CHAPTER 4

CHEMICAL DISINFESTATION OF GRAVEL SUBSTRATE USED IN RECIRCULATING HYDROPONIC SYSTEMS

Abstract

The chemical disinfectants calcium hypochlorite, chlorine dioxide + activator, dazomet, formaldehyde, glutaraldehyde, hydrogen peroxide, hydrogen peroxide + formic acid, metham-sodium, methyl bromide + chloropicrin, N-alkyl dimethyl benzyl ammonium chloride, polydimethyl ammonium chloride and sodium hypochlorite, were evaluated in one or more experiments for the eradication of *Pythium* and *Fusarium* populations in naturally-infested gravel utilised as substrate in a recirculating hydroponic system. Overall, the *Fusarium* population was more resistant to chemical treatment than the *Pythium* population. Total control of both populations could be obtained in at least one of the experiments with dazomet at 20 and 30 g m^{-2} and formaldehyde at 10 ml l^{-1}. Metham-sodium at 20, 10 and 5 ml l^{-1} reduced the *Pythium* population to zero and was also highly effective against *Fusarium*. Under semi-commercial conditions, significant, but not total control of *Pythium* was achieved with formaldehyde at 5 ml l^{-1}, hydrogen peroxide + formic acid at 25 ml l^{-1}, methyl bromide + chloropicrin at 100 g m^{-2}, polydimethyl ammonium chloride at 10 ml l^{-1} and sodium hypochlorite at 50 ml l^{-1}. Hydrogen peroxide + formic acid at 25 ml l^{-1} and polydimethyl ammonium chloride at 10 ml l^{-1} were correspondingly effective against *Fusarium*.

INTRODUCTION

Root diseases caused by *Pythium* spp. are particularly important in hydroponic systems (Jenkins & Averre, 1983; Zinnen, 1988; Cherif & Belanger, 1991; Cherif *et al.*, 1994, 1997; Stanghellini *et al.*, 1996; Sanchez *et al.*, 2000). Recirculating closed cultural systems that employ a common reservoir for distributing nutrient solution to and from various separate production units provide an ideal environment for disease spread. If the pathogen is accidentally introduced into such a

system at any site, rapid and uniform distribution is virtually guaranteed and control often difficult to achieve (Stanghellini *et al.*, 1984; Paulitz, 1997). Inoculum may be transmitted from diseased plants in run-off water into the reservoir and from there be further disseminated throughout the entire production facility (Mebals *et al.*, 1997b).

Attempts at controlling *Pythium* spp. in hydroponica have met with varying success. Disinfestation of nutrient solutions by heat, ozonation, UV-radiation, filtration, amendment with surfactants or incorporation of antagonists has proved to be effective in preventing the spread of *Pythium* in recirculating systems (Zhou & Paulitz, 1993; Runia, 1994, 1995; Wohanka, 1995; McCullagh *et al.*, 1996; Menzies & Belanger, 1996; Stanghellini *et al.*, 1996; Mebalds *et al.*, 1997b). However, the volume of water in circulation in such systems renders treatments like these rather costly. Furthermore, while zoospores are relatively sensitive, the treatments are not always effective against mycelial propagules retained in the substrate (Hendrix & Campbell, 1973).

Infected root residues that persist in the hydroponic substrate after the crop has been harvested constitute a major source of inoculum (Menzies & Belanger, 1996; Sanchez *et al*, 2000). Removal of the residues and disinfestation of the recycled substrate are essential for maintaining a pathogen-free system (Stanghellini & Rasmussen, 1994). Various compounds are available that can be used for this purpose, e.g. surfactants (Stanghellini & Tomlinson, 1987; Stanghellini *et al.*, 1996) fumigants (Garibaldi & Gullino, 1995; Mappes, 1995), and chemicals applied through irrigation or drenching (Handreck & Black, 1984; Fritsch & Huber, 1995). This paper reports on the efficacy of selected chemical disinfectants against *Pythium* and *Fusarium* spp. in naturally-infested hydroponic gravel substrate. *Fusarium* was included as reference (Runia, 1995) because it is also an important pathogenic genus in hydroponic systems and generally more resistant to chemicals than *Pythium* (Picket-Popoff & Parker, 1994; Minuto *et al.*, 1995).

