# Final report

**Project title:**

# Integrated disease management of Citrus Black Spot and 'Emperor' Brown Spot (CT20009)

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## Public summary

Australia's export mandarin industry operates under increasing production, residue and market-access pressures. Citrus Black Spot (CBS) and 'Emperor' Brown Spot (EBS) cause significant fruit quality downgrades, especially in summer-rainfall dominant subtropical regions where disease pressure builds rapidly. Reliable disease control, tight export residue requirements and a narrowing chemical base must be balanced. This project assessed new and softer, low residue fungicide options, refined and safer use of copper formulations through pH optimisation, evaluated how spray timing affects disease outcomes, and investigated whether pruning debris within the canopy acts as a source of CBS inoculum. This report provides new field-derived guidance for managing CBS and EBS under Australian conditions from research completed in commercial orchards in partnership with industry to ensure results are immediately relevant to industry.

Several new chemical formulations were identified as effective chemistries to incorporate into season-long fungicide regimes. NUL3446, a formulation with a favourable residue profile, provided reliable late-season suppression of EBS when copper and particularly dithiocarbamates cannot be used, and two succinate dehydrogenase inhibitor (SDHI) co-formulations provided promising control of CBS and EBS when alternated with multi-site protectants including copper. These findings broaden future options for growers, noting that label extensions in some cases will be required before commercial use. Progress was also made in optimisation of the safe use of copper formulations to minimise copper phytotoxicity symptoms of fruit while maintaining disease control. Trials confirmed that copper injury varies with pH, formulation, fruit stage and seasonal conditions. Acidic copper produced the highest level of rind injury in sensitive cultivars and copper hydroxide generally resulted in lowest rind injury, although no formulation was entirely risk-free. The work highlights the value of pH-adjusted programs and careful copper use after mid-summer, when rind sensitivity can increase.

Understanding canopy hygiene, sources of inoculum and infection timing of CBS was another key component of the project. Significant outcomes include confirming the critical infection period is in the early component of the season, that pruning debris and dead wood in the canopy are significant sources of CBS inoculum affecting fruit quality, and that removing pruning debris can significantly reduce CBS as seen in mature, harvested fruit. Collectively these outcomes show canopy inoculum is as an important source of inoculum of CBS as leaf litter, providing pivotal knowledge for IDM-style management of CBS. In the context of canopy hygiene, the project also examined whether pre-petal-fall and petal-fall fungicide inputs provide meaningful benefit for control of CBS and EBS. Multiple trials investigated timing of fungicide application and found that petal-fall and pre-petal-fall applications provide negligible benefits while continuous coverage and fungicides applied in critical infection periods significantly reduce disease. The results also demonstrated that lack of canopy hygiene and high seasonal disease pressure can limit the effectiveness of all fungicide regimes, and both facets are required for effective, IDM-based control of EBS and CBS.

## Technical summary

The coordinated field program undertaken here between 2022 and 2025 addressed a critical industry need arising from increasing constraints on traditional fungicides used to manage CBS (Citrus Black Spot) and EBS ('Emperor' Brown Spot). Subtropical export mandarins have historically relied on copper and particularly dithiocarbamate protectants, but industry is sometimes reticent regarding use of copper due to phytotoxicity concerns, and mancozeb is progressively being phased out in key markets and is usage is scheduled for review in Australia in 2027. These pressures leave growers with fewer fungicide options, including in the latter half of the season when fruit remains highly susceptible to rind-blemish diseases but withholding periods must be considered. The project therefore sought to identify effective low-residue chemistries, optimise copper use to minimise phytotoxicity, quantify the role of canopy hygiene and refine spray timing under Australian subtropical field conditions to build a practical integrated disease management framework that maintains export-grade fruit quality while navigating tightening residue and regulatory restrictions.

A key outcome was field-derived evidence to support incorporation of a biofungicide (NUL3446) and SDHI co-formulations within effective, export-aligned, fungicide programs. Field-applied NUL3446 provided consistent late-season suppression of EBS caused by *Alternaria* spp. in mature 'Daisy' mandarin and, in postharvest assays in 'Murcott' mandarin, prior field exposure to NUL3446 reduced the severity of new infections in mature fruit. Complementing this, other field-derived results showed systemic SDHI products applied early to mid/late season in conjunction with copper and/or mancozeb perform reliably with respect to disease control as tested in multiple seasons. Some of these products need registration or label extensions for use in mandarin.

Copper-optimisation studies advanced our understanding of the strengths and weaknesses of copper usage for disease control in mandarin and clarified how formulation, spray pH and seasonal timing influence phytotoxicity/fruit safety in subtropical conditions. Trial outcomes illustrated use of acidic copper produces more, and more distinctive, injury symptoms than neutral and alkaline solutions and, when neutral and alkaline formulations were reassessed in a second growing season, pH made little difference with respect to the type or amount of rind phytotoxicity symptoms that developed. When copper formulation was assessed in conjunction with pH, cuprous oxide performed more favourably with respect to control of CBS than copper hydroxide but generated more phytotoxicity symptoms. Comparison of copper hydroxide before or after January indicated less rind injury was induced when sprays were applied until January. Importantly, both trials showed that no particular formulation or pH entirely eliminated fruit marking and usage needs fine tuning. This work was conducted with respect to CBS and needs to be assessed with respect to EBS and across seasons with variable disease pressure.

The fungicide-timing studies conducted in multiple varieties showed that scheduling adjustments does affect the amount of disease affecting mature fruit but that efficacy is limited by the amount of inoculum in the canopy. In the first of two trials conducted in 'Imperial' mandarin, spraying through the early season reduced CBS incidence and severity in fruit but was less important than the presence of increased inoculum in the canopy. A second trial used a more detailed treatment structure incorporating fortnightly, monthly or unsprayed periods to investigate the effect of timing on CBS (inoculum as dead wood added again) and found frequent, consistent, application in the first half of the growing season were key to disease control, and under these regimes, effective in the presence of increased inoculum. The suppression of fungicide activity and loss of disease control in the presence of high levels of inoculum cannot be over emphasized.

When pre-petal-fall fungicide applications were considered as a mechanism to reduce infection by *Alternaria*, and therefore, EBS, a multi-year trial showed there was no significant benefit afforded by their application when applied as two sprays at petal-fall. These trials reinforced that consistent

fungicide application during the season was required to reduce expression of both EBS and CBS. When an extensive series of fungicides were trialed over a longer period prior to petal fall, again no significant benefit was found in terms of control of EBS. These trials provided evidence that inoculum control must continue throughout the season and target critical infection periods of each disease, especially in seasons with high disease pressure.

Canopy-hygiene studies conducted simultaneously with the timing studies described above showed that pruning debris retained within the canopy was associated with measurable differences in CBS in fruit and are a significant source of inoculum contributing to disease in mature fruit. The first trial conducted in this theme showed extended exposure to infected woody material was linked with higher incidence of CBS at harvest than were short or early-season placements. Another large trial investigated the effect of types of inoculum sources, hosts, and fungicide or sterilization treatment of pruning debris on disease. Despite reasonably low incidence of disease across all treatments, it appears that all citrus hosts tested are hosts of CBS, that a one-off sterilization of debris at the start of the season has little effect on disease and that the woody components, potentially the twigs and small debris, are the most important sources of inoculum of CBS in mandarin production systems. Understanding these outcomes in a very practical commercial sense was then investigated. Three demonstration trials were conducted over several seasons where industry-generated pruning debris was left in or removed from the canopy for the season. The results showed the response to debris removal is highly variable and depends on variety, grower, season and orchard, ranging from very large to very small reductions in CBS. However, the results from the demonstration trial as well as other canopy hygiene trials do indicate a particularly high sensitivity of 'Imperial' mandarin to the presence of dead wood within the tree canopy. The results have contributed to an essential update of understanding the infection biology of CBS in that disease-producing inoculum is produced in the canopy in addition to any produced in the leaf litter, providing a significant opportunity to improve IDM-friendly control of CBS by managing canopy hygiene.

## Keywords

*Phyllosticta citricarpa*; *Alternaria* spp.; Citrus Black Spot (CBS) ; 'Emperor' Brown Spot (EBS); integrated disease management (IDM); citrus pathology; copper fungicide; phytotoxicity; pH adjustment; canopy hygiene; fungicide residue analysis; Queensland citrus; export fruit quality; low-residue fungicides; foliar application; rind injury; copper hydroxide; cuprous oxide; mancozeb (dithiocarbamate); captan; iprodione; azoxystrobin (QoI); pyraclostrobin (QoI); imazalil; ethephon; potassium hydroxide; citric acid; SDHI–strobilurin co-formulation; mode of action (MoA).

## Introduction

Australia's citrus industry relies heavily on production from the subtropical regions of Queensland, where mandarins form the core of national mandarin export market. Unlike the winter-dominant rainfall patterns of southern growing regions, subtropical orchards experience summer rainfall and humid temperatures, extended leaf wetness and frequent summer rainfall during fruit development. These conditions create a prolonged period of susceptibility to rind-blemishing pathogens, with *Phyllosticta citricarpa* and *Alternaria* spp. causing CBS and EBS respectively. Because commercial mandarin cultivars lack resistance to both pathogens (some are resistant to one but not both), and market standards permit minimal cosmetic defects, the management of CBS and EBS remains one of the most persistent challenges facing subtropical Australian growers.

Historically, fungicide programs have formed the backbone of CBS and EBS control. However, the industry now operates in an environment shaped by tightening maximum residue limits, shorter withholding periods, and increasing scrutiny of copper use due to phytotoxicity and copper accumulation in soils. These constraints are especially significant for late-maturing mandarins that remain on trees well into the wet (summer & autumn rainfall) season, often during periods when traditional protectants cannot be safely applied frequently to reduce the disease pressure. Maintaining export-grade fruit quality therefore requires an approach that balances efficacy, crop safety and residue compliance, supported by a sound epidemiological understanding of the diseases.

These industry pressures coincide with a recognised decline in national field pathology capacity, particularly within subtropical regions where disease pressure is greatest. The need for technically robust, field-validated disease management strategies is now more critical than ever. This project was established to address that gap by redefining integrated CBS and EBS management under subtropical conditions. Its purpose was to generate practical, export-fit strategies that incorporate fungicide performance, program timing, canopy inoculum dynamics and fruit safety considerations.

To achieve this, the project focused on four complementary themes. The first theme evaluated early- and mid-season applications of new SDHI co-formulations that could be integrated into subtropical mandarin programs, as well as late-season applications of low-residue chemistries which have particularly relevance in the lead up to harvest when mancozeb and copper use is restricted. The second investigated how copper formulation, pH and application timing influence phytotoxicity and disease suppression, with the aim of improving crop safety without reducing efficacy. The third examined spray-timing strategies, including rainfall-aligned scheduling, to determine whether closer alignment between fungicide inputs and infection events delivers meaningful improvements in control. The fourth quantified the contribution of canopy-retained dead wood and other in-canopy inoculum sources to CBS incidence, generating field-based evidence for cultural practices that can support chemical programs.

Taken together, these research themes provide a clearer understanding of how chemistry selection, spray timing, tree canopy hygiene and residue constraints influence CBS and EBS outcomes under subtropical Australian conditions. The project generates new field-based evidence to support more informed decisions about chemical management, copper use and cultural practices within commercial mandarin programs. These findings strengthen the technical basis for disease management in export-oriented production and provide practical guidance for aligning control strategies with market-access requirements while maintaining fruit quality.

## Methodology

A coordinated program of field trials and glasshouse trials was conducted between 2022 and 2025 to generate export-fit management strategies for CBS and EBS in subtropical mandarin production regions.

The methodology combined scientific investigation and consistent collaboration with industry. The research centered around four major themes that reflected industry priorities raised during project co-design with industry: 1. Evaluation of new-IDM appropriate chemistries, 2. Copper optimization and phytotoxicity, 3. Better timing of fungicide application and 4. Canopy hygiene and inoculum studies.

Within the themes, field trials were conducted as a series of field-scale experiments to investigate 1. new soft, low residue compounds (biofungicide NUL3446), and SDHI co-formulations (e.g. Merivon®, Luna® Experience) with respect to EBS and CBS, 2. how copper formulation (copper hydroxide, copper sulphate and cuprous oxide) affects disease suppression of CBS and how to optimise the use of copper fungicide to minimize phytotoxicity (formulation and pH studies), 3. testing timing of fungicide application (e.g. copper, SDHI's), including in relation to rainfall events and 4. canopy-borne inoculum (impact, source, viability) with respect CBS and EBS expressed in mature fruit of the commonly grown varieties of mandarin.

All investigations, but particularly the canopy-borne inoculum of CBS component, were considered within an integrated disease management (IDM) ethos to further IDM style disease management in mandarin production. Theme-specific methodologies are presented in the next sub-section and detailed methodologies for each trial in the appendices.

Grower engagement activities were conducted throughout the project to extend project findings in an ongoing manner. Activities included collaboration with a local industry reference group, hosting farm-walks, presenting at annual regional citrus forums, the national citrus congress, and national and international pathology and citrus conferences, and were conducted annually.

The project was strategically based in Bundaberg to be within or within easy reach of the export mandarin industry located in the Central Burnett region of Queensland and almost every field trial was undertaken in a commercial orchard the region with known histories of EBS and/or CBS. Again, this was by design to ensure findings reflected local, geographical, climatic and commercial operating conditions. Key staff (Dr. Thangavel) were based at the DPI facility in Bundaberg to ensure access to DPI support and facilities, including Malcolm Smith, the well-respected DPI citrus breeder.

Data from all field trials were analysed by members of the team (Dr. Tamil Thangavel) during the project and by QDPI Senior Principal Biometrician (Dr. Carole Wright) for final report preparation. Trial specific analysis details are in Appendices B–E.

**Note: Chemical usage**

Some trials reported here have included either off-label use of fungicides registered for disease control in citrus, or use of fungicides currently not registered for disease control in citrus, as detailed below:

1. Off-label use of a registered fungicide: Merivon® - Registered for EBS and blossom blight in lemon and tangelo but not in mandarin. The use of Merivon® for mandarin in these trials has been for strictly experimental purposes only and is not an approved use.
2. Treatments incorporating unregistered fungicides have been coded where applicable.

## Theme-specific methodologies

### Theme 1: Evaluation of new IDM-appropriate chemistries

Four field trials examined low-residue fungicides and SDHI co-formulations, including NUL3446, and Merivon® and Luna® Experience respectively, all representing emerging options for disease management in citrus. Programs were structured as monthly, alternating or season-wide applications in 'Murcott', 'Daisy' and 'Imperial' mandarins. Efficacy was evaluated by the incidence and severity of CBS and/or EBS in harvested fruit. Trials were conducted in mature commercial orchards in the Central Burnett. Trial designs, methods, results and conclusions for each trial are provided in Appendix A.

### Theme 2: Copper optimisation and phytotoxicity management

Three field trials evaluated how copper formulation, pH and application timing influence rind injury and disease suppression. Copper hydroxide, cuprous oxide and copper sulphate were applied under acidic, neutral and alkaline spray conditions. Efficacy was evaluated by the incidence and severity of CBS/EBS in harvested fruit. Trials were conducted in both young hybrid blocks at BRF and mature commercial orchards in the Central Burnett. Trial designs, methods, results and conclusions for each trial are provided in Appendix B.

### Theme 3: Better timing of fungicide application

Four field trials were conducted to evaluate how fungicide spray timing influences CBS and EBS control under contrasting inoculum and seasonal pressures in commercial 'Imperial', 'Daisy' and 'Murcott' mandarin orchards. The trials incorporated early copper inputs, fortnightly and monthly intervals, rainfall-triggered applications and pre-petal-fall fungicide programs. Canopy inoculum levels were manipulated to test how timing of fungicide application interacts with background disease pressure. Efficacy was evaluated by the incidence and severity of CBS/EBS in harvested fruit. Trials were conducted in mature commercial orchards in the Central Burnett. Trial designs, methods, results and conclusions for each trial are provided in Appendix C.

### Theme 4: Canopy hygiene and inoculum source studies

Two replicated field trials and three commercial-scale demonstration studies were conducted to determine whether canopy-retained pruning debris functions as a meaningful source CBS inoculum in commercial mandarin systems. Treatments manipulated the presence, type and persistence of infected dead wood within the canopy, including timed introduction, removal or replacement of debris, and comparisons among citrus host sources. Efficacy was evaluated by the incidence and severity of CBS/EBS in harvested fruit. Trials were conducted in mature commercial orchards in the Central Burnett. Trial designs, methods, results and conclusions for each trial are provided in Appendix D.

## Results and discussion

The following sections summarize the major findings from the four research themes that investigated performance of potential new fungicides, copper fungicide safety, application timing and canopy hygiene under subtropical Australian conditions. The results for each theme are presented and how the different management approaches influenced CBS and EBS expression within commercial mandarin production systems is discussed.

### Theme 1: Evaluation of new IDM appropriate chemistries

The evaluation of new IDM-appropriate chemistries across three subtropical seasons demonstrated that low-residue fungicides and SDHI–co-formulations can play a substantial role in managing EBS and CBS when applied within integrated spray programs. NUL3446 (off-label use with manufacturer permission) reduced late-season EBS in 'Daisy' mandarin, with NUL3446 1x + surfactant or NUL3446 1x alternated with Dithane giving the greatest reductions in disease incidence at harvest. Postharvest artificial inoculation assays in 'Murcott' confirmed that residual NUL3446 from in-field application suppressed the severity of new *Alternaria* infections of mature fruit. These results position NUL3446 as an important late-season option for mid- and late-maturing mandarins, especially where mancozeb and copper are restricted by residue, withholding-period, environmental or fruit safety considerations.

To complement this late-season focus, a second series of trials assessed whether systemic SDHI–based co-formulations could strengthen disease control when applied earlier in the season and provide effective protection across the entire window of susceptibility of both CBS and EBS, in association with industry standard multi-site protectants. In the first trial in 'Murcott' mandarin, the two SDHI-based co-formulations, Merivon® and Luna® Experience, were trialled alongside Amistar® and successfully reduced CBS/EBS incidence and severity relative to untreated controls when used in rotation with mancozeb. Of the two products, Merivon® generally produced the lowest levels of disease, although both SDHI-based programs performed well overall. Multiple sprays of mancozeb alone were also highly effective against EBS and particularly CBS, while acknowledging that the off-label use pattern in this experiment (i.e. increased spray frequency of mancozeb) may have contributed to the performance of all programs incorporating mancozeb. The main take-away message is that these SDHI-based co-formulations can help to reduce reliance on mancozeb in early- or mid- season treatment windows. Canopy hygiene elements were also incorporated in this trial and showed that even high-performing chemistries must be supported by canopy-hygiene practices to achieve maximum efficacy as disease increased when dead wood was added to the canopy. Please see Theme 4 for more detailed canopy hygiene results and management insights. No phytotoxicity attributable to the SDHI-based co-formulations was observed, and residue testing of Merivon® treated fruit showed residues detected remained below Australian MRLs, and for Luna® Experience, no fruit residues of its two actives were detectable at harvest.

In the second of these trials, the evaluation of SDHI-based co-formulations was extended to 'Imperial' mandarins and examined whether these products could enhance copper-based programs if used early to mid-season in the program. Copper alone, SDHI–copper and mancozeb-copper combinations all achieved acceptable CBS suppression at harvest; however, none of the programs significantly reduced the expression of CBS post-incubation (resulting from latent field infections) compared to the control, potentially due to the high incidence and severity of CBS observed at this stage. Strategic SDHI-based sprays applied immediately after rainfall performed comparably to fixed-schedule SDHI–copper programs overall, suggesting that rainfall-aligned timing may not offer any significant practical benefit over calendar-based applications. In any case, and as prescribed on the label, these products are to be used preventatively as part of an overall IDM program for resistance management reasons. Although copper-based programs performed well in this early-maturing cultivar, reliance on repeated

copper use remains less suitable for mid- and late-maturing cultivars due to heightened phytotoxicity risk under warm, humid subtropical conditions. The strong performance of SDHI-based products + copper, at least for pre-incubation CBS, suggests that these products can extend program reliability in seasons or cultivars where copper alone is insufficient or cannot be applied at higher frequency.

In summary of Theme 1, these trials demonstrated how new low-residue chemistries and SDHI–based co-formulations can be functionally positioned across the subtropical mandarin season. NUL3446 provides a residue-compliant late-season option; SDHI–mancozeb programs deliver robust early- and mid-season suppression and SDHI–copper combinations provide additional flexibility where fruit safety or residue constraints limit conventional protectants. (See complete details in Appendix A.)

### Theme 2: Copper optimisation and phytotoxicity management

Copper optimisation studies undertaken across three seasons showed that rind phytotoxicity in subtropical mandarins is influenced by formulation, pH and the timing of application, and that these factors interact with seasonal conditions to shape both fruit safety and disease outcomes.

An exploratory study established in a densely canopied scab-orange hybrid block at the DPI research facility provided an early indication that the pH of copper formulation is closely linked with rind injury and the degree of injury recorded in mature fruit varies with pH. However, the crowded canopy of the scab-orange planting and inherently blemish-prone fruit of the variety limited detailed interpretation of the results due to high degrees of mechanical injury, rub and scab. It informed the design of subsequent trials, though, by highlighting the need to assess pH effects under commercial field conditions and on cultivars commonly grown for the export market. As importantly, the trial also showed none of the treatments impacted tree health and that it was safe to take this work into commercial orchards.

The second trial (FT2), conducted in a commercial 'Murcott' mandarin block, showed clearly that pH is a core component of copper-induced phytotoxicity in mandarin and that a dark speckling symptom appeared to be the most reliable symptom of phytotoxicity seen here. Acidic copper (pH 4) consistently produced very high levels of rind injury whereas neutral (pH 7) and alkaline copper (pH 11) had fewer and similar amounts of visible injuries in comparison. Application of neither cuprous oxide nor copper hydroxide (each applied at pH 7) incited more phytotoxicity symptoms of any kind or more disease than the other, while higher levels of CBS were noted when acidic copper hydroxide was applied suggesting that the damage to the rind needed to create visible copper injury may sometimes increase the sensitivity of fruit to infection. These results collectively confirmed that copper safety is formulation and pH dependent, and that pH management can provide a practical means of reducing fruit damage in cultivars with known sensitivity.

A third trial (FT3), also conducted in 'Murcott' mandarin, investigated the effect of formulation, pH and timing of application (early-spring to mid-summer versus mid-summer to harvest) on phytotoxicity and disease. Results showed a shift in symptom expression, where a chemical run-off pattern appeared to be the symptom indicating phytotoxicity injury this season rather than the dark speckling pattern of the previous season. They also showed cuprous oxide is more strongly associated with phytotoxicity than copper hydroxide when compared at pH 7 and 11 and that application of either product at pH 11 is more likely to cause more symptoms than at pH 7. The severe speckling symptoms seen in the trial described above were not seen, corroborating that they were associated with application of acidic copper.

Design constraints of the third trial meant only copper hydroxide at pH 7 and 11, applied until and including December, and after December, could be compared but showed application at pH 7 prior to December generated (non-statistically) lower levels of phytotoxicity than the treatments applied after

December. Timing of application with respect to immature and maturing fruit needs further investigation. Cuprous oxide provided better control of CBS than copper hydroxide but the benefits were outweighed by the greater amount of phytotoxicity symptoms it induced. These outcomes demonstrated that copper-related symptoms are expressed differently between seasons, early-season copper applications are less likely to cause rind injury, delivery of neutral copper formulations decreases the likelihood of injury and that copper hydroxide may be one of the mildest forms of copper with respect to rind injury.

The combined analysis of these two trials highlights that phytotoxicity patterns can change markedly between seasons and with formulation and pH of delivery. For example, speckling injury was more prominent under the experimental conditions of FT2 where acidified copper preparations showed a very high incidence of this symptom, whereas chemical run-off pattern was more common in FT3 assessments where only neutral and alkaline preparations were evaluated.

These results show that copper phytotoxicity in subtropical mandarins cannot be understood through formulation alone. Spray-solution pH and seasonal timing are key determinants of the degree of phytotoxicity expressed in mature fruit, with acidic conditions presenting the highest likelihood of visible rind injury as demonstrated in the second trial. Alkaline and neutral copper produced less injury than acidic copper in FT2, indicating the importance of pH management when applying copper fungicides. These findings provide a clearer understanding of how formulation, pH and season interact to influence copper behaviour, and support the development of more predictable, pH-adjusted copper programs for sensitive mandarin cultivars. (See complete details in Appendix B.)

### Theme 3: Better timing of fungicide application

The fungicide application timing studies carried out in 'Imperial', 'Daisy' and 'Murcott' mandarin showed timing of fungicide application plays an important role in control of EBS and CBS but must be considered hand in hand with the continuity of coverage as well as orchard-specific and seasonally influenced inoculum pressure.

In the first of two 'Imperial' mandarin trials, timing of fungicide application was investigated over different ranges of months and combinations of months. The treatment structure was duplicated and dead wood was inserted into the canopy of one entire complement of treatments to assess the effect of timing in relation to disease pressure. The results showed that while early season fungicide application reduced levels of CBS in 'Imperial' mandarin, the most important factor for predicting disease symptoms was the presence of elevated inoculum from dead wood in the canopy.

The second of these trials reinforced that early, consistent coverage is the key to control of CBS with copper in mandarin. Here a suite of treatments incorporating fortnightly, monthly and unsprayed periods were tested in the presence of increased inoculum (added dead wood) and disease was lowest where fortnightly sprays were applied for one if not two components of the early growing season The results also showed that an unsprayed period in the early to mid-season elevated the risk of disease equivalent to that of no coverage. CBS was higher in some fungicide treatments than an unsprayed, 'no dead wood added' control treatment, reinforcing that fungicide application can't overcome high disease pressure.

The early-season EBS evaluations in 'Daisy' mandarin provided a complementary perspective on timing. In FT3, where 19 pre-petal-fall programs were applied at frequent intervals in a 6-week lead up to petal-fall, EBS incidence remained uniformly high across all chemistries, with only small numerical differences among treatments. Although some extended or iprodione-based programs recorded lower severity, the overall range was narrow, and no treatment group provided strong suppression. These results show that extensive pre-petal-fall applications did not materially alter EBS

levels at harvest under the conditions of this trial.

In the fourth trial in this theme, a bold assessment of whether two fungicide applications at petal fall could influence EBS and CBS measured in mature 'Murcott' fruit was undertaken. Nine fungicides were evaluated in a duplicated trial structure, in which the grower applied their own season-long (non-disclosed) fungicide program to half of the trial. As there was little disease pressure in the year the trial was first conducted, at request of industry, it was repeated in the following year. Higher disease pressure was seen in the second year making this a useful exercise. Unfortunately, in neither year did the petal-fall treatments significantly affect the amount of EBS in comparison to the untreated control. In both seasons, the grower's standard in-season spray program consistently reduced EBS incidence relative to petal-fall-only treatments, confirming that petal-fall sprays alone were insufficient to maintain suppression through to harvest.

Petal-fall sprays had no effect on CBS when applied alone and minor effects when applied prior to the grower's fungicide regime, but the result again clearly showed disease control needs consistent fungicide coverage through the season with a sharp decrease in incidence and severity seen when the grower's fungicide program was applied.

For both CBS and EBS, this minimal response occurred despite a wide range of chemistries with differing modes of action being evaluated as early season sprays—including multi-sites, QoIs (quinone outside inhibitors), dicarboximides, triazoles, SDHIs (succinate dehydrogenase inhibitors) and the biofungicide NUL3446.

Collectively, these trials show that spray timing adjustments provide limited benefit when used in isolation and cannot compensate for high canopy inoculum or gaps during the fruit susceptibility period. Where inoculum was low, such as in the clean-canopy treatments of FT1, all timing programs produced similarly low CBS levels. Where CBS inoculum was high, such as in FT1 with dead wood and in FT2, early or frequent sprays moderated disease only marginally. For EBS in FT3 & 4, early season sprays provided little or no benefit. Effective management of both EBS and CBS relies on canopy hygiene and sustained coverage during the key infection periods, rather than on isolated early-season inputs. (See complete details in Appendix C.)

### Theme 4: Canopy Hygiene, Pruning and Inoculum Source Studies

The canopy hygiene studies conducted between 2023 and 2025 showed that dead wood retained within the tree canopy plays a vital role in the incidence and severity of CBS symptoms in mature mandarin fruit although the degree of the effect varied between sites, seasons and experimental design. The trials discussed here complement the results presented in Theme 3 that have already introduced an understanding of the significant effect increased canopy inoculum makes on disease in mature fruit. These trials were carried out in production systems in the Central Burnett where growers have typically focused on orchard-floor and leaf-litter management. Citrus field consultants in the region and a previous Hort Innovation project (CT13021) (Drenth, 2018; Tran *et al*., 2020) had previously proposed that inoculum within the canopy may play a more active role but had never been thoroughly investigated.

A first field trial examined the effect of attaching infected wood to 'Imperial' mandarin canopies across defined seasonal windows on inoculum seen as disease in fruit at the end of the season. Not only did the presence of the dead wood directly contribute to increased levels of disease, but longer exposure periods resulted in higher pre-incubation CBS incidence and severity than short or early-season placements as measured in mature fruit at harvest. The trial also showed that longer exposure in the early to mid-season was more influential than later in the season.

In a series of demonstration trials conducted over two growing seasons in three varieties and locations, whether removing small dead wood and fine pruning debris could reduce CBS under commercial management was assessed and found to affect the amount of disease recorded in mature fruit. The largest and exceptional result was recorded at the Mundubbera site in a mature 'Imperial' mandarin orchard with an eighteen percent reduction in the incidence of CBS where the debris had been removed. The differences between the treatments at the remaining Gayndah and Wallaville site were modest. These results show the effect of pruning debris on disease is dependent on season, variety and location but that it can be outstanding.

A last trial tested if disease measured in fruit as a proxy for different levels of canopy inoculum differed with dead wood type, host origin, surface sterilisation or timing of introduction. In all categories, trees receiving attached dead wood showed higher CBS incidence than clean-canopy controls, but only in very few cases were these differences statistically different. Dead wood from lemon, Tahitian lime or mandarin produced comparable outcomes, indicating limited influence of host origin. Chemical surface treatments did not produce durable suppression across the season, and neither replacement schedules nor delayed introduction produced clear timing responses.

Overall, this series of studies show managing leaf litter alone does not account for all sources of CBS inoculum and that canopy-retained dead wood contributes to CBS incidence and severity. These findings clarify that canopy management is a relevant component of CBS management and that leaf-litter management, while important, does not fully address infection pathways operating within subtropical mandarin canopies. (See complete details in Appendix D.)

This project delivered practical, IDM aware, outcomes for management of CBS and EBS, the two major diseases affecting production of export grade mandarin in the subtropics. The potential of new fungicide formulations was documented in relation to disease control and MRL concerns. Efficacy, usage and fruit safety in relation to copper formulations and phytotoxicity was refined, and fungicide scheduling of the new and existing products examined in relation to phytotoxicity and disease management. Lastly, the project conducted extensive evaluation of the role of canopy-derived inoculum resulting in a dramatic shift in understanding that CBS in the canopy is as or more a significant source of inoculum as leaf litter, that fungicide efficacy cannot overcome high levels of inoculum and canopy hygiene provides a practical, IDM friendly management tool to aid disease control.

