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PRACTICAL SECTION FOR GROWERS

BACKGROUND AND OBJECTIVES

Pythium species cause root rots in a wide range of pot and bedding plant species, and disease caused by *Pythium* is a major cause of losses in this sector of the industry, second only to *Botrytis*. Despite being very widespread and destructive, remarkably little was known until these studies began about the representatives of this pathogen group that cause problems in U.K. ornamental crops.

A lack of knowledge of the sources of infestation meant that growers were unable to properly target control measures and thereby maximise their productivity. Growers of ornamentals currently rely on four fungicides for the control of *Pythium* with variable results. There was a need to assess resistance levels to current fungicides and assist growers in developing a strategy for fungicide use. A knowledge of the sources of *Pythium* species and their particular properties would also allow for the optimisation of hygiene and cultural control measures.

The aim of the project was to improve our understanding of the identities, distributions and epidemiology of *Pythium* species pathogenic to pot and bedding plants and identify potential improvements in disease control. The study covered four areas of work:

1. Identification of the pathogenic *Pythium* species, their sources and modes of spread.
2. Investigation of methods for avoiding disease involving cultural measures.
3. Use and efficacy of chemical control measures.
4. Preliminary assessment of the possibilities for using biological solutions for *Pythium* control.

SUMMARY OF RESULTS

1. Sources and spread of *Pythium*

Distribution of Pythium on nurseries

Surveys of commercial nurseries have shown that *Pythium* species are widespread. Apart from on infected plants, the highest levels of contamination were seen on 'operational surfaces' such as benches, used capillary matting, troughs, floors, Danish trolleys, pipework and, importantly, footwear.

Particularly badly contaminated were capillary mats that had been used for a single crop and wooden surfaces such as bench slats and trolleys. Finally, water often contained *Pythium* spp., which were detected in holding tanks, reservoirs and in small pools on greenhouse floors. Lower levels of contamination were seen in used trays and pots, on machinery and in used growing media.

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Some sources of contamination, such as floors and workers footwear, would aid spread from one part of the nursery to another. Contaminated water has also been demonstrated to rapidly spread disease in this and other recent studies.

Unused growing media and sterilised surfaces were found to be consistently free from *Pythium.*

Identification and quantifying pathogens

Different *Pythium* species have variations in their lifecycles, formation of survival propagules and modes of spread. It's therefore important to know which species are present on an affected nursery if effective control strategies are to be implemented. For example, *Pythium* HS group spread mainly by fast-growing mycelium, which can rapidly colonise sterilised media, whilst *Pythium* Group F can readily produce large numbers of motile zoospores which spread quickly in water.

Identification of *Pythium* isolates to species level is a complex area, and for practical purposes rapid methods of distinguishing pathogenic from non-pathogenic isolates are needed. A number of procedures for detection and diagnosis of *Pythium* species have been developed at HRI. These have improved our ability to identify the presence of *Pythium.*

Identification to species level is still best achieved by either traditional plating methods or possibly by modern molecular Polymerase Chain Reaction (PCR) techniques.

Immunodiagnostic methods have been used but two of these: lateral flow dipsticks and ELISA (enzyme linked immunosorbent assay) have not proved to be sufficiently sensitive or discerning to be practically useful. On the other hand, a technique called ZTI (zoospore trapping immunoassay) has successfully been developed for detecting minute quantities of *Pythium* spores in irrigation water.

On-nursery detection and identification of pathogenic *Pythium* species is still not practical. However, the efficiency, and reliability of lab-based tests has been greatly improved.

Detection of *Pythium* is important, but unless identification to species is possible, the interpretation of such results requires care, as some species of *Pythium* such as *P. oligandrum* (a widespread mycoparasitic species) are actually beneficial to plant production. Non-pathogenic *Pythium* species are common, but their presence especially in irrigation water can be indicative of an increased risk of pathogens also being present.

Pathogenicity testing: sorting the wood from the trees

Isolations from eight different commercial bedding plant nurseries at four times during the year identified 21 different species of *Pythium.* However, pathogenicity tests of these against Antirrhinum and geranium showed that only six of these species or groups were actually serious pathogens of bedding plants. Unfortunately, these pathogenic species (*Pythium* 'Group HS', '*Pythium* Group F', *Pythium ultimum, P.*

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sylvaticum, P. intermedium and *P. irregulare*) were amongst the most commonly isolated from all nurseries. Of the *Pythium* Group F isolates tested, up to 47% were pathogenic depending on temperature and crop.

2. Avoiding disease by good hygiene and cultural measures

Surface sterilants

Isolation studies showed that in most crops, infection by *Pythium* appeared to be initiated from contaminated structures such as benching or floors, or from contaminated irrigation water.

It is therefore important to remove as much potential pathogen inoculum as possible before starting a new crop. Careful use of surface sterilants to routinely clean production structures such as beds, capillary matting, trolleys and irrigation pipework will reduce the incidence of initial infection in crops. In addition, it is a wise precaution to occasionally test irrigation water for contamination by *Pythium* at the point of application.

Five commercially available surface sterilant chemicals were compared for their efficacy at varying rates against contaminated plant debris and on used *Pythium* infested Mypex matting. These were Bleach (sodium hypochlorite), Jet 5, Panacide M, Panaclean and Ter Spezial. All but Panaclean were effective at cleaning the Mypex matting at the recommended label rates, but only Bleach and Jet 5 showed any ability to kill *Pythium* propagules contained in plant debris and even these were limited in this. It is therefore important to try and remove as much debris and spent media from the previous crop as possible before applying sterilant treatments.

Cultural methods for reducing disease

Irrigation regimes had a strong effect on the expression of *Pythium* disease in antirrhinum. Inoculated plants under 'low' (kept dry by infrequent irrigation with small amounts of water) and 'moderate' (plants allowed to dry between irrigations) irrigation regimes were little different from their uninoculated controls, whereas those receiving 'overwatering' were considerably stunted. Overwatering can increase disease both by increasing the amount of *Pythium* inoculum and in some species by stressing the plants.

3. Chemical control measures

Chemical control of *Pythium* disease on pot and bedding nurseries has often seemed poor. A major focus of this project has been to assess the range of approaches to *Pythium* control to identify potential problems and techniques of promise for the future.

Fungicides

Plate tests with pure *Pythium* cultures and tests using inoculated young plants were carried out on the efficacy of the four most frequently used fungicides for *Pythium* control: Fongarid (furalaxyl), Filex (propamocarb hydrochloride), Aaterra WP (etridiazole) and Aliette (fosetyl-aluminium).

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Plate tests revealed that some pathogenic isolates of *Pythium* spp. possess some degree of resistance or tolerance to furalaxyl (Fongarid), propamocarb hydrochloride (Filex) and etridiazole (Aaterra). Fosetyl-Al (Aliette) could not be tested in these assays, because an important part of its mode of action is to stimulate the plants natural defences. Similarly, plate tests of any fungicides need to be viewed with caution as a fungicide can be very effective in the field whilst giving poor plate test results, but these tests can give a useful indication of the potential for fungicide resistance in the pathogen.

Fungicides were tested as both drenches and growing medium incorporations, in simulation of their main uses on nurseries. Similarly, two approaches to inoculation were used:

- incorporation in the growing medium – a harsh test for a fungicide, simulating absolute worst case conditions and assessing pre-emergence damping-off.
- inoculation of a sand capillary bed on which test plant modules are placed – a more realistic simulation of the usual route for infection via contaminated surfaces or irrigation water.

In all tests, resistance to furalaxyl (Fongarid) was detected. Of the remaining fungicides, the best performance was by etridiaxole (Aaterra) which only showed disease when used as a drench in tests using incorporated inoculum of 5 particularly virulent isolates, and even in this case losses were due to pre-emergence damping-off and not post-emergence root rot. When used as a compost-incorporation, which is the method recommended on the product label, Aaterra gave good control of all isolates tested. Filex gave more variable results, with some evidence of fungicide tolerance, but still achieved reasonable control of post-emergence root rot in most cases.

Surfactants

Four surfactants were tested against *Pythium* zoospores in water. Agral and Enhance were effective at killing zoospores at low concentrations (40 µg/ml; a concentration well within the recommended ranges for cleaning operations with either of these chemicals), whereas Activator 90 and PBI Spreader required 10 times this concentration for the same effect. Current regulations state that these chemicals can be used to treat water if it is left to stand for a period of time before using for irrigation. ***Please see note * below on regulations of use of surfactants and surface sterilants.***

Copper

Direct kill of zoospores in water required concentration of copper of 0.1µg ml^{-1} but to suppress other stages of the *Pythium* lifecycle required higher concentrations (3-15µg ml^{-1}). The incidental presence of copper in irrigation water, perhaps as a component of the micronutrients in feed solution, could also be effective against the spread of *Pythium* zoospores. However, copper ions were found to readily bind to peat-based growing media and under these circumstances, considerably higher concentrations than normally present in feed solutions would be required to give control. Such levels (5-10 µg ml^{-1}) would be in excess of the European guidance level for copper in the applied feed solution (4 µg ml^{-1}), and might cause phytotoxicity problems. Levels of copper capable of achieving some measure of control may be achieved by the application of an approved copper fungicide (eg. Croptex Fungex) to the affected

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plants. However, the efficacy and possible phytotoxic effects of such a fungicide application was not investigated in this study.

Please note that the application of copper to feed solutions or irrigation water for the primary purpose of disease control is not permitted under the Control of Pesticides Regulations 1986 (COPR) and the Plant Protection Products Regulations 1995 (PPPR).

4. Biocontrol: set a thief to catch a thief!

Recent HDC funded results from media incorporation of propagules of a species of *Pythium* that parasitises and kills pathogencic *Pythiums* have been very promising. When propagules of *P. oligandrum* were incorporated in the growing medium for pelargoniums the amount of disease was reduced by nearly 80 %.

ACTION POINTS FOR GROWERS

It has been demonstrated by the activities of the nurseries involved with this project that it is possible and economically viable to substantially cut down *Pythium* losses in pot and bedding plant production by following a SERIES of simple measures.

1. Pathogenic *Pythium* propagules are widely dispersed on most nurseries but they are not found everywhere: Unused growing media and sterilised surfaces are consistently free from pathogen. Cut down pathogen inoculum before disease starts by regularly cleaning and sterilising the 'trouble spots'. These are: Danish trolleys, used matting, benches, production troughs and floors.

2. Use sterilants to kill *Pythium* on used production surfaces and on floors. Jet 5, sodium hypochlorite, Ter Special and Panacide M were all effective at the manufacturers recommended rate. Sodium hypochlorite and Jet 5 were especially good, even killing propagules in plant debris, but no sterilant will completely eliminate this potent source of disease under the constraints of commercial operation.

