Project title:	Tomato and Cucumber: An investigation into the commercial significance of *Pythium* species in the propagation and production phases of hydroponic crops.
Project number:	PC 97b
Project Leader:	Dr G Martin McPherson
Report:	Final Report, October 2001
Previous Reports:	Annual Reports (December 1998, December 1999)
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Location:	HRI Stockbridge House HRI Wellesbourne Various propagation, tomato and cucumber nurseries (confidential)
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Date project commenced:	1 January 1998
Date completion due:	31 March 2001
Keywords:	Cucumber, tomato, rockwool, hydroponic, *Pythium*, *Pythium aphanidermatum*, root rot, propagation, replanting, Filex, Proplant, propamocarb HCl, Aliette, fosetyl aluminium, Amistar, azoxystrobin, SL567A, metalaxyl-M, KIF 230

The results and conclusions in this report are based on number of laboratory and glasshouse based experiments. The conditions under which these experiments were carried out and the results have been reported with detail and 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

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

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PRACTICAL SECTION FOR GROWERS

Commercial Benefits of the Project

This project has provided new information on the prevalence and diversity of *Pythium* spp. found on tomato and cucumber nurseries in the UK. The work has given a better understanding of the role and potential importance of individual *Pythium* spp. in hydroponic production systems. Some novel fungicides have been identified with potential to control *Pythium* in hydroponic crops of tomato and cucumber.

Background and Objectives

Pythium spp., as oomycetes or 'water-moulds', are generally well adapted to the aqueous environment found in hydroponic production systems worldwide. Normal growth of the fungus is by thread-like structures known as mycelium, but multiplication and spread over large distances for many species of *Pythium* is through the production of swimming spores called zoospores. Zoospores may be produced in a short time and in very large numbers and therefore in recirculating systems such as NFT can infect every plant within hours. Modular 'run-to-waste' growing systems based on rockwool slabs undoubtedly reduce the risk of such an extensive spread though there are a number of different ways *Pythium* spp. can spread from module to module.

The visible effects of *Pythium* on cucumber and tomato crops examined in this study were quite different. In cucumber, *P. aphanidermatum* is well recognised almost everywhere cucurbits are grown. It is an aggressive pathogen consistently causing establishment problems particularly during the summer replanting period when the ambient temperature is near optimum for the fungus. In tomato, *Phytophthora* spp. have tended to be the most predominant and aggressive oomycete root pathogens. However, in the last few seasons, *Pythium* spp. have become more problematic in numerous UK tomato crops and plant establishment and subsequent yield and quality has been markedly affected on several nurseries. Whilst *Pythium* spp. are known to occur in both tomato and cucumber crops they have previously not been well documented (ie identified to species level) and their significance (with the exception of *P. aphanidermatum* in cucumber) with respect to plant establishment and subsequent crop performance has not been fully investigated.

Pythium spp. form a very diverse group (>80 species and groups) and, unlike *Phytophthora* spp., individual species may either be highly pathogenic, weakly pathogenic, non-pathogenic or even beneficial on a particular host. It is therefore unsatisfactory to know merely whether *Pythium* is present as it would be inappropriate to adopt counter measures (eg apply a fungicide) if the species present was non-pathogenic or beneficial. Also, fungicides (eg Filex/Proplant, propamocarb hydrochloride; Aaterra, etridiazole) have tended to be used prophylactically in hydroponic crops for many years and yet little monitoring of their efficacy has been undertaken. Numerous anecdotal reports suggest that they are less effective than previously thought and this indicates, perhaps, that fungicide resistance or insensitivity may have occurred in pathogen populations.

The overall objectives of this project were:

1. To determine the prevalence and diversity of *Pythium* spp. on the roots of plants from a range of cucumber and tomato nurseries including propagation facilities.
2. To determine the primary source(s) of the *Pythium* spp. on propagation and cropping nurseries.
3. To determine the pathogenicity of individual species using a laboratory bioassay followed by artificial inoculation of the most prevalent species onto young plants under commercial growing conditions.
4. To examine the sensitivity of species recovered to the fungicides propamocarb hydrochloride and etridiazole.
5. To examine whether various bio-control treatments or cultural practices can be successfully deployed to minimise the effects of the *Pythium* spp. on the host.

Summary of Results

Pevalence and diversity of Pythium spp. on the roots of plants from a range of cucumber and tomato production nurseries and propagation facilities

Propagation units

On propagation nurseries there was a greater range of *Pythium* isolates recovered from cucumber than tomato plant material, although the levels of *Pythium* isolated varied between the nurseries sampled. The majority of *Pythium* isolates were identified as *Pythium* 'mycelium only'[1] and generally were not pathogenic in the seedling laboratory bioassay.

In contrast, plant material collected from UK nurseries originating from Dutch propagators was found to have very high levels of *Pythium* comprising mainly of *Pythium* 'mycelium only' and *P. rostratum* with *P. paroecandrum* isolated at lower levels. The majority of *Pythium* 'mycelium only' isolates from Dutch sources proved pathogenic during laboratory testing.

On a positive note, *P. aphanidermatum* was not isolated from any plant material sampled from UK propagation nurseries.

Cucumber nurseries

On the cucumber nurseries the levels of *Pythium* generally built up through the season reaching high levels, with *Pythium* 'mycelium only' being present in the largest proportion, with the majority of those tested showing pathogenicity towards cucumber seedlings. On one cucumber nursery, high levels of *P. aphanidermatum* were detected towards the end of the second crop. The pathogenicity of isolates towards cucumber seedlings varied between species and from each nursery.

[1] This group identified as *Pythium* 'mycelium only' covers a range of isolates with no oogonia. It is difficult to distinguish them into individual species and so they are grouped on their physical structure. *Pythium* 'mycelium only' isolates can often be distinguished into *Pythium* Group F, *Pythium* Group T or *Pythium* Group G based on the structure and formation of the sporangia. However, this identification of *Pythium* 'mycelium only' into groups is not necessarily based on levels of pathogenicity. *Pythium* Group HS is made up of isolates with hyphal swellings and no sporangia are produced in culture.

Tomato nurseries

Pythium was consistently isolated from two of the three commercial tomato nurseries sampled. Overall, the range of *Pythium* spp. isolated was much narrower than from cucumber nurseries with *Pythium* 'mycelium only' consistently being isolated throughout the growing season; the majority of which, when screened, were pathogenic towards tomato seedlings. On one nursery no *Pythium* was detected from plant material until the last sampling date in the autumn, while another nursery had very high levels of *Pythium* during the whole season with up to 94% positive isolations for *Pythium*.

Primary source(s) of the Pythium spp. on propagation and cropping nurseries

Potential sources of *Pythium* around the nurseries were also identified with *Pythium* 'mycelium only' again the most commonly isolated. *Pythium* was isolated from a wide range of sources, some of which could be more important in leading to plant infection than others. As propagules of *Pythium* spp. can be disseminated rapidly in water, detection of *Pythium* spp. in water sources and storage tanks on each nursery is important because contaminated water could easily be applied to the crop without disinfection treatment. However, the *Pythium* spp. isolated from water sources or storage facilities sampled were not pathogenic to seedlings in the laboratory bioassay.

Pythium was isolated from sciarid flies collected from irrigation tanks on one of the propagators, although this isolate of *Pythium* 'mycelium only' was not subject to pathogenicity screening. *Pythium* isolated from key pieces of equipment on propagation nurseries such as seeder machines or from tray washer machines could potentially be important in the rapid spread of *Pythium* to plant material. However, no indication of spread was confirmed on these nurseries during the audit. *Pythium* was also readily detected around the nurseries on pathways in the crops as well as roads indicating potential sources for *Pythium* to spread to crops. Run-off puddles in paths and gutters also proved a source of *Pythium* on nurseries.

Testing the pathogenicity of Pythium species

The most prominent *Pythium* species identified in Year 1 of this project were subjected to replicated pathogenicity screening on young cucumber & tomato plants. Isolates of *P. aphanidermatum* screened severely reduced leaf area, plant height and plant quality of both tomato and cucumber plants. Isolates originating from tomato or cucumber plants were pathogenic to either tomato or cucumber hosts. Isolates of *Pythium* Group F were not pathogenic on cucumber plants in either the spring or summer propagation periods. However, both *Pythium* Group F isolates significantly reduced the growth of tomato plants although this reduction was not as great as with either *P. aphanidermatum* isolates.

In an unreplicated screen carried out in Spring, isolates of *P. tracheiphilum*, *P. ultimum* and *P. sylvaticum* reduced plant growth and plant quality of tomato and cucumber. Yet, during the summer propagation period, *P. ultimum* and *P. sylvaticum* reduced cucumber plant growth although the effect was less marked and no differences in plant dry weight were recorded. Surprisingly the isolate of *Pythium* Group HS tested, a group usually noted for its pathogenicity, had no deleterious effect on plant growth.

Pathogenicity assessments on a range of *Pythium* 'mycelium only' isolated in Year 2, which displayed pathogenicity in the seedling bioassay, were further studied on propagation plant material. There were no significant reductions in growth on cucumber compared to the uninoculated control. However, three of the four *Pythium* 'mycelium only' isolates on tomato plants led to significant reductions in leaf area, dry weight and root assessments. In addition, *P. middletonii* and *P. paroecandrum,* both isolated from cucumber caused an increase in root discoloration on tomato plants.