## Outputs

### Table 1 Output summary

| Output | Description | Detail |
|---|---|---|
| Multi-season evaluation of new IDM-appropriate chemistries for CBS and EBS | Field trials in three seasons generated evidence on the efficacy and crop safety of NUL3446, Luna® Experience, Merivon® and protectant fungicides under commercial mandarin production conditions. The work established how these chemistries function within integrated spray programs across early-, mid- and late-season windows. | Results and datasets are provided in Appendix A, including treatment structures, disease assessments, phytotoxicity observations, residue analysis, and associated tables and figures. |
| Evaluation of NUL3446 for late-season EBS management | Two field trials in 'Murcott' and 'Daisy' mandarins assessed rate responses, spray frequency, compatibility with mancozeb, and the behaviour of residual deposits under low and high late-season *Alternaria* pressure. The trials provide the first subtropical field evidence for NUL3446 efficacy against EBS in Australian mandarins. | Complete methodology, treatment lists and results are presented in Appendix A (FT1–FT2), including incidence/severity datasets, inoculation-assay outcomes and supporting figures. |
| Evaluation of two SDHI-based co-formulations for management of EBS and CBS. | Two field trials in 'Murcott' and 'Imperial' mandarin assessed SDHI-based co-formulations as components of season-long fungicide regimes including mancozeb and copper providing evidence of efficacy and that they should be considered as components of spray programs. | Complete methodology, treatment lists and results are presented in Appendix A (FT3–FT4), including incidence/severity datasets and supporting figures. |
| Multi-season evidence on copper formulation, pH and timing effects on rind phytotoxicity in mandarins | Three field trials generated consolidated evidence describing how copper formulation, spray-solution pH and seasonal timing influence rind phytotoxicity under subtropical commercial conditions. The work clarifies the conditions under which visible copper injury is more likely to occur and identifies situations where lower symptom expression was observed. | Complete trial methods and results are presented in Appendix B (FT1–FT3), including treatment structures, phytotoxicity datasets and associated tables and figures. |
| Review and grower article of copper phytotoxicity in citrus prepared | Literature review on copper fungicide phytotoxicity in citrus completed. | Literature review and article submitted to Hort Innovation. |

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| | An article summarising key messages from FT1–FT3 were consolidated into a practical evidence package describing the factors that influence copper performance and crop safety in sensitive mandarin cultivars. | |
| Multi-season field trials evaluating fungicide timing effects on CBS. | Two timing studies in 'Imperial' mandarin (Appendix C FT1 and FT2) assessed whether early copper sprays, rainfall-based application triggers, or tighter seasonal intervals influence CBS expression under contrasting canopy inoculum and seasonal pressure. The trials provide field-based evidence describing the extent to which timing refinements support disease suppression relative to canopy inoculum and continuity of protection. | Complete methods and results for FT1–FT2 are presented in Appendix C (CBS sections), including treatment structures, pre- and post-incubation datasets, incidence and severity tables, figures, and composite analyses. |
| Field trials evaluating early-season fungicide timing for EBS. | Three early-season studies in 'Daisy' and 'Murcott' mandarins (Appendix C FT3 and FT4a/b) examined whether pre-petal-fall sprays, extended early programs, or specific mode-of-action groups influence EBS suppression under low and high seasonal pressure. The work provides multi-season evidence describing the limited and pressure-dependent value of early-season inputs relative to the grower's mid-season spray program. | Complete methods and results for FT3 and FT4a/b are presented in Appendix C (EBS sections), including treatment descriptions, incidence and severity datasets, pre- and post-incubation tables, figures, and composite disease index outputs. |
| Multi-site field trials evaluating canopy-retained dead wood and canopy hygiene as factors influencing CBS | Three studies examined whether pruning debris and dead wood retained within the tree canopy contribute to early-season CBS incidence and whether simple canopy hygiene practices influence disease levels under commercial management. FT1 tested the timing and duration of infected debris within the canopy, FT2 assessed debris removal across multiple orchards, and FT3 compared infection potential across debris types, host origins, | Complete methodologies and results for FT1–FT3 are presented in Appendix D, including treatment structures, pre- and post-incubation datasets, incidence and severity tables, figures and integrated analyses describing canopy hygiene as a supplementary cultural practice. |

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| | surface treatments and seasonal introduction periods. | |
| Grower benchmarking survey report | A grower benchmarking survey was completed across Central Burnett mandarin orchards to document current CBS and EBS management practices, canopy hygiene behaviours, fungicide use patterns and perceived disease pressures | Survey report submitted to Hort Innovation (ML104). |
| Survey of citrus nursery disease and fungicide-use practices | A survey of citrus production nurseries was completed to document disease management practices, fungicide use patterns and awareness of fungicide-resistance issues. The survey results provide baseline insight into IDM practices in nurseries supplying industry. | Survey report submitted to Hort Innovation (ML106). |
| Scientific publication, conference dissemination and grower field demonstration | CT20009 findings were communicated through a peer-reviewed publication, national and international conference presentations, and industry field-walk activities. These outputs extended the project's reach to scientific, extension and grower audiences. Activities included a peer-reviewed article, an accepted abstract and poster at the 12th International Congress of Plant Pathology (ICPP 2023, Lyon), a poster presented at the 2024 Australian Citrus Congress, a field walk delivered at the Southern Queensland Regional Forum (October 2023) demonstrating canopy hygiene and its relevance to CBS management, and a second field walk held during the 2024 Australian Citrus Congress (March 2024) demonstrating copper phytotoxicity observations and integrated CBS/EBS disease-management principles. | • Peer-reviewed article (in press): Thangavel, T. et al. Tree hygiene for effective management of CBS in Australian mandarin orchards, *Acta Horticulturae*.<br>• ICPP 2023 (Lyon, France): Accepted abstract and scientific poster — An investigation of ways to reduce phytotoxicity caused by copper-based fungicides in citrus.<br>• 2024 Australian Citrus Congress: Poster presentation of CT20009 findings.<br>• Grower field walk (Oct 2023): Demonstration of canopy hygiene principles and importance of CBS management.<br>• Grower field walk (March 2024): Demonstration of copper phytotoxicity issues and discussion of integrated CBS/EBS disease management under commercial orchard conditions. |

## Outcomes

### Table 2. Outcome summary

| Outcome | Alignment to fund outcome, strategy and KPI | Description (how outcome was realised by industry / relevance to Fund level) | Evidence |
|---|---|---|---|
| Evidence of a viable late-season compound for the control of EBS in Australian mandarin (NUL3446). Data includes initial rate responses, spray frequency effects and compatibility with mancozeb under late-season EBS pressure. | SIP Outcome 1 Develop and optimise a whole-systems approach to IPDM - Adoption of whole-systems IPDM strategies that reduce crop losses and enable sustainable management of pests and diseases | Two field trials clarified that NUL3446 provides suppression of late season infection. This evidence is available to all relevant stakeholders (growers, agronomists, Citrus Australia, Hort Innovation, Nufarm) to consider and make decisions on next steps regarding potential approved uses of NUL3446 in the context of late-season EBS management programs. | Appendix A (FT1–FT2), incidence/severity datasets, inoculation assay outputs, treatment structures and statistical analyses. |
| Evaluation of new IDM-appropriate fungicides (NUL3446, Luna® Experience, Merivon® and protectants) completed and evidence of effectiveness for CBS and EBS control under commercial subtropical conditions achieved. | SIP Outcome 1 - as above | Two seasons of replicated field data describing performance, fruit-safety and limited postharvest residue information (experimental, exploratory only) of new fungicides in mandarins. This provides growers, consultants and regulators with locally generated evidence to guide spray-program decisions and inform ongoing research. | Appendix A (FT3-FT4) trial reports, incidence/severity datasets, residue summaries, disease assessments, phytotoxicity observations, statistical outputs. |
| Field-based evidence regarding how copper formulation, spray-tank pH and seasonal timing influence rind phytotoxicity and disease (CBS) in sensitive mandarin cultivars | SIP Outcome 1 - as above | Three field trials conducted over three seasons investigated when copper phytotoxicity is more likely to occur and identified conditions where injury was lower. This reduces uncertainty for growers who depend on copper while managing residue and | Appendix B (FT1–FT3), phytotoxicity datasets, pH behaviour data, incidence/severity tables, associated figures. |

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| | | fruit quality requirements | |
| Increased grower awareness of copper fungicide use in citrus and associated crop safety risks. | SIP Outcome 1 - as above | A desktop review of copper phytotoxicity in citrus and associated condensed article prepared. | Submitted to Hort Innovation. |
| Field evidence describing how copper spray timing and interval length influence CBS incidence and severity under contrasting canopy inoculum and seasonal pressure. | SIP Outcome 1 - as above | Two field trials in 'Imperial' mandarin showed the timing and interval length of copper applications does affect the level of CBS seen in mature fruit but the success of these fungicide applications is heavily reliant on the amount of available canopy inoculum in combination with the continuity of coverage. Fortnightly programs (early season) were demonstrated to be the most effective relative to reduced frequency of coverage but that they also cannot entirely overcome the effect of elevated inoculum. This reinforces the need for industry adoption of canopy hygiene in addition to leaf litter management for ongoing fungicide efficacy. | Appendix C (FT1–FT2), pre- and post-incubation incidence and severity datasets, treatment structures, rainfall-triggered schedules, and statistical analyses. |
| Evidence describing how early-season and petal-fall fungicide programs influence EBS outcomes under low and high seasonal pressure in subtropical mandarins. | SIP Outcome 1 - as above | Three trials across 'Daisy' and 'Murcott' mandarins showed that limited or extended pre-petal fall fungicide applications provided little or no suppression of EBS. Across all seasons, petal-fall programs, even when applied in conjunction with grower-applied | Appendix C (FT3, FT4a/b), EBS incidence and severity datasets, composite analyses, treatment structures and statistical outputs. |

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| | | sprays during the fruit season, did not contribute to overall disease control. These findings provide growers and consultants with evidence regarding the lack of efficacy of pre-petal-fall applications to inform fungicide scheduling recommendations. | |
| Evidence derived from multiple replicated trials demonstrating that canopy-retained dead wood/pruning debris contributes significantly to CBS incidence under subtropical conditions. | SIP Outcome 1 - as above | Detailed assessments confirmed that multiple forms of canopy dead wood can act as inoculum sources and strongly influences the effectiveness of fungicide regimes in the control of CBS. These findings also clarify that canopy-borne inoculum operates alongside orchard-floor CBS sources and that maintaining cleaner canopies provides better support within integrated CBS management programs. | Appendix D (FT1, FT3), pre- and post-incubation incidence/severity datasets, treatment structures multi-site demonstration results and statistical outputs. |
| Evidence derived from multiple demonstration-style field trials describing how simple canopy hygiene practices influence early-season symptom expression under commercial subtropical conditions. | SIP Outcome 1 - as above | Three studies simulating commercial removal of dead wood showed that retained dead wood increased pre-incubation CBS incidence relative to clean-canopies, and, although the magnitude of the effect varied across sites, seasons and debris types, at one site the result was an exceptional decrease in disease recorded in harvested fruit. Confirmation canopy debris harbours significant amounts of inoculum and influences fruit quality. | Appendix D (FT2), pre- and post-incubation incidence/severity datasets, treatment structures multi-site demonstration results and statistical outputs. |

## Monitoring and evaluation

### Table 3. Key Evaluation Questions

| Key Evaluation Question | Project Performance | Continuous Improvement Opportunities |
|---|---|---|
| 1. To what extent has the project achieved its expected outcomes? | CT20009 generated the first coordinated, multi-season field evidence linking canopy hygiene, copper timing, fungicide timing and early-season inputs to CBS and EBS expression in 'Imperial', 'Murcott' and 'Daisy' mandarins. Results confirmed that canopy inoculum load, spray-interval continuity and seasonal pressure influence disease outcomes more strongly than chemistry choice alone. These findings address the project's expected outcome of improving knowledge of effective IDM components for CBS/EBS. | Future work could focus on repeating these studies under a wider range of seasonal pressures to confirm how consistent the observed patterns are across years. Testing canopy-hygiene approaches in blocks with different pruning systems and tree ages would also help determine where dead wood removal has the most practical benefit. Additional trials that compare early-, mid- and late-season fungicide programs in years with unusually high disease pressure would improve confidence in timing recommendations. As new chemistries become available, simple field screening methods that align with commercial spray practices would help industry evaluate how they fit within existing IDM programs. Continued work in these areas would help maintain a practical evidence base for growers and consultant |
| 2. How relevant was the project to the needs of intended beneficiaries? | The project directly addressed grower, consultant and nursery questions relating to copper phytotoxicity, the role of canopy dead wood, the value of early sprays and reduced access to mancozeb. Regular engagement through field walks, advisory meetings and industry forums ensured that the work remained closely aligned with levy-payer priorities. Feedback indicated strong relevance, particularly for guidance on canopy hygiene and copper use. | Future work could include small demonstration blocks in more production regions to see how CBS and EBS behave under different orchard conditions. Continued coordination with Citrus Australia and interstate colleagues will support clear communication while recognising that disease pressure varies between districts. |
| 3. How well have intended beneficiaries been engaged in the project? | Engagement was frequent and interactive. Trial sites supported in-field demonstrations, and findings were presented at grower meetings, regional | Short, theme-specific extension modules (e.g. "Tree canopy hygiene in 5 minutes", "Safe copper use – Don't burn your fruits", "When early sprays matter for citrus diseases") |

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| | forums, national congresses and scientific events. One-on-one discussions with consultants provided additional technical clarity. Grower and consultant participation was strongest for canopy-hygiene and copper-safety themes, reflecting high practical interest. | would improve accessibility of key messages. |
| 4. To what extent were engagement processes appropriate to the learning styles and constraints of the citrus industry? | Engagement was delivered predominantly through regional forums, on-farm visits, field walks and direct discussions, aligning well with grower preferences for practical and visual learning. Several growers revisited trial blocks multiple times, indicating strong engagement. Digital summaries were provided where appropriate, improving access for those unable to attend in person. | Additional formats such as short videos, step-by-step pictorial summaries and short technical podcasts (e.g. Spotify – Citrus Australia) could support broader accessibility, particularly for time-constrained growers and consultants.<br>  <br>(Image credit: K. Bransgrove) |
| 5. To what extent did the project make effective use of resources and strengthen longer-term capability? | The project was delivered efficiently through coordinated fieldwork across Bundaberg and Brisbane, shared equipment, and use of existing commercial orchards rather than new plantings. During the project period, regional diagnostic and field-pathology capability was re-established in Bundaberg, supporting timely trial management and industry engagement. The project also produced datasets that will support future modelling, breeding evaluations and IDM research. | Ongoing coordination with the Australian Citrus Breeding Program (CT21001) and related Hort Innovation projects would strengthen efficiency by sharing field sites, pathology methods and diagnostic capacity. Continued involvement in national committees and breeding program activities will help maintain alignment across research, screening and disease-management work. |

## Recommendations

1. Refine IDM and export market compatible fungicides and programs under different seasons and canopy conditions, including ongoing exploration of novel, soft chemistries.

- Scientific, replicated field trials conducted in multiple years with differing disease infection pressures, varieties and pruning systems would elevate understanding of spray timing, copper safety, and placement of new chemistries for production of blemish-free fruit. This would build directly on methods established in and outcomes derived from this project.

2. Quantify canopy-based inoculum production and dissemination biology to support CBS risk prediction and grower decision-making.

- Studies to elucidate the canopy component of the CBS life-cycle including aspects such as timing, duration, spore dissemination. This knowledge would immediately aid industry to target fungicide applications to mirror periods of critical risk. It could generate industry-adoptable protocols such as simple spore-release monitoring (air-sampling, humid traps, twig traps, canopy microclimate sensors) that would help identify when CBS spores are most active within the tree canopy to support fungicide application decision pathways. This knowledge would further underpin refinement of modelling systems as per below.

3. Develop practical, region-ready disease-risk models that growers and consultants can use.

- Validating Citrus Research Institute (CRI, South Africa) models against local rainfall and humidity patterns and refining thresholds for Australian mandarin blocks will support more accurate spray planning, reduce ill-timed fungicide application and increase certainty of production of premium-grade fruit with lower fungicide inputs.

4. Strengthen the molecular and breeding link by improving CBS resistance screening in mandarins.

- A PhD-led program can develop rapid inoculation/symptom-expression assays and integrate pangenomic and transcriptomic markers to help the national breeding program identify resistant lines.

5. Maintain funding for regional citrus pathology capacity in Queensland to efficiently support progress in subtropical and tropical citrus disease management to deliver continuous improvements in citrus quality and productivity, whilst mitigating pathology risks.

## Referred scientific publications

1. Thangavel, T., Smith, M., Miles, A., Gallagher, B., Wallis, M., Cooke, T., Martin, E., Peressini, T., Reid, M., Bransgrove, K., & Coates, L. (in press). Tree hygiene for effective management of CBS in Australian mandarin orchards. *Acta Horticulturae*.

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## Intellectual property

None to Report

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# Appendices

## Appendix A

### Theme 1: Evaluation of new IDM appropriate chemistries

**Background**

Fungicide inputs remain a critical component of CBS and EBS management in subtropical mandarins and usage becomes increasingly constrained as fruit approach maturity. Many registered fungicides carry withholding periods of 14–28 days. This limits their suitability during the weeks before harvest when disease-sensitive cultivars such as 'Murcott' and 'Daisy' remain highly vulnerable to late infection by EBS. Compliance with maximum residue limits further narrows this window, and, although copper has a short withholding period and can technically be applied closer to harvest, the risk of rind injury in warm, humid conditions restricts its use in late-maturing cultivars. These combined constraints create a period in which fruit are vulnerable and fungicide options are limited, reinforcing the need for chemistries that provide effective suppression while meeting residue, withholding and crop-safety requirements.

At project commencement, two classes of fungicides were considered to have potential to address different components of this management gap. The biofungicide NUL3446 represented a low-residue option that could potentially be applied late in the season to suppress late infection by *Alternaria* when mancozeb cannot be used and copper usage carries phytotoxicity risk. Despite international evidence of efficacy against *Alternaria* spp., there were no field data from Australian mandarin production systems on efficacy, rate responses, program intensity, compatibility with protectants or the extent to which surface residues influence infection potential at harvest.

In contrast, SDHI–strobilurin co-formulations (Luna® Experience, Merivon®) were identified as early- to mid-season candidates that may provide persistent protection during periods of high CBS and EBS activity. Their potential value lies in their capacity to minimise disease infection early in the season, strengthen multi-site-based fungicide programs and align with resistance-management requirements. However, no independent information was available on their performance, fruit residue levels under subtropical field conditions, crop safety or fit within integrated seasonal programs under subtropical field conditions. Furthermore, the interaction between systemic fungicides and canopy inoculum dynamics, including the influence of retained dead wood, had not been investigated in mandarins.

To resolve these gaps, four field trials conducted between 2022 and 2025 evaluated the field performance and program fit of these IDM-appropriate chemistries across susceptible mandarin cultivars. Field Trials 1 and 2 assessed the efficacy of NUL3446 during the late-season infection window, focusing on rate, frequency, efficacy alone and in alternation with mancozeb and the resulting postharvest disease suppression. Field Trial 3 examined SDHI–based co-formulations across a full season under natural CBS/EBS pressure, incorporating canopy hygiene and residue analysis to understand how they may be best integrated into an existing program. Field Trial 4 extended this evaluation to compare these SDHI compounds with copper-only, copper–mancozeb and SDHI–copper combinations NUL3446 was also included in trials in Appendix C. This theme provides field-based evidence describing how low-residue and systemic fungicide options can be positioned within mandarin disease-management programs to maintain efficacy while meeting practical constraints imposed by withholding periods, residue compliance and crop-safety considerations.

## Field Trial 1 & 2 (FT1 &FT2): Late-season performance of NUL3446 (low-residue chemistry) against EBS in 'Murcott' and 'Daisy' mandarins (2022)

### Methodology

*Glasshouse evaluation of NUL3446 treatments*

Two glasshouse trials were conducted to test the efficacy of biofungicide NUL3446 to control infection of citrus leaves by *Alternaria* sp.

The first trial was undertaken at Bundaberg Research Station. Fungicides (NUL3446 and Captan) were applied to 'Murcott' mandarin seedlings (each with 2-3 new shoots) as a spray until runoff and left to dry for 24 hours. Plants were then inoculated with a conidial suspension ($10^5$ spores/mL) of *Alternaria alternata* (isolate AKM452) and incubated at 25°C and 80% humidity in the dark. Seven days after inoculation, the number of symptomatic leaves on each plant was recorded.

In the second trial, which was undertaken in a glasshouse at the Ecosciences Precinct (Brisbane), the apical shoots of young 'Murcott' hybrid mandarin trees were cut back 3 weeks prior to treatment application to stimulate a new growth flush. Plants were then treated with NUL3446 as a spray until runoff and left to dry. Forty-eight hours after treatment plants were inoculated with a conidial suspension of *Alternaria alternata* (isolate AKM452 at $10^4$ spores/mL). Inoculated plants were covered in a pre-moistened plastic bag and incubated for 24 h in the glasshouse. The bags were then removed, and plants assessed for disease symptoms (total no. of spots/symptomatic leaves/defoliated shoots per plant) at 5 days after inoculation.

Treatment details for each trial are shown in Tables B1.2 and 1.3.

*Field evaluation of NUL3446 treatments*

Two field experiments were conducted in the late-season period of the 2021/2022 growing season to evaluate the performance of NUL3446 for the late-season management of EBS in two cultivars of mandarin. The trials were established in commercial orchards in Mundubbera, Queensland in separate blocks of 'Daisy' (IRM-1) (FT1) ("888 Citrus") and 'Murcott' (FT2) ("Favco Farms") mandarins, both varieties recognised as highly susceptible to EBS. The orchards were managed according to standard commercial practices by each grower collaborator until the trials commenced in late April (autumn) when *Alternaria* pressure is typically high.

In both trials, eleven fungicide programs were evaluated to examine: 1. the effects of NUL3446 application rates (0.2, 0.4, or 0.8 g $L^{-1}$); 2. spray program intensity (three applications at 20-day intervals versus five applications at 10-day intervals); 3. compatibility with and effect of alternating with Dithane (mancozeb) programs; and 4. the impact of including a non-ionic surfactant (manufacturer recommendation) (Table A1.1). A water control and a Dithane benchmark were included. As NUL3446 is not registered for use in citrus, usage and selection of application rates and frequencies done with guidance and permission from the manufacturer.

All sprays were applied to the foliage to the point of run-off using a calibrated motorised sprayer fitted with a 2.0 mm hollow-cone nozzle, delivering approximately 15 L of spray mixture per tree (equivalent to ~5000 L $ha^{-1}$). Applications were made between 27 April and 25 June 2022 during stable weather periods, with no rainfall forecast for at least six hours after treatment to minimise loss of spray deposits due to rainfall. A randomised complete block design with five blocks and five single-tree replicates per treatment was used for each trial.

Disease assessments were undertaken at fruit maturity immediately prior to (in-field) and after

harvest to quantify the incidence and severity of EBS. The in-field assessments included leaves and fruit while the postharvest assessment was of fruit only. For FT1, the in-field assessment included immature fruit from a late flush while the postharvest assessment included mature fruit only.

In-field disease assessments were completed by assessing each tree replicate using a 0-10 severity scale (Appendix E). Severity scores were recorded separately for leaves, mature fruit and young fruit and a mean severity value was generated per tree.

For FT1 and FT2, eighteen mature fruit were collected per tree, transported to the laboratory and assessed immediately. Each fruit was inspected for typical EBS lesions, and both incidence (proportion of symptomatic fruit) and severity (mean lesions per fruit) were recorded as per Appendix E.

For FT2 (Murcott), the in-field assessment showed negligible symptoms of EBS in the entire trial and no differences between treatments could be discerned. The harvested fruit also showed almost no incidence of EBS (data not presented).

Considering this, for FT2 only, the collected fruit were utilised to investigate whether NUL3446 residues remaining on the fruit surfaces at harvest were able to prevent new infections of *Alternaria alternata* in mature fruit. A postharvest inoculation assay (equatorial strip method) (Miles et al, 2019) was used to achieve this. Five fruit per replicate (25 fruit per treatment) were left unwashed so the spray residue was intact and were inoculated using paper strips saturated with a conidial suspension of *Alternaria alternata* (isolate AKM 452; Drenth, 2014) (~1–2 × $10^4$ conidia $mL^{-1}$). The paper strips remained on the fruit for 7 days and incubated at 27 °C in complete darkness in 70-80% high humidity. After 7 days the paper strips were removed, and the resulting lesions were scored on a 0–5 ordinal scale (Table A1.5).

At harvest, leaves from trees in both FT1 and FT2 were collected to investigate the effect of NUL3446 application on the presence of endophytic *Alternaria* in the foliage. Ten leaves were collected from single-tree replicates (50 leaves per treatment), and, from each leaf, a single 10 mm disc was excised from asymptomatic leaf tissue adjacent to the midrib using a sterile cork borer. Leaf discs were surface-sterilised in 70% ethanol for 5 min, air-dried in a laminar flow cabinet, and placed onto half-strength potato dextrose agar (½ PDA) in 90 mm Petri dishes, 10 discs per plate, each plate representing a single tree replicate. Plates were sealed and incubated at ambient laboratory temperature (approximately 25 °C) for seven days under natural light. Following incubation, emerging fungal colonies were examined and identified to genus using colony and conidial morphology. The incidence of *Alternaria* was expressed as the proportion of plated discs yielding *Alternaria* colonies relative to the total number of discs per tree (Table A1.6).

Disease incidence (FT1, Daisy) was analysed with a HGLM with a fixed effect (treatment) assumed to follow a binomial distribution with a logit link and a random term (block) assumed to follow a beta distribution with a logit link. The HGLM did not converge and the variance components of the GLMM fitted next (binomial distribution, logit link function applied, terms as above) were bound so a GLM was fitted instead (terms as above). Predicted means are presented as proportions. Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Table A1.1**: Treatments evaluated in Field Trial 1 ('Daisy' mandarin) and Field Trial 2 ('Murcott' mandarin) for late-season management of EBS, including product, spray frequency, application dates, and application rate.

| Treatment | Product / Program | Spray Frequency & Number | Application Dates | Rate (g or mL per L) |
|---|---|---|---|---|
| A | Water Control | Every 20 days (3 sprays) | 27 Apr; 16 May; 5 Jun | – |
| B | Dithane (mancozeb) | Every 20 days (3 sprays) | 27 Apr; 16 May; 5 Jun | 2.0 g/L |
| C | NUL3446 0.5× | Every 20 days (3 sprays) | 27 Apr; 16 May; 5 Jun | 0.2 g/L |
| D | NUL3446 1× | Every 20 days (3 sprays) | 27 Apr; 16 May; 5 Jun | 0.4 g/L |
| E | NUL3446 2× | Every 20 days (3 sprays) | 27 Apr; 16 May; 5 Jun | 0.8 g/L |
| F | NUL3446 0.5× | Every 10 days (5 sprays) | 27 Apr; 6, 16, 26 May; 5 Jun | 0.2 g/L |
| G | NUL3446 1× | Every 10 days (5 sprays) | 27 Apr; 6, 16, 26 May; 5 Jun | 0.4 g/L |
| H | NUL3446 2× | Every 10 days (5 sprays) | 27 Apr; 6, 16, 26 May; 5 Jun | 0.8 g/L |
| I | Dithane (mancozeb) | Every 10 days (5 sprays) | 27 Apr; 6, 16, 26 May; 5 Jun | 2.0 g/L |
| J | NUL3446 1× alternated with Dithane | Every 10 days (5 sprays) | 27 Apr; 6, 16, 26 May; 5 Jun | 0.4 g/L (3 sprays) + 2.0 g/L (2 sprays) |
| K | NUL3446 1× + non-ionic surfactant | Every 10 days (5 sprays) | 27 Apr; 6, 16, 26 May; 5 Jun | 0.4 g/L + 0.5 mL/L |

**Results and Discussion**

Results from the field trial conducted on 'Daisy' (FT1) mandarin and the postharvest experiment conducted on 'Murcott' (IRM-1) (FT2) mandarin demonstrated late-season in-field application of combinations of NUL3446 treatments can reduce the incidence and severity of EBS in mature fruit and symptom expression of new infections postharvest respectively (Figure/Tables B1.1-1.6). Ongoing trialling of NUL3446 as a late-season fungicide option for control of EBS is recommended.

*Glasshouse trials*

In the first trial (Bundaberg), all treatments significantly reduced the number of infected leaves per plant compared to the control (Table A1.2). There was no difference between NUL3446 with or without addition of surfactant in this trial. While not statistically significant, plants treated with Captan had the lowest actual values for *Alternaria* infection.

While there was a lower overall disease incidence in the second trial (Brisbane), most likely due to the lower inoculum concentration used, plants treated with NUL3446 + surfactant consistently had the lowest values for number of spots, symptomatic leaves and defoliated shoots per plant (Table A1.3). However, these reductions were not statistically significant, possibly due to the low number of replications (n=3).

*Field trial (FT1, 'Daisy')*

Results from this field trial are encouraging with respect to further investigation of use of late-season NUL3446 applications for control of EBS.

All treatments, except NUL3446 0.5x (3 sprays) and Dithane (3 or 5 sprays), significantly reduced in-field visual disease scores compared to the water control (Figure A1.1). In general, NUL3446 treatments with 5 sprays had lower disease scores than those with 3 sprays, although these differences were not statistically different.

For the pre-incubation postharvest disease assessment, the incidence of EBS was very high in this trial, with an average of 91% of fruit affected in the 'water only' control (Table A1.4). Fruit treated with NUL3446 1x + surfactant or NUL3446 1x alternated with Dithane gave the greatest reductions in disease incidence compared to the control, with 40 and 34% disease incidence, respectively. Dithane (3 sprays) and NUL3446 2x (3 sprays) were the only other treatments to significantly reduce disease incidence compared to the control. Unlike Dithane (3 sprays), Dithane (5 sprays) surprisingly did not reduce disease incidence compared to the control. All NUL3446 treatments gave equivalent (or greater) reduction of disease incidence compared to Dithane treatments irrespective of spray frequency (3 sprays or 5 sprays).

All treatments reduced disease severity compared with the control, with the exception of NUL3446 (3 sprays) at the low rates (0.5x and 1x) and 5 sprays of NUL3446 2x (no surfactant) or Dithane (Table A1.4). Fruit treated with NUL3446 1x alternated with Dithane had the lowest value for disease severity overall, with an average of only 1.74 lesions per fruit, although this was not statistically different to Dithane (3 sprays) or NUL3446 1x + surfactant (5 sprays) which had 3.73 and 4.30 lesions per fruit, respectively.

Increased spray frequency with shorter intervals (5 applications at 10-day intervals compared with 3 applications at 20-day intervals) did not significantly improve disease incidence control (Table A1.4). Unfortunately, it was not possible to include a corresponding 3-spray treatment for the 1x NUL3446 + Dithane and 1x NUL3446 + surfactant treatments due to tree number constraints. However, the addition of surfactant to the NUL3446 5-spray treatment did significantly reduce disease incidence

compared to the NUL3446 5-spray treatment *without* surfactant, effectively halving disease incidence, indicating that the surfactant is an important component of the treatment.

*Inoculation assay (FT2, 'Murcott')*

In the 'Murcott' field trial (FT2), almost no disease (due to low natural field infection) was detected in any treatment. As a result, the fruit were deemed suitable for use in a postharvest inoculation experiment to evaluate the effect of field fungicide residues on postharvest infection by EBS, since there was very low risk of latent symptom expression interfering with symptoms which developed as a result of the equatorial inoculations.

When considering the proportion of fruit that developed symptoms (versus no symptoms) after inoculation, the assay showed few significant differences between treatments (Table A1.5). An exception was the NUL3446 + Dithane (5-spray) treatments having more symptomless fruit following inoculation than all treatments except NUL3446 + surfactant (5-spray) and Dithane alone (3-spray). In both the in-field control and NUL3446 0.5x (5-spray) treatments, every piece of inoculated fruit developed symptoms compared with the other treatments where there was always a proportion of fruit that did not develop symptoms.

When the severity of symptoms was considered, again few significant differences were found between treatments (Table A1.5). Symptoms were significantly less severe in fruit in all treatments compared with those in the in-field control treatment except for the NUL3446 0.5x (5-spray) treatment. The NUL3446 0.5x (5-spray) results were not different from the NUL3446 0.5x (3-spray), NUL3446 1x (5-spray) or NUL3446 2x (5-spray) treatments. While some trends were evident among the different treatments, e.g. fewer symptoms in Dithane and/or NUL3446 + Dithane treated fruit, these results were not significant.

Overall, the inoculation assay indicates in-field application of NUL3446 does not affect the ability of *Alternaria* to infect mature fruit unless a surfactant is added or the treatment is alternated with Dithane. However, most NUL3446 treatments, with or without surfactant, did impact the severity of those symptoms when they developed when compared with no late season in-field treatment at all. Of note, most of the NUL3446 treatments were as effective as the Dithane treatments in this context in terms of infection and symptom development.

*Effect of NUL3446 on endophytic* Alternaria *in leaves*

The results of isolations from asymptomatic leaf tissue also differed between field trials. In FT1 (Daisy), there was no impact of any of the treatments on the number of leaves from which *Alternaria* was isolated ($p = 0.1$, data not presented). The treatments did affect the outcome in FT2 (Murcott), however, with all treatments except NUL3446 0.5x (3-spray) and NUL3446 1x (3-spray) significantly reducing the number of leaf discs from which *Alternaria* was isolated when compared with the water-only control (Table A1.6).

While we would recommend a similar experiment being conducted to investigate the scope to reduce inoculum in the wood/twig component of the canopy, these results indicate application of NUL3446 or Dithane throughout the latter part of the growing season reduces the population of endophytic *Alternaria* in the foliage in comparison with the control treatment (water alone) and has positive implications for effective inoculum management.

*Summary and recommendations*

These results show that the biofungicide NUL3446 can provide effective late-season protection against EBS and is well worth ongoing investigation. This would address a critical gap for mid- and late-

maturing mandarins in subtropical Queensland where fewer fungicide products are eligible for use due to withholding period or phytotoxicity limitations.

The performance of the 1X rate (label rate) suggests that this rate should be the focus of further investigation. The consistent results of the NUL3446 1x + surfactant is encouraging and needs further investigation, including scientifically designed trials to understand the role of the surfactant in disease control.

The artificial inoculation results highlight in-field treatment with NUL3446 does not necessarily suppress new infections by *Alternaria* after harvest, but should infection occur, can limit the severity of expressed symptoms.

While positive, and supportive of inclusion of NUL3446 in late-season fungicide programs for EBS-susceptible mandarin cultivars, further trials are necessary to validate results in multiple seasons and in years or orchards where high disease pressure is present.

**Table A1.2**: Glasshouse trial 1 (Bundaberg) - EBS symptom development in 'Murcott' mandarin seedlings inoculated with *Alternaria alternata* ($10^5$ spores/mL) 24h after fungicide application. Disease assessments conducted at 7 days after inoculation. n=9.

| Treatment | Mean number of symptomatic leaves/plant |
|---|---|
| Water control | 6.2 a[1] |
| Captan 1.8 g/L | 0.4 b |
| NUL3446 0.4 g/L | 2.4 b |
| NUL3446 0.4 g/L + Surfactant 0.5 mL/L | 2.2 b |

[1] Means followed by the same letter are not significantly different at p≤0.05.

