM) applied at fruit set or golf ball stage was more effective in reducing creasing. Application of MGBG (1000 µM) at the golf ball stage significantly increased CI in sweet orange fruit.

Elharouny S.B., Ahmed F.K. and Abdel-Aziz R.A. (2015) The role of protein contents and enzyme activity on creasing of Washington navel orange fruits. *Egyptian Journal of Horticulture* 42(1), 1-15. DOI 10.21608/EJOH.2014.1064

Creasing percentage was increased progressively with fruit aging, and creasing incidence was relatively influenced by geographical direction (more pronounced in fruits of northern tree periphery–Northern hemisphere = Egypt). Meanwhile, the protein banding patterns of albedo and flavedo total proteins exhibit the association between some particular protein types and the changing in citrus peel tissue from healthy to crease. Moreover, the higher amount of PG-ase release was tended to be closely related to albedo taken of creased fruits compared to non creased ones.

Ahmad S. and Singh Z. and Iqbal Z. (2016) Tree and cold storage influence on incidence of albedo breakdown, textural properties of the rind and fruit quality in 'Washington Navel' orange. *Fruits* 71 (3), 131-139. DOI: 10.1051/fruits/2015057

The cold-stored fruit exhibited a higher rind hardness, rind tensile force, firmness and SCC:TA ratio, lower concentrations of citric acid, malic acid, fructose and glucose, and lower albedo breakdown incidence than the tree-stored fruit. These findings indicate a preference for cold storage over tree storage for the orange fruit quality.

Li J., Liang C., Liu X., Huai B., Chen J., Yao Q., Qin Y., Liu Z. and Luo X. (2016) Effect of Zn and NAA co-treatment on the occurrence of creasing fruit and the peel development of 'Shatangju' mandarin. *Scientia Horticulturae* 201, 230-237. https://doi.org/10.1016/j.scienta.2016.01.039

The Zn and Zn + NAA (naphthaleneacetic acid) treatments enhanced the levels of IAA, GA3, and tryptophan, promoted the division of cells, decreased variation of peel hardness. These treatments reduced creasing abnormalities in peel tissue and lowered the creasing fruit rate and cumulative abscission rate of 'Shatangju' mandarin. The Zn + NAA treatment could be recommended as a cultivation technique to lower the creasing fruit rate of Shatangju mandarin.

Sallato B., Bonomelli C. and Martiz J. (2017) Differences in quality parameters and nutrient composition in Fukumoto oranges with and without creasing symptoms. *Journal of Plant Nutrition* 40, 954-963. DOI: 10.1080/01904167.2016.1184278

Oranges with creasing had larger weight and fruit size, higher water content in the pulp and whole fruit, lower coarse rind grading, thinner peel, higher nitrogen content in the pulp and the whole fruit, higher potassium content in all fruit components and lower calcium content and concentration. Magnesium content, only in flavedo showed significant differences, being higher in fruit without creasing. Fruit size and Ca were key factors related to the expression of creasing disorder, as well as nutrient balance.

Review - Li J. and Chen J. (2017) Citrus fruit-cracking: Causes and occurrence. *Horticultural Plant Journal.* Volume 3(6), 255-260. https://doi.org/10.1016/j.hpj.2017.08.002.

This paper summarized the mechanism of citrus creasing or cracking and further explained the effects of genetic factors and environmental factors (light, temperature, humidity, mineral nutrition and plant growth regulators) on citrus fruit creasing or cracking rate.

Asim M., ul Haque E., Ashraf T., Hayat A. and Aziz A. (2018) application of plant growth regulator and potassium nitrate to improve the quality and yield in Washington Navel oranges (Citrus sinensis). *World Journal of Biology and Biotechnology*, 3(3), 209-213.

Creasing was effectively minimized by spraying plants in combination of GA_3 and KNO_3.

OMAR A.E.D.K. and EL-ENIN M.S.A. (2018) effects of different irrigation regimes on fruit quality and exportability of 'Washington' Navel orange fruit (Citrus sinensis l.). '5th International Conference on Sustainable Agriculture and Environment (5th ICSAE) October 08-10, 2018, Hammamet, Tunisia. Pages 416-420.

Deficit irrigation (80% of field capacity (FC) (T1), 70% FC (T2), 60% FC (T3)) reduced creasing.