Two long-season trials were conducted to assess the pathogenicity of the most commonly isolated *Pythium* species from both cucumber and tomato crops in the *Pythium* audit conducted during this project. *P. aphanidermatum* severely affected both crops from the time of inoculation and markedly reduced plant growth from which the cucumber plants never recovered. By the termination of the trials the yield of both tomato and cucumber was significantly reduced on plants inoculated with *P. aphanidermatum.* None of the other *Pythium* spp. introduced into either the tomato or cucumber crop had such a deleterious effect on crop development and can therefore be regarded, at best, as weak opportunist pathogens.

Occasional species, notably *P. ultimum*, *P. monospermum*, *P.* Group HS, *P. middletonii* did have a slight effect on the overall yield in the cucumber crop, but their impact was nowhere near as great as *P. aphanidermatum*. Because these trials were conducted during the summer period when ambient temperatures were relatively high we cannot necessarily assume that these other species would not have been more damaging at other times of year. Conversely, this was amply demonstrated by *P. aphanidermatum* which, when inoculated under cooler conditions during November-January, failed to cause appreciable disease. Also, it has not been possible to evaluate the potential cumulative effect of these *Pythium* spp. were they to occur simultaneously in the natural environment.

Sensitivity of Pythium spp. to the fungicides propamocarb HCl and etridiazole

In year 1 of the project, representative isolates of *Pythium* of each type/species were tested for their response to propamocarb hydrochloride (Filex/Proplant) and to etridiazole (Aaterra).

All isolates of *P. aphanidermatum* isolated from cucumber nurseries were sensitive to propamocarb hydrochloride. *Pythium* Group F isolates showed a wide range of responses. Single isolates of *P. ultimum* and *P. tracheiphilum* were also sensitive to propamocarb hydrochloride.

Isolates of *P. aphanidermatum* from tomato nurseries all showed sensitivity to propamocarb hydrochloride. However, isolates of *P. aphanidermatum* and *Pythium* Group F showed a range of sensitivities to etridiazole (Aaterra).

Evaluation of novel fungicides and biocontrol agents for the control of Pythium in hydroponic crops of tomato and cucumber

A range of fungicides including approved products (ie those with a UK registration on one or more crops) together with a range of novel fungicides (no current UK registration) were screened for their efficacy against *Pythium* spp. on cucumber plants in the propagation phase (November 2000 to January 2001). In this study, inoculation with *P. sylvaticum* caused a significant difference in plant growth, whereas inoculation with *P. aphanidermatum* did not. This is likely to be due to different optimum conditions for infection and development of each of the *Pythium* species introduced.

Propamocarb-HCl (Filex/Proplant), a fungicide approved for the control of *Pythium* in hydroponic crops, was effective in these trials. This result backs up the laboratory screening on fungicide sensitivity and to some extent, counters the anecdotal evidence suggesting insensitivity of the fungus to this chemical. Two novel fungicides, azoxystrobin (Amistar) and metalaxyl-M (SL567A) both performed well in the propagation phase. Fosetyl-Al (Aliette) although controlling the disease development caused phytotoxic symptoms on the young plants treated.

The bio-control screen was conducted on tomato plants in the propagation phase during the autumn period when glasshouse temperatures were still quite high (September to November 2000). Under these conditions *P. aphanidermatum* was highly pathogenic significantly reducing plant growth and development. A number of bio-control agents were effective in controlling disease, although not to the same extent as the fungicide treatments. BS-MBI-600, Gliomix, Companion and QRD 713 all showed potential to control *Pythium* infection in hydroponic crops. These bio-control agents would require further development work with respect to dose rate, timing, frequency of application, formulation etc before they could compete effectively with fungicide treatments in terms of overall efficacy.

Action points for growers

- It is important to ensure a high level of hygiene around the nursery prior to planting. This should involve sterilisation/disinfection of glasshouse structures, concrete floors and equipment involved in the growing crop such as irrigation pipes and drippers. Rockwool, if re-used, must be properly sterilised. New polythene floor covering should be used for each crop and laid in a manner to ensure the clean surface is not contaminated with soil and tools sterilised where appropriate.

- Hygiene during crop production is important to reduce the spread of *Pythium,* particularly *P. aphanidermatum*, around nurseries. This includes installing foot dips with disinfectants as well as limiting staff movement between different production areas around the nursery.

- The lack of detection of *P. aphanidermatum* in earlier studies on UK propagation nurseries is particularly encouraging and emphasises the fact that the initial infection by this pathogen would appear to arise on the production nurseries themselves. This re-emphasises the need for strict hygiene precautions, particularly where the site has a history of infection by this pathogen.

- Compost used on propagation nurseries should be contained in a separate designated clean area of the nursery. Levels of sciarid and shore flies, known to spread *Pythium*, should be monitored and controlled where necessary.

- Equipment used for seeding blocks and tray washing should be cleaned regularly to reduce the risk of spread of disease onto plant material carried around the site and between nurseries.

- The observation that *P. aphanidermatum* (by far the most important *Pythium* species in this study) did not cause appreciable disease during low temperature periods provides important information for growers. Firstly, in the early establishment phase of crops the pathogen may be present in a 'latent' symptomless form and therefore may be spread inadvertently around the nursery. **Growers must not assume their crops are free of this pathogen merely because symptoms are not evident.** More likely, the pathogen population in the crop is establishing and being disseminated from plant to plant as the crop matures. As the slabs are re-used for the replant cucumber crops, and susceptible young healthy plants are placed on the same slabs during June-July, when temperatures are much higher, they are more likely to succumb to the disease. Growers can no longer assume that the plants, as delivered, were necessarily already infected.

- Propagators should introduce a routine quality assurance programme which includes regular checks for *Pythium* spp. and other pathogens from water sources and water storage tanks prior to application to the crop. Although no highly pathogenic species of *Pythium* were detected on UK propagation nurseries in this survey, routine monitoring of plant material for the presence of disease prior to dispatch would be a sensible precaution.

- It is clear that within the range of *Pythium species* isolated in this project and screened on long-season crops, the pathogenicity of an individual species varies depending on a number of factors including the susceptibility of plant host, the *Pythium* species involved and the prevailing environmental conditions. As identification of the species of *Pythium* is only possible in the laboratory using microscopic techniques, it is important to ensure that suspect plant material undergoes diagnosis to ensure correct identification of the problem and also that the appropriate course of action, including fungicide treatment, is chosen. For *Pythium* species other than those renowned for causing root damage in hydroponic crops, eg *P. aphanidermatum*, it may be necessary to commission *in vitro* pathogenicity testing to determine the potential significance of the species concerned before fungicide application or alternative control strategies are deployed.

- Results from this project indicate that propamocarb-HCl (Filex/Proplant) continued to be effective against the two *Pythium* species under test and no insensitive/resistant strains or species were detected (albeit on a limited range of isolates of two different *Pythium* species). It is more likely, though by no means certain, that anecdotal reports of poor control following treatment relate more to the timing of application of the product(s) rather than insensitivity of the pathogen to a particular fungicide. It remains possible that other *Pythium* spp., not tested in this study, may be present which are insensitive to fungicide treatment. This would require specific evaluation on a case-by-case basis.

- The fungicides SL567A (metalaxyl M) and Amistar (azoxystrobin) proved effective in controlling *Pythium* in these trials when applied as a drench treatment. Discussions are to be encouraged with the chemical manufacturers regarding the on- or off-label approval for these uses in the future.

- Although a broad range of bio-control products were screened, their performance was not as good as for chemical fungicides during this project. The inherent variation in the performance of individual bio-control agents remains a significant limiting factor. Until such bio-control products can be shown to provide robust reproducible control under the varied environmental conditions experienced regularly in the UK, their use will be limited.

Anticipated practical and financial benefits from this study

In the work reported here the significance of individual *Pythium* species, with the exception of *P. aphanidermatum*, in hydroponic tomato and cucumber crops appears relatively small. Yet, because we do not know their full significance under different climatic conditions and at different times of the year it is difficult to accurately estimate overall losses due to infection by the genus *Pythium*.

However, in the case of the most important single species (*P. aphanidermatum* in cucumber) annual losses, based on the cost of replacement plants, labour and fungicides, have been estimated to be in the region of £0.5M/annum. It would therefore not be unreasonable to expect overall financial benefits in excess of £1M/annum if all pathogenic *Pythium* spp., on both tomato and cucumber, were to be eradicated or effectively controlled in the future. This estimate does not take account of any crop production losses that may occur in crops that are affected, but not killed, by *Pythium*.

The project provides improved knowledge to the industry of species diversity, significance of individual species with respect to pathogenicity, identification and elimination of initial sources of the pathogen and improved control measures. The ultimate benefits overall will allow improved establishment of the crop(s) reducing the reliance on prophylactic fungicide application leading to greater uniformity, higher yields, improved quality, fewer plant losses over the season and reduced pesticide inputs to the crop. This in turn should increase consumer acceptability of the product and benefit the environment by reducing the risk of pollution from contaminated surface waters.

SCIENCE SECTION

Introduction and objectives

In the first two years of this project a wide range of *Pythium* spp. and isolates were collected from a range of propagation and commercial cucumber and tomato nurseries. For future work type cultures were maintained in a culture collection at HRI Wellesbourne.