3. Before applying chemical sterilants, remove as much dirt, spent media, dust and previous crop debris as possible.

4. Eliminate standing water: treat leaks in roofs and find out why those niggling puddles are there and get rid of them.

5. Encourage good crop hygiene in staff and pay particular attention to footwear, as inoculum is readily transferred from dirty to clean areas on the soles of shoes. If foot baths are employed at the entrance to sensitive areas of the nursery, ensure that the sterilant in them is regularly changed otherwise they may inadvertently become sources of disease themselves. And at all times there should definitely be no muddy boots!

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6. Good hygiene, cleanliness and use of sterilants should drastically reduce the need for fungicides. If possible, use fungicides sparingly. If using fungicides as routine treatments it is vital to alternate products as there is evidence of tolerance to Fongarid and Filex and possibly even to Aaterra.

7. Application techniques and timing strongly influence fungicide efficacy. Aaterra was very effective as a compost incorporation but worked less well as a drench whilst Filex worked best as a drench application. **Use pesticides safely – Always read the label.**

8. It is important, when persistent suspected *Pythium* problems occur, to send samples to a diagnostic laboratory for correct isolation, identification and possibly fungicide resistance testing.

9. Check irrigation water at the point of application several times each year for contamination by *Pythium*.

10. Water from ponds, rivers and reservoirs or greenhouse roofs is often contaminated by pathogenic *Pythium* species. It is wise to treat water from such sources to kill pathogen spores before using it on the nursery. A number of techniques have been shown to be effective for this, in particular slow sand filtration (see HDC reports HNS 88, 88a & 88b)

11. Water stored in open tanks (no matter how clean) can easily become contaminated from debris blown in. Always cover 'clean' water tanks.

12. Avoid over-watering which creates ideal conditions for *Pythium* to multiply and infect. Use media with high air-filled-porosity (AFP) to improve aeration and give good drainage.

13. Avoid plant stress – stressed plants are generally more susceptible to *Pythium* attack.

14. Copper kills *Pythium* zoospores in water at very low concentrations and when present in the trace elements part of feed solutions may give the added benefit of some disease control. ***However, addition of copper to water or feeds primarily to control disease is illegal.***

15. The surfactants Agral and Enhance killed zoospores at a concentration of 40 ppm. Current regulations state that these chemicals can be used to treat (clean) water if it is left to stand for a period of time before it is used for irrigation. ***Please note that use of surfactants directly on plants to control Pythium (i.e. as a pesticide) is illegal.***

16. Biological solutions for the control of *Pythium* involving disease suppressive growing media and the use of biocontrol agents are under development and show some promise. However, further work is required to make these techniques more robust.

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***Note on approval of pesticides, surface sterilants, surfactants and use of copper**

Fungicides

The fungicides Aaterra, Filex, Fongarid, Aliette and Croptex Fungex, mentioned in this report have on label approval for use for the control of diseases on ornamental crops under protection (i.e. under glass or plastic).

Please note that the approval status of fungicides can change. Therefore it is important that regular checks are made with the manufacturers or with PSD [Information line (01904) 455 775] to check the current approval status for any pesticide.

Surface sterilants and surfactants

Currently there are no regulations specifically controlling the use of disinfectants or surfactants in horticulture. They will however be covered by the EU Biocidal Products Directive, due to be implemented by the HSE in GB and phased in over a 10 year period commencing 14 May 2000. Disinfectants are exempt from the 1986 Control of Pesticides Regulations, providing they are not used on plants, or the soil, compost or other growing medium (see HDC Review CP4). If a disinfectant product is a hazardous chemical, it will be covered by COSHH. Care also needs to be taken to prevent contravention of legalisation on pollution of watercourses etc; pollution incidents have occasionally occurred in recent years following use of disinfectants in commercial horticulture.

Copper

European guidance levels for copper in applied feed solutions are 4 µg per ml or 4 ppm. Copper cannot be applied directly to plants for the control of disease unless as a component of an approved pesticide. Please see notes above on fungicides.

ANTICIPATED PRACTICAL AND FINANCIAL BENEFITS

By following the action points outlined above, growers should be able to devise strategies on how to minimise losses due to *Pythium* throughout the whole production system. Cost savings will result from a reduced but more rational use of fungicides and the targeted use of sterilants to reduce the initial disease load and prevent cross-contamination. Labour costs in replacing dead seedlings and plants will be reduced and there will be an overall improvement in plant quality and grade-out, thus increasing profitability.

Over the period of this project, the nurseries involved in the surveys and the regular sampling work became more aware of the 'mechanics' of the *Pythium* problem and were able to implement strategies to reduce contamination. This is evidenced by the reduction over the period of this project in both disease and *Pythium* contamination on production structures, for example on used matting the occurrence of *Pythium* was reduced from 72 % in the period 1995 to 1997 to 19 % in the period 1998 to 1999. This demonstrates that it is possible to reduce *Pythium* disease by adopting an overall strategy of good hygiene, water treatment, regular use of sterilants and minimised, careful, alternated use of fungicides.

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EXPERIMENTAL SECTION

General Introduction

Pythium spp. cause root rots in a wide range of pot and bedding species, and disease caused by this genus is a major cause of losses in this sector of the UK horticulture industry, second only to *Botrytis*. Despite being very widespread, surprisingly little was known about the *Pythium* spp. present on nurseries and their modes of spread before HDC-funded work began in 1995 with projects PC 97/97a. The work carried out in these studies has covered a broad range of topics. The key areas of study are:

- diagnosis of the *Pythium* species present
- assessments of pathogenicity of the *Pythium* species present
- monitoring populations present with cropping and season and disease expression
- assessing modes of spread in dirt, debris, plants and irrigation water
- development of new and more sensitive diagnostic tests
- determining the impact of cleaning and sterilisation procedures on subsequent disease
- determining the effects of irrigation frequency on disease severity
- studying fungicide efficacy and the possibility of fungicide tolerance in the pathogen populations
- assessments of alternative disease control options including the use of copper salts, surfactants and biocontrol agents.

In this final report we present the results of the concluding series of experiments carried out in PC97a. These have primarily focussed on control;

a) determining the efficacy of currently used fungicides and investigating the possibility of tolerance development especially to furalaxyl.
b) investigating the use of copper salts and surfactants for treatment of contaminated irrigation water.
c) using the mycoparasitic *Pythium* species *P. nunn, P. periplocum* and *P. oligandrum* incorporated in growing media as biocontrol agents.

In addition we list the key conclusions drawn from the entire study from both PC 97 and PC97a which provide a valuable base of information for guidance in developing robust *Pythium* control/management strategies for pot and bedding nurseries.

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PART I – Culture collection

Introduction

From the first year of PC 97a, a collection of *Pythium* isolates has been preserved. Representative isolates (numbers of isolates of each species are in brackets) of fourteen different species of *Pythium* were saved. These species were: *P. aquatile* (1), *P. intermedium* (2), *P. irregulare* (1), *P. monospermum* (8), *P. paroecandrum* (5), *P. polymastum* (3), *P. rostratum* (1), *P. spinosum* (4), *P. sylvaticum* (5), *P. ultimum var. ultimum* (6), *Pythium* groups F (31), G (9), HS (15) and P (2). A number of isolates have also been saved which were not identified to species level (39), as were a number of *Phytophthora* isolates.

Materials and Methods

Isolations were usually made on cornmeal agar (CMA, Difco) amended with rifamycin (30 mg l^{-1}) and pimaricin (100 mg l^{-1}). Often these cultures would contain bacterial contaminants. Where necessary, these were eliminated by growing the *Pythium* isolates on 2 % water agar amended with rifamycin (30 mg l^{-1}). They were then transferred to CMA and V8 juice agar plates for identification. From here they were inoculated onto cornmeal agar slopes and stored at 15°C. These cultures are routinely sub-cultured at 6 monthly intervals.

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PART II – Fungicide sensitivity

a) Sensitivity to Fongarid (25 % w/w furalaxyl) of reference isolates of *Pythium*

Introduction

Over 100 isolates of *Pythium* collected in PC 97 and the current project have been assessed for their sensitivity to furalaxyl using a laboratory test. In order to establish a baseline for sensitivity of *Pythium* spp. to furalaxyl, 20 *Pythium* isolates from the HRI culture collection comprising 11 species that have never been exposed to furalaxyl, were tested in a similar assay for their responses to furalaxyl.

Materials and Methods

A stock solution of furalaxyl was prepared in sterile distilled water (SDW) using Fongarid (a.i. furalaxyl, 25 % w/w), then diluted 2- and 10-fold and mixed with CMA to achieve the required concentrations (100, 50, 10, 5, 1, 0.1, 0.01, 0 µg furalaxyl ml^{-1}). There were four replicate plates for each concentration/isolate combination. Each plate was inoculated at the side with a 5 mm diameter mycelial disc cut from V8 agar cultures. The plates were incubated at room temperature for up to 4 days. Colony growth was measured as mycelial extention (mm). Growth measurements were taken when the first colonies in the dilution series of fungicide had almost reached the furthest edge of the plate, and the data analysed using a Genstat 5 programme which fitted logistic curves and calculated the ED_{50} values for furalaxyl on the different *Pythium* isolates tested.

Results and Discussion

All of the reference isolates of *Pythium* that had not previously been exposed to furalaxyl gave low ED_{50} values for furalaxyl (Table 1). Out of a total of 158 representative *Pythium* isolates (comprising 18 species or groups), collected from nurseries during 1995-1999, and therefore likely to have been exposed to furalaxyl, over 50 % still had a low ED_{50} (<10 µg ml^{-1}). However, 19 % had a high ED_{50} (>90 µg ml^{-1}) indicating a reduction in sensitivity to furalaxyl (Figure 1).

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Table 1. ED_{50} values ($\mu g\ ml^{-1}$) of furalaxyl for isolates of *Pythium* species that had not previously been exposed to furalaxyl.

