Different Surfaces Need Different Treatments for Successful Nursery Hygiene©

Martin I. Mebalds
Agriculture Victoria, Knoxfield, Institute for Horticultural Development, Private Bag 15, Scoresby Business Centre, VIC 3175

Gordon E. Stovold
NSW Agriculture Tropical Fruit Research Station, PO Box 72, ALSTONVILLE NSW 2800

Chemical disinfestation treatments for a range of surfaces typically found in nurseries, namely; steel, plastic, capillary mat, sand bed, gravel, and concrete were assessed for their efficacy against the fungi, *Phytophthora cinnamomi* and *Chalara elegans*, the bacterium, *Xanthomonas campestris*, and the nematode *Meloidogyne* spp. No one chemical treatment (sodium hypochlorite, quaternary ammonium chloride, copper hydroxide, or copper ethanolamine) could be used for all combinations of surface or pathogen, however, effective disinfestation treatments were found for most surface-pathogen combinations.

INTRODUCTION

A review of the literature on the efficacy of surface disinfestation protocols was instigated to address concerns by the Australian nursery industry that there was little specific information available on the efficacy of common disinfestation protocols for plant pathogens. A literature review on nursery hygiene procedures was undertaken and reported to the Horticultural Research and Development Corporation in 1997 (Mebalds et al., 1997). The review showed that many widely accepted chemical disinfectants do not kill or inactivate plant pathogens from every biological group, nor were they equally effective on all surfaces. For example, iodine, sodium hypochlorite, and quaternary ammonium compounds were found to effectively control a range of fungal pathogens on pot surfaces but not on peat colonised by those pathogens (Koponen et al., 1992; Koponen et al., 1993). Similarly, quaternary ammonium chloride formulations may effectively control fungal and bacterial pathogens (Voss and Meier, 1987) but do not fully inactivate viruses (Hu et al., 1994). All disinfectants required extended contact times for disinfestation, therefore rapid disinfestation for "quick dip" procedures such as use of foot baths and disinfestation of pots, trays knives, and shears need further development.

The effectiveness of common disinfestants was therefore assessed against a range of plant pathogens on a range of surfaces found in nurseries commonly found in Australian nurseries.

MATERIALS AND METHODS

Preparation of Surfaces. All prepared surfaces were sterilised in an autoclave prior to application of pathogens, except for plastic surfaces which were sterilised with 70% ethanol.

Suspensions of fungal and bacterial pathogens were sprayed onto 10 cm × 10 cm squares of stainless steel. Fungal pathogens were spayed onto polythene film (10 cm × 10 cm) stapled onto linoleum while bacterial suspensions were sprayed onto 10cm plastic pots. Capillary mats were cut from 'Bottom up' capillary matting for fungal pathogens while bacterial and nematode pathogens were applied on squares of weed mat overlaying a geotextile base.

White quartz sand (size fractions were: <0.25 mm, 42%; 0.25 to 0.60 mm, 56%; 0.60 to 1.40 mm, 2%) and blue stone gravel (ranging from 10 to 20 mm diameter) beds were constructed in trays.

Circular cement blocks were made using 90 mm petri dishes as moulds. The concrete blocks were rinsed for 24 h under running water to remove any cement residue.

Inoculation. Chlamydospores of *Chalara elegans* grown on potato vegemite dextrose agar (PVDA) were suspended in sterile water containing 0.1% Tween and passed through a 38 μM sieve to remove microconidia. The chlamydospores were resuspended in sterile distilled water (SDW) and homogenised to break up spore clusters. The suspension was then stored at 2 to 5°C until required. Chlamydospores of *P. cinnamomi* were produced and harvested following published methods (Mircetich et al., 1968).

