Horticulture Innovation Australia

Final Report

National Citrus Postharvest Science Program

Dr Peter Taverner
South Australian Research and Development Institute (SARDI)

Project Number: CT10006

CT10006

This project has been funded by Horticulture Innovation Australia Limited using funds from the Australian Government and the following sources:

South Australian Research and Development Institute (SARDI)
Citrus (R&D Levy)

ISBN 0 7341 3778 8

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

Contents

Summary

The citrus industry has for many years supported a national citrus postharvest program to assist packers as they adjust to a changing competitive market. Packers have benefited from an outstanding level of practical research that has contributed to improved returns for growers.

Packers require timely advice and information on the latest postharvest advances. The program has a strong extension and advice component. Generally, a mix of activities, including seminars, workshops, individual shed visit and newsletters, was the approach used to maximise reach. The Packer Newsletter has developed a solid reputation for providing sound practical information.

The program also offered an advisory desk providing advice for specific issues. Generally, timely advice could be provided by phone or email. Where detailed information was not available, research trials were conducted for individual companies. Packing-shed visits provided line specific results, and formed the basis for more general reporting to regional and export packer groups.

The 'showcase' shed program was a new initiative. SARDI mentored key packers over several seasons to improve and incorporate best practice into their commercial packing lines. We were aiming to maximise the benefits in a few 'showcase' packing lines, which then become models for the whole industry. We encouraged showcase sheds to have 'open' demonstrations of new technologies adapted to their packing lines. The activities of this initiative were disseminated through our packer newsletter and other extension services.

We instigated the first systematic fungicide resistance survey in Australian citrus packing sheds. Surveys collected mould from packing lines that could not be controlled with 3 times the label rate of fungicide. As a result, we established regular monitoring in packing lines and SARDI developed a 'scorecard', which forecast the risk of fungicide resistance developing. The participating packers indicated they wanted to see fungicide resistance monitoring continued. We are working with other industry service providers to establish a modified fungicide resistance service.

New fungicide actives were registered during the life of this project. SARDI was an active participant of the working group that was successful in freeing up the restraints for these fungicide treatments on citrus fruit exported to Japan from Australia. Australian packers can now treat their fruit with fludioxonil and pyrimethanil for export markets. SARDI has improved packer knowledge in the use of these new fungicides through seminars, newsletters, info-notes and the Citrus Australia postharvest technical forum.

SARDI also worked with Syngenta Australia to evaluate a new fungicide for the control of sour rot in citrus. Evaluation over several seasons has provided data for registration of a dual active fungicide for the control of mould and sour rot. This activity was a high priority in 2011 Citrus Strategic Agrichemical Review Process (SARP) and will assist in the management of fungicide resistance.

Broad priorities for future citrus postharvest research include:

- Maintenance of a nationally focussed citrus postharvest program ensuring a technical capability relevant to citrus packers.
- Research into mixtures of fungicides, sanitisers and food preservatives for the control of postharvest diseases.
- Resistance monitoring of postharvest fungicides.

- Research into sustainable control of postharvest diseases with the lowest possible pesticide residue on produce.
- Development of seminar/workshop programs with regular regional visits by technical staff.
- Production of practical publications for citrus packers. In particular, continuation of the Packer Newsletter.

Keywords

Citrus, postharvest; fungicide resistance; generally regarded as safe (GRAS) compounds; mould; sour rot.

Introduction

The citrus industry has for many years supported a National citrus postharvest program, through the citrus R&D levy. The National program has provided the technical capacity for packers to adjust to a changing competitive market. Packers have benefited from an outstanding level of practical research that has contributed to improved returns for growers. This project supersedes CT08007 – 'The national coordinated citrus postharvest program supported through SARDI' as the core program to support a national postharvest service.

At the time, the opportunity existed to link our postharvest expertise to a citrus drought recovery project (CT08014 – 'Citrus Drought Survival and Recovery Trial') and provide some answers to the 'storage life' of citrus under irrigation deficit and after full recovery. The results will provide information about the mid- to long-term keeping characteristics of droughted citrus fruit.

Postharvest fungicides, fludioxonil and pyrimethanil, are new active ingredients with greater environmental safety ('reduced risk'), and are being introduced worldwide. These are the first new fungicide actives to be registered for postharvest use in citrus for at least 20 years. Their introduction is timely because there are concerns with declining efficacy using existing fungicides, potential resistance issues, and an increasing need to reduce the residues of these 'older' chemicals on fruit (stricter MRLs). The introduction of fludioxonil and pyrimethanil presents a rare opportunity to review our postharvest fungicide schedules and practices. It was important to evaluate these fungicides for our fruit and market conditions, to assess their compatibility with other chemicals (e.g. sanitisers) and generally integrate them into our existing practices. The result of the evaluation needed to be extended to packers and then to integrate the practices to satisfy quality, resistance and residue issues.

This proposal continued the extension functions of previous citrus postharvest programs. The team provides technical advice, conducting packers' workshops, maintains an industry review committee and publishes the Packer Newsletter.

'Showcase sheds' is a new initiative that combines research and extension. This initiative involves services to key packers over several seasons to respond, improve and incorporate best practice into their commercial packinglines. We aimed to maximise the benefits in a few 'showcase' packinglines, which then became working models for the whole industry.

This proposal met several criteria listed in the relevant Citrus Industry Strategic Plan, such as to increase demand for Australian citrus and to improve industry communication and information systems. In particular, strategy 1.3.4 'Develop and promote best practice growing and post-harvest management to increase fruit quality' and strategy 3.3 'Enhance the value and delivery of industry information. In regards to strategy 1.3.4, the proposal will provide R&D for continuous improvement of post-harvest technologies to maintain quality from orchard to consumer. In regard to strategy 3.3, the proposal has a strong extension component to deliver information that meets defined industry and commercial needs.

Methodology

Technical advice and extension service

Packers require timely advice and practical information about the latest postharvest advances. It was intended that the activities conducted in previous programs were substantially continued. Packers continue to seek technical advice from the SARDI postharvest group. The Packer Newsletter and workshops on sanitisers/fungicides have been well received in the past and would continue. The Packer Newsletter website was updated. Packing-shed visits were conducted to provide line specific results, and formed the basis for more general reporting to regional and export packer groups. Postharvest meetings with industry stakeholders were convened to discuss research results. In addition, we intended to publish a new citrus handling guide.

Activities

- Technical advice
- Cittgroup/grower presentations
- Packer Newsletter
- Sanitation Workshops
- Industry meetings
- Published revised Citrus Handling Guide

Showcase sheds initiative

This initiative involved annual services to key packers over several seasons to improve and incorporate best practice into their commercial packinglines. We were aiming to maximise the benefits in a few 'showcase' packinglines, which then became a model for the whole industry. The service for individual exporters/packers involved yearly fees for the following services:

- Packingline Audit (1 per year) Pre- or Post- Seasonal Review
- Packingline Survey (alternating each year) Sanitation Survey (Year 1) & Fungicide Survey (Year 2)
- Fungicide Resistance Testing (3 per year) DIY test kit with technical support
- Project Review Committee Membership to review of activities and latest research results
- 'On-Site' Research Trials (ad hoc) Trials designed for individual packingline lines

The results of surveys and research trials in showcase sheds were used to produce reports for distribution to all packers. We encouraged showcase sheds to have 'open' demonstrations of new technologies adapted to their packinglines. The activities of this initiative were disseminated through our packer newsletter and other extension services (see above).

Postharvest assessment of fruit from CT08014 – 'Citrus Drought Survival and Recovery Trial'

HAL project CT08014 – Citrus Drought Survival and Recovery Trial was funded as a Voluntary Contribution project from 2008 to 2013. This trial applied 2 levels of irrigation restriction to citrus trees, and a range of management strategies, to measure the impact of the management strategies on survival, yield and recovery of trees under restricted irrigation. These conditions mirrored the impacts of the recent drought in the Murray Darling Basin on citrus growers in the Riverland and Sunraysia districts. Yield and fruit quality were measured as part of project CT08014, but only at harvest. This project planned to look at postharvest impacts of drought. The results provided information about the mid- to long-term keeping characteristics of droughted citrus fruit.

Fruit were harvested each season from the CT08014 trials and then transferred to the citrus postharvest laboratories at the Waite Campus for cool storage evaluation. The fruit were treated according to best postharvest practice and evaluated for weight loss, disease and disorders over an extended 'simulated voyage'. Based on the results in the first season, a range of treatments, such as varying wax types, were used to improve storage quality. Different irrigation regimes were compared within each season, and comparisons across seasons were inferred. The results were extended with

other results from CT08014.

Activities

- Use establish sites for sources of 'droughted' and 'recovery' fruit (with Mark Skewes)
- Fruit collection, preliminary assessment and treatments applied
- Simulated 'voyage' conditions and over-storage of fruit
- 'Market' assessment of postharvest, disease, disorder and quality
- Extension of results

Integrating new postharvest fungicides & resistance management strategies into existing practices

In Australia, many traditional postharvest fungicides have been removed for use on export fruit or are being critically reviewed. New fungicide actives, such as fludioxonil and pyrimethanil, became registered during the life of this project. The registration of new fungicide groups for postharvest use is rare and should be carefully introduced to ensure the highest efficacy, comply with chemical residue limits and provide the least opportunity of developing fungicide resistance. We aimed to provide the information to allow these fungicides to be sustainably integrated into the Australian situation.

Research was undertaken to establish whether these chemicals could be enhanced by heating or combining with GRAS compounds, such as postharvest oil, sodium carbonate, sodium bicarbonate and calcium polysulphide to improve efficacy. Promising treatments conducted in the laboratory to assess the control of moulds, Rhizopus rot and sour rot should be evaluated in the packingline. The compatibility of various mixtures on fruit quality after appropriate cool storage was also assessed.

Activities

- Combination efficacy laboratory bioassays
- Combination compatibility laboratory bioassays
- Packingline evaluation
- Packingline evaluation of 'new' fungicides

Adoption strategies

The strategies for adoption were extending the information directly to the packers and growers by using the Packer Newsletter, seminars and workshops. These activities were on-going throughout the program. Workshops are well regarded and commenced early in the program and research results were distributed throughout the course of the program. The grower bodies, export groups, and individual packers were participants in these research activities and were provided with information. The results from the collaboration with CT08014 – 'Citrus Drought Survival and Recovery Trial' will be extended to growers through Regional boards/Cittgroups, articles in industry media and with extension activities of CT08014.

The progress was peer reviewed by SARDI to ensure milestones were completed. The program had a strong collaboration between the research staff and representatives of grower bodies, export groups, and individual packers. This ensured that the program continued to have a relevant industry focus. Postharvest research coordination meetings were scheduled each season to evaluate priorities and coordinate research efforts. Alternative arrangements were necessary after the disbanding of the regional marketing boards and Riversun (export group) and the program became aligned to the new Citrus Australia peak body.

The success of adoption was related to the ability to communicate the new technology to citrus packers and growers, and their willingness to 'take up' the technology. This project relied on established networks during the early part of the program to ensure that the work was relevant and well communicated throughout the citrus industry. In collaboration with the Regional Citrus Boards, Cittgroups, State Departments and packingshed personnel, the research team conducted trials and workshops in different regions. Specific activities planned to enhance the adoption of technologies are described below:

1) Any packer participating in shed trials will receive a concise confidential report with results specific to their shed.
2) Detailed reports on laboratory trials will be distributed to citrus marketing boards, Riversun Export P/L and participating chemical suppliers.
3) In addition, concise results and recommendations will be published in the Packer Newsletter and/or other industry media.
4) Workshops and seminars will be conducted in citrus regions demonstrating new technologies, with separate tailored presentations for growers and packers.

Outputs

2011

Droughted fruit report (2011)

Showcase sheds initiative (2011)

Fungicide Survey Reports (2011) – General survey report (see appendices) released through Citrus Industry Bodies and summarized in the Packer Newsletter. Individual client reports for Lochert Bros., Pacific Fresh, GoldenWest, Mildura Fruit Company, Venus Citrus & AgriExchange. The surveys assessed decay management and fungicide residues. Fungicide residues were included due to MRL breaches in 2010.

Fungicide resistance survey report (2011) – Incorporated into the general fungicide report. Individual client reports of resistance results as per fungicide survey reports.

Pre- or Post-seasonal review (2011) –Meetings held with participant packingshed managers to review packing practices and chemical use (Pre-season) and debrief of postharvest success and issues during season (post-season). Participants; Mildura Fruit Company, AgriExchange and Venus Citrus.

Client report (Syngenta) (2011) – evaluation of propiconazole was undertaken by SARDI to provide a fungicide for the control of sour rot in citrus. Sour rot was identified as a priority in 2010 industry meetings. This and other work conducted later in this program provides the data required to register the product in Australia.

Strategic Agrichemical Review Process (SARP) meeting (2011) – Provided advice on current postharvest chemical use and emerging use strategies (e.g., propiconazole) for the SARP review. There was also discussion of approaches to control quarantine pests. We were directed to coordinate the submission 'in-line approaches for the control of surface pest of concern to citrus', subsequently led by PFR New Zealand.

Pre-season seminar series (2011) – Grower/packer meetings held in the three major citrus-growing regions; Riverland, Sunraysia and Riverina. Presentation on decay control best practices.

Steering committee meeting (2011) – National industry committee of citrus growers/packers, grower/marketing bodies, researchers and HAL representatives. Presented an overview of the new project and sought feedback and direction on issues in past season.

Six extension articles published in the Packer Newsletter:
Peter Taverner (2011). Fungicide survey – is resistance blowing in the wind? Packer Newsletter 101.
Peter Taverner (2011). Strippers – how closely are you watching? Packer Newsletter 101.
Peter Taverner (2011). History never repeats. Packer Newsletter 102.
Peter Taverner (2011). Timely advice from 25 years ago. Packer Newsletter 102.

Peter Taverner (2011). 1986 revisited in 2011. Packer Newsletter 102.
Peter Taverner (2011). Anthracnose – a short precautionary tale. Packer Newsletter 102.

2012

Showcase sheds initiative (2012)

Sanitation Survey Reports (2012) – General survey report (see appendices) released through Citrus Industry Bodies and summarized in the Packer Newsletter. Individual client reports for Mildura Fruit Company, Venus Citrus & AgriExchange. The surveys assessed mould spores and overall colony forming units on fruit at different points in the packingline process.

Fungicide resistance survey report (2012) – Incorporated into the general survey report. Individual client reports of resistance results as per sanitation survey reports.

Pre- or Post-seasonal review (2012) –Meetings held with participant packingshed managers to review packing practices and chemical use (Pre-season) and debrief of postharvest success and issues during season (post-season). Participants; Mildura Fruit Company, AgriExchange and Venus Citrus.

Client report (Syngenta) (2012) – evaluation of dual active product was undertaken by SARDI to provide a fungicide for the control of sour rot and green mould in citrus.

Pre-season seminar series (2012) – Grower/packer meetings held in the three major citrus-growing regions; Riverland, Sunraysia and Riverina. Presentation on maintaining fungicides – topping up and stripping out.

Mid-season seminar series (2012) - Grower/packer meetings held in Central Burnett and Emerald regions. Presentation on decay control and new fungicides.

Steering committee meeting (2012) – National industry committee of citrus growers/packers, grower/marketing bodies, researchers and HAL representatives. Presented an overview of the new project and sought feedback and direction on issues in past season.

Peter Taverner. Citrus Postharvest Science. Oral presentation at the National Citrus Pathology Meeting August 2015, Brisbane, QLD.
Peter Taverner and Nancy Cunningham (2012). Current and emerging strategies for control of sour rot in Australia. Oral Presentation. International Citrus Congress November 2012, Valencia, Spain.
Peter Taverner and Nancy Cunningham (2012). Tips for growers to improve out-turns and their returns. Citrus Australia National Conference, October, Leeton, New South Wales.

Twelve extension articles published in the Packer Newsletter:
Peter Taverner (2012). Degreening of citrus. Packer Newsletter 103.
Peter Taverner (2012). Tips for postharvest Anthracnose control of citrus. Packer Newsletter 103.
Peter Taverner (2012). SOPP. Packer Newsletter 103.
Peter Taverner (2012). Sanitation Surveys 2012. Packer Newsletter 104.
Peter Taverner (2012). Oleocellosis. Newsletter 104.
Peter Taverner (2012). Citrus black rot. Packer Newsletter 104.
Peter Taverner (2012). Fungicide Resistance Surveys 2012. Packer Newsletter 104.
Peter Taverner (2012). Packingshed designs to reduce fungicide resistant mould. Packer Newsletter 104.
Nancy Cunningham (2012). Fungicides and sanitisers – new products; new compatibility issues. Packer Newsletter 105 (reprint).
Brian Wild (2012). Chlorine aggravates chilling injury. Packer Newsletter 105 (reprint).
Nancy Cunningham (2012). Fungicide formulations and compatibility. Packer Newsletter 105 (reprint).
Peter Taverner (2012). Measuring peracetic acid – or should that be hydrogen peroxide? Packer

Newsletter 105.

2013

Showcase sheds initiative (2013)

Fungicide resistance survey reports (2013) – Incorporated into the general fungicide report. Individual client reports of resistance results as per sanitation survey reports.

Pre- or Post-seasonal review (2013) –Meetings held with participant packingshed managers to review packing practices and chemical use (Pre-season) and debrief of postharvest success and issues during season (post-season). Participants; Mildura Fruit Company, AgriExchange and Venus Citrus.

Client report (Syngenta) (2013) – Further evaluation of dual active products was undertaken by SARDI to provide a fungicide for the control of sour rot and green mould in citrus.

Peter Taverner (2013). New fungicides. Seminars, Griffith, NSW, Mildura, Vic., and Waikerie, SA. 2013.
Peter Taverner (2013). Decay Management – fungicide application and timing. Seminars, Griffith, NSW, Mildura, Vic., and Waikerie, SA. 2013.

Thirteen extension articles published in the Packer Newsletter:
Peter Taverner (2013). Early season degreening and associated practices (condensed version). Packer Newsletter 106.
Peter Taverner (2013). Ethylene conditioning to reduce chilling injury and non-chilling injury in mature fruit. Packer Newsletter 106.
Peter Taverner (2013). Compatibility of peracetic acid with fungicides and salt mixtures. Packer Newsletter 106.
Peter Taverner (2013). Degreening mandarins (condensed version). Packer Newsletter 106
Peter Taverner (2013). Sanitation – back to basics. Packer Newsletter 107.
Peter Taverner (2013). Postharvest Pitting. Packer Newsletter 107.
Nancy Cunningham (2013). Compatibility of fungicides and fungicide mixtures in dips. Packer Newsletter 107.
Peter Taverner (2013). Brush burn. Packer Newsletter 107
Peter Taverner (2013). Water pH and chlorination. Packer Newsletter 108
Peter Taverner (2013). Age-related rind breakdown. Packer Newsletter 108
Peter Taverner (2013). The efficacy of fungicides, peracetic acid and salt. Packer Newsletter 108
Peter Taverner (2013). Drench Phytotoxicity. Packer Newsletter 108
Peter Taverner (2013). Are fungicide label rates becoming obsolete? Packer Newsletter 108
Other extension articles
Peter Taverner. Study throws lifeline to export companies. Feature article. SA Grower, February 2013.

2014

Showcase sheds initiative (2014)

Fungicide resistance survey reports (2014) –Individual client reports of resistance results 3 times during season.

Pre- or Post-seasonal review (2014) –Meetings held with participant packingshed managers to review packing practices and chemical use (Pre-season) and debrief of postharvest success and issues during season (post-season). Participants; Seven Fields, Nippys Waikerie Producers, Mildura Fruit Company, AgriExchange and Venus Citrus.

Client report (Syngenta) (2014) – Further evaluation of dual active products was undertaken by

SARDI to provide a fungicide for the control of sour rot and green mould in citrus.

Seventeen extension articles published in the Packer Newsletter:
Peter Taverner (2014). A few maintenance hints for the off season. Packer Newsletter 109.
Peter Taverner (2014). Can mouldy fruit infect nearby healthy fruit? Packer Newsletter 109.
Peter Taverner (2014). The trials and tribulations of conducting fungicide resistance surveys. Packer Newsletter 109
Peter Taverner (2014). Septoria spot. Packer Newsletter 109
Peter Taverner (2014). Degreening practices. Packer Newsletter 110
Peter Taverner (2014). Tips for the control of anthracnose. Packer Newsletter 110
Peter Taverner (2014). Caltex Prospect®— postharvest fruit treatment for control of surface pests. Packer Newsletter 110
Peter Taverner (2014). Fungicide use is heating up. Packer Newsletter 111.
Peter Taverner (2014). Flocculating water using alum. Packer Newsletter 111.
Peter Taverner (2014). Chilling injury – in its many forms. Packer Newsletter 111
Peter Taverner (2014). Wax defects. Packer Newsletter 111
Peter Taverner (2014). Integrated postharvest management (IPHM). Packer Newsletter 112
Peter Taverner (2014). Integrating 'reduce' risk' fungicides into current practice. Packer Newsletter 112
Peter Taverner (2014). Adding SBC to high pressure washers. Packer Newsletter 112
Peter Taverner (2014). Integrating Scholar®. Packer Newsletter 112
Peter Taverner (2014). Age-related breakdown and frost. Packer Newsletter 112
Peter Taverner (2014). Integrating PHILABUSTER®. Packer Newsletter 112
Peter Taverner (2014). Coolroom disinfection (the IPHM way). Packer Newsletter 113
Other extension articles
Peter Taverner (2014). Reduce risk fungicides. Citrus Postharvest Info Note. October 2014.
Peter Taverner (2014). Peracetic acid. Citrus Postharvest Info Note. November 2014.

2015

Citrus Australia Forum & Field Day, Mildura, Victoria, March 2015. – coordinated postharvest seminars and packing shed demonstrations.

Packing shed visits with David Sorenson (Fruit Growers Supply Company, California). Two days touring packing sheds in Sunraysia and Riverland areas to discuss packing line design with packers. Recent advances in sanitation. Oral presentation. Citrus Technical 2015 Forum & Field Day, Mildura, Victoria, 17th March 2015.

Client report (Syngenta) (2015) – evaluation of fungicide and sanitizer mixtures was undertaken by SARDI to provide a compatibility, phytotoxicity and efficacy on citrus.

Five extension articles published in the Packer Newsletter:
Peter Taverner (2015). Web information update. Packer Newsletter 114
Peter Taverner (2015). Sodium bicarbonate with high pressure washing – an ode to Dave. Packer Newsletter 114
Peter Taverner (2015). Zebra skin. Packer Newsletter 114
Peter Taverner (2015). Using sodium bicarbonate – understanding how and why. Packer Newsletter 114
Peter Taverner (2015). The cost of delaying the dumping of fungicides. Packer Newsletter 114

In addition, there have been numerous calls and emails every season from packers, service providers and consultants seeking advice and information on citrus postharvest problems or best practice procedures.

Outcomes

Technical advice and extension service

The program had a strong extension and advice component. Generally, a mix of activities, including seminars, workshops, individual shed visits and newsletters, was the approach used to maximise reach. The Packer newsletter is particularly effective in influencing packer behavior. Over the years, it has created a solid reputation for providing sound practical information. In a recent industry survey commissioned by Citrus Australia, packers rated SARDI's postharvest information very highly, with 76% of respondents rating the Packer newsletter as 'very good' (highest category).

Specific comments by packers in the survey included the following:

- I found Dr Taverner extremely helpful in troubleshooting fungicide residue issues we were experiencing this season. He seems to have a very practical and methodical approach to problem solving.
- Peter Taverner's advice to our business has been excellent and proven to be essential in ensuring perfect out-turn in the market.
- This research has been my bible. Without it we would be unable to keep up with competing countries product quality. It is critical as chemicals are being delisted and new ones registered.
- The newsletter in particular has been of benefit to our business
- The program has been accessible at all times. It has provided timely, accurate advice to me and my business.

The program also offered an advisory desk providing advice for specific issues. In some instances, this led to trials for individual companies. In one instance, a major packer had poor returns over several seasons due to chilling injury. After auditing the cool chain, no obvious reason was apparent. SARDI undertook trials using different chemicals, waxing and the storage conditions applicable to that packer to determine influence on chilling injury. The packer introduced new practices based on trial results and chilling injury has not re-appeared in the subsequent two seasons.

Showcase sheds initiative

This initiative involved annual services to a few key packers over several seasons to improve and incorporate best practice into their commercial packinglines. The 'showcase' packinglines benefited from the service and other packers requested similar service during the project. Unfortunately, there were not enough resources to provide direct service to more packers but the results and trends were published in the packer newsletter.

Fungicide surveys and sanitation audits were scheduled annually but were abandoned in years 3 & 4 due to lack of industry funds. Overall, the first survey results showed that packingline procedures were usually sound, with evidence of monitoring and hygiene. High-pressure washing, sanitised fungicide tanks and wash brushes are significant improvements implemented over recent years. In-line applications were normally sufficient to control decay on inoculated fruit added to the line. New practices were promoted after previous poor survey results and the improvement during this program was evidence that new guidelines were working.

Surveys indicated a trend toward fungicide resistance in packingsheds. Some isolates collected from packing lines and re-inoculated onto fruit decayed and produced spores, despite being treated with 3 times the label rate of fungicide. As a result, survey work in later years was concentrated on fungicide resistance monitoring. SARDI started a fungicide resistance service for citrus packers participating in the 'Showcase Sheds' initiative. The service was well received and other packers requested to be included in the service. SARDI developed a fungicide resistance 'scorecard', which forecast the risk of fungicide resistance developing. The participating packers indicated they wanted to see the fungicide resistance monitoring continued. SARDI is currently working with a local ag-chemical distributor (E.E. Muirs & Sons) and fungicide company (Janssen PMP) to establish a modified fungicide resistance

service.

Postharvest assessment of fruit from CT08014 – 'Citrus Drought Survival and Recovery Trial'

Grower and packer practices both contribute to market out-turn. Recent droughts highlighted a lack of knowledge about the shelf life of citrus fruit from trees recovering from drought. The citrus drought recovery trial allowed us to assess some of these effects and extend the results to packers and growers. Fortunately, citrus growers have not experienced similar drought conditions since the project was completed. However, we now have a better understanding of the resilience of citrus trees which will guide grower's decisions during any future drought.

Integrating new postharvest fungicides & resistance management strategies into existing practices

Prior to the program, fungicide MRL's on exported Australian citrus were exceeded, resulting in costly re-direction of fruit. SARDI was instrumental in providing optimal targets for fungicide residues and educating packers in appropriate methods to ensure meeting those levels. Emphasis has been on monitoring fungicide residues on fruit and using residue levels to ensure good decay control without exceeding the MRL's for importing countries. In recent years, MRL breaches are uncommon.

New fungicide actives, such as fludioxonil and pyrimethanil, were registered during the life of this project. SARDI was an active participant of the working group that was recently successful in freeing up the restraints for post-harvest fungicide treatments on citrus fruit exported to Japan from Australia. Australian packers can now treat their fruit with fludioxonil and pyrimethanil for export markets. The registration of new fungicide groups for postharvest use is rare and should be carefully introduced to ensure the highest efficacy and to comply with chemical residue limits. SARDI has improved packer knowledge in how to integrate these new fungicides into their packing operations through seminars, newsletters, info-notes and the Citrus Australia postharvest technical forum.

In some circumstances, these new fungicide actives are not as efficacious as the older fungicides they will be replacing. Research to enhance these chemicals by combining with GRAS compounds, such as postharvest oil, sodium carbonate, sodium bicarbonate and calcium polysulphide, or heated to improve efficacy was commenced during this program. This work promotes the industry objective to reduce residue on citrus fruit (ultra-low residue citrus).

Evaluation and Discussion

Influence of citrus industry restructure

During the life of this project there were significant changes to the citrus industry bodies traditionally associated with our activities. We attempted to limit the disruption to services, but some change was unavoidable.

Initially, the SARDI postharvest group continued its strong alliance with Riversun Export, which represented the major Tri-State packers exporting to the USA, and the three regional citrus boards: Riverina Citrus, Murray Valley Citrus Board and the Citrus Board of South Australia. They were an important conduit for receiving intelligence on out-turns and disseminating information to packers. Equally important to us, the boards provided industry funds, which when matched by federal funding under the voluntary contributions arrangement, provide ~50% of CT10006's annual budget. Riversun also provided 'ad hoc' funding to SARDI to respond to unexpected issues in markets. The arrangement with Riversun and the regional boards had worked well for previous projects and there was a strong expectation that it would continue to be successful during the life of this project.

Early in the project, industry restructuring meant that the regional marketing boards were considered redundant and were progressively disbanded. During this period, the USA program and Riversun's influence waned. Citrus Australia proved to be a vibrant new national peak body and quickly filled the role as conduit for market intelligence and packer dissemination. However, the voluntary contributions were lost. This required variations to CT10006 in 2012 & 2013. The loss of funding reduced the outputs in later years, as can be seen in the outputs section. To mitigate, some additional industry funding was obtained through Syngenta Australia to evaluate a new fungicide for the control of sour rot. Improved control of sour rot was rated highly during the 2011 Citrus Strategic Agrichemical Review Process (SARP).

Despite disruption, our experience and networks allowed us to maintain many planned activities. However, the value of the program was compromised, with the loss of significant industry funding. Extra industry funds obtained were tied to the laboratory evaluation of a new Syngenta fungicide and some previously agreed extension activities were reduced. A reduction in regional activity was criticised but available funds were 'tied' to other activities. We lost staff and were also less able to respond to unforeseen issues arising during the season. These were difficult choices because regional services and the capably to respond were highly regarded outputs in previous projects.

Technical advice and extension service

This project relied on established networks during the early part of the program to ensure that the work was relevant and well communicated throughout the citrus industry. In collaboration with the Regional Citrus Boards, Cittgroups, State Departments and packingshed personnel, the research team conducted trials and workshops in different regions. Later in the program the regional board and Cittgroups were disbanded, which affected the established networks. As previously mentioned, it was difficult to maintain services with less funds and some previously agreed regional extension activities were reduced. We maintained the packer newsletter because it had the widest reach and was of good reputation. The reduction in regional activities, such as workshops and individual packer visits, was criticised, which indicates they are a priority of packers.

Later in the program, Citrus Australia provided new extension opportunities. For instance, SARDI coordinated and facilitated the Citrus Australia Post Harvest Technical Forum held in Mildura, in March 2015. This event was part of the Citrus Australia Conference and helped to attract over 300 attendees. The event was extremely well received with a list of quality presenters delivering the latest in best management of post-harvest treatments.

Showcase sheds initiative

This initiative involved annual services to a few key packers over several seasons to improve and

incorporate best practice into their commercial packinglines. We were hoping to maximise the benefits in a few 'showcase' packinglines, which then become models for the whole industry. The participating packers certainly benefited and readily contributed an annual fee for the service (under the voluntary contributions arrangements). The results from the 'showcase' packinglines were disseminated through seminars and articles in the packer newsletter. We encouraged showcase sheds to have 'open' demonstrations of new technologies adapted to their packinglines. The field day at Seven Fields packing facility was very popular, which was held during the Citrus Australia postharvest technical forum. This provided information for change but packers prefer 'one on one' interaction. Packers requested more regional shed visits &/or participation in a 'showcase' type program.

The fungicide and sanitation survey results showed that packingline procedures were usually sound, with evidence of monitoring and hygiene. High-pressure washing, sanitised fungicide tanks and wash brushes are significant improvements implemented over recent years. Our guidelines were working but there were some warning signs from fungicide resistance surveys. Imazalil and thiabendazole have been used exclusively to control postharvest decay on export citrus fruit consignments. Packers are holding treated fruit for longer on premises and there are less 'quiet' periods to conduct major sanitation measures. All of which, are likely to proliferate resistance to these fungicides.

Australian citrus packers had not been systematically sampled for fungicide resistance prior to this work. The initial plate surveys were instigated in participating packing sheds, as part of the showcase sheds initiative. Through the survey, it quickly became evident that fungicide resistance isolates could be found in packing sheds at most times (see reports in appendices for more detail). From this work, we identified a need to monitor and to explain the implication of the results to packers. The ability to respond to these issues were important and the survey results meant more effort went into the 'Integrating new postharvest fungicides & resistance management strategies into existing practices' activity.

We also developed a new activity with Syngenta Australia to evaluate a new fungicide for the control of sour rot. Evaluation over several seasons has provided data for registration of a dual active fungicide for the control of mould and sour rot. This activity was a high priority in the 2011 Citrus Strategic Agrichemical Review Process (SARP) and will assist in management of fungicide resistance.

<u>Postharvest assessment of fruit from CT08014 – 'Citrus Drought Survival and Recovery Trial'</u>

The citrus drought recovery trial was opportunistic. We proposed the work because CT08014 – 'Citrus Drought Survival and Recovery Trial' was evaluating production factors but postharvest quality was ignored. The citrus fruit was available through CT08014 and it allowed us to assess some of the postharvest effects. During the drought, the production was poor and postharvest storage ability was also compromised. We discovered that citrus trees quickly recover when water was returned and that postharvest storage of fruit from those trees was excellent. Fortunately, citrus growers have not experienced similar drought conditions since the project was completed. However, we now have a better understanding of the resilience of citrus trees which will guide grower's decisions during any future drought.

<u>Integrating new postharvest fungicides & resistance management strategies into existing practices</u>

The active constituents in Scholar and Philabuster are designated food additives in Japan but Japanese supermarkets were reticent to allow new fungicide actives on citrus. SARDI was an active participant of the working group that was successful in freeing up the restraints for post-harvest fungicide treatments on citrus fruit exported to Japan from Australia. This has led to Australian packers now being able to treat their fruit with fludioxonil and pyrimethanil for export markets.

Although fungicides, such as imazalil and thiabendazole, are under regular review and potential withdrawal, the major impetus for integrating these products into the packing lines is resistance. Scholar and Philabuster are the first new postharvest fungicides since imazalil, and have actives with new modes of action. During the program, SARDI has promoted use patterns that should avoid resistance developing. It is highly recommended to alternate or mix fungicides with different modes

of action to reduce the risk of selecting for resistance. SARDI has promoted this approach and some 'showcase' packers have adopted the use of new 'reduce risk' fungicides for resistance management. Conducting fungicide resistance surveys has been a useful in creating awareness of the risk of resistance and in demonstrating the value of fungicide rotation.

Alternating with reduce risk fungicides will reduce the overall use of older fungicides. Alternating is preferred, but mixing a reduced risk fungicide with older fungicides will sometimes be the best practical option. SARDI's aim is to encourage resistance management by preferentially replacing older fungicides when practical during the season.

Recommendations

In 2014, a survey, commissioned by Citrus Australia, indicated that packers wanted to retain a National Citrus Postharvest Science Program. They particularly valued information on fungicides, sanitisers and their compatibility. Extension was also a priority and packers preferred a mix of activities. Regional seminars, workshops and individual packer visits were rated highly. In regard to printed material, the Packer Newsletter was greatly valued; as were sanitiser compatibility posters/charts.

During the current research program (CT10006), fungicide resistance surveys indicated increased levels of resistance to older fungicides (imazalil and thiabendazole). Recently, there have been two 'reduced risk' fungicides (fludioxonil and pyrimethanil) registered for postharvest use on citrus, and another fungicide (propiconazole) has commenced the registration process. There has also been interest in augmenting decay control by heating fungicides and the use of food preservatives, such as sodium bicarbonate and potassium sorbate. The trend is to reduce reliance on conventional fungicides by promoting 'reduce risk' fungicides and ultimately, using mixtures of sanitisers, 'generally regarded as safe' compounds and non-chemical means.

A national program provides linkages to associated postharvest projects. The project CT14001 'Zero residue concept – scoping study for citrus' examined the possibilities for Australian citrus to reduce chemical residues on citrus. The trend for using safer alternatives coincides with an increasing emphasis on food safety by local and export markets. Research is required for orchard and postharvest practices to meet the challenge of reducing residues on fruit.

Quarantine pests, such as Fullers rose weevil, are costly to control in orchards and are limiting exports to emerging Asian markets. In-line treatments of Caltex Prospect® and high pressure washes control Citrophilus mealybug and lightbrown apple moth on citrus and similar approaches are being evaluated in the recent project CT 13010 'In-line approaches to control surface pests of concern on export citrus'. Treatments identified by CT 13010 will require integration into current commercial practices, preferably with the assistance of researchers familiar with packinglines.

Mandarin production is increasing and they provide special challenges for export marketing. The influence of wax type and other storage parameters on taste is being investigated in CT12023 'Enhancing the export performance of Australian mandarins by improving flavour quality'. A national program provides the platform to offer postharvest technical advice to these related activities, industry bodies, exporters and government agencies.

<u>Broad priorities for future citrus postharvest research</u>

- Maintenance of a nationally focussed citrus postharvest program ensuring a technical capability in pathology, fruit physiology and surface pest disinfestation.
- Research into mixtures of conventional fungicides, sanitisers and food preservatives for the control of postharvest diseases. Resistance monitoring of conventional fungicides.
- Research into sustainable control of postharvest diseases with the lowest possible pesticide residue on produce. Treatments should be selected and applied in a manner that maintains product quality and shelf-life while minimizing risks to human health and the environment.
- Development of seminar/workshop programs with regular regional visits by technical staff.
- Production of practical publications for citrus packers. In particular, continuation of the Packer Newsletter.
- Capability to provide high level advice to government and industry groups/representatives on complex technical matters associated with the postharvest handling of citrus. This

includes advice to State, Federal and International agencies relating to food quality, food safety and market access.

- Develops and maintains a network of contacts across government, research and industry sectors. Effectively liaises with people at all levels and ensures important information is shared.
- Capability to respond effectively to diverse and urgent postharvest issues as they arise during the season.

Scientific Refereed Publications

Journal article

Peter Taverner, Nancy M. Cunningham, and Annunziata T. Leo, 2015, Current and emerging strategies for sour rot management of citrus in Australia. *Acta Hort. (ISHS*) **1065**:1555-1562.

Retrieve from http://www.actahort.org/books/1065/1065_198.htm

Intellectual Property/Commercialisation

No commercial IP generated

Acknowledgements

I would like to acknowledge my co-authors for this document and the reports in the appendices; Nancy Cunningham, Nancy Leo and Karolina Steciuk.

There are too many individuals to thank. However, I would like to acknowledge the following bodies and companies for their help and support over the life of this complex project; Citrus Australia, Syngenta Crop Protection, Mildura Fruit Company, Venus Citrus, AgriExchange, Nippys Waikerie Producers, Seven Fields, E.E. Muir & Sons Pty. Ltd., Colin Campbell (Chemicals) Pty Ltd, Riverina Citrus, Murray Valley Citrus Board, and the Citrus Board of South Australia.

Appendices

List of technical reports included

1. POSTHARVEST EXAMINATION OF DROUGHT AFFECTED FRUIT (CROSS SEASONAL ANALYSIS) (2011)
2. FUNGICIDE SURVEY OF CITRUS PACKINGSHEDS IN RIVERLAND, SUNRAYSIA AND RIVERINA (2011)
3. SANITATION SURVEY OF CITRUS PACKINGSHEDS (2012)
4. DEVELOPMENT OF FUNGICIDE RESISTANCE MONITORING SERVICE TO CITRUS PACKERS (2014)
5. FUNGICIDE RESISTANCE SURVEYS OF COMMERCIAL CITRUS PACKINGSHEDS – 2011-2014
6. EFFICACY AND COMPATIBILITY OF FUNGICIDE, ADDITIVES AND PERACETIC ACID MIXTURES (2015).

Appendix 1

Postharvest examination of drought affected fruit (cross seasonal analysis)

INTRODUCTION

A long period of drought has affected much of south eastern Australia's citrus growing areas over the past 10 years, culminating in extreme temperatures during the 2009 summer. In 2008 SARDI scientists, based in the Riverland, began to investigate the effect of reduced irrigation on citrus plantings in the Loxton area. The aim of the trial was to examine the effects of restricted irrigation on survival, yield and recovery of citrus trees. The conditions used in the trial mirrored the impacts of the current drought affecting the main citrus growing areas. However, researchers also felt that the effect of reduced irrigation on shelf life of fruit also warranted investigation.

Initial drought work for the HAL project CT08014 – Citrus Drought Survival and Recovery Trial was to apply 2 levels of irrigation restriction to citrus trees (plus control, 100% irrigation), and a range of management strategies, to measure the impact of the management strategies on survival, yield and recovery of trees under restricted irrigation. These conditions reflected the impacts of the current drought in the Murray Darling Basin on citrus growers in the Riverland and Sunraysia districts. From initial work this progressed to looking only at the irrigation restriction effects minus the management strategies from the first season's harvest.

Fruit from trees under restrictions may appear suitable for the fresh market at harvest, and may enter the fresh fruit chain, but we are not well informed about the implications of stress on the mid to long term keeping characteristics of citrus fruit. Research on irrigation effects in citrus overseas (Garcia-Tejero et al, 2010) suggested that deficit irrigation gave better organoleptic characteristics to citrus fruit after harvest, but this has not been established for fruit that has had severe deficit irrigation (<40%) here in Australia.

Fruit samples were assessed for quality following storage. With the storage program designed to match the storage conditions of an export market such as the USA.

General Methods

2009 growing season

The work for this initial season focused on reducing water allocation within experimental plots to 33% and 67% of 'normal' allocation with a range of management treatments.

Irrigation Levels

Trees were irrigated with 100% or 67% of full citrus evapotranspiration (initially 33% irrigation schedules were included in fruit collection but due to little or no fruit being harvested from affected trees they were excluded for further postharvest assessments this season). The reference level of 100% irrigation represents 'normal practice' for the property, and is scheduled based on soil water monitoring equipment (EnviroSCAN) in an adjacent planting patch. (Skewes, 2009)
The reduced irrigation rate is applied by applying a full depth irrigation event on 2 out of every 3 days on which the 100% treatment is irrigated.

Tree Management

The 100% irrigation treatment is a control, and has no tree management treatments applied.
The 67% irrigation treatment has combinations of three different treatments applied, as described below. These treatments aim to reduce water stress, and allow trees to carry a normal crop load while using less water.

Aquaboost AG30 is a polyacrylamide water retention product that slows water movement through soil, holding more water in the upper rootzone, and encouraging lateral movement of water. It was applied through the irrigation system every 5 weeks throughout the irrigation season.
Jeffries Recover (AS4454) is mulch composed of a combination of organic compost and recycled wood-chips, which reduces surface evaporation and encourages root growth near the soil surface. It was applied as a band extending a metre either side of the tree row, with the drip-line placed on top of the mulch.

Screen is a kaolin clay product that forms a film on plant surfaces, reducing leaf temperature and stress associated with high temperature and water deficit. It was applied at a high rate at the beginning of the trial, and reapplied at a lower rate on a monthly basis, or immediately prior to conditions of very high temperature.
The following combinations of these management treatments were applied:

- **100% Cont** – 100% irrigation with none of the above treatments;
- **67% Cont** – 67% irrigation with none of the above treatments;
- **67% AgSc** – 67% irrigation with 'AG30' Polyacrylamide + 'Screen' Kaolin Clay Film;
- **67% MulSc** – 67% irrigation with 'Recover' Mulch + 'Screen' Kaolin Clay Film;
- **67% All** – 67% irrigation with 'AG30' Polyacrylamide + 'Recover' Mulch + 'Screen' Kaolin Clay Film.

METHODS - POSTHARVEST

In December 2009, the trees described above were harvested, and the fruit sent to a commercial packingshed. Fruit from replicates was bulked into field treatments (100% Control, 67% Control, 67% AgSc, 67% MulSc, 67% All), and washed, sanitised and treated with two commercial postharvest fungicides, imazalil and thiabendazole, as well as a commercial food grade wax. Some fruit from each treatment were left unwaxed and untreated. Treatments can be seen in Table 1.

Table 1: Field and post harvest treatments

Irrigation Treatment	*Field Treatment*	*Postharvest Treatment*
100%	Control	Fungicide/Wax
100%	Control	Nil
67%	Control	Fungicide/Wax
67%	Control	Nil
67%	MulSc	Fungicide/Wax
67%	MulSc	Nil
67%	AgSc	Fungicide/Wax
67%	AgSc	Nil
67%	All	Fungicide/Wax
67%	All	Nil

Samples of the treated and untreated fruit were taken for assessment of postharvest storage life. The fruit samples were divided into 2 groups. One group of fruit per treatment was stored at 20 degrees Celsius for 12 weeks, and assessed weekly for mould, button health, Albedo breakdown, oleocellosis, stem end blackening and dehydration. A subset of these (5 fruit per treatment) was weighed weekly until 7 weeks and then assessed for juice content, brix, acid and taste after 8 weeks.

The second group of fruit per treatment was stored at 3 degrees Celsius for 12 weeks, and assessed weekly for mould, button health, Albedo breakdown, oleocellosis, stem end blackening and dehydration. A subset of these (5 fruit per treatment) was removed from cool storage after 5 weeks, and held at 20 degrees for a further 2 weeks, weighed weekly, and then assessed for juice, brix, acid etc. after 8 weeks. Storage treatments are demonstrated in Table 2

Table 2: Storage treatments

Week	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Gp1	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C
Subset	20°C	20°C	20°C	20°C	20°C	20°C	20°C	*				
Gp2	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C
Subset	3°C	3°C	3°C	3°C	3°C	20°C	20°C	*				

* Fruit destructively sampled for juice, brix and acid in week 8.

2010 GROWING SEASON

The work for this second season began in the recovery phase of the drought with the focus being on effects of reduced water allocation within experimental plots.

IRRIGATION LEVELS

As this was the recovery phase, postharvest assessment included 33% and 67% of 'normal' after 1 or 2 years of irrigation treatment.

TREE MANAGEMENT

The 100% irrigation treatment is a control. No tree management treatments applied during the recovery phase of the program

METHODS - POSTHARVEST

Citrus fruit trees treated with different irrigation regimes as described above were harvested and sent to a commercial packingshed. The fruit was washed, sanitised and treated with a commercial postharvest fungicides containing imazalil.