***Pythium* sp.**	**ED_{50} ($\mu g\ ml^{-1}$)**
P. irregulare	0.14 (± 0.016)
P. irregulare	0.45 (± 0.111)
P. irregulare	2.50 (± 0.152)
P. irregulare	0.72 (± 0.054)
P. intermedium	2.33 (± 0.071)
P. intermedium	1.72 (± 0.087)
P. intermedium	0.63 (± 0.123)
P. sylvaticum	0.85 (± 0.024)
P. sylvaticum	0.08 (± 0.003)
P. sylvaticum	0.07 (± 0.002)
P. ultimum var. *ultimum*	0.39 (± 0.027)
P. ultimum var. *ultimum*	0.40 (± 0.014)
P. ultimum var. *ultimum*	0.42 (± 0.019)
P. ultimum var. *sporangiiferum*	0.07 (± 0.002)
P. polymastum	0.83 (± 0.016)
P. paroecandrum	0.73 (± 0.023)
P. torulosum	0.24 (± 0.015)
P. rostratum	0.05 (± 0.002)
P. salpingophorum	0.88 (± 0.010)
P. mastophorum	0.10 (± 0.019)

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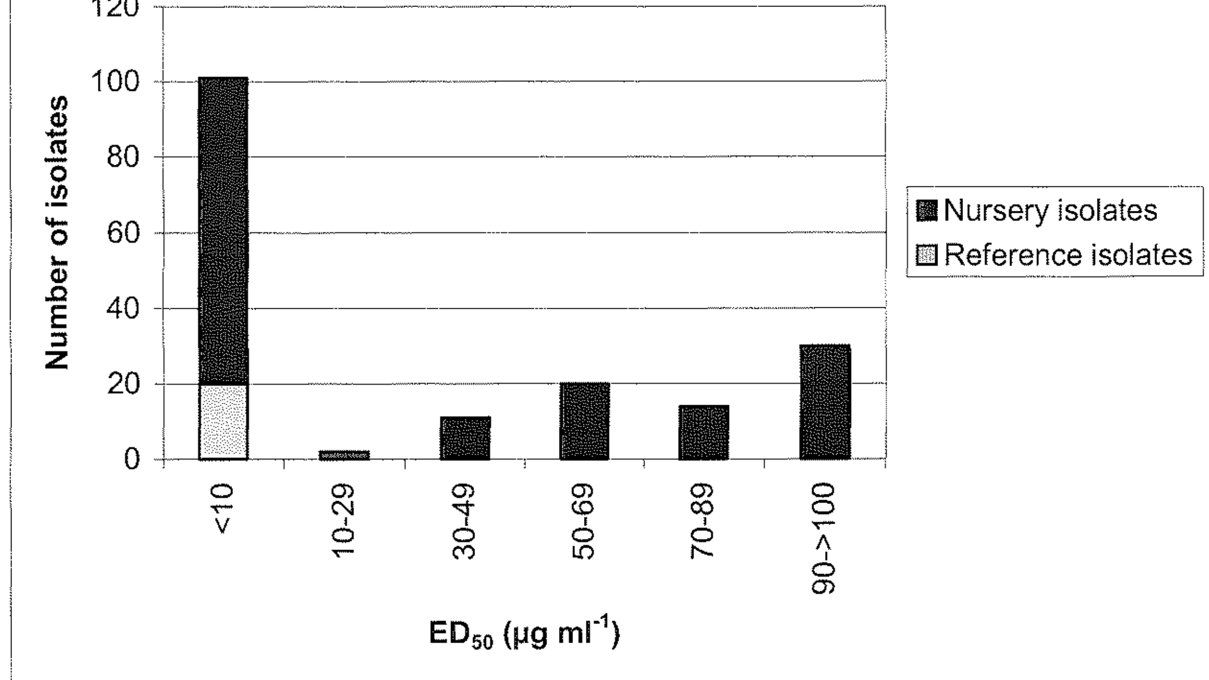

With the exception of several unidentified isolates, the group of isolates with reduced sensitivity (ED_{50} >90 µg ml^{-1}) were all from 6 *Pythium* species or groups: *P. irregulare, P. sylvaticum, P. ultimum var ultimum, P. paroecandrum,* and *Pythium* groups HS and F (Figures 2-7). Five of these species are of economic importance since they contain isolates that are pathogenic to ornamentals, and were frequently isolated from both structures and plant material on nurseries in this study. *P. paroecandrum*, although frequently isolated, was found to be less pathogenic than the other species in this group, when tested in PC97. The reduced sensitivity that some isolates of these species showed *in vitro*, suggested the possibility for poor disease control by furalaxyl *in vivo*. No reference isolates were available for groups HS and F and so the incidence of low sensitivity to furalaxyl cannot be directly inferred as a recent phenomenon although HS group has been allied with *P. ultimum* (Kageyama, Ohyama and Hyakumachi, 1997; Martin, 1990). However, as both groups were among the most frequently isolated species on nurseries and were regularly found to be pathogenic, the reduced sensitivity of many isolates to furalaxyl is of great commercial importance.

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Figure 2. ED_{50} values for furalaxyl of *P. irregulare* isolates collected from nurseries from 1995-1999 and reference isolates from HRI culture collection

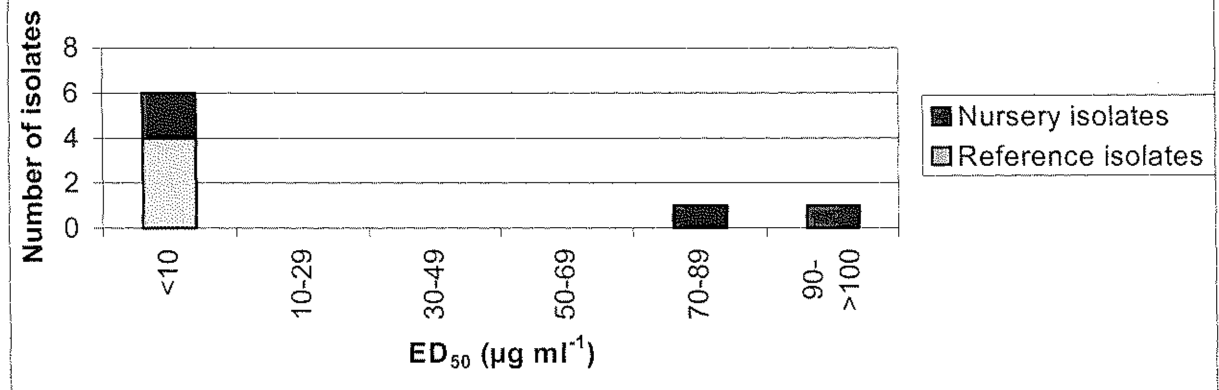

Figure 3. ED_{50} values for furalaxyl of *P. sylvaticum* isolates collected from nurseries from 1995-1999 and reference isolates from HRI culture collection

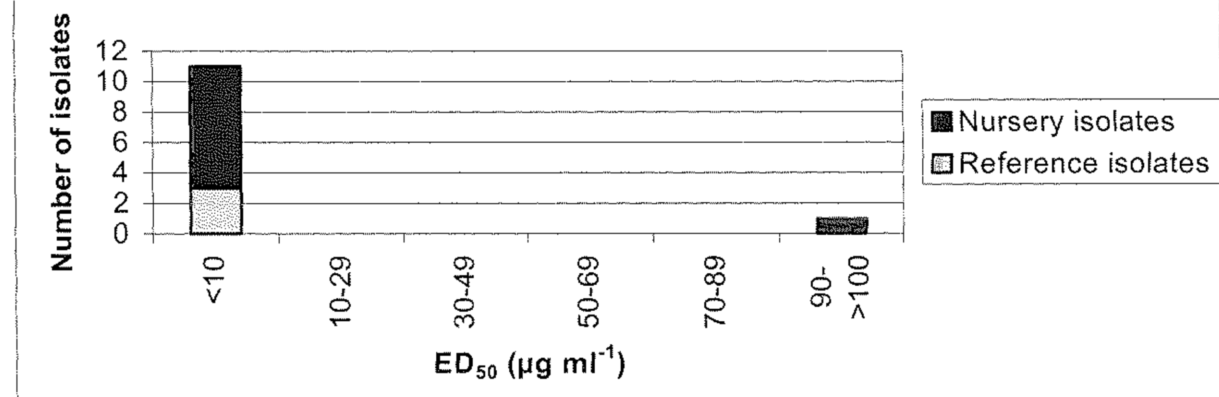

Figure 4. ED_{50} values for furalaxyl of *P. ultimum var ultimum* isolates collected from nurseries from 1995-1999 and reference isolates from HRI culture collection

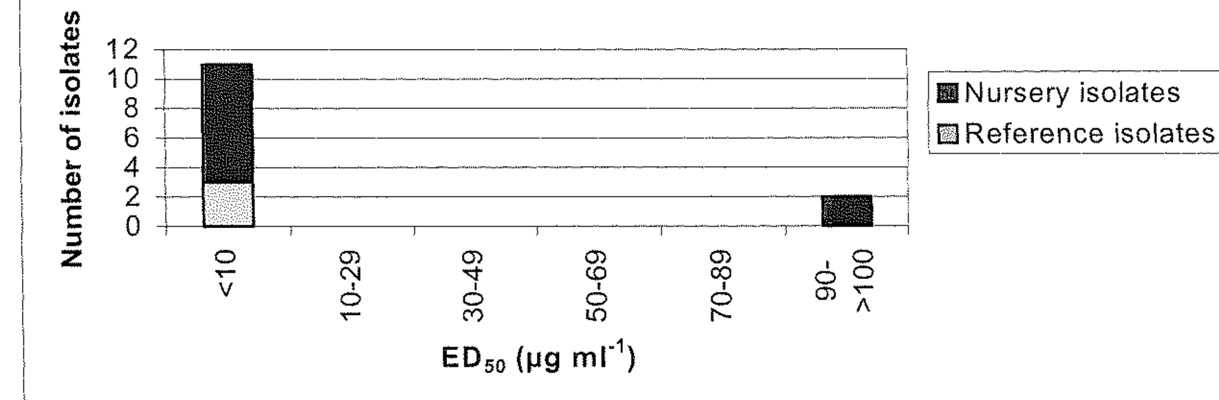

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Figure 5. ED_{50} values for furalaxyl of *P. paroecandrum* isolates collected from nurseries from 1995-1999 and reference isolates from HRI culture collection

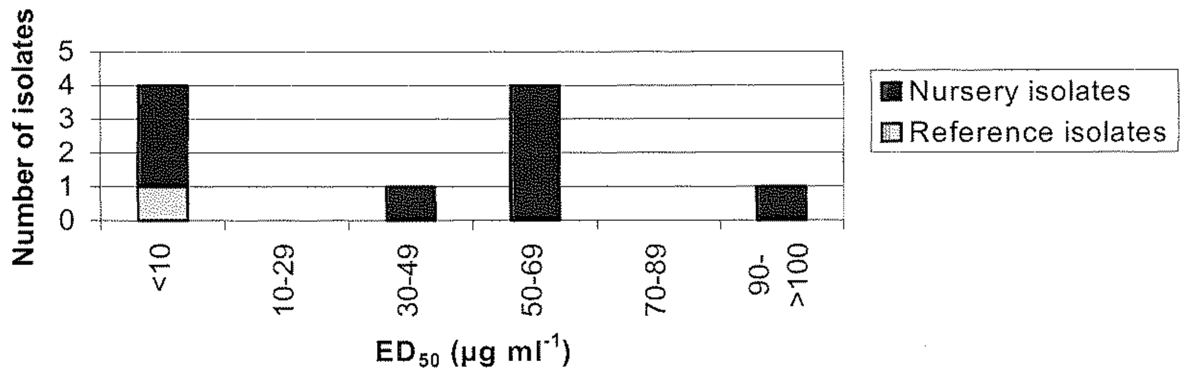


Figure 6. ED_{50} values for furalaxyl of *Pythium* Group HS isolates collected from nurseries from 1995-1999

