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Validating water disinfestation systems in nursery production

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Validating water disinfestation systems in nursery production

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Public summary

The Australian production nursery industry makes up nearly 20 % of the value of horticultural production in this country. Being dynamic and diverse, it holds a pivotal place in supporting Australian horticulture.

Water quality is a key issue as many production nurseries irrigate with water sourced from creeks, dams, or rivers, and many businesses collect and reuse irrigation water. This introduces significant risk from fungal and bacterial plant diseases that are spread in irrigation water. A variety of different disinfestation systems are used to eliminate pathogens from water. There is a high level of uncertainty that exists around the efficacy and cost-effectiveness of some disinfestation systems, particularly new technologies. To support the Australian production nursery industry in cost effectively managing these risks, this project evaluated and compared the efficacy and crop safety of current and alternative irrigation water disinfestation methods and provides detailed guidelines on their use within the Australian nursery industry. To achieve this we reviewed available mainstream, alternative and emerging water disinfestation systems and identified gaps in methods where evaluation of efficacy is required. We then evaluated the efficacy of different disinfestation systems currently used by industry.

An industry-wide survey was conducted to gauge the type of systems being used and the efficiency of these. While the uptake of this survey was low, there were participants that we were able to work with in the next phase of the project. Through this testing we were able to optimise water testing procedures for the enumeration of fungal and bacterial populations. We were also able to work with growers to determine the efficiency of their systems and retest to determine if any addressed issues were resolved. This testing highlighted the need for regular testing of disinfestation systems to maintain their efficacy.

The final phase was to test some alternate systems/products to determine their efficacy against target fungal and bacterial plant pathogens. Based on laboratory testing stabilized hydrogen peroxide and a quaternary ammonium compound showed promising potential as disinfestation products in a nursery setting based on their ability to eliminate bacteria and fungi with minimal impact on seedlings based on phytotoxicity assays.

Keywords

Nursery irrigation; water disinfestation; water-borne plant pathogens

Introduction

The Australian production nursery industry represents the largest horticultural sector with sales worth $2.79 billion in 2020-21. The majority of nursery production value occurs in the eastern states – New South Wales (30%), Queensland (30%), Victoria (28%) – with smaller values from Western Australia (8%), South Australia (2%), Northern Territory (1%) and Tasmania (<1%).

A key characteristic of the nursery industry is diversity, with nurseries operating in each region across Australia, including urban, peri urban, rural and remote settings. The industry also sold approximately 2.3 billion plants across 2020/21 covering more than 10,000 varieties of plant cultivars, with supply ranging in size from propagation stock through to advanced tree specimen stock. Supply extends to a diverse customer base including retail, landscape (private and public), primary production (fruits, nuts, vegetables, cut flowers), revegetation and forestry. This requires large volumes of plants to be routinely traded across intrastate, interstate, and international pathways. It is important to recognise that the nursery sector is an essential link in the supply chain for the broader production horticultural sector acting as the *ab-initio* input for fruit, nut and ornamental trees and shrubs, vegetable crops, cut flowers, and stock for environmental rehabilitation.

Access to high quality irrigation water is required in production nurseries to grow healthy plants. Production nurseries commonly use irrigation water sourced from open water systems or recycle and reuse irrigation water. However, these sources can increase the risk of plant diseases as many pathogens including Phytophthora, Pythium, Fusarium, Ralstonia and Clavibacter can be spread via irrigation water. These pathogens can cause root rot, wilt, damping-off, lack of vigour, decline and/or plant death in containerised nursery stock. Disease management programs are costly and fungicide/bactericide treatments often only suppress disease, such that plants appearing healthy at point of sale may remain infected and subsequently decline and die months or years later. Disease prevention makes better economic and environmental sense, and disinfestation of irrigation water, potentially contaminated with pathogens before use, is pivotal to this concept.

Hort Innovation industry consultation revealed that the presence of plant pathogens in irrigation water is a major concern, and a high level of uncertainty exists around the efficacy and cost-effectiveness of current and alternative irrigation water disinfestation practices. Historically, the nursery industry in Australia has applied chlorine as the most common chemical water disinfestation treatment. However, there has been significant uptake of alternative chemical treatments, such as copper ionisation, within the industry, for which knowledge on the efficacy and crop safety in the Australian nursery system is limited. There are not only significant differences in efficacy between current methods, but the efficacy of any individual method can be influenced by the target pathogen (largely the type of propagule, i.e., conidia vs chlamydospores) and water quality characteristics (e.g., turbidity and pH). Therefore, the efficacy of alternative methods needs to be established on a select range of economically important plant pathogens present in irrigation water with different quality characteristics.

This project builds on previous nursery industry and Hort Innovation investments of relevance, including projects delivered by the former Nursery and Garden Industry Australia (NY13003 - Increasing productivity through industry research, development and extension programs, and NY17009 - Improving pest management for the nursery industry) and draws on components previously delivered by Queensland Department of Agriculture and Fisheries (recent name change to Queensland Department of Primary Industries, and from here on in referred to as QDPI) (NY15002 - Building the resilience and on-farm biosecurity capacity of the Australian production nursery industry). These projects have produced several outcomes and developed a range of tools for use by the nursery industry to improve the knowledge base for management of pathogens in irrigation water. NSW led previous research involving monitoring and evaluating nursery irrigation water, through NY13003. In their study, the efficacy of chlorine (sodium hypochlorite), chlorine dioxide and ultraviolet radiation (UV), at a range of application rates and exposure times, in deionised water and dam water was successfully determined against propagules (spores, mycelium and cells) of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), *Alternaria alternata*, *Chalara elegans*, *Colletotrichum gloeosporioides*, *Calonectria pauciramosa*, *Fusarium oxysporum*, *Phytophthora cinnamomi* and *Pythium aphanidermatum* (Scarlett et al. 2015). Furthermore, NSW Department of Primary Industries and Regional Development (DPIRD) researchers developed water testing assays for the detection of plant pathogens. Trials were conducted through 2011 and 2012 under the Water Smart Farms project (NSW DPIRD Project based in the Hawkesbury/Nepean Catchment) to evaluate the efficacy of ultraviolet light (UV), copper ionisation and ozone technologies against Pythium, Phytophthora and Fusarium spp. in irrigation water sourced from town water, tanks and dams. In addition, NSW DPIRD researchers from this project team developed and conducted testing assays of an ultrafiltration system for horticultural industries in a Hort Innovation project VG13052 and contributed to efficacy testing of chlorine dioxide, chlorine and UV systems for the nursery industry (Scarlett et al., 2015). For several years, the NSW Plant Health Diagnostic Service (PHDS) also performed independent testing of water samples for Phytophthora spp. for businesses undergoing NIASA accreditation audit, as does the Queensland Government diagnostic service, Grow Help Australia at QDPI.

The overall project aims were to evaluate and compare the efficacy and crop safety of current and alternative irrigation water disinfestation methods and provide detailed guidelines on their use within the Australian nursery industry. The project was delivered in two years through three sequential Phases. While this project was led by NSW DPIRD it was a collaborative project with QDPI designed to deliver on project aims to best suit the industry which required adjustments along the way based on project findings.

Phase 1: To undertake a review of the available mainstream, alternative and emerging water disinfestation methods used by the nursery industry and conduct a gap analysis to identify methods where evaluation of efficacy is required.

Phase 2: To evaluate and compare efficacy and crop safety of the water disinfestation methods identified in the gap analysis, providing detailed information on the contact time, dose response, residual concentration,

cost and benefit.

Phase 3: Collaboration with Greenlife Industry Australia to update the Nursery Industry Water Management Best Practice Guidelines and NIASA Best Management Practice Guidelines

Methodology

Phase 1: Undertaking a review of the mainstream water disinfestation methods used by the nursery industry.

Activity 1: Establish a project reference group

The project reference group (PRG) was established, and meetings were held during the project. Based on discussions at the PRG the next steps for the project were taken to improve the project outcomes.

Activity 2: Project Management and Communication

This activity was conducted as outlined in the project management document included in Appendix 1.

Activity 3: Digital Survey on the types of disinfestation systems being used by nursery growers

A short survey was developed and made available to nursery growers through the national nursery e-news and state NGI newsletters. In addition, an email was directly sent to all production nursery clients with whom the QDPI team have had contact through the Grow Help diagnostic service. This survey requested information on the types of disinfestation systems currently in use, the water source type, if water is reused etc. and if growers are willing to participate in water sampling for the project. This information was vital for the PRG discussions and the design of a proper scientific study of these systems and for obtaining comparable results.

Activity 4: Review and gap analysis

A literature review was conducted in two ways. Firstly, previous project reports including, but not limited to, NY13003 (water disinfestation method validation and efficacy subproject), Nursery Industry Water Management Best Practice Guidelines (NGINA) and A Comparison of Proven Water Disinfestation Systems for Production Nurseries (NY15002 Factsheet output) were evaluated to understand current systems and recommendations yet to be validated (e.g., Aqua-Hort copper ionisation). Secondly, a critical review of the scientific literature was conducted that reviewed disinfestation systems with scientific data for both the evaluation of the disinfestation system and the methods they were using to evaluate these systems. This involved investigating new technologies and scientific methods for evaluating microorganisms in water samples.

Phase 2: Evaluation of disinfestation methods used in nurseries

Activity 1: Evaluation of the efficacy of available water disinfestation systems

All water samples collected from nurseries were tested to detect the following groups of pathogens:

1. General fungi, (including *Fusarium*, *Calonectria*, *Verticillium*, *Thielaviopsis*, *Rhizoctonia*)
2. General bacteria (including *Clavibacter michiganensis* pv *michiganensis*, *Ralstonia* sp. and *Pseudomonas* sp.)
3. *Agrobacterium* sp. (a specific bacterial pathogen)
4. Oomycetes (includes *Pythium*, *Phytophthora* and *Phytopythium*)
5. Phytophthora alone

Nursery businesses with certain types of disinfestation systems were prioritised for sampling based on the gap analysis results. Initially, it was hoped to gain multiple businesses of each disinfestation type to participate in the study. However, only 40 businesses replied to the survey and many of these could not be sampled for logistical reasons. Assistance was sought through numerous sources, but no additional water samples were received.

Each business was to have their water tested on at least two occasions in different seasons. On each occasion water was collected from at least two points (pre-treatment and post-treatment) and then analysed and compared across sampling times and different disinfestation systems. Queensland nursery water samples were processed at the Grow Help diagnostic service QDPI for the presence of fungal and bacterial pathogens based on the optimised testing methods worked up during the project. The NSW samples were collected and processed at the NSW DPIRD Plant Health Diagnostic Service. During the collection of samples, the plant diagnosticians offered the nursery a disease check on any plants that they were having issues with. Growers were also informed of free diagnostics through NY20000.