Review – Garmendia A., Beltrán R., Zornoza C., García-Breijo F.J., Reig J., Merle H. (2019) Gibberellic acid in Citrus spp. flowering and fruiting: A systematic review. *PLoS One* 14(9):e0223147. doi: 10.1371/journal.pone.0223147

This is an excellent review of GA in all citrus production. In relation to albedo breakdown, treatments likes $CaCl_2$, $Ca(NO_3)_2$, Zn, Zn + NAA (naphthaleneacetic acid), NAA, NAA + GA_3 and only GA_3 have been tested to control creasing [6,61–63]. $CaCl_2$ treatments (0.33%) cause unacceptable fruit drop and leaf damage [62]. The best results were obtained with two sequential sprays: the first with NAA in May and the second with GA_3 in August which reduced the incidence of creasing from 36% to only 3% of the fruits [6]. The early NAA spray (May) thinned 14% of the fruitlets and increased the size of the remaining fruit [6]. Gibberellic acid by itself does not seem to be able to completely control creasing [61]. In addition, late applications can affect rind quality inducing regreening of fruit [62].

Hussain Z. and Singh Z. (2020) Role of aminoethoxyvinylglycine in creasing of sweet orange [*Citrus sinensis* (L.) Osbeck] fruit. *Journal of Pure and Applied Agriculture* 5(1) 1-10. https://jpaa.aiou.edu.pk/?p=878

Aminoethoxyvinylglycine (AVG) a naturally occurring reversible ethylene inhibitor (used in apple industry as ReTain®) and was effective to reduce creasing and improve the fruit quality. The creasing was significantly reduced when AVG (60 mg/l) was sprayed at Golf ball size (28 and 24%) stage with respect to control (52 and 52%) in Washington Navel. The AVG application at fruit set stage (23%) was more effective than the control (51%) in cv. Lane Late sweet orange during second harvest season. In conclusion application of AVG significantly alleviates the creasing (%) and improves the textural properties of sweet oranges cultivars.

Hussain Z. (2020). Creasing in sweet orange: role of cobalt sulfate. *Acta Horticulturae* 1299, 133-140. DOI: 10.17660/ActaHortic.2020.1299.20

Cobalt sulfate ($CoSO_4$) was applied as an ethylene inhibitor with different concentrations. Creasing was significantly reduced when a higher concentration of $CoSO_4$ (500 mg L^{-1}) was sprayed at golf ball stage (29 and 34%) stage than control (51 and 57%) in Washington Navel sweet orange during 2011 and 2012, while similar findings were noticed in 'Lane Late'. It is concluded from the current study that the exogenous spray applications of $CoSO_4$ significantly mitigate the incidence of creasing and improve textural properties of 'Washington Navel' and 'Lane Late' fruit.

Effect of climate

Nathan Hancock (Citrus Australia) was interested in the climatic effects of albedo breakdown. This was taken from:

Bevington K., Zeng L., Falivene S., Lindhout K., Treeby M. and Storey R. (2007) Communicating the effects of production conditions on outturn quality (CT01029). Horticulture Australia Limited. 134 pages.

Relationship to seasonal conditions

Climatic factors

Monthly heat unit and rainfall data were examined to see if any trends could be related to the high incidence of rind breakdown seen during the 2000 export season and the much lower incidence recorded from 2001 – 2005. Analysis of climatic data showed that the 1999/00 growing season was characterised by above average heat unit accumulation during inflorescence development and flowering in September and October and throughout most of the Stage II cell expansion phase of fruit growth from February to April (Fig. 2.8). For both periods, observed heat unit accumulation was the highest recorded since the USA export program commenced in 1992. Data presented in Fig. 2.8 are based on temperature data recorded at Renmark in the Riverland. Similar seasonal trends were also evident for centres located in Sunraysia and the Riverina.

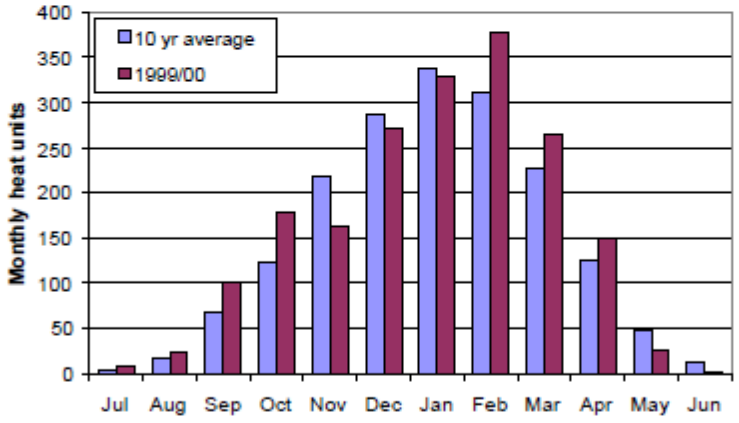


Figure 2.8. Trends in monthly heat units (degree-day sums >13°C) calculated from temperature data recorded at Renmark, South Australia. Ten year average: 1995/96 to 2004/05.