A proportion of the fruit was treated with a commercial food grade wax. Some of the fruit was left unwaxed and untreated. Fruit was divided into 2 groups, one was left at ambient (20°C ± 2°C) or placed in cool room temperatures (3°C ± 1°C). Treatments and storage temperatures can be seen in Table 3 and Table 4. After 5 weeks the fruit stored at 3°C being weighed was removed to ambient for a further 2 weeks before disposal. The rest of the fruit was left to 'overstore' at cool room temperatures (until week 12). Although official recording of fruit quality ceased after 12 weeks, fruit was held until significant deterioration was evident.

Table 3: Field and postharvest treatments.

Irrigation	Cropping treat - Recovery	postharvest treatment
100%	-	Unwaxed/untreated, fungicide/wax
33%	1 year	Unwaxed/untreated, fungicide/wax
33%	2 year	Unwaxed/untreated, fungicide/wax
67%	1 year	Unwaxed/untreated, fungicide/wax
67%	2 year	Unwaxed/untreated, fungicide/wax

Samples of the treated and untreated fruit were taken for assessment of postharvest storage life. The fruit samples were divided into 2 groups. One group of fruit per treatment was stored at 20 degrees Celsius for 12 weeks, and assessed weekly for mould, button health, oleocellosis, and general blemish. A subset of these (5 fruit per treatment) was weighed weekly until 8 weeks and then assessed for juice content, brix, acid and taste.

The second group of fruit per treatment was stored at 3 degrees Celsius for 12 weeks, and assessed weekly for mould, button health, oleocellosis, and general blemish. A subset of these (5 fruit per

treatment) was removed from cool storage after 6 weeks, and held at 20 degrees for a further 2 weeks, weighed weekly, and then assessed for juice, brix, acid etc. after 8 weeks.

Five fruit from each crate were subdivided and weighed on a weekly basis. Blemishes and mould development was also assessed on a weekly basis. Blemishes caused by tree oleocellosis, insect and wind damage were assessed in the first week only. After 8 weeks this fruit was assessed for Brix/Acid levels, juice content and taste (off flavours).

Storage treatments are demonstrated in Table 4

Table 4: storage treatments

Week	1	2	3	4	5	6	7	8	9	10	11	12
Gp1	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C
Subset	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C*				
Gp2	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C
Subset	3°C	3°C	3°C	3°C	3°C	3°C	20°C	20°C*				

* Fruit destructively sampled for juice, brix and acid in week 8.

2011 GROWING SEASON

The work for this third season was during the recovery phase of the drought with the focus being on effects of reduced water allocation within experimental plots

TREE MANAGEMENT

The 100% irrigation treatment is a control. No tree management treatments applied during the recovery phase of the program.

METHODS - POSTHARVEST

Citrus fruit trees treated with different irrigation regimes were harvested and processed in the SARDI Entomology labs. The fruit was washed, sanitised and treated with a commercial postharvest fungicide, containing imazalil. Fruit was then treated with a commercial food grade wax. However, some fruit for weight loss experiments at ambient temperatures were left unwaxed. Fruit was placed in cool room temperatures (3°C ± 1°C). Fruit for weight loss experiments were divided into 3 groups, two groups were left at ambient (20°C ± 2°C) either waxed or unwaxed and one group placed with the bulk of the other fruit at 3°C. After 6 weeks the fruit for weight loss experiments at 3°C was removed to ambient for a further 2 weeks before organoleptic tests were carried out and the fruit disposed. The rest of the fruit was left to 'overstore' at cool room temperatures (until week 12). Treatments can be seen in Table 5.

Table 5: Field and postharvest treatments.

Irrigation	Cropping treat - Recovery	postharvest treatment
100%	-	fungicide/no wax, fungicide/wax
33%	1 year	Fungicide/no wax, fungicide/wax
33%	2 year	Fungicide/no wax, fungicide/wax
67%	1 year	Fungicide/no wax, fungicide/wax
67%	2 year	Fungicide/no wax, fungicide/wax

Fruit for weight loss experiments were weighed on a weekly basis and all fruit was assessed weekly for mould, button health, oleocellosis, and general blemish. Blemishes caused by tree oleocellosis, insect and wind damage were assessed in the first week only. Organoleptic tests carried out measured Brix/Acid levels, juice content and taste (off flavours) for cold stored fruit only. Storage treatments are demonstrated in Table 6.

Table 6: Storage treatments.

Week	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Gp1	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C
Subset	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C				
Gp2	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C	3°C
Subset	3°C	3°C	3°C	3°C	3°C	3°C	20°C	20°C*				

* Fruit destructively sampled for juice, brix and acid in week 8.

RESULTS

WEIGHT LOSS 2009

It was observed that the fruit was generally still quite green, and it is likely that this provided some protection from deterioration.

There were no significant differences in weight loss among all treatments for fruit stored at 20°C. There were no significant differences in weight loss among treatments for unwaxed fruit stored at 3°C

There were significant differences in weight loss among the waxed fruit treatments stored at 3°C (Table 7, Figure 1). However average weight loss varied by only 0.3% between treatments showing the highest and lowest weight loss, and after 5 weeks there was less than 0.5% difference in weight loss between them.

Table 7: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and stored for 5 weeks at 3°C

Irrigation Treatment	*Weight loss (g)*									
	Week 1		*Week 2*		*Week 3*		*Week 4*		*Week 5*	
100% Control	1.37	cd	2.22	ab	3.22	bc	4.06	bc	5.11	bc
67% Control	1.24	d	2.04	b	2.91	c	3.61	c	4.4	c
67% MulSc	1.51	bc	2.35	ab	3.48	ab	4.33	ab	5.33	ab
67% AgSc	1.7	ab	2.54	a	3.79	a	4.85	a	6	a
67% All	1.78	a	2.6	a	3.76	ab	4.61	ab	5.62	ab

[a] Weight loss of fruit is the mean of 10 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, Randomized Complete Block AOV: Week 1 $F=12.61$, $p<0.05$, Week 2 $F=5.05$, $p<0.05$, Week 3 $F=6.92$, $p<0.05$, Week 4 $F=7.81$, $p<0.05$, Week 5 $F=8.16$, $p<0.05$.

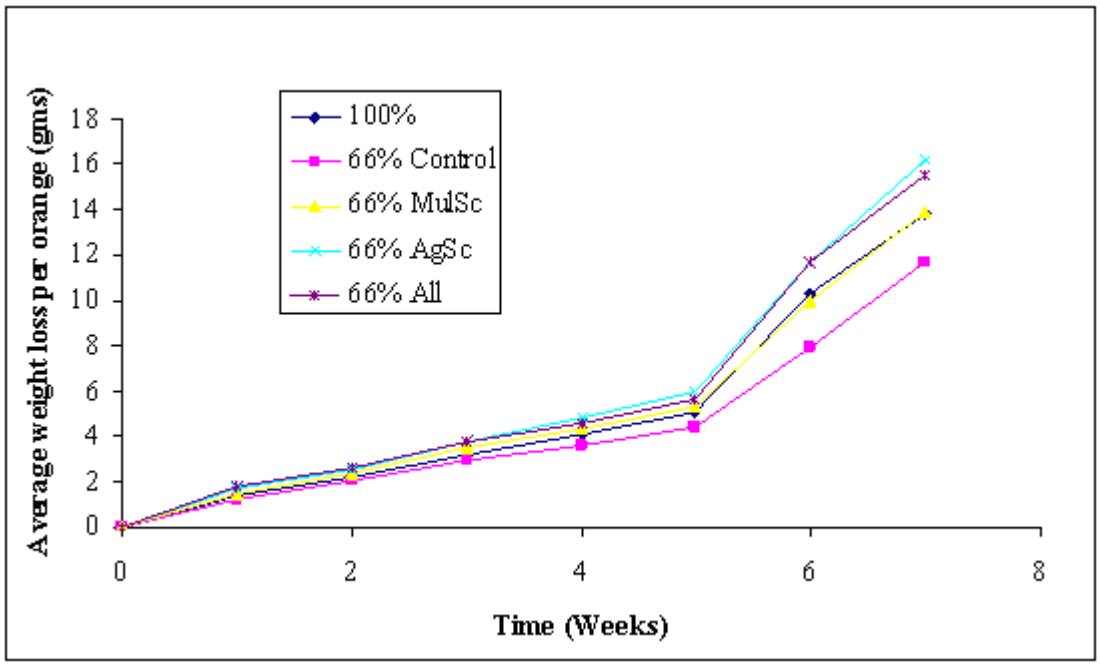
When fruit was removed to ambient (20°C ± 2°C) for a further 2 weeks, significant differences between treatments continued (Table 8). Again, the differences were small, only 3% between treatments with the highest and lowest weight loss after the second week at ambient temperature.

Table 8: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and removed from cool storage (3°C) and stored for a further 2 weeks at 20°C

Irrigation Treatment	*Weight loss (g)*			
	Week 6		*Week 7*	
100% Control	10.29	ab	13.85	b
67% Control	7.98	c	11.68	c
67% MulSc	9.98	b	13.9	b
67% AgSc	11.73	a	16.17	a
67% All	11.73	a	15.53	ab

[a] Weight loss of fruit is the mean of 10 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, Randomized Complete Block AOV: Week 6 $F=14.02$, $p<0.05$, Week 7 $F=10.94$, $p<0.05$.

Figure 1: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and stored for 5 weeks at 3°C and a further 2 weeks at 20°C



WEIGHT LOSS - 2010/2011

There were no significant differences in weight loss among all treatments for fruit stored at 20°C for 4 weeks during both seasons. This was independent of whether the fruit was waxed or remained unwaxed.

There were significant differences in weight loss among the waxed fruit treatments stored at 3°C from week 2 (see Table 9) during the 2010 season, this effect disappeared briefly in week 3 before returning. However the differences were insignificant in some instances and in commercial terms varied by only a 0.36% between treatments that showed the highest and lowest weight loss after 6 weeks. Irrigation schedule of 33% (1yr) showed the highest amount of loss (although fruit was generally larger.) After fruit was removed to ambient (20°C ± 2°C) for a further 2 weeks, significant differences between treatments continued to occur (Table 10). Again, the differences varied by only a 1.4% between treatments that showed the highest and lowest weight loss after the second week at ambient temperature. Graph of weight loss can be seen in Figure 2.

Unlike 2010, in 2011 there were no significant differences in weight loss among the waxed fruit treatments stored at 3°C. Even after fruit was removed to ambient (20°C ± 2°C) for a further 2 weeks, no significant differences were seen (see Table 11, Figure 3.)

Table 9: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and stored for 6 weeks at 3°C (2010)

Irrigation Treatment	Weight loss (gms)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
100%	1.92 a	3.68 b	4.7 a	6.38 b	8.08 b	9.94 b
33% 1 year	2.24 a	4.7 a	6.1 a	8.58 a	10.58 a	12.72 a
33% 2 year	2 a	3.7 ab	4.88 a	6.46 b	8.04 b	10.02 b
67% 1 year	1.78 a	3.62 b	5.54 a	6.58 b	8.4 b	10.12 b
67% 2 year	2.18 a	4.16 ab	5.46 a	7.26 ab	9.24 ab	11.08 ab

[a] Weight loss of fruit is the mean of 10 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, Randomized Complete Block AOV: Week 1 $F=2.82$, $p<0.05$, Week 2 $F=5.89$, $p<0.05$, Week 3 $F=2.49$, $p>0.05$, Week 4 $F=6.39$, $p<0.05$, Week 5 $F=5.84$, $p<0.05$, Week 6 $F=4.41$, $p<0.05$

Table 10: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and removed from cool storage (3°C) and stored for a further 2 weeks at 20°C (2010).

Irrigation Treatment	Weight loss (gms)	
	Week 7	Week 8
100% Control	18.26 a	25.76 a
33% 1 year	22.36 b	32.50 b
33% 2 year	18.08 a	25.18 a
67% 1 year	18.44 a	26.08 a
67% 2 year	19.08 ab	31.18 b

[a] Weight loss of fruit is the mean of 10 replicates. Means labelled with similar letters in columns are not significantly different from each other using Least Significant Difference test, One-way AOV: Week 7 $F=3.01$, $p<0.05$, Week 8 $F=3.99$, $p<0.05$.

Table 11: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and stored for 6 weeks at 3°C for a further 2 weeks at 20°C (2011)

Irrigation Treatment	Weight loss (gms)							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
33% 1 year	2.18	2.92	3.7	4.38	4.98	5.62	11.36	16.94
33% 2 year	2.32	3.1	3.86	4.46	5	5.7	11.54	16.84
67% 1 year	2.24	2.96	3.76	4.32	4.82	5.64	11.92	18.14
67% 2 year	2.14	2.94	3.82	4.44	5.04	5.78	11.98	17.64
100%	1.92	2.72	3.42	3.94	4.52	5.08	10.82	16.12

[a] Weight loss of fruit is the mean of 5 replicates. One way AOV: Week 1 $F=0.79$, $p>0.05$, Week 2 $F=0.49$, $p>0.05$, Week 3 $F=0.54$, $p>0.05$, Week 4 $F=0.65$, $p>0.05$, Week 5 $F=0.48$, $p>0.05$, Week 6 $F=0.81$, $p>0.05$, Week 7 $F=0.64$, $p>0.05$ Week 8 $F=0.66$, $p>0.05$

Figure 2: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and stored for 8 weeks (2010).

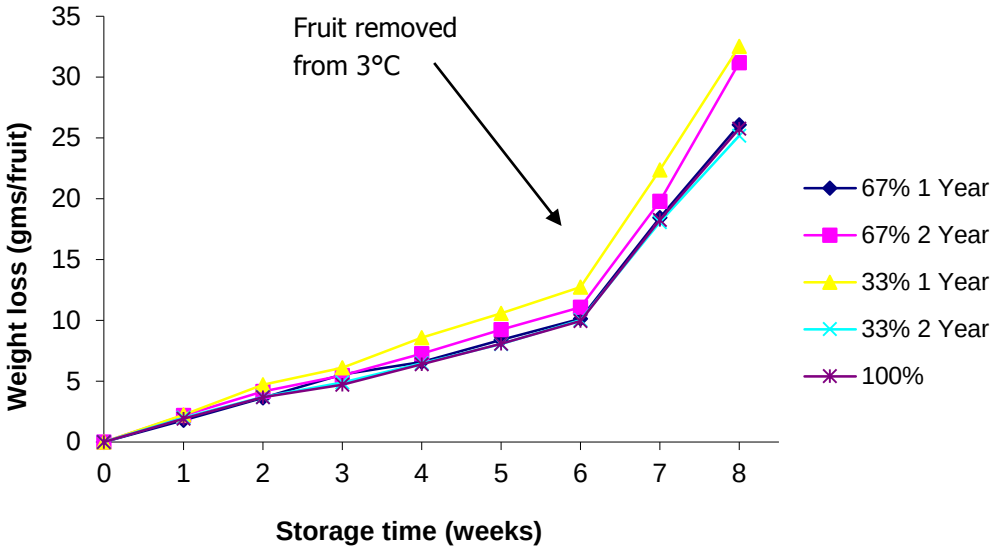
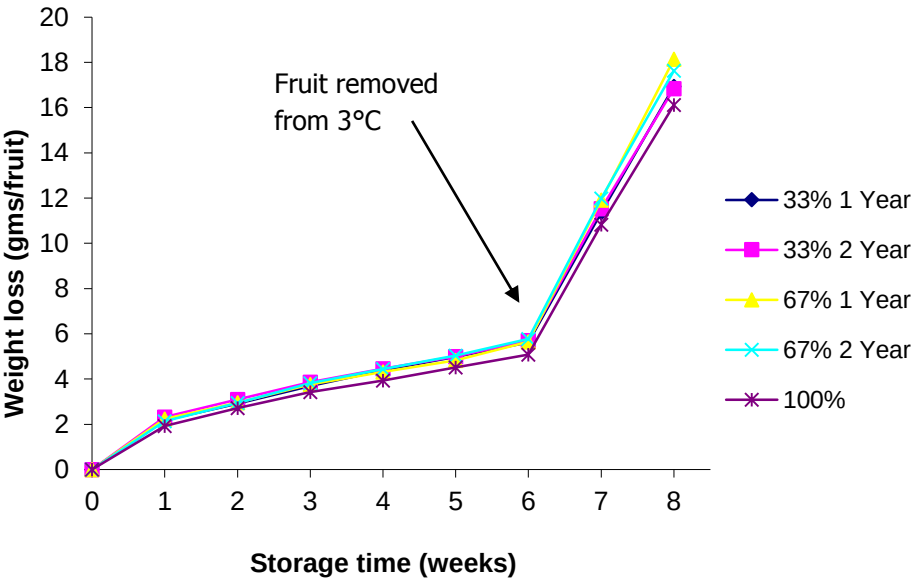


Figure 3: Mean weight loss of waxed Valencia oranges grown under different irrigation treatments and stored for 8 weeks (2011).



Overall, there appeared to be higher weight loss in fruit harvested in 2010 compared to fruit from 2011.

FRUIT QUALITY OVER SEVERAL SEASONS (OVER STORAGE)

2009 FRUIT QUALITY

At the end of the 7 weeks a subset of cold treated fruit was sent for chemical residue analysis (see Table 12). All residues were very high, compared with industry average postharvest residue levels around 1-2 mg/kg. The levels help to explain why decay incidence was significantly less than might have been expected.

Table 12: Fungicide residue levels on Valencia oranges grown under different irrigation treatments and stored for 7 weeks

Irrigation Treatment	Residue level mg/kg (ppm)	
	Imazalil	Thiabendazole
100% Control	4.4	2
67% Control	4.9	2
67% MulSc	5	2.1
67% AgSc	5.6	2.6
67% All	5	1.8

MOULD

Mould levels for both ambient and cool room stored fruit were very low and the type of mould differed depending on storage conditions (see Table 13 and 14). Ambient stored fruit was mostly affected by stem end rot (Diplodia, see Figure 4) and cool room stored fruit was affected mostly by wound pathogens (blue or green mould or sour rot, see Figure 5). No rot was recorded for 3°C fruit that had received the 100% irrigation treatment.

Table 13: Mould found on individual Valencia oranges grown under different irrigation treatments and stored for 12 weeks at 20°C

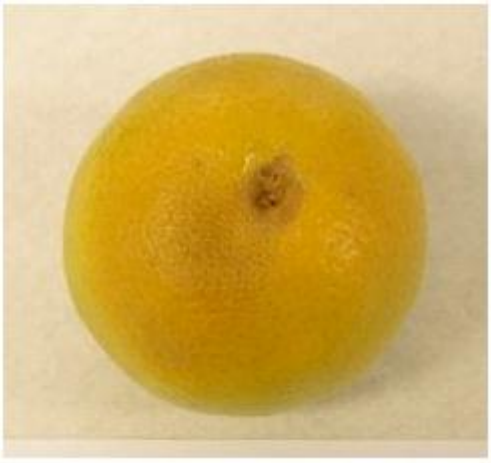
Treatment	Week of 1st appearance	Mould type	Week discarded
Unwaxed 67% All	8	diploda	9
Waxed 100%	12	diploda	12
Waxed 100%	12	diploda	12
Waxed 67% MulSc	12	diploda	12
Unwaxed 67% Control	12	diploda	12
Unwaxed 67% AgSc	12	diploda	12

Table 14: Mould found on individual Valencia oranges grown under different irrigation treatments and stored for 12 weeks at 3°C

Treatment	Week of 1st appearance	Mould type	Week discarded
Waxed 67% Control	0	sour rot	2
Waxed 67% All	5	sour rot	6
Unwaxed 67% Control	6	sour rot	7
Unwaxed 67% Control	6	blue/green	7

Figure 4: Diplodia rot on unwaxed '67% All' fruit stored at 20°C for 8 weeks

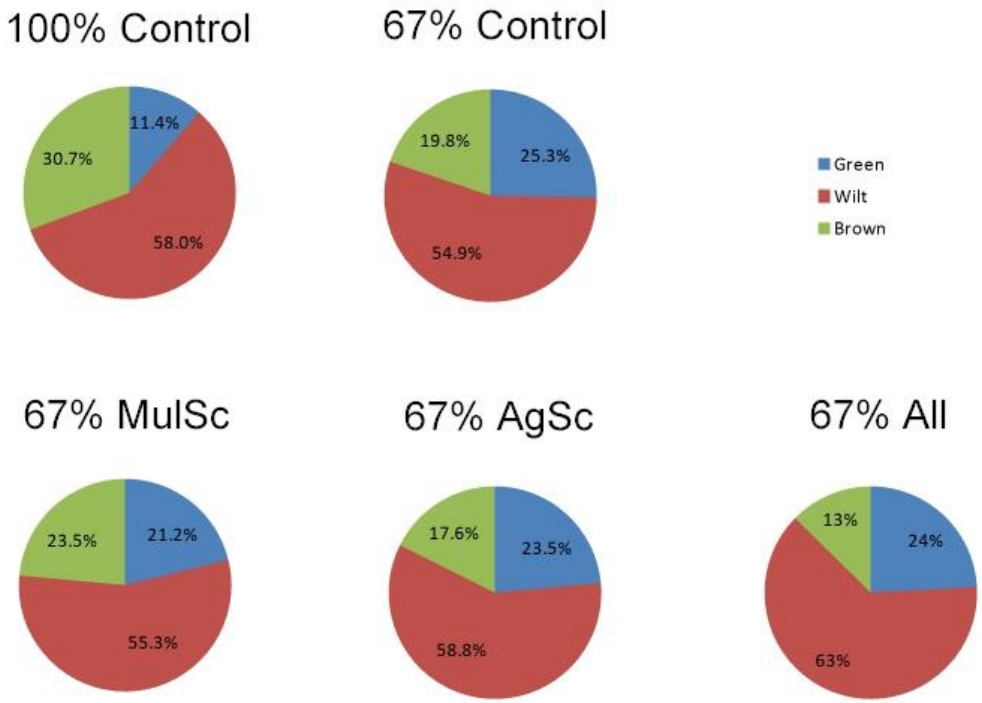
Figure 5: Sour rot in small split in waxed '67% All' fruit stored at 3°C for 5 weeks, and 1 week at 20°C



BUTTON HEALTH – WAXED FRUIT

There were no significant differences in button health after 12 weeks for waxed fruit stored at 3°C (χ^2 = 13.64, p>0.05, see Figure 6) and at 20°C (χ^2 = 8.301, p>0.05). Most calyces on fruit stored at ambient temperature (20°C) were brown after 12 weeks.

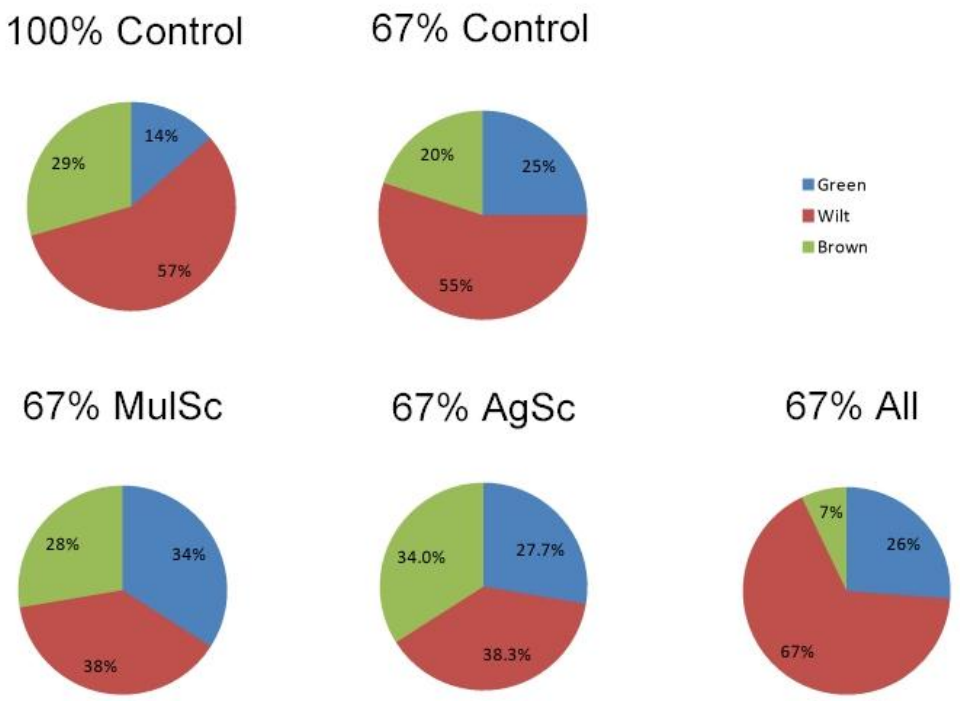
Figure 6: Percentage of waxed fruit with green, wilted or brown calyces after 12 weeks at 3°C



BUTTON HEALTH – UNWAXED FRUIT

There were significant differences in button health after 12 weeks for unwaxed fruit stored at 3°C ($\chi 2 = 17.35$, $p<0.05$, see Figure 7), but no significant differences for unwaxed fruit stored at 20°C ($\chi 2 = 8.301$, $p>0.05$). Most calyces on fruit stored at ambient temperature (20°C) were brown after 12 weeks.

Figure 7: Percentage of unwaxed fruit with green, wilted or brown calyces after 12 weeks at 3°C

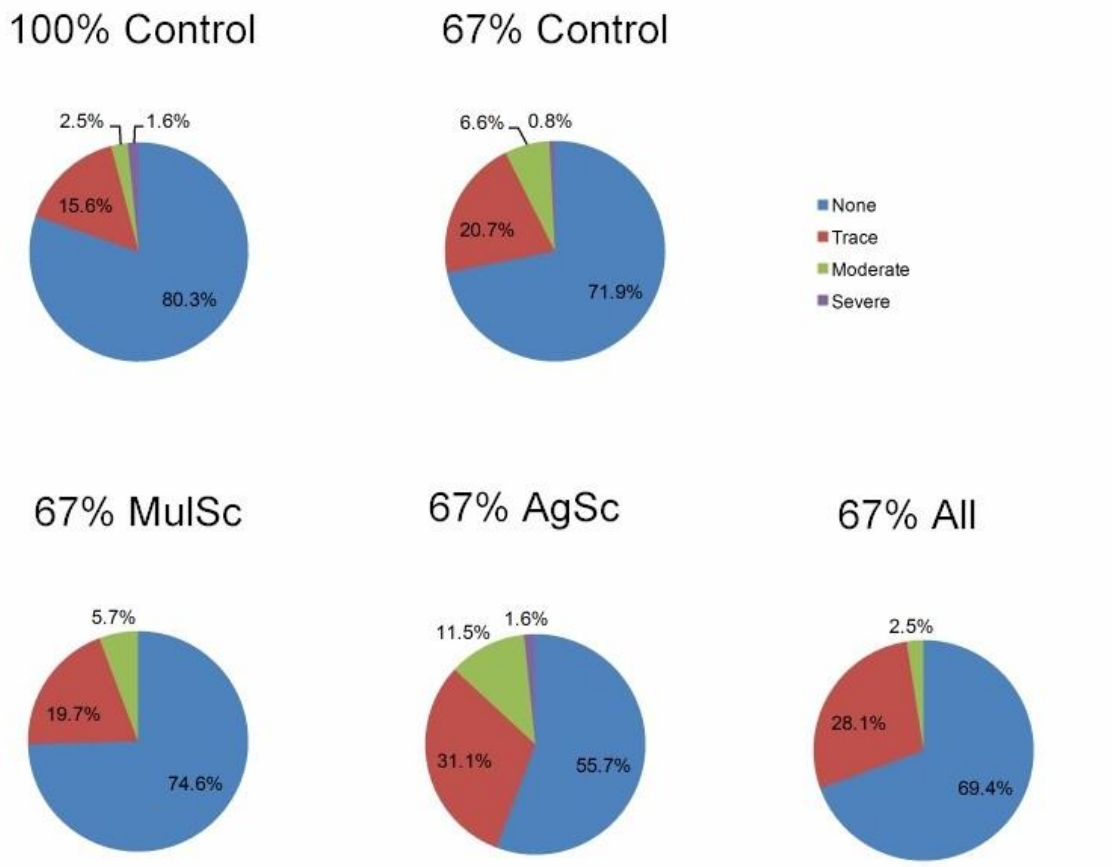


ALBEDO BREAKDOWN

Albedo breakdown is believed to be caused by a combination of factors, including crop load, stress and nutrition. Fruit under different irrigation treatments could show different levels of Albedo breakdown.

Waxed fruit stored at 3°C showed significant differences in Albedo breakdown between field treatments ($\chi 2 = 30.098$, $p<0.05$, see Figure 8. However, waxed fruit stored at 20°C showed no significant differences in Albedo breakdown ($\chi 2 = 11.38$, $p>0.05$).

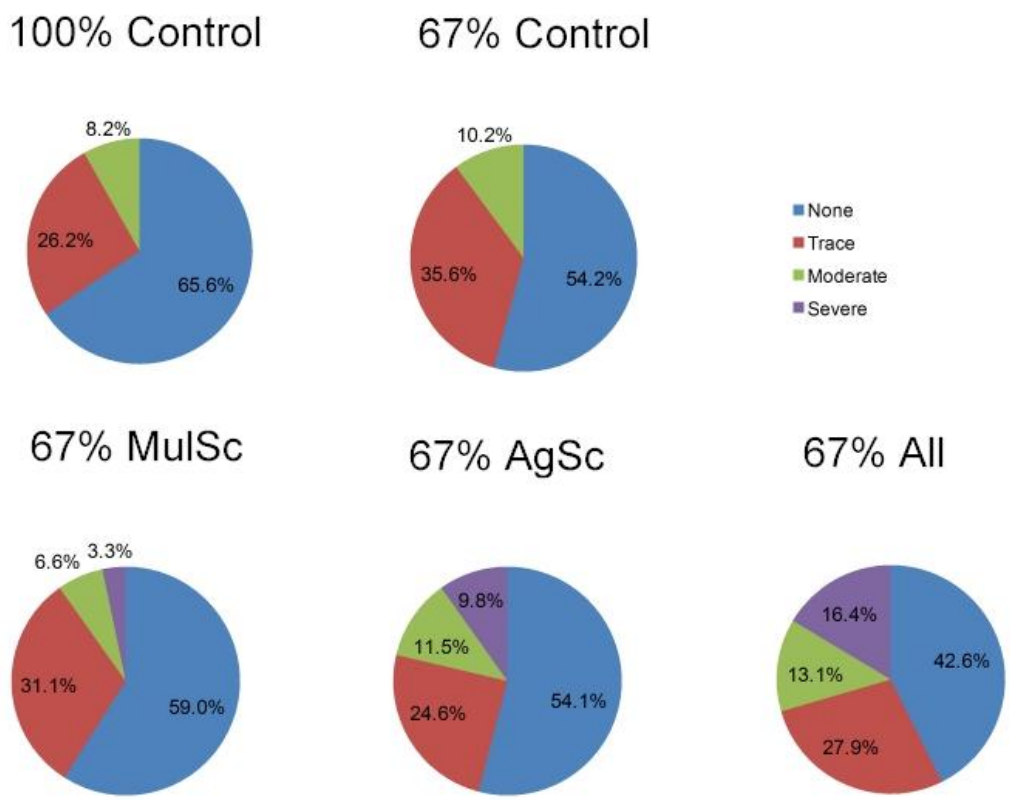
Figure 8: Percentage of waxed fruit with different amounts of Albedo breakdown after 12 weeks at 3°C



Similar development of Albedo breakdown was seen in unwaxed fruit stored at 3°C, where there were significant differences between field treatments ($\chi 2 = 26.99$, $p<0.05$, see Figure 9). However, unwaxed fruit kept at 20°C again showed no significant differences in Albedo breakdown ($\chi 2 = 13.72$, $p>0.05$).

There is some suggestion in the data that field treatments containing AG30 polymer contributed to greater incidence of moderate and severe Albedo breakdown, particularly in unwaxed fruit.

Figure 9: Percentage of unwaxed fruit with different amounts of Albedo breakdown after 12 weeks at 3°C



OLEOCELLOSIS

There were no significant differences in the amount of Oleocellosis between treatments for either waxed ($\chi 2 = 19.67$, $p>0.05$) or unwaxed fruit ($\chi 2 = 7.95$, $p>0.05$).

Little or no oleocellosis appeared on fruit kept at ambient temperature (20°C).

Stem End Blackening and Dehydration

There were only a few traces of stem end blackening on fruit stored at 3°C, and not enough to analyse. Fruit kept at 20°C showed no significant differences between field treatments ($\chi 2 = 20.08$, $p>0.05$).

Dehydration burn was only present in waxed fruit stored at 20°C, and there were no significant differences between treatments ($\chi 2 = 14.27$, $p>0.05$).

Figure 10 shows the various types of decay seen on ambient stored fruit (20°C) after 12 weeks.

Figure 10: Stem end blackening (left), stem end blackening & dehydration burn (middle), and dehydration burn (right) on fruit stored at 20°C for 12 weeks

BRIX/ACID AND TASTE ANALYSIS

Analysis of variance showed significant differences in Brix:Acid ratios ($F=11.51$, $p<0.05$), as well as percentage acid ($F=8.37$, $p<0.05$), in waxed fruit for different field treatments, irrespective of storage treatment (Table 15) . Fruit from the 100% irrigation treatment had the highest Brix:Acid ratio (lowest percentage acid), and fruit from the 67% irrigation with the addition of 'AG30' (polymer) and 'Screen' had the lowest Brix:Acid ratio (highest percentage acid).

Organoleptic tests revealed that the 2 tasters could not detect sweetness differences between treatments, but rated the highest irrigation treatment (100%) as the best tasting (highest 'good' rating).

Unwaxed fruit showed significant differences in percentage acid ($F=9.36$, $p<0.05$). The 100% irrigation treatment had the lowest percentage acid. However, there were no significant differences in Brix:Acid ratios, or in either of the organoleptic taste tests.

Table 15: Assessment of harvested fruit for citrus drought and recovery trial

Temp	Treatment	Wax	% Acid	Brix: Acid	Good[a]		Sweet[a]		% Juice
					Taste1	Taste2	Taste1	Taste2	
3°C	100 Cont	W	0.53	20.26	11.9	12	6.9	8	50.68
	67 Cont	W	0.66	16.90	8.3	12.7	9.3	9.3	52.12
	67 MulSc	W	0.71	16.39	7.7	11.3	9.6	10	45.48
	67 AgSc	W	0.97	12.43	2.8	3.7	8.1	10.8	50.84
	67 All	W	0.99	11.69	6	12.2	8.7	11.5	50.88
	100 Cont	W	0.49	20.96	10.4	13	6.2	8.6	47.75
	67 Cont	W	0.66	16.92	6.8	11.1	7.6	9.7	54.38
	67 MulSc	W	0.75	16.09	5.2	8.5	10.2	10.5	48.65
	67 AgSc	W	0.84	13.78	3.7	13.3	7.9	8.3	50.20
	67 All	W	0.65	15.93	11.1	13.8	8.4	8.9	35.85
	100 Cont	UW	0.66	15.39	10.1	10.5	9.6	12.1	51.88
	67 Cont	UW	0.72	15.00	6.4	10.1	9.2	12	53.38
	67 MulSc	UW	0.86	13.94	8	11.7	9.8	10.5	48.84
	67 AgSc	UW	0.89	12.97	5	12.3	4.2	11.3	53.03
	67 All	UW	0.96	11.91	5.7	9.5	8	9.1	52.00
20°C	100 Cont	W	0.64	18.65	10.1	11.7	9.5	13	50.72
	67 Cont	W	0.69	17.08	8	11.1	9.9	13.4	57.44
	67 MulSc	W	0.84	14.60	0.2	3.2	5.7	9.8	52.86
	67 AgSc	W	0.84	14.48	6.5	9.7	6.7	13.5	55.03
	67 All	W	0.89	13.20	7.2	10	7.4	11.3	55.36
	100 Cont	W	0.64	16.48	9	12.2	9	12.1	51.67
	67 Cont	W	0.78	15.04	8.4	12.5	8	12.6	56.33
	67 MulSc	W	0.90	13.72	4.8	5.6	5.4	14	52.69
	67 AgSc	W	0.88	13.68	0.6	2.5	6.2	10.5	53.88
	67 All	W	0.81	13.88	7.5	8.6	6.5	10.8	53.87
	100 Cont	UW	0.79	12.52	6.8	10.4	7	11	57.02
	67 Cont	UW	0.76	15.16	9.6	11	8.6	13.3	55.95
	67 MulSc	UW	1.02	11.95	8.7	13	10.8	9.2	52.55
	67 AgSc	UW	1.09	10.78	5.6	9.1	4.7	9.5	55.20
	67 All	UW	0.96	11.94	3.1	13.5	5	9.6	57.00

[a] This is a raw figure based on measurement along a 15 cm line – the higher the rating the sweeter or better (good or not good) the taste of the fruit.

2010/2011 FRUIT QUALITY

OLEOCELLOSIS

In 2010, all treatments had more fruit in the trace or no oleocellosis categories compared to the other two categories (moderate and severe) after 6 weeks in cold storage (χ^2 = 21.55, p=0.0429). This did not change even after 12 weeks overstorage at 3°C (χ^2 = 26.01, p=0.0107) see Figure 11. Further investigation showed that there were no significant differences between treatments within each rating of oleocellosis.

In 2011 After 6 weeks fruit in treatment group 33% (1YR) had more fruit in the moderate category of oleocellosis than any other treatment (χ^2 = 18.54, p<0.05). Both 33% (1YR) and 33%(2YR) had higher numbers of fruit in the moderate category than other treatments (χ^2 = 18.21, p<0.05). This was also repeated at 12 weeks (χ^2 = 21.17, p<0.05), see Figure 12. However, none of the fruit had severe levels of oleocellosis. Again, there were no significant differences between treatments within each rating of oleocellosis.

Figure 11: Percentage of fruit with no, trace, moderate or severe oleo on waxed Valencia's after 12 weeks storage at 3°C.

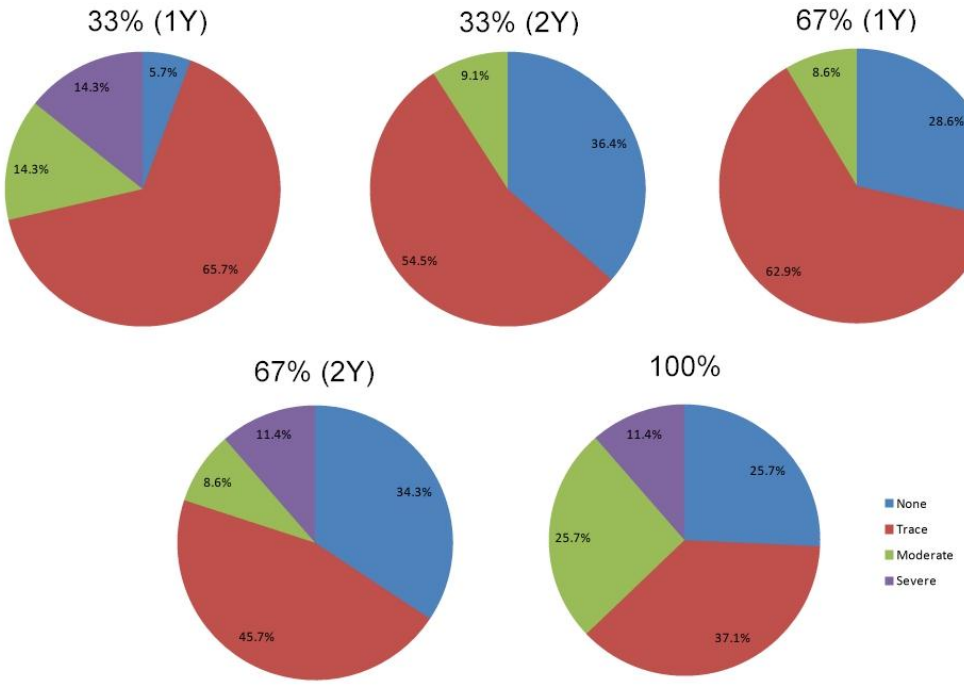
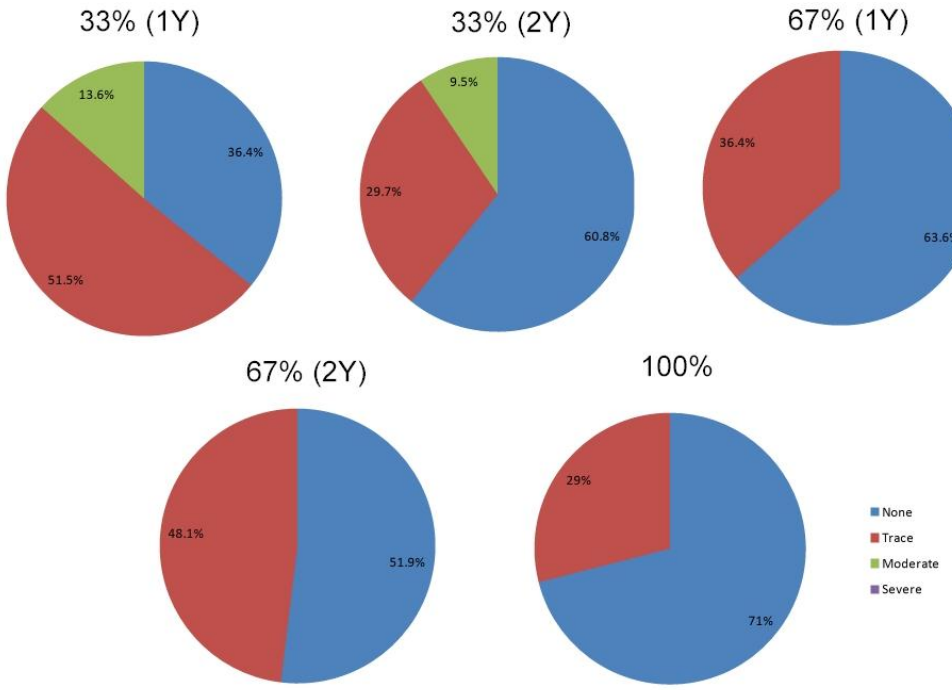


Figure 12: Percentage of fruit with none, trace, moderate or severe oleocellosis on waxed Valencia's after 12 weeks storage at 3°C.



GENERAL BLEMISH

In 2010, after 6 weeks in cold storage there were higher numbers of fruit in the no blemish (none) category – the amount of fruit in the other categories were evenly distributed across the other three categories ($\chi^2 = 13.14$, $p>0.05$). Again at 12 weeks, little blemish was recorded and the amount of fruit in each blemish rating across the other categories was similar ($\chi^2 = 12.52$, $p>0.05$). See Figure 13.

When fruit from the 2010 season was bought out to ambient and left for a further 2 weeks deterioration was rapid. Most treatments had moderate to severe blemish on fruit, ($\chi^2 = 31.05$, $p=0.0019$) but there were no significant difference between treatments.

In 2011, after 6 weeks in cold storage there appeared to be little blemish affect on fruit – with most fruit having no blemish at all ($\chi^2 = 13.77$, $p=0.088$). After 12 weeks in cold storage, again there was also very little blemish on fruit ($\chi^2 = 16.39$, $p=0.1741$) with most fruit in the 'no blemish' (none) category (Figure 14). Deterioration in fruit quality was not evident until 15-16 weeks.

Figure 13: Percentage of fruit with no, trace, moderate or severe blemish on waxed Valencia's after 12 weeks storage at 3°C (2010).

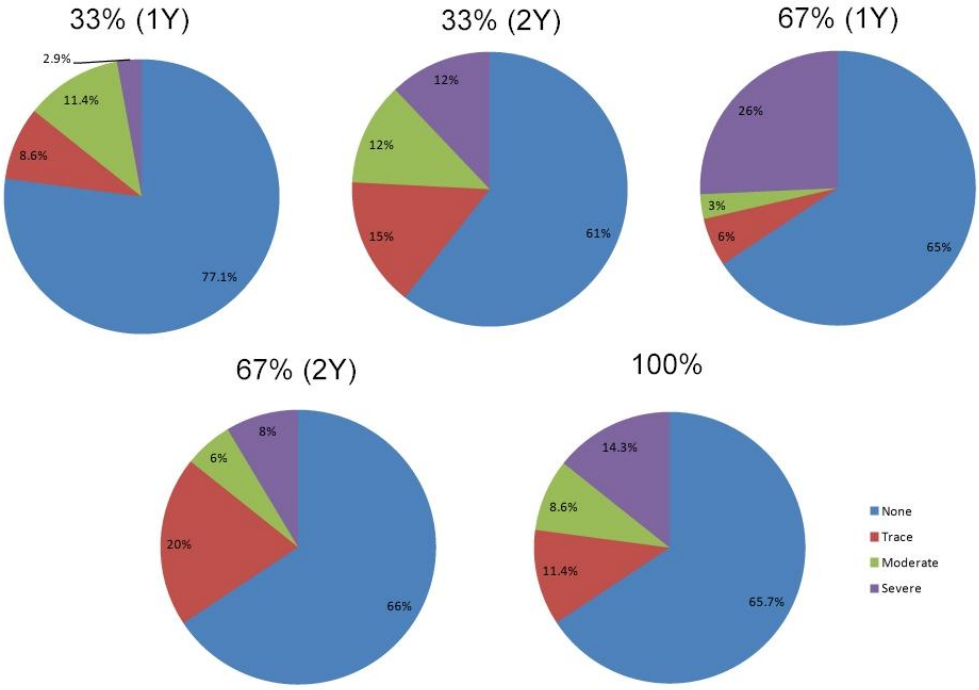
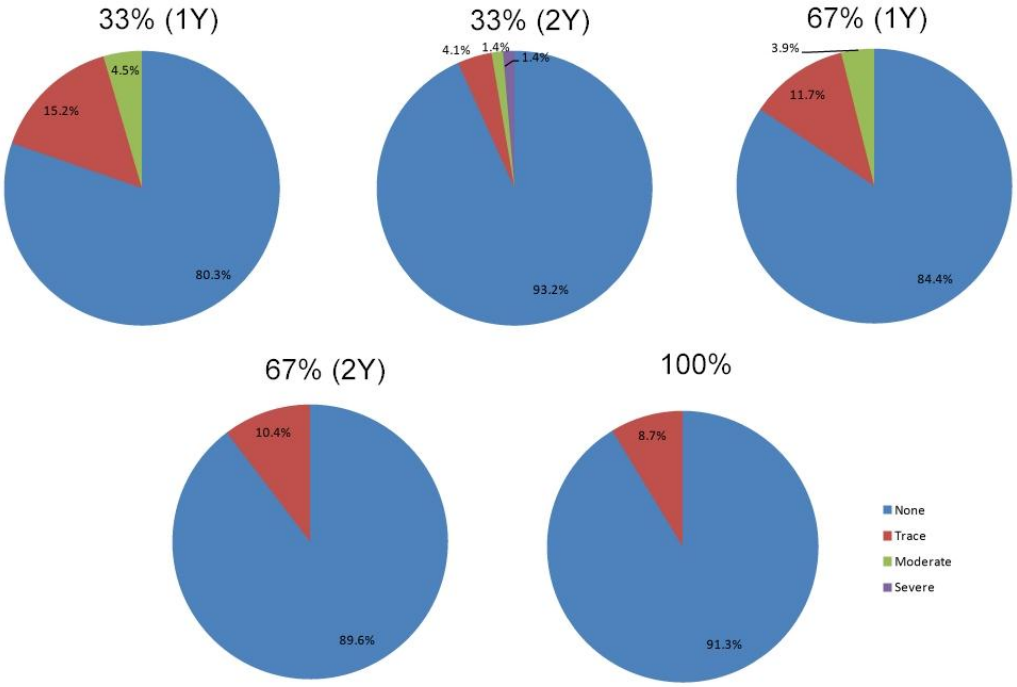


Figure 14: Percentage of fruit with no, trace, moderate or severe blemish on waxed Valencia's after 12 weeks storage at 3°C (2011).



