Project title:	Managing ornamental plants sustainably (MOPS)
Project number:	CP 124
Work package title:	Evaluation of disinfectants against *Fusarium* sp. *ex* stocks and *Pythium* sp.
Work package leader:	Dr Erika Wedgwood
Report:	Annual report, December 2014
Previous report:	None
Key staff:	Erika Wedgwood Jonny Kerley Sam Brown
Location of work:	Boxworth Pathology Laboratory
Date work commenced:	01 January 2014
Date work completed **(or expected completion date):**	12 December 2014 (18 December 2015)

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Erika Wedgwood
Plant Pathologist / Study Director
ADAS

Signature Date 19 December 2014

Report authorised by:

John Atwood
Project Leader
ADAS

Signature Date 22 January 2015

CONTENTS

GROWER SUMMARY

Headline

Three out of nine disinfectant products tested, Disolite, Unifect G and Domestos Extended Germ Kill, gave complete control of *Fusarium* even when immersion was for only five minutes at half rate, with Disolite and Unifect G also preventing *Pythium* survival

Background and expected deliverables

Factsheet 03/14 collated information from AHDB Horticulture projects on the efficacy of various disinfectants. Several actives listed in the factsheet are no longer available or permitted (e.g. dichlorophen, formaldehyde, high boiling point tar phenols). Actives currently marketed for use in other areas (e.g. amines and chlorhexidine used in veterinary hygiene and designated as product-type 3) will no longer be permitted for use in horticulture unless a successful application under the BPCR for their use as a product-type 2 active is made.

Nine disinfectants currently used or likely to be available for use in commercial horticulture will be evaluated for their effectiveness against two common ornamental plant pathogens a *Fusarium* sp. (e.g. *Fusarium oxysporum* f. sp. *mathiolae*) and an oospore producing *Pythium* sp.. Work on *Pythium* sp. is expected to be relevant to other oomycetes such as root and foliar infecting *Phytophthora* spp. and downy mildews. Products selected will be from across a range of biocide types, representing the major different chemical modes of action (e.g. hydrogen peroxide, benzoic acid, iodophor, quaternary ammonium compound).

A series of laboratory tests will be done with *Pythium* and *Fusarium* mycelium containing resting spores to determine the effect of i) disinfectant concentration ii) exposure time iii) the presence of organic matter and iv) surface type on product activity. The effect of the concentration and contact duration of the disinfectants on *Fusarium* spores (conidia) will also be examined. Methods devised to test against *Mycosphaerella melonis* and reported in AHDB Horticulture project report PE 001a will be used.

The objectives of this work are:

1. To determine the efficacy of a range of chemical disinfectants, from different active ingredient groups, against spores of *Fusarium*
2. To determine the efficacy of a range of chemical disinfectants, from different active ingredient groups, against mycelium of *Fusarium* and *Pythium*

3. To determine the efficacy of selected disinfectants for reduction of *Fusarium* and *Pythium* on five surfaces (glass, aluminium, rigid plastic, concrete and woven ground cover material).
4. To determine the effect of peat contamination of selected disinfectants against mycelium of *Fusarium* and *Pythium*

Summary of the work and main conclusions

Nine disinfectant products were selected for efficacy testing against two pathogens of ornamental plants, *Fusarium* and *Pythium*, whose resting spores can contaminate nursery beds, benches and re-used containers and lead to root infestation. Products were selected to include disinfectants with different active ingredients, some having been tested by ADAS using the same methods in previous AHDB Horticulture projects (as summarised in Factsheet 03/14) and thus included to aid comparison with the current work. The products selected for this project are shown in Table 1. All products were tested at both full rate and half rate, with contact times of both five and 30 minutes (four treatments).

Table 1. Details of products tested

Product	Active ingredient(s)	Full rate on product label
1. Jet 5	Hydrogen peroxide + peroxyacetic acid	1:125
2. Citrox P	Plant extract	1:150
3. Disolite	Phenolics	2%
4. FAM 30	Iodophor	1:125
5. Menno Florades	Benzoic acid	1%
6. Hydrocare	Hydrogen peroxide + silver	3%
7. Unifect G	Quaternary ammonium compounds + aldehyde	4%
8. Virkon S	Peroxygen compounds + organic acids	1:100
9. Domestos Extended Germ Kill	Chlorine-based bleaching agents (sodium hypochlorite)	120 ml into 5 L

The pathogens used in the work were *Fusarium oxysporum* f. sp. *mathiolae* isolated from wilted stems of column stocks (*Matthiola incana*) and *Pythium irregulare* from yew (*Taxus baccata*) roots.

Spores were collected from three-week old agar plates of *Fusarium* sp. Disinfectant was added to the spore suspension to obtain either a full or half rate product dilution before centrifuging after five or 30 minutes so that the disinfectant could be pipetted off and replaced with sterile distilled water

(SDW). Spores from each of the four treatments per disinfectant were pipetted onto agar, giving ten replications. The control treatment was SDW. The agar plates were incubated and *Fusarium* growth recorded at intervals up to seven days.

In order to treat resting spores of *Pythium* sp. (oospores) and *Fusarium* sp. (chlamydospores), mycelium was grown for three weeks on 7 mm diameter filter paper discs on agar plate that could be lifted off for immersion in the disinfectants. The same four treatments and ten replicates were used per disinfectant as for the spore testing, together with a SDW control. The discs were removed from the disinfectants after the required interval, rinsed in SDW and allowed to drip dry before incubating on agar plates for seven days to allow recording of colony growth.

The mycelium disc testing was repeated at full rate for five and 30 minutes immersion of *Fusarium* and *Pythium* with the addition of 0.1% w/v John Innes No1 with peat to the disinfectant. Organic matter can affect the efficacy of some disinfectants (AHDB Horticulture Factsheet 03/14).

For both spore and mycelium tests, disinfectant efficacy was generally the same regardless of the concentration or contact time except for the control of *Fusarium* spores by Hydrocare, where there was no control at half rate for five minutes, but complete control at full rate. Jet 5, Citrox P, Disolite FAM 30, Unifect G and Domestos Extended Germ Kill gave complete control of Fusarium spores. Efficacy of most products was, however, poor against *Fusarium* mycelium with only Disolite, Unifect G and Domestos Extended Germ Kill giving complete control, and these were still effective when organic matter was added. *Pythium* mycelium control was more variable, with Disolite and Unifect G giving complete control, but with Jet 5, Citrox P, FAM 30 giving 50% to 80% control at half rate, but some complete control at full rate. After the addition of organic matter to full rate disinfectants Jet 5, FAM 30 and Unifect G gave complete control after 5 minutes contact.

Table 2 shows the percentage control given by each disinfectant used at full rate for 30 minutes (the number of replicates out of the ten without any surviving pathogen). The most effective disinfectants against *Fusarium* and *Pythium* were Disolite, Unifect G and Domestos Extended Germ Kill.

Table 2. Summary of the control given by disinfectants used at full rate for 30 minutes contact time

Table 2. The proportion of replicates out of ten without pathogen growth after full rate for 30 minutes					
No survival	**80% - 90% control**	**50% - 70% control**	**30% - 40% control**	**10 – 20% control**	**Zero control (alive)**
+++++	++++	+++	++	+	-

Product name	***Fusarium* spores**	***Fusarium* mycelium**	***Pythium* mycelium**	***Fusarium* mycelium + peat**	***Pythium* mycelium + peat**
1. Jet 5	+++++	+++	+++++	-	+++++
2. Citrox P	+++++	-	++++	+	++++
3. Disolite	+++++	+++++	+++++	+++++	+++++
4. FAM 30	+++++	-	+++++	-	+++++
5. Hydrocare	+++++	-	++++	+	-
6. Virkon S	++++	-	+	-	-
7. Unifect G	+++++	+++++	+++++	+++++	+++++
8. Menno Florades	+++	-	+++	-	+
9. Domestos Extended Germ Kill	+++++	+++++	+++++	+++++	+++++

The type of surface being treated can affect the efficacy of some disinfectants (AHDB Horticulture PE 001a) and so materials were brought into the laboratory for testing. The selected surfaces were a glass sheet, a polypropylene grow-bag tray, an aluminium sheet, a concrete slab and woven ground cover material. These were cleaned down with running water and marked into sections for inoculation. The mycelium and spores were scraped off three-week old agar plates of *Fusarium* sp. and of *Pythium* sp. used to produce an even suspension of unfiltered inoculum. Droplets of the inoculum of one or other pathogen were dispensed over each surface and spread out over each 100 mm x 100 mm area and left to dry. Each of the nine disinfectants and a SDW control was then sprayed to the point of run-off onto the square areas of each pathogen and left for 30 minutes. Five cotton wool bud swabs were then used in turn to wipe across each surface per disinfectant and

each swab streaked over a different agar plate. The plates were incubated and examined after seven days for the presence or absence of mycelial growth.