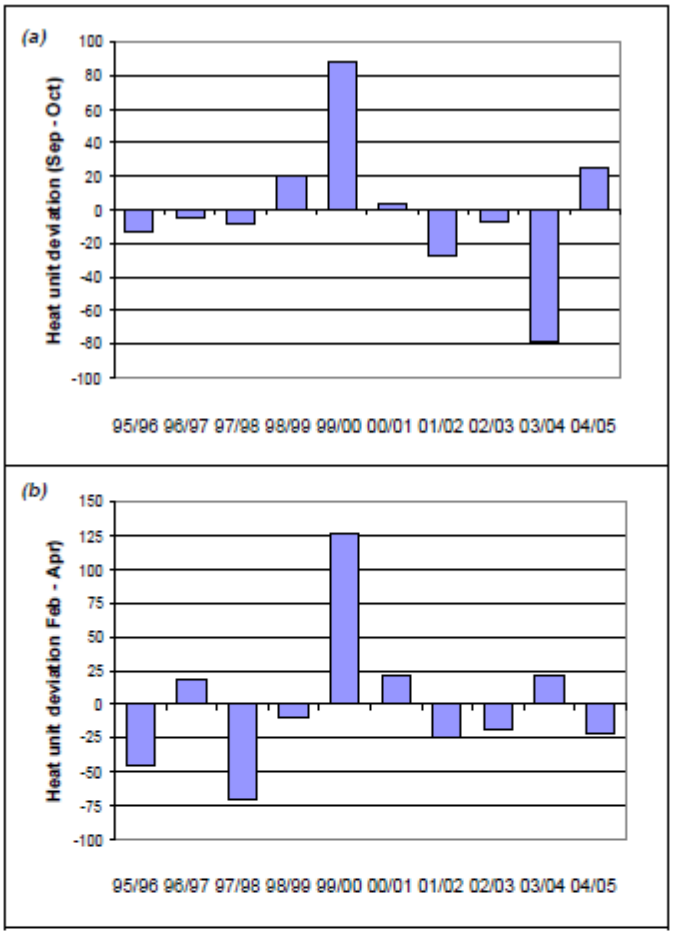


Figure 2.9. Deviation in heat unit accumulation (degree-day sums >13°C) from 10-year average during (a) September – October and (b) February – April periods from 1995/96 to 2004/05. Heat units calculated from temperature data recorded at Renmark, South Australia.

The unique pattern of heat unit accumulation observed in 1999/00 during September – October and February – April is further highlighted in Fig. 2.9 which shows annual deviations in accumulated heat units from the 10-year average. Following 1999/00, heat unit accumulation was close to average in all years during the February – April period and, with the exception of 2003/04 – characterised by a relatively cool spring, close to average during the September – October period.

In addition to above average heat unit accumulation, the 1999/00 growing season was also characterised by above average rainfall throughout most of the year (Fig. 2.10). The late summer/autumn period from February to April was the wettest since the export program to the USA began. Following 1999/00, rainfall during this period was well below average ranging from 40 mm in 2000/01 to a low of 7 mm in 2003/04. During the

five years following 1999/00 the average February – April rainfall at Renmark was only 21 mm compared to a long term average of 45 mm.

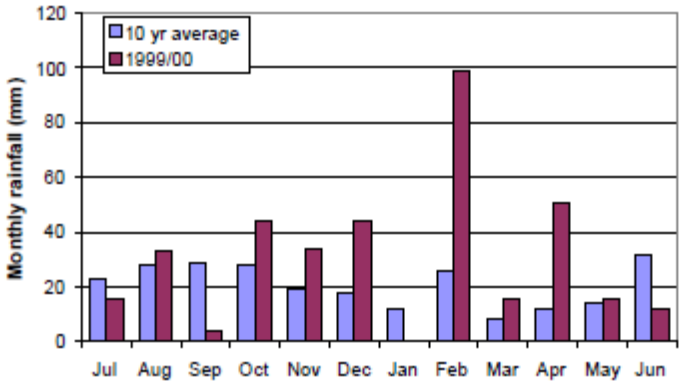


Figure 2.10. Monthly rainfall data recorded at Renmark, South Australia. Ten year average: 1995/96 to 2004/05.