During the third and final year of this project the aim was to investigate opportunities for minimising initial infection and subsequent establishment of *Pythium* spp. In addition, the epidemiological aspects of specific *Pythium* spp were also investigated.

The scientific targets of this project in the third year were to:

1. Maintain the *Pythium* culture collection collected during the first two years of this project.

2. Minimise the introduction and establishment of *Pythium* spp. in a crop by:
   (i) screening novel fungicides for activity towards oomycetes and
   (ii) introducing antagonistic microbial/bio-control populations.

3. Investigate epidemiological aspects of individual *Pythium* spp. on long-season crops of tomato and cucumber on a semi-commercial scale.

1. Screening novel fungicides for activity towards *Pythium* spp.

Objective

- To screen novel fungicides against two *Pythium* spp. on cucumber plants in propagation.

Materials and Methods

Site and crop details

Crop: Cucumber cv Enigma
Site: Glasshouse M17, HRI Stockbridge House

Treatments

	Treatment/ product	Active ingredient	Application rate[+]
1	Uninoculated control	-	-
2	Inoculated control	-	-
3	Filex	Propamocarb HCl	2.5ml /10 litres water
4	Aliette	Fosetyl Aluminium	31.25g /10 litres water*
5	Amistar	Azoxystrobin	0.5ml /10 litres water
6	SL567A	Metalaxyl-M	1.0ml /10 litres water
7	KIF 230	Experimental fungicide	2.5g /10 litres water

[+] The fungicides were applied to the rockwool blocks as a drench treatment following the label recommended rates where possible.
* Rate derived from rate for compost drenching.

Experimental design & analysis

The trials were arranged in a randomised block design with four replicates per treatment. Each replicate plot consisted of 8 plants (4 plant isolated with each *Pythium* species) raised in rockwool plugs. Each replicate plot was raised above floor level on a slab of new polystyrene and covered in clean polythene sheeting to avoid cross-transfer on inoculum between plots. Plants inoculated with each species were spatially separated by placing a narrow piece of wood under the polythene to avoid possible transfer and cross-contamination in run-off water between inoculated plants. The occurrence of sciarid, or other, flies in the experimental area were monitored using yellow sticky traps and minimised to avoid potential aerial dissemination of *Pythium* spp. Polythene covering the glasshouse floor was sprayed when required with disinfectant (Jet 5) to minimise the development of algae.

Growing environment

The experiments were conducted at the Stockbridge House blueprint temperature regimes for Cucumber at 19/23°C night and day temperatures respectively (with supplementary lighting during propagation).

Crop Diary

Cucumber cv Enigma sown:	21 November 2000
Plant spaced out in glasshouse:	29 November 2000
Cucumber plants inoculated:	30 November 2000
Fungicide treatment applied	30 November 2000
Plant height recorded:	21 December 2000
Plant vigour recorded:	22 December 2000
Plant height, leaf area recorded:	18 January 2001
Plant vigour recorded:	18 January 2001
Root discoloration/root growth assessment:	19 January 2001
Trial termination:	19 January 2001

Inoculation

Cucumber seeds were sown directly into modified propagation blocks. They were modified prior to sowing by removing a core 1 cm in diameter from one side of the rockwool block towards the central well. Around 9 days from sowing a 1cm^2 piece of agar, with mycelium of the *Pythium* isolate under test was positioned beside root tissue in the block and the rockwool core replaced.

Assessments

Agronomic measurements including plant height, stem diameter and leaf area were recorded during the trial to monitor plant vigour. At the termination of the trial root development (scale 0-3), root discoloration (scale 0-3), and the plant dry weight were recorded to measure the effect of the introduced pathogens, in the individual treatments.

The root development and discoloration indices were calculated form a 0-3 assessments as follows:

$$\frac{1(\text{No in category 1}) + 2(\text{No in 2}) + 3(\text{No in 3})}{\text{No of plants assessed}} \times \frac{100}{3}$$

The range of this index was, therefore, 0 (poor development, low discoloration) to 100 (good development, high discoloration)

Statistical Analysis

Analysis of variance on the results from the agronomic assessments was carried out by Biometrics, HRI Wellesbourne. Significance is indicated by - NS no significant difference; * = Significance at the 95% level of probability; ** = Significance at the 99% level of probability; *** = Significance at the 99.9% level of probability. Differences between treatments can be compared (at the 95% probability level) using the Standard Error of Difference.

Storage of Data

The raw data from these experiments will be stored for a period of not less than 5 years in the Archive at Stockbridge House. Access to the data can only be made via the designated Archivist.

Results and Discussion

A range of registered and novel fungicides was screened on cucumber plants in the propagation phase. Inoculation with *Pythium sylvaticum* caused significant differences in agronomic assessments recorded during the trial compared to the uninoculated control treatments. Surprisingly, treatment differences were less marked when plants were inoculated with *Pythium aphanidermatum*.

An assessment of plant height showed significant differences between the uninoculated and inoculated control treatments. Inoculation with *P. aphanidermatum* significantly reduced plant height 3 weeks after inoculation (Table 1) though 6 weeks after inoculation *P. sylvaticum* affected the inoculated plants more severely than *P. aphanidermatum* (Table 2) when compared to the uninoculated control plants. The fungicide treatments had a marked effect on overall plant vigour and even after 3 weeks it was evident that infection was suppressed by some of the fungicide applications. Interestingly, there appeared to be a difference in performance depending on the *Pythium* species concerned. Even though *P. aphanidermatum* did not cause the damage normally expected of this pathogen treatment with Filex, Amistar, SL567A and KIF 230 increased plant height compared to the inoculated control. Where *P. sylvaticum* was introduced, treatment with Amistar and Filex was very effective with measurements of plant height similar to that of the uninoculated control. SL567A, Aliette and KIF 230, although increasing the height of the cucumber plants, were not significantly greater that the inoculated control.

Measurements of leaf area, 6 weeks from inoculation, showed no significant difference between cucumber plants inoculated with *P. aphanidermatum* and the uninoculated control (Table 3), while inoculation with *P. sylvaticum* led to a significant reduction in leaf area (69% of the uninoculated control). Drenching with Filex and, in particular, Amistar following inoculation with *P. sylvaticum* performed well with leaf areas similar to the uninoculated control and significantly greater than the inoculated control (Table 3). The other fungicide treatments although increasing the leaf area compared to the inoculated control, were significantly poorer than those treated with Amistar.

P. aphanidermatum reduced the Plant Vigour Index (Tables 4 and 5), although not significantly compared to the uninoculated control. However, in an assessment conducted 3 weeks after inoculation (Table 4) all of the inoculated plants, including those treated with fungicide, displayed reduced vigour. The effect was less marked by the second assessment after 6 weeks (Table 5) except where Aliette had caused some phytotoxicity symptoms on the young plants. In both assessments *P. sylvaticum* significantly reduced the Plant Vigour Index. Following inoculation with *P. sylvaticum* the Amistar treatment provided the greatest improvement in plant vigour and was not significantly worse than the uninoculated control.

During the trial plants treated with Aliette displayed poor vigour, together with reduced plant height and leaf area. This was recorded from the earliest assessments of cucumber plants inoculated with either species of *Pythium*. It was concluded that this was likely to result from phytotoxicity from the chemical application, possibly as the plants were small when treated with the fungicide and therefore more sensitive to such chemical treatment.

At the termination of the trial, 6 weeks from inoculation, the root development and root discoloration were assessed. Cucumber plants inoculated with *P. sylvaticum* displayed the most significant differences compared to the uninoculated controls and caused a marked reduction in root development and an increase in discoloration (Table 6 and 7). Amistar treated plants developed more root that plants treated with any the other fungicides. Surprisingly, plants inoculated with *P. aphanidermatum* showed no detrimental effect on the root growth due to the low level of pathogenicity expressed in this trial. Again root development of plants treated with Aliette was low but this is relative to the poor levels of growth recorded in the earlier assessments during the trial. The level of root discoloration on control plants inoculated with *P. aphanidermatum* was lower than all the treatments including the uninoculated control (Table 7). However, on closer examination of the inoculated control treatments there were a number of plants with a stem base rot caused by this pathogen. This was not accounted for in the root discoloration score at the base of the propagation block. Treatment with Amistar, Filex or SL567A resulted in the lowest levels of root discoloration.

Both of the *Pythium* spp. used in this study had been shown to be pathogenic towards cucumber seedlings and young plant material in previous experiments in this project (Jackson *et al.* 2000). However, the genus *Pythium* is very diverse with over 80 distinct species, which often have their own optimal environmental conditions for infection and development. It is known that *P. aphanidermatum* is a particular problem during the summer months when the ambient temperature is higher (optimal 35-40°C). Whereas the optimum for *P. sylvaticum* is much lower at between 20-25°C (Van Der Plaats-Niterink, 1981). This could be the reason for the low level of pathogenicity displayed among plants inoculated with *P. aphanidermatum* compared to *P. sylvaticum* where the experiment was conducted in November-January (ie the standard commercial propagation period for new season crops). This also helps explain the increased severity of *P. aphanidermatum* observed commercially at replanting in the summer. It is hypothesised that *P. aphanidermatum* may be present in some of these crops early in the season but not expressed fully due to the low temperature conditions. During replanting it is often claimed that the infection arises as a result of infected young plants introduced onto the nursery but the evidence here suggests that it is more likely that the infection occurring is a result of symptomless infection present on the roots in the slabs from the first crop.