The failure of Citrox P, FAM 30, Hydrocare, Virkon S and Menno Florades to control *Fusarium* was again shown in the surface tests (Table 3). Disolite and Unifect G still gave very good control, although sometimes incomplete. Domestos Extended Germ Kill performed much poorer than in the immersion tests, only working on glass, but this problem was not seen with *Pythium* (Table 4). Relative product performance at full rate for 30 minutes across the surface types was similar to that seen for the same rate and duration in the *Pythium* mycelium dip tests, with Virkon S giving poor control. However, the previously complete control by Disolite was incomplete on all of the surfaces. No adverse effects on the surfaces were seen from any of the products.

In conclusion, Disolite (a phenolic currently used by the mushroom industry) and Unifect G (a quarternary ammonium compound + aldehyde) performed consistently well against both *Fusarium* and *Pythium*, giving complete control in immersion tests even with short contact time or using a half rate. Domestos Extended Germ Kill (sodium hypochlorite) gave complete control of both with a 30 minute contact period. *Fusarium* resting spores survived treatments that controlled *Pythium*. Where *Pythium* is the main concern on a nursery then Jet 5 (hydrogen peroxide + peroxyacetic acid) or Citrox P (plant extract) at full rate should be effective. Where other bacterial, fungal or virus pathogens are to be controlled then information should be sought on the likely spectrum of activity of the disinfectant types and consideration given to, for example, odour, corrosiveness and biodegradability.

Table 3. Effect of treatments on five surfaces contaminated with *Fusarium* after 7 days incubation, showing % control 30 minutes after the application of disinfectant sprays at full rate

Tables 3&4. The proportion of five replicates without pathogen growth after full rate for 30 minutes					
No survival	**80% control**	**60% control**	**40% control**	**20% control**	**Zero control (alive)**
+++++	++++	+++	++	+	-

Product name	**Surfaces contaminated with *Fusarium* prior to disinfectant spray**				
	Glass	**Plastic**	**Aluminium**	**Concrete**	**Woven ground-cover**
1. Water (untreated)	-	-	-	-	-
2. Jet 5	-	++++	-	-	-
3. Citrox P	-	-	-	-	-
4. Disolite	+++++	+++++	+++++	++++	++
5. FAM 30	-	-	-	-	-
6. Hydrocare	-	-	-	-	-
7. Virkon S	-	-	-	-	-
8. Unifect G	+++++	+++++	++++	+++++	+++++
9. Menno Florades	-	-	-	-	-
10. Domestos Extended Germ Kill	+++++	-	-	-	-

Table 4. Effect of treatments on five surfaces contaminated with *Pythium* after 7 days incubation, showing the % control 30 minutes after the application of disinfectant sprays at full rate

Product name	Surfaces contaminated with *Pythium* prior to disinfectant spray				
	Glass	Plastic	Aluminium	Concrete	Woven ground-cover
1. Water (untreated)	-	-	-	-	+++
2. Jet 5	+++++	+++++	+++++	++++	+++++
3. Citrox P	+	+++++	-	+++	+++++
4. Disolite	+++	+++	++++	++++	+++
5. FAM 30	+++++	+++++	+++++	++++	+++++
6. Hydrocare	++++	++++	+++++	+++++	++++
7. Virkon S	-	-	+	++++	++++
8. Unifect G	+++++	+++++	+++++	+++++	+++++
9. Menno Florades	+	+++++	++++	+++++	++++
10. Domestos Extended Germ Kill	+++++	+++++	+++++	+++++	+++++

Action Points

- Ensure thorough disinfection of surfaces and containers between crops, particularly if there has been a root disease problem
- Before disinfecting a surface remove as much debris as possible to remove sources of resting spores that can resists treatment and to prevent any loss of product efficacy
- Refer to product labels and information in AHDB Horticulture Factsheet 03/14 together with the results from this research to select the most effective disinfectant product for the situation
- Do not use disinfectants on plants, soil or growing substrates
- Ensure that the recommended personal protective clothing is worn when handling the product and ensure safe disposal of any waste product

SCIENCE SECTION

Introduction

Effective nursery hygiene, which generally includes a good disinfection programme between crops or batches, is an essential part of sustainable crop management and can lead to a reduction in the use of plant protection products. A “start clean, stay clean” mindset helps to maintain plant quality. Growers need information about disinfectants to be confident that the materials, concentration and contact duration used are capable of doing the job intended on the surfaces presented so that time, money and crop health are not sacrificed.

Multicell trays used for seed sowing, cuttings and weaning micropropagated plants have the highest risk factor in their re-use. Seedlings and young plants succumb more easily to infection, usually causing whole-plant death rather than infection of a plant part. The higher humidity conditions needed for seed germination and rooting also favour the spread of fungal mycelium and the germination of spores (AHDB Horticulture Factsheet 23/02). Amongst the wide range of fungal pathogens infecting ornamentals it is the soil-borne root infecting pathogens that cause the greatest problem in the re-use of containers. Species include those from the genera *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Thielaviopsis*, *Fusarium* and *Verticillium*. Foliar or stem pathogens such as *Botrytis* and *Alternaria* able to survive on plant debris can also be carried over into a new crop. The resting spores of downy mildews may enter the growing medium from leaf litter. Bacterial plant pathogens are generally relatively easy to kill by disinfection. However, the rapid multiplication of bacterial pathogens under suitable conditions means that even a low inoculum of a pathogen can rapidly increase to cause disease, and disinfection is not necessarily as effective as against fungal pathogens.

Several different chemical types of disinfectants are currently available for use in horticulture including chlorine-based, iodophors, organic acids, phenols, quaternary ammonium compounds (QAC), peroxyacetic acid and hydrogen peroxide. Some have specific recommendations for use in horticulture, others do not. The chemical type of a disinfectant is a key factor in determining disinfectant efficacy in different use situations (Factsheet 03/14). The efficacy of disinfectants is affected by many factors including level of organic matter contamination, nature of the pathogen, survival form of the pathogen, duration of exposure to the disinfectant, type of surface, temperature and water pH.

There is uncertainty as to what disinfectant products will be available to UK growers in the next few years as current products become subject to new biocides legislation. Unlike pesticides, until recently there was no specific requirement for disinfectants to be registered (approved) for a

particular use; they were however subject to several pieces of environmental and health and safety legislation. The EU Biocidal Products Directive (BPD) which came into effect in May 2000 initiated a registration requirement but the registration process was slow and few of the disinfectants commonly used in commercial horticulture in the UK were assessed for inclusion. Formaldehyde and dichlorophen were assessed and were not included for common use situations in horticulture (product-type 2). The new EU Biocides Regulation 528/2012, implemented in Britain by the Biocidal Products and Chemicals Regulations (BPCR) 2013, applies from 1 September 2013 when the BPD is revoked. There is a four year transition period for actives not assessed under the BPD to be submitted for approval at EU level under the EU Biocides Regulation. Product approval will be at national level, undertaken by the HSE in Britain. Actives / products submitted for inclusion and not supported will become unavailable; actives / products not submitted for approval (e.g. due to cost of providing a data registration package) will also become unavailable. Actives already approved under BPD will remain approved under the new Biocides Regulation. Actives used for treating surfaces in commercial horticulture will most probably need to be approved under product-type 2 (disinfectants and algaecides not intended for direct application to humans or animals). The scope for using disinfectants with a label recommendation for use in other situations (e.g. veterinary hygiene) in commercial horticulture will reduce.