Fungal chlamydospore suspensions were sprayed onto test surfaces and allowed to dry before application of disinfestant chemicals. Two-day-old cultures of *Xanthomonas campestris* bacteria isolated from *Ranunculus* sp. and grown on nutrient agar were suspended in SDW. A 0.5-ml aliquot of the suspension was sprayed onto test surfaces and allowed to stand to 5 min before disinfestant treatments were applied. Free-swimming *Meloidogyne* sp. nematodes from naturally infested soil from grape and solanaceous crops were extracted using the Whitehead method. Nematodes suspensions were applied either to capillary may, sand bed, or gravel beds and disinfestant applied immediately. Nematode suspensions in pools of water sitting on plastic were treated with disinfestants to attain a final concentration of active ingredient consistent with treatments on other surfaces. The survival of nematodes was measured every 10 minutes for 80 minutes. In all cases, there were 4 replicates of each treatment and treated surfaces were completely randomised on the laboratory bench.

Disinfestant Solutions. The disinfestants were 2000 or 4000 mg·litre^{-1} available chlorine from sodium hypochlorite, 2000 or 4000 mg·litre^{-1} quaternary ammonium compound (Phytoclean™, Avis Chemicals), copper hydroxide, 20,000 mg·litre^{-1} or 40,000 mg·litre^{-1} (applied to fungal contaminated surfaces), and 533 or 1066 mg·litre^{-1} copper (II) ethanolamine complex (Brunnings Algae and Moss Destroyer™), applied to bacteria and nematode contaminated surfaces. The copper concentrations were derived from existing label recommendations. The disinfectant solutions were applied at the rate of 160 ml·m^{-2} for each surface. For brevity, disinfestant names were abbreviated to Cl for sodium hypochlorite, QAT for quaternary ammonium chloride and Cu for the copper compounds.

Assessment. All treatments were stopped when a neutralising solution (55 ml 0.1N sodium thiosulphate and 1 ml Tween 80 diluted to 400 ml) was washed over the test surface. After expiry of the contact time the viability of the *C. elegans* inoculum was assessed using the carrot disc baiting method. Ten fresh carrot discs cut from the interior of carrot roots were pushed randomly onto the surface of each replicate then

removed and incubated on a blotter pad in a moist chamber. For the capillary matting, the upper plastic surface was first removed before applying the carrot discs. After 7 days incubation in moist chambers, the carrot discs were examined with a low-power stereoscope for evidence of active growth of *C. elegans*.

Agar plugs were placed on surfaces contaminated with *P. cinnamomi* after disinfestation treatment. After 24 h, the plugs were removed and placed (5 per plate) onto the surface of agar plates containing *Phytophthora* selective agar. The plugs were examined for evidence of active growth of *P. cinnamomi* over several days using the 10× objective of a compound microscope. Ten agar plugs were used for assessing each treatment replicate. The viability of chlamydospores used to inoculate the sand bed surface was assessed by taking ten small scoops of the sand and plating (5 per plate) to petri dishes containing *Phytophthora* selective agar. Plates were examined over several days for evidence of active growth of *P. cinnamomi*.

Surfaces treated for *X. campestris* contamination were rinsed with 9 ml saline neutralising solution. The runoff was collected and serially diluted up to 10° for controls and up to 10^4 for treated surfaces. Two subsamples of 0.5 ml suspension from each dilution was then spread over Nutrient Agar (NA) in petri dishes and incubated for 2 to 3 days at 28°C. The number of *X. campestris* colonies per plate was counted if below approximately 300 colonies/plate and number of surviving *X. campestris* /ml suspension was then estimated. *Meloidogyne* sp. nematodes were washed off surfaces with neutralising solution after treatment and immediately assessed for viability by observation using a 40× dissecting microscope.

RESULTS

Chalara elegans chlamydospores were controlled on stainless steel surfaces after 1 min exposure to 4000 mg·litre^{-1} chlorine but on plastic surfaces, a 20-min exposure was required (Table 1). Copper oxychloride (40,000 mg·litre^{-1}) controlled *C. elegans* chlamydospores on both stainless steel and plastic surfaces but was not effective at 20000 mg·litre^{-1}. QAT was not effective against *C. elegans* chlamydospores at concentrations up to 4000 mg·litre^{-1} even for exposures over 5 h. *Chalara elegans* was difficult to control on capillary mat, where 4000 mg Cl or QAT litre^{-1} for 1 h suppressed but not kill the fungus. On sand beds, 2000 mg·litre^{-1} chlorine for 1 h to completely kill, or 20000 mg·litre^{-1} of Cu for 24 h was required to suppress *C. elegans*. None of the treatments tested were effective for *C. elegans* contaminated gravel or cement.