BUTTON HEALTH

In 2010, most treatments had fallen or brown buttons after six weeks (χ^2 = 28.2, p=0.0052) with the number of fruit in the brown category increasing slightly after 12 weeks of storage (χ^2 = 21.47, p=0.044). However, there were no significant differences between treatments. See Figure 15.

However, for fruit picked in 2010/2011 and stored at 3°C for 6 weeks – the amount of fruit in either wilted, brown or fallen buttons was roughly even (χ^2 = 36.31, p=0.0003), with the most fruit falling in the 'wilted category'. After 12 weeks of storage the number of fruit in the 'brown' category increased (χ^2 = 23.17, p=0.0263) see Figure 16.

Figure 15: Percentage of fruit with green, wilted or brown calyces on waxed Valencia's after 12 weeks storage at 3°C (2010).

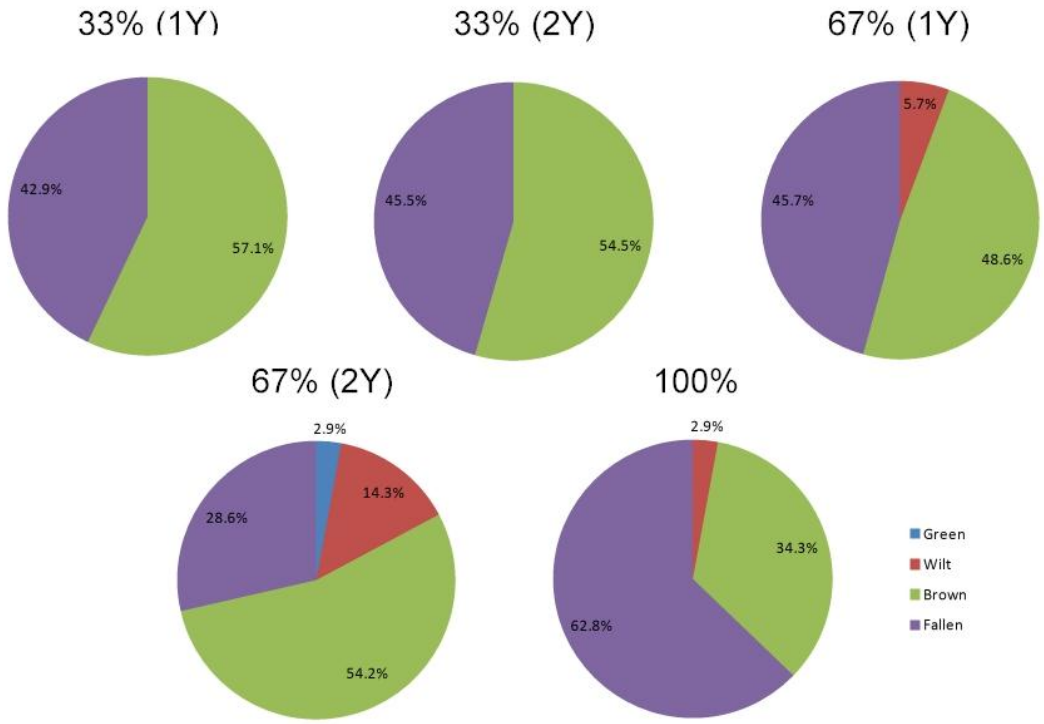
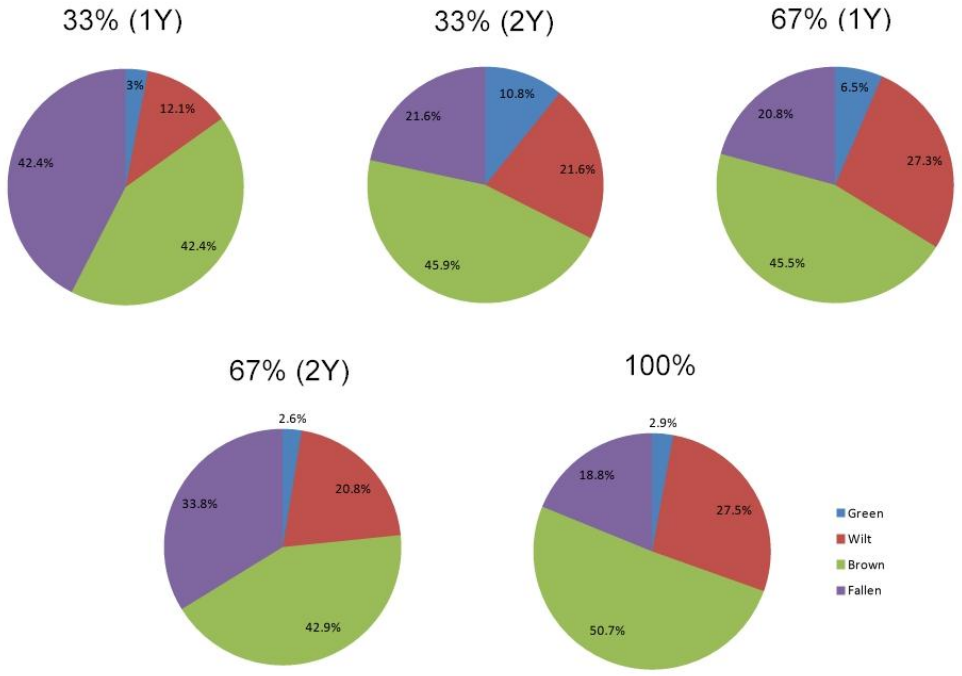


Figure 16: Percentage of fruit with green, wilted or brown calyces on waxed Valencia's after 12 weeks storage at 3°C (2011).



DISEASE/DISORDERS

In 2010, no mould or rot was observed on fruit after 6 weeks of storage at 3°C, and there were no significant differences in moulds and rots across treatments after 12 weeks of overstorage, (χ^2 =16.5, p=0.1692). However, fruit showed distinct softening after 12 weeks with all treatments having a degree of soft or dehydrated fruit, the percentage of 'softened fruit' varied, with the most softened/dehydrated seen in the 33% 1 year treatment (40% fruit affected) this lowered significantly for 33% 2yr to only 6% close to the 100% irrigated treated fruit (around 10%). Irrigation schedules of around 67% had fruit softening/dehydration levels of around 20% in both the 1yr treatment and the 2 year treatment.

When fruit was bought out to ambient and left for a further 2 weeks (14 weeks storage total) deterioration was rapid and mould and rots were evident. Although all fruit had deteriorated, there appeared to be more penicillium moulds and more softening and dehydration on fruit that had the 33% irrigation schedule than on fruit at the 67% and 100% irrigation schedule (see Figures 17-19.)

After four weeks at ambient temperature (22°C) fruit also showed suspected Septoria rot on low irrigation treatments (33%1 year) see Figure 20, and was also observed on cool stored fruit after about 7 weeks.

In 2011, no mould or rot was observed on fruit after 12 weeks of storage at 3°C.
Fruit showed distinct softening after 12 weeks with all treatments having a degree of soft or dehydrated fruit.

When fruit was bought out to ambient at 12 weeks it took until week 16 before any deterioration was evident (see Figure 21-23). Overall fruit quality this season was good with fruit appearing in excellent condition up until this time.

Figure 17: Mould development on overstored fruit (33%1year 2010)

Figure 18: Mould development on overstored fruit (67%1year - 2010)

Figure 19: Mould development on overstored fruit (Control - 2010)

Figure 20: Suspected Septoria rot on wax treated citrus (33%1yr – 2010)

Figure 21: Mould development on overstored fruit (33%1year 2011)

Figure 22: Mould development on overstored fruit (67%1year - 2011)

Figure 23: Mould development on overstored fruit (Control - 2011)

WEEK 8 BRIX/ACID AND TASTE ANALYSIS

In 2010, analysis showed no differences in Brix/Acid ratios or percentage acid, for different treatments in waxed fruit held at 3°C for 6 weeks and 22°C for a further 2 weeks.

Organoleptic tests revealed that the 2 tasters could detect some off flavours in fruit but only fruit treated with 33% (1 YR) appeared to show no off flavours to either tester. The off flavour assessment did not correlate significantly with other organoleptic factors. Table 16 shows the final organoleptic assessment for weighed fruit.

Table 16: Organoleptic results

Temp	*Treat*	*Brix*	*Perc Acid*	*Brix:Acid*	*Percent Juice Content*	*Off flavours**
3°C	33% 1 YR	8.8	0.57	15.884	46.833	0%
	33% 2 YR	9.7	0.69	14.218	47.214	30%
	67% 1 YR	9.42	0.54	17.87	48.622	60%
	67% 2 YR	8.8	0.57	15.736	39.533	50%
	100%	9.96	0.65	16.212	49.715	30%

*off flavours were based on the results of 2 separate tasters, sampling juice from 5 pieces of fruit per treatment (so total of 'off' is percentage of fruit that had that assessment.)

Results of organoleptic tests on fruit from 2011 season showed significant differences in Brix:Acid ratios between treatments ($F=5.35$, $p=0.0043$), as well as percentage acid ($F=5.4$, $p=0.0041$) in waxed fruit for different field treatments (Table 17). Fruit from the 67% (2 years) irrigation treatment had the highest Brix:Acid ratio (lowest percentage acid), and fruit from the 33% irrigation (2 year) and 67% irrigation (1 year) had equivalent lowest Brix:Acid ratio (highest percentage acid).

Organoleptic tests revealed that on average, the 4 tasters rating of 'sweet' correlated well with the Brix readings ($r^2= 0.7864$). The rating of 'good' correlated strongly to the off flavours detected ($r^2= 0.7355$). No other strong correlations were detected.

Table 17: Organoleptic results

Temp	*Treat*	*Brix*	*Perc Acid*	*Brix: Acid*	*Perc Juice*	*Good*[1]	*Sweet*[1]	*Off flavours*[2]
3°C	33% 1 YR	10.32	0.66	15.81	51.02	7.29	7.68	20.0
	33% 2 YR	10.4	0.75	13.95	52.15	7.48	7.16	13.3
	67% 1 YR	9.24	0.67	13.92	53.69	3.85	3.83	26.7
	67% 2 YR	10.52	0.57	18.71	53.96	5.7	6.17	26.7
	100%	9.52	0.63	15.08	51.56	3.34	3.36	46.7

[1] This is a raw figure based on measurement along a 15 cm line – the higher the rating the sweeter or better (good or not good) the taste of the fruit (4 tasters).

[2] off flavours were based on the results of 4 separate tasters, sampling juice from 5 pieces of fruit per treatment (so total of 'off' is percentage of fruit that had that assessment.)

DISCUSSION

Australia will continue to go through cyclic periods of drought that affect citrus production. Research on how reduced watering affects stress on trees has focussed mainly on development stages prior to harvest and has shown that water stress can strongly influence flowering and fruit set, fruit drop, fruit size, tree yields and canopy development (Falivene et al, 2006)

However, with internal quality issues – research has varied in its results. Castel and Buj (1990) showed an increase in Brix and percentage acids in fruit that has been under deficit, but this was not consistent across the three years that were examined. Sanchez Blanco et al (1989) also showed that lemons had higher Brix and acidity after deficit irrigation (although too low a schedule lead to very low production and fruit bearing in alternate years.)

Higher soluble solids in fruit was also noted by Pérez-Pérez et al (2008) in late Lanes, Garcia-Tejero (2010a) in Salustiano oranges and Gasque et al (2010) and Garcia-Tejero (2010) in Navelina oranges. Hockema and Etxeberria (2001) also noted elevated acids and soluble sugars in Hamilin oranges, but Romero et al (2006) noted some lower Brix and acid on Clemenule mandarins with certain rootstocks.

Weight loss and external quality issues can also be used as a measurement of drought effects. Severe weight loss of fruit can affect eventual shelf life (especially if fruit is going on long journeys to mainly overseas destinations.) Shrivelled dehydrated fruit, or fruit that has increased blemishes, brown or fallen buttons, oleocellosis, albedo, or increased disease is likely to cause problems for exporters on fruit arrival.

INTERNAL QUALITY

In the initial season, percentage acid levels and Brix were higher in fruit with lower irrigation deficits (67%) compared with 100% control. Ratings of sweetness of droughted fruit was also higher for both of the taste testers in this instance, more so for fruit held in commercial conditions (3°C for 5 weeks then 20°C for 2 weeks.) In the second and third season when fruit was in the recovery phase acid levels began to lower compared to the previous season and fruit was now able to be collected from severely droughted fruit (33%). During the first phase of recovery all soluble sugar and acid readings were lower with no significant differences between treatments, but the second season of recovery showed higher acid levels in 33% (2yr) and 67% (1yr) droughted fruit.

Organoleptic indicators also show that fruit with higher percentage acid had less off flavours detected than other treatments during the recovery phase and during the second year of recovery, previously droughted fruit was considered by four separate tasters, better and sweeter than control treated fruit.

Generally the influence on increased Brix and acid is thought to be through a passive dehydration of juice sacs giving a lower juice percentage but increasing the sugar and acid levels in stressed fruit (Navarro et al, 2009). Navarro also suggested that this affect in fruit could increase palatability for consumers, which in this instance for the small sample size we had, showed a trend towards greater palatability (sweeter and better) of fruit with higher percentage acid.

WEIGHT LOSS

Dehydration can affect droughted fruit with subsequent weight loss being and indicator that fruit may not last in long-term storage. The most weight loss recorded was from lower irrigation schedule treatments, suggesting that dehydration and water loss was higher from fruit under deficit irrigation treatments. Also, weight loss differences were more likely to be seen on waxed fruit, although by the time the second season of recovery was assessed this affect had also disappeared.

EXTERNAL QUALITY

In the all seasons drought and recovery phases it was difficult to detect distinct quality issues in the first 6 weeks of fruit held at commercial conditions. It was only after overstorage at 12 weeks (at 3°C) that differences could be seen between treatments.

During the first season albedo on fruit was evident, with most albedo seen on cold stored fruit (irrespective of whether the fruit was waxed or not) – in the recovery phase, hardly any albedo was seen on fruit. The first season's fruit was also relatively free of blemish and oleocellosis.

Oleocellosis was most severe during the first year of recovery with most seen on 33% (1yr) treated fruit. In the second year of recovery only trace oleocellosis was seen on 67% and 100% irrigation treatments with the 33% irrigation treatments the only ones to have any fruit in the moderate category.

Again a similar pattern was seen in the blemish category, with more fruit having severe blemish in the first year of recovery compared to little seen in treatments during the second year of recovery. However, overall little blemish was seen on fruit in either year compared to other external effects.

Although fruit from the 2010 and 2011 seasons had the same treatments – it was obvious from the results that fruit from the 2011 season had recovered to the level where postharvest influences of irrigation were minimal and most fruit (from all treatments) was in excellent condition. Fruit from the first season of recovery – especially after overstorage, looked in poor condition, with increased rots in the lower

irrigation schedules compared with control treated fruit, although by this stage all fruit was in poor condition. This compares with fruit from the second season of recovery – which even after prolonged overstorage appeared to be in excellent condition (little rots occurring).

SUMMARY

Although difficult to tease out specific differences in deficit irrigation effects on postharvest quality, as minimal effects were seen, there were definite trends towards a number of concerns that could impact the yield and quality of fruit.
These include:

- Lower yield (or none) from trees with less than 50% irrigation
- Increased weight loss in stored fruit
- More Albedo in drought seasons
- Increased oleocellosis and general blemish
- General trend towards increased disease/faster deterioration
- Higher acid and Brix levels (could increase palatability but see a decrease overall juice content).

However, what was also evident was that in a second year of recovery, fruit appeared to recover quickly from any issues that arose. Fruit appeared in better condition, with few external quality issues and little or no differences between treatments when compared using commercial times for transit and delivery.

Considering all the factors investigated, that there is general trend towards negative impacts on postharvest quality of fruit when deficit irrigation is used. However, these impacts could easily be mitigated by good quality postharvest treatments and minimising storage times or minimising time from harvest to market.

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Appendix 2

FUNGICIDE SURVEY OF CITRUS PACKINGSHEDS IN RIVERLAND, SUNRAYSIA AND RIVERINA

CT10006 'National Citrus Postharvest Science Program'

This work has been funded by HAL using the citrus levy, voluntary contributions from industry and matched from the Australian Government

July 2011

Peter Taverner, Nancy Cunningham and Karolina Steciuk

South Australian Research and Development Institute

SUMMARY

- Most packers surveyed applied two fungicides in-line, with average fruit residues of 0.5ppm IMZ and 1.5ppm TBZ for in-line application only. Generally, the in-line fungicide application resulted in good coverage and decay control.
- Packers surveyed were aiming for reduced IMZ residues in packed fruit to avoid MRL issues, but not as low as recorded in this survey (0.4-1.0ppm). The TBZ residues were usually higher than IMZ, with some packers compensating for a lower IMZ level by increasing TBZ residues (>3ppm).
- The sporulation control was variable, with both good (<10% spore coverage of fruit surfaces) and reasonably poor control (>50% spore coverage) on fruit collected just prior to packing.
- Sporulation control was highly correlated with the total fungicide rind residues. This survey suggested that a combined concentration of >3ppm (IMZ+TBZ) in rind usually resulted in good sporulation control.
- The fungicide resistance survey indicated that mould spores were common in the air around the packingline, but less common in the cool-room. An expected result for the beginning of the navel season and after cleaning/maintenance.
- Some mould spores appeared to be tolerant of low levels of fungicide. Growth occurred on 1ppm TBZ-amended plates, which is considered 'technical' resistance.
- This survey has not established if fungicide resistance is at levels sufficient to impact on out-turn but does indicate that further investigation is warranted.
- Overall, the packingline procedures were usually sound, with evidence of monitoring and hygiene. In-line applications were normally sufficient to control decay. There was a trend to increase fruit volume or flow rates over the line without altering fungicide application, which may lead to reduce decay control. Sporulation results suggest that there can be less emphasis on a high IMZ residue when the TBZ residues are high (>3ppm). There appeared to be a trend toward fungicide resistance in some packingsheds. Some practices may exacerbate this trend. For instance, extended storage of fungicide-treated fruit on premises may lead to fungicide resistance problems (see appendix).

INTRODUCTION

Excessive decay leads to concerns that inline fungicide application methods are not controlling blue/green mould effectively. It is often difficult to determine where failures in decay control occur because there are many different fungicides and application methods being used by packers.

The simple approach to solve decay problems is to increase the fungicide residues by combining fungicides, applying multiple times (e.g., drench, in-line &/or in wax) and increasing exposure time or fungicide concentration. This strategy may work in the short term but there are inherent risks. For instance, this approach has probably resulted in some fungicide MRL's being exceeded in overseas markets. Conversely, there is also a risk of fungicide resistance when application is poor or monitoring is not adequate.

The risks of resistance are greater due to a reduction in fungicide groups, consistent use of these few groups and longer on-premises storage of fruit. There is no recognition of increased or widespread fungicide resistance in citrus packing sheds in Australia. However, Brian Wild[1] did collect fungicide resistance strains from fruit packed in NSW during 1994. This is our first attempt to discern if any fungicide resistance occurs in packing sheds.

In this study, we ran inoculated fruit through the main fungicide application inline. The study was designed to provide feedback on whether inline application methods are working effectively. The in-line application is the key fungicide and should control decay by itself. However, many packing sheds also use bulk dip/drenching and fungicide in wax as part of their decay control procedures. We were interested in whether these additional applications provided the higher residues required for good sporulation control. As such, fruit was collected from the end of line for sporulation assessments.

For fungicide resistance, we chose very low rates of fungicides that control a highly sensitive mould isolate to determine if 'technical' resistance was evident. Any growth of mould spore collected in packing sheds on fungicide-amended plates will indicate a trend towards fungicide resistance. However, further testing would be required to determine if any fungicide resistance is sufficient to impact on packing operations.

[1] Wild, Brian L., 1994, Differential sensitivity of citrus green mould isolates to the fungicide imazalil. New Zealand Journal of Crop and Horticultural Science, Vol. 22: 167-171.

PACKINGSHED SURVEY METHODS

Seven commercial packing shed lines from the three major in-land citrus-growing regions were investigated. At each packing shed, fruit were infected with blue/green mould in three different tests. All tests were conducted on navel oranges.

The three different tests were designed to assess different aspects of decay control. Methods were as follows:

Standard Test – Fruit dipped in solution of blue/green mould spores and 10 shallow wounds were made around the equator of each fruit. This is the conventional test used to assess fungicide effectiveness.

Stem/Stylar Test– Fruit dipped in solution of blue/green mould spores and one shallow wound made at the stem end and one at the stylar (navel) end of each fruit. Fruit on a packing line often has a tendency to roll on its equator and it is possible that the stem and stylar ends may not get adequate coverage. This test was designed to assess fungicide coverage on fruit.

Sporulation Test – Blue/green mould solution injected into the centre of the fruit. Fruit will definitely decay but the test is designed to examine whether all fungicide applications combined is sufficient to prevent blue/green mould spores developing and potentially infecting other fruit.

In the decay tests the following controls were used:

Untreated Control - Fruit from each test were not treated with any fungicide after inoculation to test that the spore solution was viable and would cause decay in the absence of any fungicide application.

Dip Control - Fruit were dipped for 30 seconds in a sample of the fungicide that was being applied inline. Dipping fruit gives very good coverage and this test aimed to determine if the fungicide solution alone was working effectively (i.e. is the concentration of the fungicide adequate to control decay). By removing the variability of individual packing line fungicide application methods, the result from this test is comparable with a standard dip test.

In the majority of sheds, infected fruit was run through the fungicide section of the line, including any brushes following the fungicide application, and retrieved prior to the wax application. The fruit were then taken back to the lab to be incubated and assessed for decay at various time intervals. All tests were conducted on fruit that had not been previously drenched or treated with fungicide in any way.

The results from each shed were analysed and graphed. The standard test results are presented as the percentage of fruit wounds decayed i.e. 10% indicates that one of ten

wounds had decayed, 100% indicates that all of the ten wounds had decayed. For the stem/stylar test there are only two wounds per fruit so the percentage decay for an individual fruit would be 0, 50 or 100%. The percentage of wounds decayed gives us an indication of the effectiveness of the inline fungicide application.

For sporulation tests, fruit was taken after processing through the entire line (from postharvest drench to waxing). The fruit were then infected and sporulation test results are presented as the average percentage of each individual fruit covered by blue/green mould spores. The percentage of sporulation is divided into six categories; 0% (no spore development), 1-10%, 11-50%, 51-90%, 91-99% and 100% of the fruit covered in spores.

For fungicide resistance tests, PDA media plates (controls) and PDA media amended with two concentrations of fungicide (IMZ, 0.025ppm & 0.1ppm; TBZ, 0.25ppm & 1.0ppm) were uncovered for ~2 hours near the packing line and in cool rooms. In addition, swabs were collected of packing line equipment and cool room walls, and then transferred into PDA plates. All plates were incubated for up to 7 days before final assessment. The colony forming units were counted and PDA plates photographed. The photographs are presented in this report to illustrate differences.

Table 1 – Mean fungicide residues (ppm) in fruit associated with the in-line application, a 30 second dip comparison and whole of line application.

Fungicide	Application	Mean Fruit Residues (range)
IMZ	In-line	0.46 (0.3-0.6)
	Dip	0.50 (0.3-0.7)
	Whole line	0.66 (0.4-1.0)
TBZ	In-line	1.44 (0.5-3.6)
	Dip	1.66 (1.0-3.6)
	Whole line	1.69 (0.8-3.5)

- The in-line applications were consistently resulting in about 0.5ppm of IMZ, and the in-line applications are comparable to a 30 second dip.
- The in-line applications of TBZ were yielding a wider range of residues (0.5-3.6ppm), and the in-line application residues were slightly lower than a 30 second dip. The wider range is more likely due to different target residues for TBZ, with a few packers aiming for much higher residues (e.g., >3ppm).
- Two packers were postharvest drenching fruit, which resulted in only a slight increase the mean TBZ whole fruit residues for the group.
- Four of six packers included IMZ in wax, which resulted in a slight increase the mean IMZ whole fruit residues for the group.
- In all instances, whole of line fruit residues included both TBZ and IMZ. Overall, the packers surveyed were aiming for low IMZ residues, but not as low as recorded in this survey. The TBZ residues were usually higher than IMZ, with some packers trying to compensate for a lower IMZ level by increasing TBZ residues.

STANDARD TEST DECAY RESULTS & DISCUSSION

The following graphs represent the upper and lower range of decay results for the survey.

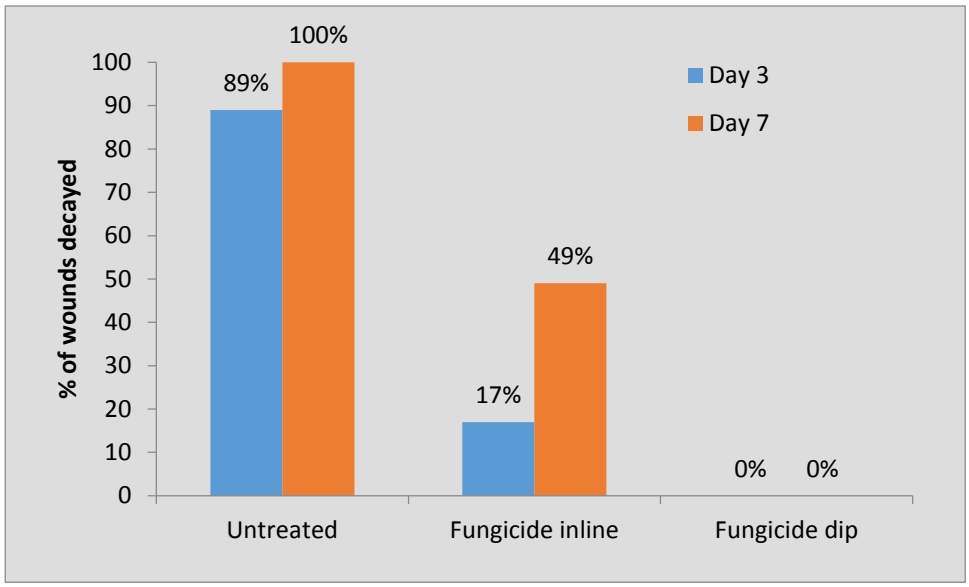


Figure 1a – Percentage of wounds decayed in Standard test after 3 and 7 days incubation (upper)

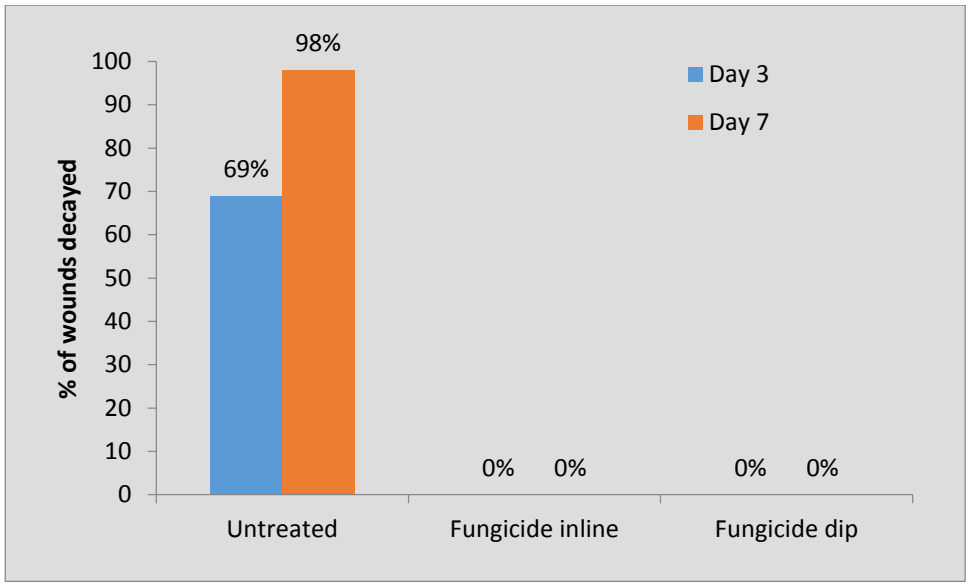


Figure 1b – Percentage of wounds decayed in Standard test after 3 and 7 days incubation (lower)

- The standard test results in figure 1a shows the percentage of wounds decayed in fruit treated inline after 7 days was 49%. Fruit dipped in the same fungicide solution rather than through the line showed no decay at all. No decay for dipped fruit suggests that the fungicide concentration is sufficient but the high level of decay inline suggests that the fungicide application is poor. It is likely that the volume is too low and/or dwell time too short.
- The standard test results in figure 1b show the percentage of wounds decayed in fruit treated inline after 7 days was 0%. Fruit dipped in the same fungicide solution rather than through the line showed no decay at all. No decay for dip and inline suggests that the fungicide concentration and fungicide application is adequate.

General discussion

- The inline fungicide is the major application but not usually the only fungicide application. Ideally, the inline application should control decay as a standalone treatment. However, additional applications in a postharvest drench or in wax will contribute to control decay.
- The standard test involves wounding fruit around the equator. Fruit usually rolls with the equator exposed; decay in the test is usually due to insufficient fungicide concentration (which would also show as decay in dipped fruit) or deficient exposure to fungicide (too little volume or short dwell).
- The results show a 'snap shot' only and repeated surveys are required to get a better understanding of the trends in packing sheds.
- Some practices we noticed in surveyed sheds that may contribute to poor application are faster flow rates and higher volumes of fruit per hour without altering the fungicide application accordingly.

STEM/STYLAR TEST RESULTS & DISCUSSION

The following graphs represent the upper and lower range of decay results for the survey.

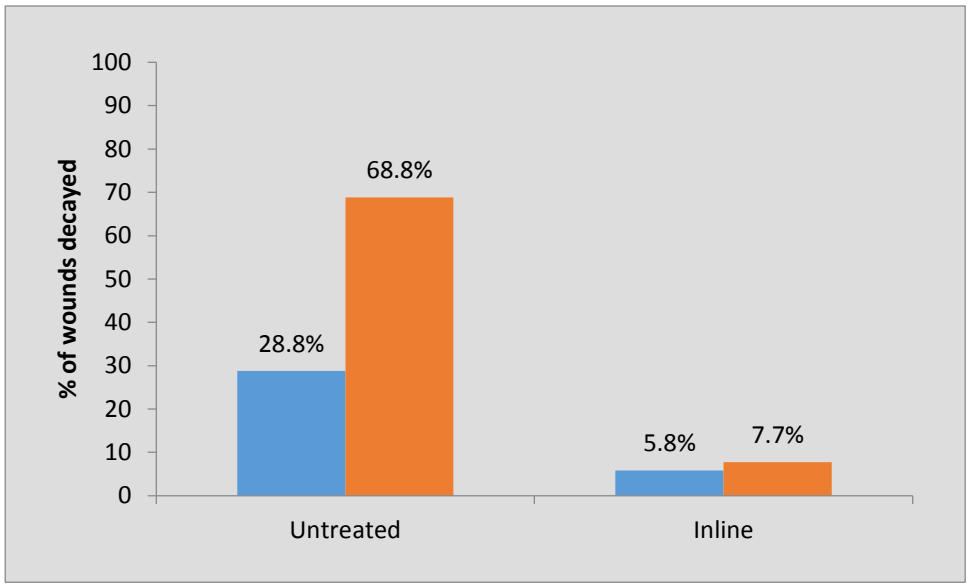


Figure 2a – Average percentage of wounds decayed in Stem/Stylar test after 3 and 7 days incubation (lower)

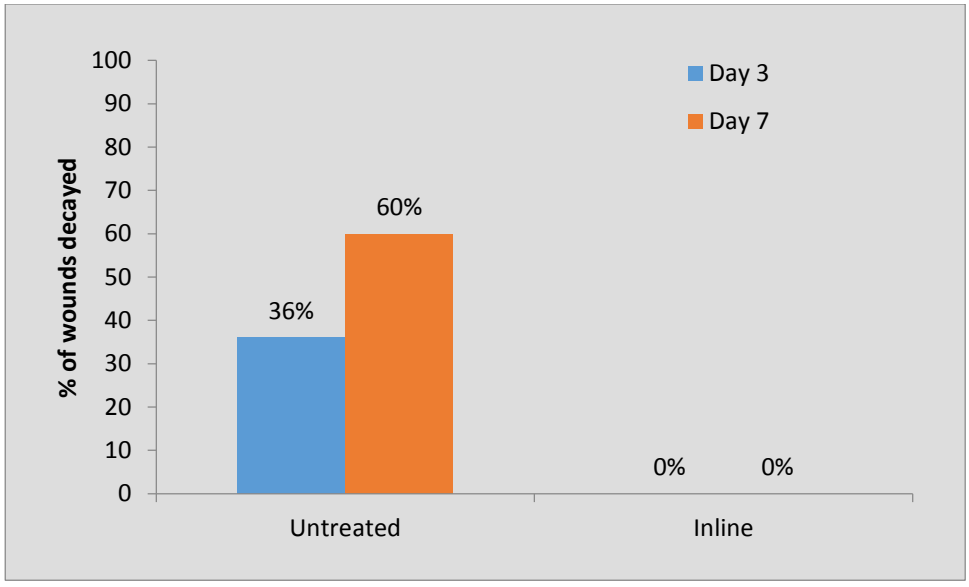


Figure 2b – Average percentage of wounds decayed in Stem/Stylar test after 3 and 7 days incubation (upper)

- The stem/stylar test results in figure 2a show the percentage fruit decay when treated inline after 7 days was 16%. Fruit dipped in the same fungicide solution rather than through the line showed no decay at all. No decay for dipped fruit (figure 1.a) suggests that the fungicide coverage is sufficient but the decay inline suggests that the fungicide application not working well enough. In this case, the standard test showed no decay; it is more likely to be a coverage issue, e.g., fruit may not be rotating under the flow of fungicide.

- The standard test results in figure 1b show the percentage of wounds decayed in fruit treated inline after 7 days was 0%. Fruit dipped in the same fungicide solution rather than through the line showed no decay at all (figure 1b). No decay for dip and inline suggests that the fungicide concentration is sufficient and fungicide application coverage is good.

General discussion

- The inline fungicide is the major application but not usually the only fungicide application. Ideally, the inline application should control decay as a standalone treatment. However, additional applications in a postharvest drench or in wax will contribute to control decay.

- The results show a 'snap shot' only and repeated surveys are required to get a better understanding of the trends in packing sheds.

- Some practices we noticed in surveyed sheds that may contribute to poor or variable coverage are non-rotating fruit under the fungicide flow.

- As indicated earlier, faster flow rates and higher volumes of fruit per hour can reduce fungicide deposit and coverage.

SPORULATION TEST RESULTS & DISCUSSION

The following graphs represent the upper and lower range of sporulation results for the survey.

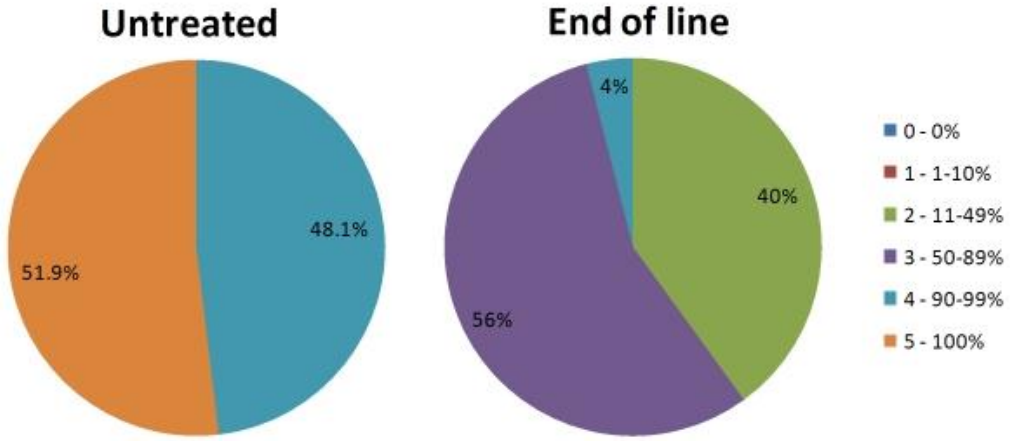


Figure 3a – Percentage sporulation on fruit in Sporulation test after 14 days incubation (lower)

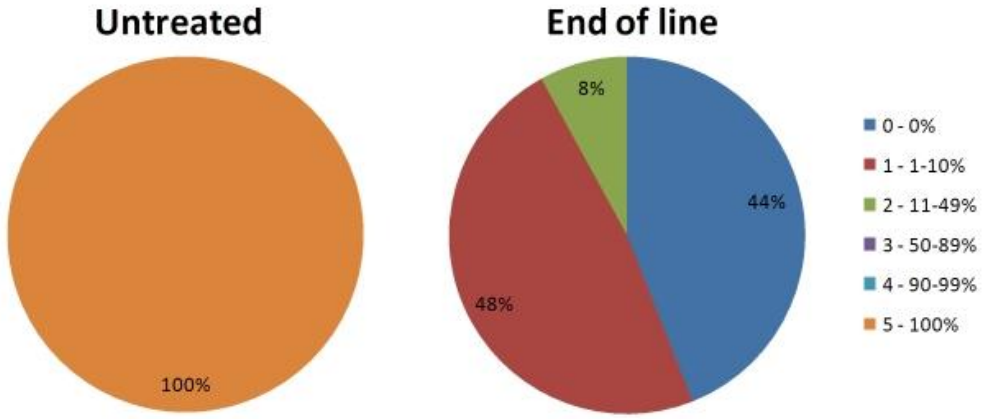
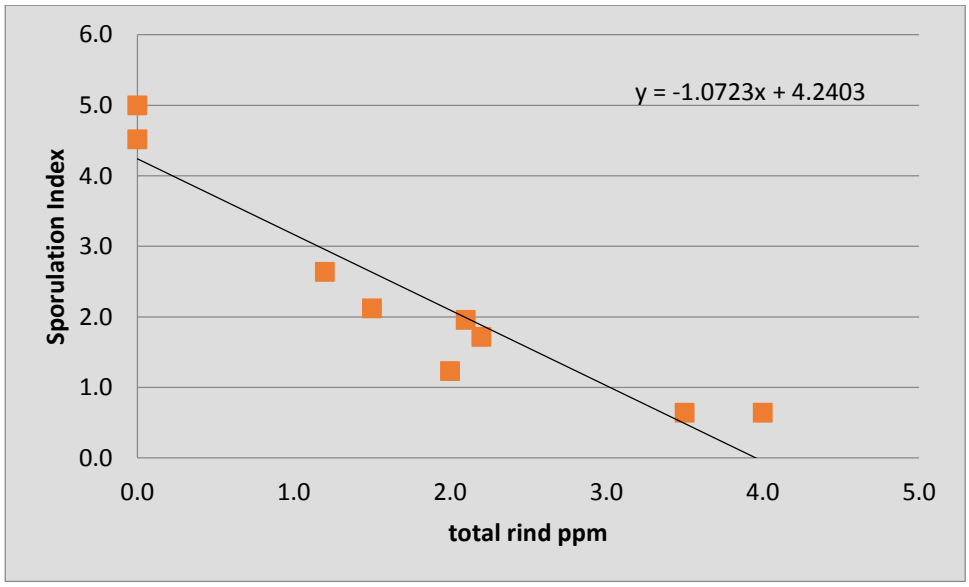


Figure 3b – Percentage sporulation on fruit in Sporulation test after 14 days incubation (upper)

- The results show the area of fruit covered with spores, expressed as a percentage. Fungicide treated and inoculated fruit developed spores after 14 days, with >50% of fruit predominately covered with spores and all fruit having at least 10% of the fruit covered with spores (figure 3a). These results suggest that the overall fungicide residues are not high enough to control sporulation.
- Fungicide treated and inoculated fruit developed relatively few spores after 14 days, with 44% of with no spores and the remainder with less than 10% of the fruit covered with spores (figure 3b). These results suggest that the overall fungicide residues are high enough to control sporulation.

General discussion

- This test involved collecting fruit from end of line (after all fungicide treatments applied). Fruit were injected with mould spores to ensure the fruit decayed. If fungicide residues in the rind are high enough fruit should develop a 'white crust' only. The development of spores in packed fruit 'foul' the carton and are a resistance risk.
- The surveyed sheds all applied IMZ and TBZ to fruit, with the combined fungicides leading to sporulation control. Generally, the IMZ were lower (0.4-1.0ppm) than TBZ residues, and the TBZ range was greater (0.8-3.5ppm). The sporulation control was highly correlated with the combined fungicide rind residues (linear regression; adjusted R^2= 0.88), with >3ppm resulting in good sporulation control.



Graph 1 – The relationship of sporulation index to total fungicide deposit in the rind (TBZ + IMZ; ppm).

RESISTANCE TEST RESULTS & DISCUSSION

The plates grow a range of fungi and yeasts after exposure. The total numbers give an indication of general hygiene but our interest is in the mould spores. They are the smaller blue/green to black circles (CFU). Disregard the brightly coloured and large gray/black CFU.

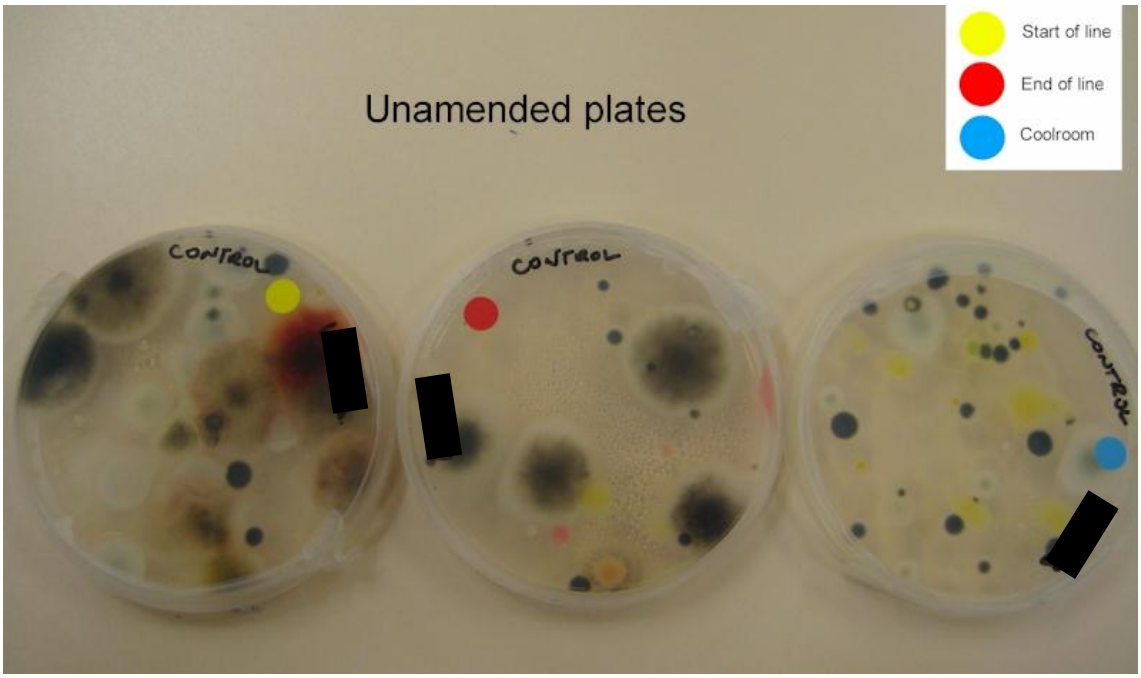


Figure 4a – Images of unamended and exposed plates (lower)

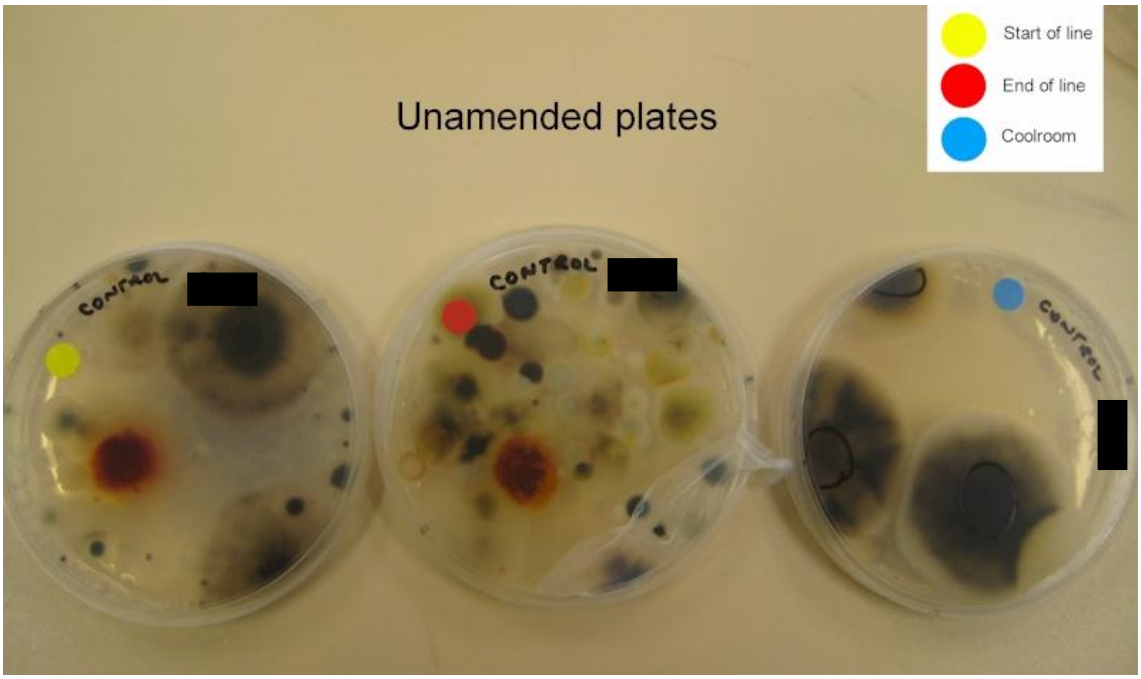


Figure 4b – Images of unamended and exposed plates (upper)

- Two packing sheds were selected to represent the lower (figure 4a) and upper range (figure 4b) for the survey.