A number of AHDB Horticulture projects have been carried out testing the efficacy of various disinfectant products against the key pathogens surviving on contaminated surfaces, but not all products are still available. AHDB Horticulture project PC186a (summarised in Factsheet 15/01) carried out laboratory tests against *Verticillium albo-atrum*. AHDB Horticulture Factsheet 16/04 on the control of economically important root, crown and basal stem rots caused by *Pythium*, *Phytophthora* and *Rhizoctonia* in container-grown hardy ornamentals recommends either Jet 5 (peroxyacetic acid) or sodium hypochlorite for use in propagating areas against *Pythium* and *Phytophthora.* Factsheet 19/02 on the control of downy mildew (*Peronospora violae)*, black root rot (*Thielaviopsis basicola)* and *Ramularia* leaf spot, notes the known risk of infection from the black root rot fungus when re-using plug trays, but that this can be minimised by soaking them in Jet 5. Panacide M (dichlorophen), Ter Spezial (QAC) or sodium hypochlorite (10 to 14% available chlorine) may alternatively be used to reduce disease incidence. Factsheets 04/04 and 12/04 suggest treating standing areas with e.g. Jet 5 or Panacide M against downy mildew of hardy nursery stock and herbaceous perennials, and also other foliar diseases of roses. Bench disinfection with 2% Antec Farm Fluid S, 1.66% Panacide M, or 1% Jet 5 was recommended in Factsheet 05/04 against *Plasmopara obducens* downy mildew on Impatiens. Disinfection of standing areas, sandbed and matting (HNS 63 on *Fusarium oxysporum* f. sp. *dianthi*, HNS 123 on *Phytophthora ramorum* and PC 107 on *Phytophthora nicotianae)* has been examined, but only PC

38c has examined directly the control of a fungus (*Thielaviopsis basicola*) on plastic trays. The effectiveness of only FAM 30 against *Fusarium oxysporum* f. sp. *narcissi* chlamydospores was shown in *in vitro* efficacy tests (BOF 71). Project PC 181 looked at disinfection of a virus. Recent work (PC 291) has tested disinfectants against three major groups of plant pathogenic bacteria and this can be supplemented by information on products for veterinary and human hygiene which are principally bactericides.

The objectives of this work were:

1. To determine the efficacy of a range of chemical disinfectants, from different active ingredient groups, against spores of *Fusarium*
2. To determine the efficacy of a range of chemical disinfectants, from different active ingredient groups, against mycelium of *Fusarium* and *Pythium*
3. To determine the efficacy of selected disinfectants for reduction of *Fusarium* and *Pythium* on five surfaces (glass, aluminium, rigid plastic, concrete and woven ground cover material)
4. To determine the effect of peat contamination of selected disinfectants against mycelium of *Fusarium* and *Pythium*

Materials and methods

Site details

All tests were carried out in the pathology laboratory at ADAS Boxworth.

Treatment details

Table 1. Detail of products tested

Product	Active ingredient(s)	Manu-facturer	Batch number	% a.i	Form-ulation type
1. Sterile distilled water. Untreated	-	-	-	-	-
2. Jet 5	Hydrogen peroxide + peroxyacetic acid	Certis UK	None given	20% hydrogen peroxide, 5% peracetic acid, 10% acetic acid, 1% ethoxylate	liquid
3. Citrox P	Plant extract	Agralan Ltd	C1145	No information on product leaflet	liquid
4. Disolite	Phenolics	Progress Products/	None	Ortho phenyl phenol, orthobenzyl, chlorophenol, iso propyl alcohol,	liquid

		Aromany	given	anionic detergent	
5. FAM 30	Iodophor	Evans Vanodine	None given	9.3% w/w sulphuric acid, 9.5% w/w/phosphoric acid, minimum available iodine 2.75% w/w at time of manufacture	liquid
6. Menno Florades	Benzoic acid	Brinkman	Not given (decanted sample from Aromany)	9% benzoic acid	liquid
7. Hydrocare	Hydrogen peroxide + silver	Intracare BV	None given	No information provided	liquid
8. Unifect G	Quaternary ammonium compounds + aldehyde	Aromany	Not given (decanted sample from Aromany)	<15% glutaraldehyde, ammonium compounds	liquid
9. Virkon S	Peroxygen compounds + organic acids	Du Pont Animal Health Solutions	12080095 Expiry 08 2015	40-50% pentapotassium bis (peroxymonosulphate bis (sulphate), 10-12% sodium C10-13-alkylbenzenesulfonate, 4-6% sulphamic acid, 7-10% malic acid, 1-5% polyphosphoric acids sodium salts, 1-5% sodium chloride, <1% dipotassium peroxodisulphate	powder
10. Domestos Prof-essional citrus fresh Extended Germ Kill with CTAC	Chlorine-based bleaching agents (sodium hypochlorite)	Unilever	None given	4.83 g sodium hypochlorite per 100g (CTAC acts like a glue)	liquid

Products were stored in an air-conditioned room at 20 °C following delivery in December 2013.

Table 2. Treatments used at both full and half rate, with 5 minutes and 30 minutes contact time

Product name	Dilution rate on label	Half rate dilution
1. Untreated	-	-
2. Jet 5 (standard control)	1:125	1:250
3. Citrox P	1:150	1:300
4. Disolite	2%	1%
5. FAM 30	1:125	1:250
6. Menno Florades	1%	0.5%
7. Hydrocare	3%	1.5%
8. Unifect G	4%	2%
9. Virkon S	1:100	1:200
10. Domestos Extended Germ Kill	120 ml into 5 L	60 ml into 5 L

Table 3. Application details for each of the four ADAS Boxworth BX coded experiments

Application	**BX 14-092 Spores**	**BX 14-093 Mycelium**	**BX 14-095 Mycelium + organic matter**	**BX 14-094 Surfaces**
Application date against *Fusarium*	18.11.2014	19.11.2014	01.12.2014	02.12.2014
Air temperature during *Fusarium* test	16.8-17.5°C	17.5–18.4°C	17.3-17.8°C	17.1–17.9°C
Application date against *Pythium*	No test	19.11.2014	01.12.2014	03.12.2014
Air temperature during *Pythium* test	n.a.	16.9-17.7°C	16.7-18.3°C	17.1-18.2°C
Application method	dip	dip	dip	spray

Target pest(s)

Table 4. Target pest(s)

Common name	Scientific Name	Host	Isolate identification
Fusarium wilt	*Fusarium oxysporum* f. sp. *mathiolae*	Column stocks (*Matthiola incana*)	ADAS AR06/56
Pythium root rot	*Pythium irregulare*	Yew (*Taxus baccata*)	RHS P71098.7.2008

Assessments

Table 5. Assessment intervals after disinfectant treatment for each experiment following incubation on agar plates

Assessment No.	BX 14-092 Spores	BX 14-093 Mycelium	BX 14-095 + organic matter	BX 14-094 Surfaces
1.	Day 1	Day 1	Day 1	n.a.
2.	Day 3	Day 3	Day 3	n.a.
3.	Day 5	Day 5	Day 5	n.a.
4.	Day 7	Day 7	Day 7	Day 7

Preparation of pathogen for spore testing and culture procedure after testing (BX 14-092)

Three-week old plates of *Fusarium* sp. were grown on potato dextrose agar incorporating the antibiotic Streptomycin to control bacterial growth (PDA+S). Pythium does not produce air-borne spores and so this pathogen was not used in this test.

Fusarium sp. plates were flooded with sterile distilled water (SDW) and mycelium plus micro conidia (spores) scraped off the surface of the agar and filtered to remove mycelia and collect the spores.

For each disinfectant product, 10 ml volumes of the spore suspension were pipetted into sterile tubes and calculated volumes per disinfectant added to obtain the correct product dilutions of full and half rate. The spores were left in contact with the disinfectant for either 5 or 30 minutes (and with SDW for 30 minutes as the untreated control). To be able to rinse off the disinfectant the spore suspensions were centrifuged to produce a pellet of spores and the disinfectant then pipetted off. The standard procedure used required a spinning-down of the spores for two minutes and then a further minute to bring the centrifuge to a gentle stop so that the spores were actually in the disinfectants for 7 or 32 minutes. The spore pellets were then immediately agitated with 5 ml of

SDW per tube to rinse and re-suspend. A 5 µl droplet was placed into the centre of each of the nominated five wells on each of the two labelled 25-well plates of PDA+S, as shown in Figure 1. Plates were then incubated at 20 °C for 7 days under a 16:8 h light:dark cycle. The cells were examined one, three, five and seven days after treatment in to record pathogen survival and any checking of growth by the products.