**Table A1.3**: Glasshouse trial 2 (Brisbane) - EBS symptom development in young hybrid 'Murcott' mandarin plants inoculated on 3-week-old flush with *Alternaria alternata* ($10^4$ spores/mL) 48 h after treatment application. Disease assessments conducted at 5 days after inoculation. n=3.

| Treatment | Mean no. of spots on plant (leaf and stem) | Mean no. of symptomatic leaves/plant | Mean no. of defoliated shoots/plant |
|---|---|---|---|
| Untreated control | 4.3 | 2.0 | 2.7 |
| Water control | 3.3 | 1.7 | 2.0 |
| Surfactant 0.5 mL/L | 7.3 | 2.0 | 2.7 |
| NUL3446 0.4 g/L + Surfactant 0.5 mL/L | 0.3 | 0.3 | 0.7 |

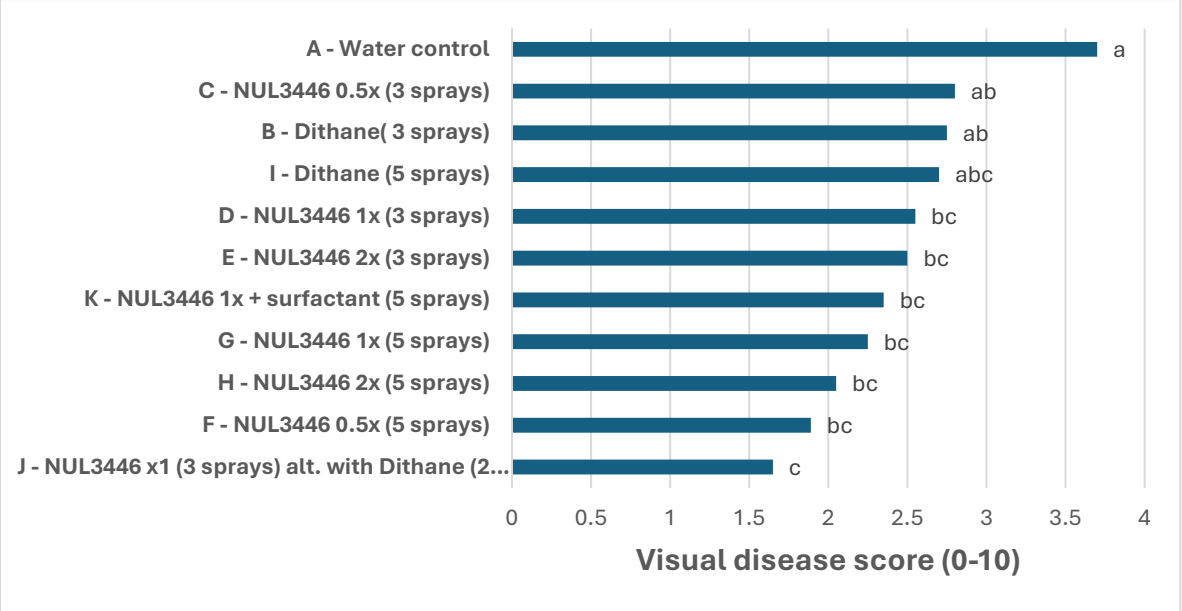


**Figure A1.1**: In-field visual disease scores (0–10 scale)[1] for EBS averaged across all trees and assessors for each treatment (FT1). Treatments surmounted by the same letter are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

[1]0=10 scale where 0 indicated no lesions and 10 indicated more than 100 lesions per fruit (0 = no lesions; 1 = 1 lesion; 2 = 2–3 lesions; 3 = 4–6 lesions; 4 = 7–9 lesions; 5 = ≥10 lesions; 6 = ≥15 lesions; 7 = ≥20 lesions; 8 = ≥30 lesions; 9 = >50 lesions; 10 = >100 lesions per fruit).

**Table A1.4**: Mean proportion of fruits with EBS lesions and mean number of EBS lesions per fruit following field treatment applications on 'Daisy' mandarin (FT1). Values are raw means (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment | No. of sprays | Disease incidence (proportion) | Disease severity (no. of lesions/fruit) |
|---|---|---|---|
| A. Water control | 3 | 0.91 (-0.09 ± 0.05) a | 6.68 (2.18 ± 0.24) a |
| B. Dithane | 3 | 0.64 (-0.37 ± 0.11) bc | 3.73 (1.27 ± 0.27) bc |
| C. NUL3446 0.5x | 3 | 0.85 (-0.15 ± 0.07) ab | 3.92 (1.53 ± 0.24) ab |
| D. NUL3446 1x | 3 | 0.77 (-0.23 ± 0.09) ab | 3.65 (1.54 ± 0.24) ab |
| E. NUL3446 2x | 3 | 0.67 (-0.33 ± 0.1) bc | 4.21 (1.49 ± 0.24) b |
| F. NUL3446 0.5x | 5 | 0.79 (-0.21 ± 0.08) ab | 3.46 (1.48 ± 0.24) b |
| G. NUL3446 1x | 5 | 0.76 (-0.24 ± 0.08) ab | 3.74 (1.5 ± 0.24) b |
| H. NUL3446 2x | 5 | 0.74 (-0.26 ± 0.08) ab | 4.31 (1.58 ± 0.24) ab |
| I. Dithane | 5 | 0.74 (-0.26 ± 0.08) ab | 4.24 (1.53 ± 0.24) ab |
| J. NUL3446 1x + Dithane * | 3+2 | 0.34 (-0.66 ± 0.1) d | 1.74 (0.7 ± 0.24) c |
| K. NUL3446 1x/surfactant | 5 | 0.4 (-0.59 ± 0.09) cd | 4.30 (1.24 ± 0.24) bc |
| LSD (predicted means) | | 0.24 | 0.68 |
| P value | | 0.002 | 0.04 |

* Alternating applications of NUL3446 and Dithane

**Table A1.5**: Mean incidence (proportion of fruit with disease) and mean lesion severity (0–5†) on 'Murcott' mandarin fruit (FT2) after equatorial-strip inoculation with *Alternaria alternata*. Values are

raw means (predicted means ± standard errors). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment | No. of sprays | Incidence of fruit with disease | Disease severity (0-5) † |
|---|---|---|---|
| A. Water control | 3 | 1.0 (0.00 ± 0.001) a | 4.46 (4.47 ± 0.45) a |
| B. Dithane | 3 | 0.8 (0.20 ± 0.1) bc | 1.9 (1.88 ± 0.45) cd |
| C. NUL3446 0.5x | 3 | 0.96 (0.04 ± 0.05) ab | 2.5 (2.52 ± 0.45) bcd |
| D. NUL3446 1x | 3 | 0.92 (0.08 ± 0.07) ab | 2.2 (2.20 ± 0.45) cd |
| E. NUL3446 2x | 3 | 0.84 (0.16 ± 0.09) abc | 1.8 (1.80 ± 0.45) cd |
| F. NUL3446 0.5x | 5 | 1 (0.00 ± 0.001) a | 3.7(3.68 ± 0.45) ab |
| G. NUL3446 1x | 5 | 0.92(0.08 ± 0.07) ab | 2.5 (2.52 ± 0.45) bcd |
| H. NUL3446 2x | 5 | 0.92 (0.08 ± 0.07) ab | 2.5 (2.52 ± 0.45) bcd |
| I. Dithane | 5 | 0.96 (0.04 ± 0.05) ab | 1.8 (1.84 ± 0.45) cd |
| J. NUL3446 1x + Dithane * | 3+2 | 0.6 (0.40 ± 0.12) c | 1.3 (1.32 ± 0.45) d |
| K. NUL3446 1x/surfactant | 5 | 0.8 (0.20 ± 0.1) bc | 2.7 (2.68 ± 0.45) bc |
| LSD (predicted means) | | 0.2 | 1.3 |
| P value | | 0.03 | <0.001 |

* Alternating applications of NUL3446 and Dithane.

† Disease severity was assessed on a 0–5 ordinal scale based on the extent of lesion development on the fruit rind at and beyond the equatorial inoculation strip. A score of 0 indicated no visible symptoms on the rind; 1 indicated very limited lesion development confined to the equatorial strip; 2 indicated discrete lesions clearly visible along the equatorial strip with minimal extension into surrounding rind tissue; 3 indicated moderate lesion development with partial coalescence of lesions along the equatorial strip; 4 indicated extensive lesion development covering most of the equatorial strip and extending into adjacent rind tissue; and 5 indicated severe disease with continuous necrosis along the equatorial strip and pronounced spread into surrounding rind tissue.

**Figure A1.2**: L and R) Mandarin with equatorial symptoms after inoculation with *Alternaria*. (Image credits: T. Thangavel)

**Table A1.6**: Mean proportion of leaves yielding *Alternaria* spp. after incubation on half-strength potato dextrose agar. Values are back transformed treatment means (predicted means ± standard errors). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment | No. of sprays | Mean proportion of leaves yielding *Alternaria* spp. |
|---|---|---|
| A. Water control | 3 | 0.79 (1.29 ± 0.44) a |
| B. Dithane | 3 | 0.26 (-1.07 ± 0.42) de |
| C. NUL3446 0.5x | 3 | 0.64 (0.57 ± 0.43) ab |
| D. NUL3446 1x | 3 | 0.59 (0.36 ± 0.4) abc |
| E. NUL3446 2x | 3 | 0.36 (-0.59 ± 0.4) cde |
| F. NUL3446 0.5x | 5 | 0.46 (-0.16 ± 0.38) bcd |
| G. NUL3446 1x | 5 | 0.3 (-0.87 ± 0.41) de |
| H. NUL3446 2x | 5 | 0.32 (-0.77 ± 0.4) de |
| I. Dithane | 5 | 0.18 (-1.55 ± 0.47) e |
| J. NUL3446 1x + Dithane * | 3+2 | 0.32 (-0.77 ± 0.4) de |
| K. NUL3446 1x/surfactant | 5 | 0.44 (-0.25 ± 0.39) bcd |
| LSD (predicted means) | | 1.06 |
| P value | | <0.001 |

## Field Trial 3 (FT3): Evaluation of new crop protection products for CBS and EBS management in 'Murcott' Mandarin (IRM-1), Wallaville, 2022–2023

### Methodology

Field Trial 3 (FT3) was conducted during the 2022–2023 growing season in a commercial 'Murcott' (IRM-1) mandarin block at Citrus Abbotsleigh Farm, Wallaville, Queensland.

The primary aim of the experiment was to evaluate the newly registered crop protection products, Luna® Experience and Merivon®, for the management of EBS and CBS when applied according to their registered use pattern and in rotation with the industry standard fungicide, Dithane (mancozeb). Luna® Experience contains the active ingredients fluopyram and tebuconazole, which are Group 7 (SDHI[1]) and Group 3 (DMI[2]) fungicides, respectively. Merivon®[3] contains the active ingredients fluxapyroxad and pyraclostrobin, which are Group 7 (SDHI) and Group 11 (QoI[4]/Strobilurin) fungicides, respectively. Treatments relevant to this component of the experiment were 5-7 (Table A2.1).

A secondary aim of the experiment was to evaluate the influence of canopy hygiene on EBS and CBS disease incidence and severity. The trial block had a documented history of CBS/EBS and was located adjacent to mature trees retaining naturally infected dead wood, providing a realistic background inoculum environment. Dead wood from these mature trees was added to selected trees in the trial to increase fungal inoculum levels within the canopy. Treatments relevant to this component of the trial were 1-4 (Table A2.1).

All treatment application details are outlined in Table A2.1 and described below.

Treatments 5-7 (use pattern applications as part of overall disease control program): One application of Luna® Experience or three applications of Merivon® were made in accordance with the respective product labels. Merivon® was applied at intervals of 7-14 days as directed, with no more than two consecutive applications made. Luna® Experience and Merivon® were applied early in fruit development and were alternated with industry standard Dithane (mancozeb) sprays. For comparison purposes, separate industry standard fungicide programs alternating two Amistar® (azoxystrobin) sprays with Dithane, or just Dithane alone, were also included in the trial. Spray applications were made at approximately four-week intervals from November 2022 to June 2023, using a dilute, foliar-directed application to the point of run-off (≈ 4–6 L per tree). Product application rates were: Luna® Experience 40mL/100L; Merivon® 25mL/100L; Amistar® 250 SC 40mL/100L and Dithane Rainshield 200g/100L.

Treatments 1-4 (tree hygiene study): Branches with dead wood sourced from 15-year-old 'Murcott' trees adjacent to the trial block were added to an additional set of untreated control trees and 'Dithane only' trees (Treatments 2 and 4, respectively) at trial commencement to elevate localised inoculum pressure. Branches with dead wood bearing fungal fruiting bodies were tied to the centre of each tree.

The experiment utilised a randomised complete block design with four single-tree replicates per

[1] SDHI = succinate dehydrogenase inhibitor

[2] DMI = demethylation inhibitor

[3] Note Merivon® is currently registered for EBS and blossom blight in lemon and tangelo, but not mandarin.

[4] QoI = quinone outside inhibitor

treatment (44 trees total).

**Disease assessment**

Field and postharvest disease assessments followed the standard procedures described in Appendix E (General Assessment Methodology). For FT3, the following trial-specific modifications applied:

- In-field incidence of disease: Five independent assessors counted the number of symptomatic fruit per tree in a 30 second pass; CBS and EBS lesions were not separated during field counts due to similarity of early rind symptoms.
- Fruit sampling: 50 fruit were harvested from each of the eastern and western canopies per tree to ensure no confounding canopy-orientation effects on disease.
- CBS/EBS assessment: Fruit were scored on the standard 0–10 scale before and after incubation (ethephon-imazalil dip followed by 21-day incubation), as described in Appendix E. At the pre-incubation stage, total disease incidence and severity was reported as it is difficult to discern EBS and CBS lesions in the 'Murcott' variety at this early stage, particularly when dealing with very large sample sizes. EBS, however, appeared to be the dominant disease expressed at harvest prior to incubation. Only CBS was assessed post-incubation due to more advanced/distinct symptom expression of CBS lesions, which masked any pre-existing EBS lesions.

**Light Detection and Ranging (LiDAR) canopy structure assessment**

To verify that canopy size and geometry did not confound spray coverage or disease outcomes, canopy height and volume were measured using a mobile LiDAR CropScan unit. Point-cloud data were compared with manual height measurements and confirmed that tree size across treatments was consistent, allowing disease and phytotoxicity results to be interpreted without canopy-related bias.

**Phytotoxicity and scale assessment**

The proportion of fruit affected by rind blemish and discolouration, and the severity of these symptoms, was assessed on fruit at harvest. Blemish/discolouration severity was rated on a 0-3 scale where 0 = no blemish or discolouration, 1= mild blemish or discolouration, 2 = moderate blemish or discolouration and 3 = severe blemish or discolouration. The proportion of fruit with visible scale insects on fruit surfaces was also recorded, due to observation of waxy scale during scoring for disease.

**Residue analysis**

A subset of the treatments representing different fungicide programs was selected for residue testing at harvest (treatments 2, 4, 5, 6 and 7). Composite samples consisting of ten whole fresh fruit per tree replicate of these treatments were submitted to Symbio Laboratories (NATA-accredited) for multi-residue analysis, which included dithiocarbamates, fluxapyroxad, pyraclostrobin, fluopyram and tebuconazole.

**Data analysis**

Disease incidence (pre-incubation) was analysed with a HGLM and assumed to follow a binomial distribution with a logit link. Treatment was fitted as the fixed effect (binomial distribution, logit link) and the block and tree fitted as the random terms (beta distribution, logit link). Post-incubation, convergence issues meant a GLMM and then a GLM was fitted (binomial distribution, logit link) and the predicted means are presented as proportions. Mean severity was analysed with a linear mixed model with effects as above (post-incubation, a log10 transformation completed to satisfy assumptions of homogeneity of variance). All significance testing was performed at the 0.05 level, and

if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Table A2.1**: Fungicide treatment structure and spray schedule for Field Trial 3 (FT3): Evaluation of Luna® Experience & Merivon® fungicide co-formulations for CBS and EBS management in 'Murcott' (IRM-1) mandarin, in comparison to industry standard fungicides. Trial conducted at Wallaville, Queensland, over the 2022–2023 season.

| Treatment no. | Treatment description | 14-Nov-22 | 28-Nov-22 | 9-Dec-22 | 12-Jan-23 | 9-Feb-23 | 14-Mar-23 | 4-Apr-23 | 2-May-23 | 7-Jun-23 |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **Water control** | Water | No spray | Water | Water | Water | Water | Water | Water | Water |
| **2** | **Water control + dead wood added** | Water | No spray | Water | Water | Water | Water | Water | Water | Water |
| **3** | **Dithane monthly** | Dithane | No spray | Dithane | Dithane | Dithane | Dithane | Dithane | Dithane | Dithane |
| **4** | **Dithane + dead wood added** | Dithane | No spray | Dithane | Dithane | Dithane | Dithane | Dithane | Dithane | Dithane |
| **5** | **Amistar/Dithane program** | Amistar | No spray | Dithane | Amistar | Dithane | Dithane | Dithane | Dithane | Dithane |
| **6** | **Luna/Dithane program** | Luna | No spray | Dithane | Dithane | Dithane | Dithane | Dithane | Dithane | Dithane |
| **7** | **Merivon/Dithane program** | Merivon | Merivon | Dithane | Merivon | Dithane | Dithane | Dithane | Dithane | Dithane |

**Notes**

Sprays were applied at approximately four-week intervals using method described in Appendix E. Each treatment was replicated four times in a randomised complete block design. Treatments 2 and 4 included placements of naturally infected dead wood in the centre of the canopy to enhance local inoculum pressure. Product application rates were: Luna® Experience 40mL/100L; Merivon® 25mL/100L; Amistar® 250 SC 40mL/100L and Dithane Rainshield 200g/100L.

**Results & Discussion**

For the visual in-field disease assessment, the Merivon®/Dithane program was the only treatment to significantly reduce the mean number of symptomatic fruit on trees compared to the 'no dead wood' control (Figure A2.1). However, despite having the lowest mean value, the Merivon®/Dithane program did not differ significantly in number of symptomatic fruit compared to any program which included Dithane sprays (including the 'Dithane only' program).

For the pre-incubation disease assessment, where EBS was predominant disease observed, all treatments significantly reduced disease incidence and severity relative to the untreated controls, except for the Dithane with dead wood program (Table A2.2). The Merivon® program had the lowest overall values for pre-incubation disease incidence and severity but had statistically similar disease incidence and severity to any program including Dithane sprays (without dead wood added).

All treatments in the post-incubation assessment, where CBS was the predominant disease observed, also significantly reduced disease incidence and severity relative to the untreated controls, except for the Dithane with dead wood program (Table A2.2). Any treatments that had Dithane sprays (without dead wood) were statistically similar for disease incidence, but for disease severity, the Luna®/Dithane program was less effective than the Dithane alone or Merivon®/Dithane programs, but statistically similar in efficacy to the Amistar®/Dithane program.

Overall, these results confirm that under the conditions of this experiment, Dithane is unsurprisingly a very effective product for CBS and EBS disease management. The results also suggest that Luna® Experience, and particularly Merivon®, can be applied in lieu of a Dithane spray without reducing the overall efficacy of a disease control program.

It should be noted that the number of Dithane applications made in this trial exceeded commercial spray application frequency. This was done to have whole of season fungicide coverage while reducing treatment complexity for this first season's evaluation of Luna® Experience and Merivon® (i.e. each disease control program only included one or two products).

Dithane treatments incorporating dead wood demonstrated significantly higher disease levels (incidence and severity) post-incubation compared to corresponding Dithane treatments without dead wood (Table A2.2). Statistically this effect was not seen pre-incubation, although the same trend was observed. A similar trend was seen when dead wood was added to untreated control trees, but once again was not significant. The stronger effect of dead wood on post-incubation disease compared to pre-incubation disease in Dithane treated trees suggests that dead wood inclusion has a greater effect on CBS compared to EBS, since CBS was the predominant disease post-incubation. The dead wood effect was also stronger on Dithane treated trees compared to untreated trees (post-incubation), most likely due to the strong efficacy of Dithane against CBS.

LiDAR-based canopy assessment confirmed that tree height did not differ among treatments, supporting the interpretation that treatment effects reflected chemistry performance rather than canopy variation (Table A2.3). Rind injury and scale damage was low across all treatments, and no phytotoxicity attributable to the fungicides was observed (data not shown).

There were no detectable residues of fluopyram or tebuconazole in fruit harvested at maturity from the Luna® Experience/Dithane program (Table A2.4). While residues of fluxapyroxad and pyraclostrobin were detected in mature fruit harvested from the Merivon®/Dithane program, residue levels remained low and within apparent regulatory limits. It should be noted that the single Luna® Experience application was made in mid-November 2022 whereas the last of three Merivon® sprays was made in mid-January 2023 – so timing of application and spray frequency undoubtedly would

have impacted residue levels of the two different products. As SDHI-based formulations can persist on fruit, leaves, and branches to provide extended protectant and curative activity, early- to mid-season application is therefore most appropriate to prevent exceeding residue limits at harvest. This is particularly relevant for export markets, where MRLs for key active ingredients are often comparatively lower than domestic MRLs. Product manufacturers should be consulted for expert advice on residues. Additionally, Citrus Australia publishes export MRLs on a regular basis for key export destinations.

Dithiocarbamate residues in all samples were found to be above the mancozeb MRL of 0.2 mg/kg, most likely reflecting the high frequency of Dithane applications in the trial, noting the strictly experimental nature of the trial. That the control samples had dithiocarbamate residues ranging from 0.2-0.3 mg/kg is most likely due to Dithane sprays applied by the grower to these trees prior to trial commencement. In any case, dithiocarbamate levels in control fruit were much lower than that for treated fruit.

Collectively, the findings of this trial support the inclusion of SDHI–co-formulations as part of integrated CBS and EBS programs in 'Murcott' mandarins, provided applications are alternated with protectants and used within label constraints (noting that Merivon's registration is currently only for lemons and tangelos). However, further field evaluations of both co-formulations in association with protectants other than Dithane are recommended, since mancozeb use is expected to be limited in the future. The results also flag the importance of canopy hygiene in citrus disease management, particularly for CBS, and highlight that even strong fungicide programs may be constrained by high inoculum pressure.

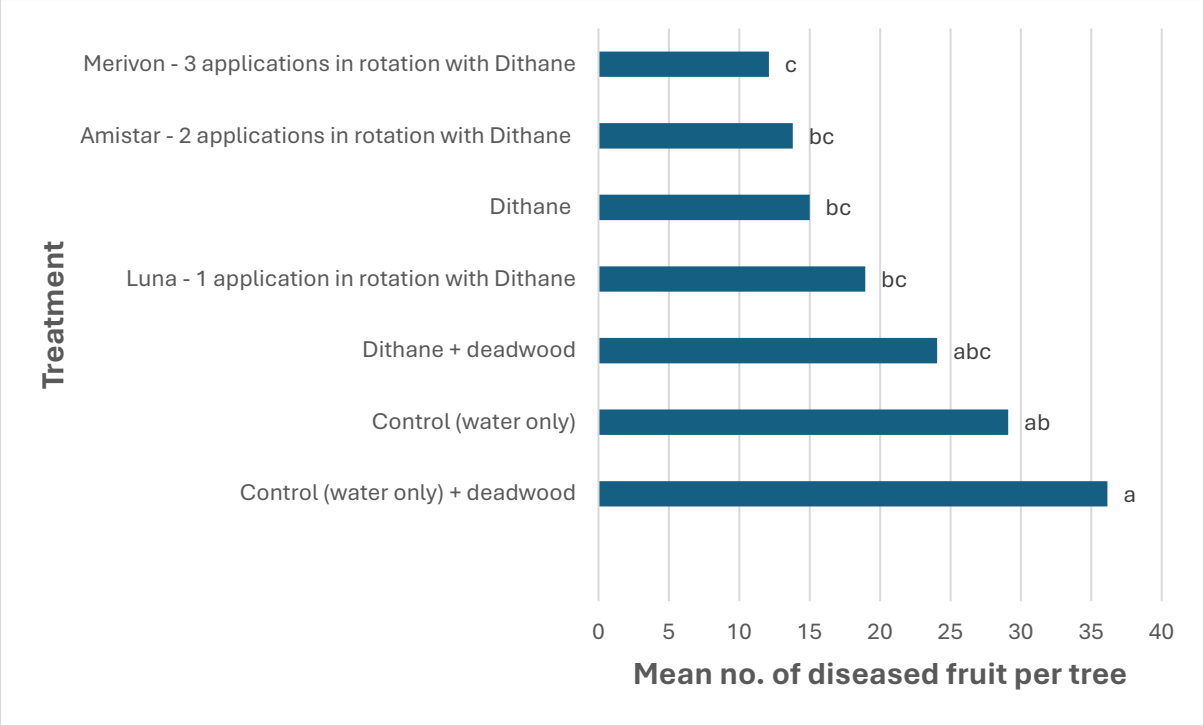


**Figure A2.1:** In-field visual disease count[1] for FT3. Treatments surmounted by the same letter are not significantly different according to Fisher's least significant difference (LSD) test at $P \leq 0.05$.

[1] Mean no. of fruit with disease counted during a 30s pass of each tree, averaged across 5 assessors.

**Table A2.2**: Pre- and post-incubation mean disease incidence and severity in mature 'Murcott' mandarins in response to seven programs, including SDHI and strobilurin fungicides (Merivon®, Luna® Experience, Amistar®) (Wallaville, 2022–23). Values are back transformed or raw* means followed by (predicted means ± standard error). Post-incubation predicted mean incidence values are expressed as proportions. Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05; CBS only.

| Treatments | Pre-Incubation Assessment[1] | | Post-Incubation Assessment[2] | |
|---|---|---|---|---|
| | Prop. of disease incidence | Mean disease severity score (0-10)[3*] | Prop. of disease incidence* | Mean disease severity score (0-10) |
| 1. Control | 0.84 (1.66 ± 0.44) a | 2.65 (2.66 ± 0.44) ab | 0.89 (-0.11 ± 0.05) a | 2.43 (0.39 ± 0.08) ab |
| 2. Control + dead wood | 0.85 (1.7 ± 0.45) a | 2.88 (2.91 ± 0.44) a | 0.9 (-0.07 ± 0.05) a | 3.49 (0.54 ± 0.11) a |
| 3. Dithane | 0.53 (0.11 ± 0.34) bc | 1.25 (1.25 ± 0.44) c | 0.41 (-0.6 ± 0.08) c | 0.65 (-0.19 ± 0.08) d |
| 4. Dithane + dead wood | 0.72 (0.95 ± 0.37) ab | 2.07 (1.97 ± 0.44) abc | 0.77 (-0.2 ± 0.07) ab | 1.96 (0.29 ± 0.1) ab |
| 5. Amistar + Dithane | 0.54 (0.17 ± 0.35) bc | 1.19 (1.18 ± 0.44) c | 0.55 (-0.45 ± 0.08) c | 0.98 (-0.01 ± 0.08) cd |
| 6. Luna Exp. + Dithane | 0.6 (0.41 ± 0.34) bc | 1.67 (1.64 ± 0.44) bc | 0.65 (-0.36 ± 0.09) bc | 1.34 (0.13 ± 0.1) bc |
| 7. Merivon + Dithane | 0.39 (0.45 ± 0.35) c | 0.77 (0.80 ± 0.44) c | 0.49 (-0.51 ± 0.08) c | 0.71 (-0.15 ± 0.08) d |
| LSD (predicted means) | 1.0 | 1.24 | 0.21 | 0.26 |
| P value | <0.001 | 0.017 | <0.001 | <0.001 |

[1] Total disease incidence and severity (primarily EBS as determined by visual assessment)

[2] CBS only as determined by the presence of distinct CBS symptoms

[3] Disease severity scores were averaged from visual ratings on a 0–10 scale, where 0 = no visible symptoms and 10 = full-surface lesion development.

**Table A2.3**: Mean canopy height of trees in FT3 measured using LiDAR-derived geometry (n = 4 trees per treatment).
Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment no. | Program | Canopy height (m) ± standard error |
|---|---|---|
| 1 | Control | 3.17 ± 0.25 a |
| 2 | Control + dead wood | 2.65 ± 0.29 a |
| 3 | Dithane | 2.65 ± 0.12 a |
| 4 | Dithane + dead wood | 3.08 ± 0.28 a |
| 5 | Amistar + Dithane | 3.07 ± 0.42 a |
| 6 | Luna Exp. + Dithane | 3.02 ± 0.30 a |
| 7 | Merivon + Dithane | 2.72 ± 0.20 a |
| **P value** | | p = 0.684 |

**Table A2.4**: Fungicide residue levels[1] detected on harvested 'Murcott' mandarin fruit across selected treatments.
Values represent the range (mg/kg) observed across four replicate composite samples per treatment. ND= no detected residues (below laboratory reporting limits).

| Treatment No. | Program | Dithiocarbamates[2] (mg/kg) | Fluxapyroxad[3] (mg/kg) | Pyraclostrobin[3] (mg/kg) | Fluopyram[2] | Tebuconazole[2] |
|---|---|---|---|---|---|---|
| 2 | Control (+ dead wood) | 0.29–0.3[4] | ND | ND | ND | ND |
| 4 | Dithane (+ dead wood) | 0.78–1.6 | ND | ND | ND | ND |
| 5 | Amistar + Dithane | 1.1–1.9 | ND | ND | ND | ND |
| 6 | Luna + Dithane | 1.3–4.7 | ND | ND | ND | ND |
| 7 | Merivon + Dithane | 1.1–1.6 | 0.02–0.03 | 0.04[5] | ND | ND |

[1] Residue values referenced in this study were derived from accredited laboratory residue analysis conducted by Symbio Laboratories (Brisbane).
[2]Australian & NZ MRL for mancozeb, tebuconazole & fluopyram=0.2mg/kg (Citrus Export MRLs 2025).
[3] MRLs not publicly available but APVMA Trade Advice Notice on pyraclostrobin and fluxapyroxad in the product Merivon® Fungicide for use on lemon and tangelo June 2023 considered 1.0-1.5 mg/kg appropriate for fluxapyroxad and 0.7-1.0 mg/kg appropriate for pyraclostrobin in the lead up to label extension by BASF.
[4]Residues detected in 2 of 4 samples only. [5]Residues detected in 1 of 4 samples only.

## Field Trial 4 (FT4): Further evaluation of new crop protectants and strategic and fixed-schedule fungicide programs for CBS in 'Imperial' mandarin, Coringa, 2024–2025

### Methodology

Field Trial 4 (FT4) was conducted during the 2024–25 growing season, to further evaluate the SDHI-based co-formulations Merivon®[5] and Luna® Experience in rotation for management of CBS in a commercial 'Imperial' mandarin orchard at Redlea Citrus, Coringa, Queensland.

The trial tested the formulations within an overarching copper fungicide program (delivered as cuprous oxide (red copper), applied either on a fixed calendar schedule or strategically triggered in response to rainfall events considered conducive to infection. Twelve fungicide programs (12 treatments) were evaluated, including monthly red copper applications, copper–mancozeb combinations, copper-systemic fungicides (Luna® Experience and Merivon®) combinations, and post-rainfall fungicide application of Luna® Experience and Merivon® (Table A3.1).

The post rainfall-triggered treatments were applied immediately after the first rain event exceeding precipitation of 10–15 mm in 72 h. Subsequent planned sprays were then resumed at the next scheduled interval. Spray intervals, therefore, ranged from 2 to 6 weeks depending on seasonal conditions, and each program comprised 5–8 applications across the season. All other aspects of orchard maintenance were conducted by the grower collaborator.

As the available orchard contained mixed tree age classes due to progressive replanting by the grower, the trial was replicated across these canopy classes using a randomised complete block design, ensuring that treatment comparisons accounted for structural variability within the block. This included four canopy categories (2yo (2M – recently planted trees, ≤ 2 years), 3 yo (3M – intermediate replants, ≈ 3 years), mature (Ideal – mature commercial trees), and in decline (Bench – declining, senescent trees)). Each treatment consisted of five single-tree replicates. At maturity, 50 fruit per tree were harvested (fewer if fruit were unavailable) and assessed for incidence and severity of CBS using a 0-5 scale as per Appendix E within 48 hours of harvest. Fruit were dipped (ethephon-imazalil) and incubated as per Appendix E prior to reassessment of incidence and severity of CBS.

Disease incidence was analysed with a HGLM and assumed to follow a binomial distribution with a logit link. Treatment was fitted as the fixed effect and the orchard row and tree type fitted as the random terms and assumed to follow a beta distribution with a logit link. The dispersion parameter was estimated. Predicted mean and standard error are presented on the logit scale. Back transformed means are expressed as a proportion. Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). Pre-incubation a $\log_{10}$ transformation was required to satisfy the assumption of homogeneity of variance. A small constant of 0.01 was added prior to transforming due to some trees having all fruit score zero. Post-incubation a square root transformation was required to satisfy the assumption of homogeneity of variance. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

[5] Note Merivon® is currently registered for EBS and blossom blight in lemon and tangelo, but not mandarin.

**Table A3.1**: Spray programs evaluated in Field Trial 4 (FT4) for CBS management in 'Imperial' mandarin during the 2024–25 season. The table summarises the timing of copper-, mancozeb-, and SDHI–strobilurin-based fungicide applications applied either on a fixed schedule or as strategic rainfall-triggered sprays. Treatments were applied using cuprous oxide–based copper (Nordox®), mancozeb (Dithane®), and systemic fungicides (Luna® Experience and Merivon®**).

| Date | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | T12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 Oct. | Water | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox |
| 5 Nov. | Water | Nordox | Nordox | Nordox | **Luna** | **Luna** | **Merivon** | **Merivon** | **Luna** | **Merivon** | Nordox | Nordox |
| 19 Nov.* | – | – | – | – | – | – | – | – | – | – | **Strategic Luna*** | **Strategic Merivon *** |
| 3 Dec. | Water | Nordox | Dithane | Nil | Dithane | Nil | Dithane | Nil | Nordox | Nordox | Nordox | Nordox |
| 15 Jan. | Water | Nordox | Dithane | Nil | Mancozeb | Nil | Dithane | Nil | Nordox | Nordox | Nordox | Nordox |
| 6 Feb. | Water | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox |
| 4 March | Water | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox | Nordox |

* Strategic applications, experimental only (label use of these products is preventative only). **Experimental treatment – Merivon® registered for lemon & tangelo only.