MATERIALS AND METHODS

The study comprised three experiments, viz. (i) a pilot trial in which gravel collected from commercial beds was transferred into smaller containers and treated with chemicals, (ii) a large-scale screening where chemicals were applied *in situ* to the gravel in commercial hydroponic

beds, and (iii) a small-scale refinement, conducted similarly to the pilot trial, to optimise dosage rates of selected chemicals. The chemicals that were tested are listed in Table 1. Treatment with sterile water (SW) served as control, except in the large-scale screening where the water was not sterilised.

(i) Pilot trial

Approximately 60 l of gravel was obtained from a *Pythium*-infested commercial hydroponic system producing butter head lettuce (*Lactuca sativa* L. var. *capitata* L.). The gravel was collected from four hydroponic beds in the system, pooled, mixed and dispensed into thirty 2 l plastic containers, each with ten 5-mm-diameter holes in the bottom. The gravel in each of three containers was treated at 23°C with one of the chemicals indicated in Table 1. Basamid was sprinkled on the surface of the gravel and then drenched into the gravel with 2 l of SW. The other chemicals were either suspended or dissolved in SW and 2 l of the suspension/solution drenched through the gravel in each container, the excess liquid draining through the holes at the bottom. Containers receiving dazomet, hydrogen peroxide, hydrogen peroxide + formic acid, and metham-sodium were sealed with tight-fitting lids to enhance the fumigative action of the chemicals. Metham-sodium was applied to gravel in the containers with and without a saucer underneath to establish if enhanced retention of the compound could increase its efficacy.

After 48 hours, the gravel in each container was flushed twice with 2 l SW to remove chemical residues. The gravel was then transferred to a clean container without holes and the root residues that remained were extricated by adding 1 l of SW to the container and swirling it until the residues had risen to the surface. The supernatant was collected in a clean Erlenmeyer flask and decanted through filter paper to retrieve the root segments. Fifteen *ca.* 5-mm-long root segments from each replicate were plated, five segments per plate, on a *Pythium*-selective medium (Roux & Botha, 1997), and a further 15 segments on RBGU medium (Van Wyk *et al.*, 1986) selective for *Fusarium*. Plates were incubated for four days at 25 °C and the number of root segments yielding *Pythium* or *Fusarium* were recorded.

(ii) Large-scale screening

Three blocks, each comprising eleven 27 x 0.5 x 0.1 m hydroponic beds, were randomly designated in the commercial recirculating gravel system from which gravel was collected for the first experiment. Temperatures in the hydroponicum varied between 13 and 27 °C. One bed in each block was treated with one of the chemicals indicated in Table 1. Dazomet was sprinkled onto the gravel in the beds and then drenched into each bed with 300 l of water, whereas methyl bromide + chloropicrin was released from pressurised canisters into the beds. The other chemicals were applied in 300 l of water to the respective beds. Beds receiving dazomet and methyl bromide + chloropicrin were covered with plastic sheeting.

After 48 hours, each bed was rinsed with 300 l of water to remove chemical residues. Ten gravel samples of approximately 700 ml each were collected along the length of each bed. The samples from each bed were pooled and transferred to a clean container. Two litres of water was added to the gravel, the container was swirled until root segments floated in the water, and the segments were collected in an Erlenmeyer flask. The presence of *Pythium* and *Fusarium* in the root segments was determined as described above.

(iii) Small-scale refinement

Approximately 60 l of gravel was randomly collected from control beds in the previous large-scale screening. The gravel was pooled, mixed, and dispensed into thirty 2 l plastic containers, each with ten 5-mm-diameter holes in the bottom. The gravel in each of three containers was treated at 25 °C with one of the chemicals indicated in Table 1. Containers receiving formaldehyde, hydrogen peroxide + formic acid and metham-sodium were sealed as before during the exposure period. The same procedures for collection of root segments and enumeration of *Pythium* and *Fusarium* were followed as in the pilot trial.

RESULTS

The dominant *Fusarium* species in all three experiments was *Fusarium oxysporum* Schltdl. emend. W.C. Snyder & H.N. Hansen, with *Fusarium solani* (Mart.) Appel. & Wollenw. the

second most prevalent. Although the *Pythium* species were not identified, it can be assumed that the population comprised mainly *Pythium coloratum* Vaartaja, *Pythium irregulare* Buisman and *Pythium* groups F, G and HS, previously (Chapter 2) shown to be present in the hydroponicum concerned.