Preparation of pathogens for mycelium testing and culture procedure after testing (BX 14-093)

Mycelium of *Fusarium* sp. and *Pythium* sp. was grown over 7 mm diameter filter paper discs placed on PDA+S plates and allowed to mature for three weeks to produce resting spores. The discs were peeled off the agar plates and placed into beakers of each disinfectant at full and half rate and left for either 5 or 30 minutes before rinsing in SDW. The discs were allowed to drip dry before placing into the centre of each of the nominated five wells on each of the two labelled 25-well plates of PDA+S, as shown in Figure 1. Plates were then incubated at 20 °C for 7 days under a 16:8 h light:dark cycle. The cells were examined one, three, five and seven days after treatment in to record pathogen survival and any checking of growth by the products.

Preparation of pathogens for mycelium testing with organic matter present and culture procedure after testing (BX 14-095)

The procedures for experiment BX 14-093 were repeated, but 100 mg of finely crumbled growing media (Vitax John Innes No 1 loam-based compost with added peat) direct from the bag without sterilising was added to 100 ml of each disinfectant at full and half rates prior to treatment of the filter paper pieces with mycelial growth. SDW contaminated with peat in the same proportion was used as the untreated control treatment (i.e. peat added at 0.1% w/v). The organic matter was swilled around in the beaker during the treatment periods (5 and 30 minutes). After rinsing, the paper discs were cultured on PDA+S in 50 mm diameter petri dishes.

Figure 1. Experiments BX 14-092 and BX 14-093. Layout of two 25-well Petri plates showing incubation positions of one pathogen after treatment with one disinfectant at recommended or half recommended rate dilutions for 5 or 30 min for replicate rows 1 to 10.

X = droplet of spore suspension or inoculated filter paper disc after treatment placed on agar in a 20 mm x 20 mm x 20 mm multiwell cell

	Disinfectant Treatment				
	0 SDW	Full 5 min	Full 30 min	½ rate 5 min	½ rate 30 min
1	X	X	X	X	X
2	X	X	X	X	X
3	X	X	X	X	X
4	X	X	X	X	X
5	X	X	X	X	X

	0 SDW	Full 5 min	Full 30 min	½ rate 5 min	½ rate 30 min
6	X	X	X	X	X
7	X	X	X	X	X
8	X	X	X	X	X
9	X	X	X	X	X
10	X	X	X	X	X

<u>Preparation of pathogens for testing on surfaces and culture procedure after testing (BX 14-094)</u>

Three-week old plates of *Fusarium* sp. and *Pythium* sp. were grown on potato dextrose agar incorporating the antibiotic Streptomycin to control bacterial growth (PDA+S). The *Fusarium* sp. plates had developed chlamydospores and *Pythium* sp. had oospores, the resting spores that can

remain on glasshouse surfaces. The inoculum was prepared by scraping off the aerial growth of each fungus using SDW into sterile beakers (without filtering). Further SDW was added and the inoculum briefly blended to break up agglomerations of mycelium.

Plastic (Stewart grow-bag tray of recycled polypropylene), glass, concrete (paving slab), woven ground-cover and aluminium surfaces were taken into the laboratory, washed with water under a running tap, dried with paper towel and left to dry for five minutes. They were then marked out with adhesive tape into 100 mm x 100 mm squares (leaving a discard area between each). Inoculum suspension (1 ml) was dispensed in drips over each area using a syringe. The inoculum was spread out across each area using a sterile loop and the surface allowed to dry. A hand-sprayer was then used to treat each surface with the full rate dilution of each product so that the area became evenly wetted to the point of run off. A four-sided cardboard shield was used to prevent disinfectant spread between the areas. The disinfectant was allowed to dry for 30 minutes. SDW was also used as a control treatment. A cotton bud moistened in SDW was then used to swab each area in a zig-zag pattern. A further four fresh cotton buds were used to swab each area giving 5 replicates. Each swab was streaked for a length of approximately 100 mm over a 90 mm diameter agar plate (PDA+S) to give five swab plates per disinfectant per pathogen. The number of swabs that resulted in growth of the pathogen after incubation of agar plates for 7 days at 20 ºC was recorded. Records were made of any visible detrimental effect of any disinfectants on the nature of the surface treated.

Results

Disinfectant dip tests (experiments BX 14-092, BX 14-093 and BX 14-095)

Table 6. Effect of treatments on *Fusarium* sp. spores, assessed after 7 days incubation. Count of multiwell cells out of ten which had colony growth

Product name	SDW (untreated)	1/2 rate 5 min	1/2 rate 30 min	Full rate 5 min	Full rate 30 min
1. Jet 5	10	0	0	0	0
2. Citrox P	10	0	0	0	0
3. Disolite	10	0	0	0	0
4. FAM 30	10	0	0	0	0
5. Hydrocare	10	10	1	0	0
6. Virkon S	10	2	0	2	1
7. Unifect G	10	0	0	0	0

8. Menno Florades	10	10	10	8	4
9. Domestos Extended Germ Kill	10	0	0	0	0

Table 7. Effect of treatments on *Fusarium* sp. mycelium, assessed after 7 days incubation. Count of multiwell cells out of ten which had colony growth

Product name	SDW (untreated)	1/2 rate 5 min	1/2 rate 30 min	Full rate 5 min	Full rate 30 min
1. Jet 5	10	10	9	10	4
2. Citrox P	10	10	10	10	10
3. Disolite	10	0	0	0	0
4. FAM 30	10	10	10	10	10
5. Hydrocare	10	10	10	10	10
6. Virkon S	10	10	10	10	10
7. Unifect G	10	0	0	0	0
8. Menno Florades	10	10	10	10	10
9. Domestos Extended Germ Kill	10	0	0	0	0

Table 8. Effect of treatments on *Pythium* sp. mycelium, assessed after 7 days incubation. Count of multiwell cells out of ten which had colony growth

Product name	SDW (untreated)	1/2 rate 5 min	1/2 rate 30 min	Full rate 5 min	Full rate 30 min
1. Jet 5	10	0	2	0	0
2. Citrox P	10	2	4	0	2
3. Disolite	10	0	0	0	0
4. FAM 30	10	5	2	2	0
5. Hydrocare	10	10	10	10	2
6. Virkon S	10	10	10	10	9
7. Unifect G	10	0	0	0	0

8. Menno Florades	10	9	8	10	5
9. Domestos Extended Germ Kill	10	9	0	10	0

Table 9. Effect of treatments with on *Fusarium* sp. mycelium when organic matter was present, assessed after 7 days incubation. Count of multiwell cells out of ten which had colony growth

Product name	SDW (untreated)	Full rate 5 min	Full rate 30 min
1. Jet 5	10	10	10
2. Citrox P	10	10	9
3. Disolite	10	0	0
4. FAM 30	10	10	10
5. Hydrocare	10	10	8
6. Virkon S	10	10	10
7. Unifect G	10	0	0
8. Menno Florades	10	10	10
9. Domestos Extended Germ Kill	10	1	0

Table 10. Effect of treatments with on *Pythium* sp. mycelium when organic matter was present, assessed after 7 days incubation. Count of multiwell cells out of ten which had colony growth

Product name	SDW (untreated)	Full rate 5 min	Full rate 30 min
1. Jet 5	10	0	0
2. Citrox P	10	6	2
3. Disolite	10	3	0
4. FAM 30	10	0	0
5. Hydrocare	10	10	10
6. Virkon S	10	10	10

7. Unifect G	10	0	0
8. Menno Florades	10	8	8
9. Domestos Extended Germ Kill	10	2	0

Control of *Fusarium* sp. spores (BX14-092) and *Fusarium* sp. and *Pythium* sp. mycelium (BX14-093) following immersion of mycelium in disinfectant without organic matter

The three-week old mycelium of both pathogens was confirmed by microscope examination to have developed thick-walled resting spores at the time of testing. The oospores of *Pythium* sp. and chlamydospores of *Fusarium* sp. were attached between hyphae embedded within the mycelium. The *Fusarium* filter paper discs also had micro conidia on them, but as these are held loosely on aerial spore-bearing structures these would have been dislodged during immersion in the disinfectants, leaving fewer on the discs when they were placed on the agar.