A number of other fungicides (eg fenamidone + mancozeb, fenamidone + fosetyl aluminium, fluazinam, iprovalicarb, trifloxystrobin and zoxamide + mancozeb) which have demonstrated efficacy towards oomycete fungi on a range of crops around the world, were sourced for inclusion in this trials work though ultimately many were not included in the final evaluation for a variety of reasons eg lack of an operator safety data package for use under protection, unlikely to be ultimately registered for use in the UK or manufacturers reluctant/unable to provide samples in time for inclusion in the work.

Filex (propamocarb HCl) performed well in these studies on cucumber plants in propagation. There did not appear to be any indication of resistance towards the *Pythium* isolates used in this study. However, it should be noted that insensitivity of a number of *Pythium* spp. towards propamocarb HCl has been reported elsewhere.

Table 1: Evaluation of novel fungicides to control *Pythium aphanidermatum* and *Pythium sylvaticum* on cucumbers in propagation. Assessment of plant height recorded on 21 December 2000.

Treatment		Plant height (cm)		
		Pythium aphanidermatum	*Pythium sylvaticum*	Treatment Mean
T1	Uninoculated control, water	7.5	6.9	7.2
T2	Inoculated control, water	5.7	6.0	5.8
T3	Filex	8.6	7.5	8.0
T4	Aliette	4.8	5.0	4.9
T5	Amistar	7.1	7.4	7.2
T6	SL567A	7.2	6.6	6.9
T7	KIF 230	8.2	6.6	7.4

To compare 2 different treatments means

Significance	*
SED (18 df)	0.80

To compare *Pythium* isolates within any treatment

Significance	NS
SED (35.2 df)	1.01

Table 2: Evaluation of novel fungicides to control *Pythium aphanidermatum* and *Pythium sylvaticum* on cucumbers in propagation Assessment of plant height on 18 January 2001.

Treatment		Plant height (cm)		
		Pythium aphanidermatum	*Pythium sylvaticum*	Treatment Mean
T1	Uninoculated control, water	28.2	22.7	25.4
T2	Inoculated control, water	22.3	7.8	15.0
T3	Filex	28.4	18.4	23.4
T4	Aliette	11.2	11.8	11.6
T5	Amistar	20.6	22.8	21.7
T6	SL567A	25.9	14.6	20.2
T7	KIF 230	23.8	10.8	17.3

To compare 2 different treatments means

Significance	*
SED (18 df)	3.32

To compare *Pythium* isolates within any treatment

Significance	*
SED (38.8 df)	4.69

Table 3: Evaluation of novel fungicides to control *Pythium aphanidermatum* and *Pythium sylvaticum* on cucumbers in propagation Assessment of leaf area on 18 January 2001.

Treatment		Leaf Area (cm^2)		
		Pythium aphanidermatum	*Pythium sylvaticum*	Treatment Mean
T1	Uninoculated control, water	110.1	83.2	96.6
T2	Inoculated control, water	95.4	26.5	60.9
T3	Filex	118.8	63.1	90.9
T4	Aliette	43.5	48.9	46.2
T5	Amistar	95.9	85.7	90.8
T6	SL567A	100.0	53.1	76.5
T7	KIF 230	97.1	41.1	69.1

To compare 2 different treatments means

Significance	*
SED (18 df)	14.87

To compare *Pythium* isolates within any treatment

Significance	*
SED (36.9 df)	19.53

Table 4: Evaluation of novel fungicides to control *Pythium aphanidermatum* and *Pythium sylvaticum* on cucumbers in propagation Assessment of plant vigour on 22 December 2000.

Treatment		Plant Vigour Index (0-100) [0=very poor vigour; 100=strong good vigour]		
		Pythium aphanidermatum	*Pythium sylvaticum*	Treatment Mean
T1	Uninoculated control, water	97.9	85.6	91.7
T2	Inoculated control, water	83.3	35.9	59.6
T3	Filex	60.9	48.4	54.7
T4	Aliette	59.4	56.2	57.8
T5	Amistar	64.1	65.6	64.8
T6	SL567A	59.4	60.9	60.2
T7	KIF 230	59.4	53.1	56.2

To compare 2 different treatments means

Significance	*
SED (18 df)	7.30

To compare *Pythium* isolates within any treatment

Significance	*
SED (37.8 df)	9.85

Table 5: Evaluation of novel fungicides to control *Pythium aphanidermatum* and *Pythium sylvaticum* on cucumbers in propagation Assessment of plant vigour on 18 January 2001.

Treatment		Plant Vigour Index (0-100) [0=very poor vigour; 100=strong good vigour]		
		Pythium aphanidermatum	*Pythium sylvaticum*	Treatment Mean
T1	Uninoculated control, water	72.0	59.0	65.5
T2	Inoculated control, water	59.5	20.2	39.8
T3	Filex	70.2	45.2	57.8
T4	Aliette	42.2	39.0	40.6
T5	Amistar	67.2	59.5	63.4
T6	SL567A	67.2	39.0	53.1
T7	KIF 230	56.2	25.0	40.6

To compare 2 different treatments means

Significance	*
SED (18 df)	9.40

To compare *Pythium* isolates within any treatment

Significance	NS
SED (38.9 df)	13.43

Table 6: Evaluation of novel fungicides to control *Pythium aphanidermatum* and *Pythium sylvaticum* on cucumbers in propagation Assessment of root development on 19 January 2001.

Treatment		Root Development Index (0-100) [0=poor root development; 100=excellent root development]		
		Pythium aphanidermatum	*Pythium sylvaticum*	Treatment Mean
T1	Uninoculated control, water	77.0	65.6	71.3
T2	Inoculated control, water	66.7	34.2	50.4
T3	Filex	77.0	50.0	63.5
T4	Aliette	31.2	27.1	29.2
T5	Amistar	62.5	60.4	61.5
T6	SL567A	66.7	43.7	55.2
T7	KIF 230	58.3	39.6	49.0

To compare 2 different treatments means

Significance	**
SED (18 df)	8.05

To compare *Pythium* isolates within any treatment

Significance	NS
SED (38.9 df)	11.49

Table 7: Evaluation of novel fungicides to control *Pythium aphanidermatum* and *Pythium sylvaticum* on cucumbers in propagation Assessment of root discoloration on 19 January 2001.

Treatment		Root Discoloration Index (0-100) [0=low discolouration; 100=high level of discolouration]		
		Pythium aphanidermatum	*Pythium sylvaticum*	Treatment Mean
T1	Uninoculated control, water	16.7	13.3	15.0
T2	Inoculated control, water	8.3	66.5	37.4
T3	Filex	12.5	27.1	19.8
T4	Aliette	43.8	25.0	34.4
T5	Amistar	12.5	16.7	14.6
T6	SL567A	12.5	12.5	12.5
T7	KIF 230	22.9	25.0	24.0

To compare 2 different treatments means

Significance	NS
SED (18 df)	9.76

To compare *Pythium* isolates within any treatment

Significance	**
SED (35.6 df)	12.45

2. Evaluation of antagonistic microbial/bio-control populations against *Pythium spp.*

Objectives

- To evaluate a range of antagonistic microbial populations and specific bio-control agents against two species of *Pythium* on tomato plants in propagation.

Materials and Methods

Site and crop details

Crop: Tomato cv Espero
Site: Glasshouse MFU Section, HRI Stockbridge House

Treatments

	Treatment/ product	Active ingredient	Application rate	Volume applied per plant (ml)[+]
1	Uninoculated control	-	-	100
2	Inoculated control	-	-	100*
3	Companion	*Bacillus subtilis* GB03	1ml per litre	100
4	BS-MBI-600	*Bacillus* sp.	1ml per litre	100
5	Gliomix	*Gliocladium virens*	2g per litre (0.2% suspension)	100
6	Stimagro	*Streptomyces*	0.5g per litre (0.05% suspension)	20
7	Deny (biological fungicide)	*Pseudomonas* (*Burkholderia cepacia*)	1.25 ml per litre	100
8	Rootshield	*Trichoderma harzianum* Strain T22	0.6ml per litre	100
9	Biomex SA	*Trichoderma* spp.	1ml per litre	100
10	QRD 713	Experimental biofungicide	5g per litre	100
11	Pseudomonas 13	*Pseudomonas corrugata* Strain 13	10 ml x 10^8 cells per plant	100[#]
12	Pseudomonas 15	*Pseudomonas fluorescens* Strain 15	10 ml x 10^8 cells per plant	100[#]

[+] Application volume and rates were based, where appropriate, on the product information or guidelines supplied with the product samples.
[*] Water was applied to control treatments T1 and T2.
[#] Treatments T11 and T12 were applied as 100 ml suspension of 10^6 cells/ml.