Treatments description: **1** water control (6 sprays); **2** copper every round (6 sprays); **3** copper with Dithane applied at Rounds 3 and 4 (6 sprays); **4** copper with no spray at Rounds 3 and 4 (4 sprays); **5** one Luna® Experience spray at Round 2 plus copper and Dithane at Rounds 3 and 4 (6 sprays); **6** one Luna® Experience spray at Round 2 plus copper and no spray at Rounds 3 and 4 (4 sprays); **7** one Merivon® spray at Round 2 plus copper and Dithane at Rounds 3 and 4 (6 sprays); **8** one Merivon® spray at Round 2 plus copper and no spray at Rounds 3 and 4 (4 sprays); **9** one Luna® Experience spray at Round 2 plus copper at all remaining rounds (6 sprays); **10** one Merivon® spray at Round 2 plus copper at all remaining rounds (6 sprays); **11** copper at every scheduled round plus one strategic Luna® Experience spray triggered by the first significant rainfall event (≥10–15 mm in 24 h) (7 sprays); and **12** copper at every scheduled round plus one strategic Merivon® spray triggered by the first significant rainfall event (7 sprays)

**Results & Discussion**

Pre-incubation CBS incidence was low overall in this trial, with only 11% of fruit affected in the control (Table A3.2). All treatments reduced CBS incidence compared to the control, except where there was a 3-month period of no fungicide application following either Luna® Experience or Merivon® application in early November (T6 & T8), suggesting that despite the long-lasting residual activity of these products, the time period without fungicide coverage was too long. Fruit from Merivon® + copper or Strategic Merivon® (T10 & T12) had the lowest values for pre-incubation CBS incidence (only 1% of fruit affected) of all the treatments, although they were not significantly lower than most other treatments, except for the control and the 3 treatments where there was a 3-month gap in fungicide application (T4, 6 & 8).

Pre-incubation CBS disease severity showed similar trends to disease incidence (Table A3.2). Treatments with a gap in fungicide application (T4, T6 & T8), as well as the Luna® Experience + copper + Dithane program (T5), being the only treatments not reducing disease severity compared to the control.

The Nil application treatments indicate the necessity of spray coverage in the November-February CBS susceptibility period, rendering an early application of Luna® Experience or Merivon® inadequate if followed by consecutive months without fungicide application. It is interesting that while the copper + Nil treatment wasn't more effective than the other Nil treatments, disease incidence was not significantly greater than where copper was applied throughout. Cuprous oxide products are deemed to last longer/'stick' better and its residue may have been effective for longer through the wet season in 2024/2025 (providing significantly more CBS suppression than the control treatment).

CBS incidence and severity scores were considerably higher post-incubation than pre-incubation (Table A3.2). Surprisingly, disease incidence was significantly higher in T6 & T8 (i.e. where there was 3-month period of no fungicide application following either Luna® Experience or Merivon® application in early November) compared to the control. Aside from that difference, there were no other differences compared to the control. For post-incubation disease severity scores, there were no differences between any of the treatments, including the control (Table A3.2).

Although copper-based programs performed well in at-harvest (pre-incubation) assessments this season, reliance on cuprous oxide as the sole or primary control option remains risky for 'Imperial' mandarins (grower communication/experience), which are known to develop rind blemishes such as speckling when exposed to repeated copper sprays. In mid- and late-maturing cultivars, extended use of copper can also increase the likelihood of phytotoxicity under warm and humid conditions, where rind injury is more common. These results therefore support copper as an effective multi-site protectant but also highlight the value of integrating SDHI–copper or copper–mancozeb combinations to maintain efficacy while reducing total copper inputs. Any reduction in copper use may offer benefits in minimising rind marking, although decisions must still consider seasonal conditions and the potential for phytotoxicity in high-risk periods.

While clearer differences between treatments is desirable, in terms of assessing new chemistries, it is encouraging to note the effectiveness of the SDHI chemistries. Merivon®, in particular, shows promise, but the control provided was not greater than most of the Luna® Experience treatments, indicating Luna® Experience also has a place in rotation as part of fungicide programs for citrus. The use of different chemistries with different modes of action (e.g. tebuconazole in Luna® Experience and pyraclostrobin in Merivon®) provides more effective fungicide resistance management.

Copper is addressed in more detail under Theme 2/Appendix B but it is worth noting that copper alone and the remainder of the treatments which were effectively applied as the minor component of a

copper spray regime were as effective as the treatments that included mancozeb. We acknowledge considerable research is needed regarding copper phytotoxicity, application scheduling and mitigation of phytotoxicity risk.

**Table A3.2**: Effect of fungicide treatments on CBS incidence and severity. Data are shown for fruit assessed immediately after harvest (pre-incubation) and after a controlled incubation period (post-incubation). Values are back-transformed means (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatments* | Pre-Incubation Assessment | | Post-Incubation Assessment | |
|---|---|---|---|---|
| | Prop. of disease incidence | Mean disease severity score (0-10) | Prop. of disease incidence | Mean disease severity score (0-10) |
| 1. Water | 0.11 (-2.05 ± 0.55) a | 0.11 (-0.92 ± 0.24) a | 0.58 (0.31 ± 0.3) bc | 1.21 (1.1 ± 0.12) a |
| 2. Copper | 0.02 (-4.12 ± 0.73) cd | 0.01 (-1.67 ± 0.24) cd | 0.54 (0.17 ± 0.31) bc | 1.23 (1.11 ± 0.11) a |
| 3. Copper + Dithane | 0.02 (-3.93 ± 0.72) cd | 0.02 (-1.58 ± 0.24) bcd | 0.56 (0.23 ± 0.32) bc | 1.20 (1.1 ± 0.12) a |
| 4. Copper + Nil | 0.05 (-2.94 ± 0.61) bc | 0.06 (-1.17 ± 0.24) ab | 0.74 (1.05 ± 0.37) ab | 1.97 (1.4 ± 0.11) a |
| 5. Luna Exp. + Copper + Dithane | 0.03 (-3.41 ± 0.64) bcd | 0.03 (-1.42 ± 0.24) abcd | 0.65 (0.63 ± 0.31) abc | 1.45 (1.21 ± 0.11) a |
| 6. Luna Exp. + Copper + Nil | 0.053 (-2.88 ± 0.61) abc | 0.05 (-1.25 ± 0.24) abc | 0.8 (1.36 ± 0.38) a | 2.12 (1.46 ± 0.11) a |
| 7. Merivon + Copper + Dithane | 0.02 (-3.85 ± 0.73) cd | 0.02 (-1.59 ± 0.24) bcd | 0.6 (0.41 ± 0.36) abc | 1.37 (1.17 ± 0.12) a |
| 8. Merivon + Copper + Nil | 0.07 (-2.55 ± 0.58) ab | 0.06 (-1.14 ± 0.24) ab | 0.81 (1.42 ± 0.41) a | 1.86 (1.37 ± 0.11) a |
| 9. Luna Exp. + Copper | 0.04 (-3.18 ± 0.62) bcd | 0.03 (-1.44 ± 0.25) bcd | 0.69 (0.82 ± 0.35) abc | 1.89 (1.37 ± 0.12) a |
| 10. Merivon + Copper | 0.01 (-4.99 ± 0.98) d | 0.01 (-1.77 ± 0.24) d | 0.53 (0.12 ± 0.32) c | 1.07 (1.03 ± 0.11) a |
| 11. Copper + Strategic Luna Exp. | 0.02 (-4.04 ± 0.73) cd | 0.01 (-1.61 ± 0.24) bcd | 0.59 (0.37 ± 0.34) bc | 1.21 (1.1 ± 0.13) a |
| 12. Copper + Strategic Merivon | 0.01 (-5.26 ± 1.14) d | 0.01 (-1.81 ± 0.27) d | 0.62 (0.5 ± 0.4) abc | 1.45 (1.21 ± 0.13) a |
| LSD (predicted means) | 1.47 | 0.52 | 0.92 | 0.3 |
| P value | <0.001 | 0.018 | 0.03 | 0.06 |

*Treatments description: **1.** water control (7 sprays); **2.** copper every round (7 sprays); **3.** copper with Dithane applied at Rounds 3 and 4 (7 sprays); **4.** copper with no spray at Rounds 3 and 4 (5 sprays); **5.** one Luna® Experience spray at Round 2 plus copper and Dithane at Rounds 3 and 4 (7 sprays); **6** one Luna® Experience spray at Round 2 plus copper and no spray at Rounds 3 and 4 (5 sprays); **7.** one Merivon® spray at Round 2 plus copper and Dithane at Rounds 3 and 4 (7 sprays); **8.** one Merivon® spray at Round 2 plus copper and no spray at Rounds 3 and 4 (5 sprays); **9.** one Luna® Experience spray at Round 2 plus copper at all remaining rounds (7 sprays); **10.** one Merivon® spray at Round 2 plus copper at all remaining rounds (7 sprays); **11.** copper at every scheduled round plus one strategic Luna® Experience spray triggered by the first significant rainfall event (≥10–15 mm in 24 h) (8 sprays); and **12.** copper at every scheduled round plus one strategic Merivon® spray triggered by the first significant rainfall event (8 sprays).

## Appendix B

### Theme 2: Copper optimisation and phytotoxicity management.

**Background**

Copper remains one of the few broad-spectrum fungicides available for CBS and EBS management in Australian mandarins, and industry uncertainty around the long-term availability of dithiocarbamates has further elevated the importance of understanding the efficacy of copper with respect to disease control and how copper can be used safely without compromising fruit quality. Concerns include phytotoxicity (fruit and tree), residue expectations, and seasonal copper-load considerations and have reinforced the need for clear, field-derived guidance on how formulation, pH and application timing influence both efficacy with respect to disease control and the risk of rind injury.

At the outset of this project, growers commonly reported variability in the degree of rind injury induced by copper application, especially under acidic spray conditions or when using particular formulations. Although cuprous oxide (New South Wales and Victorian citrus growing regions) and copper hydroxide (Queensland growing regions) are widely applied, there had been little systematic evaluation of their crop safety under subtropical conditions, nor any direct comparison of the effects of pH in different seasons or parts thereof. Previous industry observations suggested that rind sensitivity increases after mid-summer, but no structured data existed to quantify this risk or determine whether adjusting pH could provide a practical mitigation strategy. These knowledge gaps have limited growers' confidence in building copper-based programs that balance fruit safety, efficacy and regulatory compliance.

To address this, three field trials were established between 2023 and 2025 to characterise copper behaviour under commercial conditions in relation to tree and fruit phytotoxicity injury. Field Trial 1 (2023) was conducted at the Bundaberg Research Facility in an existing citrus research block to establish whether the pH of copper fungicide applied in the latter half of the growing season affects the amount of phytotoxicity expressed in mature fruit. This provided an initial understanding of pH thresholds associated with injury. This work was expanded in the 2023-2024 season in an on-farm trial (Field Trial 2) in a commercial 'Murcott' orchard, where the effect of formulation type and pH and their interaction on rind safety was evaluated when applied from January to July. Field Trial 3 (2024–25) progressed evaluation of copper products to entire-season programs (September - July) in commercially grown 'Imperial' mandarins, including effects of timing of application, pH, and copper formulation on phytotoxicity/rind injury. Here treatments included two copper products (copper hydroxide and cuprous oxide), a range of pH applications and applications made before and after January when rind tissues typically become more sensitive. The effect of copper and pH on disease control was also investigated in Field Trials 2 and 3.

These trials form a coordinated sequence that progresses from exploratory assessment to commercial validation. The work was designed to answer a practical question for industry: How copper can be positioned within integrated CBS and EBS fungicide programs if other multi-site protectants become more restricted, and which adjustments provide the lowest risk of phytotoxicity without reducing disease control? The resulting data provides growers, consultants and industry bodies with field-based evidence on how formulation, pH management and seasonal timing influence copper performance, supporting confident use of copper within integrated disease-management programs.

## Field Trial 1 (FT1): Copper pH adjustment and phytotoxicity in irradiated scab orange hybrids, Bundaberg Research Station, 2022-2023.

### Methodology

Field Trial 1 was a preliminary trial conducted at the Bundaberg Research Facility from January to June 2023 to evaluate how the pH of copper fungicides influences copper-related phytotoxicity when applied in early fruit development under subtropical field conditions. It was considered important to assess the degree of phytotoxicity risk on a research block first before undertaking trials on commercial farms.

At project commencement, it was widely assumed that the adjustment of the pH of the copper solution in the spray tank mix could enhance the performance of copper fungicides, but that acidic spray conditions might also increase the risk of phytotoxic injury to developing fruit. This trial was therefore designed to examine copper-related rind injury on trees sprayed with copper hydroxide adjusted to different pH values.

The trial was established in a uniform research block of irradiated scab-orange hybrids (*Citrus sinensis* × *C. reticulata*, grown on Rough Lemon rootstock), a citrus cross known to express rind sensitivity under certain spray conditions.

Five pH treatments were evaluated: (1) copper at pH 5; (2) copper at pH 7; (3) copper at pH 9; (4) copper at pH 11; and (5) 'soluble' copper at pH 7. These treatments were selected to quantify whether increases in acidity or alkalinity reduces the occurrence or severity of copper-induced rind injury. The treatment structure was designed to test mildly acidic to strongly basic copper pH solutions. Treatment 5 was included as a novel experimental approach to test if 'solubilising' a standard pH 7 copper hydroxide solution (which contains precipitate) by first adjusting it to pH 5 to obtain a clear solution, and then returning it to neutral pH (where it retains a clear solution free of precipitate) would result in more or less phytotoxicity than the standard neutral copper hydroxide preparation with precipitate.

For all treatments, the water used to make the spray mixture was adjusted to the target pH and, after the copper hydroxide (BlueShield®, 50% Cu) was added at 200 g 100 $L^{-1}$ and dissolved, the pH of the copper mixture re-adjusted as needed using citric acid or potassium hydroxide to achieve acidic, neutral, alkaline and highly alkaline solutions. The pH 7 'soluble' copper mixture (treatment 5) was prepared by first adjusting the desired spray volume of water to pH 7, adding copper hydroxide, lowering the mixture to pH 4 using citric acid and ensuring the copper ion particles were completely dissolved before the solution was returned to pH 7 using potassium hydroxide. Sprays were applied as dilute, foliar-directed applications to the point of run-off using a calibrated 2.0 mm nozzle delivering approximately 3.5 L $tree^{-1}$.

Three applications were made at four-week intervals between January and April 2023 during stable weather periods, i.e. when no rainfall forecast for at least six hours to reduce the risk of wash-off. The pH of the spray mixture was verified immediately before treatment, and tarpaulin shields were used during application to prevent drift between adjacent plots.

The experiment followed a randomised complete block design with six blocks and multi-tree plots (3–6 trees) with guard trees between plots. The number of trees per plot varied according to the availability of fruit on each tree, and, as some trees had very few fruit, more trees were needed to achieve the minimum sample size.

Each fruit was assessed visually for three symptom categories: Wind rub (pale or flattened areas caused by mechanical abrasion), scab-like lesions (raised or corky physiological injury), and generalised

spotting (small brown or black specks not attributable to CBS or EBS). For each category, the percentage of the fruit surface area affected was estimated (0–100%). A mean value per tree was calculated and summarised at the block level to determine whether copper pH influenced the incidence or severity of visible injury.

**Results & Discussion**

Very high levels of scab-like lesions were recorded in this trial, with an average 37% of fruit surface area affected in the untreated control treatment. As a result, it was very challenging to discern symptoms of 'speckle' and 'scar' from the scab-like lesions, particularly in the treatments which had more scab than others, such as the pH 7 and pH 11 treatments where approximately 50% of the fruit surface area was affected by scab. The treatments with the lowest % of fruit surface area affected by scar or speckle (soluble copper pH 7 and pH 9) also had the lowest amount of scab. As a result, it was decided not to proceed with statistical analysis of these results.

Despite these limitations, FT1 demonstrated that copper applications across a wide pH range can potentially be made during early fruit development without severe phytotoxic effects such as extensive defoliation, canopy dieback, or increased fruit drop under subtropical conditions. This was a positive outcome ahead of trials in subsequent seasons that were to be conducted on commercial farms. FT1 also highlighted the importance of suitable tree and site selection for phytotoxicity assessments, as factors such as varietal susceptibility, tree spacing, and background fruit can limit the suitability of such material for phytotoxicity assessment.

Rind injury was highly variable between treatments in this trial, reflecting the tightly spaced planting arrangement and subsequent, frequent mechanical abrasion between canopies, and the inherently blemish-prone characteristics of the scab–orange hybrids.

**Figure B1.1**: Scab-orange trial site (BRF) and combined phytotoxicity, scab-like lesions and general mechanical damage (Image credits: T. Thangavel).

## Field Trial 2 (FT2): Effect of copper formulation and pH on phytotoxicity and disease control in 'Murcott' mandarins, Citrus Abbotsleigh, 2023-2024

### Methodology

Field Trial 2 was conducted during the 2023–2024 growing season (January to July) to evaluate the influence of copper formulation and spray mixture pH on rind phytotoxicity and disease control in a commercial block of 'Murcott' mandarins at Citrus Abbotsleigh Farm, Wallaville, Queensland.

Eleven treatments (Table B2.1) were evaluated, structured to compare copper hydroxide, cuprous oxide, copper sulphate, Bordeaux mixture and an untreated control, together with pH-adjusted sprays at pH 4, pH 7 and pH 11 using citric acid (for pH 4) or potassium hydroxide for (pH 7 or pH 11). Also included were pH 7 'soluble' copper treatments (referred to as pH 4-7 in the results), which were prepared as previously described in FT1.

Applications were made at monthly intervals between January and June 2024 (six sprays: 16–17 January, 15 February, 22 March, 17 April, 24 May and 20 June) under dry, low-wind conditions, with no rainfall forecast for at least six hours after treatment to minimise wash-off.

The trial design was a randomised complete block design with five blocks and single-tree plots, giving a total of 55 experimental trees. All copper products were applied at 50 g 100 $L^{-1}$ (or the equivalent label rate in the case of Bordeaux mixture). Sprays were applied as dilute, foliar-directed applications to the point of run-off using a calibrated 2.0 mm hollow-cone nozzle, delivering approximately 4–5 L of spray mixture per tree.

The orchard had a documented history of CBS and EBS, and, except for fungicides, trees were maintained under the grower's standard management practices throughout the trial (no other fungicides were applied by the grower). 'Murcott' was selected as the test cultivar because it is known to show moderate sensitivity to copper injury, providing an appropriate context for formulation and pH comparisons.

**Table B2.1**: Copper fungicide treatment details for FT2. All copper treatments were applied at 50 g 100 $L^{-1}$. Spray pH was adjusted using citric acid (pH 4) or potassium hydroxide (pH 7 or pH 11). Applications were made at monthly intervals from January to June 2024.

| Treatment | Active ingredient / Formulation | pH |
|---|---|---|
| 1 | Copper sulphate pentahydrate | 7 |
| 2 | Cuprous oxide (Nordox®) | 7 |
| 3 | Copper hydroxide (BlueShield®) | 7 |
| 4 | Copper hydroxide (BlueShield®), acidified | 4 |
| 5 | Copper hydroxide (BlueShield®), alkaline | 11 |
| 6 | Copper hydroxide (BlueShield®), pH 4 followed by pH 7 | 4 → 7 |
| 7 | Copper sulphate (copper sulphate pentahydrate) acidified | 4 |
| 8 | Copper sulphate (as above), alkaline | 11 |
| 9 | Copper sulphate (as above), pH 4 followed by pH 7[1] | 4 → 7 |
| 10 | Untreated (water) | 7 |
| 11 | Bordeaux mixture (1:1:10) (copper sulphate: Lime hydrate: Water) | 11 |

At harvest, 50 fruit were collected from each tree and the primary rind injury categories associated with copper exposure, including speckling, chemical run-off streaks or roughened rind texture were assessed. Additional observations including the proportion of immature fruit and the presence of localised stem-end greening were recorded to provide supporting context for fruit quality.

Disease assessments for CBS and EBS were conducted immediately after harvest and again after the standard ethephon-imazalil and incubation method on the 0-5 scale used previously described in Appendix E.

Proportions of phytotoxicity and disease incidence were analysed with a HGLM with a fixed effect (treatment) assumed to follow a binomial distribution with a logit link and a random term (block) assumed to follow a beta distribution with a logit link. For the proportion of fruit with 'chemical pattern' symptoms and speckling, the HGLM did not converge and a GLMM was fitted (binomial distribution assumed, logit link function applied, terms as above). For the 'chemical pattern' symptoms, the variance components of the GLMM were bound, so a GLM was fitted instead (terms as above, binomial distribution, logit link, means expressed as proportions). Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). A square root transformation was applied to the EBS data to satisfy the assumption of homogeneity of variance. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Results & Discussion**

Both pH and formulation of copper spray mixtures applied here affected both the expression of phytotoxicity symptoms and the amount of EBS and CBS recorded in mature fruit. The proportion of fruit with speckling, greening at the stem-end or rough rind texture were significantly affected by pH and formulation (Table B2.2) while the proportion of fruit with immature rind or a chemical-run like symptom were not. Incidence of both EBS and CBS were affected by the treatments while the only the severity of CBS symptoms was affected (Table B2.3).

Speckling of the rind appeared to be the most consistent visual indicator of rind phytotoxicity (Figure B2.1) and is the symptom where the clearest significant differences between treatments and formulations were noted (Table B2.2). All acidic copper applications (pH 4) significantly increased speckling to 84-91% of fruit assessed in comparison with the water control treatment (2%), the pH 11 application treatments (32-33%), the copper hydroxide (BlueShield®) and cuprous oxide (Nordox®) pH 7 applications (33% each), and the pH 11 Bordeaux mixture (50%).

When comparing formulation of copper product, the amount of speckling induced by copper hydroxide and cuprous oxide at pH 4 (all 84-91%) or pH 11 (32-33%) was not affected by formulation. At pH 7, however, the amount of speckling in the copper hydroxide and cuprous oxide treatments were identical (33%) while the speckling recorded in the copper sulphate treatment was significantly higher (84%) and equal to that seen in the pH 4 treatments, most likely due to the higher solubility of copper sulphate.

Some parallels were noted in symptoms of greening at the stem end and fruit with rough rind texture (Table B2.2), but the differences are not as clear and whether these are true phytotoxicity symptoms needs to be investigated. That said, the highest incidence of stem-end greening was seen in the copper sulphate pH 4 and 4-7 treatments (61 and 79% respectively), with copper sulphate pH 7, copper hydroxide pH 4 and 4-7, and Bordeaux mixture also having significantly more stem-end greening than the control. For rough rind texture, the only treatments that didn't increase symptoms compared to the control were copper sulphate at pH 7 or 11, and copper hydroxide at pH 11.

Very low incidences of CBS and EBS indicated disease pressure was low in 2023-2024 with little EBS or CBS recorded in mature fruit irrespective of treatment (Table B2.3). There were no significant effects of any treatment on EBS severity, and only one treatment, copper hydroxide pH 11 (20%) increased EBS incidence compared to the control (3%).

The effect on incidence and severity of CBS was more nuanced and few significant differences were noted between treatments (Table B2.3). Copper hydroxide pH 4 yielded the highest incidence of CBS of all treatments with significantly more disease noted than in all other treatments except the water control. Copper hydroxide pH 4-7, pH 7 and pH 11, copper sulphate pH 4 and Bordeaux mixture pH 11 treatments conversely recorded significantly less disease than the water control treatment. Copper hydroxide pH 7, 11 and 4-7, copper sulphate pH 4, and Bordeaux pH 11 recorded no disease on any single piece of fruit.

In treatments where CBS was recorded, the severity of symptoms was again the highest in the copper hydroxide pH 4 treatment with significantly more symptoms than all treatments except the water control (Table B2.3). Copper hydroxide pH 7, 11 and 4-7, copper sulphate pH 4-7, cuprous oxide pH 7, and Bordeaux pH 11 all reduced CBS compared to the control.

These results demonstrate that copper phytotoxicity in 'Murcott' mandarins is strongly influenced by the interaction between formulation and the pH of the final spray solution, with acidic conditions significantly increasing the risk of visible rind injury and incidence of CBS (copper hydroxide) and EBS

(copper sulphate).

While alkaline applications increased the incidence of injury symptoms (speckle) above that of the water control, this increase was not statistically higher than that seen in the pH 7 treatments, and for copper sulphate, was lower than the corresponding copper sulphate pH 7 treatment. EBS and CBS were affected differently by increasing the alkalinity of spray solutions - elevated EBS symptoms (copper hydroxide pH 11) and reduced CBS symptoms (copper hydroxide pH 11). In terms of phytotoxicity, these results are consistent with grower observations that usage of high-pH copper is more favourable than acidic copper sprays under field conditions.

Bordeaux mixture, while very effective in controlling CBS in this trial, did result in moderate rind injury. The mixture, which was prepared in this trial by mixing copper sulphate, lime hydrate and water, is known to have very good 'sticking' properties but can be phytotoxic under certain conditions. It is generally recommended for dormant plant tissues rather than young growth due to the phytotoxicity risk. However, modern 'Bordeaux' formulations (tribasic copper sulphate) are generally safer and easier to use. In this trial we experienced difficulties adjusting the pH of our mixture below pH 11 and also found it difficult to agitate the mixture in the spray tank due to its high viscosity.

The results also highlight the importance of formulation-specific properties, particularly particle size and stability, in determining both safety and efficacy. Here we see that copper sulphate performs as effectively at pH 7 as copper hydroxide and cuprous oxide in terms of disease control but contributes to very high rind injury. Copper sulphate (pentahydrate) was included in this trial for research purposes only and would never be recommended for use by citrus growers. Modern copper sulphate products are formulated with alkaline agents such as calcium hydroxide to produce tribasic copper sulphate which is less phytotoxic than copper sulphate alone.

The results also indicate that cuprous oxide performed well in comparison with copper hydroxide and warrants future investigation.

There was no advantage in applying a 'soluble' pH 7 preparation (pH 4-7) for either copper hydroxide or copper sulphate, and in fact injury levels were considerably and significantly higher in the 'soluble' copper hydroxide at pH 7 compared to the largely insoluble (fixed copper) commercial copper hydroxide preparation at pH 7. Collectively, these findings support the adoption of optimised pH copper programs to maintain disease suppression while reducing the likelihood of rind damage in commercial orchards. Further study in years with higher disease pressure is needed to assess disease control in conjunction with rind injury.

**Figure B2.1**: Dark speckling phytotoxicity symptoms induced by application of acidic copper (pH 4). (Image credit: T. Thangavel.)

**Table B2.2**: Effect of copper formulation and pH on the mean proportion (incidence) of rind/blemish symptoms across copper formulation and pH treatments in 'Murcott' mandarins. Values are presented as back-transformed means or raw* means followed by (predicted means ± standard errors). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment Description | Prop. of Speckle pattern | Prop. of Stem-end Greening | Prop. of Rough rind texture | Prop. of Chemical-run off streaking pattern* | Prop. of Immature fruit |
|---|---|---|---|---|---|
| 1. Copper sulphate, pH 7 | 0.83 (1.62 ± 0.58) a | 0.48 (-0.1 ± 0.38) bcd | 0.39 (-0.46 ± 0.39) abc | 0.21 (0.21 ± 0.08) a | 0.17 (-1.61 ± 0.63) a |
| 2. Cuprous oxide, pH 7 | 0.33 (-0.7 ± 0.498 b | 0.35 (-0.61 ± 0.39) cde | 0.47 (-0.11 ± 0.38) ab | 0.11 (0.11 ± 0.06) a | 0.35 (-0.66 ± 0.58) a |
| 3. Copper hydroxide (BlueShield), pH 7 | 0.33 (-0.7 ± 0.51) b | 0.26 (-1.04 ± 0.44) e | 0.43 (-0.28 ± 0.41) ab | 0.25 (0.23 ± 0.09) a | 0.08 (-2.46 ± 0.82) a |
| 4. Copper hydroxide (BlueShield), pH 4 | 0.84 (1.66 ± 0.66) a | 0.49 (-0.05 ± 0.41) bcd | 0.41 (-0.35 ± 0.42) ab | 0.12 (0.14 ± 0.08) a | 0.18 (-1.58 ± 0.67) a |
| 5. Copper hydroxide (BlueShield), pH 11 | 0.33 (-0.72 ± 0.49) b | 0.28 (-0.97 ± 0.42) de | 0.32 (-0.73 ± 0.41) bc | 0.19 (0.21 ± 0.08) a | 0.14 (-1.87 ± 0.69) a |
| 6. Copper hydroxide (BlueShield), pH 4 → 7 | 0.89 (2.07 ± 0.66) a | 0.52 (0.09 ± 0.38) bc | 0.46 (-0.17 ± 0.37) ab | 0.37 (0.38 ± 0.1) a | 0.20 (-1.4 ± 0.6) a |
| 7. Copper sulphate, pH 4 | 0.93 (2.65 ± 0.89) a | 0.61 (0.45 ± 0.4) ab | 0.51 (0.05 ± 0.39) ab | 0.28 (0.28 ± 0.09) a | 0.15 (-1.78 ± 0.67) a |
| 8. Copper sulphate, pH 11 | 0.33 (-0.78 ± 0.49) b | 0.28 (-0.95 ± 0.42) de | 0.37 (-0.54 ± 0.4) abc | 0.16 (0.16 ± 0.08) a | 0.19 (-1.44 ± 0.64) a |
| 9. Copper sulphate, pH 4 → 7 | 0.91 (2.33 ± 0.83) a | 0.79 (1.3 ± 0.45) a | 0.63 (0.52 ± 0.42) a | 0.21 (0.19 ± 0.08) a | 0.21 (-1.38 ± 0.0.65) a |
| 10. Water control | 0.02 (-3.76 ± 1.36) c | 0.25 (-1.11 ± 0.41) e | 0.15 (-1.7 ± 0.5) c | 0.04 (0.04 ± 0.04) a | 0.21 (-1.36 ± 0.6) a |
| 11. Bordeaux mixture, pH 11 | 0.50 (0.0 ± 0.47) b | 0.53 (0.13 ± 0.4) bc | 0.56 (0.24 ± 0.4) ab | 0.31 (0.34 ± 0.1) a | 0.20 (-1.42 ± 0.064) a |
| LSD (Predicted means) | 1.9 | 0.92 | 1.33 | 0.23 | 1.44 |
| P value | <0.001 | <0.001 | 0.02 | 0.16 | 0.76 |

**Table B2.3**: Effect of pH and copper formulation on mean incidence and severity of EBS and CBS in mature 'Murcott' mandarin. Values are back transformed or raw* means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatments | EBS | | CBS | |
|---|---|---|---|---|
| | Prop. of disease incidence | Mean disease severity score (0-5) | Prop. of disease incidence | Mean disease severity score (0-5)* |
| 1. Copper sulphate, pH 7 | 0.06 (-2.73 ± 0.65) ab | 0.04 (0.19 ± 0.11) a | 0.02 (-4.15 ± 0.63) bc | 1.2 (0.03 ± 0.02) bc |
| 2. Cuprous oxide, pH 7 | 0.05 (-2.86 ± 0.68) ab | 0.08 (0.28 ± 0.11) a | 0.02 (-4.13 ± 0.63) bc | 0.8 (0.02 ± 0.02) c |
| 3. Copper hydroxide (BlueShield), pH 7 | 0.01 (-4.7 ± 1.66) ab | 0.01 (0.07 ± 0.11) a | 0.0 (-13.49 ± **) | 0.0 (0.0 ± 0.02) c |
| 4. Copper hydroxide (BlueShield), pH 4 | 0.06 (-2.7 ± 0.69) ab | 0.06 (0.25 ± 0.11) a | 0.08 (-2.49 ± 0.37) a | 3.2 (0.08 ± 0.17) a |
| 5. Copper hydroxide (BlueShield), pH 11 | 0.20 (-1.37 ± 0.44) a | 0.21 (0.46 ± 0.11) a | 0.0 (-13.58 ± **) | 0 (0.0 ± 0.02) c |
| 6. Copper hydroxide (BlueShield), pH 4 → 7 | 0.07 (-2.55 ± 0.59) ab | 0.07 (0.27 ± 0.11) a | 0.01 (-4.91 ± 0.87) c | 0.8 (0.02 ± 0.02) c |
| 7. Copper sulphate, pH 4 | 0.19 (-1.44 ± 0.45) a | 0.29 (0.53 ± 0.11) a | 0.02 (-4.07 ± 0.64) c | 1.0 (0.02 ± 0.02) bc |
| 8. Copper sulphate, pH 11 | 0.06 (-2.7 ± 0.66) ab | 0.06 (0.24 ± 0.11) a | 0.02(-3.85 ± 0.57) bc | 1.0 (0.02± 0.02) bc |
| 9. Copper sulphate, pH 4 → 7 | 0.12 (-2.0 ± 0.54) ab | 0.17 (0.41 ± 0.11) a | 0.01 (-4.69 ± 0.88) bc | 0.4 (0.01 ± 0.02) c |
| 10 Water control | 0.03 (-3.51 ± 0.87) b | 0.01 (0.08 ± 0.11) a | 0.05 (-2.96 ± 0.39) ab | 3.4 (0.06 ± 0.02) ab |
| 11. Bordeaux mixture, pH 11 | 0.13 (-1.89 ± 0.51) ab | 0.16 (0.40 ± 0.11) a | 0.0 (-13.5 ± **) | 0 (0.0 ± 0.02) c |
| LSD (predicted means) | 1.91 | 0.3 | 1.74 | 0.045 |
| P value | 0.006 | 0.053 | <0.001 | 0.019 |

** No standard error output for these treatments

## Field Trial 3 (FT3): Timing and pH effects of copper applications on rind phytotoxicity in 'Murcott' mandarins, Citrus Abbotsleigh, 2024–2025

### Methodology

Field Trial 3 was conducted during the 2024–25 season to determine the effect of copper formulation, spray timing and pH influence on development of rind phytotoxicity in a commercial block of 'Murcott' mandarins at Citrus Abbotsleigh Farm, Wallaville, Queensland.

Ten treatments that comprised (i) two copper formulations (copper hydroxide and cuprous oxide), (ii) two spray pH values (pH 7 and pH 11), and (iii) two seasonal application windows: early-season (September–December) and late-season (January–July) (Table B3.1). Water controls for both pH levels and each seasonal window were included to distinguish copper-pH injury from pH-induced injury alone. Unfortunately, too few trees were available to design a complete factorial trial structure and cuprous oxide was omitted from the September-December application window. A non-amended water only treatment was omitted for the same reason.

All copper solutions were adjusted to the target pH immediately before spraying using citric acid or potassium hydroxide. Eight sprays were applied between September 2024 and May 2025 in dry weather conditions where no rainfall was forecast for a minimum of six hours post-application to minimise wash-off. Sprays were applied as dilute foliar applications to the point of run-off using the same equipment and operating parameters described in FT2.