Both *Fusarium* sp. and *Pythium* sp. grew in all ten replicates of the SDW untreated control used for each of the disinfectant product tests. The results of the final assessments for the ten replicates at each disinfectant rate and timing show some treatments to give complete control (Tables 6, 7 and 8). Cells without growth in the multicell plates were clearly visible after 7 day's incubation (Appendix B). Earlier assessments of growth on the agar plates at one, three and five days as well as seven days are given in the Appendix C. Growth of both pathogens commenced without delay after mycelium or spores were dipped in SDW, but some disinfectants initially appeared to have worked, but growth had only initially been held back. For example, *Fusarium* sp. spore growth was delayed following full rate treatment for Extended Germ Kill 30 minutes by Virkon S and Menno Florades and half rate Hydrocare. When full rate products were used for 5 minutes against *Pythium* mycelium all products except Hydrocare had not grown by Day 1, but FAM 30, Virkon S, Menno Florades and Domestos Extended Germ Kill all had Pythium growth by Day 7. Delayed growth of *Fusarium* and *Pythium* mycelium may have been as resting spores germinated.

Most products (Jet 5, Citrox P, Disolite, FAM 30, Unifect G and Domestos Extended Germ Kill) killed *Fusarium* spores (principally microconidia) even at half rate and following only 5 minutes (+ 3 minutes pre-rinse time) immersion (Table 6). Menno Florades failed to give consistent control even at full rate and following 30 minutes immersion. Virkon S gave some level of control. Hydrocare gave control at full rate only.

Compared with spore treatment, the control of *Fusarium* mycelium with chlamydospores (resting spores) was poorer (Table 7), with only Disolite, Unifect G and Domestos Extended Germ Kill

giving control of both. Jet 5, Citrox P and FAM 30 did not control mycelium although they controlled *Fusarium* spores.

Two of the products, Disolite and Unifect G were effective at all rates and times against *Pythium* resting spores and mycelium (Table 8) and had also controlled *Fusarium* resting spores and mycelium. Jet 5 was effective against *Pythium* at full rate only at both times. Domestos Extended Germ Kill gave control if immersion was continued for 30 minutes at either rate. Citrox P and FAM 30 gave *Pythium* control at full rate, but there was some survival at different immersion times.

Control of *Fusarium* and *Pythium* mycelium following immersion in disinfectant with organic matter (BX14-095)

The addition of organic matter to full rate disinfectants did not change the level of control given by any of the products against *Fusarium* (Table 9). Disolite, Unifect G and Domestos Extended Germ Kill were the only products to give control.

When tested against *Pythium* (Table 10), Jet 5, FAM 30 and Unifect G still gave total control with organic matter present. Citrox P and Disolite became less effective at 5 minutes immersion. Hydrocare had given some control when 30 minutes immersion was given without organic matter, but no control was achieved with it present. Menno Florades also became less effective. Domestos Extended Germ Kill gave complete control in 30 minutes, regardless of the presence of organic matter.

Disinfectant surface treatment tests (experiments BX 14-094)

Control of *Fusarium* and *Pythium* by disinfectant on inoculated surfaces (BX14-094)

Table 11. Effect of treatments on five surfaces contaminated with *Fusarium* mycelium, micro conidia and resting spores after 7 days incubation, showing the proportion of five swabs infested

Product name	Glass	Plastic	Aluminium	Concrete	Woven ground-cover
1. Water (untreated)	5	5	5	5	5
2. Jet 5	5	1	5	5	5
3. Citrox P	5	5	5	5	5
4. Disolite	0	0	0	1	3
5. FAM 30	5	5	5	5	5
6. Hydrocare	5	5	5	5	5

7. Virkon S	5	5	5	5	5
8. Unifect G	0	0	1	0	0
9. Menno Florades	5	5	5	5	5
10. Domestos Extended Germ Kill	0	5	5	5	5

Table 12. Effect of treatments on five surfaces contaminated with *Pythium* mycelium and resting spores after 7 days incubation, showing the proportion of five swabs infested

Product name	Glass	Plastic	Aluminium	Concrete	Woven ground-cover
1. Water (untreated)	5	5	5	5	2
2. Jet 5	0	0	0	1	0
3. Citrox P	4	0	5	2	0
4. Disolite	2	2	1	1	2
5. FAM 30	0	0	0	1	0
6. Hydrocare	1	1	0	0	1
7. Virkon S	5	5	4	1	1
8. Unifect G	0	0	0	0	0
9. Menno Florades	4	0	1	0	1
10. Domestos Extended Germ Kill	0	0	0	0	0

Although the surfaces were left for 30 minutes in the laboratory before swabbing to pick up any surviving pathogen material, the plastic, glass and aluminium were still moist. The disinfectants had disappeared into the cement slab and the woven ground cover material.

Control of *Fusarium* mycelium and spores on surfaces (Table 11) was only complete on glass for Disolite, Unifect G and Domestos Extended Germ Kill at full rate when tested after 30 minutes, there was full survival for all other products. Only Disolite and Unifect G gave complete control on plastic (although Jet 5 gave 80% control). Disolite was the only product to give complete control on aluminium (although Unifect G gave 80% control). On concrete and woven ground cover Unifect G was the only product to give complete control (although Disolite gave reasonable control). Overall

therefore Disolite and Unifect G gave the best control across the range of surfaces tested. Citrox P, FAM30, Hydrocare, Virkon S and Menno Florades were not effective on any of the five surfaces.

Control of *Pythium* by disinfectants on surfaces was much better than it was against *Fusarium*, with only three products achieving less than 20% control (Citrox P, Virkon S and Menno Florades). Unifect G gave good control on *Pythium* across all surfaces (as on Fusarium), but Disolite control was not as good on glass, plastic and aluminium as it had been for *Fusarium*. Domestos Extended Germ Kill gave complete control of *Pythium* on all surfaces (only having been effective on glass against Fusarium).

Neither pathogen was more or less likely to be controlled on a particular surface.

No problems were encountered during mixing or application of any of the product formulations under test. There were no visible adverse changes to the nature of the surfaces tested.

Discussion

Information on the half-rate efficacy of the products could be relevant where active ingredients become lost to the atmosphere in use or become diluted by use on washed/wet surfaces. Several products gave good control with contact time of 5 minutes (mycelium) or 7 minutes (spores). On a nursery this timing would assume immediate direct contact i.e. that the disinfectant would not first need to penetrate plant debris or the remains of growing-media. Although equipment immersion or surface spraying may not normally be continued for as long as this by growers, growers would not usually rinse off disinfectants and so the pathogens would remain wetted by them until the disinfectants dried. Treated pots and trays should be wrapped to prevent re-infestation from air-borne inoculum and if this can be done by growers directly after treatment this should assist disinfection.

Jet 5 at full rate was confirmed as giving control of *Pythium* (matching the findings of PC 97), and variable control of *Fusarium* was again shown (previously tested in PC 213 and HNS 63) with spores, but not mycelium killed in the current tests. Disinfectant products were previously (PC 213) shown to be less effective against *Fusarium* mycelium than against spores. FAM 30 was confirmed as controlling *Pythium* at full rate if allowed contact for 30 minutes (previously tested in HNS 147). Unifect G was also shown to control *Pythium* and its efficacy against *Fusarium* (tested in PC 213) was confirmed. Citrox P did not control *Pythium* when tested in HNS 147, but 80% control was found in the current work and it killed *Fusarium* spores (although ineffective on mycelium). Previous work (PC 97) showed control of *Pythium* by the laboratory product sodium hypochlorite and of Fusarium (PC 213), and in the current work its closest equivalent, Domestos Extended Germ Kill, also proved effective against these two pathogens although it was ineffective against *Pythium* if only allowed 5 minutes contact time. The Domestos Extended Germ Kill product used is a relatively

new formulation that “sticks” to surfaces and this may improve its performance. Domestos Extended Germ Kill contains a declared chlorine content, while that of other bleaches is often not declared or is lower.

Disolite had not been tested in earlier projects and it was shown to be very effective against both *Pythium* and *Fusarium*. It is marketed for use in the mushroom industry, with the product label claiming proven activity against bacteria, fungi and virus.

Conclusions

- Disolite and Unifect G gave complete control of *Fusarium* and *Pythium* even when used at half rate with only 5 minutes contact time, and were unaffected by the presence of organic matter
- Domestos Extended Germ Kill gave complete control of *Fusarium* even when used at half rate with only 5 minutes contact time, but control of *Pythium* was only achieved after 30 minutes contact time at either dilution. Its efficacy was unaffected by organic matter
- Jet 5 at full rate controlled *Pythium* mycelium, even in the presence of organic matter, but gave poor control of *Fusarium* mycelium
- The other five disinfectants tested gave no control of *Fusarium* mycelium and four gave incomplete control of *Pythium* mycelium
- Products that did not give control after immersion for 5 minutes at full rate were no more effective (or only marginally more effective) after immersion at full rate for 30 minutes
- Organic matter only reduced the effectiveness of Jet 5 (to *Fusarium*).
- *Fusarium* control by disinfectant sprays failed for most products on all surfaces, with only Disolite and Unifect G giving control across glass, plastic, aluminium, concrete and woven ground-cover. Domestos Extended Germ Kill -controlled *Fusarium* on glass.
- *Pythium* control on surfaces was more effective than that of *Fusarium*, with Jet 5, FAM 30 and Domestos Extended Germ Kill showing control as well as Unifect G. Several disinfectants gave incomplete, but 50% or better, control on various surfaces.