As there were numerous newer systems identified in the gap analysis that we were not able to test in a nursery setting it was decided at the PRG meeting that laboratory testing should be performed to test systems that could be useful in a nursery setting. The systems selected included silver stabilised hydrogen peroxide (Huwa-San was selected for this work), a quaternary ammonium compound (Path-X selected for this work based on discussions with nursery growers) and nanobubbles. Attempts were made to contact industry representatives for nanobubble technology without success. Given the time restrictions remaining on the project, it was decided to omit testing of nanobubbles and focus on the two other systems. Ideally, we would have wanted to test the systems against three fungal pathogens and three bacteria, however time constraints prevented this and testing was completed using Thielaviopsis and the two bacteria *Pseudomonas syringae* and *Clavibacter michiganensis* subsp *michiganensis.* These pathogens were given priority due to their frequency of occurrence in a nursery setting and their persistence against treatments that were observed in the initial testing phase.

Disinfectant testing for bacteria

We tested the effectiveness of different concentrations of the disinfectant treatments on water containing different concentrations of bacteria. Four bacterial concentrations were prepared for each of the pathogens, $1x10^2$ CFU/mL, $1x10^4$ CFU/mL, $1x10^6$ CFU/mL, $1x10^8$ CFU/mL and each was tested against 20 ppm and 200 ppm of the Huwa-San and Path-X products, with a control treatment of calcium hypochlorite at the recommended effective dose (ED) of 3 ppm.

Huwa-San TR-50 Roam Technology Active: hydrogen peroxide 49-49.9% (w/w).
Path-X™ Nutri-Tech Solutions® Active: 120/L didecyldimethyl-ammonium chloride.
Calcium hypochlorite – HyClor Granular pool chlorine Active: 650 g/kg available chlorine.

Huwa-San TR-50 and Path-X™ were tested with contact times of 5 minutes, 2 hours and 24 hours. Calcium hypochlorite was tested at 3ppm for 20 minutes and 24 hours.

The bacterial inoculum was made to a cloudy suspension at approximately $1x10^8$ CFU/mL using freshly grown culture in 500 mL sterile water. The culture was serially diluted 1:100 to obtain the required dilution series. The cultures were plated onto King B agar and incubated at 25 ^oC for 24-48 hours to confirm CFU counts. For each culture, 100 mL was transferred to a sterile 200 mL flask for each dilution and treatment. Disinfectants were made immediately prior to use and sterile water was used as the control treatment. For 20 ppm, a 1% solution was made by adding 1mL disinfectant to 99 mL water. 0.2 mL of the 1% solution was added to the 100 mL bacterial sample in a flask making a 0.002 % solution, or 20 ppm. For the 200 ppm treatments, a 10 % solution was made using 1 mL disinfectant to 9 mL water. 0.2 mL of the 10 % solution was added to the 100 mL bacterial sample making a 0.02 % solution, or 200 ppm. Calcium hypochlorite was made using a 0.1 % solution from 0.1 g to 100 mL water. 0.3 ml of this 0.1 % solution was added to the 100 mL bacterial samples making a 0.0003 % solution or 3 ppm. 0.2 mL of water (control), 1 %, or 10 % of Huwa-San and Path-X or 0.3 mL of 1 % calcium hypochlorite were added to the flasks at separate times and timed for length of contact time. Aliquots of 150 uL times 3 replicate samples were removed from flasks into microcentrifuge tubes at the end of each time interval. Samples were serially diluted x $^1/_{10}$ (0.1 mL + 0.9 mL water) to $1x10^{-6}$ for plating on Kings B agar with incubation at 25 ^oC.

Disinfectant testing for Thielaviopsis

To test the effectiveness of the disinfectant treatments at different concentrations on water containing different

concentrations of Thielaviopsis, three concentrations were prepared for the pathogen, $1x10^2$ CFU/mL, $1x10^3$ CFU/mL, and $1x10^5$ CFU/mL, and each was tested against 20 ppm and 200 ppm of the Huwa-San and the Path-X, with calcium hypochlorite as a control at the recommended effective dose (ED) of 3 ppm.

Huwa-San TR-50 Roam Technology Active: hydrogen peroxide 49-49.9 % (w/w).
Path-X™ Nutri-Tech Solutions® Active: 120/L didecyldimethyl-ammonium chloride.
Calcium hypochlorite – HyClor Granular pool chlorine Active: 650g/kg available chlorine.

Huwa-San TR-50 and Path-X™ were tested at 20 and 200 ppm with contact times of 5 minutes, 2 hours and 24 hours. Calcium hypochlorite was tested at 3 ppm for 20 minutes and 24 hours.

Fungal inoculum was made by washing Thielaviopsis spores from ¼ PDA + Novobiocin agar plates. The spore suspension was filtered through a few layers of muslin into 500 mL sterile water to remove chlamydospores. Spore numbers (endoconidia) were quantified using a haemocytometer. The initial inoculum had a concentration of 10^5 spores /mL and was serially diluted $^1/_{100}$ to 10^{-2} (10^3 spores/mL) and then $^1/_{10}$ to 10^{-3} (10^2 spores/mL). For each aliquot, 100 mL of inoculum was measured into 200 mL sterile flasks for each dilution and treatment. Disinfectants were made just before use and sterile water was used as the control treatment. For 20 ppm, a 1 % solution was made by adding 1 mL disinfectant to 9 mL water. 0.2 mL of the 1 % solution was added to the test flask making a 0.002 % solution, or 20 ppm. For 200 ppm, a 10 % solution was made using 1 mL disinfectant to 9 mL water. 0.2 mL of the 10 % solution was added to 100 mL of spore suspension making a 0.02 % solution or 200 ppm. Calcium hypochlorite was made using a 0.1 % solution of 0.1 g to 100 mL water. 0.3 ml of the 0.1 % solution was added to the 100 mL spore suspension in the flask making a 0.0003 % solution or 3 ppm. 0.2 mL of water, 1 %, or 10 % solutions of Huwa-San and Path-X or 0.3 mL of 1 % calcium hypochlorite were added to the flasks at separate times and timed for length of contact time. Aliquots of 150 uL x 3 replicate samples were removed from flasks into microcentrifuge tubes at end of time interval. Samples were serially diluted x $^1/_{10}$ (0.1 mL + 0.9 mL) to $1x10^2$ or $1x10^1$ in 1 % water agar and plated on ¼ PDA + Novobiocin agar plates. Plates were incubated at 25°C and checked for the presence of Thielaviopsis under a dissecting microscope and counted. The counts for each sample replicate were averaged. The average count for the spores/mL were calculated for each sample replicate using the diluted or undiluted result.

Activity 2: Optimisation of current water testing assays and evaluation of any potential alternative methods

NSW DPIRD had pre-established methodologies for water testing that had been optimised for sensitivity, cost and time. Further optimisation was conducted to ensure the testing was appropriate for the specific needs of this project. In addition, the information gained from the review of the literature on other methods being used to evaluate water disinfestation systems was considered in line with the current methods and evaluated against current systems. Once methods were finalised (Appendix 2) they were shared with QDPI colleagues and were validated against QDPI current methods.

Activity 3: Evaluation of crop safety of approved and alternative water disinfestation systems

During water sampling at nurseries, we routinely asked if the current systems they were using were causing any phytotoxicity with their plants. This aspect of the testing considered the phytotoxicity of the systems/products that were identified in the gap analysis. The aim was to investigate the sensitivity of common plants to three commercially available disinfestation treatments at three increasing dose rates. Two plant types were selected due their sensitivity to chemicals; Vindicate RZ green coral lettuce and Pansy. The treatments included Huwa-San TR-50, Path-X, Calcium Hypochlorite and a control of water. The treatments were applied at half their recommended rate, the recommended rate and twice the recommended rate. Each treatment contained 20 plants per replicate, with three replicates, totaling 720 plants per variety.

The application concentrations are listed in Table 1. The products were prepared and allowed to sit overnight, mimicking storage in a tank, and then used for watering for one week. Plants were watered twice to three times a day depending on external temperatures.

Table 1: Application concentration for the phytotoxicity testing

Product	Half Recommended Dose	Recommended Dose	Twice Recommended Dose
Huwa-San	10 ppm	20 ppm	40 ppm
Path-X	10 ppm	20 ppm	40 ppm
Calcium Hypochlorite	1.5 ppm	3 ppm	6 ppm

The plants were grown on growing tables in 1 bay of a multi-bay standard plastic covered greenhouse, with fan and pad cooling, gas heating (Figure 1).

- Temperature set range 26.5 ^{o}C daytime 16 ^{o}C overnight
- Maximum/Min recorded temperatures: Maximum 32.7 ^{o}C recorded spike: Minimum 14.5 minimum spike
- Solar limits external – daily average over the course of the trial 800 to 900 w/m2

Plants were sourced from commercial wholesale nurseries and then compacted into treatment units with 20 lettuce and 20 pansy plants per tray. They were spaced in a checkerboard pattern with Pansy at one end and lettuce at the other. Prepared checkerboard trays were then placed in a propagation house to settle for three days under propagation house conditions (temperature range settings 26 ^{o}C max. to 16 ^{o}C min.). Misting heads irrigated for 2 minutes every 2 hrs. Plants were hand fed via hose and shower nozzle before moving to the greenhouse. Replicates were spread over two benches and the trial was conducted as follows:

1. 4 L buckets were dosed with appropriate treatment each day at approximately 15:00 hrs and left to sit overnight, including a control treatment.
2. Overhead irrigation was given at 10:00 and 14:00 hrs with ~ 1.6 L per application per treatment rate. The afternoon application increased to 2 L for hotter days. A medium/fine irrigation head was used.
3. Plant checks were conducted by counting the number of impacted seedlings and indicative levels of burn or chlorosis.
4. Four supplemental feeds were applied using a standard cucumber mix EC 1.2 and pH 6.8, delivered via hose and rose/shower nozzle. These feeds were applied late in the day ~17:00hrs to avoid any phytotoxic effects from the feed on the 10/03/2025 12/03/2025, 15/3/2025 and 17/03/2025

Timeline:

- Seedlings purchased and picked up on 28/02/25.
- Held in propagation house until moving to new trays 3/3/25 to 5/3/25
- Plants compacted to checkerboard pattern on 05/03/25-06/03/25 and held in propagation house.
- Feed applied once a day while in propagation house
- Moved to greenhouse on 07/03/25 to acclimatise. Hand watering to similar pattern as wholesale nursery with 2-3 times a day shower head setting.
- Initial disinfectant solutions mixed up on the 10/03/25
- Treatment application begun on 11/03/25
- 9 days of treatment applied. Last day of treatment 19/03/25
- Crop scored two times, 17/3/2015 and Final score 20/03/25

Figure 1: Seedling setup for the phytotoxicity assay.