The above average heat unit accumulation and rainfall patterns observed during 1999/00, especially during the latter stages of Stage II fruit growth, were most likely conducive to the early onset of rind senescence and reduced postharvest shelf-life of fruit. Conditions experienced during the 1999/00 growing season were in marked contrast to the seasonal conditions experienced in the five years following 1999/00, a period of relatively low rind breakdown, and would appear to have been a major factor in the outturn problems experienced in 2000.

Phenological indicators

The 1999/2000 growing season was an early season characterised by very early flowering, early fruit maturity and exceptional fruit size at harvest considering district crop loads. Based on annual observations of flowering carried out since 1979 in the Riverland, flowering for the 1999/00 growing season was the earliest recorded date of flowering over the 26 seasons data were collected. Flowering was two weeks earlier than the median flowering date of 12 October (Table 2.12). The very early flowering in combination with the climatic factors highlighted above may well have been an additional factor contributing to the outturn problems experienced during the 2000 export season. Following 1999/00, flowering was also relatively early in the 2000/01 and 2001/02 growing seasons but there were few outturn problems in those years. In the absence of climatic conditions promoting early rind senescence, early flowering per se may, therefore, not necessarily be detrimental.

Table 2.12. Dates of full bloom recorded in the Riverland for the 1999/2000 to 2004/2005 growing seasons and range in flowering dates from 1979/80 to 2004/05.

1999/00	2000/01	2001/02	2002/03	2003/04	2004/05	Range
28 Sep	2 Oct	3 Oct	10 Oct	24 Oct	10 Oct	28 Sep - 25 Oct

Source: P. Gallasch, SARDI Loxton

Flowering in 2003/04 was among the latest flowering dates (Table 2.12). The very late flowering observed in 2003/04 reflected the below average heat unit accumulation which occurred during September and October (Fig. 2.9). Interestingly, the lowest levels of quality defects at outturn were also recorded in 2004 (see Fig. 2.2). Comments by Riversun staff at the time were that the fruit were some of the best seen for many years, which may have been a reflection of the younger physiological age of the fruit at the start of harvest.

There is a strong belief among packers that once acid levels drop below a certain threshold (approximately 0.7 – 0.8%) fruit are less suitable for export, especially to long distance markets, because of an increased risk of outturn problems and reduced shelf-life. Records of early season maturity tests of district growers' fruit conducted at Dareton from 2000 to 2005 show an early decline in acid levels in 1999/00 (Fig. 2.11). The early decline in acid levels seen in 1999/00 may have been a further factor contributing to outturn problems. However, in addition to 1999/00, early fruit maturity was also evident in 2004/05, a season of relatively low rind breakdown, suggesting that in the absence of other factors a direct link between low acidity or early maturity and increased susceptibility of fruit to postharvest rind breakdown may not be that strong.

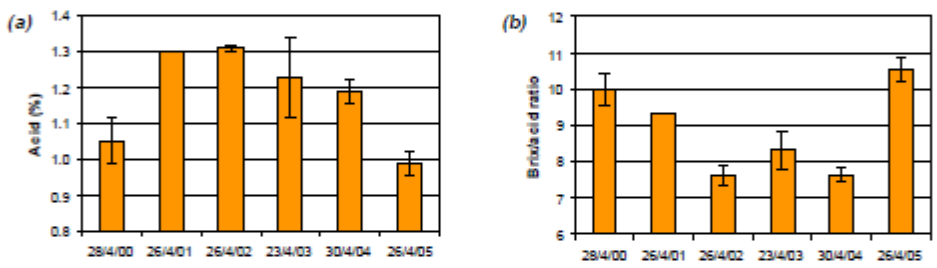


Figure 2.11. Seasonal variation in (a) acidity and (b) Brix/acid ratio of Navelina oranges in Sunraysia district orchards from 2000 to 2005.