References

Ann, D., O'Neill, T. & Atwood, J. (2004). Control of the main foliar diseases of container-grown roses. Horticultural Development Council Factsheet 12/04 Rose foliar diseases. Project No. HNS 106 & 106a.

Jackson, A., McPherson G., M. & Brough, W. (2002). Control of downy mildew, black root rot and Ramularia leaf spot diseases on pansy and viola. Horticultural Development Council Factsheet 19/02 Bedding plants. Project No. PC 143, PC 38a & PC38c.

Jones, D. & O'Neill, T. (2004). Impatiens downy mildew. Horticultural Development Council Factsheet 05/04 Impatiens.

Lole, M. (2006). Ornamentals: control of pests, pathogens and weed seeds on re-used plant containers. Horticultural Development Company Final report HNS 147.

McPherson, G.M., O'Neill, T., Kennedy, R., Wakeham, A., Wright, K., Goodson, M. and Lambourne, C. (2012). Cucumber – Improving Control of Gummy Stem Blight caused by *Mycosphaerella melonis* (*Didymella bryoniae*). Horticultural Development Company Annual report for project PE 001a.

O'Neill, T.M. (1995). Comparison of chemical disinfectants for treatment of capillary matting stood on sand contaminated with *Fusarium oxysporum* f. sp. d*ianthi.* Horticultural Development Company Final report HNS 63.

O'Neill, T. (2003). Protected tomato: sources, survival, and disinfection of Pepino Mosaic Virus (PepMV). Horticultural Development Company Final report for project PC 181.

O'Neill, T. (2004). Control of downy mildew disease on hardy nursery stock and herbaceous perennials. Horticultural Development Council Factsheet 04/04 HNS and Herbaceous Perennials. Project No. HNS 79 & HNS 53.

O'Neill, T. (2007). Protected stock: aspects of the biology and control of Fusarium wilt, a new disease problem. Horticultural Development Council Final report PC 213 and PC213a.

O'Neill, T. (2011). Protected ornamentals: evaluation of control options for bacterial diseases of pot plants. Horticultural Development Company Final report PC 291.

O'Neill, T. and Irving, R. (2014). Use of chemical disinfectants in protected ornamental plant production. Horticultural Development Company Factsheet 03/14 (a revision of 15/05).

O'Neill, T. & Ann, D. (2004). Control of Phytophthora, Pythium and Rhizoctonia in container-grown hardy ornamentals. Horticultural Development Council Factsheet 16/04 Hardy Nursery Stock.

Wedgwood, E. (2010). Narcissus: The use of FAM 30 disinfectants as a cold dip treatment for Fusarium basal cell rot. Horticultural Development Company Final report for project BOF 71.

White, J.G. (1998). Identification of sources of inoculum and the development of rapid diagnostic tests for *Pythium* infestation of ornamentals on nurseries. Horticultural Development Company Final report PC 97.

Appendix A – Study conduct

ADAS is officially recognised by United Kingdom Chemical Regulations Directorate as competent to carry out efficacy testing. The experiments reported were carried out according the internal ADAS operating procedures.

GLP compliance will not be claimed in respect of this study.

Relevant EPPO/CEB guideline(s)		**Variation from EPPO**
PP 1/152(3)	Design and analysis of efficacy evaluation trials	PP 1/152(3)

Appendix B – Photographs showing examples of treatments

Figure 1. BX 14-092. Good control of *Fusarium* spores recorded 3 days after treatment with Virkon (2nd to 5th cells left to right) full rate for 5 min, full for 30 min, half rate for 5 min and half rate for 30 min (one treatment per dish column). The left hand of the five columns shows rapid growth of the dark *Fusarium* mycelium in the untreated. Five replicates per multicell dish, one per row. Plates shown after 7 day's incubation.

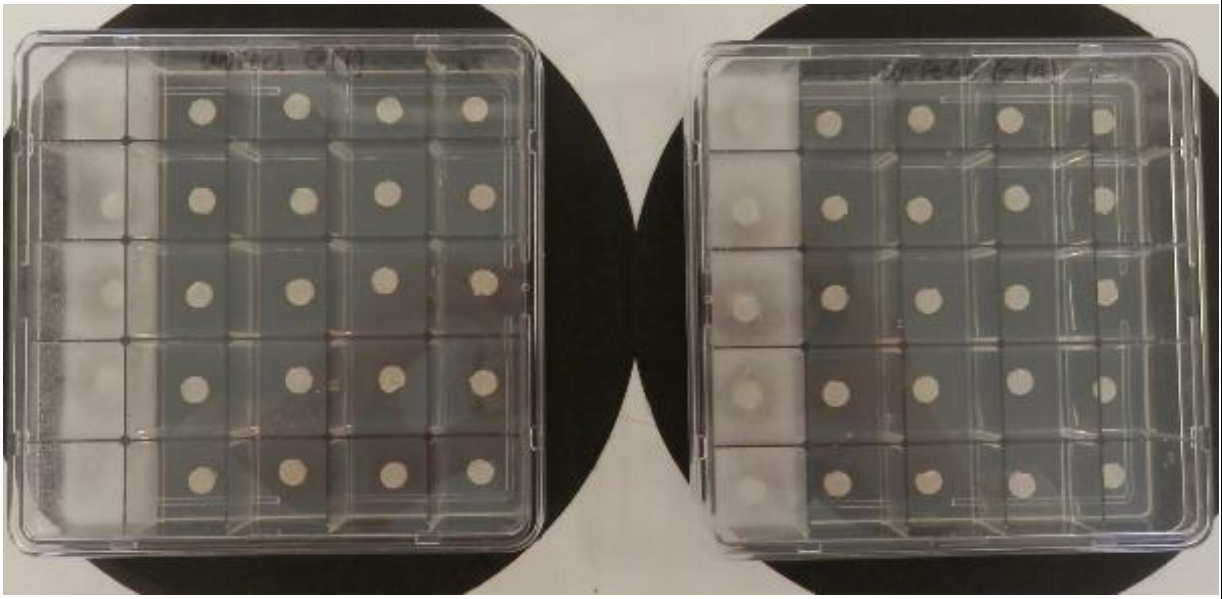

Figure 2. BX 14-093. Good control of *Pythium* mycelium recorded 3 days after treatment with half or full rate Unifect G for 5 and 30 minutes (one treatment per dish column). The left hand of the five columns shows rapid growth of the white *Pythium* mycelium in the

untreated. Five replicates per multicell dish, one per row.