Crop Diary

Tomato cv. Espero sown:	12 September 2000
Application of bio-control treatments (1):	20 September 2000
Keim plugs potted into rockwool blocks:	26 September 2000
Tomato plants inoculated:	26 September 2000
Plants spaced out in glasshouse:	26 September 2000
Application of bio-control treatments (2):	27 September 2000
Leaf area, leaf length and stem diameter recorded:	20 October 2000
Leaf area, leaf length and stem diameter recorded:	06 November 2000
Root amount /root discoloration assessment:	07 November 2000
Trial terminated:	07 November 2000

Experimental Design & Analysis

The trials were arranged in a randomised block design with four replicates per treatment. Each replicate plot consisted of 8 plants raised in rockwool plugs (4 of which were inoculated with each *Pythium* species). Each replicate plot was raised above floor level on slabs of new polystyrene and covered with a piece of new polythene sheeting to avoid cross-transfer of inoculum between plots. Tomato plants inoculated with each species were spatially separated by placing a piece of polystyrene under the polythene to avoid contamination in run-off water. The occurrence of sciarid, or other, flies in the experimental area was monitored using yellow sticky traps and minimised to avoid potential aerial dissemination of *Pythium* spp. Floors were sprayed with disinfectant (Jet 5) when required to avoid algal development. □

Growing environment

The experiments were conducted at the Stockbridge House blueprint temperature regime for tomato at 18/21°C night and day temperatures respectively.

Inoculation

Two Pythium isolates chosen for this study were *P. aphanidermatum* and *P. sylvaticum* originally isolated from cucumber and tomato crops respectively, both of which had previously been shown to be pathogenic to young cucumber plants. Tomato plants were inoculated around 14 days from sowing at the time of transfer from Keim plugs to the rockwool blocks by placing a 1 cm^2 piece of agar, with mycelium of the *Pythium* spp. under test, underneath the Keim plug at the time of potting on.

Assessments

Agronomic measurements including plant height, stem diameter and leaf area were recorded during the trial to monitor plant vigour. At the termination of the trial root development (scale 0-3), root discoloration (scale 0-3), and the plant dry weight were recorded to measure the effect of the introduced pathogens, in the individual treatments.

The root development and discoloration indices were calculated form a 0-3 assessments as follows:

$$\frac{1(\text{No in category 1}) + 2(\text{No in 2}) + 3(\text{No in 3})}{\text{No of plants assessed}} \times \frac{100}{3}$$

The range of this index was, therefore, 0 (poor development, low discoloration) to 100 (good development, high discoloration)

Statistical Analysis

Analysis of variance on the results from the agronomic assessments was carried out by Biometrics, HRI Wellesbourne. Significance is indicated by - NS no significant difference; * = Significance at the 95% level of probability; ** = Significance at the 99% level of probability; *** = Significance at the 99.9% level of probability. Differences between treatments can be compared (at the 95% probability level) using the Standard Error of Difference.

Storage of Data

The raw data from these experiments will be stored for a period of not less than 5 years in the Archive at Stockbridge House. Access to the data can be made by the designated Archivist.

Results and Discussion

Throughout the trial there were significant differences between the inoculated and the uninoculated control treatments. This is important as it shows whether inoculation with the *Pythium* species provided a stern test to assess the range of bio-control agents in this growing system and whether the methods used to inoculate the plants were successful.

There were also significant differences between the two different *Pythium* species used in this trial in the response on tomato plants. Inoculation with *P. aphanidermatum* had a significantly greater effect on the plant growth as compared to *P. sylvaticum*. These differences between the control treatments increased as the trial progressed. Inoculation with *P. sylvaticum* ultimately did not result in any significant differences between the inoculated and the uninoculated control plants.

There was a relatively large inter-plant variation within the individual experimental plots following inoculation with *P. aphanidermatum.* This was considered to be due to a variation in the effectiveness of the applied inoculum. The variation recorded applied to all inoculated treatments and therefore did not interfere with the aims and objectives of the experiment.

Agronomic assessments recorded on 20 October, 5 weeks after inoculation with *Pythium*, did not show any significant differences between the bio-control treatments under test (Tables 8-11). However, there were indications of the potential of a number of products at this stage in relation to their performance relative to the uninoculated control. Significant differences were recorded between the two *Pythium* species under test at this stage.

At the second agronomic assessment two weeks later (7 weeks from inoculation) significant differences were apparent between some on the bio-control treatments under test when inoculated with *P. aphanidermatum.* In particular, QRD 713, Companion and BS-MBI-600 all gave a significant increase in stem diameter compared to the other products (Table 15) including the inoculated control. They were not significantly worse than the uninoculated control. Plant height recorded on 6 November showed that most of the bio-control products were partially effective as the treated plants were significantly taller than the plants in the inoculated control (Table 12). Five bio-control products recorded similar levels to the uninoculated control. They were (in order of performance) BS-MBI-600, Gliomix, Companion, QRD 713 and Pseudomonas 15. Significant differences in leaf area between the controls were recorded (Table 13), although there was no significance between the bio-control treatments. QRD 713 recorded the largest leaf area. There were no significant differences in leaf length between the bio-control treatments (Table 14).

On termination of the trial, root development and root discoloration were assessed on the base of the rockwool blocks. The majority of bio-control treatments resulted in an increase in root development except for Pseudomonas 13, Rootshield and Biomex. However, Gliomix, Companion and Deny performed most successfully with levels similar to the uninoculated control (Table 16). Root development recorded between the bio-control treated plants and control treatments inoculated with *P. sylvaticum* were not significantly different.

A high level of root discoloration was recorded in the *P. aphanidermatum* inoculated control plants (Table 17). The level of root discoloration was significantly greater than all of the bio-control treatments inoculated with *P. aphanidermatum*. However, the discoloration recorded

in all of the bio-control treatments was still significantly greater than the uninoculated control (Table 17) and this suggests that only partial control of the pathogen was achieved. Levels of root discoloration on plants inoculated with *P. sylvaticum* were generally lower in bio-control treated plants compared to the inoculated control although there was no significance among any of the treatments and controls (Table 17).

The bio-control agents were applied to the plants, 7 and 14 days from sowing. This was to allow early colonisation of the plant root material in a clean environment as well as colonisation once root development throughout the block had taken place. The blocks were drenched with the capacity of the block to ensure good contact with the root system.

The results from this bio-control trial, whilst encouraging, did not provide disease control equivalent to that provided by an effective fungicide such as propamocarb-HCl. Although there were significant differences they were not as great or as consistent as might have been hoped for. However, there are considerable difficulties associated with the application of bio-control agents and it must be noted that they are dependent, and likely to require, highly specific environment conditions for establishment, growth and subsequent survival. Applied under the broad conditions used for fungicide applications they cannot necessarily be expected to provide the consistent response that has been seen with application of fungicides. Timing is crucial to allow maximum benefit. Further work is undoubtedly required to maximise their efficiency by more detailed study of their environmental requirements under commercial conditions. Finally, it should be noted that the study described here, inoculated with *P. aphanidermatum*, provided an extremely stern test for the bio-control agents under test and, in this respect, they did show considerable promise, subject to further refinement to improve their robustness in the commercial environment.

Table 8: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation
Assessment of plant height recorded on 20 October 2000.

Treatment		Plant Height (cm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	14.6	13.3
T2	Inoculated control, water	12.6	15.5
T3	Companion	13.8	15.3
T4	BS-MBI-600	14.2	14.1
T5	Gliomix	14.0	15.5
T6	Stimagro	13.4	15.7
T7	Deny	13.2	15.0
T8	Rootshield	12.4	16.0
T9	Biomex	13.6	16.7
T10	QRD 713	13.8	15.8
T11	*Pseudomonas* 13	11.0	15.7
T12	*Pseudomonas* 15	13.4	16.0

To compare 2 different bio-control treatments

Significance	NS
SED (66.6 df)	0.792

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (35.3 df)	0.426

Table 9: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation
Assessment of leaf area recorded on 20 October 2000.

Treatment		Leaf Area (cm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	90.8	90.2
T2	Inoculated control, water	65.9	89.4
T3	Companion	74.9	92.4
T4	BS-MBI-600	82.4	91.0
T5	Gliomix	72.2	112.2
T6	Stimagro	71.5	102.7
T7	Deny	67.2	95.1
T8	Rootshield	64.4	104.3
T9	Biomex	72.7	94.8
T10	QRD 713	79.0	105.1
T11	*Pseudomonas* 13	53.6	99.3
T12	*Pseudomonas* 15	102.9	97.9

To compare 2 different bio-control treatments

Significance	NS
SED (66.7 df)	7.84

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (35.4 df)	4.19

Table 10: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation Assessment of leaf length on 20 October 2000.

Treatment		Leaf Length (mm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	178.2	175.4
T2	Inoculated control, water	147.2	180.4
T3	Companion	158.6	182.2
T4	BS-MBI-600	163.6	179.3
T5	Gliomix	155.6	172.6
T6	Stimagro	151.5	188.1
T7	Deny	145.8	174.4
T8	Rootshield	140.5	190.7
T9	Biomex	151.3	181.0
T10	QRD 713	159.4	193.2
T11	*Pseudomonas* 13	125.4	187.6
T12	*Pseudomonas* 15	145.8	150.2

To compare 2 different bio-control treatments

Significance	NS
SED (62.3 df)	27.3

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (35.6 df)	0.159

Table 11: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation
Assessment of stem Diameter on 20 October 2000.

