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How efficacious are chlorine, chlorine dioxide and ultraviolet radiation as disinfectants against waterborne pathogens in irrigation water?

In this month's Nursery paper NSW Industry Development Officer Michael Danelon reviews some recently conducted levy funded research investigating the efficacy of some popular water disinfestation methods.

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Abstract
A number of disinfection treatments are available to reduce the risk of certain plant diseases in various water sources used for irrigation. Limited published studies have compared the efficacy of disinfection treatments specific to the nursery and garden industry (NGI) on a range of various life stages (propagules) of plant pathogen species and their sensitivity in different water qualities.
This nursery paper aims to summarise a levy funded study conducted by NSW Department of Primary Industries to address industry concerns about gaps in the knowledge about the efficacy of disinfection of irrigation water treatments used by the Australian Nursery Industry. Propagules of eight significant plant pathogens were exposed to chlorine (sodium hypochlorite), chlorine dioxide and ultraviolet radiation (UV-C) disinfestation treatments at a range of application rates and exposure times in deionized water and dam water.

Introduction
Plant pathogens found in irrigation water may originate from a number of sources. These source include natural occurrences in water storage reservoirs (rain water surface fed dam, creek or river), or in surrounding soil or plants, with pathogens then being washed into the nursery runoff and drainage water storage following rainfall and irrigation events. Alternatively pathogens may be introduced to the production system via externally-sourced infected propagation material, growing media or materials or workers, visitors and equipment brought onto the production site.

The reuse or recycling of nursery runoff water as an irrigation source may potentially provide a vector for pathogens. This can elevate inoculum pressure and the risk associated with infection; disease incidence and production losses. Hence effective disinfection of recycled water for irrigation is beneficial as a phytosanitary measure to reduce the risk of plant disease development.

Under the Nursery Production Farm Management System (NPFMS), all water used for irrigation from either surface supplies and nursery runoff must be disinfested with an approved treatment method as outlined in the current Nursery Industry Accreditation Scheme Australia (NIASA) Best Management Practice (BMP) Guidelines.

When considering the required effectiveness of disinfestation treatment

Chlorine dioxide generator

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of water, the log reduction of colony forming units (CFU) of the viable (potential to be infectious) pathogen propagules present prior to, and post exposure to the disinfestation technique, ie typically >99% (log 2) reduction or >99.9% (log 3) reduction post treatment is the industry measurement.

Chlorine as either sodium hypochlorite or calcium hypochlorite is commonly used to treat irrigation water, as it is easy to apply and relatively persistent. Residual concentrations can be monitored to ensure suitable germicidal dose whilst being relatively inexpensive to install. When chlorine is introduced to water, (subject to the pH), it reacts to form free chlorine species of hypochlorous acid (HOCl) pH<7 and hypochlorite (OCl-) ions pH >7 which oxidise organic materials and pathogens if present in the water. The more organic matter present in the water, the greater the rate of deactivation of free chlorine species and lower residual.

Chlorine dioxide (ClO_2) also acts by oxidising organic matter and pathogens. Chlorine dioxide exists as a dissolved gas in water and has a greater oxidising strength than hypochlorite salts. It is claimed to be at least 1.2 times more effective than sodium hypochlorite as a disinfectant. Chlorine dioxide is affected by the presence of organic matter in water, but it is effective across a wider pH range (4-10) and has the potential to offer residual post disinfestation treatment like chlorine.

Ultraviolet radiation (UV) is applied at a wavelength of 254 nm (UV-C) at a certain germicidal dose to disinfect pathogens in irrigation water. Energy discharged from the UV light reacts with the DNA and RNA of surrounding microorganisms present. This essentially eliminates the ability of vulnerable fungi, bacteria and viruses to be infectious. Effective disinfection depends on duration and intensity of UV exposure to water flow and water UV transmission (UVT) and presence of organic matter. Turbidity is measured as nephelometric turbidity units (NTU) with <2 NTU considered optimum.

The most widely used measure of water quality in relation to UV-C efficacy is UVT. Water with a UVT <50% may be disinfested with UV radiation, however, the dose needed increases greatly as UVT falls. Where plant pathogens are harboured inside organic matter or mucilage suspended in water, they may be protected from exposure to the UV and other disinfectants, highlighting the advantage of filtration prior to treatment with disinfectants.

Materials and Methods

The efficacy of the three disinfectant treatments (refer Table 1) were tested against the 22 pathogen propagules according to application/dosage rates and exposure times listed in Tables 2 and 3.