- The high numbers of mould spores on cool room plates in figure 4a are a concern. Mould spores in cool rooms early in the season indicate poor cleaning practices. The spores are also likely to be from packed (fungicide-treated) and decayed fruit stored in the cool rooms. Fungicide-treated fruit that decays are liable to be more resistance to the fungicide used.
- Significantly lower spore numbers are on cool-room plates in figure 4b suggesting that sanitation has been effective and cross-contamination limited.

General discussion

- Mould spore growth on unamended (no fungicide) plate indicates the background level in each area of the packing shed.
- The plates show a 'snap shot' only and repeated surveys are required to get a better understanding of the trends in packing sheds.
- Ideally, packinglines should be surveyed periodically over the season and for consecutive years to assess changes in background levels.
- Changes in background levels are more likely to yield useful information than a single survey.
- Sanitation procedures can be evaluated using the 'before' and 'after' results from similar surveys.

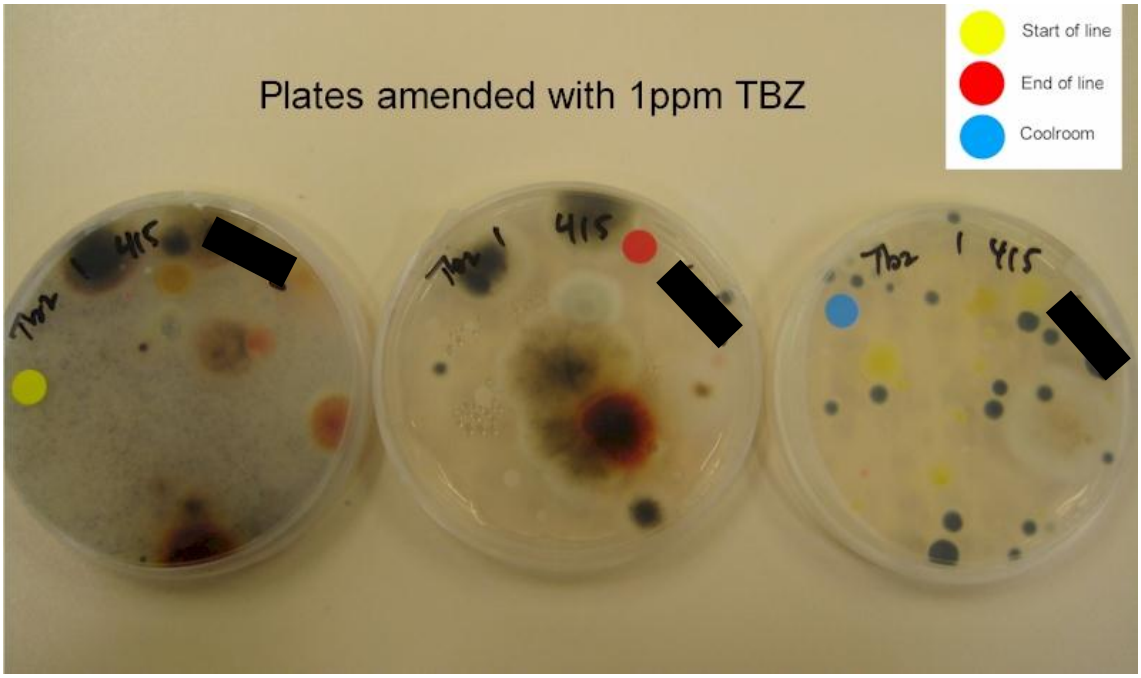


Figure 5a – Images of plates amended with fungicide and exposed (upper)

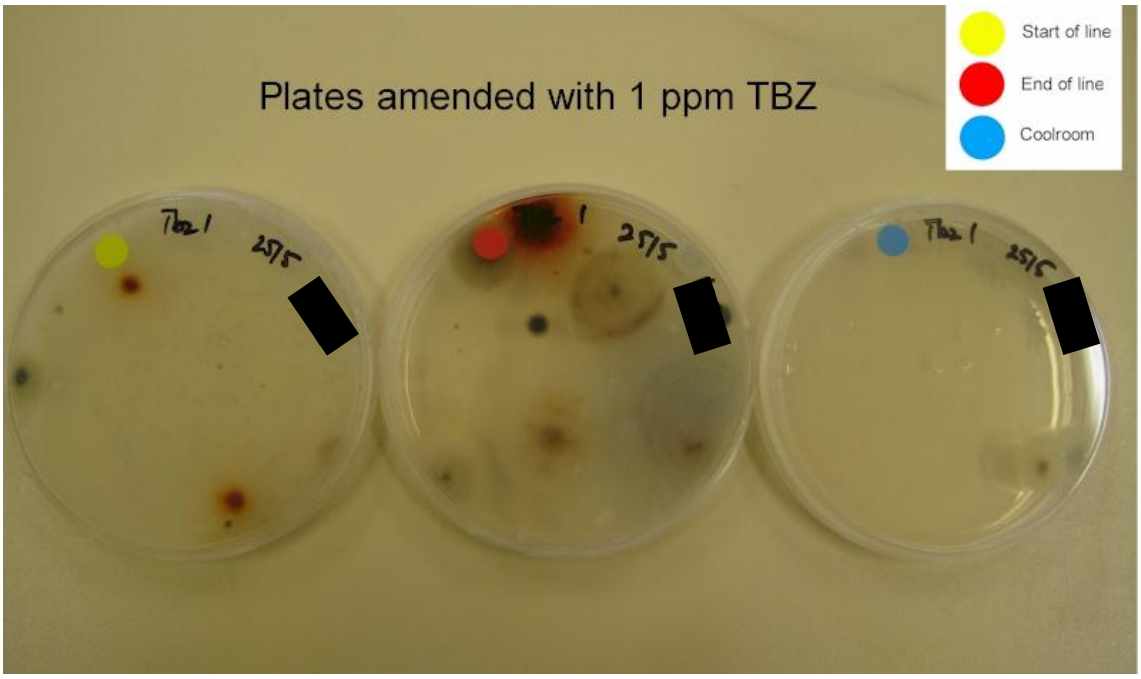


Figure 5b – Images of plates amended with fungicide and exposed (lower)

- Two packing sheds were selected to represent the lower (figure 5a) and upper range (figure 5b) for the survey.
- A comparison of the mould CFU in figure 4a and figure 5a indicates similar levels on both plates. This suggests that the majority of the spore present were resistant to thiabendazole at 1ppm. The presence of resistant spores in the cool room suggests that stored fruit is likely to have decayed, releasing spores with increased levels of TBZ resistance.
- A comparison of the mould CFU in figure 4b and figure 5b from the packing line area indicates lower levels on TBZ-amended plates. This suggests that the majority of the spores present were sensitive to thiabendazole at 1ppm. The cool rooms are virtually clear of spores suggesting that stored fruit has not decayed, either because it does not stay on premises long enough to sporulate &/or high TBZ-residues are maintained on packed fruit (no decay).

General discussion

- The concentration of 1ppm TBZ used on plates was sufficient to completely control mould spores taken from cultures in our SARDI laboratory and several 'wild' mould strains collected from citrus orchards during the survey.
- The growth of mould spores on 1ppm TBZ-amended plates indicates a trend towards resistance within the packing shed environment.
- Growth on 1ppm TBZ-amended plates is usually considered 'technical' resistance.
- This survey has not established if fungicide resistance is at levels sufficient to impact on out-turn but does indicates that further investigation is warranted.
- Packing sheds at risk need to be surveyed periodically over the season and for consecutive years to assess resistance trends.
- Changes in the levels of mould spore growth are more likely to yield useful information than a single survey.
- Resistance management procedures can be evaluated using the 'before' and 'after' results from similar surveys.

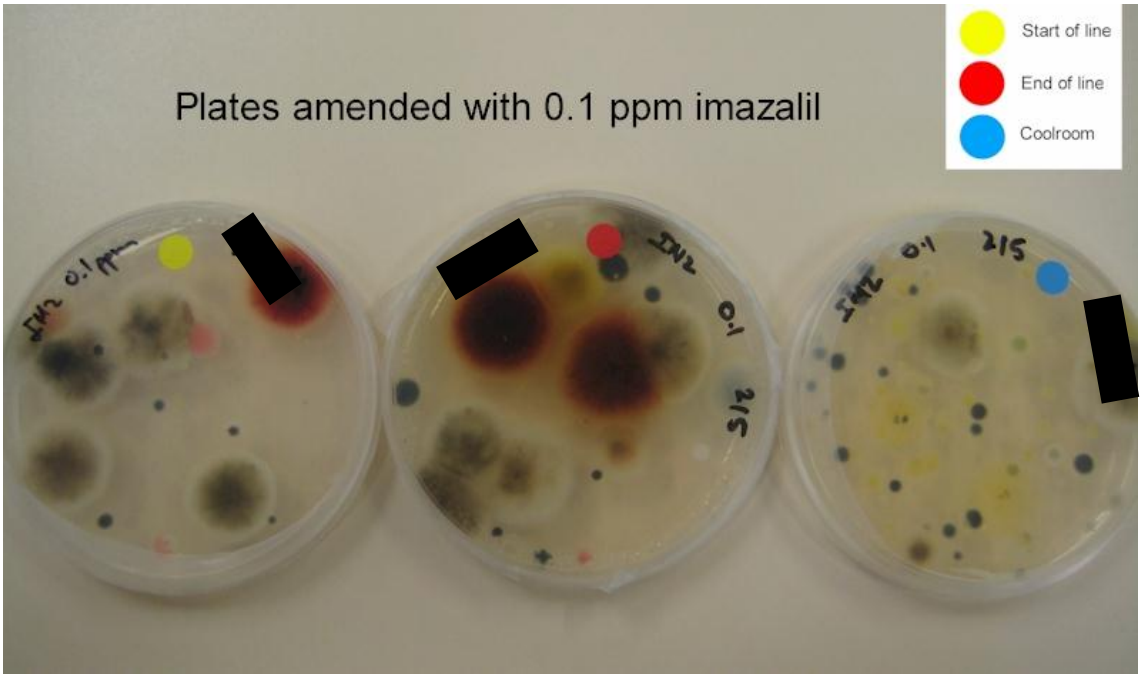


Figure 6a – Images of plates amended with fungicide and exposed (upper)

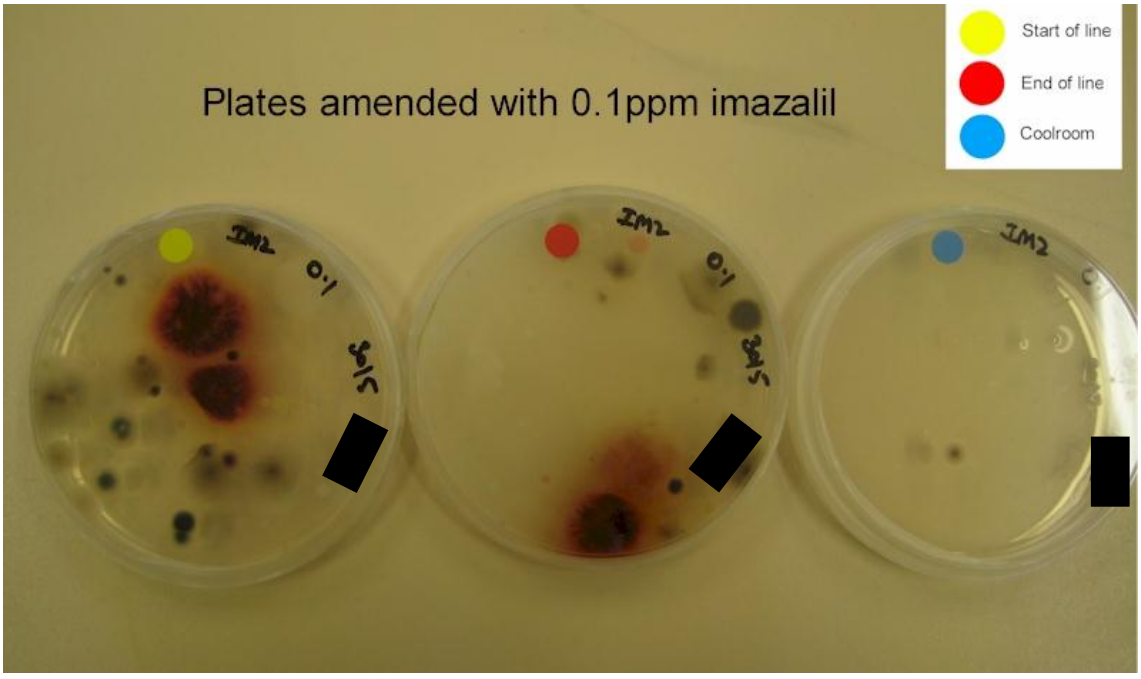


Figure 6b – Images of plates amended with fungicide and exposed (lower)

- Two packing sheds were selected to represent the lower (figure 6a) and upper range (figure 6b) for the survey.
- A comparison of the mould CFU in figure 4a and figure 6a indicates similar levels on both plates. This suggests that the majority of the spores present were tolerant to Imazalil at 0.1ppm. The presence spores in the cool room suggest that stored fruit is likely to have decayed, releasing spores into the cool room. These spores are likely to be more resistant to the fungicides used on the stored fruit. However, the rate in amended plates was too low to establish if these spores have increased levels of IMZ resistance compared to 'wild' orchard stains.
- A comparison of the mould CFU in figure 4b and figure 6b indicates similar or slightly lower levels on the IMZ-amended plates. The remaining spores forming CFU were resistant to IMZ at 0.1ppm. The cool rooms are virtually clear of spores suggesting that stored fruit has not decayed, either because it does not stay on premises long enough to sporulate &/or high IMZ-residues are maintained on packed fruit (no decay).

General discussion

- The concentration of 0.1ppm IMZ used on plates was sufficient to completely control mould spores taken from cultures in our SARDI laboratory, but did not completely control 'wild' mould strains collected from citrus orchards during the survey.
- The growth of mould spores on 0.1ppm IMZ-amended plates may indicate a trend towards resistance but 'wild' orchard strains and packing shed collected strains both showed some growth at this low concentration. Higher concentrations are necessary to discern if IMZ resistance due to packing practices is present.
- Growth on 0.1ppm IMZ-amended plates is not usually considered 'technical' resistance.
- This survey has not established fungicide resistance but does indicates that further investigation is warranted.
- Packing sheds need to be surveyed with higher IMZ concentrations periodically over the season and for consecutive years to assess resistance trends.
- Changes in levels of mould spore growth are more likely to yield useful information than a single survey.
- Resistance management procedures can be evaluated using the 'before' and 'after' results from similar surveys.

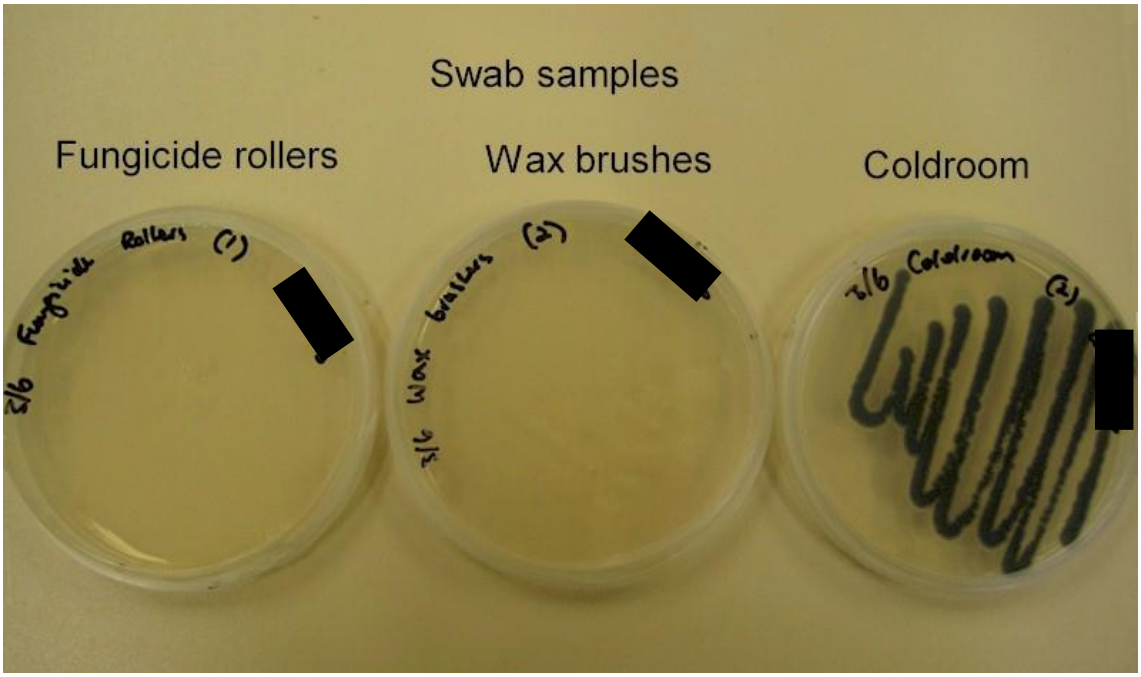


Figure 7b – Images of unamended plates from swabs taken from different parts of the packingshed (upper)

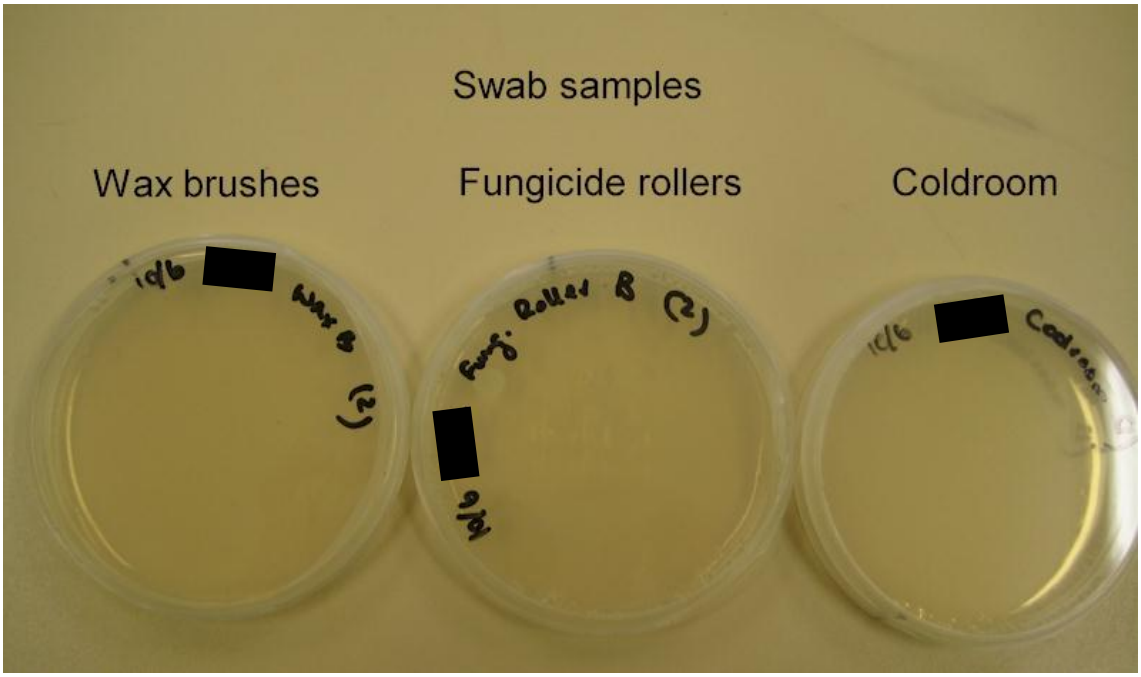


Figure 7b – Images of unamended plates from swabs taken from different parts of your packingshed (lower)

- Two packing sheds were selected to represent the lower (figure 7a) and upper range (figure 7b) for the survey.
- No viable spores were collected from fungicide or wax brushes in either packing line.
- Very high densities of mould spores were found in the cool room shown in figure 7b. However, no mould strains were present in swabs collected from the cool room shown in figure 7b. This is consistent with the other plate results presented above.

General discussion

- It would be expected that no viable spores were found on fungicide brushes or wax brushes (especially when fungicide is incorporated in wax).
- In some cases, brushes can yield high levels of yeast. The yeast is not a problem for fruit but can indicate that cleaning and sanitation is not sufficient.
- The presence of mould spores on swabs collected from the cool room is a concern. These spores are more likely to be fungicide resistant because the cool room is used to store fungicide-treated fruit. Any decay would produce spores resistant to the fungicide used on the stored fruit. Subsequent stored fruit with the same fungicide is then exposed to potentially resistant spores.
- Storing packed fruit on premises for long periods is high risk. The presence of decayed and sporulating packed fruit is a serious problem.

ACKNOWLEDGEMENTS

We would like to thank E.E. Muir & Sons and DECCO US for coordination and analysis of the liquid and fruit samples.

We would like to acknowledge Citrus Australia for endorsing the HAL funded program and our major voluntary contributors:

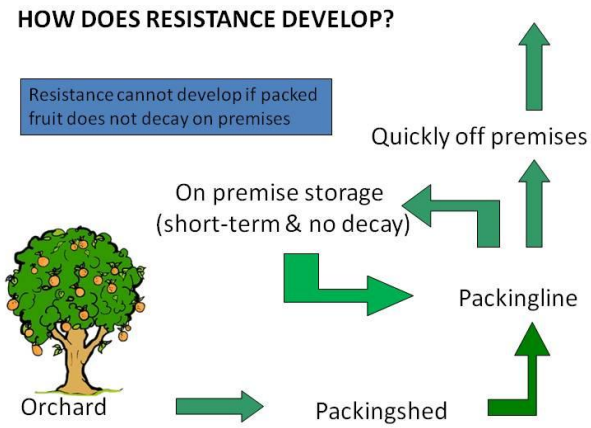
Murray Valley Citrus Board

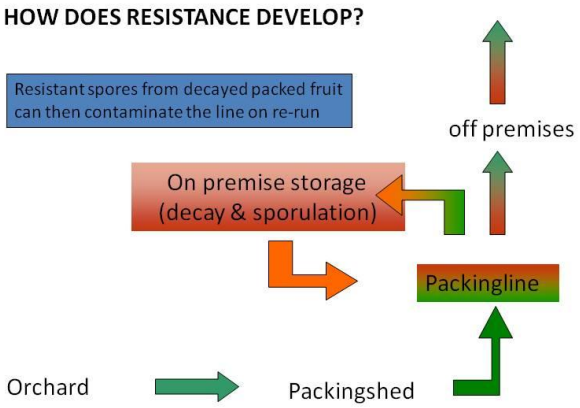
Riverina Citrus

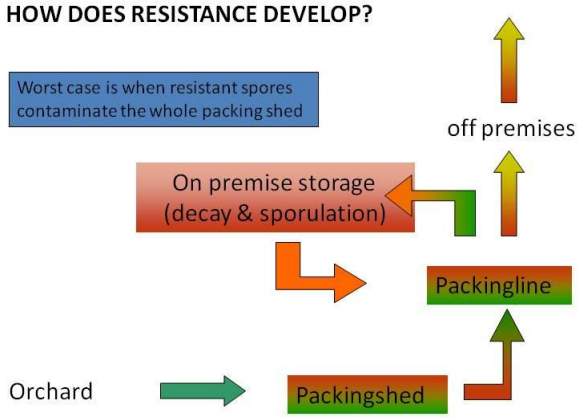
South Australian Citrus Industry Development Board.

For further information contact Peter Taverner on (08) 8303 9538 or via email at peter.taverner@sa.gov.au

Appendix



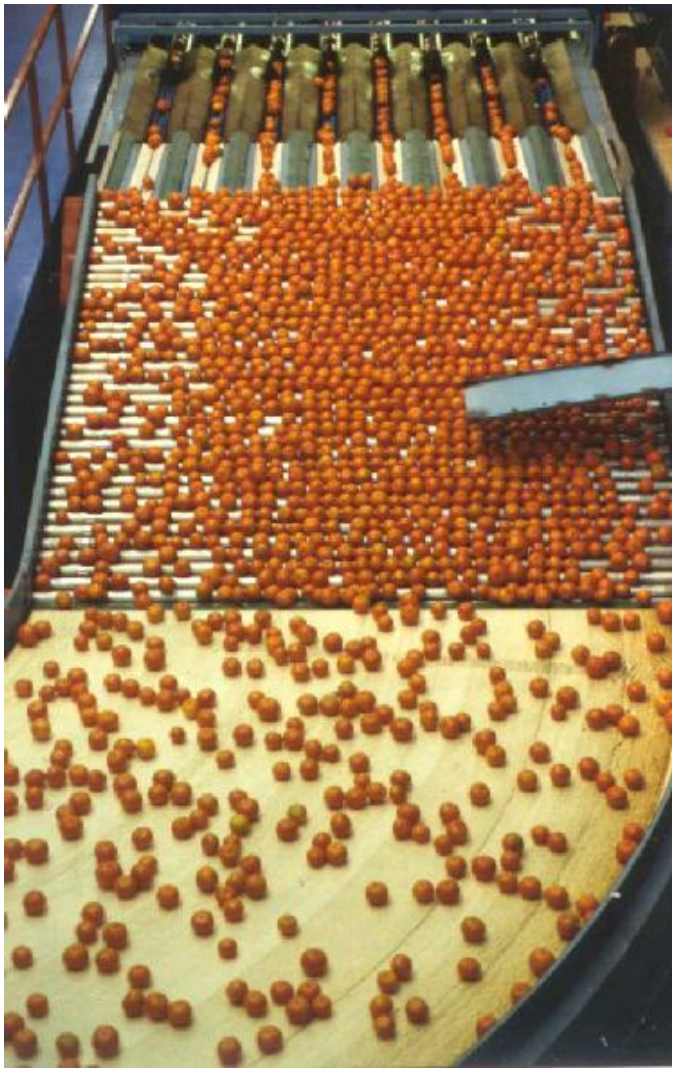




INCREASING FUNGICIDE RESISTANCE ISSUES

Appendix 3

SANITATION SURVEY OF CITRUS PACKINGSHEDS



Florida Citrus Packingline,, 2000.

(incorporating fungicide resistance survey results)

Peter Taverner, Nancy Cunningham and Karolina Steciuk

June 2012

SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

SUMMARY

This survey was conducted to determine whether packinglines are achieving good control of fungal spores on fruit. A reduction in fungal spores on fruit is desirable because the fungicides used for export do not control sour rot. We collected oranges as they past through different processing points in the packing line and measured the levels of mould spores and other microbes. In addition, we exposed media plates amended with currently registered fungicides to detect the presence of resistant spores.

To judge improvements, I have included a summary from the 2004 sanitation survey results 'side-by side' with the current 2012 summary. A comparison of the two summaries shows an overall reduction in spore counts on fruit in 2012. High-pressure washing, sanitised fungicide tanks and wash brushes are significant improvements implemented over recent years.

2004 Survey

- Fruit sampled after the chlorinated wash showed a significant reduction in total microbe levels and to a lesser extent the fungal spore load.
- After oranges pass through the chlorinated wash they still carrying viable spores. Spores are accumulating further down the packingline.
- Spores and microbes are accumulating in fungicide tank. The best approach is to frequently change the solution. Wash out the tank and treat with a sanitiser regularly to ensure that microbes in undrained residues are not re-introduced to fresh solutions.
- Non-recovery fungicides systems can carry an accumulated spore load continuously washed from fruit over the day. Regular cleaning of the brushes will be required to maintain low microbe levels.
- Wax brushes indicate a build-up of fungal spores despite earlier processes to minimise contamination. The best option to avoid contamination at this late stage is to regularly clean wax brushes.

2012 Survey

- Fruit sampled after the HP wash showed a significant reduction in surface fungal spore load and overall microbe counts.
- Sanitation of HP washes must be monitored carefully due to the high organic matter load washed off fruit.
- The spore numbers on fruit decreased as it progressed through the packing line.
- Generally, fungicide tanks had low spore counts. Microbe counts were zero when compatible sanitisers were appropriately used with fungicides.
- Wax brushes showed few spores present; this result was supported by low spore levels on waxed fruit.
- Good cleaning and sanitation practices are evident by the comparatively clean swabs collected throughout the packingline and cool room.
- The fungicide resistance plates showed growth of mould spores on TBZ fungicide amended plates. While numbers were generally low is a concern early in the season.
- Fungicide resistance can increase over a busy season. Monitoring the fungicide resistance situation in a few months would be prudent.

SANITATION SURVEY

INTRODUCTION

This survey is the second in a series of alternating annual surveys of packinglnes to assess fungicide performance and sanitation methods. The surveys are part of the 'Showcase Sheds' initiative, which is within the National Citrus Postharvest Science Program (see acknowledgements, pg. 5). This season's survey aims to assess the effectiveness of sanitation methods and general hygiene/cleaning.

For many years, packers have been using combinations of fungicides and sanitisers to minimise decay in export consignments. The limitation of chlorine products are well understood, but still cause monitoring problems due to the products' sensitivity to pH and organic matter. In addition, many sheds regularly alter their packinglines by changing sanitation products and modifing their application methods for both chlorine and fungicides. These changes have been made with the expectation of improving their packout, including decay control. However, the interactions are complex and objective measurements are rarely made to assess the effectiveness of packingline operations.

This survey of 3 major exporters was conducted to determine whether they are achieving a reduction in fungal spores (and total microbe levels) on fruit as it progresses through packingline. To assess this, we measured the levels of spores on oranges as they passed through different processing points in the packingline.

GENERAL METHODS

At each packingshed, several fruits were samples by hand (using sterile gloves) at a number of sampling points: immediately prior to dumping, after washing and in-line fungicide application, and after waxing. Wash solutions and in-line fungicide solution were sampled and sterile swabs of rollers and brushes were also collected.

All orange samples were weighed and washed with sterile solutions to remove surface microbes. Appropriate dilutions of each fruit wash solution, collected liquids and wax swab samples were surface plated onto agar and incubated. After incubation, the numbers of colonies on agar plates were counted.

The results from each area were pooled and the graphs represent the average microbial levels. The orange surface results were converted to microbial levels per gram of orange weight (eg. spores/gm). The fungicide solution and wax brush levels were converted to microbe levels per ml of solution (eg. spores/ml).

RESULTS AND DISCUSSION

POSTHARVEST DECAY IMPLICATIONS

The fungal spore levels of oranges before dumping indicates the background level of spores entering the packingline. In this survey, the average spore levels prior to dumping was 24 viable spores/gm (see figure 1). This equates to a background load of around 20,000,000 viable spores for every tonne of oranges and represents a significant challenge to the packingline over an extended period of packing. The figures in this survey are based of 'sound' oranges and do not take into account diseased fruit, which can also enter the system adding millions more spores per fruit.

Most sheds have some form of high pressure washing early in the packing process. They are sanitised using various products, including calcium Hypochlorite, peroxyacetic acid, and chlorine dioxide. All fruit sampled after the chlorinated wash showed a large reduction in the surface spore load (~98% reduction) (see figure 1). This is in stark contrast with survey results in 2004, where average spore counts were reduced by 27% after a chlorinated wash. It is important to recognise the limitations of sanitisers in reducing spore loads on the surface of fruit. This improvement was most probably due to the change to high pressure washing, which physically removes the spores from the surface of the fruit. The spores washed off are much easier to neutralise in water and do not migrate further down the line.

After oranges pass through the chlorinated wash they may still carry some viable spores. It is important to minimise the number of spores as they are washed into the recirculating fungicide solution. The fungicides used for the export markets control mould (*Penicillium sp*.), but resistant mould spores and sour rot will accumulate in the solution. For this survey, average spore levels remained low (see figure 2). Some accumulation can occur in fungicide solutions when sanitisers are not added to the tank. Generally, spore levels could be much higher and this relatively low level reflects the overall effectiveness of the shed practices.

In 2004, directly reducing spore levels by adding sanitisers in fungicide tanks was not attempted by any packingshed. Imazalil fungicides (e.g., Fungaflor®, Magnate®), commonly used in in-line fungicide systems are incompatible with most chlorine compounds. More recently, compatibility of peroxyacetic acid (e.g., Tsunami®) with imazalil fungicides has been demonstrated but it is still not commonly used for this purpose. An option many packers have adopted to overcome the accumulation of spores in the fungicide tank is frequently changing the solution (at least daily). The tank can be cleaned with a chlorinated solution between batches to sterilise the system. Smaller packinglines can run without topping up and then dump after the concentration runs down to half strength. Initially, solutions should be analysed to determine the rate of fungicide strip out to calculate top up &/or dump times. Quality assurance requires documentation of fungicide residues on fruit and this measurement can be useful in determining top up rates. The

advantages of high volume systems are that they maintain good contact of fungicides on fruit, which increases fungicide uptake.

Another option used by packers is to apply the fungicide in low volume non-recovery systems. This system has the advantage that the solution is fresh, ie. no recirculation to accumulate spores. Non-recovery systems rely heavily on the brushes to both wet fruit and distribute the fungicide evenly. An important hygiene consideration with a non-recovery system is the accumulation of spores in the brushes themselves. Although fresh solution is used, the brushes will be carrying an accumulated spore load. Low solution volumes may actually increase the concentration of spores in the brushes compared to flooding systems. Regular cleaning of the brushes will be required to maintain low microbe levels.

Swabs of wax brushes demonstrated that spores are not accumulating throughout packing system (see figure 2). Earlier processes, such as high-pressure washes, probably minimised contamination by removing spores from the surface of fruit. Packers using fungicides in wax may gain some benefit but will not be controlling sour rot spores. The best option to avoid contamination is regular cleaning of wax brushes. There is no substitution to thorough cleaning using hot water under pressure. A proprietary chlorinated detergent may provide greater reduction of microbe levels when cleaning brushes and packingline surfaces.

Overall, the packing lines surveyed achieved high fungal control. The survey shows that spore levels decline as fruit was carried through the system. The accumulation of spores in fungicide solutions and wax brushes have been addressed since earlier surveys but vigilance must be maintained. It is hoped that the packers surveyed are representative of the industry.

PUBLIC SAFETY IMPLICATIONS

The results of the survey show total microbe numbers and are not classified according to health risk. The majority of microbes on fresh produce are harmless soil-dwelling bacteria. As a consequence, high microbe numbers do not necessarily constitute a public health risk but are indicative of a potential area of risk. Use this survey to identify areas where potential problems can occur.

High populations of microbes were found on pre-dump fruit at packingsheds (see figure 3). The high pressure wash significantly reduced levels of all microbes, however, fruit collected after the fungicide solution had increased total microbe numbers. The fungicide solution had high levels of bacteria that were presumably washed off the oranges and accumulating in the system (see figure 4). As the fungicide did not control the bacteria they could proliferate. This accumulation also carried over the wax brushes where high numbers were found, probably deep in the brush-beds (see figure 4). It is important to note that the levels of fruit remained relatively low. The reasons for this were not determined in this study, but it seems likely that regular cleaning of

these areas would reduce overall microbe counts on fruit. Fungicide tanks and wax brushes were identified in pervious surveys as potential risk areas and, perhaps, mitigation measures are being applied more frequently in these areas.

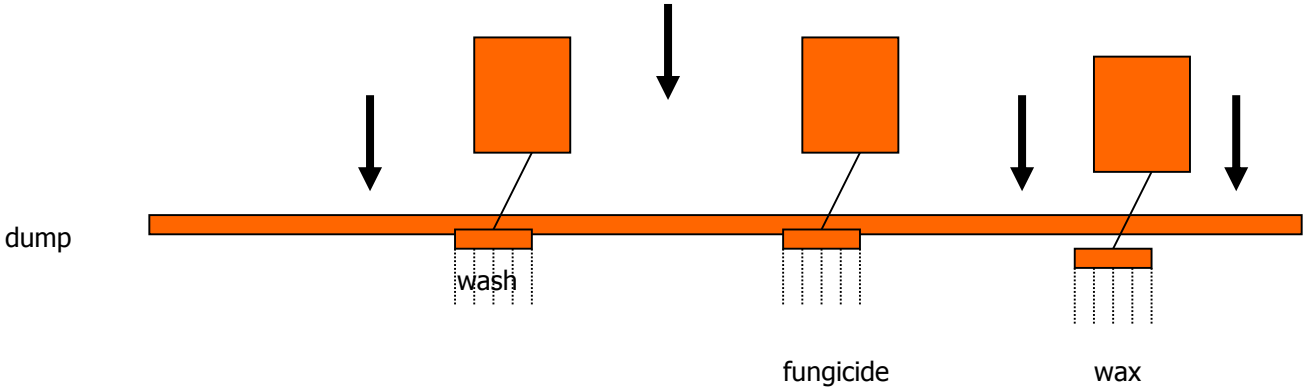
Disclaimer: This paper contains the results of a survey and the best information available to the author at publication. Mention of a commercial or propriety product does not constitute an endorsement or recommendation of its use. The South Australian Research and Development Institute (SARDI) makes no warranty of any kind expressed or implied concerning the use of technology mentioned in this document.

Acknowledgements

The National Citrus Postharvest Science Program, based at SARDI, will be facilitated by HAL in partnership with Citrus Australia for the period of June 2011–Mar 2015. It has been funded by citrus grower levies and voluntary contributions pledged from the three regional citrus marketing boards (at the time). The Australian Government provides matched funding for all HAL"s R&D activities. Several citrus packers and service providers have also provided support for the specific activities recorded in this report.

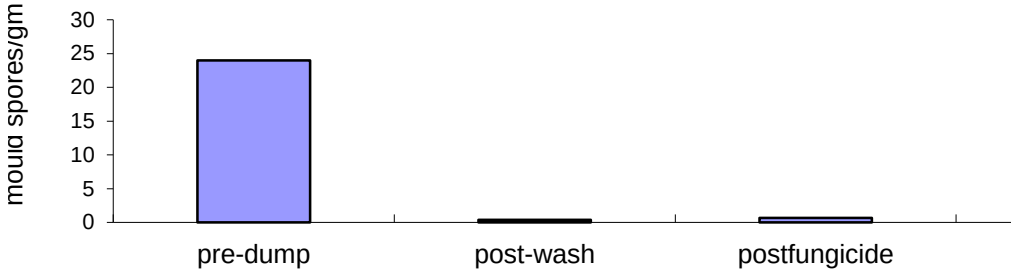
Mean mould levels on fruit surfaces and packingline solutions

Below: Schematic diagram of packingline. Arrows show where oranges were collected



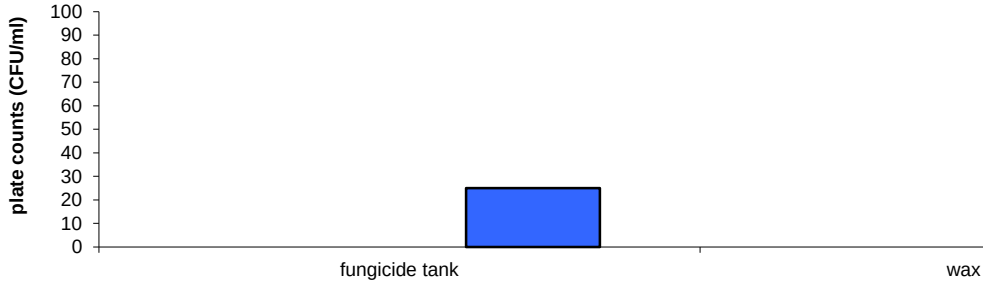
Below: Mould levels on fruit surfaces are shown. Oranges were retrieved from packinglines at 3 different points.

Figure 1



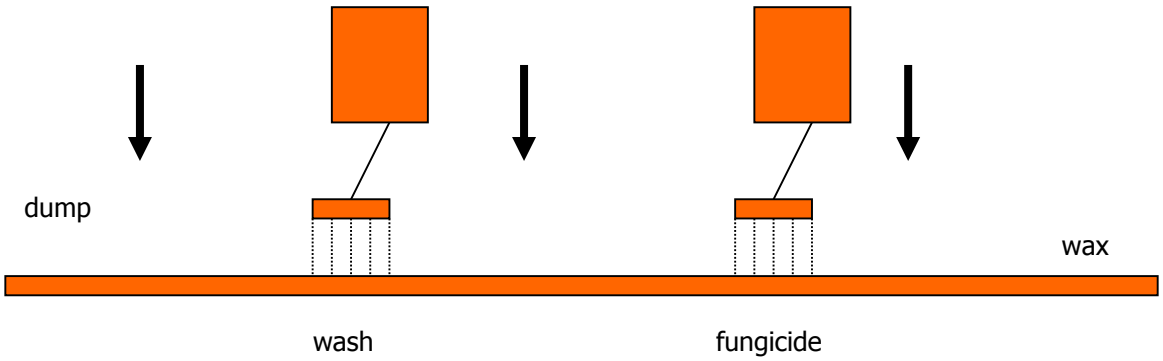
Below: Fungi levels in fungicide tanks and wax brushes are indicative of fungal spores not controlled by the fungicide used, eg., sour rot

Figure 2



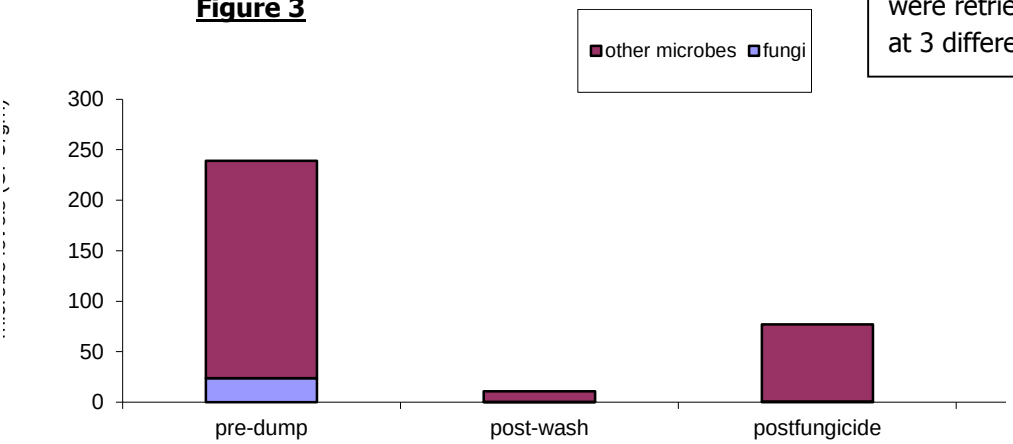
Mean total microbe levels on fruit surfaces and packingline solutions

Below: Schematic diagram of packingline. Arrows show where oranges were collected



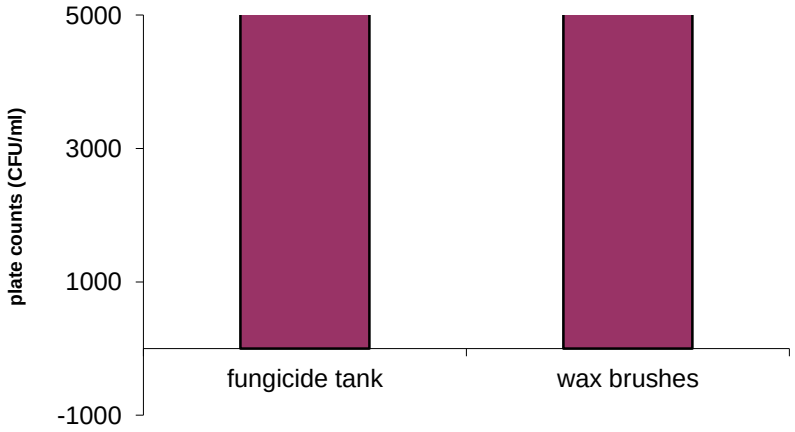
Below: Microbe levels on fruit surfaces are shown. Oranges were retrieved from packinglines at 3 different points.

Figure 3



Right: Microbe levels in fungicide tanks and wax brushes are indicative of microbes not controlled by the fungicide used. Eg. bacteria.