The *Fusarium* population was more resistant to chemical treatment than the *Pythium* population, with a mean overall reduction of 42% compared to 80% (Tables 2 & 3). In the pilot trial, all the chemicals that were evaluated, except chlorine dioxide + activator, significantly reduced the incidence of *Pythium*, whereas calcium hypochlorite, dazomet, glutaraldehyde, metham-sodium, PDAC and sodium hypochlorite were effective against *Fusarium*. Application of metham-sodium with a saucer underneath the container did not enhance the fungicidal action of the compound.

Efficacy was more pronounced in the large-scale screening, with the incidence of *Pythium* and *Fusarium* being significantly reduced by all the chemicals included in the experiment. Dazomet at 20 and 30 g m^{-2} and formaldehyde at 10 ml l^{-1} provided total (100%) control of both populations, whilst metham-sodium at 10 and 20 ml l^{-1} reduced the *Pythium* population to zero. The efficacy of metham-sodium against *Pythium* was verified in the small-scale refinement, where even the 5 ml l^{-1} application rate resulted in total control. Total control of *Pythium* in the refinement experiment was also achieved with PDAC at 5 and 10 ml l^{-1}, whereas significant control was evident with hydrogen peroxide + formic acid at 25 and 30 ml l^{-1} and formaldehyde at 10 ml l^{-1}, but not at 5 ml l^{-1}. None of the chemicals significantly reduced the incidence of *Fusarium*. Indeed, gravel treated with formaldehyde at 5 ml l^{-1} and hydrogen peroxide + formic acid at 30 ml l^{-1} yielded 54% and 60% higher *Fusarium* counts, respectively, than the control.

DISCUSSION

Pathogen colonisation of even a single plant, or plant residue for that matter, constitutes a serious threat to the entire plant population in a recirculating hydroponic system (Stanghellini *et al.*, 1990b). Spread of the pathogen from such an infection site can obviously be restricted by addition of fungicides to the nutrient solution. However, no fungicides are presently registered

for use in hydroponic systems in South Africa. It is also unlikely that any will be registered in the foreseeable future as the hydroponic industry, due to the limited area it occupies, is not a priority to chemical companies. Furthermore, the rapid succession and short duration of crop cycles do not allow sufficient time for the lag period between application and harvesting prescribed for most fungicides, hence precluding their usage.

Total eradication of the residual inoculum therefore remains the only way of rendering an infested hydroponic substrate suitable for replanting. Results of the present study indicate that relatively few of the chemical disinfectants that were tested are capable of achieving this. However, it should be kept in mind that the initial screening and eventual small-scale refinement were biased towards the pathogen, with limited contact time allowed for the chemicals. Of the compounds that did not make the grade in these experiments, hydrogen peroxide and chlorine have been evaluated before in hydroponic systems. Hydrogen peroxide has thus far been effective only under experimental conditions, in which tomato mosaic virus was inactivated at 400 ppm and conidia of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen were killed at 100 ppm (Runia, 1995; Menzies & Belanger, 1996). Even at 12 500 ppm in the present study, hydrogen peroxide provided only moderate suppression of *Pythium* and had no significant effect on *Fusarium*, despite having sporicidal activity and not readily being inactivated by organic matter. The mixture of hydrogen peroxide + formic acid was much more effective against *Pythium* than hydrogen peroxide on its own. This mixture is being marketed commercially in the Netherlands under the trade name Reciclean®, the addition of formic acid being aimed at cleaning emitters of irrigation systems from calcium deposits (Anonymous).

Chlorine compounds have been tested frequently in hydroponica. Stanghellini *et al.* (1996) used sodium hypochlorite at a rate of 10% to successfully surface-sterilise an entire hydroponic system in which the efficacy of a non-ionic surfactant was evaluated. Previously, Bates & Stanghellini (1984) reported chlorine to be effective for controlling *Pythium aphanidermatum* (Edson) Fitz. root rot of cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* Mill.), but found sodium hypochlorite at concentrations of 1 to 6 $\mu g\ ml^{-1}$ to be ineffective against *P. aphanidermatum* and *P. dissotocum* Drechsler, and phytotoxic to spinach (*Spinacea*