The experimental layout followed a randomised complete block design with the ten treatments, three single-tree replicates per treatment (Table B3.1). Each treatment plot consisted of three or four contiguous trees, with guard trees between plots. The trial was established in September 2024 at approximately 50% bloom. Trees were uniform in canopy size (approx. 2 m height) and located in Block 19, a site known to express copper sensitivity during mid-summer.

FT3 used the same sampling intensity, rating scales and assessment workflow, including all in-field phytotoxicity scoring, postharvest rind-injury assessments and CBS/EBS disease assessments as described for FT2 (Appendix B, FT2 Methods). 50 fruit per tree were collected at harvest (e.g. Figure B3.1) and assessed for copper-induced rind injury, including speckling pattern, chemical run-off streaking and rough rind texture, alongside ancillary quality variables (immature fruit and stem-end greening). Assessments were conducted at the Bundaberg Research Facility within 72 hours of harvest.

Proportions of phytotoxicity (except speckle) and disease incidence were analysed with a HGLM with a fixed effect (treatment) assumed to follow a binomial distribution with a logit link and a random term (block) assumed to follow a beta distribution with a logit link. For the speckle symptoms, the HGLM did not converge and a GLMM was fitted (binomial distribution assumed, logit link function applied, terms as above). Contrasts of higher-level comparisons (e.g. copper formulation, pH level) (proportional data) were analysed using a binomial GLMM fitted for each individual contrast.

Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). A log 10 transformation was required for the EBS data to satisfy the assumption of homogeneity of variance and a square root transformation was required for the CBS data to satisfy the assumption of normality. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Table B3.1**: Treatment structure for Field Trial 3 (FT3). Copper formulation × pH × spray timing (2024–25). All copper treatments were applied at 50 g 100 $L^{-1}$. Sprays were applied monthly from September 2024 to June 2025, with harvest in July 2025. Spray pH was adjusted immediately prior to application using citric acid (pH 4) or potassium hydroxide (pH 7 or pH 11).

| Treatment No. | Formulation | pH | Application Schedule |
|---|---|---|---|
| 1 | Copper hydroxide (BlueShield®) | 7 | Monthly, September–December 2024 |
| 2 | Copper hydroxide (BlueShield®) | 11 | Monthly, September–December 2024 |
| 3 | Water | 7 | Monthly, September–December 2024 |
| 4 | Water | 11 | Monthly, September–December 2024 |
| 5 | Water | 7 | Monthly, January–July 2025 |
| 6 | Water | 11 | Monthly, January–July 2025 |
| 7 | Copper hydroxide (BlueShield®) | 7 | Monthly, January–July 2025 |
| 8 | Copper hydroxide (BlueShield®) | 11 | Monthly, January–July 2025 |
| 9 | Cuprous oxide (Nordox®) | 7 | Monthly, January–July 2025 |
| 10 | Cuprous oxide (Nordox®) | 11 | Monthly, January–July 2025 |

**Results & Discussion**

*Rind injury*

Copper formulation and solution pH significantly affected the proportion of mature fruit displaying 'chemical pattern' rind injury at harvest (Table B3.2). A higher incidence of this symptom type was recorded when cuprous oxide was applied from January-July at pH 11 (89%) than all treatments except cuprous oxide pH 7 January-July (72%), water pH 11 January-July (72%) and copper hydroxide pH 11, September-December (80%) (Table B3.2).

Copper hydroxide pH 7 September-December (47%) had the fewest symptoms but was only significantly lower than the four treatments mentioned above (Table B3.2). When comparing the two copper hydroxide treatments applied September-December, the pH 11 treatment induced almost twice as much rind injury as the pH 7 treatment. No significant differences were noted between the other paired treatments including the water pH control treatments. As per the methodology section, it was not possible to include a non-amended water treatment (grower sourced, natural occurring pH water) so it is not possible to explain the high rate of chemical pattern symptoms in both water treatments, but it may imply this symptom is not a true phytotoxicity symptom and was caused by another factor.

There were no significant effects of treatments on the incidence of immature fruit harvested (5-14%), stem-end greening (31-70%), rough textured rind (43-61%) and rind speckling (2-8%) in this trial (Table B3.3). This suggests that these symptom types may not be true phytotoxicity symptoms, although rind speckling was strongly affected by copper fungicide treatments in FT2 (previous trial above), where all copper treatments at pH 4, 7 and 11 increased this symptom type, but particularly so in the low pH treatments or in the highly soluble copper sulphate treatments. The similarity of the incidence of speckling symptoms in this trial between treatments implies that phytotoxicity symptoms are also dependent on seasonal factors. While these results imply application of copper at higher (more basic) pH values is safe in terms of phytotoxicity, we recommend utilising spray mixtures closer to neutral as per manufacturer's recommendations.

As indicated in the Methodology, subsets of data were extracted from the main data set for statistical analysis. For 'chemical pattern' symptoms, where the cuprous oxide and copper hydroxide were applied in the same period (i.e. January-July), the mean of these treatments (pH 7 plus 11) indicated cuprous oxide applied from January onwards induced chemical pattern rind injury in a significantly higher proportion of fruit than copper hydroxide applied in the same period (January-July) (Table B3.4).

When the same comparison was conducted as pH, overall, application of formulations at pH 11 recorded significantly more chemical pattern injuries than when applied at pH 7 (Table B3.5).

*Disease*

Copper formulation, application timing or pH did not affect the incidence or severity of CBS or EBS in the overall dataset analysis (Table B3.6), although the lowest incidence and severity of EBS (21% and 0.25 mean severity score, respectively) was noted in the copper hydroxide pH 7 September-December treatment.

For the subset data analysis (Table B3.7), however, less EBS (incidence and severity) was detected in treatments where copper hydroxide was applied in September-December compared with January-July. This may reflect the importance of copper coverage during the petal fall/early fruit growth stages of development which are known EBS susceptibility periods. Late autumn can also be problematic for EBS during favourable weather, although January-July sprays were not effective against EBS in this trial. For CBS, the mean incidence and disease severity scores for copper hydroxide application between the two time periods were not significantly different from each other, although there was a trend of lower CBS in fruit treated from September to December compared to January-July, particularly for disease severity. A stronger effect from early season sprays would have been expected given the key CBS susceptibility period aligning with the September-December sprays.

CBS was significantly affected when comparing application of copper hydroxide and cuprous oxide in January to July in the subset data analysis, with less disease noted in cuprous oxide treatments (T9 and 10) than copper hydroxide treatments (T7 and 8) (Table B3.8). While there was no significant difference noted for EBS incidence for this comparison, EBS disease severity score, like CBS, was lower in the cuprous oxide treatments (T9 and 10) compared to the copper hydroxide treatments (T7 and 8).

In conclusion, the low incidence of rind speckling injury in FT3 contrasts with the stronger speckling observed in FT2 and indicates that phytotoxicity is seasonally dependent, with acidic conditions likely to be a more important driver of speckling than alkaline conditions. The relatively high incidence of chemical runoff patterns in the water controls of FT3 suggests that this symptom may not be a true phytotoxicity symptom and may be caused by another currently unknown factor. That said, cuprous oxide (January-July) did increase the incidence of chemical runoff pattern in FT3 compared to corresponding copper hydroxide treatments (averaged over pH) but did give better control of EBS and CBS than copper hydroxide. However, cuprous oxide sprays (January-July) did not increase speckling injury in FT2 any more than copper hydroxide. Collectively, FT3 reinforces that copper fungicide performance is shaped by formulation, pH and seasonal timing, and supports the recommendation that red copper products be used cautiously after January when rind sensitivity increases and residue accumulation becomes more apparent.

**Table B3.2**: Proportion of fruit assessed showing chemical pattern symptoms across copper treatments. Values are back transformed means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment | Application Schedule | Chemical pattern proportion |
|---|---|---|
| 1. Copper hydroxide* pH 7 | Monthly, Sep. – Dec. | 0.47 (−0.12 ± 0.34) c |
| 2. Copper hydroxide* pH 11 | Monthly, Sep. – Dec. | 0.8 (1.36 ± 0.41) ab |
| 3. Water pH 7 | Monthly, Sep. – Dec. | 0.61 (0.43 ± 0.36) bc |
| 4. Water pH 11 | Monthly, Sep. – Dec. | 0.62 (0.49 ± 0.36) bc |
| 5. Water pH 7 | Monthly, Jan. – July | 0.65 (0.63 ± 0.35) bc |
| 6. Water pH 11 | Monthly, Jan. – July | 0.72 (0.93 ± 0.36) ab |
| 7. Copper hydroxide pH 7 | Monthly, Jan. – July | 0.65 (0.61 ± 0.36) bc |
| 8. Copper hydroxide pH 11 | Monthly, Jan. – July | 0.59 (0.38 ± 0.36) bc |
| 9. Cuprous oxide** pH 7 | Monthly, Jan. – July | 0.72 (0.99 ± 0.43) ab |
| 10. Cuprous oxide** pH 11 | Monthly, Jan. – July | 0.89 (2.12 ± 0.61) a |
| LSD (predicted means) | | 1.09 |
| P value | | 0.01 |

*Applied as *BlueShield® or **Nordox®

**Figure B3.1**: Citrus team rating phytotoxicity symptoms and disease incidence and severity at the Bundaberg Research Facility (Image credit: T. Thangavel).

**Table B3.3**: Effect of copper treatments on the proportion of immature fruit, stem greening, rough rind and speckling symptoms in mature fruit. Values are back transformed means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment | Immature fruit proportion | Stem greening proportion | Rough rind proportion | Speckling pattern proportion |
|---|---|---|---|---|
| 1. Copper hydroxide* pH 7 | 0.05 (−2.91 ± 0.72) a | 0.35 (−0.63 ± 0.61) a | 0.54 (0.15 ± 0.41) a | 0.02 (−3.90 ± 0.64) a |
| 2. Copper hydroxide* pH 11 | 0.06 (−2.79 ± 0.58) a | 0.7 (0.84 ± 0.63) a | 0.55 (0.19 ± 0.41) a | 0.06 (−2.81 ± 0.39) a |
| 3. Water pH 7 | 0.14 (−1.82 ± 0.44) a | 0.35 (−0.63 ± 0.63) a | 0.45 (−0.21 ± 0.42) a | 0.08 (−2.52 ± 0.36) a |
| 4. Water pH 11 | 0.12 (−2.02 ± 0.49) a | 0.5 (0.00 ± 0.62) a | 0.43 (−0.28 ± 0.43) a | 0.06 (−2.78 ± 0.41) a |
| 5. Water pH 7 | 0.07 (−2.59 ± 0.54) a | 0.43 (−0.30 ± 0.59) a | 0.45 (−0.22 ± 0.41) a | 0.03 (−3.41 ± 0.51) a |
| 6. Water pH 11 | 0.09 (−2.28 ± 0.56) a | 0.33 (−0.72 ± 0.60) a | 0.44 (−0.25 ± 0.4) a | 0.05 (−2.97 ± 0.41) a |
| 7. Copper hydroxide pH 7 | 0.09 (−2.33 ± 0.50) a | 0.36 (−0.58 ± 0.61) a | 0.51 (0.06 ± 0.42) a | 0.05 (−3.02 ± 0.44) a |
| 8. Copper hydroxide pH 11 | 0.09 (−2.30 ± 0.51) a | 0.32 (−0.75 ± 0.64) a | 0.55 (0.18 ± 0.42) a | 0.06 (−2.79 ± 0.41) a |
| 9. Cuprous oxide** pH 7 | 0.07 (−2.66 ± 0.62) a | 0.31 (−0.81 ± 0.72) a | 0.49 (−0.03 ± 0.45) a | 0.03 (−3.42 ± 0.59) a |
| 10. Cuprous oxide** pH 11 | 0.14 (−1.79 ± 0.44) a | 0.63 (0.51 ± 0.70) a | 0.61 (0.43 ± 0.47) a | 0.03 (−3.60 ± 0.59) a |
| LSD (predicted means) | 1.42 | 1.68 | 0.96 | 1.37 |
| P value | 0.81 | 0.43 | 1.03 | 0.65 |

*Applied as *BlueShield® or **Nordox®

**Table B3.4:** Effect of copper formulation on incidence of chemical pattern, speckle, immature fruit, stem greening, rough rind and symptoms in copper treatments applied January - July, when averaged over pH of spray mixture. Values are back-transformed means. Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment code | Incid. chem pattern (proportion) | Incid. speckle (proportion) | Incid. immature fruit (proportion) | Incid. stem-end greening (proportion) | Incid. rough rind (proportion) |
|---|---|---|---|---|---|
| Copper hydroxide (Jan-July) | 0.62 a | 0.06 a | 0.09 a | 0.34 a | 0.53 a |
| Cuprous oxide (Jan-July) | 0.81 b | 0.03 a | 0.11 a | 0.47 a | 0.55 a |
| **P value** | **0.013** | **ns** | **ns** | **ns** | **ns** |

**Table B3.5:** Overall effect of spray mixture pH on incidence of chemical pattern, speckle, immature fruit, stem greening, rough rind and symptoms in copper treatments, when averaged over all application times and formulation type. Values are back-transformed means. Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment code | Incid. chem pattern (proportion) | Incid. speckle (proportion) | Incid. immature fruit (proportion) | Incid. stem-end greening (proportion) | Incid. rough rind (proportion) |
|---|---|---|---|---|---|
| pH 7 treatments | 0.62 a | 0.04 a | 0.08 a | 0.36 a | 0.49 a |
| pH 11 treatments | 0.72 b | 0.05 a | 0.10 a | 0.50 a | 0.52 a |
| **P value** | **0.020** | **ns** | **ns** | **ns** | **ns** |

**Table B3.6**: Effect of copper treatments on the proportion of CBS and EBS affected fruit (incidence) and mean CBS and EBS severity scores. Values are back-transformed means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment code | Proportion CBS | Proportion EBS | CBS mean severity score (0-5) | EBS mean severity score (0-5) |
|---|---|---|---|---|
| 1. Copper hydroxide* pH 7 | 0.47 (-0.12 ± 0.35) a | 0.21 (-1.34 ± 0.49) a | 0.71 (0.84 ± 0.12) a | 0.25 (−0.60 ± 0.16) a |
| 2. Copper hydroxide* pH 11 | 0.39 (-0.45 ± 0.36) a | 0.33 (-0.72 ± 0.40) a | 0.57 (0.76 ± 0.12) a | 0.31 (−0.51 ± 0.14) a |
| 3. Water pH 7 | 0.51 (0.02 ± 0.35) a | 0.46 (-0.16 ± 0.38) a | 0.85 (0.92 ± 0.12) a | 0.60 (−0.22 ± 0.14) a |
| 4. Water pH 11 | 0.46 (-0.14 ± 0.35) a | 0.34 (-0.66 ± 0.50) a | 0.69 (0.83 ± 0.12) a | 0.41 (−0.39 ± 0.18) a |
| 5. Water pH 7 | 0.39 (-0.43 ± 0.36) a | 0.36 (-0.55 ± 0.38) a | 0.56 (0.75 ± 0.12) a | 0.51 (−0.30 ± 0.14) a |
| 6. Water pH 11 | 0.47 (-0.10 ± 0.35) a | 0.47 (-0.10 ± 0.37) a | 0.76 (0.87 ± 0.12) a | 0.66 (−0.18 ± 0.14) a |
| 7. Copper hydroxide pH 7 | 0.45 (-0.18 ± 0.35) a | 0.43 (-0.26 ± 0.38) a | 0.78 (0.88 ± 0.12) a | 0.63 (−0.20 ± 0.14) a |
| 8. Copper hydroxide pH 11 | 0.49 (-0.04 ± 0.36) a | 0.46 (-0.18 ± 0.39) a | 0.86 (0.93 ± 0.12) a | 0.69 (−0.16 ± 0.14) a |
| 9. Cuprous oxide** pH 7 | 0.33 (-0.70 ± 0.37) a | 0.4 (-0.41 ± 0.39) a | 0.44 (0.66 ± 0.12) a | 0.45 (−0.35 ± 0.14) a |
| 10. Cuprous oxide** pH 11 | 0.28 (-0.95 ± 0.38) a | 0.32 (-0.77 ± 0.41) a | 0.35 (0.59 ± 0.12) a | 0.31 (−0.50 ± 0.14) a |
| LSD (Predicted means) | 0.86 | 1.91 | 0.27 | 0.36 |
| P value | 0.36 | 0.45 | 0.23 | 0.17 |

*Applied as *BlueShield® or **Nordox®

**Table B3.7:** Effect of application timing on incidence and severity of EBS and CBS in copper hydroxide treatments, when averaged over pH of spray mixture. Values are back-transformed means. Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment code | Incidence EBS (proportion) | EBS mean severity score | Incidence CBS (proportion) | CBS mean severity score |
|---|---|---|---|---|
| Copper hydroxide (Sep-Dec) | 0.27 a | 0.28 a | 0.43 a | 0.64 a |
| Copper hydroxide (Jan-July) | 0.45 b | 0.66 b | 0.47 a | 0.82 a |
| **P value** | **0.033** | **0.005** | **ns** | **ns** |

**Table B3.8:** Effect of copper formulation on incidence and severity of EBS and CBS in copper treatments applied January - July, when averaged over pH of spray mixture. Values are back-transformed means. Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment code | Incidence EBS (proportion) | EBS mean severity score | Incidence CBS (proportion) | CBS mean severity score |
|---|---|---|---|---|
| Copper hydroxide (Jan-July) | 0.45 a | 0.66 a | 0.47 a | 0.82 a |
| Cuprous oxide (Jan-July) | 0.36 a | 0.38 b | 0.31 b | 0.40 b |
| **P value** | **ns** | **0.049** | **0.022** | **0.005** |

## Appendix C

### Theme 3: Better timing of fungicide application for the control of CBS and EBS

**Background**

Understanding the importance and effectiveness of fungicide timing is becoming increasingly critical as practical constraints on fungicide use continue to grow. Copper phytotoxicity risk, residue compliance, and limitations on mancozeb availability have all contributed to uncertainty about how many sprays are necessary to simultaneously control disease, limit phytotoxicity and when they should be applied within the season.

Fungicide timing is thought to play a key role in suppression of CBS and EBS in mandarin grown in warm, humid subtropical conditions but growers currently lack clear field-based guidance with respect to whether meaningful increases in packout of premium-grade fruit can be made by adjusting spray timing, tightening spray intervals, or applying products earlier in the season.

Two major knowledge gaps persist: 1) whether early-season copper applications provide measurable benefit with respect to disease control under differing levels of canopy inoculum, e.g. between orchards; and (2) whether pre-petal-fall fungicides targeting canopy/orchard inoculum levels can reduce the incidence of EBS or CBS later in the mature fruit. The latter is of particular importance with respect to EBS where risk of infection is high throughout the end of the growing season.

The work therefore aimed to establish if changes in the timing of fungicide inputs could overcome biological constraints such as inoculum load and peak infection periods, or whether these factors ultimately limit the value of timing adjustments. The trials presented in this section preceded or were conducted simultaneously with trials incorporating copper presented in Appendix B.

The amount of inoculum present in the canopy and orchard also plays a large role in the efficacy of any fungicide applied. Field observations in Australian commercial orchards compliment findings from the Joint Florida and Australian Citrus Black Spot Research Initiative (CT13021) that indicated canopy-borne inoculum, particularly that from retained dead wood, may influence CBS development more than refinements in copper spray timing (Drenth, 2018). This needed to be investigated under subtropical growing conditions. Similarly, industry interest in early-season EBS management raised whether fungicides applied at petal fall could meaningfully reduce *Alternaria* inoculum levels and/or infection before the onset of the main infection period.

To resolve these gaps, four field trials were established in 'Imperial' (FT1 & FT2), 'Daisy' (FT3) and 'Murcott' (FT4) mandarins to test how spray timing affects canopy inoculum and seasonal disease pressure, measured by the amount of disease recorded in mature fruit. Field Trials 1 and 2 examined copper application schedules under contrasting canopy inoculum conditions (dead wood added versus no dead wood), focusing on whether early-season copper, fortnightly intervals, or rainfall-triggered sprays provide advantages with respect to disease control when canopy inoculum levels vary. Field Trial 3 evaluated if intensive pre-petal-fall programs could reduce EBS and CBS disease at harvest in 'Daisy' mandarin by focussing sprays before and/or during the main infection window. Field Trial 4 extended this question by evaluating the same petal-fall programs in two separate growing seasons in 'Murcott' mandarin, comparing sprays applied alone or embedded within a grower program to determine whether early inputs alter EBS or CBS outcomes at harvest.

Together, these trials were designed to determine the practical value of early copper sprays, the benefit of tightening spray intervals during periods of high susceptibility, and the extent to which early-season fungicides can influence harvest outcomes when disease pressure differs across seasons. The coordinated structure of these studies provides a comprehensive framework for interpreting

fungicide timing within subtropical citrus systems and clarifies which components of the spray calendar contribute meaningfully to disease suppression.

## Field Trial 1 (FT1): Effect of timing of early-season copper application and canopy management on CBS in 'Imperial' mandarin Part I (Wallaville, 2022–2023)

### Methodology

Field Trial 1 (FT1) was conducted during the 2022–23 season to evaluate the influence of canopy hygiene and early-season copper fungicide timing on CBS in mature fruit. The trial was established in a commercial 'Imperial' mandarin block at Spencer Ranch, Wallaville, Queensland, within a large rootstock trial site.

Fourteen treatments were evaluated; two overarching canopy hygiene treatments to investigate the effect of canopy inoculum on disease and seven copper fungicide application timing treatments to investigate the efficacy of copper fungicides on control of CBS as measured in mature fruit (Table C1.1).

Canopy hygiene treatments, the presence or absence of dead wood, were used to create contrasting inoculum conditions. In treatments 1–7, freshly harvested, living, lemon branches (approximately 0.75 m long) were inserted into the centre of the canopy to simulate retained pruning debris acting as an inoculum source. Treatments 8–14 had no added dead wood and served as the clean-canopy comparison. The lemon branches were harvested from an adjacent lemon orchard with a known high level of incidence of CBS. Therefore, whether canopy components are sources of CBS inoculum is also tested here.

Copper fungicide application timing treatments were used to begin investigation of whether timing of copper application affects infection by CBS as measured by disease in fruit at the end of the growing season. Copper treatments included combinations of monthly applications from August to November including a pre-rainfall application triggered by forecasts of >25 mm rainfall to target early season fruit susceptibly and growth. Copper hydroxide (Blue Shield® DF; 50% metallic copper equivalent) was applied at the label rate 100 g 100 $L^{-1}$ as dilute foliar sprays delivered to the point of run-off using a calibrated air-blast sprayer.

The investigation of the pre-rainfall application in this trial also represents trialling of the South African (Citrus Research International) modelling system CBS CRI-PhytRisk under Australian conditions in mandarin production.

The trial used a randomised block design with five single-tree replicates per treatment. Trees were eight years old and uniform in canopy structure. The 'Imperial' mandarin scions used here were grafted onto a range of rootstocks but previous analysis of disease in this trial site had indicated there were no differences in resistance to CBS inferred by the different rootstocks.

At fruit maturity, in-field disease assessments were completed prior to harvest to quantify natural CBS expression. Five assessors independently scored each tree by circling the canopy for approximately one minute and recording the number of fruits exhibiting CBS symptoms. Assessors were unaware of treatment identity to eliminate treatment bias.

50 fruit per tree were harvested at fruit maturity from evenly around the canopy. Fruit were assessed for disease (CBS) within 24 hours of harvest on the 0-10 rating scale as per Appendix E. After assessment, fruit were processed as per the standard ethephon–imazalil protocol described in Appendix E before reassessment to quantify latent infection expression.

Disease incidence was analysed with a HGLM with main effect (treatment) and fixed effect (interaction of dead wood and copper treatment) assumed to follow a binomial distribution with a logit link, and a random term (block) assigned and assumed to follow a beta distribution with a logit link. Pre-

incubation, the HGLM did not converge, so a GLMM was then fitted (binomial distribution assumed, logit link function applied, terms as above) but the variance components of the GLMM were bound, so a GLM was fitted instead (terms as above, binomial distribution, logit link, predicted means presented as proportions). Pre- and post-incubation, no significant interaction of the interaction of dead wood and copper and the terms were dropped from the respective models. Mean severity was analysed with an ANOVA with the terms as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). Pre-incubation a log10 transformation was conducted to satisfy the homogeneity of variance assumption and a small constant of 0.1 was added prior to the transformation to account for the trees with no disease. No transformation was required post-incubation. The analyses were repeated as above ignoring the factorial component. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Table C1.1**: Treatment structure for early-season copper timing and canopy hygiene in Field Trial 1 (Wallaville, 2022-2023)

| Treatment | Dead wood | Copper applications |
|---|---|---|
| 1 | Present | August, September, October |
| 2 | Present | September, October |
| 3 | Present | September, October, November |
| 4 | Present | November |
| 5 | Present | Pre-rainfall (January > 25mm) |
| 6 | Present | September, October, pre-rainfall (January > 25mm) |
| 7 | Present (untreated control) | None |
| 8 | Absent | August, September, October |
| 9 | Absent | September, October |
| 10 | Absent | September, October, November |
| 11 | Absent | November |
| 12 | Absent | Pre-rainfall (January > 25mm) |
| 13 | Absent | September, October, pre-rainfall (January > 25mm) |
| 14 | Absent (untreated control) | None |

**Results & Discussion**

This field trial successfully demonstrated canopy material can be a source of CBS inoculum and, where it is present, the incidence and severity of CBS in mature fruit is significantly greater. The trial also showed copper fungicides can effectively reduce CBS in 'Imperial' mandarin fruit but, depending on the timing of copper application, may not necessarily reduce disease more than having low levels of canopy inoculum alone. This is a significant initial contribution in understanding the importance of CBS inoculum in the canopy and of canopy hygiene with respect to disease-free, mature, harvested fruit.

For the in-field disease assessment, there was a highly significant effect of dead wood on the mean number of fruit with CBS on each tree (Figure C1.1). Where dead wood was present in the canopy, regardless of copper spray program, the mean number of diseased fruit was significantly higher than those without dead wood. The only exception to this was between Treatment 1 (trees with dead wood receiving sprays in August, September & October) and Treatment 13 (trees without dead wood receiving sprays in September, October & pre-rainfall in January), which were not significantly different from each other. Among trees without dead wood, there were no effects of different copper spray programs on CBS levels. However, for trees with dead wood, the highest disease levels were unsurprisingly seen in trees either receiving no copper sprays in the treatment period (Treatment 7) or only a single spray (Treatments 4 & 5). The best spray programs overall among trees with dead wood were those receiving three copper sprays in the treatment period, particularly when an early spray in August was included (Treatment 1).

In harvested mature fruit, the presence of dead wood in the canopy also consistently and significantly increased incidence of disease and disease severity pre- and post-incubation (Table C1.2, C1.3). When the effect of dead wood itself was considered irrespective of copper treatment, the presence of dead wood in the canopy significantly increased the incidence of CBS in fruit both at harvest and post-incubation ($p<0.001$) (Table C1.2). When copper treatments were considered irrespective of dead wood treatments, however, there was no significant impact of copper treatment (timing) pre- or post-incubation ($F_{(6,58)} = 2.13$; $p = 0.063$; $L_{(6)} = 2.74$; $p = 0.841$ respectively).

When dead wood and copper were analysed together (Table C1.3), the results again indicated the most important factor for the presence of disease in harvested fruit in the 2022-2023 growing season was the inclusion of dead wood in the canopy. In the presence of dead wood, the incidence of CBS ranged from 10 to 51% compared with 5 to 14% when dead wood was absent. The control treatments (dead wood added versus no dead wood, nil copper fungicide application, treatments 7 and 14) also illustrated the effect of increased inoculum in the canopy with incidence and severity increasing significantly from 12% and 0.14 lesions per fruit respectively to 51% and 1.21 lesions respectively when dead wood was added.

Despite few significant differences between individual treatments within the dead wood present component, and none in the dead wood absent component, adequate disease was recorded when dead wood was present to glean insights into effective application windows of copper for the control of CBS (Table C1.3).

Trends indicated early season application of copper provided the most effective control of CBS with the incidence of CBS at harvest lower in treatments 1, 2 3 and 6 (12, 14, 12 and 10% respectively) than in the remainder of the treatments (42 – 51%) including the water control treatment. One-off sprays applied in November (T4) or in early January (T5), however, had no impact on the incidence of CBS in fruit at harvest in comparison with the control treatment (42 and 50% versus 51% respectively). In terms of disease severity, the only significant difference was copper applied in August, September and October having significantly lower disease severity than the November application, January

application or control treatment.

Rain-forecast sprays did not provide consistent reductions compared with adjacent timing programs. The intended November pre-rainfall application was not applied because the CRI-PhytRisk CBS-risk did not register rainfall events adequately and output indicated the risk remained low during this period despite the local area forecasts for rain. The later January spray was therefore scheduled manually using local rainfall forecasts. As this application occurred outside the primary susceptible window, its performance does not reflect the effectiveness of a true pre-rainfall timing. Use of copper and other protectants in relation to fungal infection is a well-known disease protection strategy and requires further investigation in mandarin in the subtropics. If the system and modelling behind the programs could be updated and tested for Australian conditions, it would be a useful tool for IDM-based control of CBS

These results show that canopy inoculum level had a stronger influence on CBS outcomes than the copper timing programs assessed here. The consistently higher disease levels in dead wood treatments confirm that increased inoculum can negate the effectiveness of fungicides with respect to disease control and suggests tree hygiene is a priority. Early-season copper programs reduced disease relative to the untreated and late-season only treatments, although none provided strong suppression under elevated inoculum pressure, indicating early fungicide application is key to CBS control.

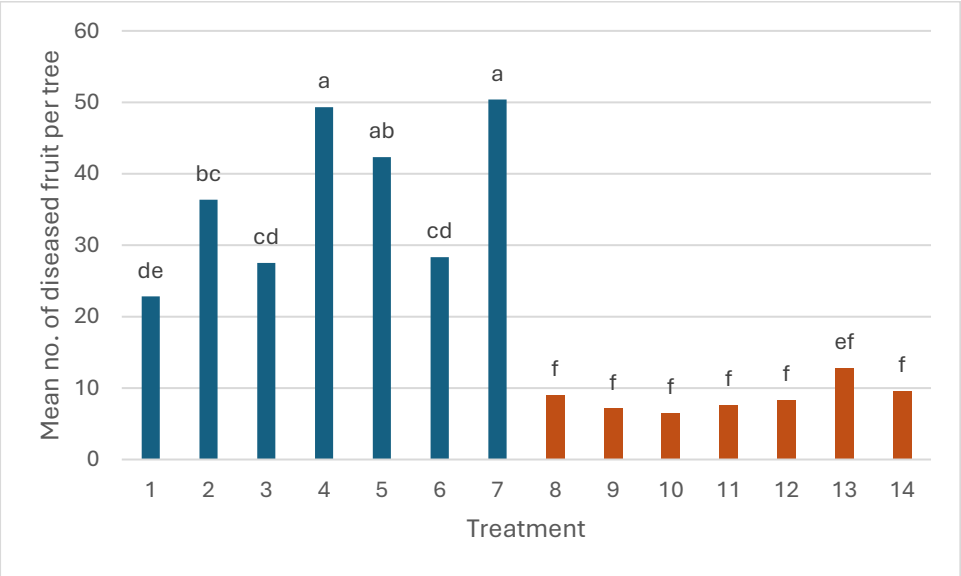


**Figure C1.1**: In-field visual disease count (mean no. of fruit with disease counted during a 30s pass of each tree, averaged across 5 assessors). Treatments surmounted by the same letter are not significantly different according to Fisher's least significant difference (LSD) test at $P \leq 0.05$.

Treatments 1–7 (blue bars) included dead wood and treatments 8–14 (orange bars) had no dead wood. T1-7 copper spray programs: **T1** August–September–October program, **T2** September–October program, **T3** September–October-November program, **T4** November-only program, **T5** January pre-rainfall program, **T6** September–October- January pre-rainfall program, **T7** no copper. **Treatments 8–14** mirrored Treatments 1-7 spray programs, but without dead wood (Full treatment details: Table C1.1).