Figure 4



APPENDIX – FUNGICIDE RESISTANCE TESTS

INTRODUCTION

In 2011, SARDI introduced fungicide resistance testing; it involved exposing media plates amended with low concentrations of fungicide in various parts of the packing operations. Initially, we chose very low rates of fungicides that controlled a highly sensitive mould isolate to determine if 'technical' resistance was evident. We found high mould spore growth on plates amended with thiabendazole (TBZ), and some growth of imazalil (IMZ) plates. A trend was established but we were unsure if fungicide resistance on these plates was sufficient to impact on packing operations.

After review of the literature[2], we increased the concentrations of TBZ to 5ppm and 15ppm, and IMZ to 0.5ppm and 1.5ppm for monitoring resistance in packing sheds. In addition, we included fludioxonil (FLU) at 1.0ppm and 2.5ppm to provide a baseline prior to commercial use in Australian citrus packing. This June, fungicide resistance surveys were conducted in 4 packingsheds.

The results presented are representative of the sheds evaluated. However, there were significant variations between each shed. These results do not necessarily represent the situation in other citrus packing operations.

RESULTS AND DISCUSSION

The images on the following page are from plates collected during the survey. They show many different fungi, yeast and bacteria. Our interest is with the mould spores only, which are dark circles.

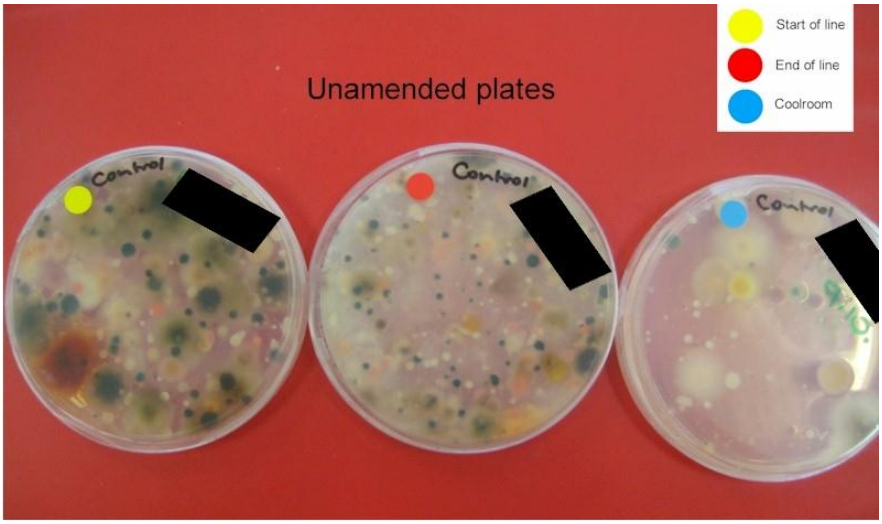
The unamended (control) plates contained no fungicide and indicate overall level of microbes. The plates exposed in the cool room shows no mould (dark circles) and fewer colonies indicating a cleaner environment. A comparison of the control and 15ppm TBZ plates indicate similar mould growth on both sets of plates suggesting a high portion of mould spores are resistant to TBZ. In contrast, a comparison of the controls with the FLU plates reveals no mould growth on any FLU plates. Ideally, all fungicide-amended plates should have no mould growth (i.e., susceptible mould spores). However, the consistent use of TBZ and IMZ appears to be leading to increased resistance.

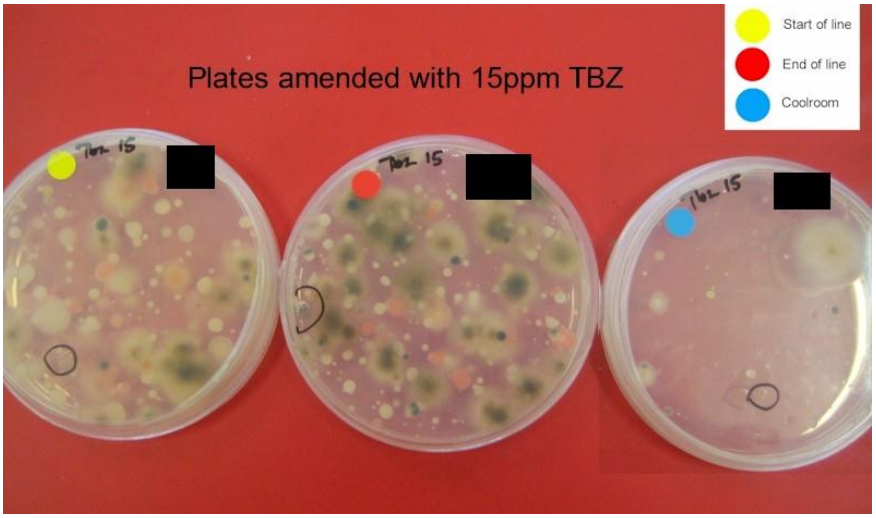
Mould spores have been isolated from fungicide-amended plates collected during this survey. Although rarer, some IMZ resistant spores were collected during the survey. Further work is planned to verify resistance by inoculating and treating fruit with label rates of fungicides.

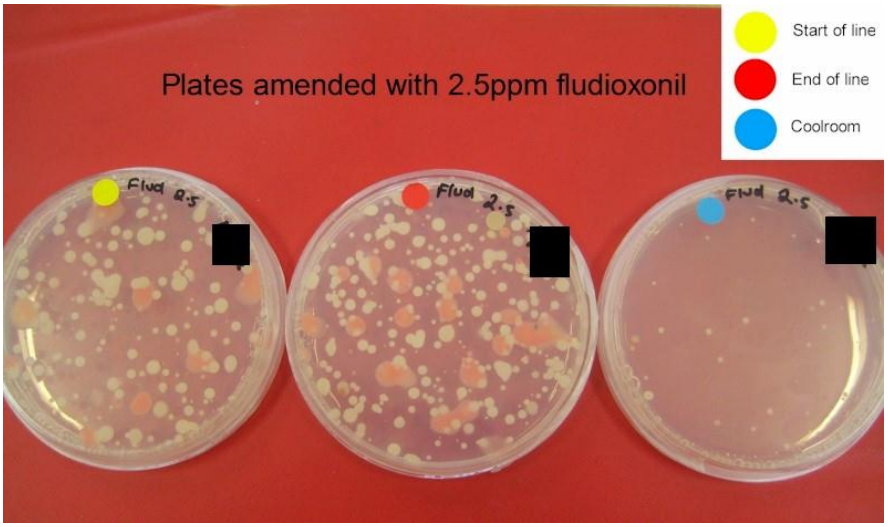
[2] Thanks to Andrea Pastore for kindly providing a useful reference on monitoring IMZ resistance in citrus packinghouses in Uruguay. [E. Perez et al., Postharvest Biology and Technology 60 (2011) 258-262].

Isolates will be exposed to different fungicides, which should allow evaluation of possible multiple resistance.

Evidence of fungicide resistance early in the season (after summer break and cleaning) is a concern because resistance is likely to increase during the season. Periodic monitoring of packinglines is recommended.






Plates amended with 2.5ppm fludioxonil
Start of line
End of line
Coolroom
Flud 2.5
Flud 2.5
Flud 2.5

Appendix 4

Development of Fungicide Resistance Monitoring Service to Citrus Packers

Introduction

Imazalil (IMZ) and thiabendazole (TBZ) have been used exclusively to control postharvest decay on export citrus fruit consignments. Packers are holding treated fruit for longer on premises and there are less ‘quiet’ periods to conduct major sanitation measures. All of which, are likely to proliferate resistance to these fungicides.

To my knowledge, Australian citrus packers have not been systematically sampled for fungicide resistance. As such, plate surveys were instigated in participating packing sheds, as part of the National Citrus Postharvest Science Program. We were interested in determining if resistance mould isolates could be detected and whether they were likely to cause decay failure under commercial conditions. It quickly became evident that fungicide resistance isolates could be found in packing sheds at most times. From this work, we identified a need to monitor and to explain the implication of the results. This chapter follows the development a survey test and assessment sheet for fungicide resistance risk.

Material and methods

Initially, the SARDI Citrus Postharvest Group used a range of methods and fungicide rates to establish if resistance to IMZ and TBZ was present. In 2012, SARDI developed a test kit to assess the resistance of air-borne spores to 3 current fungicides, and assess general hygiene trends. The kits included potato dextrose agar (PDA) amended with fungicide, resulting in nil fungicide or 5 or 15 mg/L TBZ (Syngenta Australia, Macquarie Park, NSW), 0.5 or 1.5 mg/L IMZ (Janssen PMP, Beerse, Belgium) or 1, 2.5 mg/L fludioxonil (FLU) (Syngenta Australia, Macquarie Park, NSW).

Petri dishes (9cm diameter) containing amended agar for each fungicide concentration were exposed in 3 areas of commercial packing operations for ~60 minutes. Typically, plates of each fungicide and rate were exposed near where fruit was dumped onto the line, near the waxing or packing area, and inside the cool rooms.in addition, two swabs were taken of surfaces in three comparable areas; e.g. dump rollers, wax brushes and cool room walls. Swabs and plates

were sealed before returning to the SARDI laboratories. The contents of swab were plated onto PDA plates. All plates were incubated for 3 days at 25^0C before assessment.

Results and Discussion

1.1.1 2011 Season

During the first season, agar plate were exposed in packing facilities during a scheduled fungicide survey (see appendix X; Fungicide Survey of Citrus Packingsheds in Riverland, Sunraysia and Riverina, July 2011).) This was our first attempt to discern if any fungicide resistance occurred in packingsheds. We chose very low rates of fungicides to determine if 'technical' resistance was evident. The following are an excerpt (in italics) from one packer's results.

Case study; July 21011

Agar plates were exposed in 2 areas of the packing line and the cool room. Swabs were taken in comparable areas.

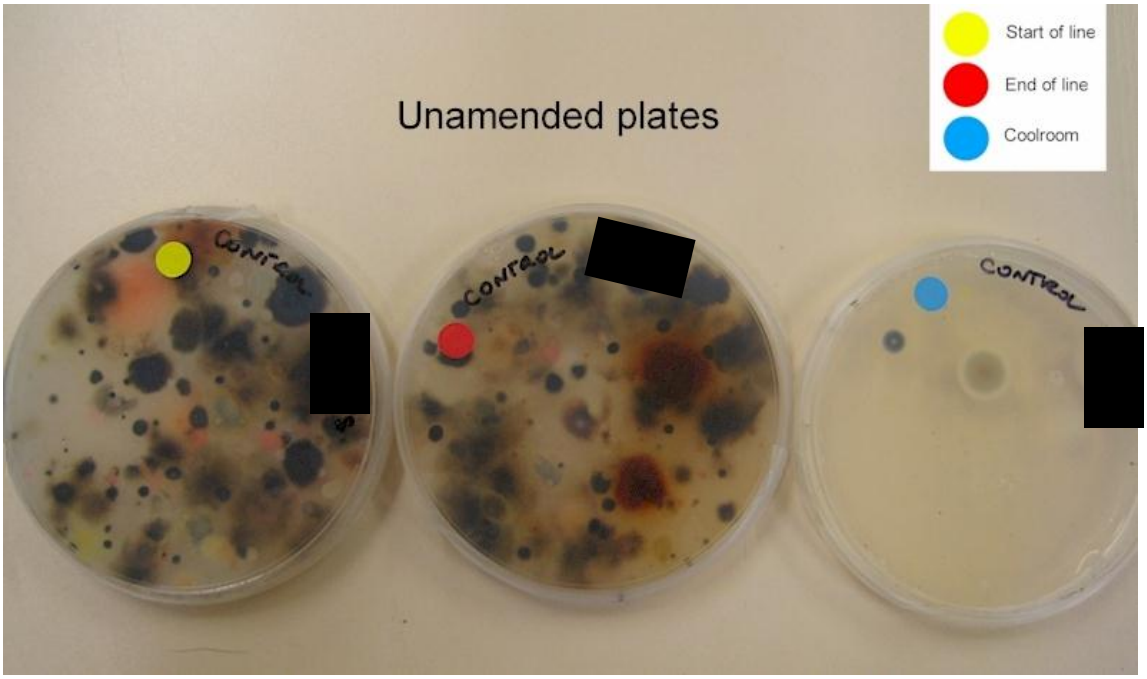


Figure 1 – Images of unamended plates exposed in packingshed

Mould spore growth on unamended (no fungicide) plate indicates the background level in each area.

Significantly lower spore numbers are on cool-room plates suggests sanitation has been effective and cross-contamination limited.

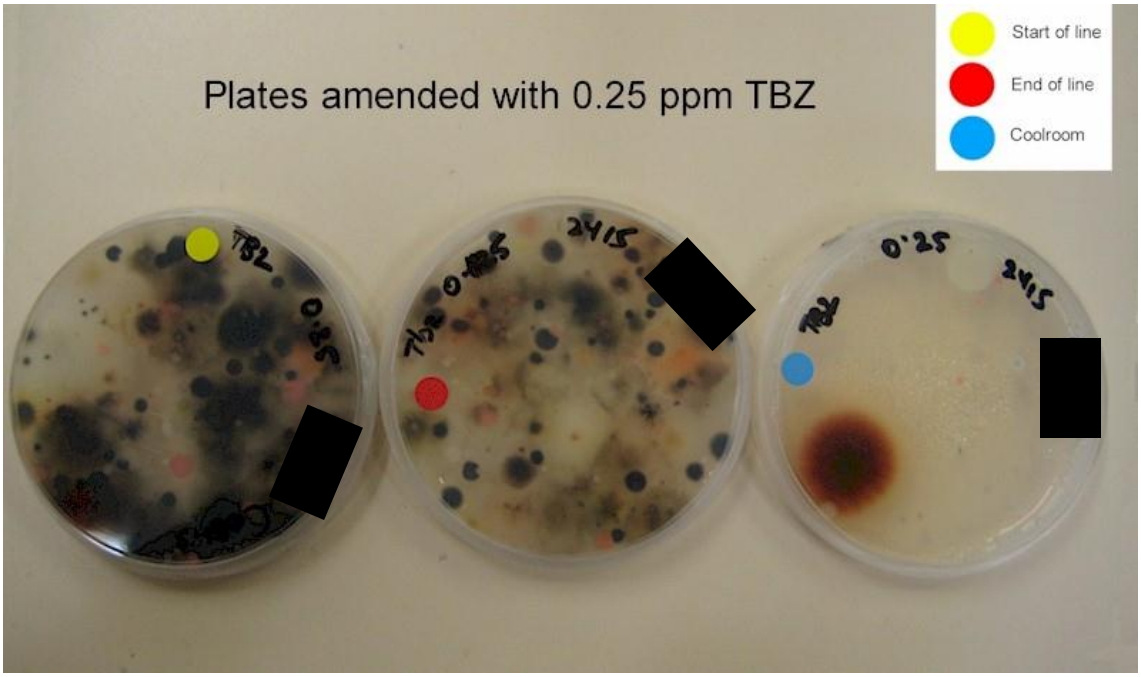


Figure 5 – Images of plates amended with fungicide and exposed in packingshed

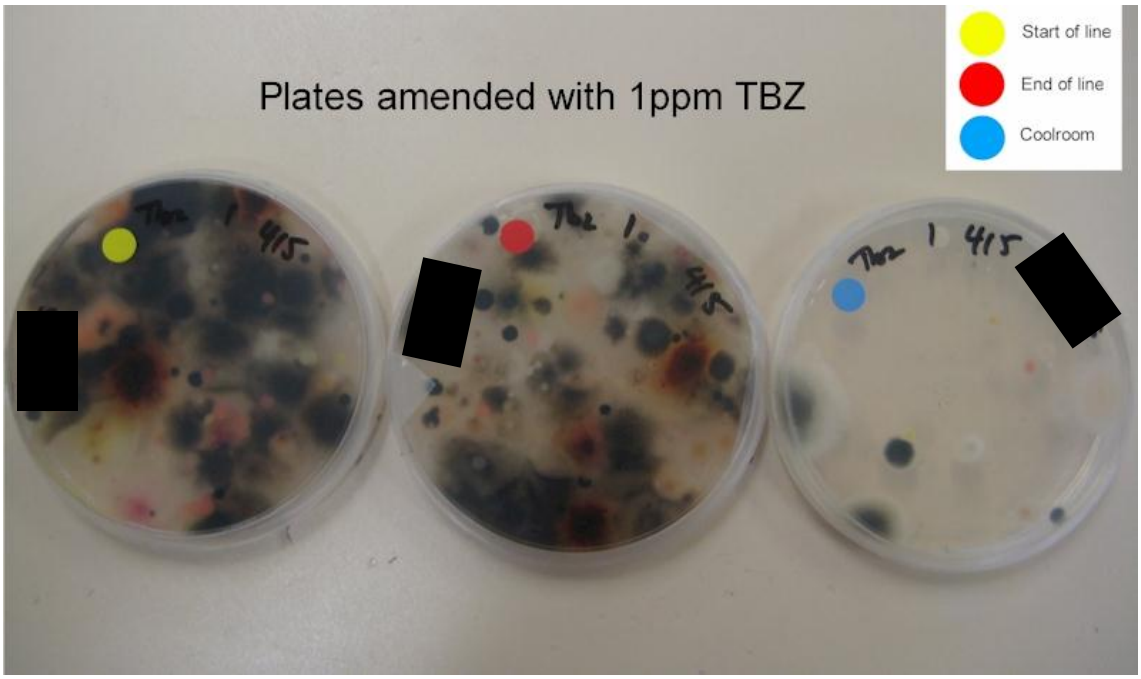


Figure 6 – Images of plates amended with fungicide and exposed in packingshed

The mould spore growth on plates exposed in your shed indicates the presence of mould spores with a greater level of tolerance to thiabendazole than our highly sensitive laboratory isolates.

The numbers of spores were similar to unamended plates suggesting most mould spores were tolerant to thiabendazole at these low concentrations.

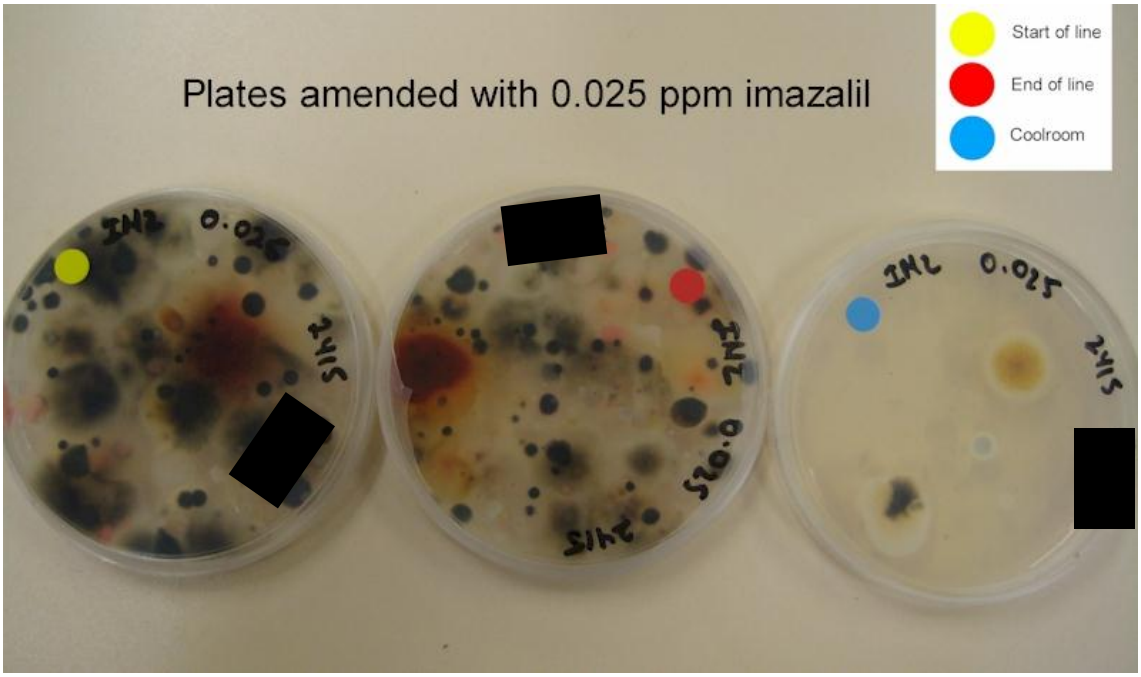


Figure 7 – Images of plates amended with fungicide and exposed in packingshed

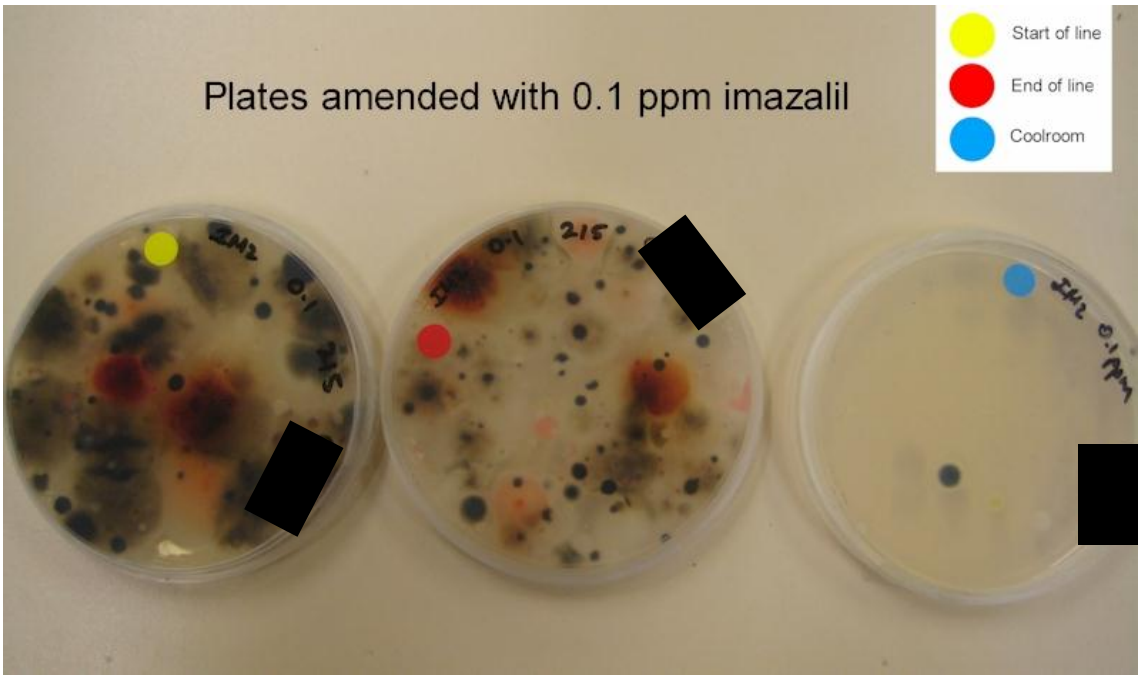


Figure 8 – Images of plates amended with fungicide and exposed in packingshed

The mould spore growth on plates exposed in your shed indicates the presence of mould spores with a greater level of tolerance to imazalil than our highly sensitive laboratory isolates.

The numbers of spores were similar or slightly lower than on unamended plates suggesting most mould spores were tolerant to imazalil at these low concentrations.

The swab from cool-room walls and wax brushes showed no mould growth. Results from fungicide rollers were inconclusive. Some mild yeast growth (seen a lines on the plate surface) was observed on wax brushes indicating that cleaning and sanitation at the end of the day is not penetrating deep into the brush beds.

The results from the July survey indicated that 'technical' resistance was widespread. Further plate surveys were conducted in November, using increased concentrations of IMZ and TBZ amended in plates, and adding fludioxonil (FLU) amended plates to the survey.

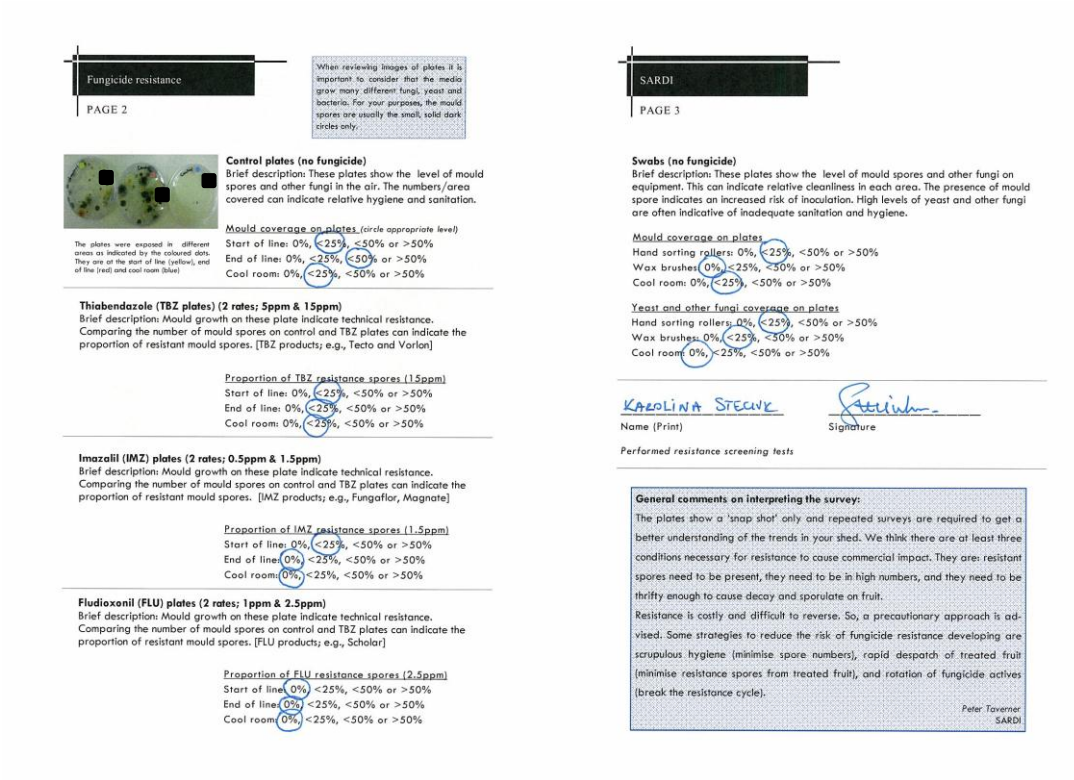
1.1.2 2012 Season

During 2012, agar plates were exposed in packing facilities during a scheduled sanitation survey (see appendix X; Sanitation Survey of Citrus Packingsheds, June 2012). We standardised the methods and concentrations of fungicides for all future fungicide resistance surveys (see Materials and methods). The June survey highlighted the importance of cleaning and hygiene during the 'off-season'. TBZ resistance isolates was detected using fungicide amended plates. The levels of IMZ resistance was considerably lower than TBZ. The survey also confirmed the susceptibility of all isolates to the new active, fludioxonil.

Similar fungicide resistant surveys were conducted in August and October to better assess the fungicide resistance as the season progressed. However, three comprehensive survey reports per year were too much for busy packers to digest and feedback was too slow for timely remedial action. Information needed to be more 'stream-lined' and in a consistent format to allow packers to compare survey periods quickly. This lead to the development of the survey sheet in October 2012 (see Image 1).

The survey sheet included an image of the control plates and provided ratings for all exposed plates. The control plates were assessed according to area covered, indicating overall hygiene. The area covered by spores in fungicide amended plates was compared to the control plates in each area, which provided the proportion of resistant spores. The rating system was 0%, <25%, <50% & >50% for proportional resistance. Swabs were also taken of packing surfaces to assess overall cleaning and hygiene.

Image 1 Survey sheet from fungicide resistance surveys given to packers



Fungicide resistance

PAGE 2

When reviewing images of plates it is important to consider that the media grow many different fungi, yeast and bacteria. For your purposes, the mould spores are usually the small, solid dark circles only.

Control plates (no fungicide)
Brief description: These plates show the level of mould spores and other fungi in the air. The numbers/area covered can indicate relative hygiene and sanitation.

The plates were exposed in different areas as indicated by the coloured dots. They are at the start of line (yellow), end of line (red) and cool room (blue)

Mould coverage on plates *(circle appropriate level)*
Start of line: 0%, <25%, <50% or >50%
End of line: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

Thiabendazole (TBZ plates) (2 rates; 5ppm & 15ppm)
Brief description: Mould growth on these plate indicate technical resistance. Comparing the number of mould spores on control and TBZ plates can indicate the proportion of resistant mould spores. [TBZ products; e.g., Tecto and Vorlon]

Proportion of TBZ resistance spores (15ppm)
Start of line: 0%, <25%, <50% or >50%
End of line: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

Imazalil (IMZ) plates (2 rates; 0.5ppm & 1.5ppm)
Brief description: Mould growth on these plate indicate technical resistance. Comparing the number of mould spores on control and TBZ plates can indicate the proportion of resistant mould spores. [IMZ products; e.g., Fungaflor, Magnate]

Proportion of IMZ resistance spores (1.5ppm)
Start of line: 0%, <25%, <50% or >50%
End of line: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

Fludioxonil (FLU) plates (2 rates; 1ppm & 2.5ppm)
Brief description: Mould growth on these plate indicate technical resistance. Comparing the number of mould spores on control and TBZ plates can indicate the proportion of resistant mould spores. [FLU products; e.g., Scholar]

Proportion of FLU resistance spores (2.5ppm)
Start of line: 0%, <25%, <50% or >50%
End of line: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

SARDI

PAGE 3

Swabs (no fungicide)
Brief description: These plates show the level of mould spores and other fungi on equipment. This can indicate relative cleanliness in each area. The presence of mould spore indicates an increased risk of inoculation. High levels of yeast and other fungi are often indicative of inadequate sanitation and hygiene.

Mould coverage on plates
Hand sorting rollers: 0%, <25%, <50% or >50%
Wax brushes: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

Yeast and other fungi coverage on plates
Hand sorting rollers: 0%, <25%, <50% or >50%
Wax brushes: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

KAROLINA STECYK
Name (Print)

Signature

Performed resistance screening tests

General comments on interpreting the survey:
The plates show a 'snap shot' only and repeated surveys are required to get a better understanding of the trends in your shed. We think there are at least three conditions necessary for resistance to cause commercial impact. They are: resistant spores need to be present, they need to be in high numbers, and they need to be thrifty enough to cause decay and sporulate on fruit.
Resistance is costly and difficult to reverse. So, a precautionary approach is advised. Some strategies to reduce the risk of fungicide resistance developing are scrupulous hygiene (minimise spore numbers), rapid despatch of treated fruit (minimise resistance spores from treated fruit), and rotation of fungicide actives (break the resistance cycle).

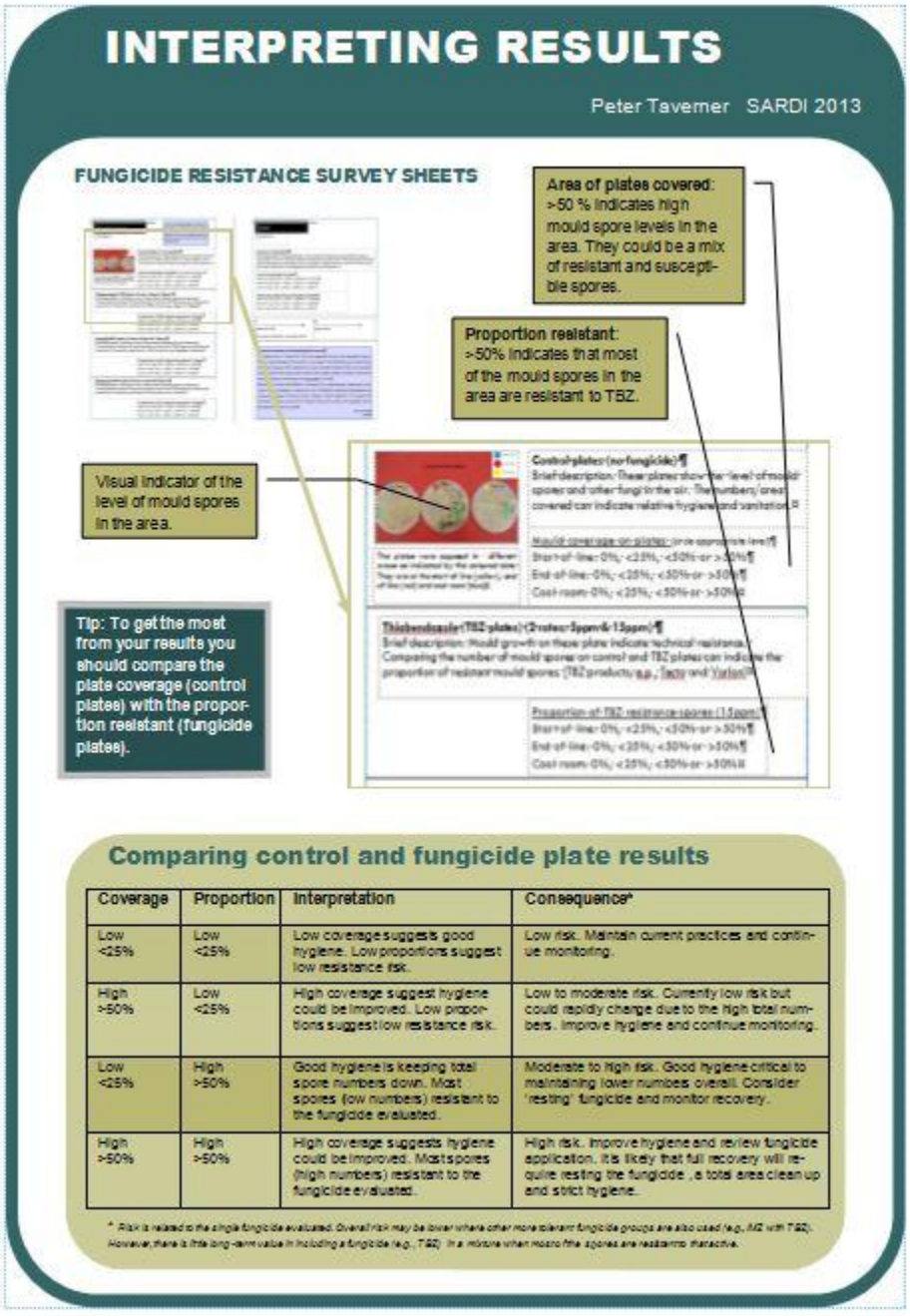
Peter Taverner
SARDI

2013 & 2014 Seasons

In 2013, we developed a sheet for packers to use in interpreting the results from the survey (see Image 2). The important comparisons for packers are between the coverage on control plates and the proportion resistance on the fungicide-amended plates. The risk is low when the coverage and proportion resistant is low: the risk is high when the coverage and proportion resistant is high. Intermediate levels have a different interpretation and lead to different levels of risk, as outlined.

The survey sheets have been given to participating packers during the 2013 & 2014 season. Generally, packers have not sought additional information on the results for a particular survey.

Image 2 Information given to packer to help interpret fungicide resistance surveys results



Conclusion

The fungicide resistance surveys has been a useful service. It has provide useful information for those participating packers and the wider industry. The consistency of sampling in the same sheds has provided robust trends. Reporting the results through the Packer Newsletter has increased industry awareness of the risk of fungicide resistance. Since the surveys commenced, there has been an

increase in packers requesting more information on resistance monitoring from both our laboratory and other service providers. Some service providers/fungicide manufacturers have instigated their own fungicide resistance service to satisfy the increased interest in monitoring.

Recommendations

- A fungicide resistance service for packers should be maintained. Chemical suppliers/manufacturers would ideally provide this service to optimise the use of their products.
- An independent survey of resistance should be conducted every five years to establish the trends of resistance to register fungicides. This should include all major citrus-growing regions.
- A resistance management strategy, including optimal integration practices for new fungicide actives, should be developed and extended to packers.

Appendix 5

Fungicide resistance surveys of commercial citrus packingsheds – 2011-2014

Introduction

Fungicide resistance and its management has a long history in citrus packing. This includes the current major fungicides used in Australian citrus packingsheds. Thiabendazole (TBZ) was introduced California citrus packing in the 1970's. Mould resistant to TBZ was discovered in lemon packinghouses within 2 years of use (Harding 1972), followed by many more cases in both blue and green mould (Houck 1977). The introduction of imazalil (IMZ) in the 1980's was an important aid in resistance management. However, resistance to IMZ was discovered within 6 years of use (Eckert 1987). It became imperative to understand and manage resistance for these major fungicides due to a lack of alternative postharvest fungicides with different modes of action.

Effective management requires monitoring the occurrence, distribution and the level of resistance (Bus 1992). Wild (1980) conducted early work on TBZ resistance in green mould and categorised resistance levels according to mean ED_{50}[3] levels. Others have subsequently determined baseline sensitivity levels for TBZ and IMZ, and suggested discriminating doses for monitoring resistance in packinghouses (Brown 1989; Holmes and Eckert 1995; Holmes and Eckert 1999; Kiney *et. al*. 2006; Perez *et.al.* 2011). To monitor, agar plates containing discriminating doses of fungicide are exposed in the packing operations and colony forming units are counted after incubation. The number of colony forming units on fungicide–treated plate can provide a warning of resistance developing.

Two new fungicide actives, fludioxonil and pyimethonil, are registered for postharvest use on citrus. History suggests that these fungicides will lose their usefulness if not judiciously used. It is recommended that these new fungicides are integrated into a program with other fungicides, i.e., not used exclusively (Shirra *et. al* 2005; Smilanick *et.al*. 2010).

In Australia, no systematic surveys or monitoring have been conducted to detect resistance in citrus packingsheds but resistance isolates have been identified in overseas consignments (Trevor Warren, Riversun Export Pty Ltd, 2007, pers. comm.). There is continuous long term use of IMZ and TBZ for export consignments. Packers are tending to hold packed fruit for longer on premises and

[3] Effective Dose required to control 50% of the strains evaluated

there are less ‘quiet’ periods to conduct major sanitation measures. All of which, are likely to proliferate resistance.

In order to obtain some longitudinal data on fungicide resistance, plate surveys were planned for 4 seasons in participating packingsheds, as part of the National Citrus Postharvest Science Program. We were interested in determining if the plate system provided an effective early warning system, i.e., were these identified isolates likely to cause decay failure under commercial conditions. We aimed to develop bioassays to verify the sensitivity/resistance of mould isolates collected on fungicide-amended plates by re-inoculating fruit, treating with fungicide and assessing for decay and sporulation.

Material and methods

1.2.1Plate survey methods

Potato dextrose agar (PDA) was amended with fungicide, resulting in nil fungicide or 5 or 15 mg/L TBZ (Syngenta Australia, Macquarie Park, NSW), 0.5 or 1.5 mg/L IMZ (Janssen PMP, Beerse, Belgium) or 1, 2.5 mg/L fludioxonil (FLU) (Syngenta Australia, Macquarie Park, NSW).

Petri dishes (9cm diameter) containing amended agar for each fungicide concentration were exposed in 3 areas of commercial packing operations for ~60 minutes. Typically, plates were exposed near where fruit was dumped onto the line, near the waxing or packing area, and inside the cool rooms. Two replicates were exposed in each area.

The exposed plates were incubated for 3 days at 25^0C before isolates were collected.

1.2.2General bioassay methods

The methods were adapted from Holmes and Eckert 1999 & Perez et. al. 2011.

Selection of isolates:

Petri dishes with fungicide amended PDA returned after exposure were incubated in the laboratory (~25^0C) and assessed for mould growth. Samples were collected when colonies formed on the lower or higher fungicide rate plates; if on both plates, the isolate was select from the higher rate. ‘Resistant’ strains were collected from both IMZ and TBZ plates. A ‘susceptible’ laboratory strain (SUS), IMZ resistant laboratory strain and a TBZ resistant laboratory strain were used for comparisons.

Preparation of isolates:

Spores were collected and stored at 4^0C on PDA plates/slants until required. Five days before fruit inoculation the spores were cultured and incubated at 25^0C to

ensure spores are uniform age. Spores collected from the agar surface were diluted to an appropriate concentration ($1x10^6$ spores/ml) for inoculation.

Bioassay method:

1. Collect fruit per treatment (plus extra 1kg fruit when residue testing required)
2. Wash and surface sterilize fruit with dilute chlorinated cleaner.
3. After dry, apply fungicide treatments to fruit. All fruit waxed.
4. After dry, inoculate with 200 µl spore suspension using a syringe to inject into the albedo of the treated fruit, and leave 10 untreated control fruit.
5. Place inoculated fruit into separate, labelled paper bags and store at 20^0C for 14 days.
6. Assess decay &/or sporulation at 7 and 14 days, rating each fruit according to the following index: **0** - No sporulation; **1** - 1-10%; **2** – 11-50%; **3** – 51-90%; **4** – 91-99%; **5** - 100%

1.2.2Quick verification bioassay

Aim: Verify that moulds collected on fungicide-amended plates were pathogenic and resistant, i.e., will decay and sporulate on 3x label rate fungicide-treated fruit.

Treatments:

<u>3 inoculated fruit per treatment; record presence or absence of sporulation.</u>

From TBZ plates[4]

- 3,000 mg/L TBZ @ 30 sec dip (~20^0C), then waxed (no fungicide)
- Untreated, then waxed (no fungicide)

From IMZ plates[5]

- 1,500 mg/L IMZ @ 30 sec dip (~20^0C), then waxed (no fungicide)
- Untreated, then waxed (no fungicide)

1.2.3Quantification bioassay

Aim: Determine if moulds collected on fungicide-amended plates were resistant to a standard commercial treatment (expected to normally control sporulation) and double resistance.

Treatments:

<u>10 inoculated fruit per treatment; record decay and sporulation index.</u>

[4] This TBZ concentration was initially 3,000ppm and adjusted to 5,000ppm TBZ after the first (June 2012) survey.
[5] This IMZ concentration is the rate used by Perez *et. al.* (2011) to verify isolates collected on 1ppm IMZ PDA plates.

Isolates collected from TBZ plates[6]

1. 3,000 mg/L TBZ @ 30 sec dip (~20^0C), then waxed (no fungicide)
2. 1,000 mg/L TBZ @ 30 sec dip (~20^0C), then waxed (2,500 mg/L IMZ)
3. 1,500 mg/L IMZ @ 30 sec dip (~20^0C), then waxed (no fungicide)
4. Untreated, then waxed (no fungicide)

Isolates collected from IMZ plates[7]

1. 1,500 mg/L IMZ @ 30 sec dip (~20^0C), then waxed (no fungicide)
2. 500 mg/L IMZ @ 30 sec dip (~20^0C), then waxed (2,500 mg/L IMZ)
3. 3,000 mg/L TBZ @ 30 sec dip (~20^0C), then waxed (no fungicide)
4. Untreated, then waxed (no fungicide)

Samples of fungicide treated fruit were collected periodically and residue levels assessed.

Results and Discussion

In 2011, the survey methodology was evaluated and adapted. Survey results were then collected over 3 seasons, from 2012-2014. Sheds A, B and C were sampled regularly for the entire period. Typically, 3 times per season at June, August and November. Some isolates collected from plate surveys were subjected to fruit bioassays.

The quick verification bioassays compared the sporulation coverage of the shed collected isolates and reference laboratory isolates. In 2012, the laboratory isolates include susceptible (SUS), a TBZ resistant and imazalil resistant examples. In subsequent years, only the SUS laboratory isolate was used for comparisons.

The quantification bioassays were used to determine if mould isolates collected on fungicide-amended plates were resistant to standard commercial treatments (dip and in wax application) and high rates of either TBZ or IMZ dips. These treatments should indicate if resistance has developed to both actives.

1.1.3 2012 Season

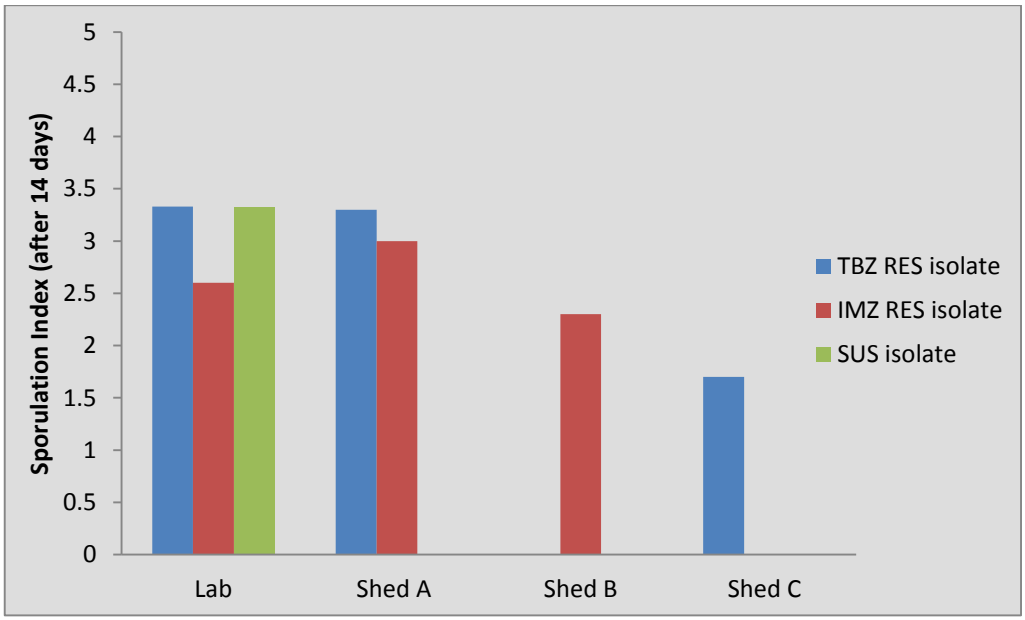
Quick verification bioassay

For the June survey, the laboratory susceptible isolate exhibited a high level of thrift, resulting in a relatively high sporulation index rating at 14 days after treatment (see graph 1). The resistant laboratory isolates level of sporulation was similar to the laboratory susceptible isolate, indicating similar thriftiness. The sporulation of isolates collected from TBZ amended plates (TBZ RES) were higher than isolates collected from IMZ amended plates (IMZ RES) from the same shed, suggesting higher thrift in the TBZ RES. In this case, Shed A had the

[6] Treatment 1 = high rate verification; treatment 2 = standard treatment test; treatment 3 = IMZ resistance test.
[7] Treatment 1 = high rate verification; treatment 2 = standard treatment test; treatment 3 = TBZ resistance test.