Phytophthora cinnamomi, was killed in all surfaces tested, usually after 1 h, however, in gravel, a 20-min treatment time with 4000 mg litre^{-1} chlorine or a over a 5-h treatment with 20,000 mg·litre^{-1} copper was required.

Xanthomonas campestris contaminated stainless steel and plastic surfaces were successfully disinfected with 2000 mg·litre^{-1} chlorine after 1 min (average 99.999% reduction on steel and total control on plastic). QAT disinfected both steel and plastic surfaces after 1-min exposure to a 2000 mg·litre^{-1} solution. Copper oxychloride solution did not wet the steel surface effectively in the first experiment so a liquid copper formulation was as copper (II) ethanolamine assessed at label rates of 533 and 1066 mg·litre^{-1}. The copper formulations failed to disinfect steel or plastic surfaces from *X. campestris*.

Meloidogyne sp. juvenile nematodes were quite resistant to all disinfectants in water, surviving lengthy contact times but 2000 mg·litre^{-1} of Cl , 2000 mg·litre^{-1} of QAT, and 533 mg Cu litre^{-1} killed all juveniles after 80 min. Where nematodes are

Table 1. Effective treatments, expressed as $mg \cdot l^{-1}$ (ppm) of active ingredient per minutes or hours contact time, for disinfection of common nursery surfaces from plant pathogens.

Surface	*Phytophthora cinnamomi*	*Chalara elegans*	*Xanthomonas campestris*	*Meloidogyne* spp.
Steel	2000 mg·litre^{-1} Cl/min	2000 mg·litre^{-1} Cl/20 min	2000 mg·litre^{-1} Cl/1 min	-
	2000 mg·litre^{-1} QAT /1 min	4000 mg·litre^{-1} Cl/1 min	2000 mg·litre^{-1} QAT/1 min	
	20000 mg·litre^{-1} Cu/18 h	20,000 mg·litre^{-1} Cu/18 h		
Plastic	2000 mg·litre^{-1} Cl/1 min	4000 mg·litre^{-1} Cl/20 min	2000 mg·litre^{-1} Cl/1 min	-
	2000 mg·litre^{-1} QAT/1 min	20,000 mg·litre^{-1} Cu/18 h	2000 mg·litre^{-1} QAT/1 min	
	40000 mg·litre^{-1} Cu/18 h	-		
Capillary mat	2000 mg·litre^{-1} Cl/1 h	None of the tested treatments	2000 mg·litre^{-1} Cl/20 min	4000 mg·litre^{-1} QAT/1 h
	2000 mg·litre^{-1} QAT/1 h	were effective	2000 mg·litre^{-1} QAT/20 min	
	20000 mg·litre^{-1} Cu/1 h			
Sand bed	2000 mg·litre^{-1} Cl/1 h	2000 mg·litre^{-1} Cl/24 h	2000 mg·litre^{-1} Cl/30 min	4000 mg·litre^{-1} QAT/20 min
	2000 mg·litre^{-1} QAT/1 h		4000 mg·litre^{-1} Cl/5 min	
	20000 mg·litre^{-1} Cu/1 h			
Gravel	4000 mg·litre^{-1} Cl/20 min	None of the tested treatments	4000 mg·litre^{-1} Cl/5 min	4000 mg·litre^{-1} Cl/1 h
	20000 mg·litre^{-1} Cu >5 h	were effective		
	2000 mg·litre^{-1} QAT/1 h			
Concrete	4000 mg·litre^{-1} QAT/1 min	None of the tested treatments	4000 mg·litre^{-1} Cl/1 h	-
	20000 mg·litre^{-1} Cu >5 h	were effective	4000 mg·litre^{-1} QAT/1 h	

Cl = Sodium hypochlorite (chlorine).
QAT = quaternary ammonium compound (Phytoclean™ used in experiments).
Cu = copper either as copper oxychloride (used on *Phytophthora* and *Chalara*) or copper (II) ethanolamine complex (used on *Xanthomonas campestris* and *Meloidogyne* spp.).

a problem in nurseries, a 40-minute soak in 2000 mg·litre^{-1} of Cl or QAT is recommended. The juveniles are the least resistant to the action of disinfectants and nematode eggs and cysts will require other methods of control such as heat treatments.