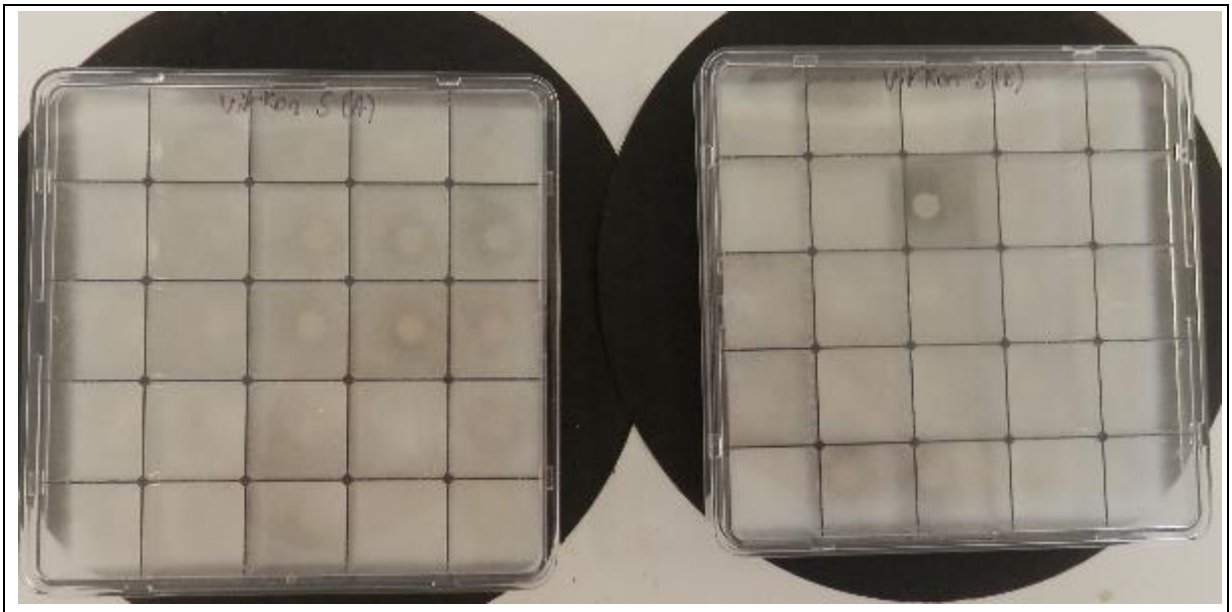

Figure 3. BX 14-093. Poor control of *Pythium* mycelium recorded 3 days after treatment with half or full rate Virkon S for 5 and 30 minutes

Figure 4. BX 14-093. Poor control of *Pythium* mycelium recorded 3 days after treatment with half or full rate Hydrocare for 5 minutes and half rate for 30 minutes. Central column in each dish of five replicates shows control of *Pythium* on eight of the inoculum discs treated at full rate for 30 minutes.

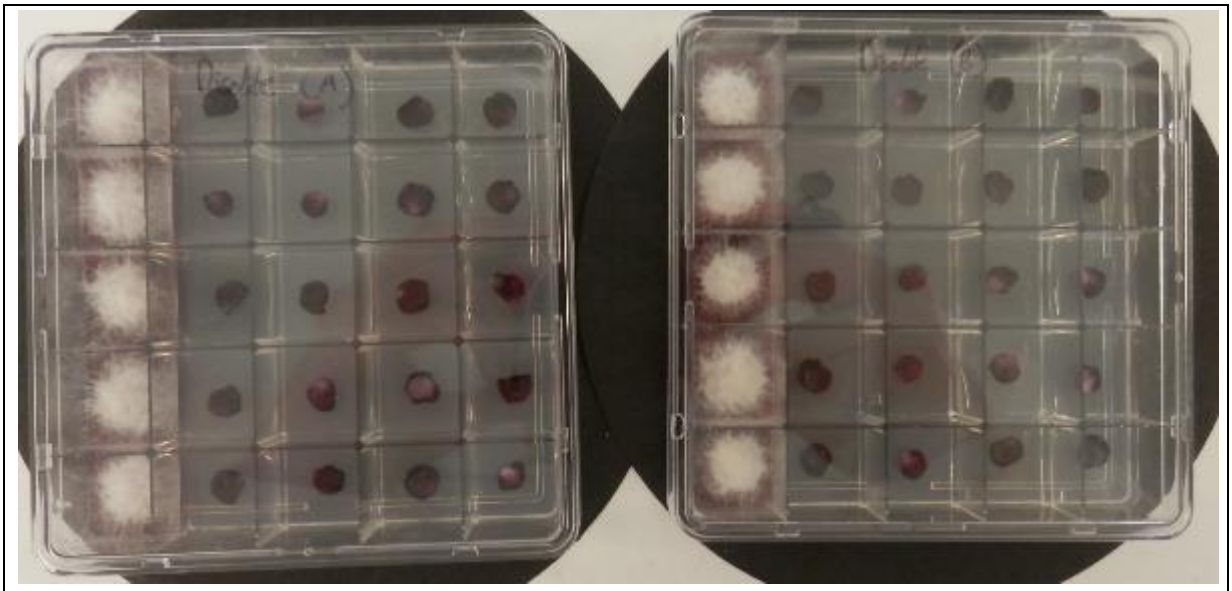

Figure 5. BX 14-093. Good control of *Fusarium* mycelium recorded 3 days after treatment with half or full rate Disolite for 5 and 30 minutes. Untreated in left hand columns.

Figure 6. . BX 14-093. Poor control of *Fusarium* mycelium recorded 3 days after treatment with half or full rate FAM 30 for 5 and 30 minutes. There was only a slight a delay in growth following treatment compared with that of the untreated in the left hand column

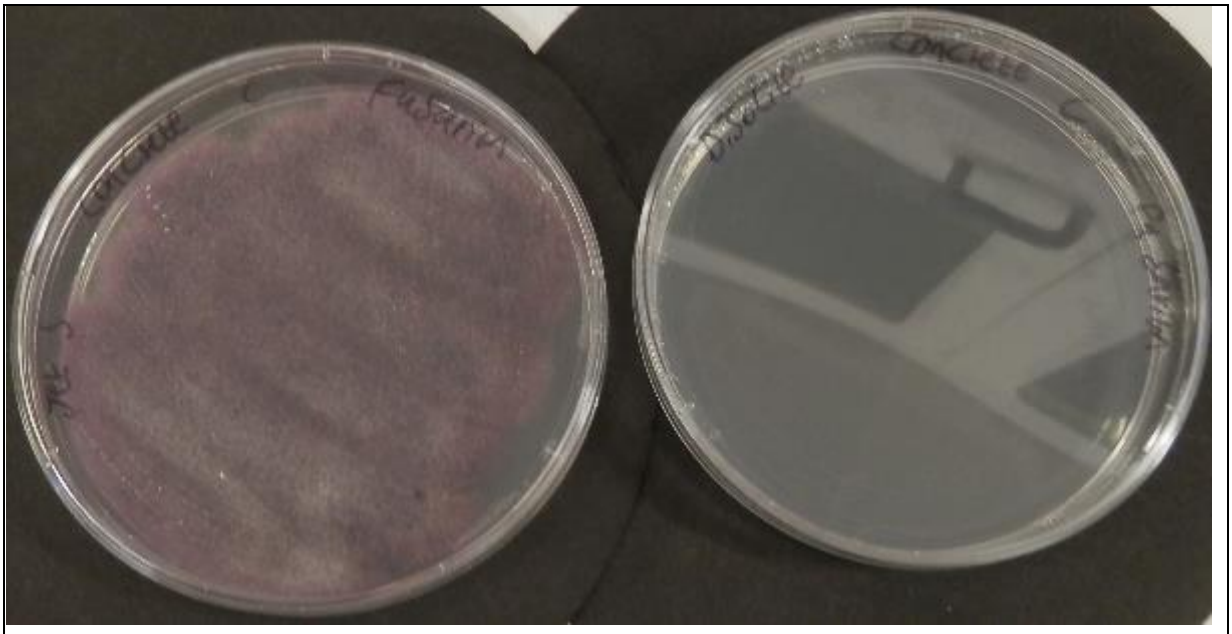

Figure 8. BX 14-094. Agar plates made with a swab taken from a concrete surface after treating with two different disinfectants. The left hand plate shows growth of *Fusarium* (seen in five out of the five Jet 5 swabs) and the right shows no growth after Disolite (although one out of five swabs produced growth).

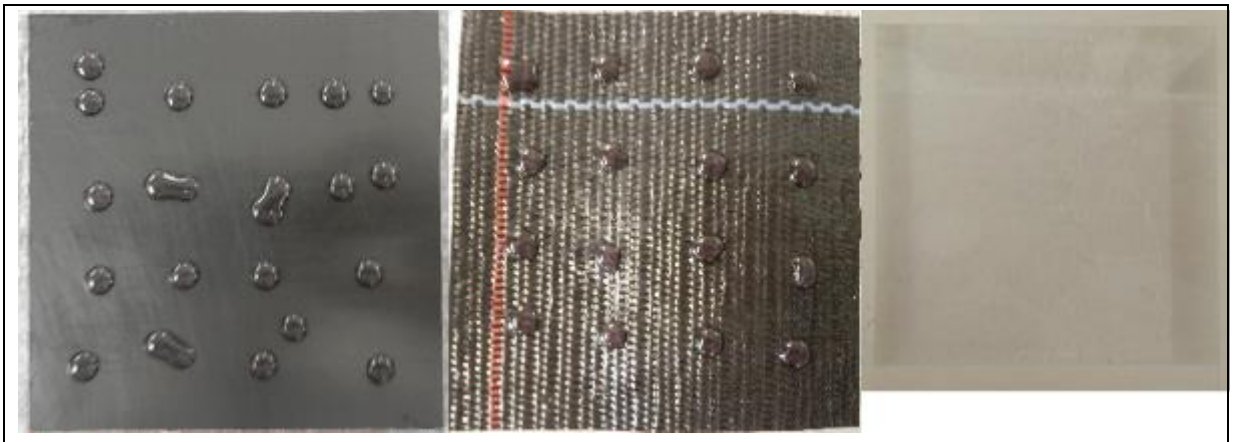

Figure 9. BX 14-094. Inoculum drops on plastic and woven ground cover before spreading out and treating with disinfectant. Other surfaces used were glass, concrete and aluminium.