Activity 4: Assessment of cost-benefit of available and emerging water disinfestation systems

During water sampling at nurseries, we routinely asked the growers the costs associated with their disinfestation system including setup and running. In addition, prices were sourced for the new products being tested.

Phase 3: Updating the water disinfestation best management practice and NIASA guidelines

Activity 1: Collaboration with Greenlife Industry Australia to update the water disinfestation best management practice and NIASA guidelines.

Post submission of this report, the results will be shared with Greenlife Australia to determine the next steps forward in the updating of the water disinfestation best management practice and NIASA guidelines.

Results and discussion

Activity 3: Digital Survey on the types of disinfestation systems being used by nursery growers

QDPI staff in consultation with NSW DPIRD staff designed and circulated an online survey for growers using existing contacts established with the GIA project NY20001. Some 40 nurseries completed the 16 question survey that covered topics such as: what raw water source type they use; if water is recycled; what types of disinfestation systems are currently being used and any issues with these; whether they regularly test their system efficacy; how is treated water stored; how long they have had their current system and if they are happy it; and if they are willing to participate in water sampling for the project. The results are presented in Appendix 3 *Nursery water disinfestation survey results*. Survey results show a mix of raw water sources are used by industry with dam water the most common, followed by town water, rainwater, bore water and river/creek water respectively. Approximately 30 % of respondents collected and recycled water through the nursery.

Results also show 12 different disinfestation systems are used regularly in the nursery industry of which inline chlorination is the most popular, followed by other chlorine-based technologies. These systems also tend to be the cheapest to install and run. Filtration (ultra and sand), UV systems, osmosis, and ionisation were also used regularly in nurseries. Results of the survey highlighted several areas where nurseries could improve and reduce the risk of water getting contaminated with pathogens. Storage tanks were used by most nurseries to hold both treated and untreated water but 60 % of respondents didn't clean these tanks at all, which was a concern. Water issues relating to water quality, algal blooms, phytotoxicity and dam water levels were generally rare in nurseries, but did occur. About 80 % of respondents believed their disinfestation system to be working effectively based on water chemistry and plant health in the nursery, but none tested their systems for the presence of pathogens on a regular basis. The occurrence of disease symptoms was experienced by 83 % of respondents indicating there may be issues with contaminated water in these nurseries.

Activity 4: Review and gap analysis

A literature review of water disinfestations systems that would be suitable for use in the nursery was conducted in partnership with QDPI and the written report is given in Appendix 4 *Water disinfestation systems*.
Through this literature review, a gap analysis was conducted and information included in the milestone report, including any recommendations either for inclusion in this project or in future studies.

Research Gaps
Several of the newer disinfestation techniques covered in this review require more research, including plasma activated water, benzoic acid, chlorobromine, iodine, ionisation systems, silver hydrogen peroxide and nanobubbles. These methods, and even some of the more established methods, require more field trial data because much of the existing literature is based on *in vitro,* artificial systems, or hospital systems, which do not resemble the environment where the irrigation system is ultimately going to be used (Stewart-Wade 2011). The last significant nursery survey in NSW was conducted in 1999 and only looked at a few nurseries that had disinfestation systems (Tesoriero et al 2002). In addition, the prospect of combining certain techniques could be examined further as there is "no single treatment that effectively manages all types of contaminants" (Ristvey et al 2019). This project addresses some of these issues by testing systems (including some coupled systems) in place in nurseries, thereby considering real-world conditions.

Another area of research that would ultimately enhance our disinfestation capacity is looking more closely at the interactions of abiotic factors with nursery disinfestation systems and the impact on pathogen survival and infectivity. Some data exists for the commonly used systems, such as UV and chlorine dioxide, and their interaction with the environment. However, the four-way interaction between environment, disinfestation system, pathogen and host should be explored in much greater depth, experimenting with different combinations of factors. To optimise disinfestation systems, an understanding of how environmental factors influence their efficacy is clearly necessary.

The biology and epidemiology of pathogens in water is another area where much more work is needed (Zappia et al. 2014). Although we have a good understanding of how some pathogens operate in the water (for example, some oomycetes with water-borne spores), many gaps remain. Questions would include survival and longevity in water, influence of environmental factors on survival (e.g., oxygen levels, pH, turbidity, flow rate), interactions with other microbes and pathogens (Hong and Moorman 2005), infectivity (Zappia et al. 2014), and inoculum potential for different crops (Stewart-Wade 2011). For example, when studying the survival of *Fusarium oxysporum* f. sp. *cubense* (Foc) spores in

irrigation water, Ullah et al (2021) questioned whether other factors absent under laboratory conditions such as solar radiation (Sichel et al. 2007), extreme temperatures (Hong and Moorman 2005), and the presence of aquatic biota (Cateau et al. 2014) could be influencing pathogen survival. Biological and epidemiological data would allow for some risk modelling which could inform grower choices regarding nursery disinfestation.

Information from the literature review and systems currently in use highlighted some newer systems that would be worth assessing for use in the nursery industry. Given that a large proportion of nurseries rely on raw water (i.e., dam or creek water) and organic material commonly found in such water is likely to significantly reduce the effectiveness of most disinfestation systems, some will require prefiltration. The systems/treatments that have potential and appear to be easily adaptable to a nursery setting include silver hydrogen peroxide (HSP) and nanobubbles as these both offer more simplicity and reduced environmental and product impact. As per the review the advantages and disadvantages of these are included in Table 2, with sodium and calcium hypochlorite and chlorine dioxide commonly used throughout the nursery industry also included.

Table 2: An exert from the review identifying disinfestations systems from the gap analysis selected for laboratory testing.

Treatment	How it Works	Advantages	Disadvantages
Sodium Hypochlorite	Oxidising Agent	• Highly effective, stable residual that keeps disinfesting • Cleans out algal and bacterial slime • Relatively safe and non-phytotoxic • Precipitates iron and manganese • Chemical readily available and relatively cheap • Test kits inexpensive • Equipment costs relatively inexpensive • Extensive scientific data available on its effectiveness	• pH to be monitored and adjusted within small range (5.5–7.5) • Highly corrosive and an irritant at high concentration so requires careful handling • Injection equipment requires regular maintenance • Requires pre-filtration as Cl rapidly used up by impurities • Requires regular testing (weekly) of residual chlorine • Requires storage tank to achieve contact time and residual (in-line systems must still achieve required contact time and residual) • Limited shelf life (1 month), reduced by sunlight and heat • Never combine with fertilisers or other chemicals containing ammonium • Problematic for water with > 0.5ppm iron due to iron precipitation (settling) — consideration needed in managing precipitated iron • Not effective against all plant pathogens
Calcium Hypochlorite	Oxidising Agent	• Available Cl about 65% > sodium hypochlorite • Less phytotoxic and corrosive to pipes and equipment than sodium hypochlorite. • As CaOCl, Calcium is available for plant uptake • Maintains its stability and efficacy during storage better than sodium hypochlorite	• Insoluble components (calcium carbonate) at higher concentrations • It can be a hazard if subjected to heat or stored in or near an easily oxidized organic material or in metal. • Calcium hypochlorite costs more than sodium hypochlorite
Chlorine Dioxide	Oxidising Agent	• Potent oxidant (more than 2x as strong an oxidant as Cl) • Not affected by nitrogenous compounds • Effective at broader pH (<10), good for Australian production nurseries with high water pH • Requires shorter contact time • Residual activity is longer than Cl • Effective against a broad range of pathogens • Extensive scientific data available on its effectiveness	• Human health and environmental hazards • Unstable gas that must be generated onsite with specialised equipment • Equipment is relatively expensive • Equipment requires regular maintenance • Requires accurate and regular testing of residual level • High residual levels can be toxic to plants • Does not have efficacy against all plant pathogens or life stages • Stock solution should be used within 15 days to minimise loss due to volatilisation • Limited scientific research available on its effectiveness as a disinfectant of irrigation water
Nanobubbles	Spherical packages of gas within a liquid with a diameter of less than 1000nm. With a negatively charged surface they can carry out oxidation reactions and reduce surface tension of water	• Negatively charged so they repel each other keeping them evenly distributed in water • Long lasting • Hydroxyl radical (HO) is one of the strongest known oxidisers • Effective against bacteria, fungi, viruses and biofilms • Enhances oxidation without chemicals • Environmentally friendly • Not phytotoxic	• A lack of scientific research on efficacy available
Silver Hydrogen Peroxide (HSP)	The use of silver to stabilise hydrogen peroxide, which in turn increases its antimicrobial activity against microorganisms while reducing the required concentration of hydrogen peroxide.	• Biodegradable and leaves no residual in the end product • Prevents blockages in irrigation systems • Reduces biofilm and prevents regrowth • pH neutral • Prevents growth of algae • Can be used to dose large bodies of water and as an inline product • HSP is safe for humans and the environment • Effective at lower dose concentrations • Stable at a wide range of temperatures • There is no known resistance from microorganism, germicidal against bacteria, fungi, viruses, spores and algae	• Toxicity can occur if dosing is too high

Phase 2: Evaluation of disinfestation methods used in nurseries

Activity 1: Evaluation of the efficacy of available water disinfestation systems

It was agreed during a PRG meeting, to ensure the quality of the samples laboratory staff would sample nurseries in person and therefore only nurseries close to the laboratory would be selected for testing. In some instances, in NSW, when samples could be collected by the nursery on a Monday, and they could be received in the lab by the Tuesday and processed immediately this would be a feasible option.

For the Queensland sampling, QDPI attempted to contact 14 local nurseries, who had previously indicated their willingness to be involved in the project via the online survey. Of the 14 nurseries, nine agreed to have their water sampled (Table 1). Of these nine nurseries the following water disinfestation systems were represented: chlorine inline systems (4); chlorine in a tank treatment (1); chlorine treatment unknown (1); chlorine dioxide treatment (1); filtration, osmosis and UV (1); and no disinfestation (1). As with the survey results, chlorination inline was the most popular system used - followed by chlorine dioxide and other chlorine-based treatments.