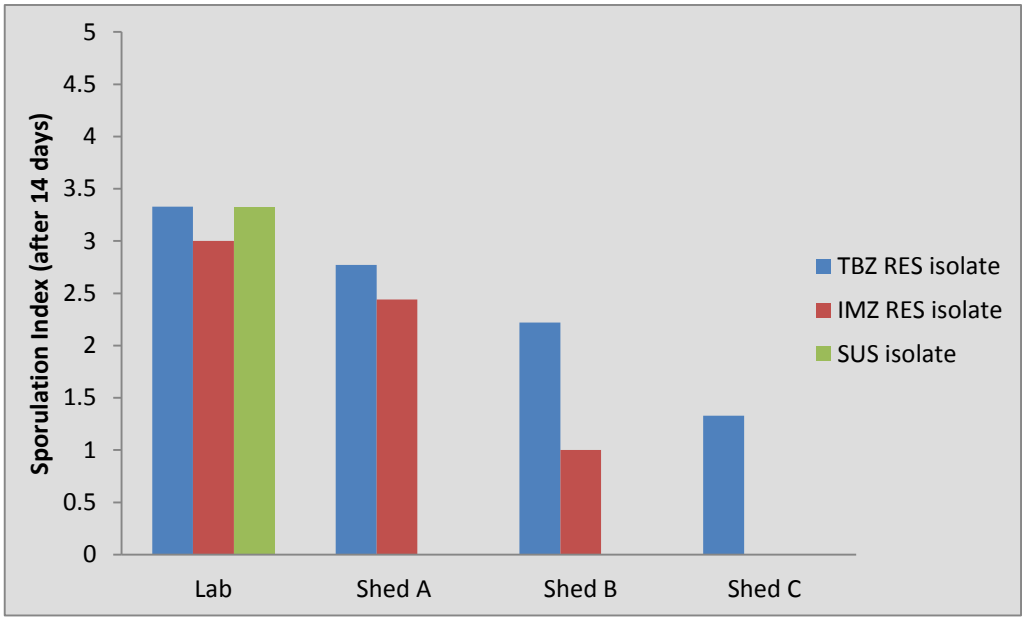
most thrifty fungicide resistance isolates and Shed C the least thrifty. For shed C, the IMZ RES were not present on survey plates and, therefore, could not be evaluated. Shed B was the reverse, with reasonably high thrift in the TBZ RES and no IMZ RES present.

Graph 1. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from three packing sheds (June 2012), and laboratory reference isolates.



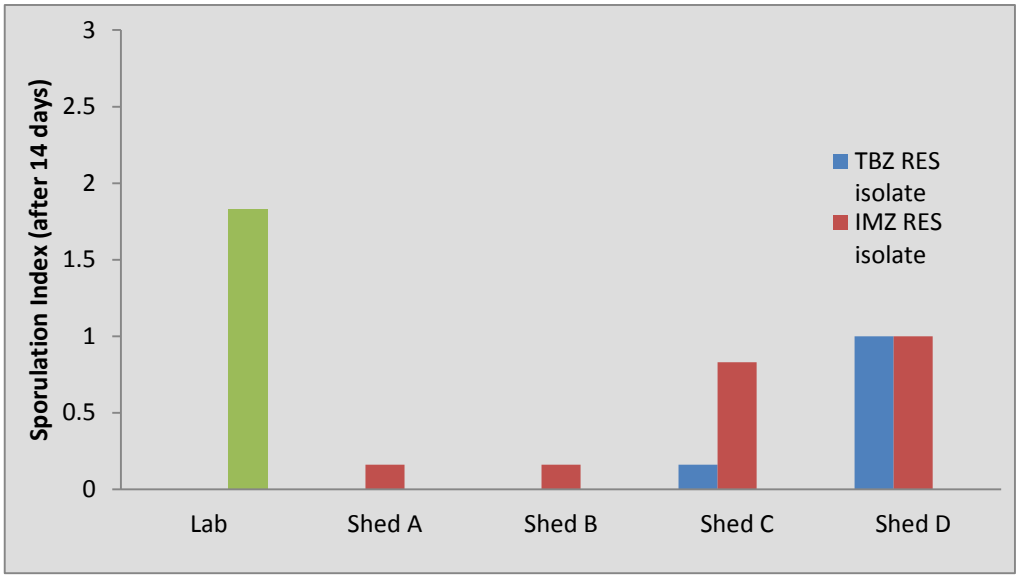
For the August survey, the results were similar to the June survey for the reference isolates and isolates from Shed A & C (see Graph 2). Shed B had changed from June, with reasonably high thrift in the TBZ RES and a lower thrift in the IMZ RES.

Graph 2. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from four packing sheds (August 2012), and laboratory reference isolates.



For the October survey, the susceptible isolate exhibited a moderate level of thrift, resulting in a relatively low sporulation index rating at 14 days after treatment (see graph 3). The sporulation of TBZ RES was lower than for IMZ RES from the same shed, except for Shed D. In this case, Shed A & B isolates had the lowest thrift and Shed C had a relatively high thrift in their IMZ RES. A fourth shed (Shed D) was included in this survey, and had relatively high thrift in both fungicide resistant isolates.

Graph 3. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from four packing sheds (October 2012), and laboratory reference isolates.



The August survey result shows the TBZ RES produce more spores (i.e., thriftier) earlier in the season than IMZ RES. There are differences in the sporulation levels of isolates collected at different sheds, which may be due to other operational factors, e.g., hygiene, sanitation or fungicide practices. The situation appears to change by the October survey, with a low level of sporulation; this makes comparisons more difficult. All TBZ RES, including the laboratory isolate, showed poor sporulation capacity and thrift. The IMZ RES also had reduced thrift in Sheds A & B. Shed C did not record any IMZ resistance during the August survey, but the October survey revealed a relatively low thrift isolate. In Shed D, TBZ RES and IMZ RES produced more spores but overall spore production was low.

The results for this survey are for one isolate per shed, which may not represent the vigour or sporulation capacity of other isolates in that same shed. Further work evaluating more isolates would be required to give greater assurance of the thrift of resident fungicide resistant spores in a particular packingshed. However, trends across all three sheds are likely to be more representative. The reason for reduced sporulation in October is unclear. However, it is interesting that the resistant isolates collected in the sheds produce more spores than the laboratory resistant isolates. Overall, the reduce sporulation in October may be due to changes in fruit maturity or cultivars used during that assessment period. It may also be due to inadvertent changes in laboratory conditions. Replicated trials over several seasons are required to establish trends.

Quantification bioassay

Fungicide residues from laboratory-treated fruit are presented in Table 1. These treatments routinely control decay caused by susceptible isolates but higher residues are required to control sporulation. Experience suggests that 2-3 mg/Kg of IMZ should provide a high level of sporulation control. In June, a dip using 3,000 mg/L of TBZ (TBZ 3000) was used but the 2.8ppm fruit residue was probably insufficient to control sporulation: A fruit residue of > 4 ml/Kg of TBZ is considered necessary to control sporulation. As such, the 'above label rate' treatments of IMZ 1500 and TBZ 5000 would be expected to control sporulation for susceptible isolates. The residues required for combinations of fungicides, such as IMZ and TBZ, to control sporulation are unknown.

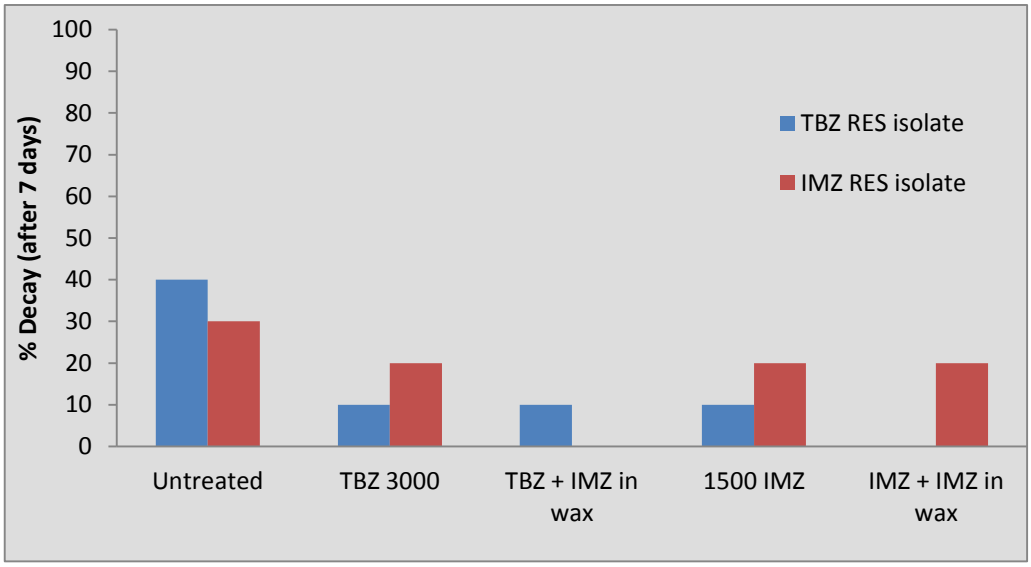
Table 1. Mean fungicide residues (mg/kg) on fruit treated with imazail (IMZ) and thiabendazole (TBZ) applied in 30 sec dips or in wax, at rates used for the qualitative bioassays.

Fungicide treatments	IMZ residues	TBZ residues	Total residues
IMZ 500 + 2500 IMZ in wax	1.8		1.8
IMZ 1500	2.8		2.8
TBZ 1000 + 2500 IMZ in wax	0.7	1.0	1.7
TBZ 3000		2.8	2.8
TBZ 5000		4.1	4.1

The IMZ RES and TBZ RES isolates were collected from Shed A during the June, August and October surveys. Comparisons between decay and sporulation rates of the isolates collected during the different surveys are discussed below.

In June, the resistant isolates collected caused modest levels of decay on untreated fruit (see graph 4). The decay in fungicide treated fruit inoculated with shed isolates supports the results from the fungicide amended plates and verification bioassays. However, fruit may decay without sporulation, and spore development is necessary for resistant isolates to build up in the packing shed.

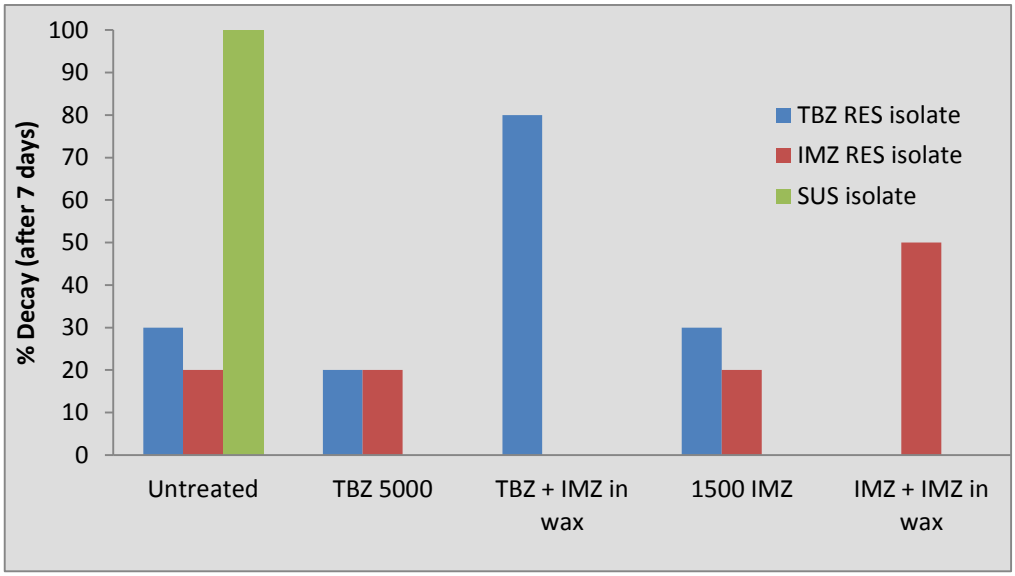
Graph 4. Percentage decay on citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from Shed A (June 2012), and treated with fungicide or dipped in water (untreated).



In June, spore development on fruit inoculated with resistance isolates was assessed 14 days after treatment. Mean sporulation is the average spore coverage on fruit surfaces and is the result of reduced decay and spore suppression. A combination of low decay and sporulation resulted in only a trace of spore coverage for most treatments. The untreated TBZ RES and IMZ RES inoculated fruit was higher, with a rating of 1.4 and 0.7 respectively. The TBZ 3000 treatment fruit had a relatively higher sporulation rating of 0.5. However, the fruit analysis indicated the TBZ residues may have been less than required to control sporulation in susceptible isolates The TBZ 3000 treatment was replaced with TBZ 5000 for subsequent bioassays

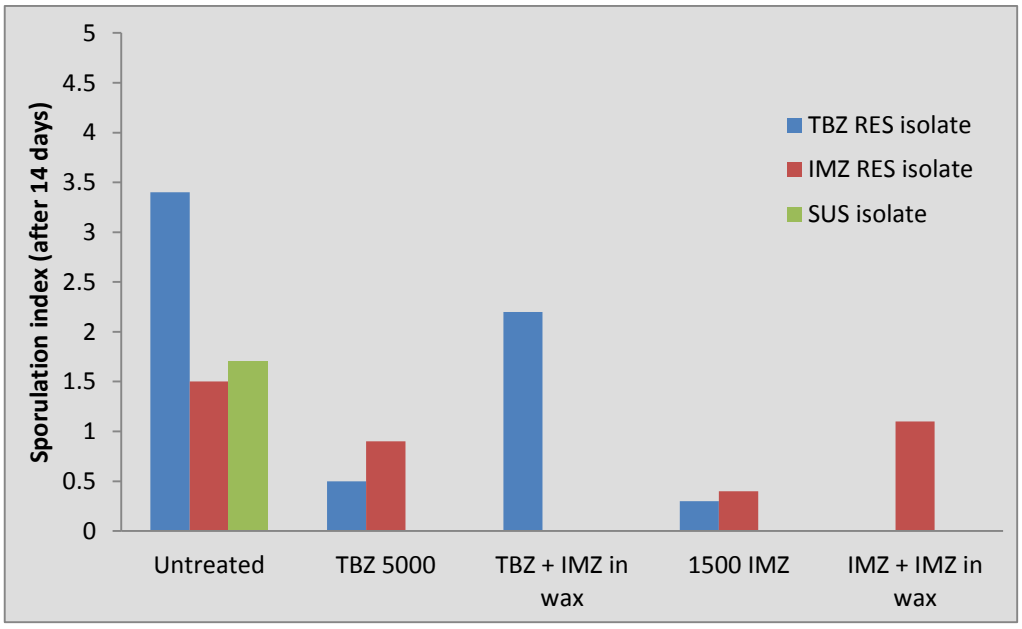
In August, the resistant isolates were less pathogenic than the laboratory susceptible isolate, suggesting that there is a cost to fitness for being resistant (See graph 5). The relatively high rates of decay in fungicide treated fruit inoculated with shed isolates is concerning, especially decay in fruit treated with above label rates.

Graph 5. Percentage decay on citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from Shed A (August 2012) or a laboratory susceptible isolate, and treated with fungicide or dipped in water (untreated**).**



In August, IMZ 1500 and TBZ 5000 had low average sporulation rates (See graph 6), mostly by suppressing decay, i.e., they did not produce spores. However, this graph does not necessarily indicate if the fungicide has arrested sporulation *per sae*. The direct effect of the fungicide treatment on sporulation is better demonstrated in table 2, where only the decaying fruit were included in the analysis.

Graph 6. Mean Sporulation on citrus fruit inoculated with green mould isolates collected from Shed A (August 2012) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



A comparison of the sporulation rates in the susceptible and two resistance isolates indicate that the TBZ RES isolate produced more spores than the susceptible isolate (see table 2) (one-way AOV: df = 2,25; F = 14.0; P<0.001). The IMZ RES isolate produce similar spore coverage to the susceptible isolate. This suggests that both resistance isolates could favourably compete with a susceptible green mould in spore production.

Table 2. Sporulation index (after 14days) on decayed citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from Shed A (August 2012) or a laboratory susceptible isolate (SUS) treated with fungicide or dipped in water (untreated).

Treatment	*n*[y]	IMZ RES	*n*	TBZ RES	*n*	Lab SUS
Untreated	8	1.9 (±0.23)	10	3.4 (±0.27)	8	1.8 (±0.25)
IMZ 500 + IMZ 2500 in wax	8	1.4 (±0.18)				-
IMZ 1500	4	1.0 (±0.0)	3	1.0 (±0.0)		-
TBZ 1000 + IMZ 2500 in wax		-	10	2.2 (±0.13)		-
TBZ 5000	8	1.1 (±0.13)	4	1.3 (±0.25)		-

[Y] number of decayed fruit (maximum 10 fruit)

In October, the resistant isolates were less pathogenic than the laboratory susceptible isolate but the TBZ RES isolate still produced 50% decay (See graph 7). The decay in fungicide treated fruit inoculated with shed isolates was modest but indicated some resistance. The decay rates of fruit treated with resistance spores was similar for laboratory and shed-collected isolates.

The sporulation was low for TBZ RES and no spores occurred on treated fungicide fruit 14 days after treatment: Most decayed fruit produced spores, but the decay rates were low leading to a low average sporulation rate.

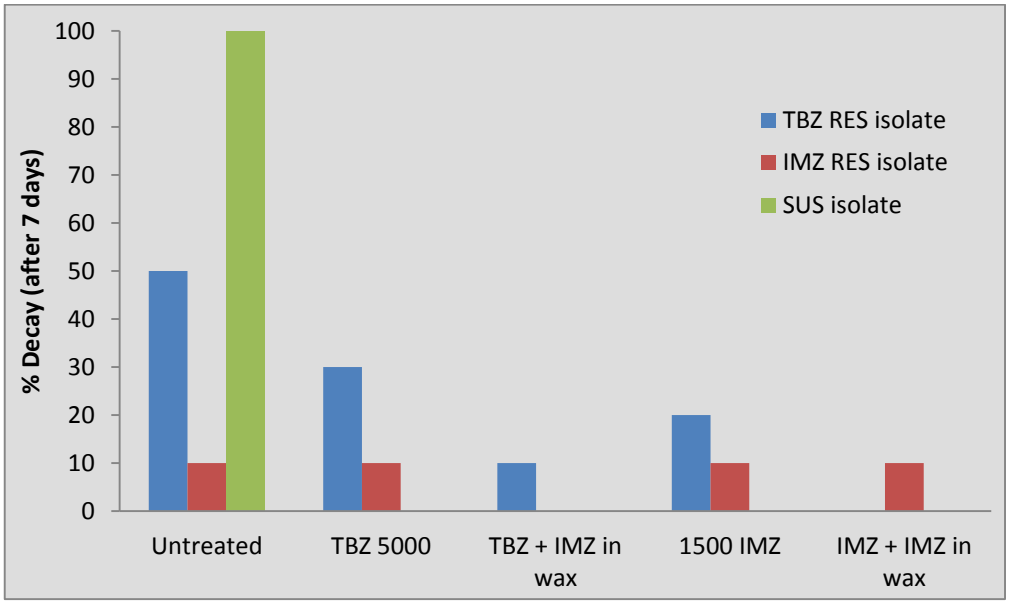
Spore development was slow in all treatments and fruit were given more time to produce spores. Fruit that had not decayed by 28 days after treatment were removed from the analysis to compare only decayed fruit. After 28 days, the resistant isolates produced significantly less spores than the susceptible isolate on untreated fruit (one-way AOV: df = 2.21; F = 4.02; P<0.05) (see table 3).

The response for the TBZ and IMZ resistance isolates was different. The sporulation rates for fungicide-treated fruit inoculated with the IMZ RES isolate were significantly lower than the untreated IMZ RES isolate, except the standard IMZ 500 + IMZ 2500 in wax (one-way AOV: df = 3,25; F = 3.65; P<0.05). The

sporulation rates for fungicide-treated fruit inoculated with the TBZ RES isolate were comparable to the untreated TBZ RES isolate, except TBZ 5000 was significantly lower (one-way AOV: df = 3,25; F = 3.65; P<0.05). These result indicate that label rates, including combinations of fungicide dips and wax applications, may not reduce sporulation in isolates identified as resistant in plate surveys.

Although the sample size is small, the results suggest that isolates identified though the agar plates surveys are resistance and are capable producing spores. If in sufficient numbers, resistant isolates cause decay in standard fungicide treated fruit.

Graph 7. Percentage decay on citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from Shed A (October 2012) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 8. Mean Sporulation on citrus fruit inoculated with green mould isolates collected from Shed A (October 2012) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).

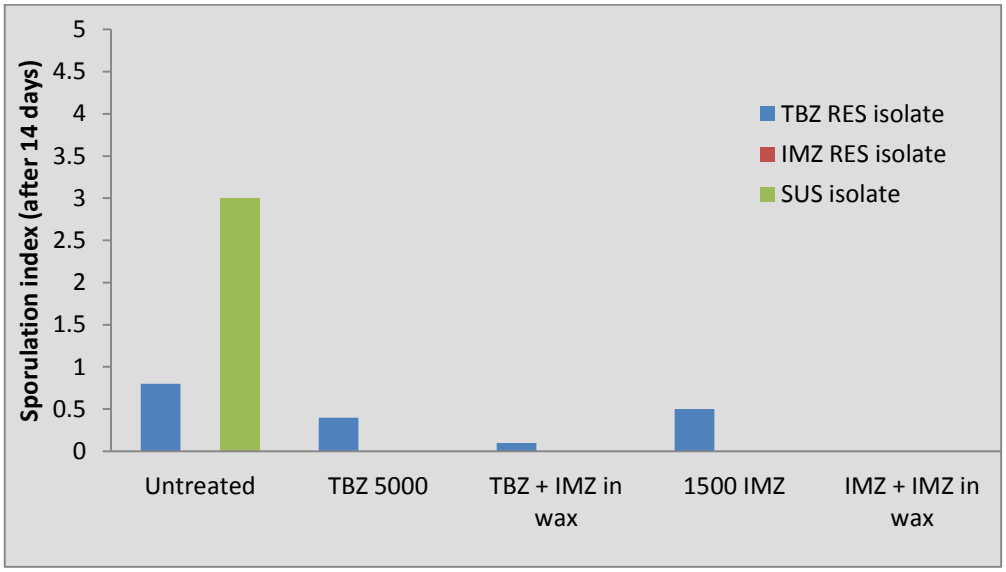


Table 3. Sporulation index (after 28 days) on decayed citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from Shed A (October 2012) or a laboratory susceptible isolate (SUS) and treated with fungicide or dipped in water (untreated).

Treatment	*n*[y]	IMZ RES	*n*	TBZ RES	*n*	Lab SUS
Untreated	6	2.5 (±0.76)	6	2.5 (±0.42)	10	4.0 (±0.26)
IMZ 500 + IMZ 2500 in wax	4	1.0 (±0.41)				-
IMZ 1500	3	0.7 (±0.33)	7	1.3 (±0.18)		-
TBZ 1000 + IMZ 2500 in wax		-	7	1.3 (±0.36)		-
TBZ 5000	5	0.8 (±0.20)	6	1.0 (±0.36)		-

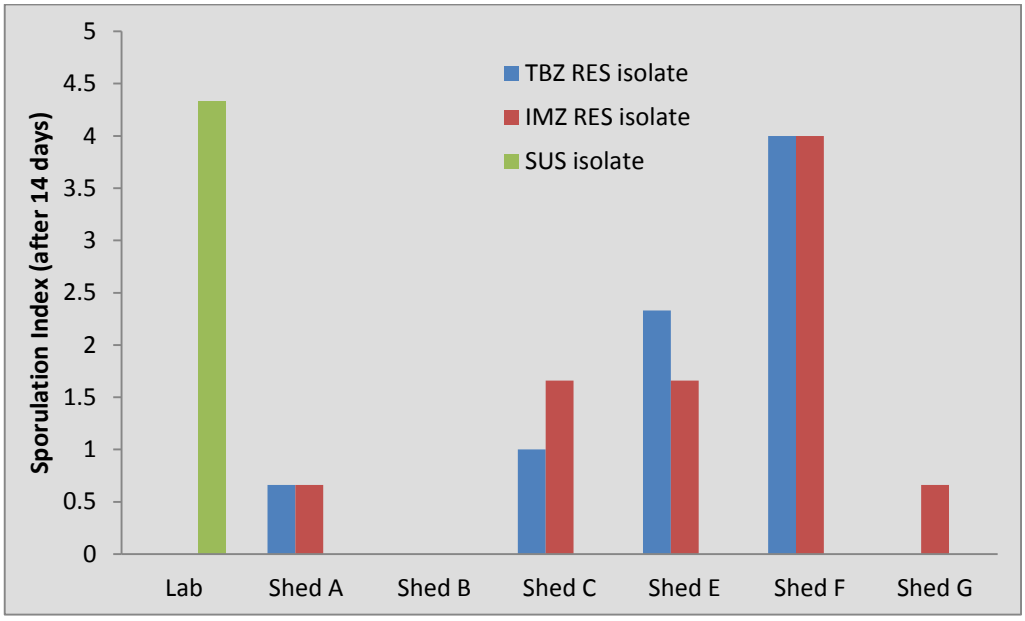
[Y] number of decayed fruit (maximum 10 fruit)

1.1.4 2013 Season

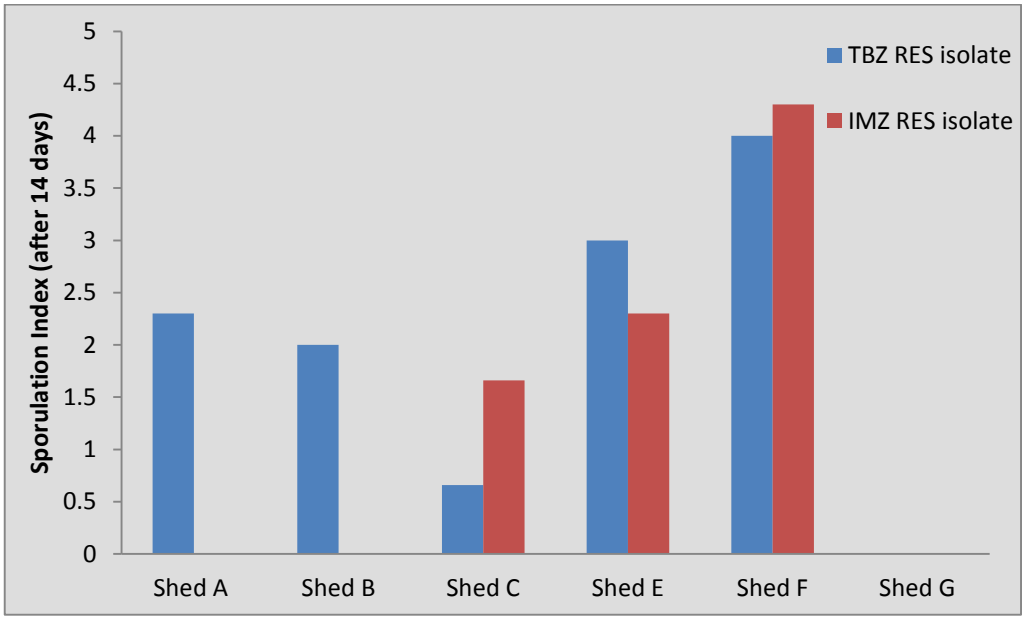
Quick verification bioassay

For the June survey, the laboratory susceptible isolate exhibited a high level of thrift, resulting in a relatively high sporulation index rating at 14 days after treatment (see graph 8). The sporulation of shed TBZ- and IMZ- resistant isolates from sheds were less than the laboratory isolate, except Shed F. Shed B & G were the least thrifty and the remaining sheds intermediate.

Graph 8. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from six packing sheds (June 2013), and laboratory reference isolate.



Graph 9. Mean sporulation on citrus fruit either dipped in 1500ppm IMZ after inoculation with green mould isolates collected from IMZ amended (IMZ RES isolate) or dipped in 5000ppm TBZ after inoculation with green mould isolates collected from TBZ amended (TBZ RES isolate) plates, from six packing sheds (June 2013).

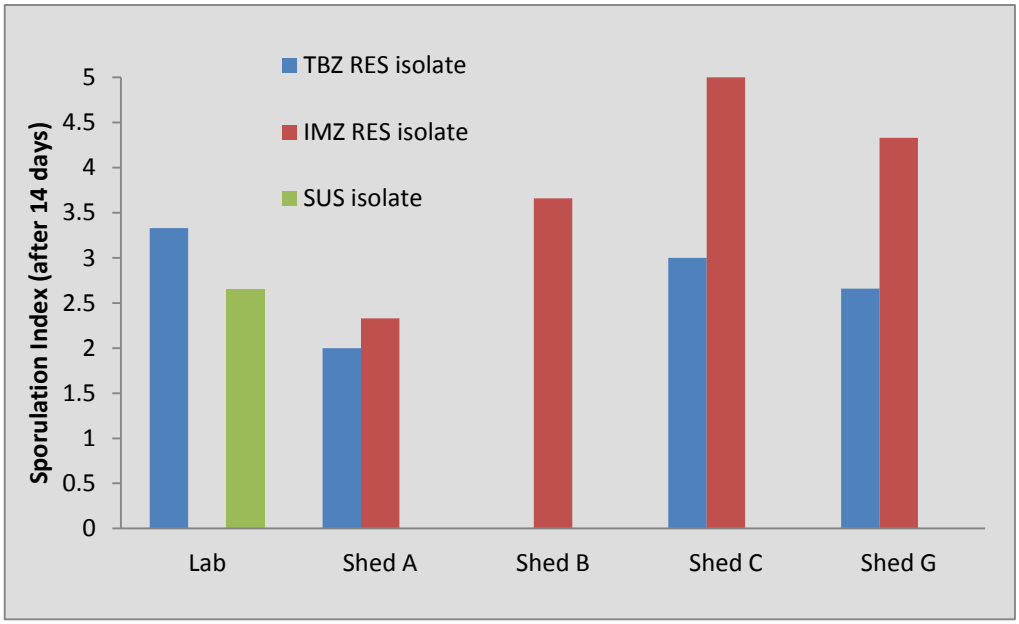


Most fungicide resistant isolates collected from survey plates and inoculated onto fungicide treated fruit produced spores (see graph 9). The isolate response varied with different sheds; only the TBZ- resistance isolate produced spores in Shed B, whereas, the TBZ- resistance isolate was dominant in Shed C isolates. The isolates collected from Shed F were the most aggressive spore producers. Shed G isolates did not produce spores on either TBZ- or IMZ- treated fruit. Interestingly, the TBZ isolate from Shed B produced spores on treated fruit but was unable to sporulate on untreated fruit.

In August, the sporulation tests were inconclusive. The laboratory isolate could not be induced to produce greater than 20% sporulation and the majority of isolates collected from sheds did not decay. Repeated attempts produced similar results.

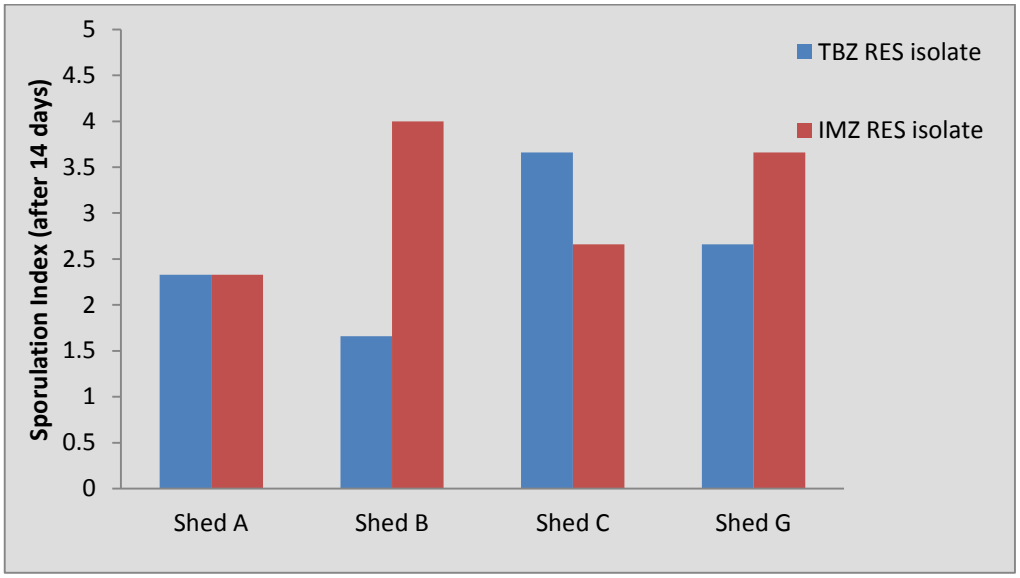
In November survey, the susceptible isolate exhibited a moderate level of thrift, resulting in a moderate sporulation index rating at 14 days after treatment (see graph 10). The sporulation of TBZ resistance isolates was similar to a laboratory TBZ-resistant isolate. IMZ resistant isolates showed high sporulation capacity. The TBZ resistance isolate from Shed B did not produce any spores. Shed A, B & C IMZ resistant isolates had a progressively higher thrift, culminating in 100% spore coverage by the Shed C isolate. A fourth shed (Shed G) also had relatively high thrift in both fungicide resistant isolates.

Graph 10. Mean sporulation on citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from four packing sheds (November 2013), and laboratory reference isolates.



All fungicide resistant isolates collected from survey plates and inoculated onto fungicide treated fruit produced spores (see graph 11). Both TBZ- and IMZ isolates sporulated well on treated fruit. Shed C had the highest sporulation of TBZ isolates. IMZ isolates from sheds B & G had the highest sporulation. Swabs were taken from sporulating fruit from under the Shed G line during the survey visit. Isolates from this fruit grew well on TBZ- and IMZ- amended plates, and had similar sporulation rates to air-borne isolates. TBZ- and IMZ- isolates from swabs and inoculated onto treated fruit yielded indexes of 3.0 and 2.66, respectively.

Graph 11. Mean sporulation on citrus fruit either dipped in 1500ppm IMZ after inoculation with green mould isolates collected from IMZ amended (IMZ RES isolate) or dipped in 5000ppm TBZ after inoculation with green mould isolates collected from TBZ amended (TBZ RES isolate) plates, from four packing sheds (November 2013).

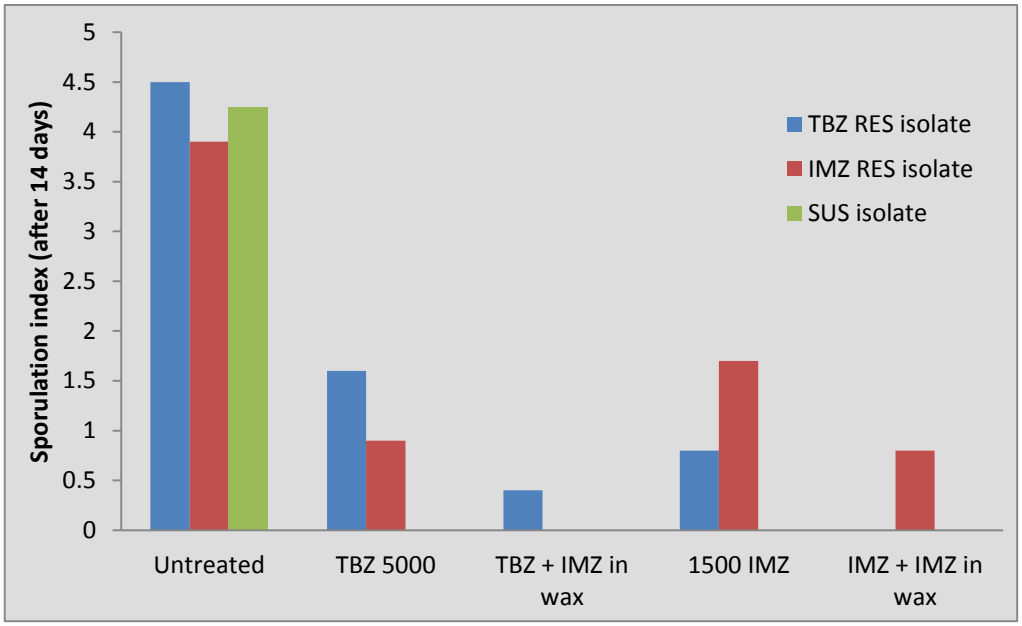


Early in the season (June), air-bourne spores grew on fungicide amended plates. However, their thrift and sporulation was relatively poor, except for Shed F, which took no further part in the program. In August, isolates were again collected but the tests were inconclusive. By the October survey, sporulation capacity and thrift of isolates had increased. High levels of spore were being produced on fruit treated with either TBZ or IMZ, which suggests a trend for increasing fitness of the isolates from fungicide amended plates during the season. The reduction in fungicide sensitivity over the season was similar in all sheds regardless of the initial survey results. Sheds with low sporulation levels early in the season, such as Shed B and G, still had highly sporulating isolates by the end of the season. The results for this survey are provisional. They are based on one isolate per shed, which may not represent the vigour or sporulation capacity of other isolates in that same shed.

Quantification bioassay

Fruit bioassays were conducted using isolates collected from Shed A in August. The IMZ RES and TBZ RES isolates produced high levels of spores on untreated fruit. . Some spore production occurred on all fruit treated with fungicide. Typically, high IMZ rates controlled sporulation on the TBZ RES isolate better than the IMZ RES isolate, and high TBZ controlled the IMZ RES isolate better. The fungicide dip plus IMZ in wax was the most efficacious, with 10% or less spore coverage on fruit.

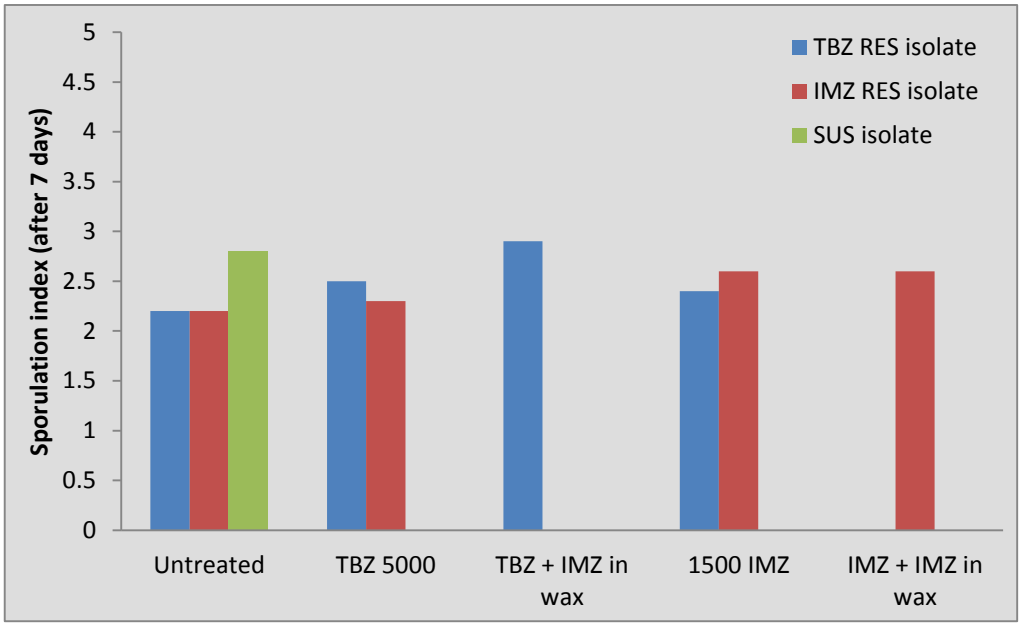
Graph 12. Mean Sporulation (14 days) on citrus fruit inoculated with green mould isolates collected from Shed A (August 2013) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



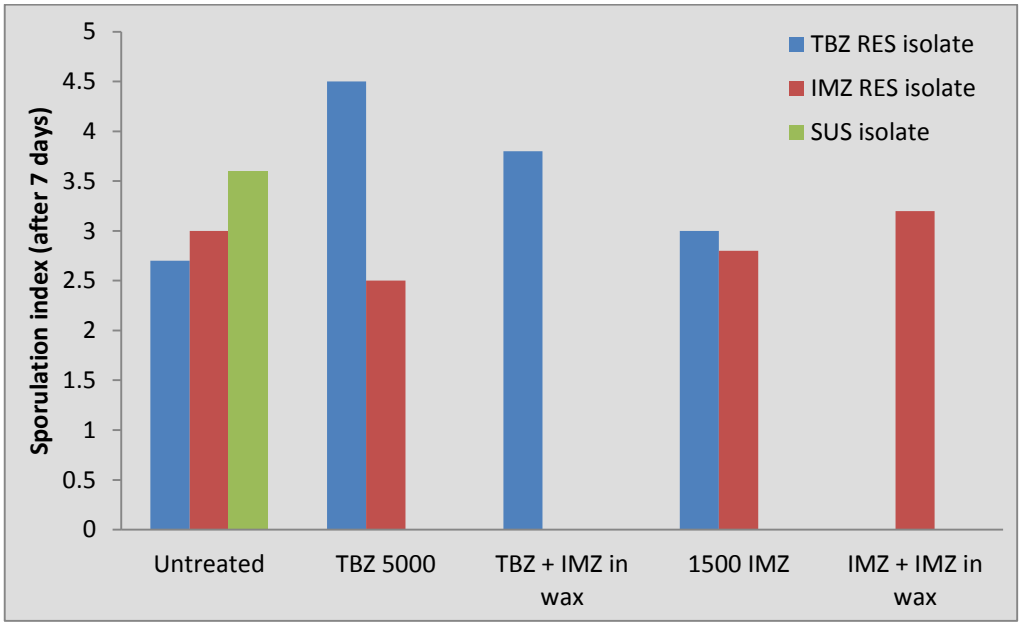
In November, the isolates collected from Shed A sporulated prolifically (100% coverage) after 14 days, regardless of the fungicide treatments. Spore production was rapid with relatively high rates in all treatment 7 days after inoculation (see graph 13). The sporulation rate of isolates was similar regardless of the fungicide used, suggesting reduced sensitivity to both IMZ and TBZ in shed collected isolates.

Isolates collected from Sheds C & G showed similar or greater thrift and reduced sensitivity to fungicides (see graphs 14 & 15). Overall, TBZ RES isolates produce more spores, and were more aggressive on fruit treated with TBZ.

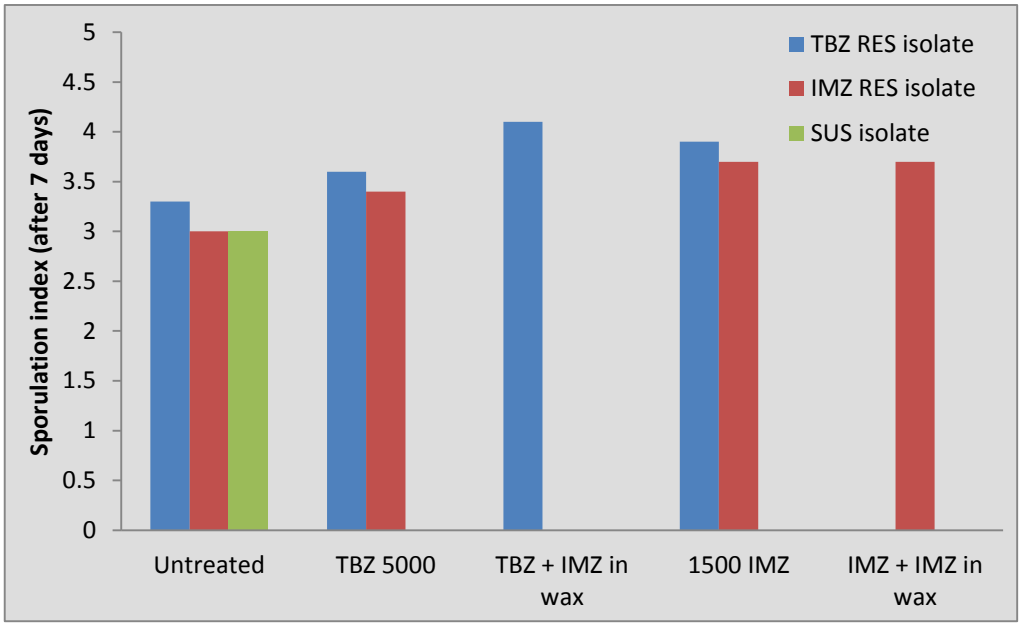
Graph 13. Mean Sporulation (7 days) on citrus fruit inoculated with green mould isolates collected from Shed A (November 2013) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 14. Mean Sporulation (7 days) on citrus fruit inoculated with green mould isolates collected from Shed G (November 2013) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 15. Mean Sporulation (7 days) on citrus fruit inoculated with green mould isolates collected from Shed C (November 2013) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



1.1.5 2014 Season

Quick Verification Bioassay

June 2014 Isolates

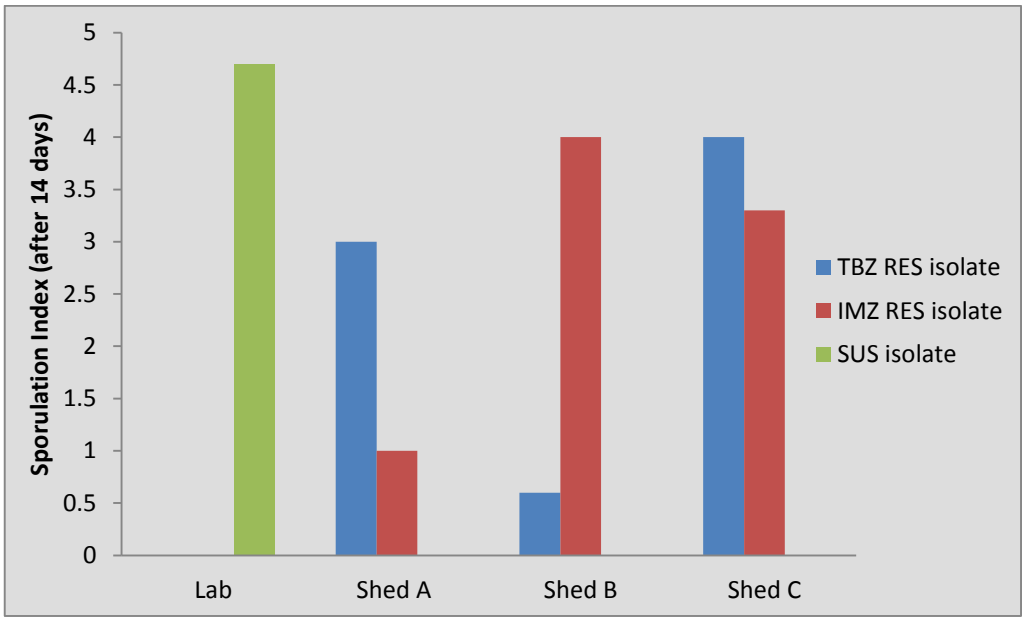
For the June survey, the laboratory susceptible isolate exhibited a high level of thrift, resulting in a relatively high sporulation index rating at 14 days after inocultion (see graph 16). The sporulation of shed TBZ- and IMZ- resistant isolates from sheds were only slightly less than the laboratory isolate, except the IMZ – resistant isolate from Shed A and the TBZ – resistant isolate from Shed B, which were less thrifty. All fruit were untreated.