Deionised water (laboratory control – pH 6.5 and 0.32 NTU) and dam water (field) were used in the experiments. The pH of the dam water ranged between 7.8 and 8.0 which are considered suboptimal for chlorine (HOCl) disinfestation. The turbidity of the dam water ranged between 20 and 87 NTU, with a pH between 7.8 and 8.0 at the different sampling times. Dam water was diluted with deionised water to achieve 50% UVT prior to use in the UV tests, whilst the dam water used in the chlorine and chlorine dioxide tests had a turbidity of 20 NTU and was not adjusted to 50% UVT.

To determine the effectiveness of exposure to each disinfestation treatment on propagule survival, the propagule suspension was sampled at required times (Table 2) or post UV treatment (Table 3). Propagules were then cultured and the number of viable propagules (CFU) determined by comparing the number of growing colonies from treated samples with those in the untreated (control) samples.

Results

The disinfestation efficacy (>99% kill of CFU) of the three disinfection treatments tested varied between pathogens and propagules types with application rate/dosage, time and water quality characteristics (pH and turbidity, likely organic matter load) – refer Tables 2 and 3.

Of the disinfection treatments tested in this study, chlorine dioxide applied at 5ppm for 10 minutes (residual 2.7 ppm) was the only effective disinfectant in dam water against all pathogen propagules in this study. In

Flitering water pre treatment is essential

New UV generator bening installed

deionised water, chlorine dioxide applied at 5ppm for 4 minutes was required for effective disinfestation of all pathogen propagules.

Chlorine applied at 5ppm for 30 minutes (residual 4.6 ppm) was the only effective disinfectant in deionised water against all pathogen propagules in this study. Chlorine was ineffective against all pathogen propagules in dam water.

In this study, residual chlorine dioxide rates were only measured after the 10 minute treatment rates, whilst chlorine residuals were only measured after 30 minute treatments – refer Appendix II of the full report.

UV was effective against all pathogen propagules except *Calnectria pauciramosa (Cylindrocladium spp.)* chlamydospores in deionised water. In dam water, UV was ineffective against all propagules of: *Alternaria alternata, Calnectria pauciramosa* and *Fusarium oxysporum* but effective against all pathogen propagules.

Discussion

Water quality is one of the factors affecting the efficacy of water disinfection treatments and longer exposure times or higher exposure rates/dosage were generally required to kill propagules in dam water compared with deionised water, however in some instances the highest rates were ineffective against certain pathogens and propagules – refer Table 2 and 3.

These results highlight the importance of ensuring the disinfection treatment and "dosage" selected is suitable for the water quality available and the importance of achieving a minimum residual chlorine and chlorine dioxide concentration for complete exposure for the contact time where these treatments are applied.

Therefore, both pH and turbidity may have affected the efficacy of the chlorine treatments tested, and turbidity of the dam water reduced the efficacy of the UV treatment for some propagules, such that higher rates or exposure times were required to kill many of the pathogen propagules, when compared with those required for deionised water.

Table 1. Exposure times and residual application rates for the disinfection treatments tested

Treatment	Time (min)	DOSAGE Rate/Concentration
Chlorine (sodium hypochlorite)	0, 10, **20**, 30	0, 1, **2**, 5 ppm
Chlorine dioxide	0, 4, **8**, 10	0, 1, **3**, 5 ppm
UV-C transmission (254 nm)	-	0, **113**, **250** mJ/cm^2

Table 2. Calculated minimum application rate and residual rate (where measured) and exposure time required to kill >99% CFU of propagules tested following exposure to chlorine and chlorine dioxide. A '–' indicates that propagules were not killed at the rates tested.