While diagnosticians from both states collected water samples from the nurseries in person, for future testing QDPI (in consultation with NSW DPIRD) prepared a document to inform growers on how to sample water in the nursery and then send samples to the laboratory for testing. This is given in Appendix 2 *Collecting water samples from the nursery.*

Figure 2: Typical water samples tested

Table 3: Nurseries sampled during the project

Nursery	Disinfestation system	Sampling one	Repeat sample[1]	Sampling two	Sampling three
NSW01	Sand filter, UV, Hydrogen peroxide	7/02/2024		27/05/24	8/10/24
NSW02	Chlorination inline	17/10/2024		27/05/2024	13/05/2024
NSW03	Particle filter / Chlorination tank dosage with Chlorine tablets	17/10/2023		27/02/2024	
NSW04	Slow sand filtration, Chlorine dioxide & Cloth filter	18/10/2023		2/04/2024	
NSW05	Chlorination inline	25/10/2023		2/04/2024	
NSW06	Chlorine dioxide	4/12/2023		8/04/2024	
NSW07	Slow sand filtration, UV	25/10/2023		8/04/2024	
NSW08	Chlorination inline, Vibrex system chlorine dioxide, Hydrochloric acid and sodium chlorite treatment	17/10/2023		15/04/2024	
NSW09	Slow filtration	25/10/2023		15/04/2024	
NSW10	Chlorination tank dosage Chlorine tablets	4/12/2023		14/05/2024	
NSW11	Ozone	4/12/2023		14/05/2024	
NSW12	No treatment	4/12/2023		13/05/2024	
NSW13	Path-X	13/05/2024			
VIC01	Chlorination tanks Wetland, biological filtration	9/01/2024			
VIC02	Mini Vibrex chlorination direct dosing inline	10/01/2024		30/04/2024	
QLD01	Chlorine -inline	7/11/2023		22/04/2024	23/07/2024
QLD02	Chlorine -inline	7/11/2023	14/11/2023		
QLD03	Chlorine -inline	14/11/2023		not done	18/06/2024
QLD04	Chlorine -inline	28/11/2023		not done	
QLD05	Chlorine tank treatment	14/11/2023		23/04/2024	
QLD06	Chlorine type unknown	9/01/2024	22/01/2024	not done	
QLD 07	Chlorine dioxide	not done		30/04/2024	
QLD 08	Filtration, osmosis and UV	7/11/2023		30/04/2024	
QLD 09	No disinfestation	7/11/2023		not done	

[1] Sample retested after recommended line and storage tank cleaning performed.

*Nurseries were deidentified to maintain confidentiality.

Results of this testing are given in Appendix 5. Water testing results summary and a condensed version is in Table 4.

The QDPI staff collected and tested water samples from nine nurseries based in Queensland. Seven of the nine nurseries were sampled and tested on the first sample date in November 2023 (early summer). An additional nursery was sampled later in January 2024 (mid-summer). Two nurseries (QLD02 and QLD06) where initial test results showed their disinfestation system was not working effectively and had subsequently followed the recommendations given by the QDPI team were retested after remedial actions had been taken. In both instances, results improved. For nursery QLD06, the chlorine disinfestation was working well, but water was getting re-contaminated in the lines. Flushing the lines with a high dose of chlorine improved this, but more flushing was needed to completely clean the system. For nursery QLD02 an increase in their chlorine dosage improved their fungal contamination, but a new bacterial contamination issue arose and remained persistent, indicating a recontamination problem. They were scheduling a tank and line clean to try to rectify this.

Only four of the original seven first date sampled nurseries were also sampled on the second sampling date of April 2024 (late summer). One additional new nursery was sampled on the second date giving a total of five nurseries tested on the

second sampling date. The uptake of the second sampling was not as good as the first, with fewer nurseries responding to requests for the second sampling.

QDPI also performed an additional nine water samples for nurseries as part of their Grow Help diagnostic service (representing seven additional businesses to those in the project). These samples could not be included in the current project as they were sent in by the growers and not sampled in the same way. Recommendations were still given to these businesses based on test results and they were sent a copy of the water collection method document (Appendix 2).

Comparison of disinfestation system efficacy results across systems show the filtration, osmosis and UV system (nursery QLD08) performed the best over both testing times. Four out of five nurseries with chlorine inline systems failed to disinfest the water effectively all the time, indicating that while it is the cheapest option it may not be the most reliable. This highlights the importance of regular testing of systems for pathogens. Some nurseries were not able to get clean water results even after making significant changes to their systems and QDPI is still working with them as indicated by the additional testing.

Nursery QLD07 chlorine dioxide system did not appear to be working. However, when collecting the post treatment sample it was noticed that it was from a tank situated on the top of the hill some distance from the treatment, increasing the chances of recontamination in the line. Also, the tank roof was not airtight, potentially allowing entry of airborne contaminants. It was therefore not clear if the disinfestation system was not working effectively or if clean water was subsequently getting contaminated. The nursery was informed of the results and recommendations were made to resample closer to the point of treatment so the system itself could be assessed, and actions were recommended to reduce the risk of recontamination of clean water in the lines and storage tanks.

The nursery with no disinfestation system (Nursery, QLD09) had high levels of bacteria, fungi and oomycetes (including Phytophthora) indicating their water needed to be treated. This nursery grew advanced trees making the presence of Phytophthora (an aggressive tree root rot pathogen) concerning. The nursery was informed of the results and advised to install a disinfestation system. They were sent a copy of the factsheet comparing different disinfestation systems for nurseries prepared as part of the QDAF NY20000 nursery project.

Results from nurseries QLD02and QLD05 indicate sampling at different times of the year may influence microbe make up – as at both nurseries, oomycetes were present in untreated dam water in summer but not in winter. Additional testing across different seasons was beyond the budget of this project, but this should be conducted in the future to determine if this is in fact true as seasons may indicate high risk periods for nurseries based on what crops they grow.

The NSW DPIRD team collected and processed samples from 13 NSW nurseries and three Victorian nurseries. Sampling was planned for a spring/summer and autumn/winter collection.

NSW01 was sampled on three separate occasions. This nursery had a sand filter, followed by a 100-um filter, UV treatment for 30 mins and then hydrogen peroxide for 30 mins at 1 L/20,000 L. There was a large difference in the number of bacteria present in pre- and post-treatment samples, with upwards of 1.1 million to upwards of 1000 CFU per litre detected respectively. Subsequent treatment with hydrogen peroxide appeared to remove the remaining bacteria. The fungal numbers were significantly lower, however treatment was effective against Pythium and Phytophthora with residual Fusarium remaining. The second sampling revealed the treatment on the fungal population was not as efficient. Following system modifications to address the issue, a third round of sampling was conducted and results were similar to those from the first sampling, but in addition Fusarium was not detected.

NSW06, which has a chlorine dioxide system, has a dam with significant levels of bacteria and fungi. Treatment appeared to significantly reduce the levels of bacteria, with around 760,000 to upwards of 1 million CFU per litre detected in pre-treatment samples and around 200,000 to upwards of 230,000 CFU per litre and detected in post-treatment samples. There was an overall reduction in detection of fungi between pre- and post-treatment samples and Pythium was not detected post-treatment but, Fusarium was. Filtration to remove organic materials and particulates prior to treatment with chlorine dioxide may improve the efficiency of this system.

NSW07 is another nursery with filtration (slow sand) and UV. In the first samples collected, there was nil detection of bacteria in post-treatment samples and a tenfold reduction in detection of fungi between pre- and post-treatment samples. However, there were no target fungal taxa detected in either pre- or post-treatment samples. Following the

second sampling the number of bacteria and fungi detected pre-treatment was double that of the first sampling, and this time Fusarium was detected. Half the number of bacteria were detected in samples collected following sand filtration, but the number of fungi detected was similar. Bacterial and fungal detection post-sand treatment was similar to that post sand and UV treatment, indicating UV treatment had no impact at the time. Time did not permit re-sampling of this system to determine if the issue was resolved.

Nursery NSW08 used an inline chlorination with chlorine dioxide (Vibrex®). In the first lot of samples collected there was a very low level of bacteria, and no fungi detected. This could have been a sampling or transport issue, or simply a low microorganism population to begin with (perhaps due to seasonal conditions, which would be consistent with several other samples collected from nurseries in the same season). The second round of samples revealed significant levels of bacteria and fungi present pre-treatment, and while the post treatment bacterial levels were reduced by a third, the fungal levels hadn't changed. All three target fungal taxa were also present.

NSW11 had an ozone system. As with nursery NSW08, there was a low microbial presence in the first lot of samples collected and a significantly higher presence the second time samples were collected; while the ozone treatment appeared to have had no impact on the overall bacterial numbers the fungal population declined from >530 CFU per litre in the pre-treatment samples to >145 CFU per litre in the post-treatment samples. Fusarium and Pythium were not detected in the post-treatment samples, but Phytophthora was not detected in either pre- or post-treatment samples.

NSW13 treated their rainwater with Path-X, which appeared to result in a reduction of the bacterial population from >600,000 to >300,000 CFU per litre and fungal population from >745 to >95 CFU per litre.

The results of this work highlight that continued monitoring of water disinfestation systems is essential for maintaining their efficacy and therefore reducing the risk of diseases caused water-borne plant pathogens.

Table 4: Results from water sampling at each of the nurseries.

Nursery	Date	WATER SOURCE FOR IRRIGATION	Treatment	Sample Tested	Bacterial CFU/L	Fungal CFU/L	Fusarium (+/-)	Pythium (+/-)	Phytophthora (+/-)
NSW01-1	7/2/2024	Dam	Sand filter, UV, Hydrogen peroxide	Pretreatment	>1,100,000 - 600,000	>750	+	+	-
				Post treatment (Filtered + UV)	>1000 - 667	>350	+	-	-
				Post treatment (H_2O_2)	0	>175	+	-	-
NSW01-2	27/5/2024	Dam	Sand filter, UV, Hydrogen peroxide	Pretreatment	>26,000 – 16,000	>1,035	+	-	-
				Post treatment (Sand)	>8,300 – 3,000	>550	-	-	-
				Post treatment (UV)	>6,000 – 5,000	>60	-	-	-
				Post treatment (H_2O_2)	>7,600	>1,250	+	-	-
NSW01-3	8/10/2024	Dam	Sand filter, UV, Hydrogen peroxide	Pretreatment	>300,000	>350	+	+	-
				Post treatment (Sand filter)	>270,000	>15	-	-	-
				Post treatment (Chlorine)	0	>10	-	-	-
NSW02-1	17/10/2023	Dam	Chlorination inline	Pretreatment	>100	0	-	-	-
				Post treatment	>100	0	-	-	-
NSW02-2	27/2/2024	Dam	Chlorination inline	Pretreatment	>130,000	>745	+	+	-
				Post treatment	>1,000,000	>110	+	-	-
NSW02-3	13/5/2024	Dam	Chlorination inline	Pretreatment	>330,000 – 230,000	>1,250	+	-	-
				Post treatment	>8,600 – 2,000	>1,250	+	-	-
NSW03-1	17/10/2023	Dam	Particle filter / Chlorination tank dosage with Chlorine tablets	Pretreatment	>143,000	0	-	-	-
				Post treatment	0	>5	+	-	-
NSW03-2	27/2/2024	Dam	Particle filter / Chlorination tank dosage with Chlorine tablets	Pretreatment	>800,000 - 360,000	>270	-	-	-
				Post treatment	>500,000 – 430,000	>535	+	-	-
NSW04-1	18/10/2023	Town & Dam Recycled Rainwater	Slow sand filtration, Chlorine dioxide & Cloth filter	Pretreatment	>360,000 – 166,666	0	-	-	-
				Sand	>33,000	0	-	-	-
				Post treatment (Chlorine)	0	0	-	-	-
NSW04-2	2/4/2024	Town & Dam Recycled Rainwater	Slow sand filtration, Chlorine dioxide & Cloth filter	Pretreatment	>400,000 – 230,000	>750	+	-	-
				Post treatment (Sand)	>260,000 – 230,000	>100	-	-	-
				Post treatment (SSF + Cloth)	>30,000	>225	-	-	-
NSW05-1	25/10/2023	Dam Recycled Rainwater	Chlorination inline	Pretreatment	>700,000	>750	+	-	+
NSW05-2	2/4/2024	Dam Recycled Rainwater	Chlorination inline	Pretreatment	>360,000 – 200,000	>635	+	-	-
NSW06-1	4/12/2023	Creek	Sand filtration	Pretreatment	>100	0	-	-	-
				Post treatment (Sand)	>100	0	-	-	-
NSW06-2	8/4/2024	Dam	Chlorine dioxide	Pretreatment (lot 4 DAM)	>100	>1,250	+	+	-
				Pretreatment (lot 5 DAM)	>1,000,000 – 760,000	>1,250	+	+	-
				Post treatment (ClO2)	>230,000 – 200,000	>1,250	+	-	-
NSW07-1	25/10/2023	Town / Bore & Recycled	Slow sand filtration & UV	Pretreatment	>200,000 - 40,000	>750	-	-	-
				Post treatment (Sand)	0	>75	-	-	-