Treatment		Stem Diameter (cm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	3.0	3.1
T2	Inoculated control, water	2.4	3.2
T3	Companion	2.4	3.2
T4	BS-MBI-600	2.8	3.3
T5	Gliomix	2.8	3.1
T6	Stimagro	2.5	3.5
T7	Deny	2.3	2.9
T8	Rootshield	2.8	3.5
T9	Biomex	2.3	3.0
T10	QRD 713	3.0	3.2
T11	*Pseudomonas* 13	2.2	3.4
T12	*Pseudomonas* 15	2.2	3.4

To compare 2 different bio-control treatments

Significance	NS
SED (62.3 df)	0.273

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (35.6 df)	0.159

Table 12: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation
Assessment of plant height recorded on 6 November 2000.

Treatment		Plant Height (cm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	32.9	30.6
T2	Inoculated control, water	23.0	30.0
T3	Companion	29.7	33.3
T4	BS-MBI-600	30.2	31.1
T5	Gliomix	30.1	33.3
T6	Stimagro	28.7	34.4
T7	Deny	28.6	32.8
T8	Rootshield	25.9	33.5
T9	Biomex	28.5	35.5
T10	QRD 713	29.6	34.0
T11	*Pseudomonas* 13	23.1	35.1
T12	*Pseudomonas* 15	29.4	35.1

To compare 2 different bio-control treatments

Significance	**
SED (65.07 df)	1.715

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (34.4 df)	0.958

Table 13: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation
Assessment of leaf area recorded on 6 November 2000.

Treatment		Leaf Area (cm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	125.4	129.2
T2	Inoculated control, water	86.1	129.6
T3	Companion	96.1	125.4
T4	BS-MBI-600	101.6	122.9
T5	Gliomix	94.2	106.9
T6	Stimagro	101.8	115.4
T7	Deny	103.4	125.9
T8	Rootshield	95.5	125.9
T9	Biomex	89.8	125.9
T10	QRD 713	110.5	135.0
T11	*Pseudomonas* 13	76.6	127.6
T12	*Pseudomonas* 15	87.6	121.8

To compare 2 different bio-control treatments

Significance	NS
SED (62.5 df)	12.34

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (33.6 df)	7.16

Table 14: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation Assessment of leaf length on 6 November 2000.

Treatment		Leaf Length (cm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	217.3	226.3
T2	Inoculated control, water	185.8	229.3
T3	Companion	197.8	219.6
T4	BS-MBI-600	197.2	220.0
T5	Gliomix	194.1	211.3
T6	Stimagro	201.3	220.9
T7	Deny	194.5	224.9
T8	Rootshield	180.1	224.8
T9	Biomex	185.9	224.8
T10	QRD 713	198.5	198.0
T11	*Pseudomonas* 13	173.8	224.9
T12	*Pseudomonas* 15	193.1	224.0

To compare 2 different bio-control treatments

Significance	NS
SED (66.4 df)	12.89

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (33.1 df)	6.98

Table 15: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation Assessment of stem Diameter on 6 November 2000.

Treatment		Stem Diameter (cm)	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	5.43	5.55
T2	Inoculated control, water	4.04	5.71
T3	Companion	5.20	5.64
T4	BS-MBI-600	5.00	5.41
T5	Gliomix	5.17	5.65
T6	Stimagro	4.74	5.51
T7	Deny	4.99	5.49
T8	Rootshield	4.73	5.86
T9	Biomex	4.85	5.81
T10	QRD 713	5.18	5.46
T11	*Pseudomonas* 13	4.22	5.69
T12	*Pseudomonas* 15	5.08	5.51

To compare 2 different bio-control treatments

Significance	*
SED (59.24 df)	0.275

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (33 df)	0.164

Table 16: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation
Assessment of root development on 7 November 2000.

Treatment		Root Development Index (0-100) [0=low discolouration; 100=high level of discolouration]	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	67.2	70.3
T2	Inoculated control, water	46.9	65.6
T3	Companion	56.4	68.8
T4	BS-MBI-600	54.7	57.8
T5	Gliomix	57.8	73.4
T6	Stimagro	50.0	68.8
T7	Deny	56.3	67.2
T8	Rootshield	42.2	71.9
T9	Biomex	45.3	73.4
T10	QRD 713	53.1	75.0
T11	*Pseudomonas* 13	42.2	71.9
T12	*Pseudomonas* 15	51.6	68.8

To compare 2 different bio-control treatments

Significance	NS
SED (65.4 df)	8.70

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (df 34.5)	4.83

Table 17: Evaluation of bio-control agents to control *Pythium aphanidermatum* and *Pythium sylvaticum* on tomato in propagation
Assessment of root discoloration recorded on 7 November 2000.

Treatment		Root Discoloration (0-100) [0=low level of dicolouration; 100=high level of discolouration]	
		Pythium aphanidermatum	*Pythium sylvaticum*
T1	Uninoculated control, water	6.3	10.9
T2	Inoculated control, water	65.3	18.8
T3	Companion	31.6	12.5
T4	BS-MBI-600	29.7	20.3
T5	Gliomix	35.9	4.7
T6	Stimagro	40.6	7.8
T7	Deny	26.6	6.3
T8	Rootshield	28.1	7.8
T9	Biomex	35.9	6.3
T10	QRD 713	23.4	9.4
T11	*Pseudomonas* 13	40.6	10.9
T12	*Pseudomonas* 15	39.1	12.5

To compare 2 different bio-control treatments

Significance	NS
SED (61.9 df)	10.98

To compare effect of *Pythium* isolates per treatment

Significance	***
SED (33.5 df)	6.40

3. Epidemiological aspects of *Pythium* spp. on long-season crops of tomato and cucumber

Objectives

- To investigate the pathogenicity of the most predominant *Pythium* spp. isolated from tomato and cucumber crops during the *Pythium* audit (years 1 & 2 of this project) in long-season commercial crops

Materials and Methods

Site and crop details

Crop:	Tomato cv Espero	Cucumber cv Enigma
Site:	MFU Section 11	MFU Section 9
	HRI Stockbridge House	HRI Stockbridge House

Treatments

The same treatments (ie *Pythium* species inoculations) were included in both the tomato and cucumber trial:

Treatment		**Source (Isolate code)**
T1	Uninoculated control	-
T2	Inoculated, *Pythium aphanidermatum*	Cucumber (C2/3/C1)
T3	Inoculated, *Pythium* 'mycelium only'	Cucumber (C3/1/C4)
T4	Inoculated, *Pythium* 'mycelium only'	Tomato (T2/1/T2)
T5	Inoculated, *Pythium middletonii*	Cucumber (C1/4/C1)
T6	Inoculated, *Pythium ultimum*	Cucumber (C3/3/C1)
T7	Inoculated, *Pythium paroecandrum*	Cucumber (C3/1/C10)
T8	Inoculated, *Pythium monospermum*	Cucumber (C3/4/C11)
T9	Inoculated, *Pythium* Group HS	Cucumber (C3/3/C12)
T10	Inoculated, *Pythium coloratum*	Tomato (T3/4/T4)

Diary

TOMATO

Tomato cv Espero sown:	09 May 2000
Keim plugs potted into rockwool blocks:	24 May 2000
Tomato plant inoculated:	24 May 2000
Plant spaced out in the glasshouse:	24 May 2000
Plant height recorded:	09 June 2000
Leaf area, leaf length and stem diameter recorded (1):	12 July 2000
Leaf area, leaf length and stem diameter recorded (2):	08 August 2000
Leaf area, leaf length and stem diameter recorded (3):	04 September 2000
Leaf area, leaf length and stem diameter recorded (4):	11 October 2000
Root amount/root discoloration recorded:	18 October 2000
Trial termination	18 October 2000

CUCUMBER CROP (I)

Cucumber cv Enigma sown:	02 June 2000
Plants spaced out in glasshouse:	12 June 2000
Cucumber plants inoculated:	12 June 2000
Leaf area, leaf length and plant height recorded:	12 July 2000
Plant vigour (Quality) assessment:	18 July 2000
Trial terminated:	20 July 2000

CUCUMBER CROP (II)

Cucumber cv Enigma sown:	18 July 2000
Plants spaced out in glasshouse:	28 July 2000
Cucumber plant inoculated:	02 August 2000
Plant height recorded:	11 August 2000
Leaf area and leaf length recorded:	30 August 2000
Root amount/root discoloration recorded:	31 October 2000
Trial terminated:	31 October 2000

Experimental Trial Design

There were 10 treatments each with four replicate plots, randomised by the Biometrics department, HRI Wellesbourne. Each plot was raised up from ground level and supported by two polystyrene sheets (2.5 cm thick). Individual plots raised on polystyrene were covered with a sheet of new polythene to separate each of the plots. The polythene sheet covering the slabs in a plot was raised and clipped at either end and in the middle. One drainage slit was made in this polythene sheet to ensure that the drainage liquid from the slabs flowed away from the plots and out of the glasshouse. This was to avoid the possibility of run-off solution disseminating between plots and leading to cross contamination between treatments.

For the tomato trial each plot comprised of 2 rockwool slabs with 4 plants per slab (8 plants/plot in total) while for the cucumber trial each plot comprised of 2 rockwool slabs with 2 plants per

slab (4 plants/plot in total).

Inoculation

The tomato trial was inoculated using agar plugs ($1cm^2$) of each *Pythium* isolate cut and placed in the plug of the rockwool block prior to the transfer of the tomato seedling in the Keim plug to ensure contact between the agar plug and root material. Fresh (uninoculated agar was placed in the control treatment.