oleracea L.), when applied to the nutrient solution. Activity of chlorine dioxide towards *Pythium* and *Fusarium* has been reported by Mebalds *et al.* (1997b). Chlorine, regardless of being applied as chlorine gas, sodium hypochlorite or calcium hypochlorite (all of which yield hypochlorous acid [HClO] and then atomic oxygen), nevertheless remains one of the most effective and safest chemical disinfectans of water sources, and is also employed in the dairy and food industries. Chlorine has an oxidative action and destroys cellular material of vegetative bacteria and fungi, though not spores. Death of almost all microorganisms usually occurs within 30 minutes but, in the presence of organic material, an excess of chlorine has to be applied to ensure microbial destruction since the organic material interferes with the action of chlorine by reacting with it (Runia, 1994; Mebalds *et al.*, 1997b). It was interesting to note that the efficacy of calcium hypochlorite and sodium hypochlorite in the present study did not differ significantly, despite the almost 17 times lower application rate of the former. It must be noted however that disinfestation of the infected root residues that persist in the substrate after the crop has been harvested (as in the present study) is a much more difficult to achieve than disinfestation of irrigation water alone. Chlorine, incapable of penetrating plant tissue effectively, therefore proved to be ineffective in the present study.

Dazomet, formaldehyde and metham-sodium were the only chemicals which provided total control of *Pythium* in the large-scale screening, whereas application of PDAC resulted in a 93% reduction, as well as total control of *Pythium* in the pilot trial and small-scale refinement. Dazomet and formaldehyde also eradicated *Fusarium*, while significant reductions of 93% and 76% in *Fusarium* populations were evident with metham-sodium and PDAC, respectively. PDAC is a quaternary ammonium compound (QAC) commonly used for disinfecting purposes in the horticultural and flower industries. For a QAC, PDAC performed exceptionally well, particularly when considering that it was applied at a lower dosage than the commercially recommended rate. As a rule, QACs are effective only against fungal structures not containing a cell wall, e.g. zoospores, but in the present study PDAC obviously also killed walled propagules such as mycelium, conidia and chlamydospores. This wide spectrum of activity is in accordance with the results of a study conducted by the ARC-Plant Protection Research Institute (unpublished data) which showed PDAC to eradicate sclerotia of *Sclerotium rolfsii* Sacc. However, with *Rhizoctonia solani* J.G. Kühn, Muller & Wehner (1999) obtained a reduction in

sclerotial viability of between 30% and 60%, depending on the concentration of PDAC used.

Formaldehyde, dazomet and metham-sodium all have a fumigative action. Formaldehyde is commonly used for sterilising gravel, pipelines and reservoirs, but should not come in contact with living plants during media sterilisation. (Harris, 1992). Dazomet and metham-sodium are both rapidly converted to methyl isothiocyanate (MITC) when released into the environment (Tomlin, 1994). MITC is a general biocide with activity against many fungi, insects and nematodes, but unfortunately also plants. Treated substrates can therefore not be planted until free of MITC, usually some time after application (Handreck & Black, 1984; Tomlin, 1994; Fritsch & Huber, 1995). Dazomet had the advantage over metham-sodium in that it eradicated *Fusarium*, and furthermore is less expensive to apply at recommended rates. However, in terms of cost-effectiveness, formaldehyde should be the preferred choice. The compound is highly soluble in water, self-dispersing and relatively easy to use, though extremely irritating to mucous membranes and toxic to virtually all forms of life (Buckle, 1981). Based on the results of this study formaldehyde has nevertheless been used successfully and safely as sterilant in some commercial gravel recirculating hydroponic systems at a rate of 10 ml l^{-1}.

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TABLE 1. Chemicals screened in the various experiments

Chemical	Product name	Rate (product)	Experiment		
			(i)	(ii)	(iii)
Calcium hypochlorite	HTH	3 g l^{-1}	√		
Chlorine dioxide + activator	Purogene	2 ml l^{-1}	√		
Dazomet (98%)	Basamid	20 g m^{-2}		√	
		30 g m^{-2}	√	√	
Formaldehyde	Formalin	5 ml l^{-1}		√	√
		10 ml l^{-1}		√	√
Hydrogen peroxide + formic acid *	Reciclean	25 ml l^{-1}	√	√	√
		30 ml l^{-1}			√
Glutaraldehyde	EcoSanitizer	10 ml l^{-1}	√		
Hydrogen peroxide		12.5 ml l^{-1}	√		
Metham-sodium	Herbifume	5 ml l^{-1}			√
		10 ml l^{-1}		√	√
		20 ml l^{-1}	√	√	
Methyl bromide + chloropicrin	Methyl bromide	100 g m^{-2}		√	
N-alkyl dimethyl benzyl ammonium chloride 5% (NDBAC)	Desogerme	500 ppm	√		
Polydimethyl ammonium chloride 12% (PDAC)	Sporekill	5 ml l^{-1}			√
		10 ml l^{-1}	√	√	√
Sodium hypochlorite	Jik	50 ml l^{-1}	√	√	

* 35% hydrogen peroxide mixed with 15% formic acid in a 1:1 ratio.