				Post treatment (UV)	0	>40	-	-	-
NSW07-2	8/4/2024	Town / Bore & Recycled	Slow sand filtration & UV	Pretreatment (tank)	>400,000 – 330,000	>1,250	+	-	-
				Post treatment (Sand)	>230,000 – 160,000	>1,250	+	-	-
				Post treatment(UV + Sand)	>230,000 – 200,000	>1,250	+	-	-
NSW08-1	17/10/2023	Town / Dam & Recycled	Chlorination inline, Vibrex system chlorine dioxide, Hydrochloric acid and sodium chlorite treatment	Pre-treatment	>100	0	-	-	-
				Post-treatment	>100				
NSW08-2	15/4/2024	Town / Dam & Recycled	Chlorination inline, Vibrex system chlorine dioxide, Hydrochloric acid and sodium chlorite treatment	Pretreatment	>900,000 - 460,000	>1,250	+	-	+
				Post-treatment	>630,000 – 530,000	>1,250	+	+	+
NSW09-1	25/10/2023	Dam	Slow filtrations	Pre-treatment	>100	>750	-	-	+
				Post-treatment	>100	>150	+	+	-
NSW09-2	15/4/2024	Dam	Slow filtrations	Pretreatment	>2,100,000 – 1,430,000	>1,250	+	+	+
				Post treatment	>1,400,000 – 960,000	>1,250	+	-	-
NSW10-1	4/12/2023	Dam	Chlorination tank dosage using Chlorine tablets	Pretreatment	>100	>750	+	-	-
				Post treatment	>100	>750	+	-	-
NSW10-2	14/5/2024	Dam	Chlorination tank dosage using Chlorine tablets	Pretreatment	>300,000	>385	+	+	-
				Post treatment	>70,000	>130	-	-	-
NSW11-01	4/12/2023	Dam	Ozone	Pretreatment	>100	>750	-	-	-
				Post treatment	>100	>750	-	-	-
NSW11-02	14/5/2024	Dam	Ozone	Pretreatment	>300,000	>530	+	+	-
				Post treatment	>300,000	>145	-	-	-
NSW12-01	4/12/2023	Tank & Town	No treatment	Pretreatment (Top Tank)	>200 - 66	>225	-	-	-
				Pretreatment (Bottom tank)	>1,500 - 100	>490	-	-	-
NSW12-2	13/5/2024	Tank & Town	No treatment	Pretreatment (Tank)	>3,000 - 900	>235	-	-	-
				Pretreatment (Untreated town)	0	>10	-	-	-
NSW13-1	13/5/2024	Rainwater	Path X	Pretreatment (Tank)	>60,000	>745	-	-	-
				Post treatment	>300,000	>95	-	-	-
VIC01-1	9/1/2024	Dam	Chlorination tanks, Wetland, biological filtration	Pretreatment	>100	>750	+	+	+
				Post treatment	>24,000-21000	>750	+	+	-
VIC01-2	9/1/2024	Dam	Chlorination tanks Wetland, biological filtration	Pretreatment	>100	>750	+	+	+
				Post treatment	>100	>750	+	-	-
VIC02-1	10/1/2024	Dam water feed by a Weir	Mini vibrex chlorination direct dosing inline	Pretreatment	>100	>750	+	+	-
				Post treatment	>100	>750	+	-	-
QLD01-1	7/11/2023	Town / Dam Recycled Rainwater	Chlorine	Pre-treatment	>100	>500	-	-	+
				Post-treatment	>100	>55	-	-	-
QLD01-2	30/4/2024	Town / Dam Recycled Rainwater	Chlorine	Pre-treatment	>1,600,000 – 1,000,000	>1,000	-	-	+
				Post-treatment	>200	>180	-	+	-
QLD02-1	7/11/2023	Bore / Dam	Slow Sand Filtration, Chlorine (tank dosage)	Pre-treatment	>100	>500	-	-	-
QLD02-2	14/11/2023	Bore / Dam	Slow Sand Filtration, Chlorine (tank dosage)	Post-treatment chlorine	0	>10	-	-	-
				Pre-treatment	>100	>500	-	-	+
				Post-treatment	>166,000	0	-	-	-
QLD02-3	22/4/2024	Bore / Dam	Chlorine tablets	Pre-Dam	>2,830,000 -1,900,000	>1,000	-	-	+
				Post-treatment	>33,000	>360	-	-	-

QLD03-01	14/11/2023		Chlorine	Pre-treatment	>100	>500	-	-	+
				Post-treatment	>100	>50	-	-	-
QLD04-1	28/11/2023	Bore River/Creek	Chlorine	Pre-treatment	>1,800,000 – 530,000	>500	-	-	+
				Post-treatment	0	>40	-	-	-
QLD04-2	18/6/2024	Bore River/Creek	Chlorine	Pre-treatment	>200	>1,000	-	-	-
				Post-treatment	>30,000	>200	-	-	+
QLD05-1	14/11/2023	Bore / Dam	Chlorine	Pre-Bore	>100	>500	-	-	-
				Pre-Dam	>1,900,000 – 1,300,000	>10	-	+	+
				Post-treatment	>100	>190	+	+	-
QLD05-2	23/4/2023	Bore / Dam	Chlorine	Pre-Dam	>700,000 -433,000	>500	-	-	-
				Pre-Bore	>133,000 – 33,000	>500	-	-	-
				Post-treatment	>100	>500	-	-	-
QLD06-1	9/1/2024	Dam	Chlorine	Pre-Dam 1	>1,200,000 – 900,000	>435	-	-	-
				Pre-Dam 2	>4,100,000	>35	-	-	-
				Post-treatment 3	0	0	-	-	-
				Post-treatment 4	0	0	-	-	-
				Post-treatment5	>200	>1,000	-	-	-
QLD06-2	22/1/2024	Dam	Chlorine	Pre-Dam 1	>200	>500	+	-	+
				Pre-treatment 2	>266,000	>120	-	-	-
				Post-treatment 3	0	0	-	-	-
				Post-treatment 4	>33,000	0	-	-	-
				Post-treatment 5	0	0	-	-	-
				Post-treatment 6	0	0	-	-	-
				Post-treatment 7	0	0	-	-	-
				Post-treatment 8	>133,000	0	-	-	-
QLD07-1	30/4/2024	Dam/ Recycled	Chlorine dioxide, Hydrochloric acid 6% and sodium chlorite 5%	Pre-treatment	>2,000,000 – 1,000,000	>1,000	-	-	+
				Post-treatment	>100	>130	-	-	-
QLD08-1	7/11/2023	Town water Rainwater	Chlorine (tank dosage), Chlorine (inline), Reverse osmosis & UV	Pre-treatment	>100	>135	-	-	-
QLD08-2	30/4/2024	Town water Rainwater		Pre-treatment	>66,000 – 33,000	>1,000	-	-	-
				Post-treatment filtration and uv	0	>5	-	-	-
				Post-treatment	0	0	-	-	-
QLD09-1	7/11/2023		No system	Pre-treatment	>100	>300	-	-	-
				Post-treatment	>100	0	-	-	+

Laboratory Testing of Disinfestation Systems

Our gap analysis of the literature revealed several systems that could be beneficial in a nursery setting. These systems included silver stablised hydrogen peroxide and nanobubble. Based on a tight timeframe we could not get access to the nanobubble system or water generated from a system for testing against the target pathogens. We would recommend testing this system alongside further testing of the systems with the results contained in this report.

Disinfectant testing for bacteria

Huwa-San TR-50 was tested at 20ppm and 200ppm for 5 mins, 2 and 24 hours against *Clavibacter michiganensis* pv *michiganensis* (Cmm) and *Pseudomonas syringae.* Table 5 provides the testing results for both bacteria and the two occasions the experiment was conducted on, while Figure 3 shows the images of the agar plates. While 20ppm for 24 hrs was effective on low levels of bacteria the results achieved at 200ppm for 2 and 24hrs were substantially more effective. From the results it appears that at high levels of bacteria the Huwa-San is not effective. Based on these results we would recommend further testing with additional plant pathogens and testing a wider range of concentrations of the product to determine what the effective does (ED) rate is. We would also recommend testing the product with alternative 'raw' creek/dam water sources to determine if this is a viable standalone alternative or an alternative that requires prefiltration.