Figure 1. "Light" and "dark" green mould colonies growing on the same fungicide amended agar plate.

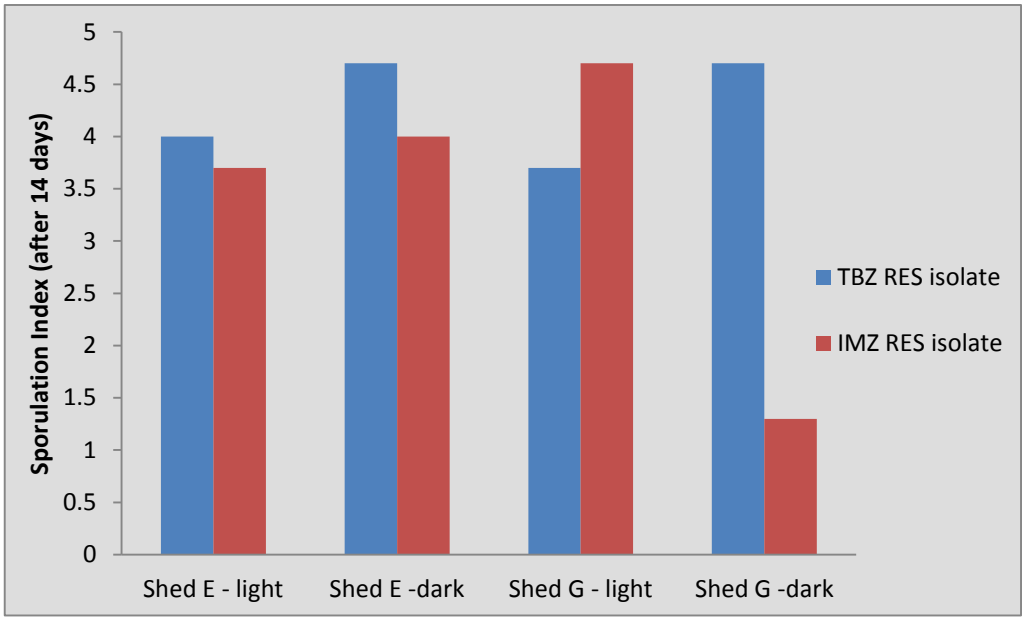
In addition, two visually different isolates of green mould ('light' and 'dark'; see figure 1) were collected from Sheds E & G. The unusual 'light' variant was most common in Shed G. A comparison of light and dark isolates from each packer yielded similarly high sporulation of both isolates on untreated fruit, except the

IMZ – resistance isolate from Shed G where the dark isolate was less thrifty (graph 17). This suggest that there can be differences in sporulation rates between the light and dark isolates when growing on untreated fruit. Numerous Penicillium species have been isolated from fruit surfaces and these isolates may be different species (Amiri and Bompeix 2005). Different Penicillium species' spores can be visually similar and difficult to identify by morphological features and growth patterns. In this study, we collected isolates from each packer with 'typical' growth patterns of either green or blue mould. We did not undertake further techniques, such as electrophoresis, to separate other Penicillium species.

Graph 16. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from three packing sheds (June 2014), and susceptible laboratory reference isolate.

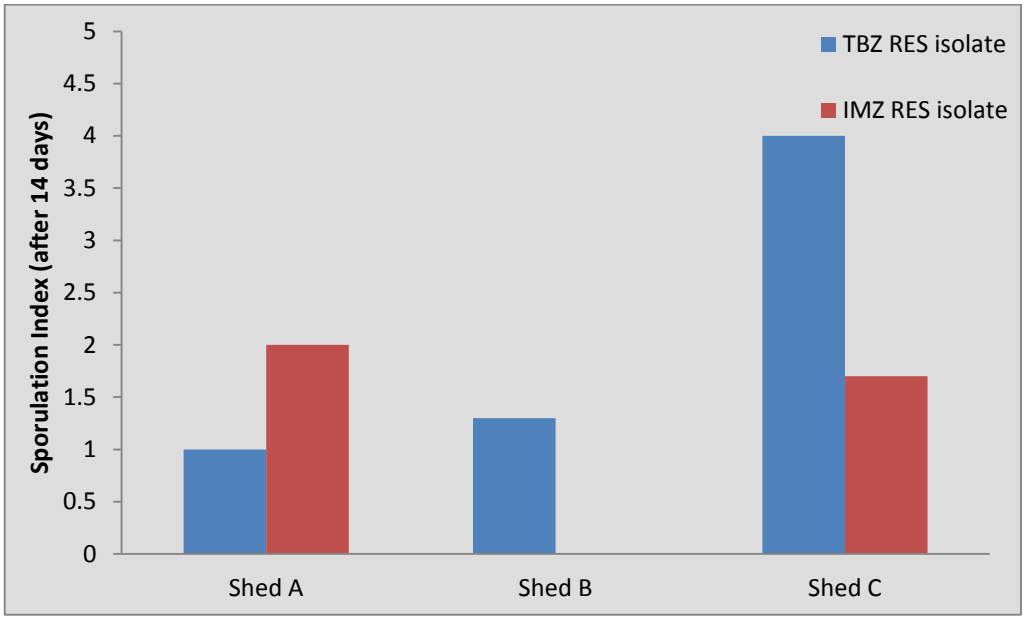


Graph 17. Mean sporulation on untreated citrus fruit inoculated with either 'light' or normal 'dark' green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from two packing sheds (June 2014).



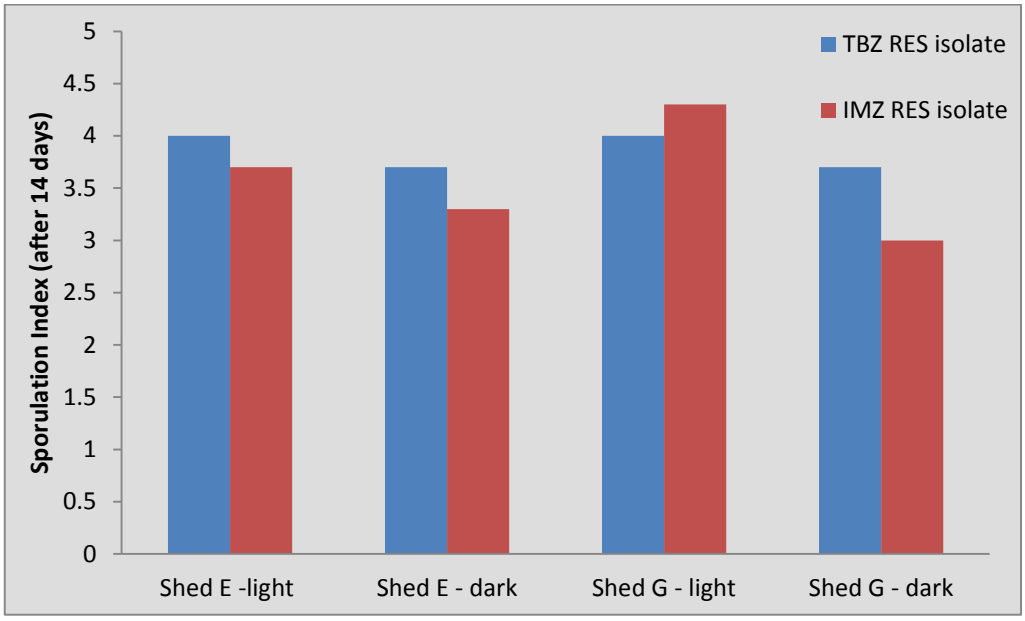
All TBZ - resistant isolates collected from survey plates and inoculated onto TBZ fungicide treated fruit produced spores, with the isolate from Shed C producing highest spore levels (see graph 18). The IMZ-resistant isolates tended to produce less spores than TBZ-resistant isolates in Sheds A, B & C. The IMZ-resistant isolate collected from Shed B caused decay but did not produce spores on IMZ fungicide treated fruit. This isolate produced prolific spores on untreated fruit.

Graph 18. Mean sporulation on citrus fruit either dipped in 1500ppm IMZ after inoculation with green mould isolates collected from IMZ amended (IMZ RES isolate) or dipped in 5000ppm TBZ after inoculation with green mould isolates collected from TBZ amended (TBZ RES isolate) plates, from three packing sheds (June 2014).



Light and dark green mould isolates from Sheds E &G were used to inoculate fungicide-treated fruit. All isolates inoculated onto fungicide treated fruit produced prolific spores (see graph 19). There were no strong differences between the light and dark isolates collected from either shed.

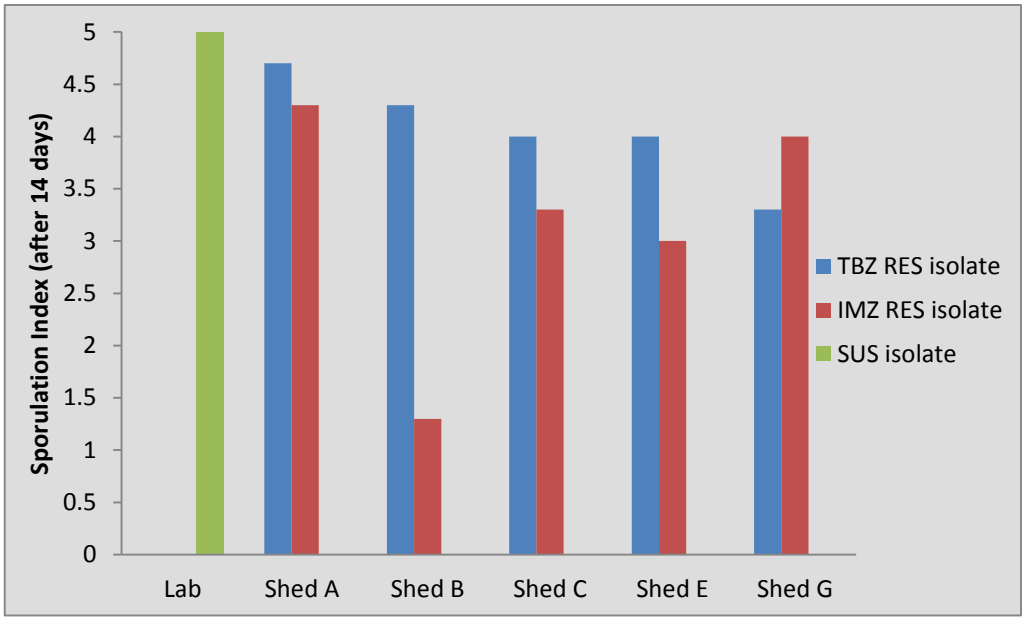
Graph 19. Mean sporulation on citrus fruit either dipped in 1500ppm IMZ after inoculation with light and dark green mould isolates collected from IMZ amended (IMZ RES isolate) or dipped in 5000ppm TBZ after inoculation with light and dark green mould isolates collected from TBZ amended (TBZ RES isolate) plates, from two packing sheds (June 2014).



August 2014 isolates

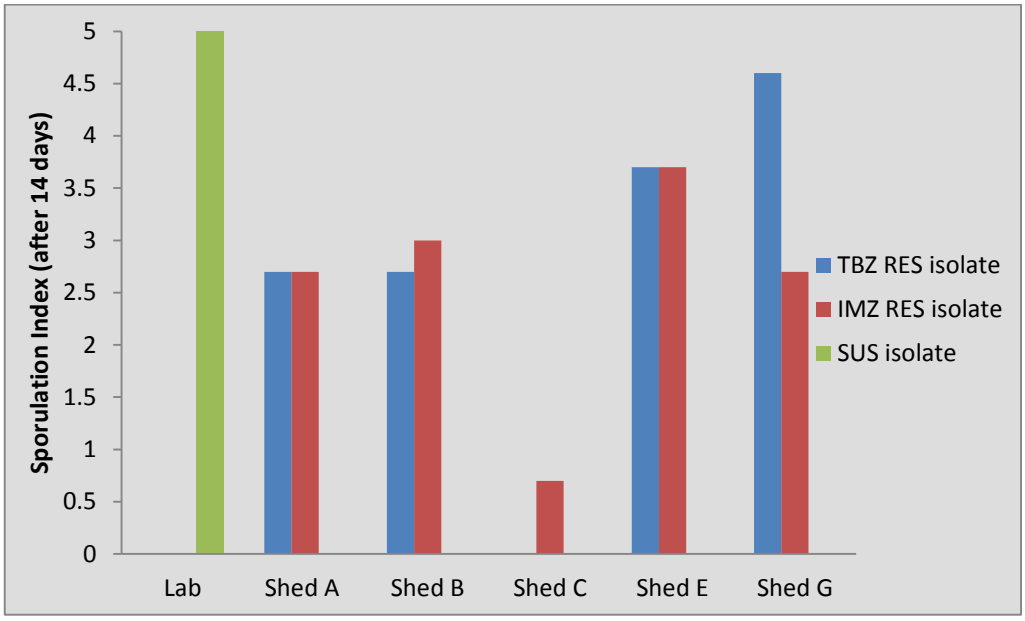
In August, both 'light' and 'dark' isolates were identified but only normal 'dark' green mould isolates were assessed. The laboratory susceptible isolate exhibited a high level of thrift, resulting in a relatively high sporulation index rating at 14 days after inoculation (see graph 20). The sporulation of shed TBZ- and IMZ-resistant isolates from sheds were only slightly less than the laboratory isolate, except the IMZ – resistant isolate from Shed B, which was less thrifty. All fruit were untreated.

Graph 20. Mean sporulation on fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from five packing sheds (August 2014), and susceptible laboratory reference isolate.



The laboratory susceptible isolate exhibited a high level of thrift, resulting in a relatively high sporulation index rating at 14 days after treatment (see graph 21). The sporulation of shed TBZ- and IMZ- resistant isolates from sheds were less than the laboratory isolate, except the TBZ – resistant isolate from Shed G. The lowest thrift was in the IMZ-resistant isolate from Shed B. The TBZ-resistant isolates from Shed B were morphologically atypical and not assessed. They showed characteristics similar to Whisker Mould (*Penicillium ulaiense*), which is much slower growing than the major citrus moulds and more capable of developing resistance to fungicides (Timmer et.al. 2000.)

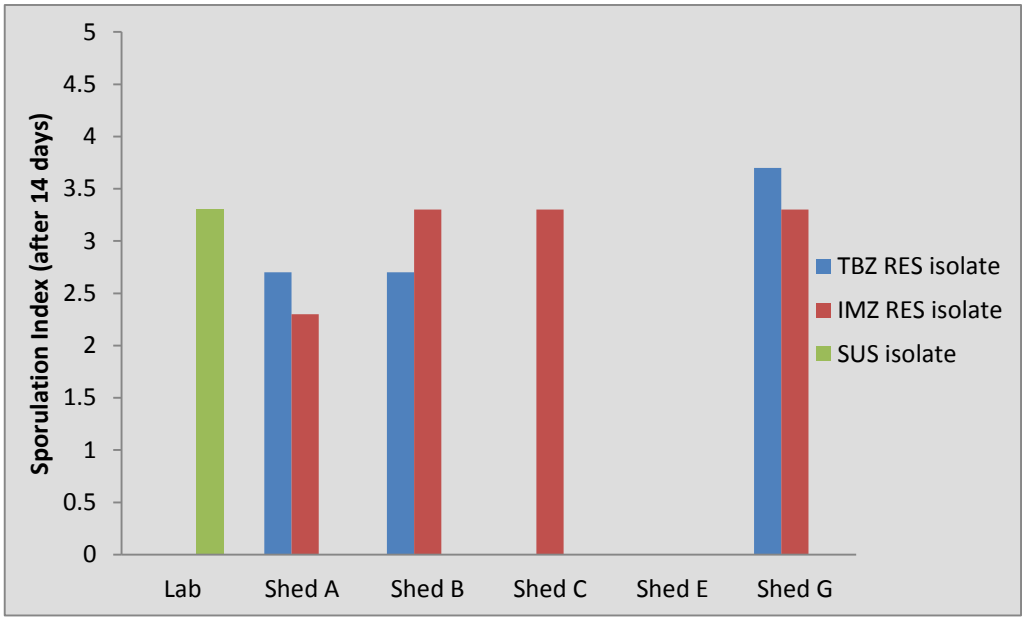
Graph 21. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) plates and dipped in 1,500ppm IMZ or from TBZ amended (TBZ RES isolate) plates and dipped in 5,000ppm TBZ, from five packing sheds (August 2014), and susceptible laboratory reference isolate.



November 2014 isolates

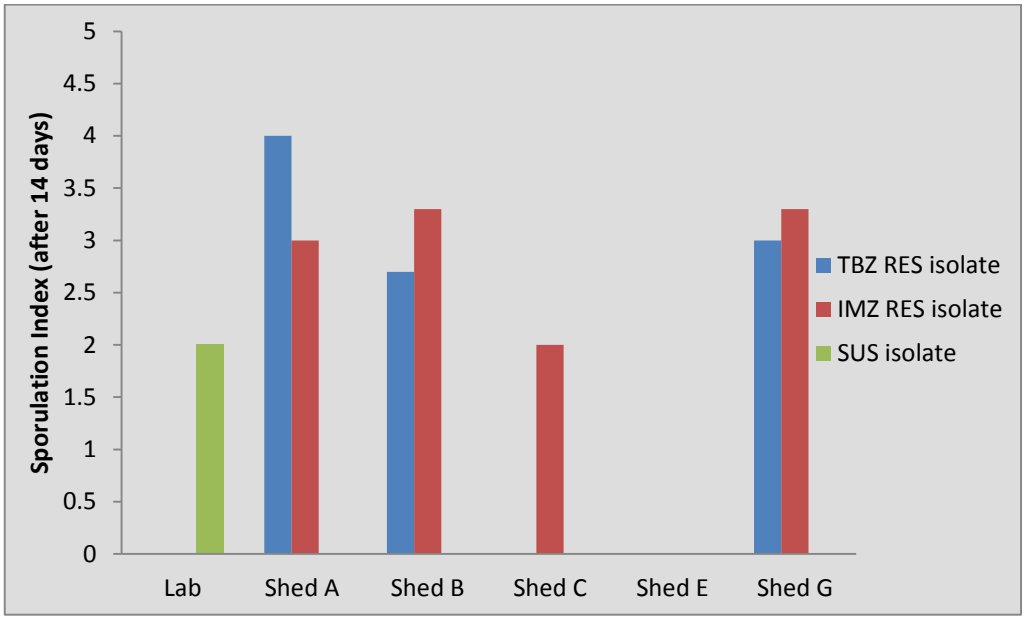
In November, only normal 'dark' green mould isolates were assessed. The laboratory susceptible isolate exhibited a high level of thrift, resulting in a relatively high sporulation index rating at 14 days after inoculation (see graph 22). The sporulation of shed TBZ- and IMZ- resistant isolates from sheds were similar to the laboratory isolate, except the TBZ – resistant isolates from Shed C & E, which decayed but produced no spores. There was no IMZ-resistant isolates identified from Shed E. In addition, the TBZ-resistant isolate from Shed B was assessed as green mould but it showed characteristics similar to Whisker Mould (*Penicillium ulaiense*) on fruit. Whisker mould is much slower growing than the major citrus moulds and more capable of developing resistance to fungicides (Timmer et.al. 2000.). A similar Whisker-type mould was isolated from Shed B in August this year.

Graph 22. Mean sporulation on fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) and TBZ amended (TBZ RES isolate) plates from five packing sheds (November 2014), and susceptible laboratory reference isolate.



The laboratory susceptible isolate exhibited a high level of thrift, resulting in a relatively high sporulation index rating at 14 days after treatment (see graph 23). The sporulation of shed TBZ- and IMZ- resistant isolates from sheds were similar or higher than the laboratory isolate, except the TBZ – resistant isolates from Shed C & E, which did not produce spores. There were no IMZ-resistant isolates identified from Shed E. In addition, the TBZ-resistant isolate from Shed B was initially assessed as green mould on plates but it showed characteristics similar to Whisker Mould (*Penicillium ulaiense*) on fruit. A similar Whisker-type mould was isolated from Shed B in August this year.

Graph 23. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) plates and dipped in 1,500ppm IMZ or from TBZ amended (TBZ RES isolate) plates and dipped in 5,000ppm TBZ, from five packing sheds (August 2014), and susceptible laboratory reference isolate.



Quantification Bioassay

In 2014, fruit bioassays were conducted using isolates collected from Shed E and Shed G.

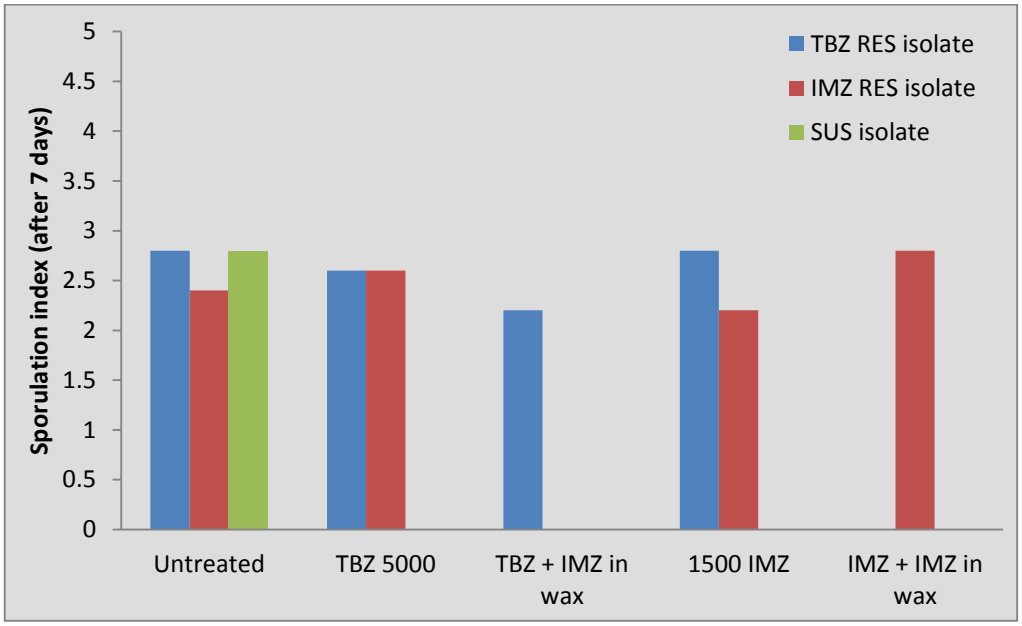
June Isolates

In June, light and dark green mould isolates were assessed from each shed. Spores were injected into fungicide-treated fruit to assessed fungicide resistance. Initially, the light green mould isolates produced spore levels comparable to the laboratory susceptible isolate, by 7 days after inoculation (see graphs 24 & 25). Whereas, the dark green mould isolates were much less thrifty (see graphs 25 & 26). In particular, Shed G where the average sporulation index rating for the light and dark green mould isolates after 7 days was different, at 3.22±0.12SE and 0.45±0.11SE, respectively (ANOVA: df=1,83, F=268, P<0.0001). Overall, the IMZ-resistant isolates were less thrifty, producing fewer or no spores 7 days after inoculation.

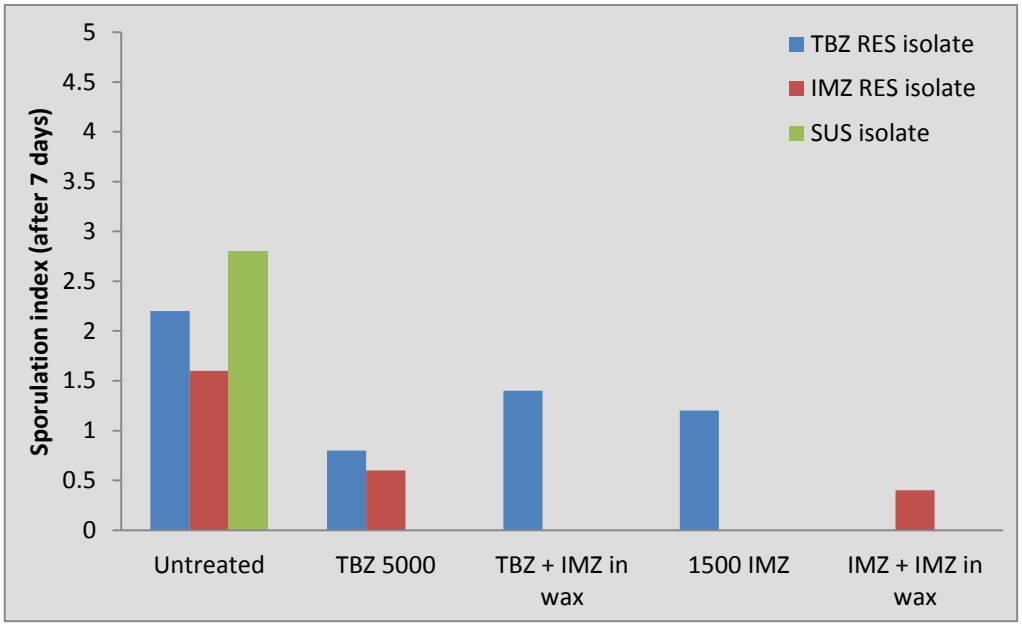
However, all treated fruit inoculated with light or dark isolates produced some spores after 14 days. Fruit treated with light green mould isolated were completely covered with spores. The sporulation index varied with the less thrifty dark isolates (see graphs 27 & 28). TBZ-resistant dark isolates produced more spores than IMZ-resistant dark isolates. The standard treatment of label rate TBZ in dip followed by IMZ in wax provide little or no protection against

sporulation. Low levels of spores were produced on fruit treated with label rates of IMZ in dip followed by IMZ in wax. Some spores were produced on fruit treated with 3x label rate IMZ in a dip and 5x label rate IMZ in a dip. The production of spores on both these treatments indicates double resistance to IMZ and TBZ. At this stage, there appears to be a fitness cost to resistance in the dark green mould isolates. However, the light green mould isolates are resistant to both fungicides tested at 3x label rate and as thrifty as the laboratory susceptible isolate evaluated.

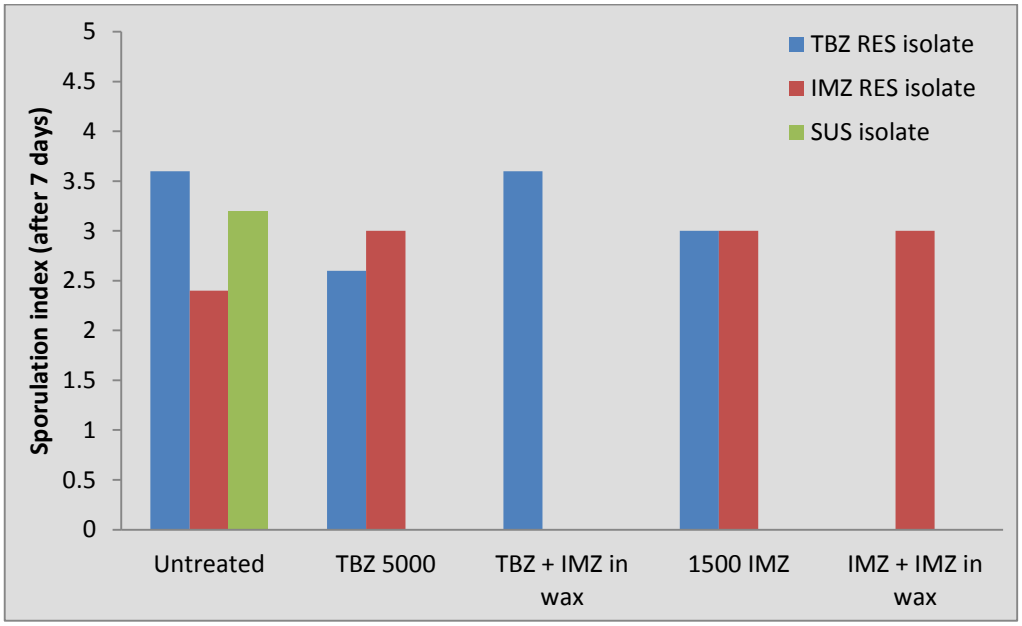
Graph 24. Mean Sporulation (7 days) on citrus fruit inoculated with light green mould isolates collected from Shed E (June 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



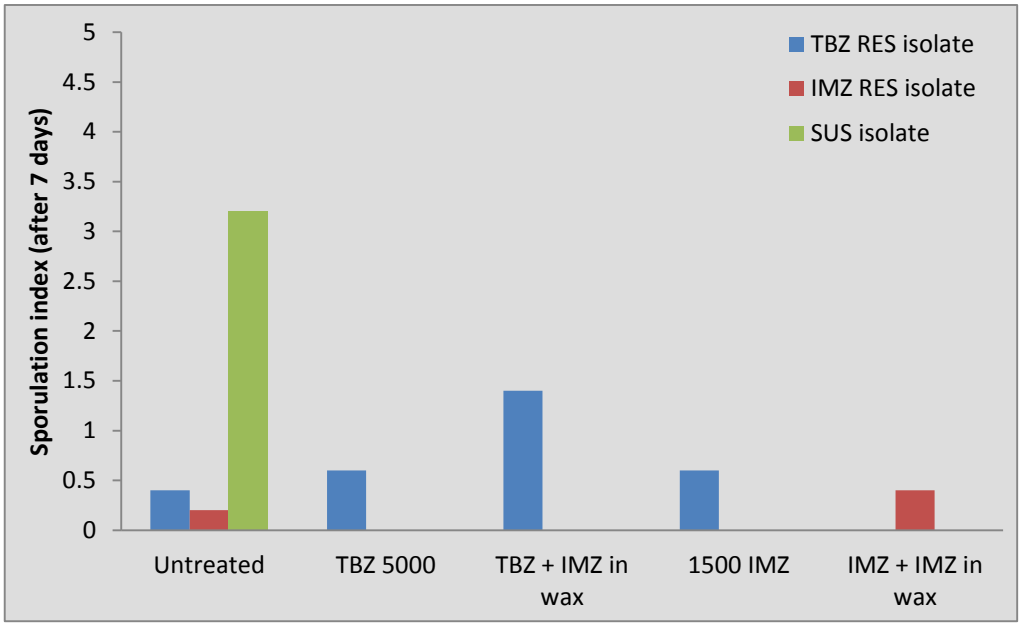
Graph 25. Mean Sporulation (7 days) on citrus fruit inoculated with dark green mould isolates collected from Shed E (June 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



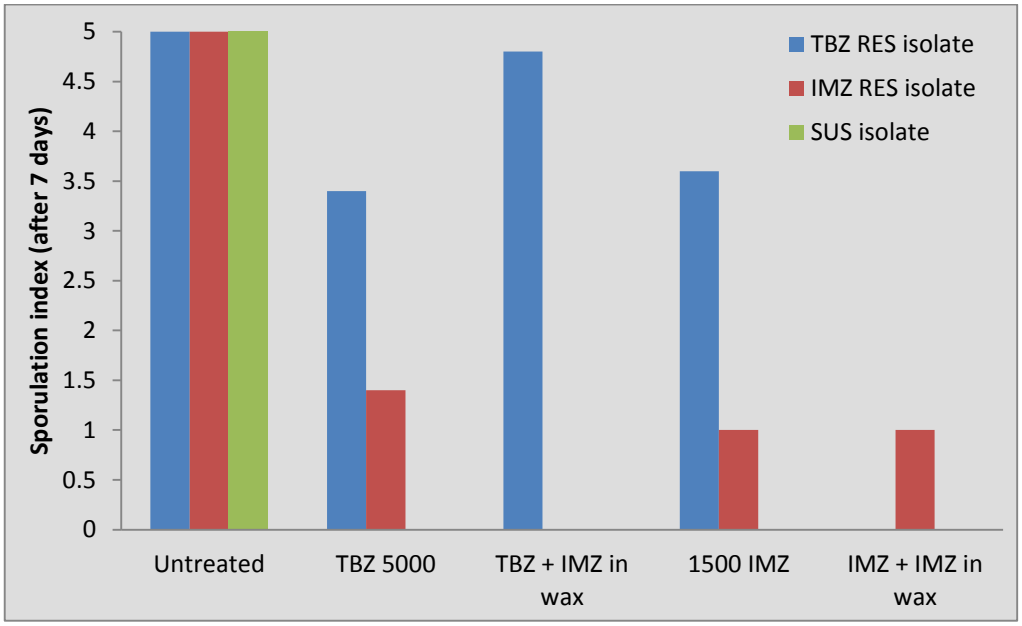
Graph 26. Mean Sporulation (7 days) on citrus fruit inoculated with light green mould isolates collected from Shed G (June 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



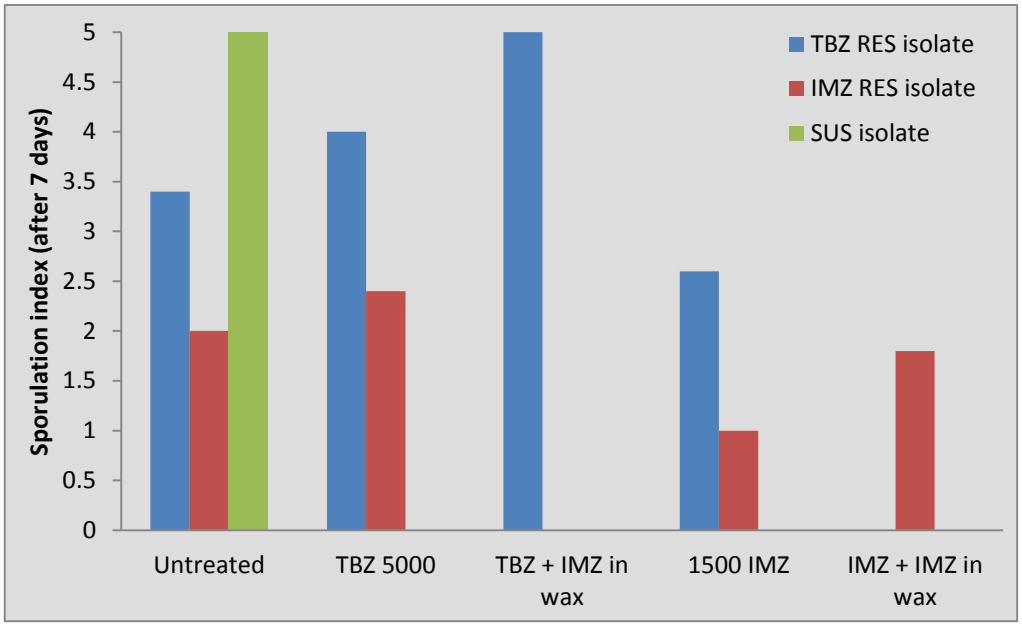
Graph 27. Mean Sporulation (7 days) on citrus fruit inoculated with dark green mould isolates collected from Shed G (June 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 28. Mean Sporulation (14 days) on citrus fruit inoculated with dark green mould isolates collected from Shed E (June 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 29. Mean Sporulation (14 days) on citrus fruit inoculated with dark green mould isolates collected from Shed G (June 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



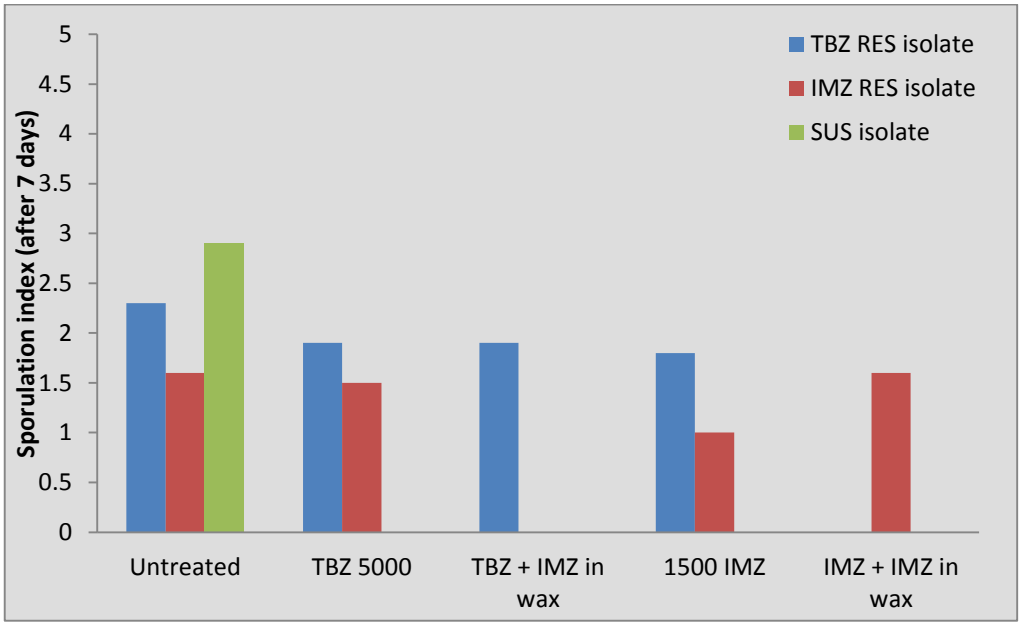
August Isolates

In August, green mould isolates were assessed from each shed; dark and light green mould spore isolates were present in Shed G. Spores were injected into fungicide-treated fruit to assessed fungicide resistance. The untreated green mould isolates from Shed E sporulated relatively well compared to the laboratory susceptible isolate, by 7 days after inoculation (see graphs 30). The untreated light green isolates from Shed G produced similar levels of spores to the susceptible isolate (ANOVA:. df=2,17, F=0.68, P=0.5) but the dark green mould isolates were less thrifty (ANOVA: df=2,17, F=11.04, P<0.001) (see graphs 31 & 32). A comparison of the average sporulation index rating for the untreated TBZ-resistant and IMZ-resistant green mould isolates after 7 days indicated they were not different, at 1.5±0.34SE and 1.6±0.27SE, respectively (ANOVA: df=1,18, F=0.5, P=0.82).

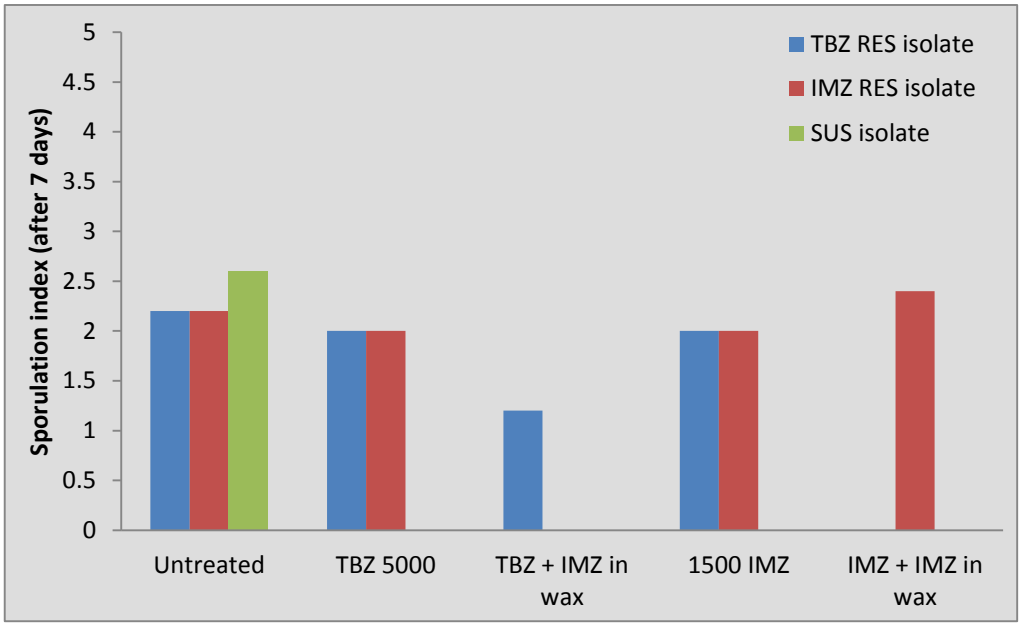
After 14 days, sporulation levels were higher in all fruit inoculated with isolates from either shed (see graphs 33, 34 & 35). Fruit treated with green mould isolated from Shed E were largely covered with spores, regardless of treatment. The sporulation index fruit inoculated with light green mould isolates from Shed G were also consistently high. Dark green mould isolates produced lower IMZ-resistant dark isolates. The standard treatment of label rate TBZ in dip followed by IMZ in wax provide little or no protection against sporulation. Low levels of spores were produced on fruit treated with label rates of IMZ in dip followed by

IMZ in wax. Some spores were produced on fruit treated with 3x label rate IMZ in a dip and 5x label rate TBZ in a dip. The production of spores on both these treatments indicates double resistance to IMZ and TBZ. At this stage, there appears to be a fitness cost to resistance in the dark green mould isolates. However, the light green mould isolates appear to be double resistant and as thrifty as the laboratory susceptible isolate evaluated.