**Table C1.2**: Effect of dead wood in the canopy on incidence and severity of CBS. Values are back transformed or raw* means followed by (predicted mean ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| | Pre-Incubation | | Post-Incubation | |
|---|---|---|---|---|
| **Dead wood Treatments** | **Prop. of disease incidence*** | **Mean disease severity score (0-10)** | **Prop. of disease incidence** | **Mean disease severity score* (0-10)** |
| Present | 0.32 (-0.67 ± 0.03) a | 0.6 (0.15 ± 0.06) a | 0.67 (0.75 ± 0.19) a | 2.45 (2.44 ± 0.14) a |
| Absent | 0.09 (-0.91 ± 0.02) b | 0.1 (-0.70 ± 0.06) b | 0.47 (-0.12 ± 0.18) b | 0.93 (0.97 ± 0.14) b |
| LSD** | 0.08 | 0.17 | 0.42 | 0.39 |
| P value | <0.001 | <0.001 | <0.001 | <0.001 |

**Predicted means

**Table C1.3**: Effect of copper fungicide spray timing and canopy inoculum level (dead wood added *vs.* not added) on CBS incidence and severity in 'Imperial' mandarin (Wallaville, Queensland; 2022–2023). Values are back transformed or raw* means followed by (predicted mean ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| | | Pre-Incubation Assessment | | Post-Incubation Assessment | |
|---|---|---|---|---|---|
| **Treatments** | **Dead wood** | **Prop. of disease incidence** | **Mean disease severity score (0-10)** | **Prop. of disease incidence** | **Mean disease severity score (0-10)*** |
| 1.August, September, October | Present | 0.12 (-2.00 ± 0.53) cde | 0.17 (-0.57 ± 0.16) bcd | 0.67 (0.73 ± 0.41) abc | 2.04 (2.04 ± 0.37) abc |
| 2. September, October | Present | 0.14 (-1.15 ± 0.48) abcde | 0.49 (-0.23 ± 0.16) abc | 0.77 (1.24 ± 0.46) a | 2.82 (2.86 ± 0.37) a |
| 3. September, October, November | Present | 0.12 (-1.01 ± 0.41) abc | 0.57 (-0.17 ± 0.16) ab | 0.67 (0.69 ± 0.46) abcd | 2.26 (2.23 ± 0.37) ab |
| 4. November | Present | 0.42 (-0.33 ± 0.39) ab | 0.8 (-0.05 ± 0.16) a | 0.62 (0.49 ± 0.40) abcd | 2.52 (2.43 ± 0.37) a |
| 5. Pre-rainfall (January > 25mm) | Present | 0.5 (0.01 ± 0.40) ab | 1.18 (-0.11 ± 0.16) a | 0.66 (0.65 ± 0.4) abc | 2.63 (2.6 ± 0.37) a |
| 6. September, October, pre-rainfall (January > 25mm) | Present | 0.1 (-1.14 ± 0.43) bcd | 0.44 (-0.27 ± 0.16) abc | 0.66 (0.67 ± 0.41) abc | 1.95 (1.99 ± 0.37) abc |
| 7. None | Present | 0.51 (0.03 ± 0.39) a | 1.21 (0.12 ± 0.16) a | 0.68 (0.78 ± 0.41) ab | 2.9 (2.92 ± 0.37) a |
| 8. August, September, October | Absent | 0.14 (-1.84 ± 0.5) de | 0.17 (-0.56 ± 0.16) bcd | 0.42 (-0.31 ± 0.38) cde | 0.81 (0.79 ± 0.37) d |
| 9. September, October | Absent | 0.05 (-2.99 ± 0.8) e | 0.04 (-0.85 ± 0.16) d | 0.39 (-0.44 ± 0.38) de | 0.75 (0.76 ± 0.37) d |
| 10. September, October, November | Absent | 0.06 (-2.81 ± 0.74) de | 0.06 (-0.79 ± 0.16) d | 0.32 (-0.75 ± 0.40) e | 0.57 (0.57 ± 0.37) d |
| 11. November | Absent | 0.1 (-2.24 ± 0.58) cde | 0.13 (-0.65 ± 0.16) cd | 0.53 (0.1 ± 0.38) abcde | 1.24 (1.27 ± 0.37) bcd |
| 12. Pre-rainfall (January > 25mm) | Absent | 0.09 (-2.32 ± 0.61) cde | 0.12 (-0.66 ± 0.16) cd | 0.61 (0.44 ± 0.39) abcd | 1.32 (1.36 ± 0.37) bcd |
| 13. September, October, pre-rainfall (January > 25mm) | Absent | 0.08 (-2.44 ± 0.72) cde | 0.07 (-0.77 ± 0.16) d | 0.48 (-0.08 ± 0.43) bcde | 0.92 (1.11 ± 0.37) cd |
| 14. None | Absent | 0.12 (-1.97 ± 0.88) cde | 0.14 (-0.63 ± 0.16) d | 0.53 (0.11 ± 0.38) abcde | 0.93 (0.94 ± 0.37) d |
| LSD (predicted means) | | 1.55 | 0.44 | 1.11 | 1.04 |
| P value | | <0.001 | <0.001 | 0.009 | <0.001 |

Disease incidence was calculated as the proportion of fruits exhibiting CBS symptoms. Disease severity was determined using a 0–10 scale, where 0 = no visible lesions and 10 = severe rind damage with coalescing necrotic tissue.

## Field Trial 2 (FT2): Effective application schedules for managing CBS using copper fungicides (fortnightly versus monthly application) (Timing plus dead wood Part II) (Wallaville, 2023–2024)

### Methodology

Field Trial 2 (FT2) was conducted during the 2023–24 growing season to determine whether the efficacy of copper with respect to control of CBS in mandarin could be improved by increased frequency of applications during the early season when fruit are susceptible to CBS infection compared with standard monthly applications. The trial was established in a commercial 'Imperial' mandarin orchard at Spencer Ranch, Wallaville, Queensland.

Nine treatments were used to evaluate fortnightly and monthly copper applications throughout the season under high inoculum pressure (added dead wood) to manage CBS in 'Imperial' mandarin (Table C2.1). Sprays were applied fortnightly or monthly in the three seasonal phases of the growing season: spring (September–November), summer (December–February), and autumn (March–April). Dead wood was added to the canopies to increase the available inoculum and simulated unpruned trees or trees where pruning debris is not been removed which is typical in commercial canopies.

Copper hydroxide (BlueShield®, UPL; 50% metallic copper equivalent) was applied at the label rate (100 g 100 $L^{-1}$) in all copper treatments. Dead wood was added into the centre of the canopy in the form of small lemon branches harvested from a lemon orchard with documented high levels of CBS. Trees were approximately 2.5 m in height, uniform in canopy structure, and maintained under standard grower practice (except fungicide). All applications were delivered as dilute foliar sprays to the point of run-off using a calibrated air-blast sprayer. The trial followed a randomised complete block design with nine treatments and five single-tree replicates per treatment.

At harvest, 50 fruit per tree were collected from across the canopy and scored for CBS symptoms using the standard 0–5 severity scale described in the Appendix E. Post-incubation assessments followed the ethephon-imazalil based incubation procedure in Appendix E to allow expression of latent lesions before re-scoring.

Disease incidence was first analysed with a HGLM with main effect (treatment) assumed to follow a binomial distribution with a logit link, and a random term (block) assigned and assumed to follow a beta distribution with a logit link. For pre- and post-incubation results, the HGLM did not converge, so a GLMM was then fitted (binomial distribution assumed, logit link function applied, terms as above). For pre-incubation data, the variance components of the GLMM were bound, so a GLM was fitted instead (terms as above, binomial distribution, logit link, predicted means presented as proportions). Mean severity was analysed with a linear mixed model with treatment as the fixed effect and block as the random term (diagnostic plots used to confirm assumptions of normality and homogeneity of variance were satisfied). For pre-incubation data, a square root transformation was conducted to satisfy the assumption of homogeneity of variance. No transformation was required post-incubation. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Table C4.1**: Seasonal spray scheduling and dead wood (DW) status for CBS copper timing treatments in Field Trial 2 (Wallaville, 2023–2024).

| Treatment | Spring (Sep.–Nov.) | Summer (Dec.–Feb.) | Autumn (Mar.–Apr.) | Dead wood |
|---|---|---|---|---|
| 1. FFM +DW | ✓ Fortnightly | ✓ Fortnightly | ✓ Monthly | Present |
| 2. FMM +DW | ✓ Fortnightly | ✓ Monthly | ✓ Monthly | Present |
| 3. FUM +DW | ✓ Fortnightly | ✗ Untreated | ✓ Monthly | Present |
| 4. FFU +DW | ✓ Fortnightly | ✓ Fortnightly | ✗ Untreated | Present |
| 5. MFM +DW | ✓ Monthly | ✓ Fortnightly | ✓ Monthly | Present |
| 6. MUM +DW | ✓ Monthly | ✗ Untreated | ✓ Monthly | Present |
| 7. UFM +DW | ✗ Untreated | ✓ Fortnightly | ✓ Monthly | Present |
| 8. UUU +DW | ✗ Untreated | ✗ Untreated | ✗ Untreated | Present |
| 9. UUU | ✗ Untreated | ✗ Untreated | ✗ Untreated | Absent |

All copper sprays consisted of copper hydroxide (BlueShield®; 50% metallic copper equivalent) applied at 100 g 100 $L^{-1}$. Spray applications commenced on 28 September 2023 and concluded on 1 April 2024. Fortnightly and monthly intervals are shown in the table. Dead wood (DW) refers to small lemon branches (approximately 0.75 m long) manually placed into the centre of the canopy to simulate pruning debris retained after pruning.

**Results & Discussion**

Results of this trial showed early, frequent, copper application is effective for control of CBS, that spring and summer are the most important periods for copper application and that fungicide alone cannot compensate for increased CBS inoculum in the canopy. This builds upon the initial understanding of the role and importance of dead wood in the canopy and early season fungicide application for disease control established in FT1.

The incidence of CBS in mature fruit at harvest (before incubation) was low in most treatments, ranging from 2-18% in seven of the nine treatments and up to 43% in the remainder (Table C2.2). Significantly more infected fruit were recorded in the control treatment (T8, UUU + dead wood, 43%) where no fungicide was applied than in all other treatments except treatment 6 (MUM, 32%).

This dead wood control treatment (T8) also recorded significantly more CBS than the paired no added dead wood control treatment (T9, UUU, no dead wood, 16%), a clear indication that a) the presence of dead wood in the canopy is an inoculum source that contributes significantly to fruit disease and b) that fungicide applications cannot necessarily overcome high inoculum loads in the canopy.

Fortnightly coverage from September to February (T4, FFU, 2%) recorded significantly less disease than all treatments except treatments 1 (FFM, 5%) and 2 (FMM, 3%). While there was considerably less disease recorded in treatments 1 and 2 than in all treatments except treatment 4, they were not significantly different from any treatment except treatments 6 and 8 mentioned above. These trends indicate consistent copper coverage in the early to mid- portion of the season is most important for control of CBS, aligning with current understanding of the CBS infection period, and needs further exploration in other seasons under high disease pressure conditions.

While monthly-fortnightly-monthly coverage (T5, MFM, 12%) showed higher disease incidence than treatments 1, 2 or 4 described above, the difference was only significant in comparison with treatment 4 (FFU, 2%) and supports early season coverage being the key to disease control.

The results also indicate an unsprayed period in the early or mid-season, before or after fortnightly spray applications (e.g. treatments 3 (FUM, 17%) and 7 (UFM, 18%)), is less effective for control of CBS than consistent fortnightly early and mid-season applications, although again the results were not significantly different from treatments 1 or 2. Where the early applications are monthly and followed by an unsprayed period (T6, MUM, 32%), incidence of disease was equivalent to not applying copper at all.

Post-incubation assessments resulted in increased symptom expression across all treatments, with incidence reaching up to 68% in treatment 8 (UUU + DW). Again the lowest incidence was recorded in treatment 4 (FFU, 34%) but this wasn't significantly lower than treatments 1, 2, or 5. Treatment 1, 2 and 4 were, however, less than treatments 3 (FUM), 6 (MUM), 7 (UFM) and 8 (UUU+DW), again indicating early and consistent coverage is of primary importance for control of CBS.

Mean disease severity ratings (number of lesions per fruit) followed similar trends with few significant differences between many of the treatments. The lowest severity ratings were recorded in treatments 1, 2, 4 and the highest in treatments 6 and 8. The recordings for treatments 1, 2 and 4 were very low and indicate that diseased fruit still had very few spots per fruit and the majority would have been saleable.

After incubation, the effect of addition of dead wood to the untreated control was very clearly seen with significantly higher disease severity than all other treatments including the counterpart control treatment. Again, severity was lowest in treatments with consistent early season coverage and higher

disease recorded when mid-season coverage was omitted, even after early fortnightly coverage.

These results show that increased spray frequency during the spring–summer susceptibility period can significantly improve CBS suppression relative to reduced or no-spray programs. The lower disease levels in treatments receiving fortnightly coverage indicates maintaining regular copper protection during periods of high fruit susceptibility can significantly limit lesion development in mature fruit.

The post-incubation data also indicates that this is carried through incubation with fewer lesions developing in storage when fortnightly copper was applied in the early season if it was followed by fortnightly or monthly copper applications. In contrast, treatments with reduced or absent summer coverage consistently recorded higher post-incubation severity, confirming that coverage gaps during this period allow infections to progress even when spring protection is in place.

For growers, the findings indicate that fortnightly programs can provide additional suppression under elevated pressure, but that maintaining inoculum-free canopies is essential to achieving commercially meaningful reductions. These results also support the practical refinement of adopting tighter intervals during peak susceptibility, while three-week intervals may represent a compromise where operational constraints limit true fortnightly coverage.

**Table C2.2**: Effect of copper fungicide spray timing programs on CBS incidence and severity in 'Imperial' mandarin trees (Wallaville, Queensland; 2023–2024) (0-5 scale). Values are back-transformed or raw* means followed by (predicted means +/- standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| | **Pre-Incubation Assessment** | | **Post-Incubation Assessment** | |
|---|---|---|---|---|
| **Treatments** | **Proportion of disease incidence*** | **Mean disease severity score (0-5)** | **Prop. of disease incidence** | **Mean disease severity score (0-5)*** |
| 1. FFM +DW | 0.05(-0.95 ± 0.04) cd | 0.08 (0.28 ± 0.14) c | 0.39 (-0.44 ± 0.21) de | 2.6 (1.23 ± 0.21) cd |
| 2. FMM +DW | 0.03 (-0.97 ± 0.03) cd | 0.01 (0.11 ± 0.14) c | 0.39 (-0.46 ± 0.21) de | 2.52 (1.20 ± 0.21) cd |
| 3. FUM +DW | 0.17 (-0.83 ± 0.07) bc | 0.26 (0.51 ± 0.14) bc | 0.55 (0.21 ± 0.2) abc | 4.03 (2.07 ± 0.21) b |
| 4. FFU +DW | 0.02 (-0.98 ± 0.03) d | 0.01 (0.12 ± 0.14) c | 0.34 (-0.67 ± 0.21) e | 1.62 (0.80 ± 0.21) d |
| 5. MFM +DW | 0.12 (-0.88 ± 0.04) c | 0.14 (0.37 ± 0.098) bc | 0.44 (-0.26 ± 0.14) cde | 2.34 (1.24 ± 0.16) cd |
| 6. MUM +DW | 0.32 (-0.68 ± 0.08) ab | 0.51 (0.72 ± 0.14) ab | 0.62 (0.49 ± 0.2) ab | 4.18 (2.23 ± 0.21) ab |
| 7. UFM +DW | 0.18 (-0.82 ± 0.07) bc | 0.28 (0.53 ± 0.14) bc | 0.54 (0.17 ± 0.2) abc | 4.04 (2.04 ± 0.21) b |
| 8. UUU +DW | 0.43 (-0.56 ± 0.09) a | 0.92 (0.96 ± 0.14) a | 0.68 (0.77 ± 0.22) a | 4.73 (2.68 ± 0.21) a |
| 9. UUU | 0.16 (-0.84 ± 0.07) bc | 0.21 (0.45 ± 0.14) bc | 0.53 (0.11 ± 0.2) bcd | 3.58 (1.73 ± 0.21) bc |
| LSD** | 0.17 | 0.41 | 0.57 | 0.57 |
| P value | 0.001 | 0.004 | <0.001 | <0.001 |

** Predicted means. DW – Dead wood added to canopies.

## Field Trial 3 (FT3): Evaluation of extensive pre-petal fall fungicide applications for the control of 'Emperor' brown spot (EBS) in 'Daisy' mandarin

### Methodology

Field Trial 3 (FT3) was conducted during the 2022-2023 growing season to determine the effect of fungicide application prior to petal fall with respect to EBS control in mandarin. The trial was established in a young commercial 'Daisy' mandarin orchard in 888 Citrus, Mundubbera, Queensland.

Nineteen treatments were used to investigate the effect of existing and emerging fungicides on disease when applied prior to petal fall, with no further fungicide application during the entire growing season. These treatments included strobilurin fungicides, SDHI co-formulations, multi-site protectants including copper fungicides and a low residue biologically derived fungicide. As the purpose of this trial was to screen potential treatments for future field evaluation, fungicide application timing and frequency were contrary to prescribed use patterns for most active ingredients tested. For this reason, treatments have been coded.

Fungicide applications were made prior to petal fall at 7–10-day intervals. Treatments 1-16 received six spray applications between 17 August and 22 September 2022, except for the untreated control (T4) which received no sprays. Treatments 17-19 received six spray applications between 17 August and 22 September 2022 with the same compounds used in Treatments 11, 12 and 1 (respectively), but then received two additional sprays on 2 and 25 November 2022 using the same active ingredients again. Treatments 6, 7, 8 and 11 included combinations of 2-3 active ingredients.

All applications were delivered using a calibrated high-pressure handgun operating at approximately 200 psi and applying 3.2 L per tree. All fungicides were applied at label or industry-standard rates, and no grower-applied sprays were applied during the trial period.

Trees were three years old and maintained under standard grower practice (bar fungicide application). The experiment followed a randomised complete block design with five single-tree replicates per treatment.

50 fruit per tree were collected where available and examined for EBS incidence and severity using the standard 0–10 scale described in Appendix E within 48 hours of harvest. Following assessment, fruit were dipped in an ethephon-imazalil solution, incubated and reassessed as per Appendix E. Pre-incubation assessments recorded visible EBS lesions (as verified by isolation from representative symptom types), while post-incubation assessments recorded only CBS (visually verifiable by the presence of distinct symptom expression of CBS lesions at this advanced stage, which masked any pre-existing EBS lesions).

Pre-incubation disease incidence was analysed with a HGLM with treatments as a fixed effect and assumed to follow a binomial distribution with a logit link. For the post-incubation incidence data, the HGLM did not converge, so a GLMM was fitted (binomial distribution, logit link function applied). Here the variance components were bound, so a GLM was fitted instead. Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Results & Discussion**

EBS incidence (proportion) was uniformly high across all treatments, ranging from 0.75 to 0.93 (Table C3.1), and severity scores (0-10 rating scale) showed a similar narrow range (1.43–2.62). No significant differences were observed among treatments, but the untreated control (T4) had the second highest value recorded for disease incidence (0.91). Only five treatments recorded EBS incidence values below 0.80 – two of these were 6 sprays of single active ingredients (T10 & 13); one was 6 sprays of two active ingredients (T11); one was 8 sprays of a single active ingredient (T18); and one was 8 sprays of two active ingredients (T17).

There were also no significant effects of treatments on EBS severity (Table C3.1). The untreated control (T4) had the highest value recorded for disease severity (2.62), and the same five treatments which had the lowest values for EBS incidence (T10, 11, 13, 17 & 18) also had the lowest EBS severity values. Disease severity results have also been presented graphically in descending order to assist with visualisation of results (Figure C3.1).

CBS incidence values were also very high among all treatments, ranging from 0.71 to 0.89, with no significant treatment effects (Table C3.1). CBS disease severity ranged from 1.35 to 2.53, and again, no significant treatment effects (Table C3.1). The untreated control (T4) had the second highest and highest values for CBS incidence and severity, respectively. Six treatments had CBS incidence values below 0.8 (T3, 10, 12, 13, 16 & 17), and of these, three treatments were also amongst the best for EBS (T10, 13 & 17). For CBS severity, six treatments had values below 1.8 (T3, 10, 12, 13, 17 & 19), with only one treatment (T19) differing from the lowest CBS incidence values. Once again, disease severity results have also been presented graphically (Figure C3.2).

While these outcomes do not show statistical differences among pre-petal fall fungicide treatments applied to 'Daisy' mandarin, there were some treatments that consistently demonstrated lower values across incidence and severity for both EBS and CBS, particularly T10, 13 & 17. Furthermore, the untreated control consistently had the highest or second highest disease levels of all the treatments evaluated, giving further validity to the results in the absence of statistical differences. Given that no sprays were applied to these trees post petal fall, it is not surprising that such high levels of EBS and CBS were recorded in this trial. The consistently high disease incidence across all of the fungicide treatments suggests that infection likely occurred outside the protective window achieved by these early sprays, or that natural inoculum pressure remained sufficient to overwhelm differences in chemistry. Both of these explanations are quite possible given that no further fungicides were applied for the entire season following the pre-petal fall sprays. Developing fruit were therefore not protected with fungicide at any stage. It was therefore decided to undertake additional field trials in 2023/24 and 2024/25, where pre-petal fall sprays were applied either with or without grower-applied fungicide sprays during the fruit development phase. Best performing chemistries from this preliminary trial (despite no significant differences) formed the basis for selecting a reduced number of active ingredients with the greatest potential for evaluation in subsequent trials.

**Table C3.1**: Effect of pre-petal fall fungicide applications on disease incidence and severity in 'Daisy' mandarins (2022/23). Results are presented for pre-incubation (EBS at harvest) and post-incubation (CBS expression following laboratory incubation). Values show the proportion of symptomatic fruit and mean severity score (0–10) and are back transformed or raw* means followed by (predicted means +/- standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Treatment code | Pre-Incubation Assessment (EBS) | | Post-Incubation Assessment (CBS) | |
|---|---|---|---|---|
| | Proportion of disease incidence | Mean disease severity score (0-10)* | Proportion of disease incidence* | Mean disease severity score (0-10)* |
| T-1 (6 sprays) | 0.83 (1.57 ± 0.4) a | 1.99 (2.15 ± 0.17) a | 0.85 (0.14 ± 0.05) a | 2.41 (2.49 ± 0.21) a |
| T-2 (6 sprays) | 0.87 (1.86 ± 0.44) a | 1.96(1.98 ± 0.17) a | 0.89 (0.13 ± 0.05) a | 2.31 (2.42 ± 0.26) a |
| T-3 (6 sprays) | 0.93 (2.64 ± 0.64) a | 2.38 (2.33 ± 0.214) a | 0.73 (0.3 ± 0.08) a | 1.74 (1.83 ± 0.33) a |
| T-4 Untreated | 0.91 (2.30 ± 0.49) a | 2.62 (2.62 ± 0.17) a | 0.87 (0.12 ± 0.05) a | 2.53 (2.86 ± 0.28) a |
| T-5 (6 sprays) | 0.86 (1.77 ± 0.41) a | 2.06 (2.03 ± 0.17) a | 0.84 (0.12 ± 0.06) a | 2.22 (2.67 ± 0.32) a |
| T-6 (6 sprays) | 0.85 (1.70 ± 0.4) a | 1.92 (1.92 ± 0.17) a | 0.85 (0.15 ± 0.05) a | 2.05 (2.06 ± 0.21) a |
| T-7 (6 sprays) | 0.86 (1.81 ± 0.41) a | 1.93 (1.93 ± 0.17) a | 0.83 (0.15 ± 0.05) a | 1.95 (1.96 ± 0.23) a |
| T-8 (6 sprays) | 0.81 (1.44 ± 0.37) a | 1.83 (1.80 ± 0.17) a | 0.81 (0.19 ± 0.05) a | 1.82 (1.82 ± 0.21) a |
| T-9 (6 sprays) | 0.87 (1.86 ± 0.44) a | 2.18 (2.12 ± 0.184) a | 0.86 (0.14 ± 0.04) a | 2.21 (2.21 ± 0.21) a |
| T-10 (6 sprays) | 0.79 (1.34 ± 0.36) a | 1.55 (1.55 ± 0.17) a | 0.74 (0.32 ± 0.08) a | 1.51 (1.39 ± 0.32) a |
| T-11 (6 sprays) | 0.77 (1.22 ± 0.35) a | 1.65 (1.65 ± 0.17) a | 0.82 (0.18 ± 0.06) a | 1.9 (2.02 ± 0.26) a |
| T-12 (6 sprays) | 0.84 (1.65 ± 0.39) a | 1.78 (1.78 ± 0.17) a | 0.75 (0.29 ± 0.07) a | 1.79 (1.67 ± 0.26) a |
| T-13 (6 sprays) | 0.75 (1.07 ± 0.36) a | 1.54 (1.65 ± 0.17) a | 0.74 (0.27 ± 0.08) a | 1.77 (2.05 ± 0.3) a |
| T-14 (6 sprays) | 0.9 (2.22 ± 0.48) a | 2.3 (2.27 ± 0.17) a | 0.83 (0.14 ± 0.05) a | 2.32 (2.54 ± 0.26) a |
| T-15 (6 sprays) | 0.88 (2.01 ± 0.45) a | 2.15 (2.11 ± 0.17) a | 0.86 (0.14 ± 0.04) a | 1.94 (1.91 ± 0.21) a |
| T-16 (6 sprays) | 0.88 (1.96 ± 0.43) a | 1.89 (1.89 ± 0.17) a | 0.77 (0.23 ± 0.05) a | 1.91(1.92 ± 0.21) a |
| T-17 (8 sprays) | 0.76 (1.14 ± 0.34) a | 1.43 (1.45 ± 0.17) a | 0.71 (0.29 ± 0.06) a | 1.35 (1.39 ± 0.21) a |
| T-18 (8 sprays) | 0.77 (1.2 ± 0.35) a | 1.57 (1.62 ± 0.17) a | 0.85 (0.15 ± 0.05) a | 1.96 (2.02 ± 0.21) a |
| T-19 (8 sprays) | 0.82 (1.5 ± 0.41) a | 1.71 (1.78 ± 0.18) a | 0.82 (0.18 ± 0.05) a | 1.66 (1.63 ± 0.23) a |
| LSD (predicted means) | 1.15 | 0.48 | 0.16 | 0.73 |
| P value | 0.41 | 0.22 | 0.21 | 0.17 |

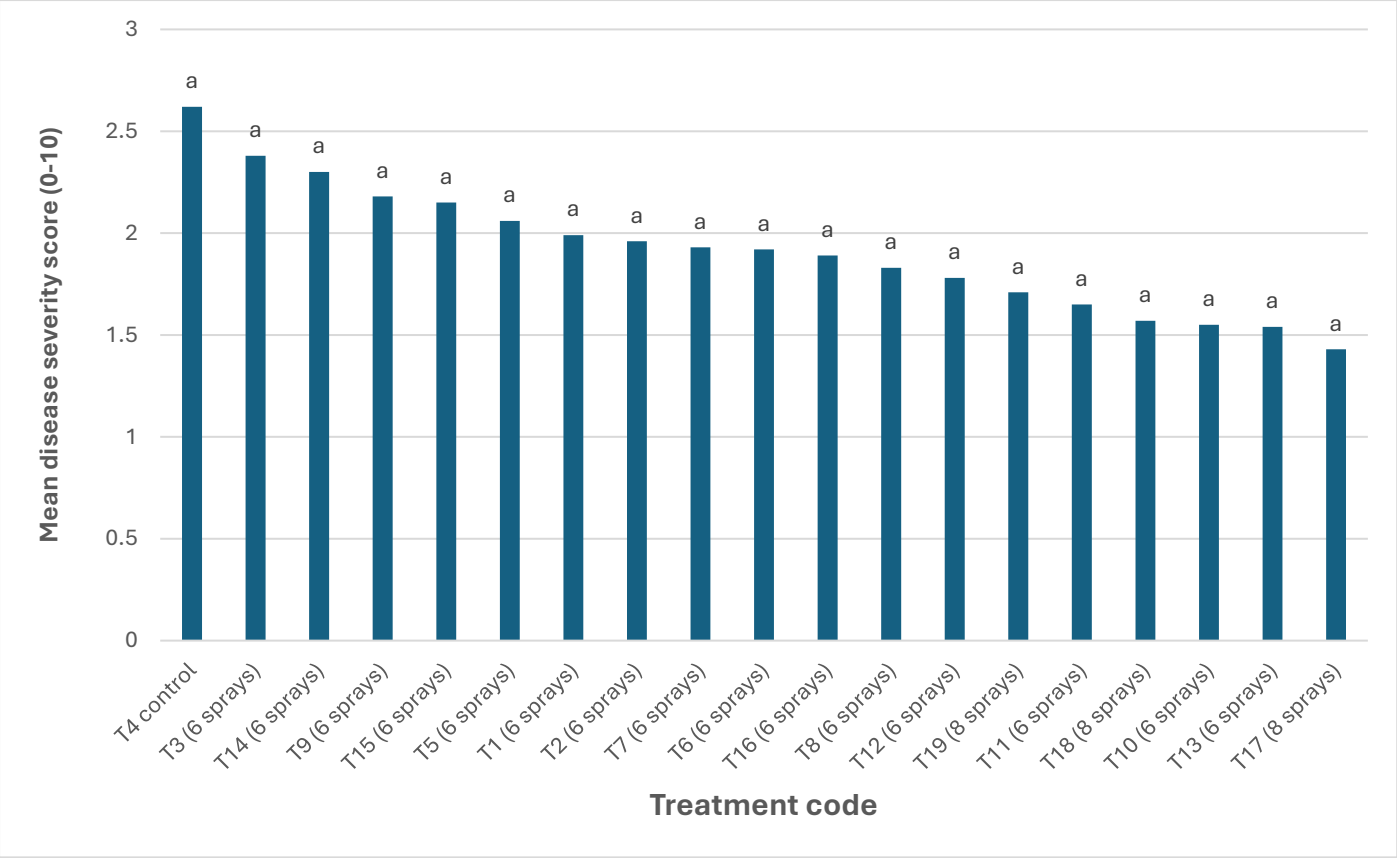


**Figure C3.1:** Effect of pre-petal fall fungicide applications on pre-incubation EBS severity (0-10 score) in 'Daisy' mandarins (2022/23), presented in descending order. Means surmounted by the same letter are not significantly different at P = 0.05.

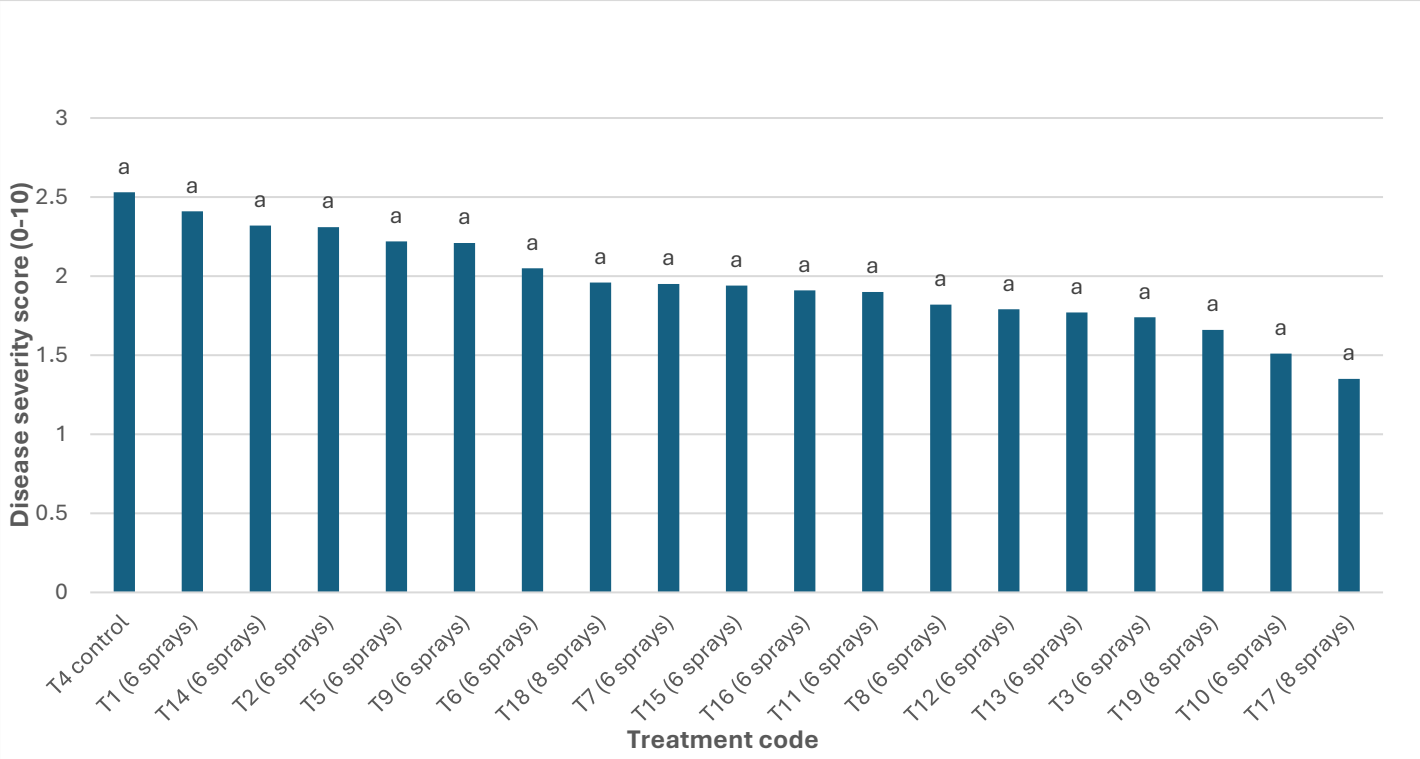


**Figure C3.2:** Effect of pre-petal fall fungicide applications on post-incubation CBS severity (0-10 score) in 'Daisy' mandarins (2022/23), presented in descending order. Means surmounted by the same letter are not significantly different at P = 0.05.

## Field Trial 4A, B (FT4A/B): Evaluation of limited pre-petal fall fungicide applications for the control of 'Emperor' brown spot (EBS) and CBS in 'Murcott' Mandarin

### Methodology

Two field trials (Trial A: 2023/24; Trial B: 2024/25) were conducted to evaluate whether limited fungicide applications at petal fall influence the expression of EBS and CBS in 'Murcott' mandarin fruit at harvest. The trials were established in an established commercial orchard at Citrus Abbotsleigh, Wallaville, Queensland and represented further trialing of promising fungicide treatments at flowering. It differs from the previous trial in the timing and frequency of fungicide application and the incorporation of industry derived fungicide programs into the treatment structure. In the previous trial, all fruit were unprotected from the last experimental fungicide application until harvest, but also received more pre-petal fall sprays (6-8 sprays) than in the current trial (2 sprays). This trial was repeated in 2024/2025 at the request of industry.

Ten fungicide treatments were applied twice at flowering, each application two weeks apart, and were duplicated in blocks that did or did not receive the farm's standard spray program (Figure C4.1, Table C4.1). Trial A was established on 29 September 2023, and Trial B on 3 September 2024. Both trials were established in Block 19, a 'Murcott' mandarin block with a documented history of CBS and EBS. Sprays were applied using a calibrated hand-held sprayer to the point of run-off. Each trial followed a randomised complete block design with five single-tree replicates per treatment.