TABLE 2. Effect of chemical treatment on the survival of *Pythium* species in root residues in gravel used as substrate in a recirculating hydroponic system

Chemical	Rate	Incidence (%) [a]		
		(i) [b]	(ii) [c]	(iii) [d]
Calcium hypochlorite	3 g l^{-1}	26.7 bc		
Chlorine dioxide + activator	2 ml l^{-1}	75.6 a		
Dazomet (98%)	20 g m^{-2}		0 b	
	30 g m^{-2}	4.4 c	0 b	
Formaldehyde	5 ml l^{-1}		17.8 b	71.1 a
	10 ml l^{-1}		0 b	37.8 b
Hydrogen peroxide + formic acid	25 ml l^{-1}	2.2 c	6.7 b	28.9 b
	30 ml l^{-1}			35.6 b
Glutaraldehyde	10 ml l^{-1}	4.4 c		
Hydrogen peroxide	12.5 ml l^{-1}	44.4 b		
Metham-sodium	5 ml l^{-1}			0 c
	10 ml l^{-1}		0 b	0 c
	20 ml l^{-1}	8.9 c	0 b	
Metham-sodium with saucer	20 ml l	6.7 c		
Methyl bromide + chloropicrin	100 g m^{-2}		17.8 b	
NDBAC [e]	500 ppm	26.7 bc		
PDAC [f]	5 ml l^{-1}			0 c
	10 ml l^{-1}	0 d	4.4 b	0 c
Sodium hypochlorite	50 ml l^{-1}	11.1 c	24.4 b	
Control		77.8 a	66.8 a	84.4 a

TABLE 2 (continued)

[a] Mean of three replicates of 15 root segments each; values in columns followed by the same letter do not differ significantly according to Fisher's protected t-test ($P \leq 0.05$).

[b] Pilot trial.

[c] Large-scale screening.

[d] Small-scale refinement.

[e] N-alkyl dimethyl benzyl ammonium chloride.

[f] Polydimethyl ammonium chloride.

TABLE 3. **Effect of chemical treatment on the survival of *Fusarium* species in root residues in gravel used as substrate in a recirculating hydroponic system**

Chemical	Rate	Incidence (%)[a]		
		(i)[b]	(ii)[c]	(iii)[d]
Calcium hypochlorite	3 g l^{-1}	33.3 bc		
Chlorine dioxide + activator	2 ml l^{-1}	93.3 a		
Dazomet (98%)	20 g m^{-2}		0 d	
	30 g m^{-2}	15.6 e	0 d	
Formaldehyde	5 ml l^{-1}		64.4 ab	51.1 a
	10 ml l^{-1}		0 d	26.7 a
Formic acid + hydrogen peroxide	25 ml l^{-1}	91.1 a	22.2 cd	24.4 a
	30 ml l^{-1}			53.3 a
Glutaraldehyde	10 ml l^{-1}	37.8 bc		
Hydrogen peroxide	12.5 ml l^{-1}	77.8 a		
Metham-sodium	5 ml l^{-1}			13.3 a
	10 ml l^{-1}		4.4 d	28.9 a
	20 ml l^{-1}	2.2 cd	4.4 d	
Metham-sodium with saucer	20 ml l	2.2 cd		
Methyl bromide + chloropicrin	100 g m^{-2}		80.0 a	
NDBAC[e]	500 ppm	73.3 ab		
PDAC[f]	5 ml l^{-1}			33.3 a
	10 ml l^{-1}	51.1 b	15.6 cd	31.1 a
Sodium hypochlorite	50 ml l^{-1}	37.8 bc	40.0 bc	
Control		95.6 a	64.4 ab	33.3 a

TABLE 3 (continued)

[a] Mean of three replicates of 15 root segments each; values in columns followed by the same letter do not differ significantly according to Fisher's protected t-test ($P \leq 0.05$).

[b] Pilot trial.

[c] Large-scale screening.

[d] Small-scale refinement.

[e] N-alkyl dimethyl benzyl ammonium chloride.

[f] Polydimethyl ammonium chloride.