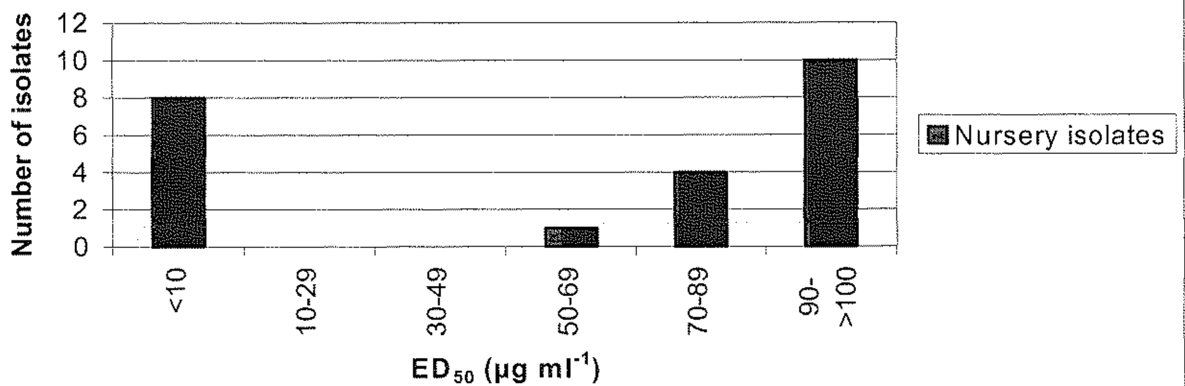
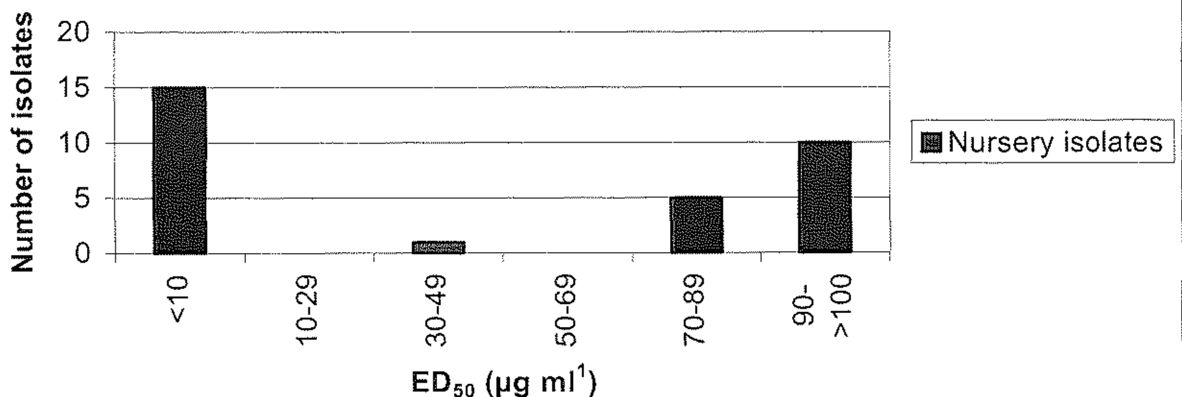


Figure 7. ED_{50} values for furalaxyl of *Pythium* Group F isolates collected from nurseries from 1995-1999



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b) Sensitivity to Aaterra WP (35 % w/w etridiazole) of *Pythium* isolates *in vivo*

Introduction

Previous trials in this project on fungicide sensitivity have shown varying responses of *Pythium* isolates to commercially available fungicides. Of some concern were results from using 'Aaterra' WP (a.i. 35 % w/w etridiazole) which was effective as a drench for both pre- and post-emergence damping-off against many *Pythium* isolates but appeared ineffective against some aggressive isolates. 'Aaterra' WP works best and is recommended as a compost-incorporation. This trial examined its effectiveness as a compost incorporation against the same *Pythium* isolates as tested in year 1 of PC 97a. Both the fungicide and the *Pythium* inoculum were incorporated into the compost. For a single selected isolate a drench treatment was also applied for comparison.

Materials and Methods

The same 16 *Pythium* isolates used in PC97a year 1's compost tests were used for this trial, and for comparison the numbers identifying them remain the same (Ref Yr. 1 report). The 16 *Pythium* isolates were cultured in 3 % v/v maize meal / sand flasks. Flasks were incubated at 20°C for 33 days.

John Innes No 2 loam compost was dried at 80°C for 2 days. The compost was then re-wetted with distilled water; 4 (compost): 1 (water) by volume. 'Aaterra' WP was incorporated into half of the compost at a rate of 75 g m^{-3} as recommended by the manufacturer. Inoculation of compost with *Pythium* was carried out for each isolate by mixing 53 g of maize meal / sand inoculum with 1 l of compost in polythene bags. This was done for both types of compost (i.e. with and without 'Aaterra' WP). The compost / inoculum mixture was left overnight and then distributed evenly between 4 FP7 pots.

For comparison with year 1 work, where 'Aaterra' WP was applied as a drench, 4 extra pots were also set-up with isolate 2 for drench treatment with 'Aaterra' WP. The drench was applied at a rate of 2.5 g m^{-2} (0.01225 g/pot in 20 ml of water).

Other treatments were uninoculated, untreated control (4 replicates); uninoculated, 'Aaterra' WP incorporated control (4 replicates); and uninoculated, 'Aaterra' WP drench control (4 replicates).

Eight geranium seeds were sown per pot. Twenty ml of distilled water were gently poured over all pots except the 'Aaterra' WP drench treatments. For each isolate, the pots were separated into 4 replicate trays. Each tray was lined with fleece and plastic. The trays were then arranged in randomised blocks in a growth room maintained at 20°C±4, with 16 hours light per day cool white fluorescent lamps supplemented with 60 Watt. tungsten light giving a total PAR of 186 μm E m^{-2}). The seedlings were watered as required.

Seedlings which had damped-off during the course of the experiment were removed

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and noted. After 42 days, the emergence and percentage incidence of disease in seedlings were assessed.
Data were analysed by analysis of variance using the Genstat 5 programme.

Results and Discussion

This trial was a rigorous test of the fungicide with *Pythium* propagules being present in the compost from sowing. Four of the 16 isolates proved to be non-pathogenic, having had no effect on emergence in inoculated control treatments. These were isolates 4, 12, 13 and 15 (Table 2). Of the remaining 12 isolates only 3 showed an improvement in % emergence with the incorporation of 'Aaterra' WP into the compost (isolates 7, 8 and 10). 'Aaterra' WP was not significantly better at increasing the percentage emergence of seeds in the presence of isolate 2, either as a compost-incorporation or as a drench.

Table 2. Effect of 'Aaterra' WP as a compost incorporation or drench on the percentage emergence of geranium seeds in *Pythium*-infested composts

***Pythium* isolate**	**% Emergence**		
	Untreated control	**'Aaterra' WP-Incorporated Compost**	**'Aaterra' WP-Drenched Compost**
1	56.3 (48.7)	65.6 (54.3)	-
2	15.6 (16.9)	18.8 (25.4)	25.0 (29.6)
3	37.5 (37.6)	53.1 (46.8)	-
4	75.0 (60.4)	71.9 (61.9)	-
5	37.5 (37.6)	50.0 (44.9)	-
6	25.0 (29.6)	25.0 (29.6)	-
7	12.5 (20.7)	56.3 (49.3)	-
8	25.0 (25.9)	65.6 (54.8)	-
9	6.3 (10.4)	15.6 (23.0)	-
10	12.5 (15.0)	40.6 (39.6)	-
11	12.5 (17.9)	28.1 (28.1)	-
12	75.0 (63.6)	65.6 (54.8)	-
13	62.5 (52.9)	50.0 (45.0)	-
14	31.3 (29.5)	46.9 (43.2)	-
15	68.8 (57.1)	65.6 (54.2)	-
16	53.1 (47.5)	53.1 (50.6)	-
Pythium-free control	84.4 (69.8)	93.8 (79.6)	90.6 (77.3)
LSD P 0.05*		(18.26)	

*arcsine transformation of percentages to which LSD applies in parentheses

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Only isolates 2 and 7 caused significant post-emergence damping-off and incorporating 'Aaterra' WP into the compost significantly reduced this (Table 3). No reduction in post-emergence damping-off was observed with the drench treatment on isolate 2.

Table 3. Effect of 'Aaterra' WP as a compost incorporation or drench on the percentage of healthy geranium seedlings in *Pythium*-infested composts

***Pythium* isolate**	**% of healthy seedlings**		
	Untreated control	**'Aaterra' WP-Incorporated Compost**	**'Aaterra' WP-Drenched Compost**
1	100.0 (90.0)	100.0 (90.0)	-
2	66.7 (64.5)	100.0 (90.0)	79.2 (69.9)
3	91.7 (81.2)	100.0 (90.0)	-
4	100.0 (90.0)	84.4 (70.0)	-
5	91.7 (81.2)	100.0 (90.0)	-
6	87.5 (78.7)	100.0 (90.0)	-
7	25.0 (22.5)	72.6 (62.1)	-
8	100.0 (89.8)	100.0 (90.0)	-
9	100.0 (88.1)	100.0 (90.0)	-
10	100.0 (87.6)	100.0 (90.0)	-
11	100.0 (90.1)	100.0 (88.7)	-
12	100.0 (90.0)	100.0 (90.0)	-
13	100.0 (90.0)	100.0 (90.0)	-
14	86.7 (77.0)	87.5 (78.7)	-
15	100.0 (90.0)	100.0 (90.0)	-
16	100.0 (90.0)	100.0 (90.0)	-
Pythium-free control	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
LSD P 0.05*		(18.16)	

*arcsine transformation of percentages to which LSD applies in parentheses

c) Efficacy of Aaterra WP and Filex

Introduction

Whilst providing a useful indication of potential shortfalls of fungicide activity, *in vivo* tests using *Pythium* inoculum incorporated into the growing medium before sowing did not provide 'commercially realistic' tests of efficacy. Results from previous years work on this project have shown that fresh unused growing media and containers are a very unlikely source of *Pythium* inoculum. The majority of infections tend to be initiated from contaminated production surfaces such as benches and old matting or from contaminated irrigation water. This type of infection route is best simulated using a sand tray

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inoculation system developed in HDC-funded project PC 157 (Carver, 2000). Representative isolates of *Pythium,* which had given indications of fungicide tolerance in previous experiments using the incorporated inoculum technique, were assessed using the more realistic assay conditions to help with the immediate interpretation of the more rigorous incorporation assay results.