Table 5: Huwa-San results for Cmm and *Pseudomonas syringae.* *1 colony in 1 replicate

Huwa-San rate	INOCULUM CFU/mL	Contact Time	Cmm Test 1 + positive/- negative	Cmm Test 2 + positive/- negative	*P. syringae* Test 1 + positive/negative	*P.syringae* Test 2 + positive/- negative
0	10^8	5 min	+	+	+	+
0	10^6	5 min	+	+	+	+
0	10^4	5 min	+	+	+	+
0	10^2	5 min	+	+	+	+
0	10^8	2 hours	+	+	+	+
0	10^6	2 hours	+	+	+	+
0	10^4	2 hours	+	+	+	+
0	10^2	2 hours	+	+	+	+
0	10^8	24 hours	+	+	+	+
0	10^6	24 hours	+	+	-	+
0	10^4	24 hours	+	+	+	+
0	10^2	24 hours	+	+	-	-
20ppm	10^8	5 min	+	+	+	+
20ppm	10^6	5 min	+	+	+	+
20ppm	10^4	5 min	+	+	+	+
20ppm	10^2	5 min	+	+	+	-
20ppm	10^8	2 hours	+	+	+	+
20ppm	10^6	2 hours	+	+	-	+
20ppm	10^4	2 hours	+	+	-	+
20ppm	10^2	2 hours	+	-	+	-
20ppm	10^8	24 hours	+	+	+	+
20ppm	10^6	24 hours	+	-	-	+
20ppm	10^4	24 hours	-	-	-	-
20ppm	10^2	24 hours	-	-	-	-
200ppm	10^8	5 min	+	+	+	+
200ppm	10^6	5 min	+	+	-	+
200ppm	10^4	5 min	+	+	-	+ *
200ppm	10^2	5 min	+	+	-	-
200ppm	10^8	2 hours	+	+	+	+
200ppm	10^6	2 hours	-	-	-	-
200ppm	10^4	2 hours	+	-	-	-
200ppm	10^2	2 hours	-	-	-	-
200ppm	10^8	24 hours	+	+	+	+
200ppm	10^6	24 hours	-	+	-	-
200ppm	10^4	24 hours	-	-	-	-
200ppm	10^2	24 hours	-	+	-	-

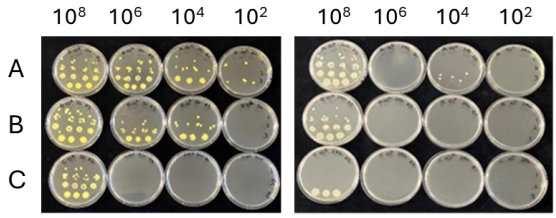

Figure 3: Agar plates for Cmm (left picture) and P. *syringae* (right picture) with Huwa-San TR-50 at both dose rates after 2 hours. 'A' labelled plates are controls, 'B' are 20 ppm and 'C' 200 ppm. From left to right are decreasing bacterial concentrations.

The Path-X treatment (Table 6, Figure 4) was more effective against bacteria than Huwa-San at the 20ppm with 2hrs contact time being more efficient. The Path-X at 200ppm is nearly 100% effective at 2hrs against all bacterial concentrations except $1x10^8$ CFU/ml for *P. syringae* in one test, with almost 100% effectiveness at 200ppm at 24hrs. We would recommend testing additional bacteria to test the broadness of the effective dose. The final assessment/control group being the recommended 3ppm of calcium hypochlorite (Table 7, Figure 5) which has similar results to the Huwa-San at 24hrs with the treatment not being effective on $1x10^8$ CFU/ml but effective at the lower doses.

Table 6: Path-X results for Cmm and *Pseudomonas syringae*

Path-X rate	INOCULUM CFU/ml	Contact Time	Cmm Test 1 + positive/- negative	Cmm Test 2 + positive/- negative	*P. syringae* Test 1 + positive/- negative	*P. syringae* Test 2 + positive/- negative
0	10^8	5 min	+	+	+	+
0	10^6	5 min	+	+	+	+
0	10^4	5 min	+	+	+	+
0	10^2	5 min	+	+	+	+
0	10^8	2 hours	+	+	+	+
0	10^6	2 hours	+	+	+	+
0	10^4	2 hours	+	+	+	+
0	10^2	2 hours	+	+	+	+
0	10^8	24 hours	+	+	+	+
0	10^6	24 hours	+	+	+	+
0	10^4	24 hours	+	+	-	+
0	10^2	24 hours	+	-	-	-
20ppm	10^8	5 min	+	+	+	+
20ppm	10^6	5 min	-	-	+	+
20ppm	10^4	5 min	-	-	-	-
20ppm	10^2	5 min	-	-	+	-
20ppm	10^8	2 hours	+	+	+	+
20ppm	10^6	2 hours	-	-	-	+
20ppm	10^4	2 hours	-	-	-	+
20ppm	10^2	2 hours	-	-	-	-
20ppm	10^8	24 hours	+	+	+	+
20ppm	10^6	24 hours	-	-	-	-
20ppm	10^4	24 hours	-	-	-	-
20ppm	10^2	24 hours	-	-	-	-
200ppm	10^8	5 min	-	-	+	-
200ppm	10^6	5 min	-	-	-	-
200ppm	10^4	5 min	-	-	-	-
200ppm	10^2	5 min	-	-	-	-
200ppm	10^8	2 hours	-	-	+	+
200ppm	10^6	2 hours	-	-	-	-
200ppm	10^4	2 hours	-	-	-	-
200ppm	10^2	2 hours	-	-	-	-
200ppm	10^8	24 hours	-	-	+	-
200ppm	10^6	24 hours	-	-	-	-
200ppm	10^4	24 hours	-	-	-	-
200ppm	10^2	24 hours	-	-	-	-

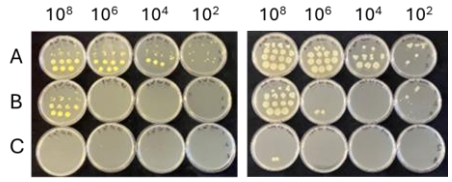

Figure 4: Agar plates for Cmm (left picture) and P. *syringae* (right picture) with Path-X treatment at both dose rates for 5 min. 'A' labelled plates are controls, 'B' are 20ppm and 'C' 200ppm. From left to right are decreasing bacterial concentrations.

Table 7: Calcium hypochlorite (chlorine) results for Cmm and *Pseudomonas syringae.* *only 1 colony was isolated from 1 replicate.

Chlorine rate	INOCULUM CFU/ml	Contact Time	Cmm Test 1 + positive/ - negative	Cmm Test 2 + positive/ - negative	*P. syringae* Test 1 + positive/ - negative	*P. syringae* Test 1 + positive/ - negative	*P.syringae* Test 2 + positive/ - negative
0	10^8	5 min (Cmm 1 20min)	+	+	+	+	+
0	10^6	5 min (Cmm 1 20min)	+	+	+	+	+
0	10^4	5 min (Cmm 1 20min)	+	+	+	+	+
0	10^2	5 min (Cmm 1 20min)	+	+	+	+	+
0	10^8	24 hours	+	+	+	+	+
0	10^6	24 hours	+	+	+	+	+
0	10^4	24 hours	+	+	-	+	+
0	10^2	24 hours	+	+ *	-	-	-
3ppm	10^8	20 min	+	+	+	+	+
3ppm	10^6	20 min	-	+ *	-	-	-
3ppm	10^4	20 min	-	-	-	-	-
3ppm	10^2	20 min	-	-	-	-	-
3ppm	10^8	24 hours	-	+ *	+	+	+
3ppm	10^6	24 hours	-	-	-	-	-
3ppm	10^4	24 hours	-	-	-	-	-
3ppm	10^2	24 hours	-	-	-	-	-

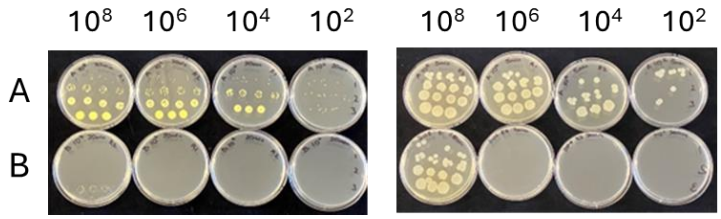

Figure 5: Agar plates for Cmm (left picture) and P. *syringae* (right picture) with chlorine at 3ppm for 20 min. 'A' labelled plates on top row are controls. 'B' are 3ppm chlorine treatment. Plates from left to right are decreasing bacterial concentrations.

Disinfectant testing for Thielaviopsis

The testing of the disinfestation products against the fungal pathogens was more time consuming and fraught with a few

more challenges compared to the bacterial testing. Due to limited time only Thielaviopsis was tested against the three products. The Huwa-San was ineffective against the Thielaviopsis at 20ppm and 200ppm at 2hrs, however at 200ppm for 24 hrs there was no remaining fungi present (Table 8, Figure 6). It would be recommended to test additional fungi. The Path-X (Table 9, Figure 7) was also highly effective at 24 hrs at both 20 and 200 ppm. At 200 ppm 5 mins was all that was required for the removal of Thielaviopsis. The calcium hypochlorite (Table 10, Figure 8) was effective at the lower concentration levels of fungi. Given the results from this single experiment, it would be recommended repeating this experiment to ensure consistent results and it would be recommended testing of further target fungi. In addition, it would be worth testing 'raw' creek/dam water with the products also. The final stage would be testing both bacteria and fungi in the same experiment.

Table 8: Huwa-San results for Thielaviopsis. *Test 1 had Penicillium contamination.
**Test 3 had Penicillium and Aspergillus contamination and are therefore not included in this table.

Huwa-San rate	INOCULUM CFU/ml	Contact Time	Thielaviopsis Test 2 + positive/- negative
20 ppm	10^5	5 min	+
20 ppm	10^3	5 min	+
20 ppm	10^2	5 min	+
20ppm	10^5	2 hours	+
20ppm	10^3	2 hours	+
20ppm	10^2	2 hours	+
20ppm	10^5	24 hours	+
20ppm	10^3	24 hours	+
20ppm	10^2	24 hours	+
200ppm	10^5	5 min	+
200ppm	10^3	5 min	+
200ppm	10^2	5 min	+
200ppm	10^5	2 hours	+
200ppm	10^3	2 hours	+
200ppm	10^2	2 hours	+
200ppm	10^5	24 hours	-
200ppm	10^3	24 hours	-
200ppm	10^2	24 hours	-

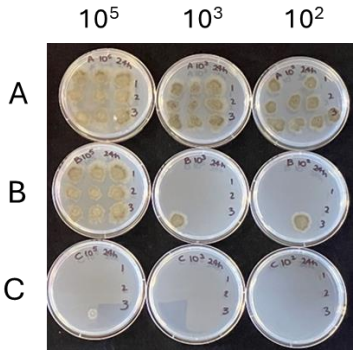

Figure 6: Agar plates for Thielaviopsis with Huwa-San at both rates for 24 hours for replicates 1-3. 'A' labelled plates are controls, 'B' 20 ppm and 'C' 200 ppm. Plates from left to right are decreasing fungal concentrations in spores/mL.