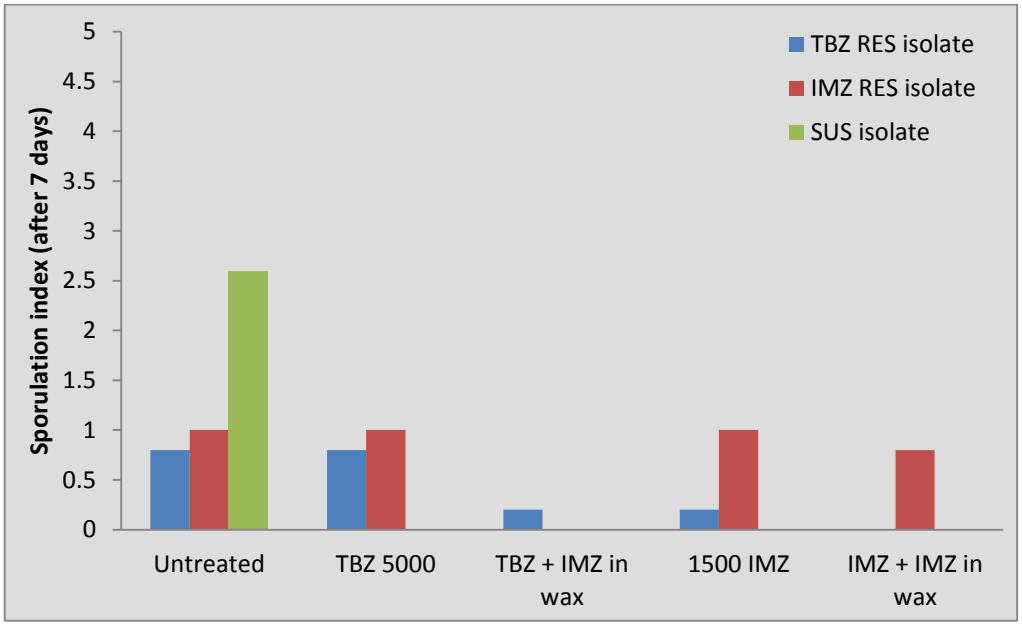
Graph 30. Mean Sporulation (7 days) on citrus fruit inoculated with green mould isolates collected from Shed E (August 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



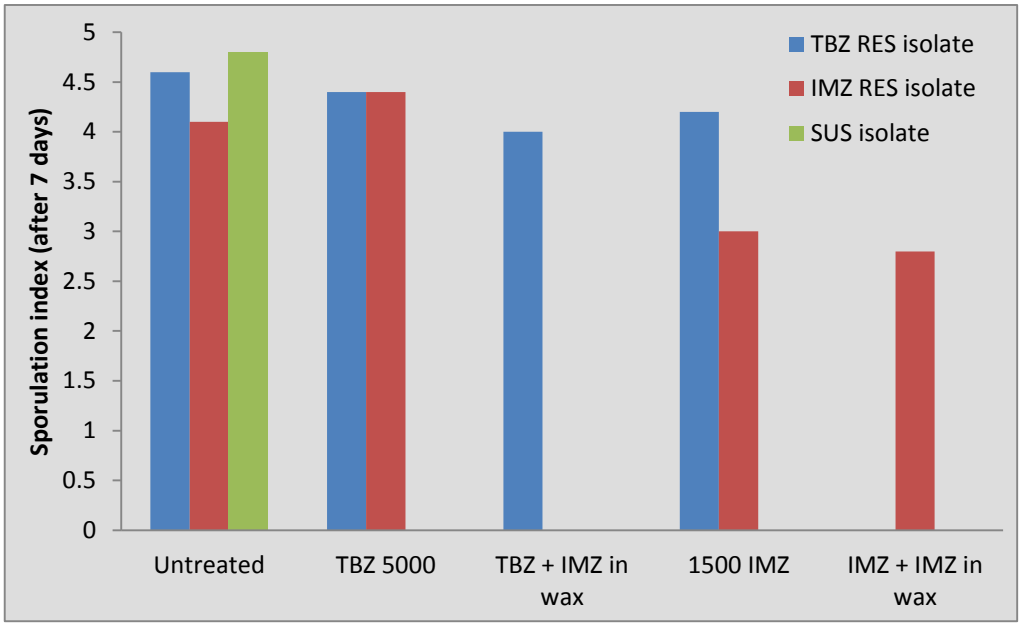
Graph 31. Mean Sporulation (7 days) on citrus fruit inoculated with light green mould isolates collected from Shed G (August 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



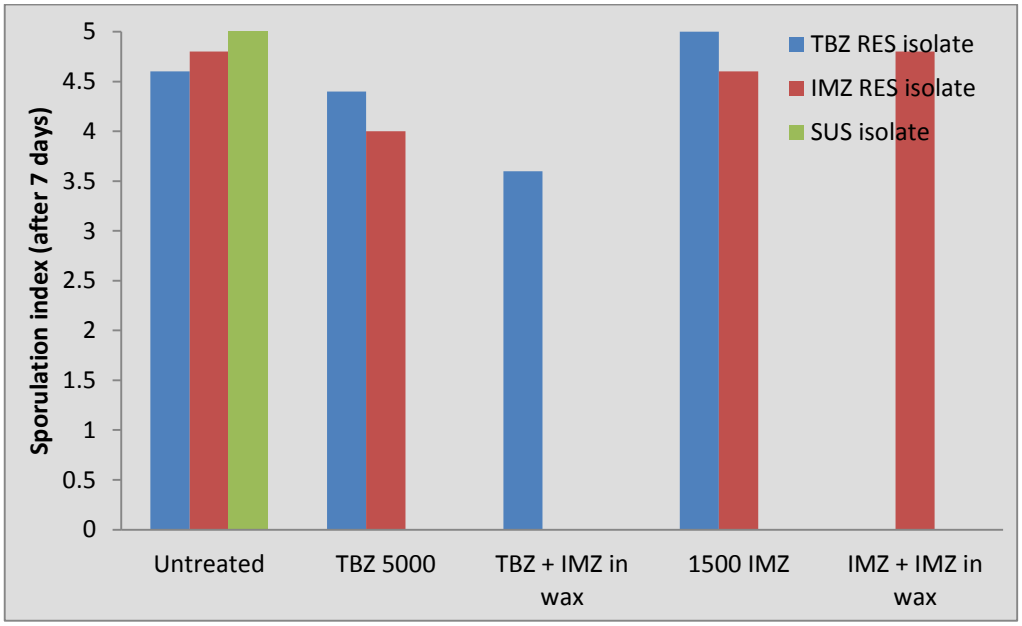
Graph 32. Mean Sporulation (7 days) on citrus fruit inoculated with dark green mould isolates collected from Shed G (August 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



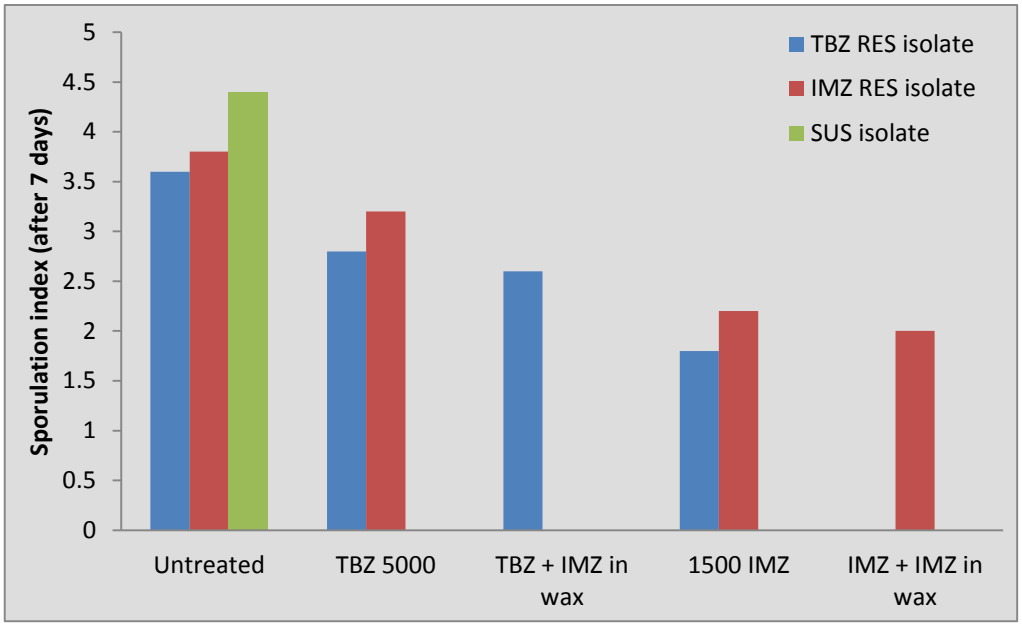
Graph 33. Mean Sporulation (14 days) on citrus fruit inoculated with green mould isolates collected from Shed E (August 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 34. Mean Sporulation (14 days) on citrus fruit inoculated with light green mould isolates collected from Shed G (August 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 35. Mean Sporulation (14 days) on citrus fruit inoculated with dark green mould isolates collected from Shed G (August 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).

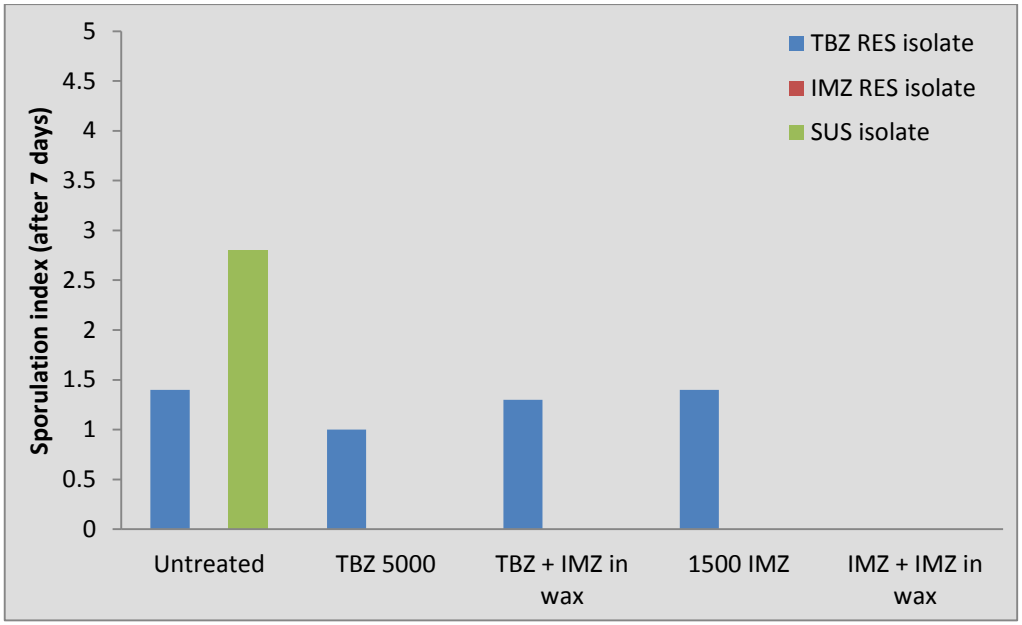


November Isolates

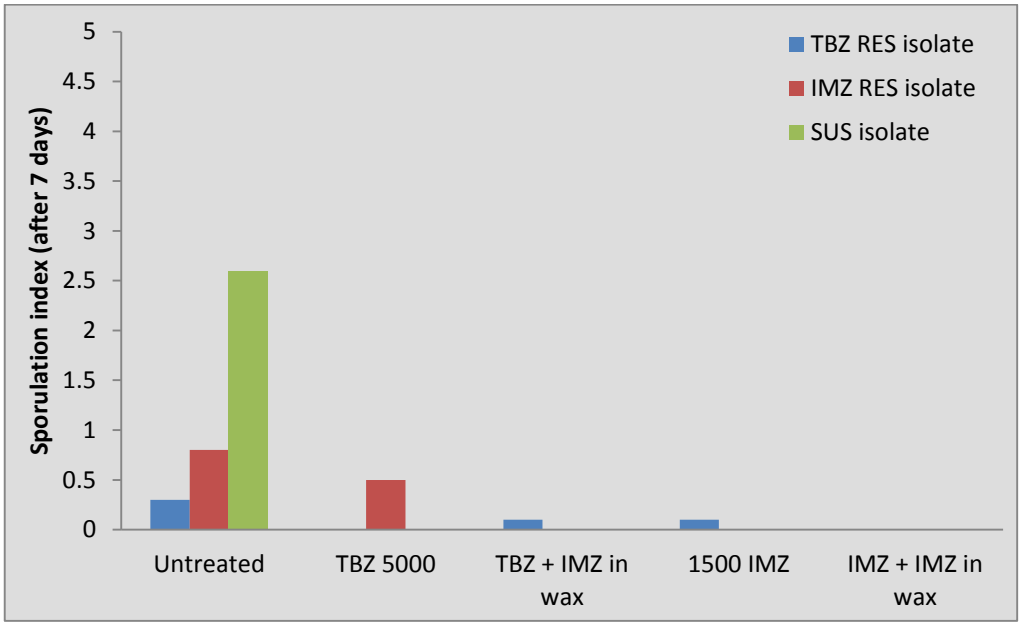
In November, green mould isolates were assessed from each shed; the typical dark green mould spore isolates were present in Shed E & G. Spores were injected into fungicide-treated fruit to assessed fungicide resistance. The untreated green mould isolate from Shed E & G produced fewer spores than the laboratory susceptible isolate, by 7 days after inoculation (see graphs 36 & 37). The fungicide-treated fruit inoculated with the TBZ RES isolate from Shed E produce similar levels of spore to the untreated fruit suggesting some level of resistance despite low thrift. The isolates from shed G sporulated poorly on untreated and fungicide treated fruit.

After 14 days, sporulation levels were higher in all fruit inoculated with isolates from either shed (see graphs 38 & 39). Fruit treated with TBZ RES green mould isolated from Shed E were largely covered with spores, fruit treated with label rates of IMZ in dip followed by IMZ in wax. The same trend occurred in the TBZ Res isolate from Shed G. The IMZ RES isolated collected from Shed G sporulated when treated with the IMZ only fungicide treatments, including 3x label rate IMZ dips. However, both treatments containing TBZ controlled sporulation. Interestingly, the level of fungicide resistance in the isolates collected in November appear to be lower than those collected in August. The results are based on one isolate per shed, which may not represent the vigour or sporulation capacity of other isolates from that same shed.

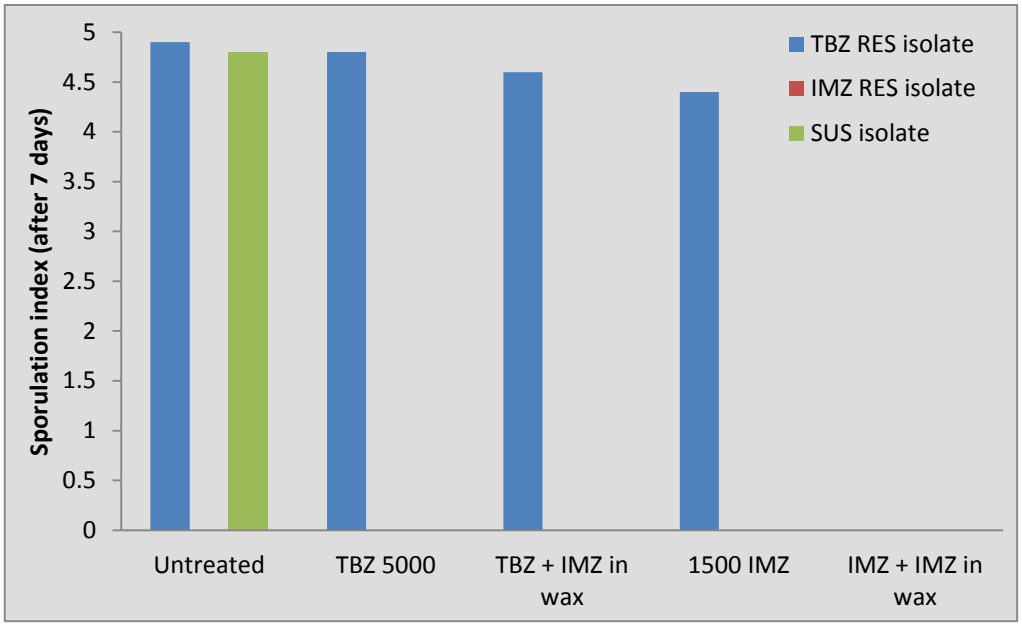
Graph 36. Mean Sporulation (7 days) on citrus fruit inoculated with green mould isolates collected from Shed E (November 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



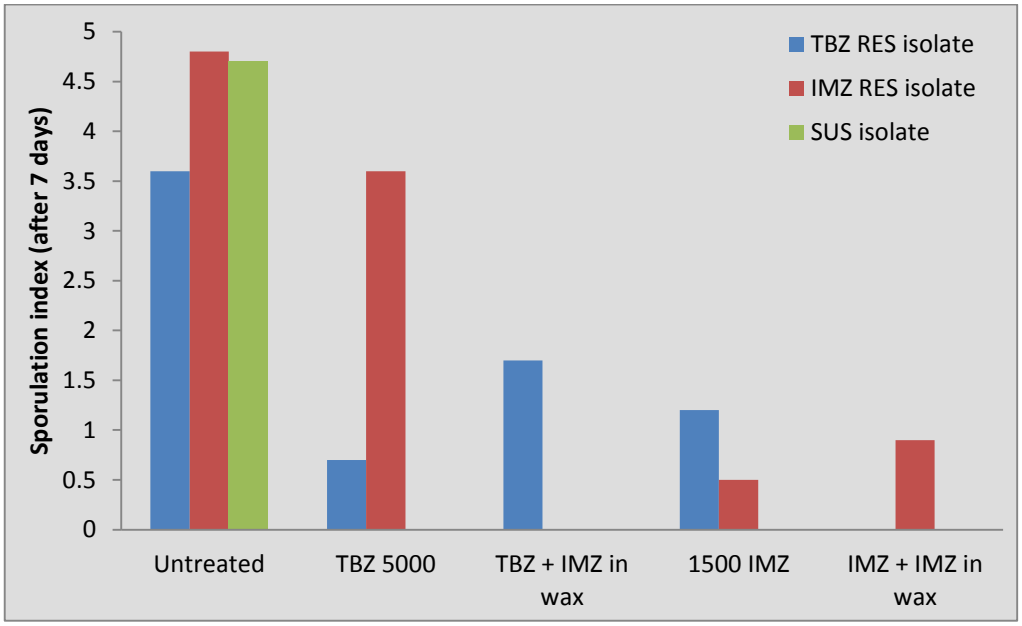
Graph 37. Mean Sporulation (7 days) on citrus fruit inoculated with green mould isolates collected from Shed G (November 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 38. Mean Sporulation (14 days) on citrus fruit inoculated with green mould isolates collected from Shed E (November 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).



Graph 39. Mean Sporulation (14 days) on citrus fruit inoculated with green mould isolates collected from Shed G (November 2014) or a laboratory susceptible isolate, and then various fungicide treatments applied or dipped in water (untreated).

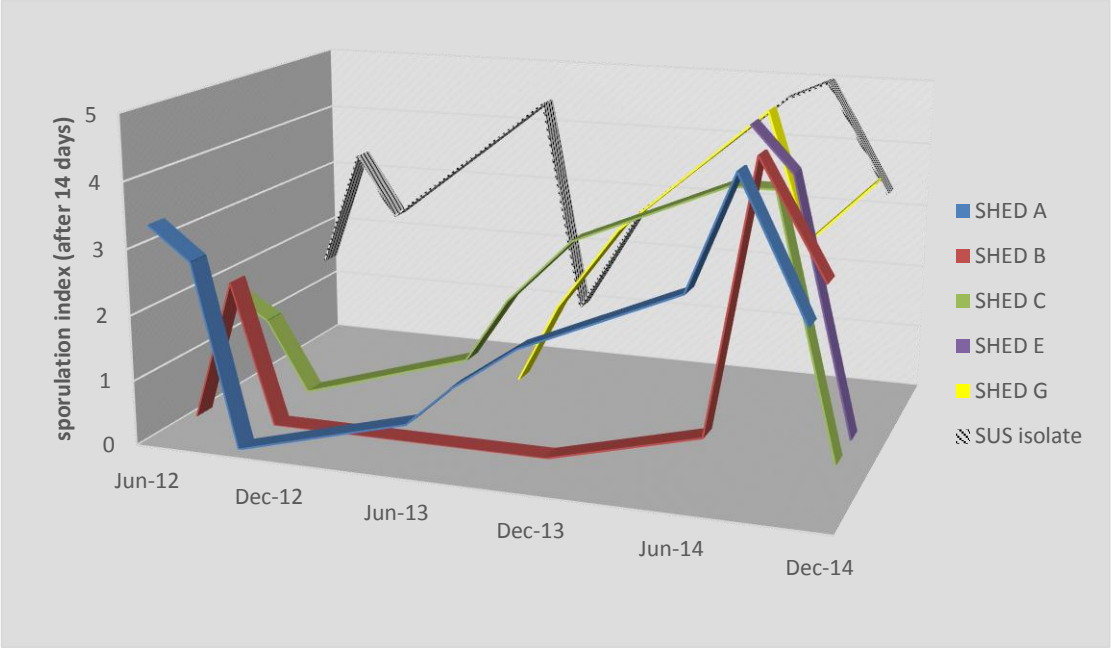


1.1.6 Seasonal trends

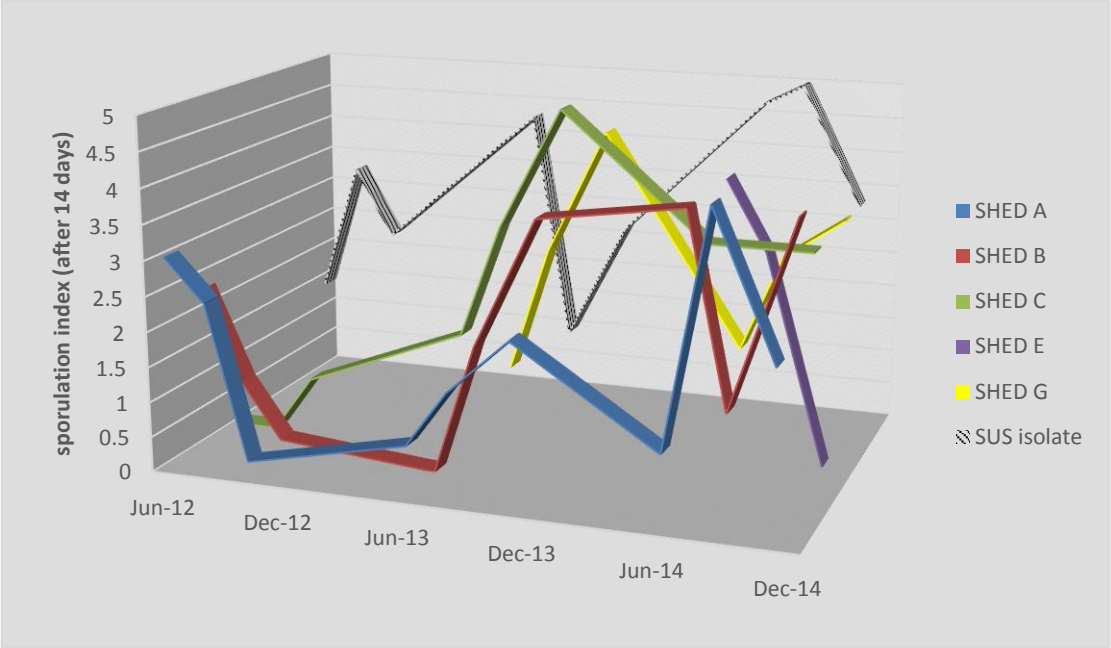
The seasonal trends were difficult to interpret unless evaluating general trends. The mean sporulation index of isolates on untreated fruit indicated the overall thrift. This varied noticeably over the seasons (Graph 40 & 41). The susceptible laboratory isolate's thrift should have been consistent from season to season but was occasionally irregular, suggesting factors beyond our control. The general trend for resistant isolates collected from sheds was more exaggerated than the susceptible isolate, with lower thrift compared to the susceptible laboratory mould isolate during the early survey years and similar in thrift throughout the later seasons. This trend suggests that there was a loss of fitness due to resistance initially but it was gradually overcome in subsequent seasons. The other interesting comparisons was the relative isolate fitness between different sheds. The loss of fitness in collected isolates persisted in some sheds longer than others (e.g., Graph 40; Shed B). This suggests differences in fungicide resistance pressure between sheds, which may be due to environmental factors or management practices; such as hygiene and sanitation.

The mean sporulation index of isolates on fungicide treated fruit indicate the level of resistance and the capacity to proliferate. The trend was more consistent in sheds, with resistant isolates sporulating more in later seasons (Graph 42 & 43). However, there was an inexplicable loss of thrift in the laboratory samples and shed collected isolates during one sample period in 2013. Excluding this period, the rate of sporulation appears similar regardless of fungicide treatment. The isolates collected from TBZ amended plates are not controlled by IMZ and vice versa.

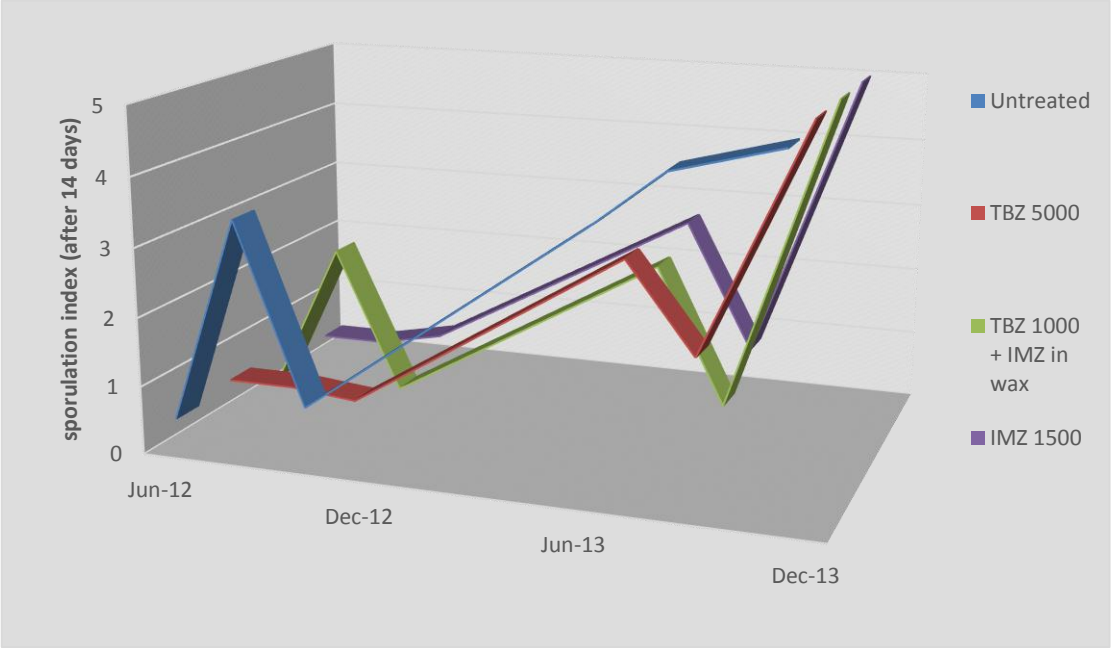
Graph 40. Mean sporulation index on untreated citrus fruit inoculated with green mould isolates collected from TBZ amended (TBZ RES isolate) plates from packing sheds from June 2012 to November 2014.



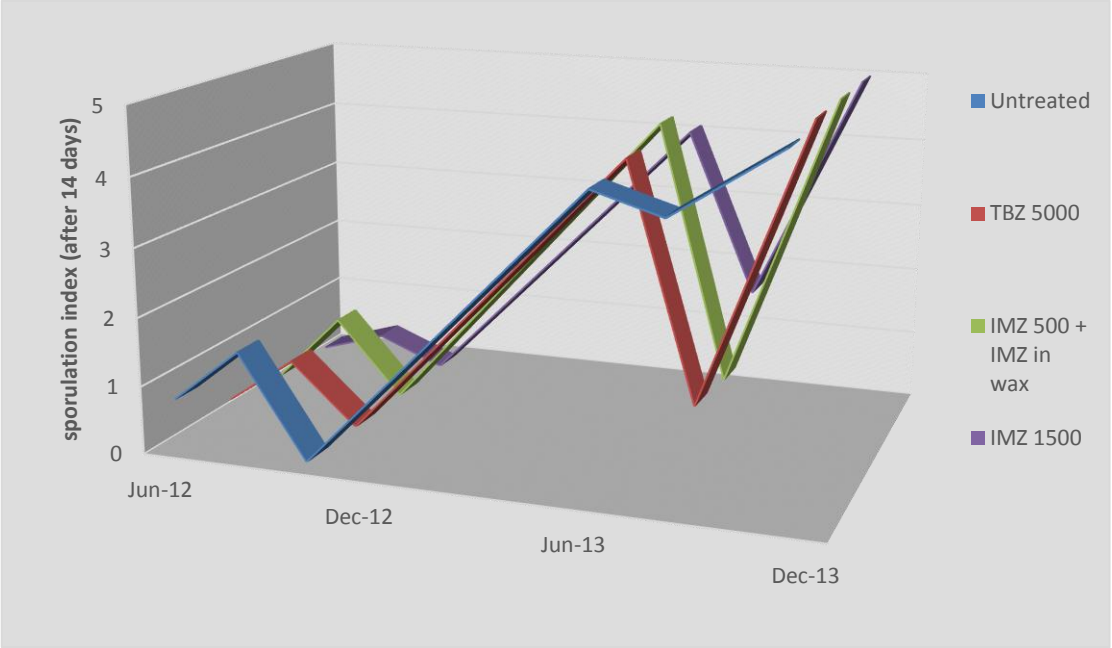
Graph 41. Mean sporulation on untreated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) plates from packing sheds from June 2012 to November 2014.



Graph 42. Mean sporulation index on fungicide-treated citrus fruit inoculated with green mould isolates collected from TBZ amended (TBZ RES isolate) plates from Shed A from June 2012 to November 2013.



Graph 43. Mean sporulation index on fungicide-treated citrus fruit inoculated with green mould isolates collected from IMZ amended (IMZ RES isolate) plates from Shed A from June 2012 to November 2013.



Conclusions

Prior to these surveys, the extent of fungicide resistance in commercial packing sheds was unknown. These surveys indicated that it was very easy to collect 'technically' fungicide resistant using amended agar plates. However, the detection of 'technically' fungicide resistant isolates occurred in packing sheds that were successfully marketing citrus. Clearly, there were other factors that contributed to commercial failure by decay, which were not measured.

We proposed that other contributing risk factors included;

- frequency,
- virulence, and
- sporulation capability.

We developed the following assessment criteria to measure the above factors;

- Compare numbers of colonies in amended and unamended plates
- Assess the decay incidence on inoculated fruit
- Assess sporulation on fungicide treated and untreated fruit

We aimed to provide a comprehensive testing regime to assess risk more accurately. However, interpretation was still difficult. There were morphologically different colonies with different fungicide sensitivities, which are likely to representing different Penicillium mould species. We concluded that the sensible approach was assessing changes in relative thrift and spore frequency over time rather than passing judgement on single assessment periods. Assessing several packers also allowed us to pool data to predict a regional risk, which was communicated to all packers through the Packer Newsletter.

During the surveys, it was apparent that some sheds were under less fungicide resistance pressure than others. This may be have been due to certain practice conducted by the sheds but no comparison of shed practices was undertaken.

After receiving the survey results, packers became interested in employing fungicide resistance management strategies. The recent registration of new fungicides allows packers to rest 'resistant' fungicides. However, the optimal length of time a fungicide must be rested is unknown. It would be useful to survey during 'resting' to assess when the mould populations return to being susceptible to the rested fungicide.

Despite the limitations, the participating packers became reliant on the survey results and were concerned when the service was discontinued. It seems likely that a fungicide resistance service would be valued by packers.

Recommendations

- Support a fungicide resistance testing service for packers.
- Monitor packers using new fungicides (e.g., Scholar® and PHILABUSTER®) to determine base-line levels and signs of fungicide resistance.
- Continue research to determine the factors indicating the development of fungicide resistance and integrate into improved monitoring services
- Assess optimum time period required to rest a 'resistant' fungicide and incorporate into fungicide resistance management strategies.
- Survey and taxonomic identification of the Penicillium species associated with decay in Australian citrus packing sheds.

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Appendix 6

Efficacy and compatibility of fungicide, additives and peracetic acid mixtures.

Introduction

Significant losses can occur during the storage of citrus fruit due to sour rot, caused by Galactomyces citri-aurantii E.E. Butler. In Australia, sour rot emerged as a serious issue during an expansion of navel orange (Citrus sinensis (L.) Osbeck) exports during in the 1990's. Sour rot can rapidly spread to nearby fruit in cartons creating a 'nesting' effect (Brown and& Eckert 2000). Most problems were associated to 'Leng' navel oranges (Citrus sinensis (L.) Osbeck 'Leng'), which remains an important early export cultivar. This cultivar has a tightly adhering rind prone to splitting at the navel end (Gallasch and Staniford, 2004), which provides entry wounds for postharvest pathogens. Postharvest application of guazatine is used for fruit destined for local consumption, but is not allowed for many overseas markets. Imazalil and thiabendazole are used for controlling green mould, caused by Penicillium digitatum (Pers.) Sacc., and blue mould, caused by P. italicum Weh. However, they are relatively ineffective against sour rot (Wild et al. 1976; Schachai and Barash 1982). Sodium ortho-phenylphenate (SOPP) has some activity against sour rot but its use in Australia is very limited. Fludioxonil and pyrimethanil were recently registered in Australia for the postharvest control of green and blue mould on citrus but their activity on sour rot is poor.

The Australian citrus industry has been able to maintain export markets despite the lack of a fungicide to control sour rot. This has primarily been due to strict hygiene and the diligent use of sanitisers in recirculated aqueous solutions and dip tanks. This use of sanitisers aims to control sour rot spores in water suspensions, and thereby, reduce the spread of disease to otherwise healthy fruit that are washed or dipped in these solutions. However, chemical incompatibility is common and sanitisers can quickly lose activity rendering them ineffective. The response varies with sanitiser active, fungicide active and fungicide formulation, making evaluation an on-going task as new actives and formulations are commercialised. In general, chlorine-based sanitisers tend to be more reactive and peroxyacetic acid (PAA) is relatively stable with a range of postharvest fungicides (Taverner and Cunningham 2004; Kanetis et. al. 2008).

Sanitisers can be used to control spores in water but the control of sour rot on fruit is more difficult. Although some GRAS compounds can retard symptoms, the level of control is not considered acceptable for commercial use. Combinations of carbonate salts or potassium sorbate mixed with fungicides have shown more promise (Smilanick et al. 1999, 2005 & 2008; Palou 2001 & 2002; Cunningham and Taverner, 2007; Montesinos-Herrero 2009). Our recent progress in combining chemicals for sour rot control on citrus is outlined in this chapter.

Material and methods

Products

The registered fungicides (a.i. rate used) (Product; Supplier) were formulated products of imazalil (500 ppm) (Fungaflor 500EC; Janssen-Cilag Pty Ltd, NSW, Australia), imazalil sulphate (500 ppm) (Magnate 750WG; Colin Campbell Chemicals, NSW, Australia), thiabendazole (1000 ppm) (Tecto SC; Scholar, Syngenta Crop Protection Pty Ltd, NSW, Australia), fludioxonil (600 ppm) (Scholar; Syngenta Crop Protection Pty Ltd, NSW, Australia), pyrimethanil (400 ppm) (Pembotec; Janssen PMP, Beere, Belgium) and a pyrimethanil + imazalil mixture (400 ppm + 400 ppm) (Philabuster SC; Janssen-Cilag Pty Ltd, NSW, Australia).

The GRAS compounds, sodium bicarbonate and potassium sorbate, were supplied by Ace Chemical Company, South Australia, Australia. The fungicide/fertiliser used was Phospot 400 pH 7.2 (400g/L phosphorous acid present as mono and di potassium phosphite; Accensi Pty Ltd, Queensland, Australia). The sanitiser used was Tsunami-On-Farm Biocide (110 g/l hydrogen peroxide and 160 g/l peroxyacetic acid (PAA); Ecolab Pty Ltd, South Australia, Australia).

Compatibility of peracetic acid (PAA) in mixtures

The compatibility of PAA was evaluated by measuring concentration over a period of 24 hours. Various mixtures of Tsunami (80 ppm PAA) with fungicides and GRAS compounds were compared with Tsunami alone. The fungicides and rates are listed in the Products section. The GRAS compounds, sodium bicarbonate and potassium carbonate, were evaluated at 1.0% (wt/vol). The fungicide/fertiliser used was Phospot 400 pH 7.2 evaluated at 0.04% (vol/vol).

Separate solutions of sanitiser, fungicide and GRAS compound were prepared in 500 ml of reverse osmosis (RO) water (pH 6.0-6.8). The solutions were combined in a 2 L glass beaker and mixed vigorously with a glass rod to achieve the desired concentration of each active. The concentrations of PAA were measured immediately on mixing and at 15 seconds, 30 seconds, 5 minutes, 1 hour, 4 hours and 24 hours using iodide test strips (Insta-Test, La Motte, Chestertown, MD, USA). Each experiment was repeated 3 times.

The solutions were readjusted to 80 ppm if the PAA concentration was reduced after 5 minutes or 1 hour. This was to account for any 'PAA demand' of the solution. The PAA concentration should be stable after any initial 'PAA demand' is satisfied.

Efficacy of mixtures against sour rot

Mature 'Leng' oranges from the Riverland region of South Australia were collected from field bins prior to any commercial postharvest treatments being applied. Fruits were inoculated using a method adapted from Eckert and Brown (1986). Fruits were dipped into a mixed pathogen inoculation mixture and punctured ten times around the equatorial region of the fruit using a steel nail (diameter 2 mm) to a depth of 3 mm into the albedo.

Sour rot and green mould were cultured on Potato Dextrose Agar (PDA) plates. A sterile paintbrush and a 10 ml volume of sterile water were used to wash spores from the surface of the culture plates. This solution was passed through sterile nylon voil to remove large fragments of hyphae. A Neubauer

haemocytometer was used to count the number of spores/ml and the solution was then adjusted to approximately 1×10^6 spores/ml for each pathogen. The sour rot and green mould spores were mixed (90:10 ratio) for inoculation. Sour rot is weakly pathogenic and the presence of Penicillium spores enhance disease development (Morris, 1982).

Fruits were inoculated with mixed pathogens and left for 2-3 hours before treatment. Ten inoculated fruits were dipped in each treatment solution for 30 seconds, placed in plastic bags to maintain high humidity and held at 30ºC±1ºC. The high temperatures were used to encourage the development of sour rot (Brown and Eckert, 2000). Each treatment was repeated three times.

Each inoculation point was assessed for decay at 72 hours and 7 days after treatment. Percentage infection for each treatment was transformed using Arcsine before ANOVA (randomised complete block design), with mean separation using Tukey's Honestly Significant Difference test.

Results

Compatibility of PAA in mixtures

The stability of PAA in mixtures with registered fungicides, additives (GRAS compounds, sodium bicarbonate and potassium sorbate, and fertiliser/fungicide, potassium phosphite), was variable (Table 1). A PAA (80ppm) solution in RO water remained stable for up to 24 hours. The additives reacted with PAA, with potassium sorbate losing all PAA activity within 24 hours. Generally, the sanitiser and fungicide combinations were more stable, except Philabuster where the concentration of PAA dropped to 60 ppm in 5 minutes. The solution was re-adjusted to 80 ppm where it remained stable for 1 hour then reducing to 40ppm after 24 hours.

The PAA mixtures with additives demonstrated losses after 1-4 hours. Potassium sorbate was most reactive, with topping required after 1 hour and total loss of PAA after 24 hours. The rate of loss with potassium phosphite was least, with no topping up require in the first hour. The sodium bicarbonate and PAA solution was readjusted after 1 hour and remained stable at 80 ppm PAA for 4 hours.

The combinations of PAA, registered fungicides and additives varied. Generally, loses followed the trends for the additives. Losses of PAA were significant over a 4-24 hour period but no gross incompatibility was demonstrated, i.e., no total losses of PAA within 5-60 minutes.

Adding 80 ppm PAA to slightly acidic (pH 6.0-6.8) RO water results in a more acidic solution (pH 4.0). The addition of potassium sorbate counteracts some acidity (pH 6.5) and sodium bicarbonate buffers more alkaline (pH 7.7). The trend is similar for the various fungicide and GRAS compound mixtures. The comparison of fungicides with the same active indicates that Magnate (imazalil sulphate) remains more acidic than Fungaflor (imazalil emulsifiable concentrate) after the PAA addition. The PAA remains stable over a wide range of pH values and there does not appear to be a strong relationship between the pH of the solution and PAA stability over time.

Table 1. Peracetic acid (PAA) concentration and pH of Tsunami combined with registered fungicides alone or combined with additives, over a 24-hour period.

Fungicide	*Additive*	*pH*	*PAA concentration (ppm)*			
			5 minutes	1 hour	4 hours	24 hours
No	No Salt	4.0	80	80	80	80
Fungicide	Na Bicarbonate	7.7	80	60[z]	80	20
	K Sorbate	6.5	80	60 [z]	40	0
	K Phosphite	6.5	80	80	60	20
Magnate	No salt	3.3	80	80	80	80
750WG	Na Bicarbonate	7.5	80	40 [z]	20	5
	K Sorbate	6.2	80	60 [z]	40	0
	K Phosphite	6.4	60[z]	60	40	40
Fungaflor	No salt	4.8	80	80	60	60
500EC	Na Bicarbonate	7.8	80	60 [z]	60	40
	K Sorbate	6.6	80	60 [z]	40	0
	K Phosphite	6.5	60[z]	80	80	20
Scholar SC	No salt	4.0	80	80	80	70
	Na Bicarbonate	7.9	80	80	60	5
	K Sorbate	6.4	80	70 [z]	35	0
	K Phosphite	6.5	80	70 [z]	60	5
Tecto SC	No salt	4.3	80	80	80	80
	Na Bicarbonate	7.6	60[z]	80	40	5
	K Sorbate	6.4	80	60	30	0
	K Phosphite	6.5	80	70 [z]	70	40
Philabuster	No salt	4.4	60[z]	80	50	40
	Na Bicarbonate	8.0	80	60 [z]	60	50
	K Sorbate	6.4	80	60 [z]	50	0
	K Phosphite	6.6	80	80	60	30
Penbotec	No salt	4.4	80	80	80	80
	Na Bicarbonate	7.7	80	60 [z]	60	50
	K Sorbate	6.5	80	80	20	0
	K Phosphite	6.5	80	80	70	40
Imazagard	No salt	5.0	80	80	80	60
800 EC	Na Bicarbonate	7.9	80	60[z]	60	15
	K Sorbate	6.7	80	60[z]	40	0
	K Phosphite	6.5	60[z]	80	80	40

[z] Tsunami concentration topped up to 80 ppm after measurement recorded.

Efficacy of mixtures against sour rot

The efficacy work consisted of twelve experiments with registered fungicides, additives and peracetic acid in mixtures. For convenience, the mean decay, seven days after treatment, of fruit dipped in all mixtures have been collated in table 2. Panoctine was included as a standard sour rot control and consistently resulted in very high control of sour rot. Panoctine was omitted from further analysis to improve the comparisons between our test mixtures. Magnate and potassium sorbate provided the lowest sour rot decay (4.0%), which was approaching commercial acceptability. The addition of PAA to Magnate and potassium sorbate resulted in higher decay rates but not significantly greater

(Kuskal-Wallis one-way ANOVA; p> 0.10). The addition of PAA did not significantly alter the efficacy of any treatment mixture.

The strongest overall trend was that the registered fungicides improved sour rot control (Table 3). Tecto provided the least improvement and the imazalil fungicides (Fungaflor and Magnate) the highest control, but there was no difference between imazalil formulations (EC vs sulphate).

The trend for additives was less significant than with fungicides. Overall, adding an additive improved control. Sodium bicarbonate, potassium sorbate and potassium phosphite provided similar levels of improvement.

Table 2 Mean percentage sour rot decay of fruit dipped in various combinations of fungicide, GRAS compounds and peracetic acid (PAA), 7 days after treatment.

		% Sour rot infection			
Fungicide	PAA[x]	Additive[y]			
		Nil	Na bicarbonate	K sorbate	K phosphite
Nil	-	72.3	45.5	49.0	40.0
	+	-	46.8	46.8	47.3
Magnate	-	32.0	18.0	4.0	23.0
	+	22.0	18.0	30.0	14.0
Fungaflor	-	30.7	17.0	26.0	27.0
	+	21.7	16.0	38.0	20.0
Scholar	-	33.0	20.0	49.0	31.0
	+	41.7	26.0	30.0	36.0
Tecto	-	58.7	26.0	55.0	49.0
	+	61.7	39.0	46.0	60.0
Panoctine	-	0.33			

x 80ppm peracetic acid
y 1% sodium bicarbonate, 1% potassium sorbate, 0.04% potassium phosphite

Table 3 Brief presentation of analysis of variance for pooled data (Panoctine omitted)

Source	*df*	*SS*	*F*
Fungicide	4	229	10.4***
Additive	3	53.2	3.2**
PeraceticAcid	1	0.5	0.08
Fungicide x Additive	12	38.7	0.6
Orthogonal Contrasts			
No fungicide vs fungicide	1	171	7.8***
Tecto vs other fungicides	1	98.7	4.5**
Imazalil vs other fungicides	1	87.0	4.0**
Imazalil EC vs imazalil sulphate	1	2.3	0.1
No additive vs additive	1	52.4	3.2*
Sodium bicarbonate vs other salts	1	16.6	1.1
Error	140		

*, ** & *** significantly different at P< 0.05, 0.01 & 0.001, respectively.

Discussion

Fungicide, sanitiser and sodium bicarbonate mixtures have been proposed for managing citrus green mould (Kanetis et. al. 2008). We propose a similar strategy for the control of sour rot on susceptible citrus cultivars, such as 'Leng' navel oranges. These experiments evaluated the compatibility peroxyacetic acid (PAA) and the efficacy of various chemical combinations for controlling sour rot.

PAA with fungicides were stable but the PAA concentration slowly decreased when GRAS compounds were added. Some combinations stabilised after the first adjustment, which may have satisfied the initial 'PAA demand' of the solution for at least 4 hours. The relatively slow losses of PAA in the mixtures suggest that rates could be periodically adjusted by packers to provide consistent levels of sanitiser. The sanitiser is required to control spores in solution, and thereby, minimise the inoculation of otherwise healthy fruit. PAA levels may be maintained but there may be other factors that influence the efficacy of PAA in solution, such as temperature, pH and total soluble solids (Mehmet 2003).

Sodium bicarbonate and potassium sorbate buffer the PAA and fungicide solutions resulting in more alkaline solutions. Other studies have shown that high alkalinity in imazalil sulphate solutions from 3% sodium bicarbonate buffering leads to significantly higher IMZ rind residues, and improved control of resistant green mould (Erasmus et. al., 2011). They recommended that imazalil sulphate buffered to pH 8 or above should not be used commercially due to its limited

solubility in water and the risk of exceeding minimum residue limits. Earlier, Kanetis et. al. (2008) recommended fungicides and PAA combinations buffered with 3% sodium bicarbonate for green mould control but they evaluated the emulsifiable concentrate (EC) form of imazalil. There is likely to be a significant difference in the response of the different forms of imazalil (Erasmus, pers comm.). The effect of buffering on residue levels or efficacy for different imazalil formulation is not clear. The situation for mixtures is complex; sodium bicarbonate levels vary from 0.5 to 3% in Australian packingsheds, which would affect the buffering capacity. We used 1% sodium bicarbonate in our compatibility work in an attempt to maintain the pH<8.

Previous studies indicate that sodium bicarbonate and fungicide mixtures are efficacious against sour rot of navel oranges (Cunningham and Taverner, 2008). In this study, imazalil EC and fludioxonil with potassium sorbate demonstrated high sour rot control, which complements earlier work showing improved control of sour rot on lemons with potassium sorbate and fungicide (Smilanick et al, 2008). In our efficacy work, the imazalil EC formulation was evaluated and sanitisers were not added.

Currently, sanitisers, fungicide and sodium bicarbonate mixtures are applied to citrus in post-harvest dips or drenches in Australia. The results from samples collected from commercial fungicide tanks show that combinations used are effective against sour rot. However, only the imazalil EC formulation was used in the packingsheds and in laboratory trials. Considerably more work is required to evaluate the efficacy of various fungicide actives and formulations when combined with sanitisers and GRAS compounds.

Ideally, solutions in contact with fruit during packing should be sanitised and also control disease on fruit. Sanitising solutions are problematic, with the choice of fungicide, GRAS compound and sanitiser influencing compatibility and efficacy. The response may also vary with the fungicide formulation and be dependent on other factors, such as pH, making evaluation time consuming. However, there is strong impetus to provide ‘safer’ alternatives to the synthetic fungicides currently used. We suggest that the approach of combining fungicides, sanitisers and GRAS compounds provides a transition to less reliance on fungicides. The use of fungicides mixed with GRAS compounds is acceptable to packers. It provides experience and, hopefully, more confidence in the commercial use of ‘safer’ alternatives. The next goal is to progressively reduce the fungicide residues required in these mixtures through an iterative process of adding and subtracting various ‘safer’ chemicals and&/or altering physico-chemical factors, such as pH and temperature.

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