Materials and Methods

Four isolates of *Pythium* were chosen based on pathogenicity and fungicide responses in Year 1 of PC 97a. These isolates were cultured in 10 % oatmeal / sand flasks, grown at room temperature for 4-7 days. (Isolate identification numbers remain the same as in year 1 growth room trial). Each culture was mixed 1:3 with autoclaved sand (134°C / 30 min. on two consecutive days), and 300 g of this inoculum was used to line a plastic tray (163 x 125 x 52 mm). Levington F1 compost was wetted at a rate of approx. 80 ml / l, to ensure it was moist before application of fungicides. 'Aaterra' WP was incorporated into Levington compost at a rate of 75 g m^{-3} as recommended by the manufacturer. For the control treatments and Filex treatments, Levington compost was wetted up as above. The compost was used to fill blocks of 6 modules (module size 50 ml, cut from a 40 module tray, Plantpak 40) and 1 set of modules was placed into each tray. There were 4 replicates per treatment per isolate. Two geranium seeds (cv. F2 'Palladium Mixed') were sown per module (12 per tray). A Filex drench was applied to relevant modules at a rate of 10 ml m^{-2} in 4 l water, ensuring that compost was thoroughly drenched. 5.8 ml of tap water was pipetted onto each module of all treatments except Filex treated modules (equivalent to 4 l m^{-2}). Trays were arranged in randomised blocks in a growth room set at 20°C±4, with 12 hours light per day as described above.

Emergence counts began on day 3 and continued for 3 weeks. Any seedlings which damped-off at the start of the experiment were plated onto CMA amended with rifamycin (30 mg l^{-1}) and pimaricin (100 mg l^{-1}), to confirm *Pythium* infection. After 3 weeks the emergence and percentage incidence of disease in seedlings was assessed, and the heights of the healthy seedlings were measured from the base of the stem to the tip of the highest leaf. Data were analysed by analysis of variance using the Genstat 5 programme.

Results and Discussion

None of the isolates significantly affected the emergence of seedlings and there were therefore no differences between the fungicide treatments (Table 4). All isolates were pathogenic to Pelargoniums, and caused different severities of post-emergence damping-off, ranging from 14.8 % - 93.2 %. Aaterra WP gave excellent control of all the *Pythium* isolates tested, with no seedlings showing disease symptoms, even when the highly pathogenic *Pythium* isolate 7 was used. Filex didn't perform as well, but gave some control of isolate 2, increasing the number of healthy seedlings.
Height reductions were seen in seedlings inoculated with isolates 2, 7 and 9, although these reductions were only significant ($P \leq 0.05$) with isolates 2 and 7. When used in the presence of isolates 2, 7 and 9 Aaterra increased the height of seedlings, but when it was used with less virulent isolate 10, seedling height was reduced. These results

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firstly demonstrate the efficacy of the chemical against virulent isolates, but the results from isolate 10 also indicate that control is achieved at a small cost. Stunting is also a potential hazard of overdosing with Aaterra. Generally the results of these tests showed that under commercially more realistic conditions Aaterra worked well against four pathogenic *Pythium* isolates that showed some indications of tolerance to it in more rigorous tests. However, under the same conditions Filex did not perform so well although it did achieve some control of isolate 2. The results of this work demonstrate that to get good results with fungicides, they need to be applied with care and that there is a cause for some concern over the development of fungicide tolerance in the *Pythium* populations present on U.K. ornamentals nurseries.

Table 4. Effect of 'Aaterra' WP as a compost incorporation and Filex drench on the percentage emergence and percentage of healthy seedlings in *Pythium*-infested composts

Isolate	Treatment	% Emergence	% Healthy
Uninoculated	Untreated	93.7 (79.8)	100.0 (90.0)
	Aaterra WP	89.6 (73.8)	100.0 (90.0)
	Filex	91.7 (75.6)	100.0 (90.0)
2	Untreated	97.9 (85.8)	48.9 (44.3)
	Aaterra WP	93.7 (77.4)	100.0 (90.0)
	Filex	91.7 (75.6)	72.8 (58.7)
7	Untreated	91.7 (73.2)	6.8 (13.2)
	Aaterra WP	87.5 (75.8)	100.0 (90.0)
	Filex	85.4 (68.1)	11.6 (16.9)
9	Untreated	89.6 (77.0)	65.5 (54.5)
	Aaterra WP	95.8 (84.0)	100.0 (90.0)
	Filex	91.7 (78.0)	70.8 (57.8)
10	Untreated	97.9 (85.8)	85.2 (70.6)
	Aaterra WP	91.7 (78.3)	100.0 (90.0)
	Filex	93.7 (79.8)	89.4 (73.9)
	LSD P 0.05	16.79	11.74
	P 0.01		15.71
	P 0.001		20.63

*arcsine transformation of percentages to which LSD applies in parentheses

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Table 5. Effect of 'Aaterra' WP as a compost incorporation and Filex drench on the height (mm) of healthy seedlings in *Pythium*-infested composts

Isolate	Treatment		Mean height (mm)
Uninoculated	Untreated		51.3
	Aaterra WP		47.1
	Filex		56.6
2	Untreated		42.0
	Aaterra WP		45.7
	Filex		40.5
7	Untreated		38.5
	Aaterra WP		48.9
	Filex		46.6
9	Untreated		45.7
	Aaterra WP		52.7
	Filex		42.6
10	Untreated		52.0
	Aaterra WP		48.5
	Filex		52.5
		LSD P 0.05	9.26
		P 0.01	12.38

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PART III – Efficacy of surfactants in reducing water-borne inoculum of *Pythium*

Introduction

Four surfactants were tested in 1999 for their potential against *Pythium* zoospores in water. The efficacy of these surfactants varied greatly at the given exposure time (1 hour), therefore, the effect of increased exposure time at concentrations lower than those previously found to be effective were investigated.

Materials and Methods

The *Pythium* isolate used for this work was obtained from Mypex matting at a commercial grower's holding and belonged to *Pythium* Group G. Zoospores were produced by flooding a V8 juice agar (V8) culture in a Petri dish with sterile distilled water and incubating overnight at room temperature. Appropriate concentrations of each surfactant (see Table 6 for surfactants tested) were prepared in 250 ml aliquots of water and then zoospores were added at the rate of $>1x10^4$ ml^{-1}. There were three replicates of each concentration. A sample (25 ml) was taken from each concentration 1, 4 and 24 hours after inoculation and filtered through a 5.0 µm cellulose nitrate membrane filter. The filter was then cut into 16 pieces and the pieces were plated on corn meal agar amended with pimaricin (100 mg l^{-1}) and rifamycin (30 mg l^{-1}). The viability of zoospores was recorded as percentage of pieces of filter that produced mycelial growth.

Table 6. Surfactants tested against *Pythium* zoospores.

Surfactant	Active ingredient (a.i.)	Concentration of a.i. (g l^{-1})	Maximum dose (µg ml^{-1}) according to product labels	Supplier
Activator 90	alkyphenyl hydroxypolyoxyethylene	750	1000	Newman
Agral	alky phenol ethylene oxide	948	1000	Zeneca
Enhance	phenol ethylene oxide condensate	900	600	Techsol
PBI Spreader	nonylphenol ethylene oxide condensate	information not supplied	1250	PBI

Results and Discussion

The results were similar to those obtained in year 2 of this project. Agral and Enhance were effective at 40 µg ml^{-1}, whereas Activator 90 and PBI Spreader were only effective at 400 µg ml^{-1} (Table 7). Prolonged exposure to lower concentrations (20 µg ml^{-1} or less) of Agral and Enhance generally increased the number of zoospores killed, although this never was increased to 100 %. The efficacy of lower concentrations of Activator 90 and PBI Spreader was not improved by longer exposure time.

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Where there is a risk of disease spread from contaminated water sources, the addition of the surfactants Agral and Enhance at rates of 40 μg ml^{-1} or more would be expected to eliminate zoospores. This concentration is well within the range recommended for cleaning purposes by the suppliers of these chemicals (see Table 6). However, these chemicals can only be used to treat (*i.e.* clean) water that is subsequently allowed to stand for some time before use on plants. Their use directly for disease control on plants (*i.e.* as a pesticide) is illegal. In addition the phytotoxicity of water treated with these chemicals is unknown as the study of this was beyond the remit of the current project.

Table 7. Effects of surfactants at different concentrations for different time periods on the survival of zoospores produced by an isolate of *Pythium* Group G.

Surfactant	Concentration of surfactant (μg ml^{-1})	Survival of zoospores (%)		
		1 h exposure	4 h exposure	24 h exposure
Activator 90	0	100	100	100
	50	96 (± 2.4)	100	96 (± 2.4)
	100	4 (± 2.4)	25 (± 8.3)	21 (± 2.4)
	200	8 (±4.8)	0	0
	400	0	4 (± 2.4)	4 (± 2.4)
Agral	0	96 (± 2.4)	100	100
	10	79 (± 12.0)	83 (± 2.4)	33 (± 2.4)
	20	67 (± 15.8)	79 (± 4.8)	54 (±2.4)
	40	0	0	0
	80	0	0	0
Enhance	0	100	100	100
	5	100	100	100
	10	100	75 (± 14.4)	100
	20	100	67 (± 2.4)	8 (± 4.8)
	40	0	4 (± 2.4)	4 (± 2.4)
PBI Spreader	0	100	88 (± 7.2)	100
	50	100	100	100
	100	100	79 (± 12.0)	100
	200	4 (± 2.4)	29 (± 4.8)	25 (± 4.2)
	400	8 (± 2.4)	8 (± 2.4)	0

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PART IV – Efficacy of elemental copper in reducing water-borne inoculum of *Pythium* spp.

Introduction

The potential of using copper as a water treatment to kill zoospores of *Pythium* was investigated in 1999. Copper was found to be effective against *Pythium* zoospores in water at low levels; there was a reduction in the viability of zoospores after exposure to 0.05 $\mu g\ ml^{-1}$ for 1 h and 0.025 $\mu g\ ml^{-1}$ for 24 h. These concentrations are well below the European guidance levels for copper in the applied feed solution (0.004 $g\ l^{-1}$ = 4 $\mu g\ ml^{-1}$). This year the work on copper was extended to look at the effects on other stages of the life cycle of *Pythium* i.e. mycelial growth and sporangial development as measured by zoospore production. Preliminary tests to assess any sequestration of copper to growing medium using atomic absorption analysis were also conducted. Copper was applied in the form of copper sulphate ($CuSO_4$), as this was the most likely form of copper to be present in pot and bedding irrigation systems as a component of the micronutrients in feed solutions.

Materials and Methods

Effect of copper on the viability of zoospores in water:

The *Pythium* isolate used in the experiment was obtained from Mypex matting at a commercial grower's holding and belonged to *Pythium* Group G. Zoospores were produced by flooding a V8 culture in a Petri dish with sterile distilled water and incubating overnight at room temperature. Appropriate concentrations of copper sulphate (Table 8) were prepared in 200 ml of SDW and zoospores were then added at the rate of $3x10^4\ ml^{-1}$. There were three replicates of each concentration. Samples were taken from each concentration at 1 and 24 hours after addition of copper, for colony forming unit (cfu) counts and filtration. The samples (0.1 and 0.5 ml) for cfu counts were spread directly on CMA amended with pimaricin (100 $mg\ l^{-1}$) and rifamycin (30 $mg\ l^{-1}$). The plates were then dried in a laminar flow hood and incubated at room temperature over night. Colonies per plate were counted and from these mean cfu counts per concentration were determined. Samples for filtration (20 ml) were passed through a 5.0 µm cellulose nitrate membrane filter which was then cut into 4 pieces for plating on CMA amended with the antibiotics as described above. After incubation at room temperature over night, the number of pieces of filter that produced mycelial growth was recorded.