Table 9: Path-X results for Thielaviopsis

Path-X rate	INOCULUM CFU/ml	Contact Time	Thielaviopsis Test 2 + positive/- negative
20ppm	10^5	5 min	+
20ppm	10^3	5 min	+
20ppm	10^2	5 min	-
20ppm	10^5	2 hours	+
20ppm	10^3	2 hours	-
20ppm	10^2	2 hours	-
20ppm	10^5	24 hours	-
20ppm	10^3	24 hours	-
20ppm	10^2	24 hours	-
200ppm	10^5	5 min	-
200ppm	10^3	5 min	-
200ppm	10^2	5 min	-
200ppm	10^5	2 hours	-
200ppm	10^3	2 hours	-
200ppm	10^2	2 hours	-
200ppm	10^5	24 hours	-
200ppm	10^3	24 hours	-
200ppm	10^2	24 hours	-

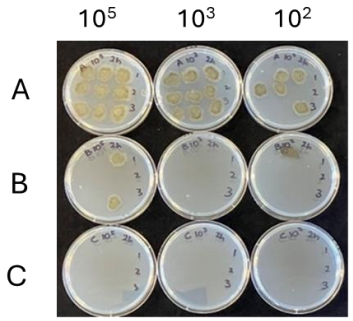

Figure 7: Agar plates for Thielaviopsis with Path-X treatment at both rates for 2 hours for replicates 1-3. 'A' labelled plates are controls, 'B' 20 ppm and 'C' 200 ppm. Plates from left to right are decreasing fungal concentrations.

Table 10: Calcium hypochlorite (chlorine) results for Thielaviopsis. *Test 1 had Penicillium contamination.

Chlorine rate	INOCULUM Cfu/ml	Contact Time	Thielaviopsis Test 2 + positive/- negative
3ppm	10^5	20 min	+
3ppm	10^3	20 min	-
3ppm	10^2	20 min	-
3ppm	10^5	24 hours	+
3ppm	10^3	24 hours	-
3ppm	10^2	24 hours	-

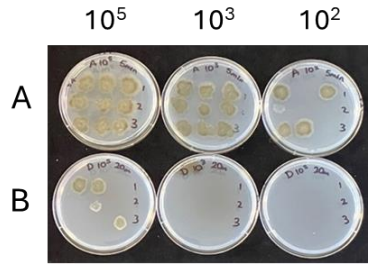

Figure 8: Agar plates for Thielaviopsis with chlorine at 3 ppm for 20 min. Plates labelled 'A' on top row are controls. Plates labelled D, are 3 ppm chlorine treatment. Plates from left to right are decreasing fungal concentrations.

The two tested products show great potential as in tank treatment options and we would recommend further testing to determine effective does rates against a wider range of pathogens.

Activity 2: Optimisation of current water testing assays and evaluation of any potential alternative methods

QDPI in collaboration with NSW DPIRD worked to compare and validate methods for testing water for each of the five target groups (general bacteria, Agrobacterium, general fungi, Oomycetes and Phytophthora). The final optimised methods for testing irrigation water are presented in Appendix 2 Water testing methods.

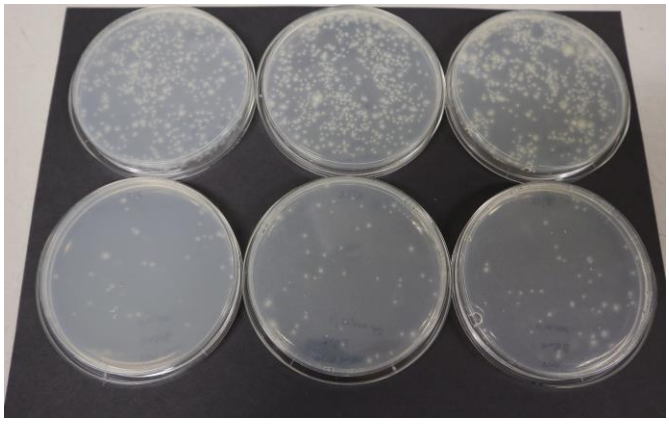

Figure 9: Comparison of fungal (Fusarium) colony growth restriction agents to produce colonies easy to count on a plate. Top plates = 1/1000 dilution, bottom plates = 1/10,000 dilution of Fusarium spores.

Activity 3: Evaluation of crop safety of approved and alternative water disinfestation systems

To test the possible phytotoxic properties of each disinfestation product we selected two plant species that are generally susceptible to chemicals, these being lettuce and pansy seedlings. The testing was conducted at half the recommended concentration of the product, the recommended dose and double the concentration. After seven days of watering there was burning occurring at low levels on the lettuce with no chlorosis, while in the pansy's the burn was negligible to non-existent while the chlorosis was observed in all groups. At the recommended dose, the Huwa-San has minimal effect on the seedlings while the Path-X and the chlorine have a similar impact at the recommended dose rate. While the maximum number is 33.3% in the lettuce with Path-X and chlorine. The two times dose concentration of all treatments did increase the damage to the plants. However as exhibited in the figures the phytotoxicity on the plants is minimal.

All three treatments have a negligible negative effect on the plants as seen from this trial. Longer term use may have a

more pronounced impact on the plants constantly exposed to the higher rates of disinfectant, longer term use may also lead to a build-up effect. A long-term study may yield more definitive results. However, given the softer nature of the seedlings and shorter throughput times in a nursery situation, long term exposures may not be reflective of actual practices.

Table 11: Phytotoxicity results from each of the treatments

Treatment	Lettuce % Burn	Lettuce % Chlorosis	Pansy % Burn	Pansy % Chlorosis
Control	6.7	0	0	6.7
Huwa-San Half Conc	3.3	0	1.7	6.7
Huwa-San Rec	0	0	1.7	3.3
Huwa-San 2x Conc	26.7	0	1.7	28.3
Path-X Half Conc	11.7	0	0	1.7
Path-X Rec	33.3	0	0	6.7
Path-X 2x Conc	58.3	0	1.7	11.7
Chlorine Half	3.3	0	0	15
Chlorine Rec	18.3	0	0	20
Chlorine 2x	33.3	0	0	20

Figure 10: Lettuce and pansy seedlings, control group at 7 days.

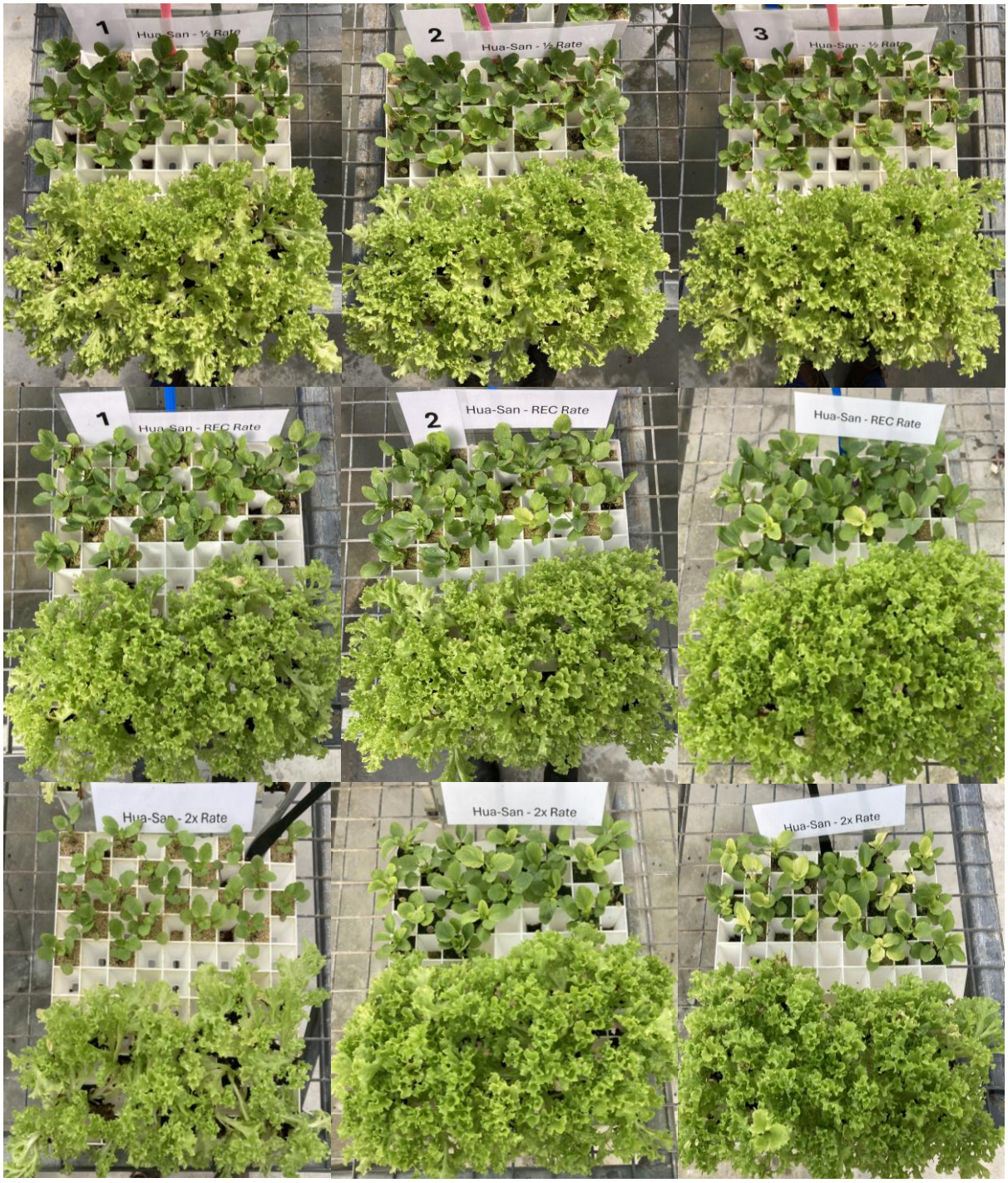

Figure 11: Lettuce and pansy seedlings treated with Huwa-San for phytotoxicity assessment. Three replicates were included for each of the doses of half, recommended and two times the dose at 7 days.

Figure 12: Lettuce and pansy seedlings treated with Huwa-San for phytotoxicity assessment. Close up of examples of damage caused with the 2x dose rate at 7 days.

Figure 13: Lettuce and pansy seedlings treated with Path-X for phytotoxicity assessment. Three replicates were included for each of the doses of half, recommended and two times the dose at 7 days.

Figure 14: Lettuce and pansy seedlings treated with Path-X for phytotoxicity assessment. Close up of examples of damage caused with the 2x dose rate at 7 days.

Figure 15: Lettuce and pansy seedlings treated with Chlorine for phytotoxicity assessment. Three replicates were included for each of the doses of half, recommended and two times the dose at 7 days.

Figure 16: Lettuce and pansy seedlings treated with chlorine for phytotoxicity assessment. Close up of examples of damage caused with the 2x dose rate at 7 days.