For the cucumber trial a corer (size 10) was used to remove a core of rockwool in the sides of the block prior to sowing. An agar plug ($1cm^2$) of each treatment isolate was cut and placed in the plug of the rockwool block and then filled with the rockwool core to ensure contact between the agar plug and root material. Fresh (uninoculated) agar was placed in the control treatment.

Methods

Tomato and cucumber plants were raised in isolation according to standard operating procedures (SOP's) for propagation of hydroponic plants at Stockbridge House. Tomato seeds were sown in modules containing vermiculite and pricked out into rockwool blocks after about 10 days. At the time of potting the plants were inoculated if appropriate following the details above. Cucumber seeds were sown directly into propagation blocks containing vermiculite. After around 10 days the plants were transferred to the glasshouse where they were inoculated according to the treatment list.

Growing environment

The experiments were conducted following the Stockbridge House blueprint temperature regimes [Tomato - $18^{O}C/21^{O}C$ and cucumber - $19^{O}C/23^{O}C$ night and day temperatures respectively].

Minimising spread of fungal inoculum within the trial

To reduce cross-contamination the rockwool slabs were elevated on a layer of polystyrene (2 x 2.5cm in height) to try and avoid run-off solution wicking up into the adjacent slabs. All run-off solution from this trial drained directly into the gutter and outside the glasshouse. Staff were careful to avoid transferring *Pythium* between plots on hands or equipment - disposable gloves were worn when working in the crop.

To control sciarid and shore flies, to minimise the potential spread of *Pythium* between treatments, a high number of sticky traps were maintained throughout the house to monitor population levels to determine the most effective means of control. The tops of the propagation rockwool blocks were covered with polythene squares to reduce algal growth. In addition, the glasshouse floor was sprayed with Jet 5, when required, to reduce algal growth in any gathering puddles.

Plant Health Policy on Containment of Disease Risk

CLASSIFICATION – ORANGE

In order to try to reduce contamination of other crops on site, there was a high level of hygiene precautions taken during this trial.

- The glasshouses were disinfected with Jet 5 thoroughly before establishing the experiment.
- Foot dips were maintained inside the glasshouse for use by all staff entering and leaving these houses.
- Disposable gloves and over-shoes were provided and worn by all staff working around the root area of the crop.
- Lab coats were worn by staff while working on the crop and were removed before leaving the house. The lab coats were regularly laundered.

Assessments

Agronomic measurements including plant height, leaf area and stem diameter and plant vigour score (scale 0-3) were recorded during the trial to monitor plant growth as appropriate. At the termination of the trial root development (scale 0-3), root discoloration (scale 0-3), and the plant dry weight were recorded to measure the effect of the introduced pathogens, in the individual treatments.

The root development and discoloration indices were calculated form a 0-3 assessments as follows:

$$\frac{1(\text{No in category 1}) + 2(\text{No in 2}) + 3(\text{No in 3})}{\text{No of plants assessed}} \times \frac{100}{3}$$

The range of this index was, therefore, 0 (poor development, low discoloration) to 100 (good development, high discoloration)

Statistical Analysis

Analysis of variance on the results from the agronomic assessments was carried out by Biometrics, HRI Wellesbourne. Significance is indicated by - NS no significant difference; * = Significance at the 95% level of probability; ** = Significance at the 99% level of probability; *** = Significance at the 99.9% level of probability. Differences between treatments can be compared (at the 95% probability level) using the Standard Error of Difference.

Storage of Data

The raw data from these experiments will be stored for a period of not less than 5 years in the Archive at Stockbridge House. Access to the data can only be made via the designated Archivist.

Results and Discussion

Tomato

Throughout the trial a range of agronomic parameters were assessed. Plant height recorded 14 days from inoculation showed that there was a significant reduction in plant height when inoculated with *P. aphanidermatum* compared to both the uninoculated control and other *Pythium* treatments (Table 18). Significant differences in stem diameter, leaf area and leaf length were recorded between the treatments on the earlier assessments (Table 19-21). However, by the end of the trial no significant differences between any of the *Pythium* inoculated treatments and the uninoculated control (Table 19-21) were detected.

At trial termination, the root development was significantly lower among plants inoculated with *P. aphanidermatum* and *P. ultimum* compared to the uninoculated control (Table 23). The amount of root development was generally lower in all other treatments though these were not significantly different from the uninoculated control. An assessment of root discoloration showed that the treatment inoculated with *Pythium* Group HS (Table 23) caused a significant increase in discoloration compared to the uninoculated control. All of the other treatments, including *P. aphanidermatum* surprisingly, were not significantly different from the uninoculated control.

Measurements of total mean yield recorded during the trial showed a significant reduction in yield when tomato plants were inoculated with *P. aphanidermatum* (Table 24), compared to all of the other *Pythium* species inoculated in the trial. One or two of the isolates resulted in slight increases in yield, relative to the uninoculated control, although they were not significant.

Cucumber

During this cucumber experiment a feed unit pump failed unexpectedly and unfortunately its failure coincided with a period of hot dry weather. This placed considerable water stress on the plants, albeit for a few hours only, but it led to a significant amount of wilting in the experimental area. As we were unable to differentiate between this symptom and the effect of *Pythium* root infection (and because we anticipated a subsequent loss of the most severely wilted plants) we made a decision to abandon the experiment. Rather than risk trying to rescue the experiment by replacing wilting plants it was decided to restart the trial with fresh plants and to repeat the artificial inoculation. As there were significant results developing in the first trial before it was abandoned these results are presented also (see data for cucumber Trial I and cucumber Trial II respectively).

Agronomic measurements recorded on 12 July (Cucumber Trial I : Table 25) show that inoculation with *P. aphanidermatum* caused a significant reduction in plant height, leaf area and leaf length. Inoculation with all the other *Pythium* isolates failed to cause any significant reduction in the agronomic performance of the plants compared to the uninoculated control treatments.

Similar assessments carried out in the repeat experiment (Cucumber Trial II : Table 26) again show that inoculation with *P. aphanidermatum* caused a significant reduction in plant height, leaf area and leaf length. Again, there were no significant differences between any of the other *Pythium* species and the uninoculated control except perhaps for *Pythium* Group HS (T9).

On termination of Cucumber Trial II root development was measured in detail. Treatment 2 (inoculated with *P. aphanidermatum*) caused a highly significant reduction in the root development relative to all other treatments (Table 27). There were no significant reductions in root development where the other *Pythium* species had been introduced, relative to the uninoculated control. Inoculation with *P. coloratum* did cause a slight reduction in root growth at the base of the slabs, although this was not significantly different from the uninoculated control. Surprisingly, differences in root discoloration between several treatments were recorded at the termination of Cucumber Trial II (Table 27) though these seemed to have had relatively little impact on the plant performance. Unusually, the level of root discoloration in the *P. aphanidermatum* treatment was not significantly greater than the uninoculated control. However, it should be noted that due to the relatively severe infection of the plants by this pathogen there was only a small amount of fresh root to assess and this is likely to have biased the result. Inoculation with *P. ultimum*, *P. monospermum*, *P.* Group HS and *P. middletonii* all caused a significant increase in root discoloration. Although the levels of discoloration were also higher with *P. coloratum* this was not significant.

Yield recorded during the second cucumber crop shows that inoculation with *P. aphanidermatum* caused a severe loss of yield (Table 28). Although all of the other treatments led to a reduction in yield they were not significantly different from the uninoculated control and interestingly the reduction in yield did not correspond very closely with the level of root discoloration recorded. In this respect, therefore, the significance of the various *Pythium* spp, with the exception of *P. aphanidermatum*, in the crop remains undetermined and open to considerable debate.

Table 18: Pathogenicity of a range of *Pythium* species on the growth and development of tomato plants - An assessment of plant height on 9 June 2000.

Treatment		Plant Height (cm)
T1	Uninoculated control	15.4
T2	*Pythium aphanidermatum*	10.3
T3	*Pythium* 'mycelium only'	15.3
T4	*Pythium* 'mycelium only'	15.4
T5	*Pythium middletonii*	15.9
T6	*Pythium ultimum*	16.2
T7	*Pythium paroecandrum*	15.6
T8	*Pythium monospermum*	15.2
T9	*Pythium* Group HS	15.5
T10	*Pythium coloratum*	16.1

To compare 2 different *Pythium* species

Significance	***
SED (23 df)	0.71

Table 19: Pathogenicity of a range of *Pythium* species on the growth and development of tomato plants - An assessment of stem diameter.

Treatment		Stem diameter (mm)			
		12 July	8 August	4 September	11 October
T1	Uninoculated control	13.8	10.0	8.6	7.5
T2	*Pythium aphanidermatum*	6.2	6.8	9.6	7.4
T3	*Pythium* 'mycelium only'	13.6	9.5	8.6	6.8
T4	*Pythium* 'mycelium only'	13.8	9.8	9.2	7.6
T5	*Pythium middletonii*	13.9	9.9	9.2	8.0
T6	*Pythium ultimum*	13.4	10.0	9.0	7.1
T7	*Pythium paroecandrum*	13.3	9.3	8.8	6.9
T8	*Pythium monospermum*	13.9	9.9	9.0	7.7
T9	*Pythium* Group HS	13.4	9.0	8.9	7.2
T10	*Pythium coloratum*	12.6	9.1	8.6	7.3

To compare 2 different *Pythium* species

Significance	***	***	NS	NS
SED (23 df)	0.52	0.52	0.50	0.41

Table 20: Pathogenicity of a range of *Pythium* species on the growth and development of tomato plants - An assessment of leaf area.