Effect of copper on the production of zoospores:

The same isolate of *Pythium* Group G as described above was used for this study. The isolate was grown on V8 agar (9 ml) and the cultures were flooded with solutions (40 ml) containing different concentrations of copper sulphate (Table 8). There were three replicate plates for each concentration. Samples were taken from each plate for haemocytometer and cfu counts 1 and 24 hours after the production of zoospores had started in the control treatment. The samples for cfu counts were diluted if necessary and then spread on CMA amended with the antibiotics as described above. The plates

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were then dried in a laminar flow hood and incubated at room temperature over night. Colonies per plate were counted and from these mean cfu counts per concentration were determined.

To determine the actual concentration of available copper in each solution used for zoospore production, samples were taken from each solution, filtered through filter paper (Whatman No. 1) and frozen at –20°C for later determinations of copper concentrations by atomic emission spectrophotometry.

To assess the effect of copper on the survival of the mycelium that had been used for zoospore production, samples were taken in two ways. Firstly, 3 plugs were cut out and secondly 3 samples of mycelium were scraped off each plate after the 24 hour sampling and plated on CMA amended with antibiotics as above.

Effect of copper on the growth of mycelium:

Six *Pythium* isolates (*Pythium sylvaticum*, *Pythium intermedium*, *Pythium ultimum* var. *ultimum* and *Pythium* Groups F, G and HS) were tested for their sensitivity to copper in agar plate tests. The isolates were obtained either from plant material or structures at commercial growers' holdings. A stock solution of copper was prepared in SDW using copper sulphate, then diluted 2- and 10-fold and mixed with CMA to achieve the required concentrations (100, 50, 10, 5, 1, 0.1, 0.01, 0 μg copper ml^{-1}). There were four replicate plates for each concentration/isolate combination. Each plate was inoculated at the side with a 5 mm diameter mycelial disc cut from V8 cultures. The plates were incubated at room temperature for up to 4 days. Colony growth was measured when growth of controls had almost reached the furthest edge of the plate. The amount of available copper in the agar was determined by incubating pieces of agar (5 g) of each concentration in SDW (12 ml) over night at room temperature. After incubation the agar was removed by filtering the solutions through filter paper (Whatman No. 1). The filtrates were frozen at –20°C for later determinations of copper concentration by atomic emission spectrophotometry. The data was analysed, using the concentrations of available copper, with the Genstat 5 programme which fitted logistic curves and calculated the ED_{50} values of copper for the *Pythium* isolates.

Binding of copper in growing media:

Aliquots of Levington F1 compost (80 ml) were placed in glass funnels (160 ml) lined with Nylon gauze. Solutions with different concentrations of copper (0, 0.01, 0.05, 0.1, 0.5, 1, 5 μg copper ml^{-1}) were prepared in distilled water (DW) using copper sulphate. The solutions were passed through compost at the speed of 80 ml in 10 min. The flow rates were adjusted by using a bulldog clip to restrict the aperture in a silicone rubber outlet tube attached to each funnel. Samples of each suspension were collected and filtered through filter paper (Whatman No. 1). The composts in the funnels were resuspended in DW (160 ml), shaken thoroughly and left for 20 min. The resuspended composts were then filtered as above and samples of each suspension collected. All the samples were frozen at -20°C for later determinations of copper concentration by atomic emission spectrophotometry. There were three replicates for each concentration.

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Results and Discussion

Effect of copper on the viability of zoospores in water:

As seen in year 2 experiments, copper was effective against zoospores of *Pythium* Group G at very low concentrations in pure water (Table 8). At concentrations down to 0.025 µg copper ml^{-1} it killed zoospores within 24 h, and only at 0.5 µg ml^{-1} was there any indication of zoospore survival after just 1 h exposure. After 24 hours, no viable zoospores were detected in any of the copper solutions tested (Table 8), whilst many viable zoospores were detected in the untreated controls.

Effect of copper on the production of zoospores:

The onset of zoospore release was delayed and the rate of release reduced at 1.13 µg ml^{-1} available copper (2 µg ml^{-1} total copper) (Table 9). No zoospores were released at 1.88 µg ml^{-1} available copper. All the agar plugs and mycelial samples taken after treatments with copper were able to grow when transferred to fresh agar plates indicating that mycelium was able to survive exposure to these concentrations of copper.

The rate of copper binding to V8 agar, as determined by differences between the total copper and available copper concentrations (Table 9), appeared to vary between concentrations; up to 64 % of the copper applied was bound at some concentrations tested.

Effect of copper on the growth of mycelium:

Higher concentrations of copper were required to inhibit mycelial growth than was required to kill zoospores or prevent their production. ED_{50} values of copper for suppressing mycelial growth ranged from 3.3 to 7.5 µg ml^{-1} for the *Pythium* isolates tested (Table 10).

Binding of copper in growing media:

Preliminary tests with Levington F1 compost showed that copper was quickly bound to the compost (Table 11). Small amounts of copper were released into the wash solution indicating that the copper was quite strongly bound. The rate of binding was dependent on the concentration of copper applied. More copper was lost at the higher concentrations than at the lower ones.

These results indicate that the incidental presence of copper in irrigation water as part of the micronutrients component of a nutrient feed, may be effective in controlling the spread of *Pythium* zoospores in water. Copper compounds may not be legally added to irrigation water or feed solutions for the sole purpose of disease control in plants. In addition to this, if the fungus is already present in compost, it is likely that much higher concentrations of copper would be necessary to give control than was effective in water alone. However, levels of copper capable of achieving some measure of control may be achieved by the application of an approved copper fungicide (eg.

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Croptex Fungex) to the affected plants. The efficacy and possible phytotoxic effects of copper fungicide applications was not investigated in this study.

Table 8. Effects of copper (as copper sulphate) on survival of zoospores of *Pythium* Group G in water measured by membrane filtration and direct plating.

	Survival of zoospores			
	1 h exposure		24 h exposure	
Concentration of copper (μg ml^{-1})	Membrane filtration (%)	Direct plating (cfu ml^{-1})	Membrane filtration (%)	Direct plating (cfu ml^{-1})
0	100	602 (±42.0)	100	317 (±81.9)
0.025	0	0	0	0
0.05	8	0	0	0
0.1	0	0	0	0
0.25	0	0	0	0
0.5	0	0	0	0
2	0	0	0	0
4	0	0	0	0

Table 9. Effects of copper (as copper sulphate) on production of zoospores of *Pythium* Group G in water measured by direct plating.

		Production of zoospores			
		1 h exposure		24 h exposure	
Concentration of total copper (μg ml^{-1})	Concentration of available copper (μg ml^{-1})	Haemocyto-meter count Zoospores (x 10^5) ml^{-1}	Direct plating Cfu (x 10^4) ml^{-1}	Haemocyto-meter count Zoospores (x 10^5) ml^{-1}	Direct Plating Cfu (x 10^4) ml^{-1}
0	0	1.8 (± 0.21)	1.3 (± 0.87)	8.8 (± 2.84)	3.4 (± 0.84)
0.025	0.02	*	0.6 (± 0.28)	7.6 (± 3.15)	1.9 (± 1.02)
0.05	0.03	*	0.8 (± 0.48)	5.9 (± 1.73)	1.1 (± 0.95)
0.1	0.04	*	1.1 (± 0.80)	6.3 (± 1.68)	1.7 (± 0.80)
0.25	0.12	*	0.7 (± 0.17)	2.2 (± 1.41)	0.6 (± 0.35)
0.5	0.18	*	1.1 (± 0.87)	*	0.5 (± 0.29)
2	1.13	0	0	*	0.009 (± 0.007)
4	1.88	0	0	0	0

* < 1.8 x 10^5 zoospores

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Table 10. Effects of copper on mycelial growth of *Pythium* Group F on agar.

Species/Group of *Pythium*	ED_{50} (µg ml^{-1})
Pythium Group G	4.02 (± 0.079)
Pythium Group F	3.34 (± 0.021)
Pythium Group HS	7.52 (± 0.000)
P. sylvaticum	4.83 (± 0.107)
P. intermedium	6.33 (± 0.055)
P. ultimum var. *ultimum*	6.13 (± 0.063)

Table 11. Concentration of copper (µg ml^{-1}) in water after passing through 80 ml of Levington F1 compost (mean of 3 reps.).

Concentration of copper in inflow (µg ml^{-1})	Concentration of copper (µg ml^{-1}) in	
	Elution [a]	Wash [b]
0	0.009	0.004
0.01	0.033	0.000
0.05	0.035	0.008
0.1	0.055	0.009
0.5	0.227	0.013
1	0.224	0.011
5	0.852	0.020

a Solution after passing through Levington F1 compost

b Following elution the compost was washed in 160 ml of distilled water and filtered through filter paper

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PART V – Potential for biological control of *Pythium* with mycoparasitic *Pythiums*

Introduction

As the current fungicides are not always effective against *Pythium* in ornamentals production and there is pressure to reduce chemical applications in general, the industry needs other methods to exclude plant pathogenic pythia from crop production systems. Treatment of surfaces with sterilants will decrease inoculum levels but this may not be enough to provide protection for the life of the crop. The use of biological control fungi as compost additives may have considerable potential in the relatively controlled environment of the glasshouse.

The fungal Genus *Pythium* contains a number of mycoparasitic species (i.e. fungi which parasitise other fungi). Plaats-Niterink (1981) lists *P. oligandrum*, *P. periplocum* and *P. acanthicum* as mycoparasites of various *Pythium* species including *P. ultimum*. *P. nunn* was identified as a new species in 1984 (Lifshitz *et al.*, 1984), isolated from a *Pythium*-suppressive soil. Other species of *Pythium* for which some degree of mycoparasitism has been observed are *P. acanthophoron* and *P. mycoparasiticum* (Lodha and Webster, 1990; Mulligan, Jones and Deacon, 1995).

In year 2 of PC97a, it was observed that many crops started off *Pythium*-free but became infected after a number of weeks in the nursery. To mimic this situation, a system was devised to sow seed into pathogen-free compost containing mycoparasitic *Pythium* and then expose the compost to external sources of pathogenic *Pythium*. This was done using an inoculated sand tray system similar to that described in the fungicide efficacy tests above. Modular trays (6 x 50 ml cells per tray) containing compost and mycoparasitic *Pythium* spp. were placed on an inoculated sand mixture containing *Pythium* Group HS (identified as a frequently occurring pathogenic species in year 1 of PC 97a).