Pathogen	Propagule	Chlorine (NaClO)				Chlorine dioxide			
		DI		Dam		DI		Dam	
		Rate/Residual (ppm)	Time (min)	Rate/Residual (ppm)	Time (min)	Rate/Residual (ppm)	Time (min)	Rate/Residual (ppm)	Time (min)
Clavibacter michiganensis	Bacterial cells	1	10	1	10	1	4	1	4
Alternaria alternata	Conidia	5	20	-	-	5	4	5	4
	Mycelium	5	20	**5/3.8**	30	3	4	5	4
Chalara elegans	Chlamydospores	2	20	5	20	3	4	5	4
	Endoconidia	**5/4.6**	30	-	-	5	4	**1/0.5**	10
	Mycelium	**5/4.3**	30	-	-	5	4	3	8
Colletotrichum gloeosporioides	Conidia	1	10	5	10	1	4	1	4
	Mycelium	5	10	-	-	1	4	3	4
Calnectria pauciramosa	Conidia	2	20	**5/2.5**	30	1	4	**3/1.3**	10
	Chlamydospores	2	20	**5/3.1**	30	3	4	**5/2.7**	10
	Mycelium	**1/0.4**	30	**5/3.2**	30	3	4	**3/1.6**	10
Fusarium oxysporum	Conidia	1	10	5	10	1	4	1	4
	Chlamydospores	5	20	-	-	1	4	5	4
	Mycelium	5	10	-	-	1	4	3	4
Phytophthora cinnamomi	Zoospores	1	10	1	10	1	4	1	4
	Cysts	1	10	1	10	1	4	1	4
	Oospores	2	10	**1/0.4**	30	3	4	3	4
	Sporangia	2	10	1	20	3	4	3	4
	Mycelium	5	10	**2/1.4**	30	3	4	3	4
	Zoospores	1	10	5	10	1	4	3	4
	Chlamydospores	5	20	-	-	1	4	3	4
	Mycelium	5	20	-	-	1	4	3	4

To use high concentrations (5 ppm initial/free) of chlorine and chlorine dioxide treatments to effectively disinfest irrigation water, further work is required to investigate potential phytotoxicity associated with residual concentrations in irrigation water and the effect of residuals on beneficial microbial organisms in the plant rhizosphere. A critical aspect which must be considered with water disinfesting treatments which leave residuals, is the original dosage rates and the residual concentration post the effective treatment duration (where known) and the potential phytotoxicity with residuals of 2.7 ppm post 10 minutes (chlorine dioxide) and 4.6 ppm post 30 minutes (chlorine) in the treated irrigation water to effectively disinfest the water – which in most instances of this study were unknown.

Recommendations

Selecting the appropriate disinfection system will depend on:

- current hygiene practices in the nursery
- water quality
- plant species grown in the nursery
- pathogens present and
- the resources available to the nursery.

Based on the outcomes from this study and the full reports literature references:

- Good nursery hygiene practices will reduce the risk of pathogens and disease being introduced and establishing
- Use initial water free of plant pathogens and prevent pathogen entry into the water source and the nursery
- When selecting a disinfection method for irrigation water, the water quality and pathogens present in the water and nursery must be carefully considered and done with a level of independent technical support to achieve best outcome
- Chlorine dioxide (with residuals) and UV were the most effective of the three treatments tested
- Where water quality can be maintained at a consistently high level with low organic matter and turbidity, UV provides good disinfection against most pathogen propagules tested
- Where water quality is lower or pH is likely to be variable, chlorine dioxide provides good disinfection against most pathogens tested
- Particulate matter can influence the efficacy of the disinfection treatment and
- Pathogens with pigmented or melanised cell walls are less likely to be susceptible to UV treatment.

Conclusion

This study has begun to address the gaps that exist in the available data for the effectiveness of disinfection treatments on different life stages, or propagules of a given pathogen, and the role of water quality characteristics.

Ultimately, the selection of a disinfection system for any given situation will depend on a number of factors including; current hygiene practices in the nursery, water quality, plant species grown, pathogens present, targeted pathogens and their propagules and the cost to treat and resources available to the nursery.

Table 3. Calculated minimum exposure required to kill >99% of CFU propagules tested following treatment with UV-C radiation.

A '– ' indicates that propagules were not killed at the rates tested.

Pathogen	Propagule	DI water	Dam water
		(mJ/cm^2)	(mJ/cm^2)
Clavibacter michiganensis	Bacterial cells	113	113
Alternaria alternata	Conidia	250	-
	Mycelium	250	-
Chalara elegans	Chlamydospores	113	113
	Endoconidia	113	250
	Mycelium	113	113
Colletotrichum gloeosporioides	Conidia	113	113
	Mycelium	113	113
Calonectria pauciramosa	Conidia	250	-
	Chlamydospores	-	-
	Mycelium	250	-
Fusarium oxysporum	Conidia	250	-
	Chlamydospores	250	-
	Mycelium	250	-
Phytophthora cinnamomi	Zoospores	113	113
	Cysts	113	113
	Oospores	113	113
	Sporangia	113	113
	Mycelium	113	113
Pythium aphanidermatum	Zoospores	113	113
	Oospores	113	113
	Mycelium	113	113

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Reference: Nursery Industry Accreditation

Reference: Final Report NY13003 - Increasing Productivity through Industry Research, Development and Extension Programs

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