Activity 4: Assessment of cost-benefit of available and emerging water disinfestation systems

During nursery visits, we asked about the cost of their current disinfestation system and associated running costs. While some systems were newer, many systems were old. Some nurseries knew the cost of their system and what it cost to run, while many did not, and this made it impossible to compare systems. The confounding factor was that most systems were custom setups specifically designed for the nursery's needs. Given the challenges around this part of the project, the relative costs of the newer products, tested both for their phytotoxicity impact on plants and their ability to kill both fungal and bacterial phytopathogens, alongside the chlorine used in comparison product testing, were determined. It should be noted that the volume requirements of a nursery would be greater, so they would have negotiating-power for bulk rates.

Table 12: Base cost of each purchased product and price at each concentration per 1000L.

Product	List Price	Cost @ 20 ppm/1000L	Cost @ 200 ppm/1000L	Cost @ 3 ppm/1000L
Huwa-San	25 kg for $336.49 (incl GST)	$0.3182	$3.182	
Path-X	20 L for $318.20 (incl GST)	$0.269	$2.691	
Calcium hypochlorite	10 kg for $70.60 (incl GST)			$0.021

Phase 3: Updating the water disinfestation best management practice and NIASA guidelines

Activity 1: Collaboration with Greenlife Industry Australia to update the water disinfestation best management practice and NIASA guidelines.

This part of the project will be addressed post-acceptance of the final report by Hort Innovation to update the best management practice and NIASA guidelines. We need to determine if the data is sufficient or if further testing is required.

Outputs

Provide a detailed list of extension activities conducted over the project life and any associated links to other projects and documentation.

Table 13: Output summary

Output	Description	Detail
Project Planning	Delivery of a project management document	Project management document is contained in Appendix 1
Linking and discussing with industry representatives and growers	Industry representatives engaged through PRG meetings. Growers engaged at time of sampling. Both industry representatives and growers engaged at workshop.	Industry workshop presentation provided in Appendix 6
Results from the digital survey on water disinfestation systems	The survey was advertised through several nursery communication networks. The results have been collected and included in this report.	The survey results are provided in Appendix 3
Literature review on mainstream water disinfestation systems including all NIASA approved systems and emerging systems.	Report summarising information gathered	Literature review is provided in Appendix 4
Gap analysis informing industry of areas where data is insufficient, questionable or required to better evaluate the efficacy of disinfestation systems used by Australian production nurseries	Report summarising information gathered	Gap analysis review is provided in Appendix 4
Results from experimental evaluation of efficacy of disinfestation systems	The experimental evaluation of the disinfestation systems in place in nurseries has occurred during this project.	Water collection instructions for nursery growers are provided in Appendix 2 Experimental evaluation results are provided in Appendix 5 Experimental evaluation results for product/treatments are contained in the results section and Appendix 7
A standard method that has been validated in two labs for the detection of fungal and bacterial pathogens in water samples	These methods have been tested and optimised over the project lifetime. The final method has now been updated.	The method is provided in Appendix 2
Project Final Reports	QDPI final report as per the subcontract requirement Final Report	Appendix 8 Final Report provided by QDPI This Final Report

Outcomes

Table 14: Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Greater knowledge and understanding of nursery industry water disinfestation systems and their efficacy	Improve industry productivity (inputs/outputs) to maintain competitiveness and viability, and sustainability of supply.	Review of different water disinfestation systems available for use in nurseries. Survey results from growers on systems currently in use in Australian nurseries Review of the gaps identified in knowledge regarding water disinfestation systems	Report summarising information on disinfestation systems available for the nursery industry (Appendix 4) Report on survey of nursery water disinfestation systems currently in use (Appendix 3)
Optimised methods for the detection of bacterial and fungal pathogens in water samples	Improve industry productivity (inputs/outputs) to maintain competitiveness and viability, and sustainability of supply.	Review on methods for analysis of water samples for bacteria and fungi Growers have more reliable water testing available through QDPI Grow Help service and NSW DPIRD PHDS lab.	Document for nursery growers on how to collect water samples for testing (Appendix 2). Adaptation of this is available on Grow Help website. Validation of a shared method for testing of water samples that will then be available to growers after this project via a fee-for-service diagnostic from the two state government labs. NSW DPIRD Plant health diagnostic service laboratory https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/plant-health QDAF Grow Help Australia diagnostic laboratory https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/crops/test/grow-help-australia Greater diagnostic capacity allows all production nurseries to complete ongoing testing of their water to check that their water is free of pathogens.

Monitoring and evaluation

This project has provided great insights into the water disinfestation systems that are being used in the nursery, provided valuable results to growers that took the opportunity to be involved in the project and provided promising data on alternative disinfestation treatments. However, the project moved more slowly than anticipated and the participants would like to have achieved a little more in the time available. The project took longer than anticipated to contract, which delayed the start of activities including the collection of the first round of samples for testing, which occurred over the December/January period. The response to the survey was also significantly lower than anticipated, with only 40 participants, resulting in lower engagement with the industry than we had hoped. This is despite using several different approaches, including: contacting each state NGI association to include articles in their print media about the project and requested completion of the survey; including an article in the national e-newsletter; and, sending emails directly to hundreds of nursery businesses informing them of the project and the opportunity to participate. We also contacted Greenlife for assistance by identifying and contacting businesses with uncommon/unconventional disinfestation systems and requesting their participation. However, this did not result in any additional samples. Overall, an insufficient number of nurseries were tested to enable general conclusions to be drawn about the relative performance of some types of disinfestation systems (e.g. chlorine, osmosis and UV). We proposed to move forward with an alternative plan for testing newer disinfestation systems hitting the market and developed methods for this purpose, but time constraints, thwarted access to some systems and completion of the required number of experiments. Nonetheless, the results achieved are promising and indicate this aspect warrants further inquiry. These products/systems also provided promising results from their use in the phytotoxicity testing.

The NSW DPIRD team also experienced several impacts that delayed the project sampling. These risks were identified in the project register early on, and the project continued despite these issues. For documentation purposes these included:

Loss of key project personnel: One of the key personnel who was 0.2 FTE on this project went on maternity leave in 2023 and then moved to a position with another organisation. We tried to backfill this position; however, this was unsuccessful and as a result, this work had to be distributed to the remaining project members.

Diversion of project personnel due to Biosecurity incursion: During this project, the EMAI plant biosecurity team had many suspect exotic detections, as well as endemic and exotic biosecurity incidents to attend to. The greatest impact was caused by the Varroa mite incursion, which had most of the project team diverted to various eradication efforts during different periods of time. Another significant impact was crown-gall, which has recently had a widespread impact on the winegrape industry, which diverted the teams at NSW DPRID and QDPI for a substantial period of time developing diagnostic methods and processing samples to provide answers for both the nursery and production sectors of the winegrape industries. Another incident was the detection of rice blast, which required a substantial amount of the Plant Health Diagnostic staff time. In our risk register, we identified that this could have minor to medium impact, but we could not have anticipated the scale of the varroa mite response and the number of grapevine submissions for crown-gall.

Table 15: Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
1. To what extent has the project achieved its expected outcomes? *a. In what form has the project provided information specific to disinfestation systems and their effectiveness on bacterial and fungal pathogens.* *b. To what extent has the project improved knowledge and awareness of disinfestation systems being used in the nursery industry* *c. To what extent has the project been able to optimise methodologies to detect bacterial and fungal pathogens in water samples.*	a. The project has tested disinfestation systems present in nurseries for their ability to neutralize fungi and bacteria. The project has also conducted laboratory testing with two additional products under laboratory testing conditions against the traditionally used chlorine. b. The project has reviewed disinfestation systems that are either used or recommended for use in nursery systems and additional disinfestation systems that could be applied to a nursery setting. This document identified gaps that should be investigated further for their ability to disinfest water for nursery usage. c. The project has optimised collection and testing of water samples for the detection of fungi and bacteria and these services are now available to the nursery industry as a fee-for-service.	a. We recommend additional testing with additional bacteria and fungi and further testing with the use of raw water including dam and creek/river water. b. We would recommend additional testing with systems like the nanobubble given its potential for use in the nursery industry. c. We would recommend nursery growers utilise the water testing methods optimised in this project to routinely check the efficacy of their water disinfestation systems.
2. How relevant was the project to the needs of intended beneficiaries? *To what extent has the project met the needs of industry levy payers?*	Given the time and budget associated with this project we have managed to highlight potential disinfestation systems that require further testing and identify potential issues with current systems being used within the industry.	We would recommend further investment in this area to continue this work.
3. How well have intended beneficiaries been engaged in the project? *a. To what extent were the target engagement levels of industry levy payers achieved?* *b. Have regular project updates been provided through linkage with the industry communication project?* *c. Did the project engage with industry levy payers through their preferred learning style?* *d. How accessible were the results to stakeholders?*	a. This project successfully engaged with industry levy payers through the delivery of workshops and through the testing and feedback on their disinfestation systems. In addition, where issues were identified with a disinfestation system, we worked with the growers with additional testing to ensure the improvement of their system. b. Project updates have been delivered through milestone reports, industry workshops and discussions with growers through the delivery of results. c. We believe we engaged with levy payers through their preferred learning style through workshops and one-on-one discussions while on farm and providing testing results. d. The results were provided as reports and to some as verbal discussions also.	a-d We would recommend further workshops on the results obtained from this project.
5. What efforts did the project make to improve efficiency? *a. What efforts did the project make to improve efficiency?* *b. Were recommendations made during the PRG meetings to suggest that efficiencies need to be improved?* *c. Were any efficiencies in project management or experimental designs identified and incorporated?*	a. During this project we optimised sample collection, water testing procedures and analysis of results. We also optimised the delivery of workshops suitable to nursery growers. b. Recommendations were made during the PRG meetings, and these were extremely helpful in providing contacts, directions for investigations and directions for testing. c. These efficiencies were incorporated into the project.	We would recommend the continual monitoring of nursery disinfestation systems and the utilization of the methods developed in this project.

Recommendations

- Given the promising results of the new disinfectant treatments it is recommended that the experiments are repeated with additional fungal pathogens and bacteria. It is also recommended to complete this testing with raw water, which is representative of dams and creeks, with pre and post filtering to remove organic material.
- Given the potential of nanobubble, we would recommend testing it, initially with the smaller panel of bacteria, and fungal pathogens and, if these results are promising, with an expanded panel of pathogenic bacteria and fungi.
- Given the testing results, it would be recommended to nurseries that they test their water annually at a minimum to ensure their systems are working efficiently.

Refereed scientific publications

This project did not generate any scientific publications.

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Intellectual property

No project IP or commercialisation to report

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Appendices

Appendix 1: Project Management document

Appendix 2: Water testing protocol

Appendix 3: Nursery water disinfestation digital survey results

Appendix 4: Water disinfestation systems and gap analysis

Appendix 5: Water testing results summary

Appendix 6: Industry workshop presentations

Appendix 7: Results from disinfestation product testing

Appendix 8: Final Report provided by QDPI