In both seasons, 50 fruit were harvested from each replicate tree, with fewer fruit collected when yield was low. Disease assessments (incidence and severity) of CBS and EBS were conducted as per Appendix E. Fruit were treated and incubated using the standard ethephon–imazalil protocol as per Appendix E.

In general, disease incidence was analysed with a HGLM with a fixed effect (treatment) assumed to follow a binomial distribution with a logit link and a random term (block) assumed to follow a beta distribution with a logit link. If the HGLM did not converge, a GLMM was fitted (binomial distribution, logit link function applied, terms as above). If the variance components of the GLMM were bound, a GLM was fitted instead (terms as above). FT4a – HGLM: Pre-incubation CBS with and without commercial spray, post-incubation CBS without commercial spray, GLM: EBS with and without commercial spray, Post-incubation CBS with commercial spray. FT4b – HGLM: EBS with commercial spray, CBS pre-and post-incubation with and without commercial spray, GLM: EBS without commercial spray.

Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). A transformation was applied if the assumptions were not met. FT4a – pre-incubation CBS with commercial spray required a log10 transformation to satisfy the assumption of homogeneity of variance. FT4b – EBS without commercial spray required a square root transformation to improve the homogeneity of variance and CBS pre-incubation without commercial spray required a log10 transformation for the same. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD). Due to the trial layout prescribed by tree availability and grower requirements, treatments receiving grower fungicide application and treatments without grower fungicide applications were analysed separately for statistical integrity.

**Table C4.1**: Treatment structure for petal-fall fungicide applications evaluated in Trials A and B. The table lists the ten fungicide programs applied at petal fall, with each treatment assessed in two versions: A (with commercial spray program) and B (without commercial spray program).

| Treatment | Product / Program | Rate (/100 L) |
|---|---|---|
| **1** | RB33* | 100 mL |
| **2** | Amistar® 250 SC | 40 mL |
| **3** | RB33* + Amistar® | 100 mL + 40 mL |
| **4** | Merivon® | 40 mL |
| **5** | NUL3446* (+ non-ionic surfactant) | 40 g + 50 mL |
| **6** | Captan | 125 g |
| **7** | Dithane® Rainshield NecTec | 200 g |
| **8** | Rovral® New Aquaflo | 100 mL |
| **9** | BlueShield® | 100 g |
| **10** | Untreated Control or Grower Application Only Control | – |

Petal-fall sprays were applied twice per season at fortnightly intervals: **Trial A (2023/24):** 29 September and 13 October 2023; **Trial B (2024/25):** 4 September and 18 September 2024

* Indicates product name has been coded (unregistered chemical)

**Results & Discussion**

Under the conditions of this experiment, limited petal-fall fungicide applications (two sprays) did not demonstrate significant benefit for the control of EBS or CBS overall, as measured in mature fruit after harvest.

EBS

In 2023/2024, incidence of EBS was low in almost all treatments and no significant differences were seen between any treatment including the fungicide and control treatments (Table C4.2). When the grower fungicide sprays were applied in addition to petal-fall treatments, disease incidence ranged from 4-11% in all treatments except for the NUL3446 treatment (18%). Where only petal-fall fungicides were applied, disease incidence fell within the range 7-14% for most treatments, with Amistar®, Merivon® and Captan treatments showing higher values (20, 20 & 23 %, respectively).

In contrast, in 2024/2025, while all treatments were again not significantly different from each other, higher levels of EBS were recorded in harvested fruit in all treatments than 2023/2024 (Table C4.2). Here EBS incidence ranged from 13-33% in petal-fall plus grower fungicides and 17-33% in petal-fall only treatments.

In 2023/2024, a non-statistical comparison of the paired petal-fall and petal-fall plus grower fungicide spray treatments showed the addition of the grower fungicide program to the trial treatment program affected the incidence and severity of EBS (Table C4.2). Adding the grower fungicide program to the petal-fall Amistar® (T2), Amistar® + RB33 (T3), Merivon® (T4) or Captan (T6) treatments helped to overcome the higher EBS disease levels in these treatments when applied at petal fall only (Table C4.2). When made in addition to petal-fall Dithane® (T7), Rovral® (T8) or RB33 alone (T1), however, no change was noted. Even more unexpectedly, the incidence and severity of EBS was increased by the application of the grower sprays to the NUL3446 treatment (T5). Incidence and severity in the control treatments were low and showed no or minor differences indicating minimal impact of sprays during fruit development on EBS under the conditions of this trial. Note, differences between with and without grower fungicides are observational only as they were not analysed as per the methods section.

In 2024/2025, despite much more EBS noted in the harvested fruit in general, most of these trends were not repeated (Table C4.2). The results are still useful, however, as they provide an understanding of how the treatments influenced disease expression in a growing season with higher disease pressure. The addition of the grower's sprays to the Amistar® (T2) reduced the incidence but not the severity of EBS while the disease expressed in most other treatments were not greatly influenced by the additional spray regime. In this season, the difference between the NUL3446 treatments was minimal. Once again, there was very little difference between EBS in mature untreated control fruit receiving no treatment at all compared to control fruit which received only grower sprays with no petal fall treatments.

As grower-applied sprays during fruit development are an essential component of EBS management, the question here is – do petal fall fungicide applications significantly enhance current industry standard spray programs that are applied during the fruit season for EBS management? On the basis of these results, and under the conditions of this experiment, the answer is most likely that only limited or no benefit is to be expected. As mentioned previously, however, experimental design constraints (see Methods) have prevented a full statistical analysis of these trial results. If a future trial could overcome these practical constraints then insights regarding EBS in this experiment could be further investigated.

CBS

The impact of the petal-fall and grower applied fungicide treatments on the development of CBS was more evident than in EBS, particularly in 2023/2024.

Pre-incubation results from 2023/2024 show that fungicide application significantly affected disease incidence (but not severity) within the petal-fall only and the grower plus petal fall fungicide applications (Table C4.3). After incubation, significant differences were noted in incidence and severity in the petal-fall only program but in neither parameter in petal-fall plus grower treatments (Table C4.4).The post-incubation results indicate the grower fungicides controlled latent infections more effectively than petal-fall only applications and the differences seen in the petal-fall only applications were eliminated by the season long grower program.

When only the two petal-fall applications were made, pre-incubation results show RB33 and NUL3446 had the lowest disease incidence (T1 & T5, both 12%), but were only significantly less than Captan® (T6, 35%), and Merivon® (T4, 39%) treatments (Table C4.3). Conversely, Merivon® had significantly higher incidence of CBS (T4, 39%) than RB33 (T1, 12%), RB33+Amistar® (T3, 17%), NUL3446 (T5, 12%), copper (T9, 15%) and the untreated control (T10, 19%).

When the grower's program was added, NUL3446 (T5, 6%) had the highest incidence of CBS and significantly more disease than all treatments except for Dithane® (T7, 5%). That aside, there were very few differences. Merivon® (T4), Captan (T6) and copper (T9) treatments had no CBS at all but were not significantly more effective than the control which received no petal fall sprays at all. There was very little disease seen in most of these treatments (8 of 10 treatments had 0-1% disease incidence) demonstrating the efficacy of grower sprays for CBS management.

After incubation, the only significant differences were seen where petal-fall treatments had been applied alone. Merivon® again had the highest disease incidence and severity and here RB33+Amistar® had the least (Table C4.4). While RB33+Amistar® had significantly lower CBS incidence and severity compared to the untreated control, it was not significantly different to Amistar® alone but did improve post-incubation control of CBS compared to RB33 alone

Large differences in the degree of CBS incidence and severity between treatment pairs were seen between the petal-fall and the petal-fall plus grower programs, with a sharp decrease in pre-incubation data in all treatments from 12-39% down to 0-6% (incidence) and 0.16-0.65 down to 0-0.03 mean lesions (severity) after the grower program was added (Table C4.4). These decreases were more pronounced after incubation, decreasing from 81-98% to 27-38% (incidence; excluding outlier NUL3446 T5 at 90%) and 1.77-2.82 to 0.37-0.54 mean lesions (severity). The increase in symptoms after incubation in both treatment programs indicates background latent infection had occurred despite fungicide application, and the difference between the two groups indicates that, overall, the application of petal-fall fungicides does not improve CBS control above that achieved by the grower sprays.

In 2024/2025, irrespective of petal-fall or added grower fungicides, no significant differences between treatments in either metric were noted at harvest or after incubation (Tables C4.3 and D4.4). The differences between the control treatments were again present, demonstrating the efficacy of the grower sprays against CBS.

Despite this, when doing a non-statistical pairwise comparison of means, the addition of the grower's program to the petal-fall treatments reduced the incidence and severity in most of the treatment's pre-incubation, the exception being disease incidence in the NUL3446 treatment (Table C4.3). Fewer differences were noted after incubation (Table C4.4).

Collectively, these findings show that applications of fungicides at the petal-fall stage are unlikely to provide significant suppression of either EBS or CBS in fruit at harvest. Effective management of CBS was seen when grower fungicides were applied after the petal-fall window. Grower sprays after petal fall did not have the same magnitude of effect on EBS.


**Figure C4.1**: Example of trial signage with 'no spray' instructions.

**Table C4.2**: Effect of petal-fall fungicide applications on **EBS** incidence and severity in 'Murcott' mandarins across two seasons (2023/24 and 2024/25). Results for with and without the commercial grower spray program applied after petal fall are presented separately. Values are back transformed or raw* means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| **EBS 2023-2024** | | **With commercial spray program** | | **Without commercial spray program** | |
|---|---|---|---|---|---|
| **Petal-fall treatment** | **Year** | **Prop. of EBS incidence*** | **Mean disease severity score (0-5)*** | **Prop. of EBS incidence*** | **Mean disease severity score (0-5)*** |
| 1. RB33[1] | 23/24 | 0.1(-0.89 ± 0.03) a | 0.16(0.15 ± 0.04) a | 0.12(-0.88 ± 0.05) a | 0.19 (0.19 ± 0.1) a |
| 2. Amistar® 250 SC | 23/24 | 0.06 (-0.94 ± 0.02) a | 0.07 (0.07 ± 0.04) a | 0.2 (-0.8 ± 0.06) a | 0.25 (0.27 ± 0.1) a |
| 3. RB33[1]+ Amistar® | 23/24 | 0.05 (-0.95 ± 0.02) a | 0.05 (0.05 ± 0.04) a | 0.14 (-0.86 ± 0.05) a | 0.2 (0.20 ± 0.1) a |
| 4. Merivon® | 23/24 | 0.09 (-0.91 ± 0.03) a | 0.12 (0.12 ± 0.04) a | 0.2 (-0.79 ± 0.07) a | 0.37 (0.39 ± 0.1) a |
| 5. NUL3446[1] (+ non-ionic surfactant) | 23/24 | 0.18 (-0.82 ± 0.04) a | 0.25 (0.25 ± 0.04) a | 0.07 (-0.93 ± 0.04) a | 0.1 (0.10 ± 0.1) a |
| 6. Captan | 23/24 | 0.11 (-0.89 ± 0.03) a | 0.14 (0.14 ± 0.04) a | 0.23 (-0.77 ± 0.06) a | 0.42 (0.41 ± 0.1) a |
| 7. Dithane® Rainshield NecTec | 23/24 | 0.14 (-0.86 ± 0.04) a | 0.16 (0.17 ± 0.04) a | 0.11 (-0.89 ± 0.04) a | 0.22 (0.21 ± 0.1) a |
| 8. Rovral® New Aquaflo | 23/24 | 0.09 (-0.91 ± 0.03) a | 0.15 (0.16 ± 0.04) a | 0.11 (-0.89 ± 0.05) a | 0.2 (0.21 ± 0.1) a |
| 9. Copper hydroxide (BlueShield®) | 23/24 | 0.04 (-0.96 ± 0.02) a | 0.09 (0.09 ± 0.04) a | 0.1 (-0.9 ± 0.05) a | 0.1 (0.1 ± 0.1) a |
| 10. Untreated Control | 23/24 | 0.09 (-0.91 ± 0.03) a | 0.11 (0.11 ± 0.04) a | 0.11 (-0.9 ± 0.05) a | 0.16 (0.17 ± 0.1) a |
| LSD (p <= 0.05) (predicted means) | | 0.09 | 0.13 | 0.15 | 0.3 |
| P value | | 0.07 | 0.17 | 0.4 | 0.45 |
| **EBS 2024-2025** | | **With commercial spray program** | | **Without commercial spray program** | |
| **Petal-fall treatment** | **Year** | **Prop. of EBS incidence** | **Mean disease severity score (0-5)*** | **Prop. of EBS incidence*** | **Mean disease severity score (0-5)** |
| 1. RB33[1] | 24/25 | 0.27 (-0.97 ± 0.28) a | 0.36 (0.35 ± 0.08) a | 0.29 (-0.72 ± 0.08) a | 0.39 (0.63 ± 0.12) a |
| 2. Amistar® 250 SC | 24/25 | 0.23 (-1.19 ± 0.3) a | 0.34 (0.34 ± 0.08) a | 0.33 (-0.66 ± 0.09) a | 0.34 (0.58 ± 0.12) a |
| 3. RB33[1]+ Amistar® | 24/25 | 0.3 (-0.83 ± 0.29) a | 0.4 (0.40 ± 0.08) a | 0.32 (-0.65 ± 0.11) a | 0.35 (0.59 ± 0.14) a |
| 4. Merivon® | 24/25 | 0.16 (-1.63 ± 0.35) a | 0.22 (0.22 ± 0.08) a | 0.13 (-0.87 ± 0.06) a | 0.11 (0.33 ± 0.11) a |
| 5. NUL3446[1] (+ non-ionic surfactant) | 24/25 | 0.21 (-1.34 ± 0.32) a | 0.22 (0.22 ± 0.08) a | 0.18 (-0.82 ± 0.06) a | 0.21 (0.45 ± 0.11) a |
| 6. Captan | 24/25 | 0.32 (-0.76 ± 0.28) a | 0.44 (0.44 ± 0.08) a | 0.27 (-0.74 ± 0.08) a | 0.37 (0.61 ± 0.11) a |
| 7. Dithane® Rainshield NecTec | 24/25 | 0.16 (-1.66 ± 0.35) a | 0.19 (0.19 ± 0.08) a | 0.25 (-0.76 ± 0.07) a | 0.27 (0.52 ± 0.11) a |
| 8. Rovral® New Aquaflo | 24/25 | 0.13 (-1.88 ± 0.38) a | 0.18 (0.17 ± 0.08) a | 0.17 (-0.83 ± 0.07) a | 0.16 (0.40 ± 0.11) a |
| 9. Copper hydroxide (BlueShield®) | 24/25 | 0.15 (-1.72 ± 0.36) a | 0.19 (0.18 ± 0.08) a | 0.22 (-0.77 ± 0.08) a | 0.24 (0.49 ± 0.12) a |
| 10. Untreated Control | 24/25 | 0.27 (-0.99 ± 0.29) a | 0.4 (0.39 ± 0.08) a | 0.31 (-0.71 ± 0.09) a | 0.42 (0.65 ± 0.12) a |
| LSD (p <= 0.05) (predicted means) | | 0.9 | 0.23 | 0.24 | 0.35 |
| P value | | 0.08 | 1.4 | 0.59 | 0.65 |

[1] Indicates product name has been coded (unregistered chemical)

**Table C4.3**: Effect of petal-fall fungicide applications on **CBS** incidence and severity in 'Murcott' mandarin at harvest (pre-incubation) in two consecutive seasons (2023/24 and 2024/25). Results for with and without the commercial grower spray program applied after petal fall are presented separately. Values are back transformed or raw* means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| **CBS 2023-2024** | | **With commercial spray program** | | **Without commercial spray program** | |
|---|---|---|---|---|---|
| **Petal-fall treatment** | **Year** | **Proportion of CBS incidence** | **Mean disease severity score (0-5)** | **Proportion of CBS incidence** | **Mean disease severity score (0-5)*** |
| 1. RB33[1] | 23/24 | 0.01 (-5.13 ± 1.05) c | 0.01 (-1.76 ± 0.17) a | 0.12 (-2.02 ± 0.47) c | 0.18 (0.19 ± 0.12) a |
| 2. Amistar® 250 SC | 23/24 | 0.01 (-5.15 ± 1.05) c | 0.01 (-1.81 ± 0.17) a | 0.22 (-1.24 ± 0.39) abc | 0.34 (0.36 ± 0.12) a |
| 3. RB33[1] + Amistar® | 23/24 | 0.01 (-5.20 ± 1.05) c | 0.01 (-1.81 ± 0.17) a | 0.17 (-1.57 ± 0.40) bc | 0.23 (0.23 ± 0.12) a |
| 4. Merivon® | 23/24 | 0.00 (-5.84 ± 1.40) c | 0.00 (-1.90 ± 0.17) a | 0.39 (-0.43 ± 0.35) a | 0.65 (0.69 ± 0.12) a |
| 5. NUL3446[1] (+ non-ionic surfactant) | 23/24 | 0.06 (-2.68 ± 0.56) a | 0.03 (-1.40 ± 0.17) a | 0.12 (-1.99 ± 0.48) c | 0.16 (0.18 ± 0.12) a |
| 6. Captan | 23/24 | 0.00 (-5.93 ± 1.40) c | 0.00 (-1.91 ± 0.17) a | 0.35 (-0.64 ± 0.35) ab | 0.61 (0.6 ± 0.12) a |
| 7. Dithane® Rainshield NecTec | 23/24 | 0.05 (-2.96 ± 0.58) ab | 0.03 (-1.38 ± 0.17) a | 0.27 (-1.00 ± 0.35) abc | 0.43 (0.45 ± 0.12) a |
| 8. Rovral® New Aquaflo | 23/24 | 0.01 (-4.21 ± 0.76) bc | 0.01 (-1.64 ± 0.17) a | 0.26 (-1.03 ± 0.36) abc | 0.45 (0.47 ± 0.12) a |
| 9. Copper hydroxide (BlueShield®) | 23/24 | 0.00 (-12.89 ± *) ** | 0.00 (-2.00 ± 0.17) a | 0.15 (-1.73 ± 0.46) bc | 0.28 (0.29 ± 0.12) a |
| 10. Untreated Control | 23/24 | 0.01 (-4.64 ± 0.90) bc | 0.01 (-1.69 ± 0.17) a | 0.19 (-1.47 ± 0.40) bc | 0.29 (0.32 ± 0.12) a |
| LSD (p <= 0.05) (predicted means) | | 2.5 | 0.47 | 1.12 | 0.34 |
| P value | | <0.001 | 0.14 | 0.03 | 0.054 |
| **CBS 2024-2025** | | **With commercial spray program** | | **Without commercial spray program** | |
| **Petal-fall treatment** | **Year** | **Prop. of CBS incidence** | **Mean disease severity score (0-5)*** | **Prop. of CBS incidence** | **Mean disease severity score (0-5)** |
| 1. RB33[1] | 24/25 | 0.22 (-1.25 ± 0.33) a | 0.29 (0.29 ± 0.1) a | 0.42 (-0.3 ± 0.42) a | 0.55 (-0.26 ± 0.14) a |
| 2. Amistar® 250 SC | 24/25 | 0.28 (-0.95 ± 0.35) a | 0.36 (0.37 ± 0.11) a | 0.34 (-0.67 ± 0.43) a | 0.42 (-0.37 ± 0.14) a |
| 3. RB33[1] + Amistar® | 24/25 | 0.22 (-1.27 ± 0.34) a | 0.29 (0.29 ± 0.1) a | 0.34 (-0.68 ± 0.39) a | 0.47 (-0.33 ± 0.13) a |
| 4. Merivon® | 24/25 | 0.25 (-1.11 ± 0.32) a | 0.35 (0.36 ± 0.1) a | 0.29 (-0.87 ± 0.44) a | 0.33 (-0.48 ± 0.14) a |
| 5. NUL3446[1] (+ non-ionic surfactant) | 24/25 | 0.52 (-1.09 ± 0.33) a | 0.35 (0.35 ± 0.1) a | 0.34 (-0.68 ± 0.38) a | 0.53 (-0.27 ± 0.13) a |
| 6. Captan | 24/25 | 0.19 (-1.44 ± 0.36) a | 0.27 (0.28 ± 0.1) a | 0.55 (0.19 ± 0.39) a | 0.81 (-0.09 ± 0.13) a |
| 7. Dithane® Rainshield NecTec | 24/25 | 0.28 (-0.96 ± 0.31) a | 0.4 (0.38 ± 0.1) a | 0.48 (-0.09 ± 0.42) a | 0.67 (-0.17 ± 0.14) a |
| 8. Rovral® New Aquaflo | 24/25 | 0.19 (-1.47 ± 0.35) a | 0.3 (0.28 ± 0.1) a | 0.4 (-0.41 ± 0.49) a | 0.45 (-0.34 ± 0.16) a |
| 9. Copper hydroxide (BlueShield®) | 24/25 | 0.29 (-0.88 ± 0.3) a | 0.39 (0.39 ± 0.1) a | 0.47 (-0.10 ± 0.38) a | 0.78 (-0.11 ± 0.13) a |
| 10. Untreated Control | 24/25 | 0.17 (-1.58 ± 0.42) a | 0.18 (0.18 ± 0.11) a | 0.51 (-0.04 ± 0.37) a | 0.63 (-0.20 ± 0.13) a |
| LSD (p <= 0.05) (predicted means) | | 0.95 | 0.504 | 1.07 | 0.34 |
| P value | | 0.92 | 0.93 | 0.41 | 0.37 |

**All fruit displayed zero CBS symptoms. [1] Indicates product name has been coded (unregistered chemical)

**Table C4.4:** Effect of petal-fall fungicide applications on **CBS** incidence and severity in 'Murcott' mandarin after incubation harvest in two consecutive seasons (2023/24 and 2024/25). Results for with and without the commercial grower spray program applied after petal fall are presented separately. Values are back transformed or raw* means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| **CBS 2023-2024** | | **With commercial spray program** | | **Without commercial spray program** | |
|---|---|---|---|---|---|
| **Petal-fall treatment** | **Year** | **Proportion of CBS incidence*** | **Mean disease severity score (0-5)*** | **Proportion of CBS incidence** | **Mean disease severity score (0-5)*** |
| 1. RB33[1] | 23/24 | 0.29 (-0.71 ± 0.06) a | 0.39 (0.39 ± 0.11) a | 0.95 (2.89 ± 0.45) abc | 2.52 (2.51 ± 0.24) ab |
| 2. Amistar® 250 SC | 23/24 | 0.27 (-0.74 ± 0.06) a | 0.39 (0.39 ± 0.11) a | 0.89 (2.08 ± 0.37) cde | 2.16 (2.16 ± 0.24) bcd |
| 3. RB33[1]+ Amistar® | 23/24 | 0.32 (-0.68 ± 0.06) a | 0.42 (0.43 ± 0.11) a | 0.81 (1.47 ± 0.33) e | 1.77 (1.76 ± 0.24) d |
| 4. Merivon® | 23/24 | 0.3(-0.7 ± 0.06) a | 0.45 (0.44 ± 0.11) a | 0.98 (3.69 ± 0.59) a | 2.82 (2.81 ± 0.24) a |
| 5. NUL3446[1] (+ non-ionic surfactant) | 23/24 | 0.9 (-0.64 ± 0.06) a | 0.53 (0.53 ± 0.11) a | 0.96 (3.33 ± 0.51) ab | 2.5 (2.5 ± 0.24) ab |
| 6. Captan | 23/24 | 0.27 (-0.72 ± 0.06) a | 0.37 (0.37 ± 0.11) a | 0.91 (2.29 ± 0.4) bcd | 2.42 (2.38 ± 0.24) abc |
| 7. Dithane® Rainshield NecTec | 23/24 | 0.38 (-0.62 ± 0.06) a | 0.54 (0.55 ± 0.11) a | 0.94 (2.69 ± 0.42) abc | 2.29 (2.28 ± 0.24) abcd |
| 8. Rovral® New Aquaflo | 23/24 | 0.29 (-0.71 ±0.06) a | 0.39 (0.39 ± 0.11) a | 0.94 (2.82 ± 0.43) abc | 2.39 (2.39 ± 0.24) abc |
| 9. Copper hydroxide (BlueShield®) | 23/24 | 0.3 (-0.69 ± 0.06) a | 0.48 (0.49 ± 0.11) a | 0.84 (1.68 ± 0.34) de | 1.96 (1.95 ± 0.24) cd |
| 10. Untreated Control | 23/24 | 0.3 (-0.7 ± 0.06) a | 0.45 (0.44 ± 0.11) a | 0.92 (2.47 ± 0.4) abcd | 2.19 (2.20 ± 0.24) bcd |
| LSD (p <= 0.05) (predicted means) | | 1.66 | 0.3 | 0.99 | 0.54 |
| P value | | 0.94 | 0.96 | <0.001 | 0.02 |
| **CBS 2024-2025** | | **With commercial spray program** | | **Without commercial spray program** | |
| **Petal-fall treatment** | **Year** | **Prop. of CBS incidence** | **Mean disease severity score (0-5)*** | **Prop. of CBS incidence** | **Mean disease severity score (0-5)*** |
| 1. RB33[1] | 24/25 | 0.87 (1.94 ± 0.78) a | 1.56 (1.49 ± 0.3) a | 0.91 (2.38 ± 0.68) a | 2.0 (2.02 ± 0.29) a |
| 2. Amistar® 250 SC | 24/25 | 0.63 (0.54 ± 0.74) a | 0.87 (0.95 ± 0.3) a | 0.76 (1.18 ± 0.57) a | 1.4 (1.38 ± 0.29) a |
| 3. RB33[1]+ Amistar® | 24/25 | 0.81 (1.45 ± 0.82) a | 1.57 (1.55 ± 0.31) a | 0.81 (1.48 ± 0.61 a | 1.59 (1.65 ± 0.32) a |
| 4. Merivon® | 24/25 | 0.73 (0.99 ± 0.74) a | 1.13 (1.05 ± 0.3) a | 0.8 (1.39 ± 0.6) a | 1.38 (1.32 ± 0.29) a |
| 5. NUL3446[1] (+ non-ionic surfactant) | 24/25 | 0.78 (1.26 ± 0.75) a | 1.23 (1.26 ± 0.3) a | 0.88 (2.01 ± 0.6)) a | 1.68 (1.69 ± 0.29) a |
| 6. Captan | 24/25 | 0.66 (0.64 ± 0.76) a | 0.95 (1.05 ± 0.31) a | 0.89 (2.15 ± 0.67) a | 2.35 (2.35 ± 0.32) a |
| 7. Dithane® Rainshield NecTec | 24/25 | 0.75 (1.1 ± 0.74) a | 0.93 (0.97 ± 0.3) a | 0.8 (1.39 ± 0.61) a | 1.57 (1.57 ± 0.32) a |
| 8. Rovral® New Aquaflo | 24/25 | 0.7 (0.84 ± 0.74) a | 0.87 (0.87 ± 0.3) a | 0.84 (1.66 ± 0.6) a | 1.5 (1.48 ± 0.2) a |
| 9. Copper hydroxide (BlueShield®) | 24/25 | 0.7 (0.86 ± 0.75) a | 1.02 (1.06 ± 0.31) a | 0.9 (2.24 ± 0.65) a | 1.89 (1.91 ± 0.29) a |
| 10. Untreated Control | 24/25 | 0.74 (1.04 ± 0.74) a | 1.19 (1.24 ± 0.3) a | 0.88 (2.0 ± 0.66 a | 1.61 (1.67 ± 0.32) a |
| LSD (p <= 0.05) (predicted means) | | 1.12 | 0.49 | 1.3 | 0.77 |
| P value | | 0.35 | 0.1 | 0.53 | 0.24 |

[1] Indicates product name has been coded (unregistered chemical)

## Appendix D

### Theme 4: Canopy hygiene, pruning and inoculum source studies

**Background**

CBS, caused by *Phyllosticta citricarpa*, has traditionally been understood as a disease driven by airborne ascospores released from pseudothecia that develop on decomposing leaf litter. This leaf-litter based model has dominated international literature for several decades, and most cultural recommendations have therefore focused on orchard floor sanitation and the removal of fallen leaves and pruning debris. Many orchards routinely clear plant material from the inter-row area on the assumption that reducing ground litter limits the development of spore producing material.

However, observations from Australian mandarin orchards, including earlier work (Hort Innovation Project: CT13021), raised questions regarding whether leaf litter is the primary inoculum source in subtropical systems. In some investigations, ascospores were not detected in ground level litter at times when fruit infection was occurring, suggesting that inoculum may originate from within the canopy rather than from the soil surface. Canopy-retained pruning debris, including senescent twigs, pruned stubs and small dry branches that remain lodged within the canopy, can persist for long periods in commercial blocks, yet this material has received very little investigation.

Although field observations indicated *P. citricarpa* may colonise these materials, there is little quantitative information on whether canopy-borne inoculum contributes meaningfully to fruit infection, whether its activity is seasonally restricted or whether its behaviour differs from leaf litter derived inoculum. This gap is important because canopy debris accumulates readily in mandarin orchards and growers already operate chemical programs close to practical and regulatory limits.

To address this gap, a series of three field trials was established to investigate the behavior, persistence and epidemiological relevance of inoculum associated with pruning debris retained in the canopy.

Field Trial 1 examined whether the timing and duration of when infected debris was present in the canopy influenced early-season infection pressure and disease in fruit with the goal to simultaneously ascertain the peak period of infection of developing fruit by CBS. This was achieved by attaching infected material at defined seasonal intervals to identify periods when fruit were most susceptible.

Field Trial 2 assessed the very practical but highly relevant question – does removing small pruning debris and dry branches from canopies produce measurable reductions in diseased orchards comprised of different varieties, canopy structure and management style? This was achieved in commercial orchards where pruning debris remaining after commercial pruning services had occurred were retained or removed from the canopy manually.

Field Trial 3 provided a more detailed assessment of inoculum potential by comparing debris sources of different *Citrus* species, branch sizes, applying surface sterilant treatments, and altering the introduction and replacement schedule to determine whether viable inoculum persisted across the entire season and whether persistence differed among debris types.

By determining the key infection period of CBS, if canopy retained pruning debris (size, type and host) functions as an epidemiologically meaningful inoculum reservoir and whether practical canopy hygiene can influence pre-harvest CBS expression in commercial mandarin production systems, this work will provide evidence for an alternative inoculum pathway within the canopy and practical, IDM, oriented management strategies in regions where CBS pressure is high.

## Field Trial (FT1): Investigation of the critical infection period of CBS in 'Imperial' mandarin using canopy inoculum as an inoculum source (Wallaville, 2023–2024)

### Methodology

Field Trial 1 was established in the 2023-2024 growing season to identify the effect of canopy inoculum with respect to infection of 'Imperial' mandarin fruit by CBS by manipulating canopy borne inoculum, and, in doing so, investigate the critical infection window of CBS within a mature commercial orchard at Spencer Ranch, Wallaville, Queensland.

Eight treatments designed to confirm the infection period of CBS in 'Imperial' mandarin is in the early portion of the growing season were conducted (Table D1.1). Dead wood (naturally infected lemon branches simulating pruning debris) was introduced to and/or removed from the mid-canopy at the beginning of each September, November, January and March (Figure D1.1).

These periods were chosen to mimic proposed periods of susceptibility and development of CBS symptoms in mandarin and allowed investigation of the effect of adding inoculum in individual or sequential seasons, continuous exposure or nil exposure (no added inoculum control treatments) on symptoms in mature fruit (Table D1.1).

Trees were mature, approximately 2.5 metres tall and uniform in canopy structure. The experiment utilised a randomised complete block design with six single-tree replicates per treatment. All trees received the grower's commercial fungicide program throughout the season.

At harvest, 50 fruit were collected from each tree and assessed for incidence and severity of CBS pre- and post-incubation (0-5 scale) following the procedures described in Appendix E. Fruit were dipped in an ethephon-imazalil solution and incubated as per Appendix E prior to reassessment of incidence and severity of CBS.

Disease incidence was analysed with a HGLM with a fixed effect (treatment) assumed to follow a binomial distribution with a logit link and a random term (block) assumed to follow a beta distribution with a logit link. The dispersion parameter was estimated. Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). A square root transformation was applied to satisfy the assumption of homogeneity of variance for the pre-incubation data. For post-incubation data, the residuals were skewed so the mean score was subtracted from the maximum score and a square-root transformation applied. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Table D1.1**: Seasonal presence of canopy-borne inoculum in Field Trial 1 (FT1), Wallaville, 2023–2024.

| Treatment No. | September to October | November to December | January to February | March to April | Description |
|---|---|---|---|---|---|
| 1 | ✓ | ✓ | ✓ | ✓ | Continuous exposure to identify full seasonal infection window |
| 2 | ✗ | ✓ | ✓ | ✓ | Mid to late season exposure |
| 3 | ✗ | ✗ | ✓ | ✓ | Late season exposure |
| 4 | ✗ | ✗ | ✗ | ✓ | Very late season exposure |
| 5 | ✓ | ✗ | ✗ | ✗ | Early season only |
| 6 | ✓ | ✓ | ✗ | ✗ | Early to mid-season |
| 7 | ✓ | ✓ | ✓ | ✗ | Early to late season |
| 8 | ✗ | ✗ | ✗ | ✗ | Control (No added inoculum) |

Naturally infected lemon pruning debris was attached to 'Imperial' mandarin trees during defined seasonal periods to determine the infection window for CBS and the effect of additional inoculum in the canopy on disease recorded in mature fruit. Treatments represent early, mid, late and full-season exposure periods, together with no inoculum control. Pruning debris was introduced or removed at the beginning of each seasonal period (September, November, January and March), and the final exposure period concluded at harvest in April 2024. All trees received the grower's commercial copper-based fungicide program, and no trial-specific sprays were applied. ✓ indicates periods when infected pruning debris was present; ✗ indicates no debris was present.

**Figure D1.1**: Example of replacing simulated pruning debris throughout the trial period. (Image credit: K. Bransgrove.)

**Results & Discussion**

In the 2023-2024 growing season, varying the duration that infected pruning debris were retained within the canopy significantly affected the severity of CBS symptoms expressed in fruit at both harvest and after incubation, but not the number of fruit with disease (incidence) (Table D1.2).