The bacterium *Xanthomonas campestris* and the free-swimming form of the root knot nematode *Meloidogyne* sp. were killed within 1 h on most surfaces with 4000 mg·litre^{-1} of Cl or QAT . Copper did not kill the nematodes completely within that time. However observations of the nematodes indicated that, after 1 h exposure to 1066 mg Cu litre^{-1}, the remaining live nematodes were severely affected and probably died later. *Meloidogyne* sp. nematodes also form cysts that are more resistant to chemical disinfection and will require longer treatment times for control

DISCUSSION

There was no one, clear, all-purpose, disinfestation treatment for all surfaces and pathogens. The recommended protocols therefore are more complex than is desirable for easy adoption in a busy nursery setting, where there are numerous surface types and pathogens of concern that require control. Generally sodium hypochlorite and the QAT product Phytoclean™, were comparatively fast-acting disinfectants but were not recommended on all surfaces and pathogens.

There are no specific sodium hypochlorite products that have label recommendations for the disinfection of the surfaces tested in this project. Phytoclean™ however is a product registered for the control of *Phytophthora* spp. as contaminants and the rates applied in the project experiments conform to label recommendations for surfaces and footbaths.

The copper compounds used in this project required longer treatment times than the chlorine or QAT to be effective. The longer treatment times should not present problems in situations were long term protection from pathogen contaminants is required as it is already generally applied in many nurseries as a long-term control for moss and algae. At present there are no specific label recommendations for the use of copper as a general disinfectant however, copper oxychloride is registered to control a wide range of fungal and bacterial diseases on plants. The use of copper should be restricted to surfaces listed on product labels as these recommendations would have been tested for environmental impact, especially with regard to the impacts of runoff. In experiments where copper ethanolamine complex (Brunnings Moss and Algae Destroyer™) was applied, the label rates were used and conform to manufacturers' specifications.

Aknowledgements. The research project was funded by the Australian nursery industry research and development levy, the Horticulture Australia Limited, NSW Agriculture and Agriculture Victoria. We thank Steven Muldoon, Suzanne Hayward, Michelle Bankier, and Bret Henderson for their support in this research project.

LITERATURE CITED

Hu, J.S., S. Ferreira, M.Q. Xu, M. Lu, M. Iha, E. Pflum, and M. Wang. 1994. Transmission, Movement, and inactivation of cymbidium mosaic virus and odontoglossum ringspot viruses. Plant Dis. 78:633-636.

Koponen, H., M. Manninen, P. Harju, H. Avikainen, and **R. Tahvonen.** 1992. The effect of disinfectants on *Clavibacter michiganensis* subsp. *sepedonicus* and *Erwinia carotovora* subsp. *atroseptica* on different surface materials. Agric. Science in Finland 1:597-602.

Koponen, H., H. Avikainen, and **R. Tahvonen.** 1993. The effect of disinfectants on fungal diseases of potato and vegetables. Agric. Science in Finland. 2:169-177.

Mebalds, M.I., W. Tregea, and **A. van der Linden.** 1997. Disinfestation protocols for the nursery industry. Literature Rev. Rpt. for HRDC Project NY612.

Mircetich, S.M., G.A. Zentmeyer, and **J.B. Kendrick** 1968. Physiology of germination of chlamydospores of *Phytophthora cinnamomi*. Phytopathol. 58(5):666-671.

Voss, von J. and **U. Meier.** 1987. The efficacy of pesticides used as disinfectants against the resting structures of plant parasite fungi. Nachrichtenbl. Deutsch. Pflanzenschutz. 39:179-182.