Although the 1999/2000 season was an on-crop year, average fruit size at harvest was much better than might be expected on the basis of tree crop loads. As part of its annual crop forecasting program, the Murray Valley Citrus Board (MVCB) collects data on tree crop loads (fruit density counts) and fruit size distribution for each of the major citrus varieties. Washington navel data collected by the board from 1991 to 2003 show a negative linear relationship between average fruit diameter at the end of the Stage II growth in May and fruit density count (Fig. 2.12). Within the data set, the season showing the greatest departure from the relationship is 1999/2000. The enhanced fruit growth seen in 1999/2000 reflects the very early flowering and the above average temperatures and rainfall evident throughout much of the Stage II period of growth. Although there are no experimental data linking an extended period of active fruit growth in navel oranges with postharvest rind breakdown, the possibility of such an association can not be entirely ruled out.

Discussion

Over the course of the project the high outturn losses seen in 2000 due to postharvest rind breakdown did not occur again. The average incidence of skin defects detected at arrival inspection decreased from 4.0 % in 2000 to 1.4 % in 2005 and serious skin defects from 1.1 % to 0.3 %. Some further outturn problems were experienced in 2003 and 2005 but losses were no where near as great as recorded in 2000, and other quality issues, in particular decay, were also involved.

The high incidence of rind breakdown seen in 2000 can be directly attributed to the unusual growing conditions experienced during the 1999/00 growing season. Rather than being the result of a single factor it is more likely that a combination of factors were responsible including above average spring temperatures leading to early flowering and rapid early fruit growth and the warm autumn conditions combined with above average rainfall which may have promoted early rind senescence. The conclusion of an overriding influence of seasonal conditions is supported by the observation that all packing sheds experienced problems to some degree in 2000, irrespective of growing area, and that seasonal trends following 2000 were basically the same for all sheds. Importantly, the precise seasonal conditions experienced in 1999/00 have not occurred since and had not been seen prior to 1999/00. Although no objective data are available from 2000, the hypothesis of the early onset of rind senescence is supported by the increasing incidence of rind breakdown seen as the season progressed. Photographic records of damaged fruit from 2000 also clearly indicate signs of over maturity.

In addition to climate, on-farm factors that may have contributed to the outturn problems experienced in 2000 were reports of low gibberellic acid (GA) use and poor effectiveness of copper sprays. GA sprays are essential to maintain rind condition and extend the effective harvest period. However, unconfirmed reports suggest that GA use may not have been as widespread as usual because of good early fruit size and the expectation that fruit could be harvested early. If this in fact was the case, then low GA use would have

Current recommendations (from NSW DPI)

Current understanding and recommendations are summarised in the NSW DPI Citrus plant protection guide (Creek and Falivene, 2021):

Creek A. and Falivene S. (2021). Citrus plant protection guide 2021–22. NSW DPI Management Guide. (August 2021). 98 pages. www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/1187654/Citrus-plant-protection-guide-2021.pdf

Creasing (albedo breakdown)

Symptoms

Creasing is also known as albedo breakdown. It is often a seasonal issue that can reduce packouts and increase the risk of fruit breakdown during transit. Fruit with albedo breakdown have a weak rind that is susceptible to cracking (Figure 95). Cracked rinds allow disease pathogens to establish and decay the fruit. Fruit with creasing are more likely to split when packed (Figure 96).

Cause

Creasing is where the underlying albedo layer collapses, giving the fruit rind a wrinkled appearance. The causes of creasing are not fully understood. The signs normally appear at the later stages of fruit maturity and are often worse on the inward facing, shaded part of the fruit. Albedo breakdown appears to be more a physiological disorder rather than a nutrient-deficiency problem.

Management

Choose varieties that are not susceptible; generally, early-maturing varieties are more susceptible than late-maturing varieties. Growers can often recognise blocks in their orchard that are more susceptible to albedo breakdown than others.

A high cropping level is thought to be associated with albedo breakdown. However, this could be a factor of the same climatic conditions that induced the high crop load. It is also associated with thin rinds; rinds normally become thinner at high crop loads.

Applying preharvest gibberellic acid (GA) and calcium foliar sprays reduces creasing. Susceptibility differs between scion varieties and rootstocks. Pruned trees have less fruit with creasing. Mature fruit rinds are more prone to creasing (Figure 97).

Calcium sprays can also help, however, research indicates that multiple sprays (i.e. 5–10) are required to provide the same level of control as a single GA application. The best time to apply foliar calcium sprays is in January and early February.

Although calcium is associated with the disorder, research shows that applying extra calcium fertiliser to soils already having sufficient levels of calcium does not alleviate the problem.

Spray coverage is essential, especially for the shaded part of the fruit that tends to exhibit worse symptoms than the unshaded side of the fruit.