At harvest, disease severity was significantly greater in treatments where debris was present for four months in the early season window (T6, 1.1 means lesions) or 6 months from November to season end (T2, 0.98 mean lesions) than no debris (T8, 0.28 mean lesions), two months early in the season (T5, 0.15 mean lesions), four months from January to season end (T3, 0.24 mean lesions) or two months at season end (T4, 0.29 mean lesions). While not significantly different from each other, the incidence of disease pre-incubation disease also showed these trends.

Following incubation, CBS incidence exceeded ninety six percent in all treatments with no significant differences between incidence in any treatment (Table D1.2). This indicates that all fruit were exposed to background inoculum from within the orchard to establish latent infections regardless of canopy exposure treatment. Differences were still detected in post incubation disease severity. Where infected pruning debris was retained for at least four or more months that encompass a proportion of the early growing season, disease severity was significantly greater than where it was retained for only two early season months, four mid to late season months or where no debris inserted at all.

These results indicate that canopy borne inoculum can contribute to CBS symptoms at harvest and after incubation, and that, while duration of exposure is important, exposure during the early to mid-season windows is particularly influential with respect to disease. That few differences in disease incidence between treatments were detected here was unexpected and may be due to high in-field inoculum levels.

**Table D1.2**: Effect of seasonal exposure to infected canopy-retained pruning debris on CBS incidence and severity in 'Imperial' mandarin (Wallaville, 2023–2024). Values are back transformed means followed by (predicted means +/- standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| | **Pre-incubation** | | **Post -incubation** | |
|---|---|---|---|---|
| **Treatment** | **Prop. of disease incidence** | **Mean disease severity score (0-5)** | **Prop. of disease incidence** | **Mean disease severity score (0-5)** |
| 1. Sept-Apr | 0.29 (-0.89 ± 0.5) a | 0.43 (0.66 ± 0.17) ab | 0.96 (3.1 ± 0.59) a | 4.29 (0.84 ± 0.14) a |
| 2. Nov-Apr | 0.39 (-0.46 ± 0.47) a | 0.98 (0.99 ± 0.17) a | 0.96 (3.3 ± 0.61) a | 4.48 (0.72 ± 0.14) a |
| 3. Jan-Apr | 0.14 (-1.8 ± 0.61) a | 0.24 (0.49 ± 0.17) b | 0.97 (3.6 ± 0.69) a | 3.49 (1.23 ± 0.14) b |
| 4. Mar-Apr | 0.24 (-1.15 ± 0.52) a | 0.29 (0.54 ± 0.17) b | 0.97 (3.5 ± 0.66) a | 3.43 (1.25 ± 0.14) b |
| 5. Sept-Oct | 0.16 (-1.66 ± 0.59) a | 0.15 (0.39 ± 0.17) b | 0.97 (3.4 ± 0.61) a | 3.20 (1.34 ± 0.14) b |
| 6. Sept-Dec | 0.42 (-0.31 ± 0.47) a | 1.10 (1.05 ± 0.17) a | 0.99 (4.5 ± 1.16) a | 4.40 (0.78 ± 0.15) a |
| 7. Sept-Feb | 0.3 (-0.87 ± 0.5) a | 0.56 (0.75 ± 0.17) ab | 0.99 (4.15 ± 0.8) a | 4.34 (0.81 ± 0.14) a |
| 8. None | 0.18 (-1.5 ± 0.56) a | 0.28 (0.53 ± 0.17) b | 0.97 (3.3 ± 0.6) a | 3.29 (1.31 ± 0.14) b |
| LSD (predicted means) | 1.36 | 0.45 | 1.77 | 0.34 |
| P value | 0.151 | 0.04 | 1.0 | <0.001 |

## Field Trial 2 (FT2 A, B, C): Effect of canopy hygiene on CBS incidence (Gayndah, Mundubbera and Wallaville, 2023/2024 and 2024/2025)

### Methodology

FT2 trials A, B and C were conducted as demonstration style field trials conducted over two growing seasons to test practical canopy hygiene under commercial management conditions with respect to the amount of disease expressed in harvested fruit. The trials represented the commercial scenario where mechanical hedging generates loose debris left within the tree canopy and inter-row and where manual removal of accessible debris could be incorporated into existing orchard routines. The intent was not to recreate Field Trial 1 where debris was added, but to examine if removing of small pruning debris could yield measurable reductions in CBS in orchards in and under different regions, varieties and management styles.

FT 2A, B and C were established in three commercial orchards in the Central Burnett and Bundaberg regions over two growing seasons (2023-2024 and 2024-2025), representing a range of varieties, canopy structures and management systems. Site A and B each had a history of CBS and site C a history of CBS and EBS.

At each site, two treatments were established: a) Clean canopies (debris removed) and b) Standard canopies (debris retained). Clean canopy treatments were established by manually removing loose pruning debris (dead leaves, small branches, twigs and dry branch fragments lodged within the canopy remaining after commercial pruning), taking two minutes or less per tree. No structural pruning or removal of major limbs occurred. Standard canopy (debris retained) treatments were left unmodified after commercial pruning. All sites were subject to the grower's routine (non-disclosed) commercial fungicide spray program throughout the season, and no trial specific fungicides were applied.

Trial locations were as follows:

A. Will McKay, Gayndah: Seeded 'Murcott', approximately thirty-year-old trees. Treatments were established in October 2023 and harvested and assessed in July 2024.
B. Blue Rocks, Mundubbera: 'Imperial' mandarin, approximately thirty-year-old trees. Treatments were established in October 2023 and harvested and assessed in April 2024.
C. Citrus Abbotsleigh, Wallaville: 'Murcott' mandarin, twenty-year-old trees Block 14. Treatments were established in August 2024 and harvested and assessed in July 2025.

At harvest, 50 fruit were collected from each tree and assessed for incidence and severity of CBS pre- and post-incubation (0-5 scale) following the procedures described in Appendix E. There was almost no disease recorded in either treatment at Site A and Site B at harvest (2023/2024) and therefore only the post-incubation data is presented. Pre- and post-incubation data is presented for the 2024/2025 trial at Wallaville.

Disease incidence (EBS and CBS) was initially analysed with a HGLM with a fixed effect (treatment) assumed to follow a binomial distribution with a logit link. A single factor representing pairs of trees down the row (blocks) was fitted as the random term and assumed to follow a beta distribution with a logit link. As the model would not converge, a GLMM was fitted (random model, normal distribution, identity link, terms as above, dispersion parameter estimated). The variance components were bound and the denominator degrees of freedom over-estimated so a GLM was used instead (dispersion parameter estimated, predicted means presented as proportions). Post-incubation, EBS, the HGLM converged and HGLM output used. Post-incubation, CBS, a GLMM was used as above.

Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). Pre-incubation, a log 10 transformation for EBS data was required to satisfy the assumption of normality. Pre- and post-incubation, no transformations were needed for CBS data. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (Fisher's Protected LSD).

**Results & Discussion**

These results illustrate removal of pruning debris can be an effective means of reducing CBS in mature fruit in commercially managed settings but is highly dependent on the locality of the orchard, variety and age of trees, existing canopy management systems and the disease history of each individual orchard.

Here, in only three orchards that differed either by locality or variety, removing small dead wood and pruning debris from canopies produced from minimal to exceptional reductions in CBS incidence and severity in harvested mature fruit (Table D2.1).

An outstanding response to pruning debris removal was recorded in 'Imperial' mandarin at Mundubbera with significantly higher disease levels in fruit where dead wood was retained (41% compared with 23%, Table D2.1). This translated to a large gain in the pack-out of premium grade fruit and farm-gate income. It also indicates that, when the inoculum pressure is lowered by removal of debris, existing fungicide treatments are highly effective. This corroborates earlier experimental field trial findings.

The differences in CBS recorded in fruit from the 'Murcott' mandarin trial at Gayndah (2023/2024) when debris was removed were minimal and not significantly different, but were slightly lower in fruit harvested from trees from which debris was removed. This site was known to have high CBS disease pressure and it is possible the inoculum load within the canopy was at such a level that it masked the effect of removing the pruning debris. This shows, in the presence of high levels of inoculum, fungicide efficacy is compromised.

Removing debris in the 2024/2025 trial at Wallaville also had no significant impact on CBS or EBS in fruit at harvest or after incubation assessments with almost identical levels of disease between treatments in both assessments. CBS increased considerably after incubation, however, indicating similar latent infection levels regardless of canopy status. This may indicate high levels of inoculum in the orchard as a whole masking treatment responses or that the fungicide program employed by the grower (non-disclosed) controlled CBS irrespective of debris removal.

These findings indicate that removal of small dead wood and leftover pruning debris can contribute to highly measurable reductions in CBS expression under commercial field conditions and that canopy hygiene may complement fungicide programs, particularly in orchards where dead wood accumulation is high. Varietal differences may also be important, as 'Imperial' fruit showed a stronger response to the removal of pruning debris compared to 'Murcott'.

We would highly recommend future work to test this in a replicated trial in an orchard with a known CBS history in conjunction with multiple pruning regimes and targeted fungicide treatments.

**Table D2.1**: Effect of canopy hygiene (debris removed vs debris retained) on CBS incidence and severity across three commercial-growing mandarin orchards (Gayndah, Mundubbera and Wallaville; 2023–2025). Values represent treatment means with standard errors. Values are raw* or back transformed means followed by (predicted means ± standard errors). LSD values correspond with predicted means. Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05.

| Trial Location | Year | Disease / Assessment Stage | Treatment | Prop. of disease incidence | Mean disease severity score (0-5) | LSD (Incidence) & P value | LSD (Severity) & P value |
|---|---|---|---|---|---|---|---|
| A. Gayndah | 2023/24 | CBS (Post-incubation) | Debris Removed | 0.35* ± 0.03 a | 0.58* ± 0.06 a | 0.10 p=0.21 | 0.19 p=0.22 |
| | | | Debris Retained | 0.41* ± 0.04 a | 0.69* ± 0.07 a | | |
| B. Mundubbera | 2023/24 | CBS (Post-incubation) | Debris Removed | 0.23* ± 0.02 b | 0.46* ± 0.06 b | 0.09; p=0.001 | 0.002; p=0.004 |
| | | | Debris Retained | 0.41* ± 0.04 a | 1.01* ± 0.13 a | | |
| C. Wallaville | 2024/25 | CBS (Pre-incubation) | Debris Removed | 0.06 (-0.94 ± 0.02) a | 0.07 (0.07 ± 0.02) a | 0.05; p=0.7 | 0.05; p=0.65 |
| | | CBS (Pre-incubation) | Debris Retained | 0.07 (-0.93 ± 0.01) a | 0.08 (0.08 ± 0.01) a | | |
| | | CBS (post-incubation) | Debris Removed | 0.31 (-0.79- ± 0.16) a | 0.46 (0.43 ± 0.06) a | 0.0; p=1.0 | 0.17; p=0.58 |
| | | CBS (post-incubation) | Debris Retained | 0.33 (-0.73 ± 0.17) a | 46 (0.47 ± 0.06) a | | |
| | | EBS (Pre-incubation) | Debris Removed | 0.03 (-0.97 ± 0.01) a | 0.02 (-1.5 ± 0.01) a | 0.032; p=0.14 | 0.33; p=0.41 |
| | | | Debris Retained | 0.04 (-0.95 ± 0.01) a | 0.03 (-1.37 ± 0.01) a | | |

All orchards were treated by the grower with the grower's non-disclosed fungicide spray program. Fifty fruits were assessed per tree before and after incubation following the General Assessment Methodology (Appendix E). Trial A and B, analysed by Dr. Thangavel, Trial C analysed by Dr. Wright and each trial was analysed separately.

## Field Trial 3 (FT3): Evaluating sources and persistence of CBS inoculum within canopy dead wood (Wallaville, 2023–2024)

### Methodology

FT3 was designed to test whether different forms of pruning debris are viable sources of *P. citricarpa* inoculum, if infection varies with type or timing of introduction of debris or if inoculum production continues on the debris after chemical sterilisation, assessed by disease recorded in mature fruit. The trial was conducted in a commercial 'Imperial' mandarin block at Spencer Ranch, Wallaville, Queensland, from petal fall in September 2023 to harvest in March 2024.

Twenty treatments representing five biological hypotheses, namely, that disease expressed in mature fruit is affected by (a) dead wood source and size, (b) surface chemical suppression, (c) citrus host species of the debris, (d) fortnightly replacement schedules and (e) delayed introduction of new branches were compared with a clean-canopy control treatment (Table D3.1).

Branches of lemon, Tahitian lime and 'Imperial' mandarin were collected from adjacent orchards and selected branches were introduced without treatment or surface-sterilised or treated with copper hydroxide, pyraclostrobin or quaternary ammonium prior to attachment (treatments 5-7). Introduction or replacement events occurred from October to March to test both persistence and seasonal activity of inoculum.

The lemon and mandarin branches were collected from orchards with known histories of CBS. The lime branches were also collected from a nearby orchard, but, as lime is traditionally thought to be resistant to CBS, it was deemed to be a zero or low inoculum treatment. Trees were relatively uniform and approximately 2.5 metres tall. A randomised complete block design was used with four single-tree replicates per treatment (Table D3.1). Excepting the trial treatments, the trial site was managed commercially by the grower collaborator (maintenance, insecticide, fungicide).

A randomised complete block design was used, consisting of twenty treatments with four single-tree replicates per treatment (Table D3.1). At harvest, 50 fruit were collected from each tree and assessed for incidence and severity of CBS pre- and post-incubation (0-5 scale) following the procedures described in Appendix E.

Disease incidence was analysed with a HGLM with a fixed effect (treatment) assumed to follow a binomial distribution with a logit link and a random term (block) assumed to follow a beta distribution with a logit link. The dispersion parameter was estimated. Predicted means and standard errors are presented on the logit scale. BT means are expressed as proportions. Mean severity was analysed with a linear mixed model with effects as above (diagnostic plots used to confirm assumptions of normality and homogeneity of variance). Pre-incubation, a square root transformation was applied to satisfy the assumptions. All significance testing was performed at the 0.05 level, and if significant, pairwise comparisons were conducted using the 95% least significant difference (LSD).

**Table D3.1**: Treatment structure for Field Trial 3 evaluating canopy dead wood as a potential source of CBS inoculum. Treatments were grouped into five categories representing different biological hypotheses: dead wood type and size, chemical surface treatments, citrus host species of the dead wood, fortnightly replacement schedules and delayed introduction of branches.

| Treat (T) | Category | Dead wood source | Size | Chemical status | Exposure timing | Movement / Replacement | Biological purpose |
|---|---|---|---|---|---|---|---|
| 1 | A: Dead wood type | Lemon branch, no leaves | All | Untreated | Present all season | None | Test whether leaves are required as inoculum source |
| 2 | A: Dead wood type | Leaves only (bagged) | Leaves | Untreated | Present all season | None | Confirm leaves alone do not act as inoculum source |
| 3 | A: Dead wood size | Lemon twigs (<20 mm) | <20 mm | Untreated | Present all season | None | Evaluate effect of small-diameter wood |
| 4 | A: Dead wood size | Lemon branches (>20 mm) | >20 mm | Untreated | Present all season | None | Evaluate effect of large-diameter wood |
| 5 | B: Chemical suppression | Lemon whole branch | All | Bleach + copper | Present all season | None | Test whether chemical sanitation reduces viable inoculum |
| 6 | B: Chemical suppression | Lemon whole branch | All | Agriquat + copper | Present all season | None | Same as above, QAC[1]-based |
| 7 | B: Chemical suppression | Lemon whole branch | All | Pyraclostrobin + copper | Present all season | None | Test QoI[2] + copper suppression |
| 8 | B: Chemical suppression | Lemon whole branch | All | Untreated | Present all season | None | Baseline control for chemical suppression |
| 9 | C: Citrus host species | Tahitian lime branch | All | Untreated | Present all season | None | Compare inoculum potential of resistant vs susceptible hosts |
| 10 | C: Citrus host species | Imperial mandarin branch | All | Untreated | Present all season | None | Compare inoculum potential of alternative host |
| 11 | D: Replaced every 2 weeks | Fresh lemon branch | All | Untreated | Present all season | New branch every 2 weeks | Test whether healthy bark becomes a reservoir over time |
| 12 | E: Moved at week 6 | Lemon whole branch | All | Bleach + copper | Present weeks 0–6 | Moved to T16 tree after 6 weeks (i.e. no debris after move) | Test if spores reappear after early sanitation |
| 13 | E: Moved at week 6 | Lemon whole branch | All | Agriquat + copper | Present weeks 0–6 | Moved to T17 tree after 6 weeks | Same as above |
| 14 | E: Moved at week 6 | Lemon whole branch | All | Pyraclostrobin + copper | Present weeks 0–6 | Moved to T18 tree after 6 weeks | Test suppression longevity |
| 15 | E: Moved at week 6 | Lemon whole branch | All | Untreated | Present weeks 0–6 | Moved to T19 tree after 6 weeks | Untreated movement control |
| 16 | F: Introduced late | Lemon whole branch | All | Bleach + copper | First introduced at week 6 | Present week 6–end | Test delayed introduction (no dead wood early season) |
| 17 | F: Introduced late | Lemon whole branch | All | Agriquat + copper | First introduced at week 6 | Present week 6–end | Test delayed introduction |
| 18 | F: Introduced late | Lemon whole branch | All | Pyraclostrobin + copper | First introduced at week 6 | Present week 6–end | Test delayed introduction |
| 19 | F: Introduced late | Lemon whole branch | All | Untreated | First introduced at week 6 | Present week 6–end | Untreated delayed introduction control |
| 20 | Control | No branch | - | - | None all season | None | Clean-canopy positive control |

[1] QAC = Quaternary Ammonium Compounds; [2] QoI = Quinone outside inhibitor (strobilurin fungicide)

**Results & Discussion**

This trial was comprised of 20 treatments in five categories representing inoculum source, size, host, timing and the ability to suppress inoculum production with early chemical intervention. While significant differences were found in the incidence and severity of CBS at harvest, and in severity post-incubation treatments, there were few overall differences between treatments.

At harvest, incidence in all treatments including the negative control was quite low (6-26%) (Table D3.2). Introduction of most types or size of debris, or time of introduction of debris (beginning of trial versus 6 weeks), had no impact on the incidence of CBS recorded in mature fruit. Only the insertion of an 'Imperial' mandarin branch (T10, 21%), lemon branches treated with Agriquat® (T6, 26%), branches left untreated (T8, 21%), or branches moved at 6 weeks (T14, 21%) showed significantly increased levels of disease compared to the negative control (natural canopy, no branch inserted, T20, 7%).

*Dead wood type (treatments 1-4)*

The size (lemon branch versus twigs) or type (branch versus leaves) did not significantly affect incidence and severity of CBS at harvest or incidence after incubation but indicated that twigs (<20 mm in diameter) may contribute more inoculum than larger branches (pre-incubation, T3, 15% versus T1, 2 and 4, 6-9%).

The post-incubation data supports this with the severity of disease in fruit in the twigs treatment (T3) showing significantly greater disease severity than T 1, 2 or 4 (3.15 versus 2.57, 2.25 and 2.27 mean lesions respectively) and the negative control treatment (T20, 1.62 mean lesions). These results indicate further investigation is warranted with respect to the disease cycle of CBS within the canopy and to understand where the greatest proportion of infective spores are being produced.

*Chemical suppression (treatments 5-8, 12-15, 16-19)*

The type of chemical treatment when the branch was treated and remained in place all season (T5-8) did not significantly affect incidence pre- or post-incubation or severity of CBS pre-incubation, but the results were informative. Agriquat® proved the least effective of the chemical treatments (T6, 26%). Disease recorded in the bleach (T5, 18%) and pyraclostrobin (T7, 16%) treatments, however, was not significantly different from the negative control treatment where no branch was added (T20, 7%) implying treatment with bleach or pyraclostrobin reduced the inoculum load and therefore infection whereas Agriquat® did not. Treatment did affect the severity of symptoms after incubation, with significantly higher severity in the Agriquat® (T6, 3.69 mean lesions) and bleach (T5, 3.79 mean lesions) than the two remaining treatments.

When branches were treated as above and moved after 6 weeks (T12-15), few significant differences in disease were noted at harvest and none post-incubation. In this scenario, treatment with pyraclostrobin was the least effective (T14, incidence and severity 21% and 0.38 mean lesions respectively) with significantly higher incidence and severity than treatment with bleach and the negative control treatment (T20). After incubation, the severity observed in all four treatments was significantly greater than the negative control treatment.

In the counterpart treatments (T16-19) where the branches were inserted at week 6, the chemical treatment had no impact on incidence or severity at harvest or post-incubation when compared with each other. When compared with the negative control, the severity of CBS at harvest was significantly greater in the bleach treatment (T16, 0.3 mean lesions) and the untreated branch treatment (T19, 0.36 mean lesions) than the negative control treatment. After incubation, the severity of disease in all

treatments were significantly greater than the negative control treatment.

*Host species*

The insertion of mandarin branches (T10, 21%) significantly increased the incidence of disease in comparison with the negative control but not in comparison with insertion of Tahitian lime branches (T9, 14%) or most of the treatments where lemon branches were inserted. The exceptions were lemon leaves (T2, 6%), lemon branches replaced fortnightly (T11, 6%) or treatment with bleach (T12, 6%). This implies that all three citrus species host CBS and that inoculum production may take longer than two weeks. Again, this warrants further investigation with respect to the life cycle and spore production of CBS in the canopy.

When examining disease severity at harvest, the presence of both mandarin and Tahitian lime significantly affected disease compared with the negative control but not more than any other treatment except than the lemon leaves only treatment (T2, 6%). Mandarin and Tahitian lime branches both facilitated higher levels of disease severity at harvest than the negative control. The severity was not significantly different from if lemon branches were added or any other treatment combination except the lemon leaves-only treatment (T2, 6%).

Host species had no impact on post-incubation incidence or severity but the presence of mandarin branches significantly increased the severity of disease in comparison with the negative control alone (T20, 1.62 mean lesions)

*Summary*

Overall, the results indicate dead wood rather than leaves are the primarily source of inoculum of CBS and that twigs may be the most important component. Many other diseases of citrus fruit reside and produce inoculum in the twigs, so this would not be unexpected. It needs to be verified, however, and further investigation is recommended.

The species of citrus does not appear to affect the incidence of disease indicating CBS is present in lemon, mandarin and lime branches and needs management in all scenarios. This is interesting with respect to lime citrus being considered resistant to CBS by much of the industry.

The replacement schedules trialled here provided no real insights with respect to inoculum production and infection. Delayed introduction of infected wood generated incidence levels comparable to those observed when branches were present from the beginning of the season. Disease recorded when fresh lemon branches were introduced every two weeks equalled the negative control treatment implying that inoculum may take more than two weeks to be produced and dispersed. This needs further investigation and is significant with respect to timing of fungicide application and practical disease control. The negative or 'clean-canopy' control consistently produced the lowest incidence, confirming that while underlying orchard inoculum was present, attaching dead wood increased infection and can be an effective method for inoculum experimentation.

Chemical treatment of debris was not effective with respect to incidence of CBS in comparison with the untreated chemical control treatment indicating that treatment may need to be ongoing to significantly reduce disease. This again indicates a more complete understanding is needed to determine where CBS resides and produces inoculum from. The postharvest disease severity measures indicate that while treatment with pyraclostrobin did lower disease compared with bleach and Agriquat® it was no more effective than not treating at all, again indicating a one-off treatment of debris is not an effective disease control strategy.

Collectively, these findings highlight that canopy dead wood functions as an inoculum reservoir but

that its contribution to fruit infection is influenced by multiple interacting factors not fully resolved within this trial. Further work that directly quantifies viable spore release from different dead wood types, rather than relying on fruit incidence alone, would be required to determine the relative importance of dead wood source, treatment and persistence.

**Table D3.2**: Effect of dead wood source, diameter, and chemical surface treatments on CBS incidence and severity in 'Imperial' mandarin under commercial orchard conditions (Wallaville, Queensland; 2023–2024). Values represent back transformed or raw* means followed by (predicted means ± standard error). Means followed by the same letter within columns are not significantly different according to Fisher's least significant difference (LSD) test at p≤0.05. (B – Bleach, Ag – Agriquat, P – pyraclostrobin, Un – untreated control.)

| | | Pre-incubation | | Post-incubation | |
|---|---|---|---|---|---|
| **Treatment** | **Dead wood source** | **Prop. of disease incidence** | **Mean disease severity score (0-5)** | **Prop. of disease incidence** | **Mean disease severity score (0-5)*** |
| 1. A: Dead wood type | Lemon branch, no leaves | 0.09 (-2.27 ± 0.47) fgh | 0.11 (0.33 ± 0.11) cdefgh | 0.84 (1.69 ± 0.44) a | 2.57 (2.61 ± 0.4) cdefg |
| 2. A: Dead wood type | Leaves only (bagged) | 0.06 (-2.82 ± 0.59) h | 0.06 (0.24 ± 0.11) gh | 0.8 (1.37 ± 0.41) a | 2.25 (2.2 ± 0.4) efg |
| 3. A: Dead wood size | Lemon twigs (<20 mm) | 0.15 (-1.72 ± 0.39) efgh | 0.25 (0.5 ± 0.11) abcdefg | 0.9 (2.16 ± 0.57) a | 3.15 (3.37 ± 0.4) abc |
| 4. A: Dead wood size | Lemon branches (>20 mm) | 0.08 (-2.39 ± 0.49) fgh | 0.07 (0.26 ± 0.11) fgh | 0.81 (1.43 ± 0.48) a | 2.27 (2.25 ± 0.43) defg |
| 5. B: Chemical suppression (B) | Lemon whole branch | 0.18 (-1.51 ± 0.37) efgh | 0.45 (0.67 ± 0.11) ab | 0.94 (2.82 ± 0.73) a | 3.79 (3.87 ± 0.4) a |
| 6. B: Chemical suppression (Ag) | Lemon whole branch | 0.26 (-1.03 ± 0.32) e | 0.50 (0.7 ± 0.11) a | 0.92 (2.37 ± 0.6) a | 3.69 (3.75 ± 0.4) ab |
| 7. B: Chemical suppression (P) | Lemon whole branch | 0.16 (-1.66 ± 0.41) efgh | 0.30 (0.55 ± 0.12) abcdefg | 0.81 (1.42 ± 0.47) a | 2.6 (2.58 ± 0.43) cdefg |
| 8. B: Chemical suppression (Un) | Lemon whole branch | 0.21 (-1.31 ± 0.34) ef | 0.41 (0.64 ± 0.11) ab | 0.88 (1.97 ± 0.54) a | 2.9 (2.92 ± 0.4) abcdef |
| 9. C: Citrus host species | Tahitian lime branch | 0.14 (-1.86 ± 0.44) efgh | 0.34 (0.59 ± 0.11) abcde | 0.75 (1.07 ± 0.4) a | 1.96 (1.97 ± 0.4) fg |
| 10. C: Citrus host species | Imperial mandarin branch | 0.21 (-1.34 ± 0.38) ef | 0.33 (0.57 ± 0.12) abcdef | 0.9 (2.14 ± 0.66) a | 3.24 (3.05 ± 0.43) abcdef |
| 11. D: Replaced every 2 weeks | Fresh lemon branch | 0.06 (-2.81 ± 0.64) gh | 0.08 (0.28 ± 0.12) efgh | 0.8 (1.36 ± 0.48) a | 2.1 (2.01 ± 0.43) fg |
| 12. E: Moved at week 6 (B) | Lemon whole branch | 0.06 (-2.8 ± 0.58) h | 0.09 (0.3 ± 0.11) defgh | 0.7 (0.86 ± 0.39) a | 2.32 (2.46 ± 0.4) cdefg |
| 13. E: Moved at week 6 (Ag) | Lemon whole branch | 0.11 (-2.09 ± 0.44) fgh | 0.24 (0.49 ± 0.11) abcdefgh | 0.84 (1.68 ± 0.48) a | 2.97 (3.16 ± 0.4) abcde |
| 14. E: Moved at week 6 (P) | Lemon whole branch | 0.21 (-1.32 ± 0.37) ef | 0.38 (0.62 ± 0.11) abc | 0.86 (1.85 ± 0.55) a | 3.32 (3.28 ± 0.4) abcd |
| 15. E: Moved at week 6 (Un) | Lemon whole branch | 0.15 (-1.7 ± 0.38) efgh | 0.28 (0.53 ± 0.11) abcdefg | 0.81 (1.43 ± 0.42) a | 2.75 (2.79 ± 0.4) bcdef |
| 16. F: Introduced late (B) | Lemon whole branch | 0.17 (-1.57 ± 0.39) efgh | 0.30 (0.55 ± 0.11) abcdef | 0.92 (2.38 ± 0.58) a | 3.09 (3.18 ± 0.37) abcde |
| 17. F: Introduced late (Ag) | Lemon whole branch | 0.13 (-1.89 ± 0.44) efgh | 0.21 (0.46 ± 0.12) abcdefgh | 0.95 (2.9 ± 0.82) a | 3.67 (3.73 ± 0.43) ab |
| 18. F: Introduced late (P) | Lemon whole branch | 0.07 (-2.58 ± 0.59) fgh | 0.12 (0.35 ± 0.12) bcdefgh | 0.85 (1.73 ± 0.47) a | 2.93 (2.94 ± 0.4) abcdef |
| 19. F: Introduced late (Un) | Lemon whole branch | 0.2 (-1.4 ± 0.36) efg | 0.36 (0.6 ± 0.11) abcd | 0.84 (1.68 ± 0.49) a | 3.13 (3.33 ± 0.4) abcd |
| 20. Negative Control | No branch | 0.07 (-2.66 ± 0.55) gh | 0.04 (0.2 ± 0.11) h | 0.74 (1.02 ± 0.38) a | 1.62 (1.61 ± 0.4) g |
| LSD (predicted means) | | 1.24 | 0.3 | 1.42 | 1.07 |
| P value | | 0.008 | 0.01 | 0.12 | <0.001 |

# Appendix E

## General Assessment Methodology

### Spray application method

Spray applications across all field trials were conducted using a high-pressure handgun spray unit (AR30-type pump coupled to a 5-HP four-stroke Honda motor). Applications were undertaken under low wind conditions to minimise spray drift between adjacent trees. Spray volumes were calibrated at the beginning of each season and maintained to ensure uniform canopy coverage across sites and seasons. Operating pressure was adjusted as required to maintain a stale spray pattern under commercial field conditions. Fungicide spray applications were directed throughout the canopy from lower to upper sections in a consistent pattern to achieve thorough wetting and ceased at the point of incipient runoff (approximately 3-6 L of spray mixture per tree depending on tree size). Unless otherwise indicated, all fungicides were applied at label or industry-standard rates according to the respective treatment rate, treatment structure, and program design. Tank mixes were prepared fresh for each spray event, with spray volumes typically prepared in 100 L or 200 L batches depending on treatment requirements and the number of trees to be treated.

### Fruit assessments

Fruit disease assessments were conducted using two approaches: in-field assessments and postharvest assessments.

*In-field fruit assessment*

In-field assessments were conducted at fruit maturity. Four to five independent assessors counted the number of fruit per tree showing visible EBS or CBS symptoms, defined as the presence of at least one lesion. Each tree was assessed for approximately 30 seconds to one minute (trial dependent). Disease incidence was expressed as the proportion of fruit with visible symptoms per tree.

*Postharvest fruit assessment*

Unless stipulated otherwise, for each harvest, 50 fruit were collected per tree. Each individual fruit was examined for the presence of symptoms of EBS and/or CBS lesions, and disease incidence was recorded as presence or absence of visible spots. Fruit were assigned a disease severity score using an ordinal rating scale, with the scale applied depending on the trial.

In 2022/2023, disease was assessed using a 0–10 ordinal scale based on lesion counts per fruit, where 0 indicated no lesions and 10 indicated more than 100 lesions per fruit (0 = no lesions; 1 = 1 lesion; 2 = 2–3 lesions; 3 = 4–6 lesions; 4 = 7–9 lesions; 5 = ≥10 lesions; 6 = ≥15 lesions; 7 = ≥20 lesions; 8 = ≥30 lesions; 9 = >50 lesions; 10 = >100 lesions per fruit).

In the 2023/2024 onwards, disease was assessed using a 0–5 ordinal scale, where 0 represented no visible lesions, 1 = 1–2 spots, 2 = <5 spots, 3 = <10 spots, 4 = <20 spots, and 5 = >20 spots per fruit. Within each trial, the same severity scale was applied consistently.

Fruit were assessed twice, once before and once after incubation. Pre-incubation assessments were conducted within two days of harvest. After assessment, fruit were immersed in a solution of ethephon (2.10 g $L^{-1}$) and imazalil (0.25 g $L^{-1}$) for one minute and allowed to dry in the shade before incubation for 21 days and reassessment.

Fruit were incubated at the BRF for 21 days at 27 °C under continuous light to allow symptom expression. Where there were large numbers of fruit (multiple trials harvested simultaneously), fruit were incubated at ambient temperatures under filtered sunlight and artificial light at night for 21 days. Following incubation, fruit were re-assessed using the same disease rating scale applied at pre-incubation.

**Pathogen confirmation**

To confirm CBS diagnosis, symptomatic fruit were collected and fungal isolations were made from lesion tissues in the laboratory. Isolations were conducted on half-strength potato dextrose agar (PDA) alone or ½ PDA amended with streptomycin and incubated under controlled conditions for one week. *P. citricarpa* colonies were identified using spore and colony morphology, including dense, dark mycelial growth and characteristic pycnidiospore shape and prolific production. Representative isolates were purified and sequenced to confirm species identity.

To confirm EBS diagnosis, the above was repeated and colonies of *Alternaria* identified using spore morphology.

**Data analysis**

Detailed methods for statistical analysis are provided for each trial in the appropriate index. During the project analyses were conducted by Dr. Tamil Thangavel (project member) and for the final report by Senior Principal Biometrician Dr. Carole Wright, DPI.