Materials and methods

Production of mycoparasite inocula:

Three *Pythium* species known for their mycoparasitic ability were used in this study: *P. periplocum* (202312) and *P. oligandrum* (PO14) were obtained from the HRI culture collection, and *P. nunn* was obtained from Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. The cultures were maintained on V8 juice agar plates amended with rifamycin (30 mg l^{-1}) and for inoculum production, the isolates were grown in a solid-substrate consisting of a 1:2:1 (v/v) maize-meal (Natural World, Birmingham): perlite (Silvaperl Standard Grade) : tap water medium, with 3 % rapeseed oil (Goldenfields, Merseyside). Extra medium was made up for uninoculated controls, for use in the growth room trial. The medium was autoclaved at 121°C for 15 minutes on two consecutive days and allowed to cool. The medium was inoculated with the *Pythium* species at intervals to allow for their different growth rates. *P. nunn* was grown for 6 weeks, *P. periplocum* and *P. oligandrum* for 3 weeks. Each bottle (containing 480 ml medium in total) was inoculated aseptically with three

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1 cm^2 plugs of agar taken from the edge of an actively growing colony on V8 juice agar, and incubated at room temperature.

***Production of plant pathogenic* Pythium *inoculum*:**

One isolate of *Pythium* Group HS (from PC 97a culture collection) was used to assess the ability of the mycoparasites to suppress disease. This isolate was previously shown to be highly pathogenic to Pelargonium, reducing emergence from 69 % to 13 % in the PC 97a year 1 growth room trial. This isolate was grown in 3 % (v/v) maize meal (Natural World, Birmingham) / sand (Hepworth Minerals and Chemicals, Cheshire) flasks for 3 weeks at room temperature.

***Growth room trials*:**

Before setting-up the experiment, colony forming unit counts were carried out on all three mycoparasites and the phytopathogenic *Pythium* inocula. A ten-fold dilution series (2 replicates) of each inoculum was prepared from 10 g in 100 ml (10^{-1}) down to 10^{-5}. The 10^{-1} dilutions were shaken for 10 minutes on a wrist action shaker, all other dilutions were shaken for 5 minutes. An aliquot (0.2 ml) of each replicate was pipetted onto 2 cornmeal agar (Difco) plates amended with rifamycin (30 mg l^{-1}) and pimaricin (100 mg l^{-1}) distributed on the agar with a spreader. Plates were allowed to dry before storing upsidedown at room temperature. Colonies were counted within 48 hours and the number of cfu g^{-1} inoculum was determined.

Treatments for the growth room experiment were as follows (4 replicate trays per treatment):

1. Uninoculated compost on uninoculated sand
2. *P. nunn*-inoculated compost on uninoculated sand
3. *P. oligandrum*-inoculated compost on uninoculated sand
4. *P. periplocum*-inoculated compost on uninoculated sand
5. uninoculated compost on HS-inoculated sand
6. *P. nunn*-inoculated compost on HS-inoculated sand
7. *P. oligandrum*-inoculated compost on HS-inoculated sand
8. *P. periplocum*-inoculated compost on HS-inoculated sand

Mycoparasitic *Pythium* species were added to compost (Levington F1) at 2.0 x 10^3 cfu g^{-1} fresh compost (equivalent to 5.6 x 10^3 cfu g^{-1} dry compost) where possible. Uninoculated maize meal / perlite was added to compost as a control (uninoculated compost). The compost mixtures were placed into the modular trays (6 cells per tray) and 2 seeds were sown per cell. The external pathogenic *Pythium* source consisted of sand (dried silica sand (Hepworth Minerals and Chemicals, Cheshire), autoclaved at 134°C for 30 minutes) mixed with *Pythium* Group HS maize meal / sand inoculum at 3.7 x 10^2 cfu g^{-1} dry sand. The controls had an equal quantity of uninoculated maize meal / sand. The sand mixtures were levelled out in plastic trays, and 1 set of modules placed into each tray. Trays were arranged in randomised blocks in a growth room set at 20°C±4, with 12 hours light per day.

Emergence counts began on day 3 and seedlings which became diseased (damped-off) during the course of the experiment were removed and plated onto CMA amended with rifamycin (30 mg l^{-1}) and pimaricin (100 mg l^{-1}). After 45 days plant heights

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were measured (from base of stem to tip of highest leaf), and combined dry weights of all plants in each replicate treatment assessed.

At the end of the experiment colony forming unit counts were carried out in the compost and in the sand, for all replicates of each treatment. 15 g compost (wet weight) or 40 g sand (wet weight) were mixed in 100 ml of sterile distilled water on a wrist action shaker for 10 min, and 4 spread plates prepared per replicate, as before. For each replicate of each treatment, two spread plates were stored at 20°C (to produce group HS colonies) and two at 35°C (for *P. nunn, P. periplocum* and *P. oligandrum* colonies). The water content of the compost and sand in each replicate was also assessed, for calculating cfu per g dry weight.

Results and Discussion

Colony forming unit counts of Group HS and *P. oligandrum* in inoculum, and in compost and sand were successfully carried out at the beginning and end of the experiment (Tables 12 and 13). However no colonies were retrieved from *P. nunn* or *P. periplocum* inoculum at the start of the experiment, despite mycelial growth being observed in the inoculum. For these two species the same quantities of inoculum were used as for *P. oligandrum*. The colony count results showed that *Pythium* group HS progressed upwards from the sand into the compost. However, the number of group HS colonies in the compost was highly significantly (P 0.001) reduced in the presence of each of the three mycoparasites compared with the group HS only treatment (treatment 5). There were no significant differences in the *P. oligandrum* populations in the presence of HS-inoculated sand compared with uninoculated sand. *P. nunn* was isolated from the compost at the end of the experiment, probably due to the higher incubation temperature of the agar plates.

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Table 12. Colony forming unit counts of *Pythium* Group HS in both compost and sand at the end of the experiment (mean of all replicate treatments)

Treatment	Number of cfu of *Pythium* Group HS (/g dry weight) In compost	In sand
Uninoculated compost on uninoculated sand	0	0
P. nunn-inoculated compost on uninoculated sand	0	0
P. oligandrum-inoculated compost on uninoculated sand	0	0
P. periplocum-inoculated compost on uninoculated sand	0	0
Uninoculated compost on HS-inoculated sand	3074	122
P. nunn-inoculated compost on HS-inoculated sand	1937	89
P. oligandrum-inoculated compost on HS-inoculated sand	1772	63
P. periplocum-inoculated compost on HS-inoculated sand	1354	138
LSD P 0.05	522.2	
P 0.01	698.7	
P 0.001	917.5	

Table 13. Colony forming unit counts of mycoparasitic *Pythiums* in both compost and sand at the end of the experiment (mean of all replicate treatments)

Treatment	Number of cfu of mycoparasitic *Pythiums* (/g dry weight) In compost	In sand
Uninoculated compost on uninoculated sand	0	0
P. nunn-inoculated compost on uninoculated sand	61	0
P. oligandrum-inoculated compost on uninoculated sand	315	0
P. periplocum-inoculated compost on uninoculated sand	0*	0*
Uninoculated compost on HS-inoculated sand	0	0
P. nunn-inoculated compost on HS-inoculated sand	71	0
P. oligandrum-inoculated compost on HS-inoculated sand	412	2
P. periplocum-inoculated compost on HS-inoculated sand	0*	0*
LSD P 0.05	270.4	
P 0.01	361.8	
P 0.001	475.1	

*unable to count colonies using spread plate method

Emergence of seedlings began on day 2 and was largely completed by day 21 (Figure 8). The first seedlings began to damp-off after 6 days (in treatment 5). No new disease symptoms were observed in the last 2 weeks of the experiment. The presence of *Pythium* group HS alone (treatment 5) caused a significant increase (P 0.05) in the time to 50 %

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emergence compared to all other treatments (Figure 8). The number of healthy plants in this treatment was also significantly lower (P 0.05) than the other treatments (Figure 9).

All three mycoparasite treatments produced an improvement in seedling health compared to treatment 5.