Figure 95. Typical albedo breakdown in a navel orange.

Figure 96. A close up of the rind separation.

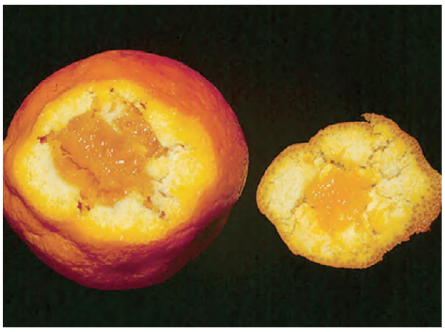


Figure 97. Severe creasing in an over-mature mandarin.

S

WA albedo breakdown PhD study (Pham, 2009)

Pham T.T.M. (2009) Pre-harvest factors affecting fruit quality in sweet oranges with an emphasis on albedo breakdown. PhD thesis. School of Agriculture and Environment. Curtin University of Technology. 192 pages. http://hdl.handle.net/20.500.11937/2300

Abstract. Albedo breakdown known as creasing, a physiological disorder, due to abnormal separation of cells leading to the formation of irregular fractures in the white tissue (albedo) causing the creases of sweet orange rind. It causes serious economic losses to the sweet orange growers in Australia and in other orange producing areas of the world. Fruit quality, particularly albedo breakdown has been influenced by various factors such as plant water relations, genetic factors, plant nutritional status and plant growth regulators. My research investigated the development of the incidence and the severity of albedo breakdown during fruit maturation and ripening, the effects of severity of albedo breakdown on fruit quality among locations and cultivars of 'Navel' sweet orange. I also elucidated the influence of deficit regulated irrigation, exogenous application of surfactants added in calcium solution, exogenous application of boron and the role of ethylene in the incidence of albedo breakdown, textural properties of the rind and fruit quality of 'Navel' sweet oranges. The incidence and the severity of albedo breakdown increased rapidly after commercial harvest. The incidence and severity of albedo breakdown in 'Washington Navel' orange differed from location to location, with the lowest at Harvey as compared to three other locations. Regardless of locations and cultivars, the severity of albedo breakdown did not affect juice content, soluble solids concentration, titratable acidity, ascorbic acid, citric acid and malic acid except for decreasing succinic acid and increasing tartaric acid. Locations and cultivars significantly influenced these fruit quality parameters. The application of deficit irrigation (50% and 75% water supply of control trees) improved fruit quality in terms of increased soluble solids concentrations and acidity levels without affecting percentage of juice, pH of juice, ascorbic acid and individual organic acids in 'Navelina' sweet orange. The enhancement of the uptake of Ca in leaf, rind, and pulp of the fruit and the reduction in the incidence of albedo breakdown were obtained with the application of different surfactants added into aqueous solutions of 2% $Ca(NO_3)]_2$ starting from 81 days after full bloom (DAFB) at 10-day intervals. Among four tested surfactants, 'Tween 20' (0.05%) was the most effective in enhancing Ca uptake, reducing albedo breakdown and improving textural properties of rind and fruit firmness while maintaining the other important fruit quality attributes in 'Washington Navel' sweet orange. The foliar application of boron enhanced the concentration of boron and calcium in the leaf, rind and pulp. The single spray application of boron in early summer at 600 mg.L^{-1} was the most effective in increasing boron concentration in the leaf, rind and pulp of fruit, reducing the incidence of albedo breakdown and improving textural properties of rind and fruit firmness without affecting any the other fruit quality attributes in 'Washington Navel' sweet orange. Rind of fruit with albedo breakdown produced the higher ethylene production than the normal fruit. The exogenous application of ethylene inhibitors including AVG (200 mg.L^{-1}) and $CoSO_4$ (300 mg.$L^{-1)}$ reduced the incidence of albedo breakdown and improved the rind textural properties in 'Washington Navel' sweet orange. Ethylene seems to be involved in the incidence of albedo breakdown. In conclusion, the severity of albedo breakdown did not affect the major attributes of fruit quality in 'Navel' sweet oranges. The applications of deficit irrigation, exogenous 2% $Ca(NO_3)_2$ containing 'Tween 20' as a surfactant and the exogenous spray application of boron (600 mg.L^{-1}) influenced the incidence and severity of albedo breakdown without affecting other fruit quality parameters. Ethylene seems to be associated with the incidence of albedo breakdown in 'Washington Navel' sweet orange.