Appendix C – Interim assessments of colony growth

Results for multicell agar incubation trays with 10 replicate cells per product rate and duration tested against *Fusarium* spores and mycelium, and *Pythium* mycelium.

BX 14-092 spores	***Fusarium* colonies after sterile distilled water dip**			
Product name	**Day 2**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	10	10	10	10
2. Citrox P	10	10	10	10
3. Disolite	10	10	10	10
4. FAM 30	10	10	10	10
5. Hydrocare	10	10	10	10
6. Virkon S	10	10	10	10
7. Unifect G	10	10	10	10
8. Menno Florades	10	10	10	10
9. Domestos Extended Germ Kill	10	10	10	10

BX 14-092 spores	***Fusarium* colonies after full rate product 5 min**			
Product name	**Day 2**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	0	0
2. Citrox P	0	0	0	0
3. Disolite	0	0	0	0
4. FAM 30	0	0	0	0
5. Hydrocare	0	0	0	0
6. Virkon S	0	1	2	2
7. Unifect G	0	0	0	0
8. Menno Florades	0	4	6	8

9. Domestos Extended Germ Kill	0	0	0	0

BX 14-092 spores	***Fusarium* colonies after full rate product 30 min**			
Product name	**Day 2**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	0	0
2. Citrox P	0	0	0	0
3. Disolite	0	0	0	0
4. FAM 30	0	0	0	0
5. Hydrocare	0	0	0	0
6. Virkon S	0	1	1	1
7. Unifect G	0	0	0	0
8. Menno Florades	0	4	4	4
9. Domestos Extended Germ Kill	0	0	0	0

BX 14-092 spores	***Fusarium* colonies after half rate product 5 min**			
Product name	**Day 2**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	0	0
2. Citrox P	0	0	0	0
3. Disolite	0	0	0	0
4. FAM 30	0	0	0	0
5. Hydrocare	0	0	10	10
6. Virkon S	0	2	2	2
7. Unifect G	0	0	0	0
8. Menno Florades	10	10	10	10
9. Domestos Extended Germ Kill	0	0	0	0

BX 14-092 spores	***Fusarium* colonies after half rate product 30 min**			
Product name	**Day 2**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	0	0
2. Citrox P	0	0	0	0
3. Disolite	0	0	0	0
4. FAM 30	0	0	0	0
5. Hydrocare	0	0	1	1
6. Virkon S	0	0	0	0
7. Unifect G	0	0	0	0
8. Menno Florades	10	10	10	10
9. Domestos Extended Germ Kill	0	0	0	0

BX 14-093 mycelium	***Fusarium* colonies after sterile distilled water dip**			
Product name	**Day 1**	**Day 4**	**Day 5**	**Day 7**
1. Jet 5	10	10	10	10
2. Citrox P	10	10	10	10
3. Disolite	10	10	10	10
4. FAM 30	10	10	10	10
5. Hydrocare	10	10	10	10
6. Virkon S	10	10	10	10
7. Unifect G	10	10	10	10
8. Menno Florades	10	10	10	10
9. Domestos Extended Germ	10	10	10	10

Kill				

BX 14-093 mycelium	***Fusarium* colonies after full rate product 5 min**			
Product name	**Day 1**	**Day 4**	**Day 5**	**Day 7**
1. Jet 5	0	9	10	10
2. Citrox P	0	10	10	10
3. Disolite	0	0	0	0
4. FAM 30	0	10	10	10
5. Hydrocare	0	10	10	10
6. Virkon S	0	10	10	10
7. Unifect G	0	0	0	0
8. Menno Florades	0	10	10	10
9. Domestos Extended Germ Kill	0	0	0	0

BX 14-093 mycelium	***Fusarium* colonies after full rate product 30 min**			
Product name	**Day 1**	**Day 4**	**Day 5**	**Day 7**
1. Jet 5	0	2	3	4
2. Citrox P	0	10	10	10
3. Disolite	0	0	0	0
4. FAM 30	0	10	10	10
5. Hydrocare	0	10	10	10
6. Virkon S	0	10	10	10
7. Unifect G	0	0	0	0
8. Menno Florades	0	10	10	10

9. Domestos Extended Germ Kill	0	0	0	0

BX 14-093 mycelium	***Fusarium* colonies after half rate product 5 min**			
Product name	**Day 1**	**Day 4**	**Day 5**	**Day 7**
1. Jet 5	0	10	10	10
2. Citrox P	0	10	10	10
3. Disolite	0	0	0	0
4. FAM 30	0	10	10	10
5. Hydrocare	0	10	10	10
6. Virkon S	0	10	10	10
7. Unifect G	0	0	0	0
8. Menno Florades	0	10	10	10
9. Domestos Extended Germ Kill	0	0	0	0

BX 14-093 mycelium	***Fusarium* colonies after half rate product 30 min**			
Product name	**Day 1**	**Day 4**	**Day 5**	**Day 7**
1. Jet 5	0	5	9	9
2. Citrox P	0	10	10	10
3. Disolite	0	0	0	0
4. FAM 30	0	10	10	10
5. Hydrocare	0	10	10	10
6. Virkon S	0	10	10	10

7. Unifect G	0	0	0	0
8. Menno Florades	0	10	10	10
9. Domestos Extended Germ Kill	0	0	0	0

BX 14-093 mycelium	***Pythium* colonies after sterile distilled water dip**			
Product name	**Day 1**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	10	10	10	10
2. Citrox P	10	10	10	10
3. Disolite	9	10	10	10
4. FAM 30	9	10	10	10
5. Hydrocare	10	10	10	10
6. Virkon S	10	10	10	10
7. Unifect G	10	10	10	10
8. Menno Florades	10	10	10	10
9. Domestos Extended Germ Kill	10	10	10	10

BX 14-093 mycelium	***Pythium* colonies after full rate product 5 min**			
Product name	**Day 1**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	0	0
2. Citrox P	0	0	0	0
3. Disolite	0	0	0	0
4. FAM 30	0	0	0	2
5. Hydrocare	8	10	10	10
6. Virkon S	0	8	10	10

7. Unifect G	0	0	0	0
8. Menno Florades	0	7	10	10
9. Domestos Extended Germ Kill	0	3	6	10

BX 14-093 mycelium	***Pythium* colonies after full rate product 30 min**			
Product name	**Day 1**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	0	0
2. Citrox P	0	0	1	2
3. Disolite	0	0	0	0
4. FAM 30	0	0	0	0
5. Hydrocare	0	2	2	2
6. Virkon S	0	5	9	9
7. Unifect G	0	0	0	0
8. Menno Florades	0	2	5	5
9. Domestos Extended Germ Kill	0	0	0	0

BX 14-093 mycelium	***Pythium* colonies after half rate product 5 min**			
Product name	**Day 1**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	0	0
2. Citrox P	0	0	1	2
3. Disolite	0	0	0	0
4. FAM 30	0	0	4	5
5. Hydrocare	10	10	10	10

6. Virkon S	0	9	10	10
7. Unifect G	0	0	0	0
8. Menno Florades	0	3	6	9
9. Domestos Extended Germ Kill	0	6	9	9

BX 14-093 mycelium	***Pythium* colonies after half rate product 30 min**			
Product name	**Day 1**	**Day 3**	**Day 5**	**Day 7**
1. Jet 5	0	0	1	2
2. Citrox P	0	0	0	4
3. Disolite	0	0	0	0
4. FAM 30	0	0	2	2
5. Hydrocare	5	10	10	10
6. Virkon S	0	4	10	10
7. Unifect G	0	0	0	0
8. Menno Florades	0	5	7	8
9. Domestos Extended Germ Kill	0	0	0	0