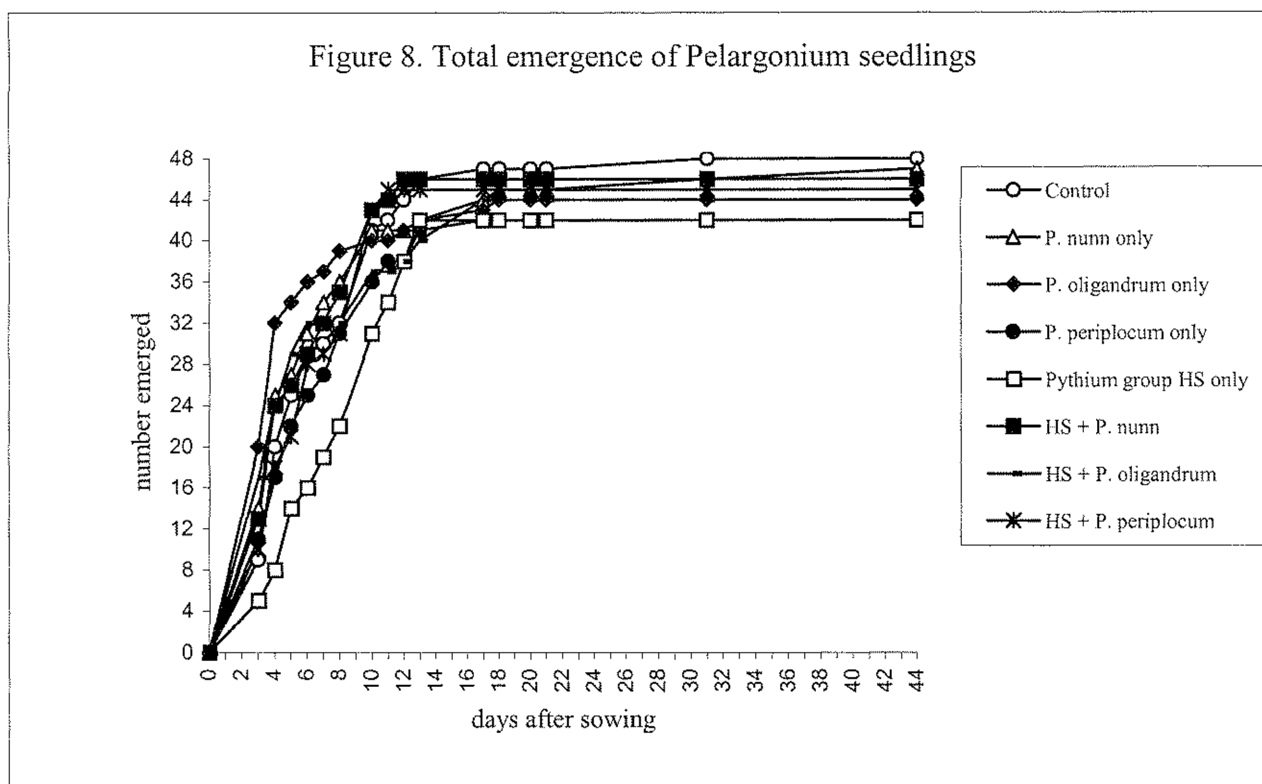

Figure 8. Total emergence of Pelargonium seedlings

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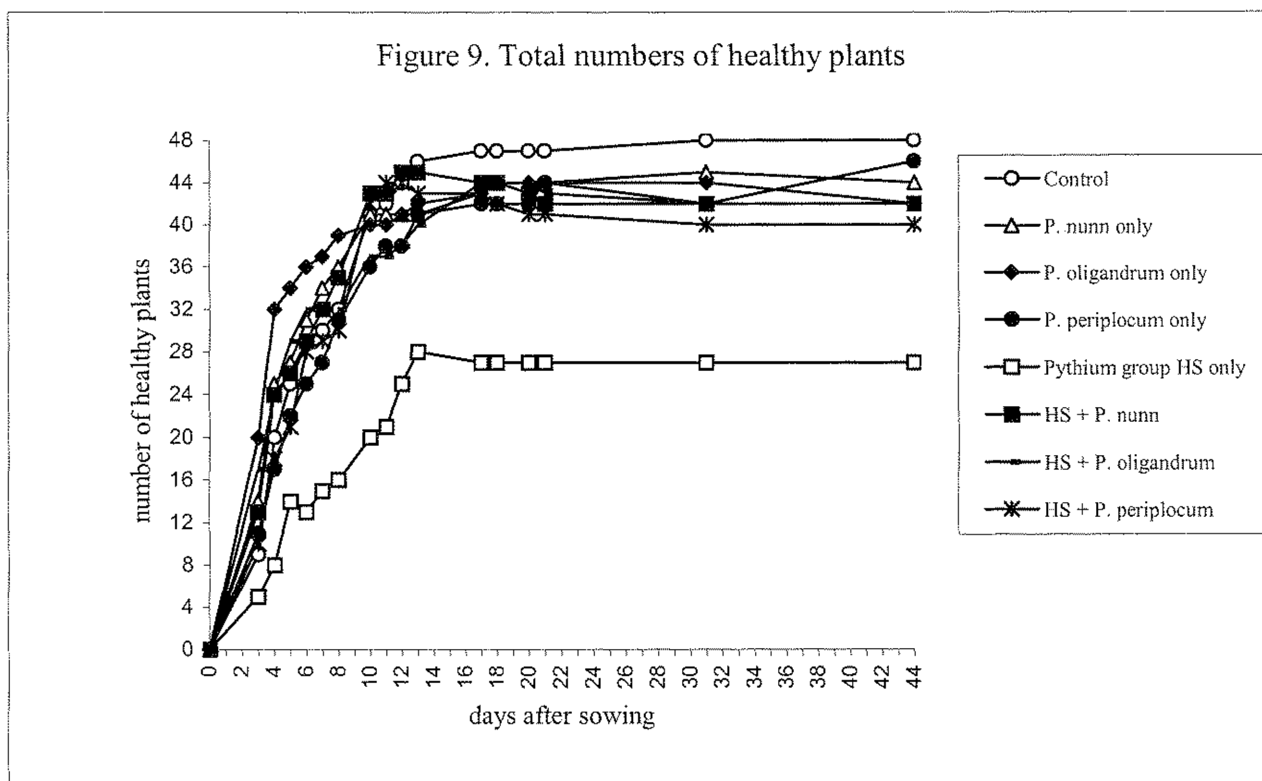

Figure 9. Total numbers of healthy plants

There was no difference in either the plant heights or plant weights between the uninoculated control and the HS infested control. However, *P. nunn* incorporated into the compost produced an increase in both these factors (Table 14).

Table 14. Mean height (mm) and weight (g) of seedlings in each treatment

Treatment	Mean Height (mm)	Mean weight (g)
Uninoculated compost on uninoculated sand	46.19	0.0982
P. nunn-inoculated compost on uninoculated sand	55.73	0.1381
P. oligandrum-inoculated compost on uninoculated sand	48.02	0.1046
P. periplocum-inoculated compost on uninoculated sand	51.34	0.1025
Uninoculated compost on HS-inoculated sand	44.31	0.1024
P. nunn-inoculated compost on HS-inoculated sand	48.63	0.1212
P. oligandrum-inoculated compost on HS-inoculated sand	45.64	0.1047
P. periplocum-inoculated compost on HS-inoculated sand	43.80	0.0961
LSD P 0.05	4.497	0.0234
P 0.01	6.121	0.0318
P 0.001	8.257	0.0430

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Plate 1. Comparison of single replicate trays containing (left) 'uninoculated compost on uninoculated sand', (centre) 'uninoculated compost on HS-inoculated sand' and (right) '*P. oligandrum*-inoculated compost on HS-inoculated sand'.

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Plate 2. Comparison of single replicate trays containing (left) 'uninoculated compost on uninoculated sand', (centre) 'uninoculated compost on HS-inoculated sand' and (right) '*P. nunn*-inoculated compost on HS-inoculated sand'.

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Plate 3. Comparison of single replicate trays containing (left) 'uninoculated compost on uninoculated sand', (centre) 'uninoculated compost on HS-inoculated sand' and (right) '*P. periplocum*-inoculated compost on HS-inoculated sand'.

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CONCLUSIONS

Sources and spread of *Pythium* spp.

- *Pythium* species are very widespread on UK pot and bedding plant nurseries.

- 21 different species and groups of *Pythium* were identified during the course of these studies. The most frequently detected species were: *Pythium sylvaticum, P. paroecandrum, Pythium* group F, and *Pythium* group HS (this latter group are generally considered to be asexual isolates of *P. ultimum*).

- Of the isolates collected in this work, 46% were pathogenic to pelargonium, and 56% to antirrhinum at 25°C.

- The most virulent isolates under the test assay conditions used were from *Pythium* group HS, *P. ultimum* var. *ultimum, P. intermedium, P. sylvaticum, P. ultimum* var. *sporangiferum* and *P. irregulare.* However, the virulence of each species/isolate did vary according to temperature, irrigation regime and host species.

- Surveys of nurseries showed that the main locations where contamination with pathogenic *Pythium* propagules was regularly detected were: Danish trolleys, floors, used capillary matting, benches & production troughs, water in puddles and in open reservoirs.

- Some areas of production on nurseries were consistently found to be clean. Importantly these included: unused growing media, unused containers and matting, freshly cleaned and sterilised benches and treated water (mains, chlorine- or Slow Sand Filter treated water).

- Over the entire study *Pythium* spp. were isolated from 38% of all root samples assessed.

- The frequency of isolations peaked in spring and early summer and declined to its lowest point during the winter months.

- The progress of *Pythium* infection was monitored on nurseries in 3 host species: cyclamen, pelargonium and poinsettia. Crops of all three species started clean. However, at the point-of-sale in 2 out of 3 crop species, more than 50% of plants had *Pythium* infections in their roots (cyclamen = 19%; pelargonium = 85%; poinsettia = 63%). A high proportion of these infections were sub-clinical, but this could still have strong quality and time-in-production consequences plus a high risk of symptoms of disease developing once plants are in the retail chain.

- Three immunodiagnostic procedures were assessed for rapid detection of pathogenic *Pythium* propagules: ELISA, dipstick assays and ZTI. ELISA gave disappointing results for two reasons: a) the antibodies raised bound to some non-pathogenic as well as pathogenic *Pythium* spp. and also did not recognise members of the pathogenic *P. aphanidermatum* complex, and b) extracts from *Pythium*-free growing media gave high background absorbance readings, making

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the procedure impractical for on-nursery tests. Dipstick tests were found not to be sensitive enough and often failed to detect *Pythium* propagules when present. The Zoospore Trapping Immunoassay (ZTI) is a very sensitive assay developed to test water samples. This technique was compared favourably with conventional membrane filtration/resuspension plating, giving greater confidence in the sensitivity of both techniques.

Avoiding disease by good hygiene and cultural measures

- Five commercially available surface sterilant chemicals were compared for their efficacy at varying rates against contaminated plant debris and on used *Pythium* infested Mypex matting. These were Bleach (sodium hypochlorite), Jet 5, Panacide M, Panaclean and Ter Spezial. All but Panaclean were effective at cleaning the Mypex matting, but only Bleach and Jet 5 showed any ability to kill *Pythium* propagules contained in plant debris and even these were limited in this.

- Where *Pythium* was present, control of symptoms and a reduction in infection was achieved in antirrhinums by reducing the frequency of irrigation. In the absence of the pathogen, high irrigation frequencies did not appear to stress these plants, but when *Pythium* was present, this irrigation treatment resulted in increased disease and plant loss.

Chemical control measures

- In plate tests, tolerance to furalaxyl was detected in one or more isolates of each of the most frequently isolated *Pythium* spp. from pot and bedding nurseries. These plate tests were confirmed in seedling screens. Reduced sensitivity to furalaxyl is now present on most nurseries and its frequency appears to have increased during the course of this study.

- Results for propamocarb hydrochloride (Filex) were variable. It was the best drench-applied treatment for the control of pre-emergence damping off and achieved reasonable control against post-emergence damping off caused by the majority of *Pythium* isolates in pelargonium seedling screens. However, a small number of isolates did appear to show tolerance to this fungicide in these tests.

- In pelargonium seedling tests, etridiazole (Aaterra) applied according to label recommendations as a growing medium incorporation, gave excellent control of post-emergence damping off. Aaterra proved less effective when used as a drench treatment.

- Four surfactants were tested against *Pythium* zoospores in water: Agral, Enhance, Activator 90 and PBI Spreader. Agral and Enhance were effective at killing zoospores at low concentrations (40ppm), whereas Activator 90 and PBI Spreader required 10 times this concentration for the same effect.

- Direct kill of zoospores in water requires concentration of 0.1 $\mu g\ ml^{-1}$ copper ions, but to suppress other stages of the *Pythium* lifecycle required higher concentrations (3-15 $\mu g\ ml^{-1}$). In peat-based growing media, the majority of the

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copper ions in solution rapidly bound to the organic matter of the medium. Copper compounds may not be legally added to irrigation water or feed solutions for the purpose of disease control in plants. However, the incidental presence of copper in the micronutrient component of a nutrient feed solution (subject to phytotoxicity levels and the EEC maximum guidance level of 4 $\mu g\ ml^{-1}$) may have some impact in controlling the spread of *Pythium* zoospores. Applications of copper-based commercial fungicides may also achieve some control, although the study of this was beyond the scope of the present project.

Biological control measures

- In pelargonium seedling tests, the presence of the mycoparasitic *Pythium* species *P. oligandrum, P. periplocum* or *P. nunn* reduced damping off. Further work is required to develop a commercially applicable control method using these organisms.

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