Treatment		Leaf Area (cm^2)[+]			
		12 July	8 August	4 September	11 October
T1	Uninoculated control	452.9 (2.63)	306.7 (2.47)	296.6 (2.45)	200.7 (2.28)
T2	*Pythium aphanidermatum*	81.9 (1.81)	243.2 (2.35)	320.9 (2.48)	218.1 (2.28)
T3	*Pythium* 'mycelium only'	466.3 (2.62)	302.1 (2.45)	253.6 (2.39)	195.2 (2.25)
T4	*Pythium* 'mycelium only'	478.8 (2.66)	274.6 (2.41)	295.4 (2.44)	224.3 (2.33)
T5	*Pythium middletonii*	456.3 (2.63)	322.3 (2.48)	305.1 (2.47)	233.8 (2.35)
T6	*Pythium ultimum*	529.4 (2.70)	299.3 (2.45)	267.1 (2.41)	233.6 (2.32)
T7	*Pythium paroecandrum*	440.4 (2.60)	276.0 (2.41)	287.9 (2.44)	201.6 (2.28)
T8	*Pythium monospermum*	479.4 (2.65)	310.1 (2.48)	295.8 (2.46)	220.2 (2.31)
T9	*Pythium* Group HS	337.3 (2.50)	301.0 (2.45)	252.8 (2.38)	201.9 (2.27)
T10	*Pythium coloratum*	441.3 (2.60)	309.4 (2.46)	250.3 (2.38)	240.2 (2.35)

To compare 2 different *Pythium* species

Significance	(***)	(NS)	(NS)	(NS)
SED (23 df)	(0.050)	(0.060)	(0.051)	(0.062)

[+] Data in parentheses transformed by log prior to analysis

Table 21: Pathogenicity of a range of *Pythium* species on the growth and development of tomato plants - An assessment of leaf length.

Treatment		Leaf Length (cm)			
		12 July	8 August	4 September	11 October
T1	Uninoculated control	393.9	335.6	365.9	349.2
T2	*Pythium aphanidermatum*	198.4	323.2	382.2	349.2
T3	*Pythium* 'mycelium only'	391.1	332.4	343.7	337.1
T4	*Pythium* 'mycelium only'	383.3	334.5	358.1	357.1
T5	*Pythium middletonii*	389.5	350.0	364.5	362.4
T6	*Pythium ultimum*	410.6	336.0	343.4	339.8
T7	*Pythium paroecandrum*	383.7	332.6	353.5	344.1
T8	*Pythium monospermum*	395.8	337.5	366.0	352.5
T9	*Pythium* Group HS	367.2	325.9	347.5	340.1
T10	*Pythium coloratum*	382.3	325.1	340.2	348.1

To compare 2 different *Pythium* species

Significance	***	NS	NS	NS
SED (23 df)	14.45	16.03	15.26	13.26

Table 22: Pathogenicity of a range of *Pythium* species on the growth and development of tomato plants - An assessment of root development and root discoloration on termination of the tomato crop on 18 October 2000.

Treatment		Root Development Index (0-100)	Root Discoloration Index (0-100)
T1	Uninoculated control	82.5	44.0
T2	*Pythium aphanidermatum*	45.3	51.4
T3	*Pythium* 'mycelium only'	85.6	53.5
T4	*Pythium* 'mycelium only'	77.6	39.4
T5	*Pythium middletonii*	79.7	32.5
T6	*Pythium ultimum*	57.5	52.4
T7	*Pythium paroecandrum*	70.2	47.2
T8	*Pythium monospermum*	73.2	45.1
T9	*Pythium* Group HS	73.4	76.8
T10	*Pythium coloratum*	71.4	45.0

To compare 2 different *Pythium* species

Significance	***	NS
SED (8 df)	7.54	12.68

Table 23: Pathogenicity of a range of *Pythium* species on the growth and development of tomato plants - An assessment of total crop yield during the tomato trial.

Treatment		Total yield (kg/m^2)
T1	Uninoculated control	36.0
T2	*Pythium aphanidermatum*	14.8
T3	*Pythium* 'mycelium only'	37.1
T4	*Pythium* 'mycelium only'	37.0
T5	*Pythium middletonii*	39.3
T6	*Pythium ultimum*	36.8
T7	*Pythium paroecandrum*	37.0
T8	*Pythium monospermum*	38.2
T9	*Pythium* Group HS	34.7
T10	*Pythium coloratum*	32.7

To compare any 2 different treatments

Significance	***
SED (23 df)	3.17

To compare 2 different treatments other than T2[#]

Significance	NS
SED (23 df)	3.15

[#] Due to the extreme treatment effect of T2 (*Pythium aphanidermatum*) from all other treatments, an ANOVA was carried out between the remaining treatments to examine for significant treatment differences.

Table 24: Pathogenicity of a range of *Pythium* species on the growth and development of cucumber plants in Cucumber Trial I - An assessment of plant height, Leaf area and leaf length on 12 July 2000.

Treatment		Plant Height (cm)	Leaf Area (cm^2)	Leaf Length (cm)
T1	Uninoculated control	47.6	117.7	14.1
T2	*Pythium aphanidermatum*	2.0	4.9	0.7
T3	*Pythium* 'mycelium only'	46.4	136.1	15.1
T4	*Pythium* 'mycelium only'	46.4	123.9	14.4
T5	*Pythium middletonii*	47.1	118.1	14.1
T6	*Pythium ultimum*	47.3	114.3	14.0
T7	*Pythium paroecandrum*	50.0	116.6	14.2
T8	*Pythium monospermum*	43.4	108.7	13.8
T9	*Pythium* Group HS	46.7	129.9	14.7
T10	*Pythium coloratum*	48.1	102.4	13.4

To compare 2 different treatments other than T2[#]

Significance	NS	NS	NS
SED (24 df)	2.42	15.96	0.77

[#] Due to the extreme treatment effect of T2 (*Pythium aphanidermatum*) from all other treatments, an ANOVA was carried out between the remaining treatments to examine for significant treatment differences.

Table 25: Pathogenicity of a range of *Pythium* species on the growth and development of cucumber plants in Cucumber Trial II - An assessment of plant height, leaf area and leaf length on 11 and 30 August 2000.

Treatment		Plant Height (cm) 11 August	Leaf Area (cm^2) 30 August	Leaf Length (cm) 30 August
T1	Uninoculated control	18.9	228.9	224.7
T2	*Pythium aphanidermatum*	4.7	7.9	11.1
T3	*Pythium* 'mycelium only'	16.1	189.0	201.9
T4	*Pythium* 'mycelium only'	18.3	186.8	203.2
T5	*Pythium middletonii*	19.3	190.9	202.3
T6	*Pythium ultimum*	15.3	212.8	219.5
T7	*Pythium paroecandrum*	16.8	187.1	208.2
T8	*Pythium monospermum*	19.9	219.6	222.5
T9	*Pythium* Group HS	17.2	157.6	194.1
T10	*Pythium coloratum*	18.8	222.9	221.4

To compare 2 different treatments other than T2[#]

Significance	NS	NS	NS
SED (24 df)	2.87	28.39	14.30

[#] Due to the extreme treatment effect of T2 (*Pythium aphanidermatum*) from all other treatments, an ANOVA was carried out between the remaining treatments to examine for significant treatment differences.

Table 26: Pathogenicity of a range of *Pythium* species on the growth and development of cucumber plants in Cucumber Trial II - An assessment of root development and root discoloration at trial termination on 31October 2000.

Treatment		Root Development Index (0-100)	Root Discoloration Index (0-100)
T1	Uninoculated control	83.3	41.7
T2	*Pythium aphanidermatum*	21.9	45.8
T3	*Pythium* 'mycelium only'	79.2	45.8
T4	*Pythium* 'mycelium only'	83.3	58.3
T5	*Pythium middletonii*	70.8	66.7
T6	*Pythium ultimum*	75.0	95.8
T7	*Pythium paroecandrum*	79.2	41.7
T8	*Pythium monospermum*	75.0	75.0
T9	*Pythium* Group HS	75.0	66.7
T10	*Pythium coloratum*	66.7	62.5

To compare 2 different treatments other that T2[#]

Significance	NS	**
SED (24 df)	8.43	14.06

[#] Due to the extreme treatment effect of T2 (*Pythium aphanidermatum*) from all other treatments, an ANOVA was carried out between the remaining treatments to examine for significant treatment differences.

Table 27: Pathogenicity of a range of *Pythium* species on the growth and development of cucumber plants in Cucumber Trial II - An assessment of total crop yield during the cucumber trial.

Treatment		Total yield (kg/m^2)
T1	Uninoculated control	30.7
T2	*Pythium aphanidermatum*	1.5
T3	*Pythium* 'mycelium only'	28.6
T4	*Pythium* 'mycelium only'	25.0
T5	*Pythium middletonii*	26.9
T6	*Pythium ultimum*	25.2
T7	*Pythium paroecandrum*	27.7
T8	*Pythium monospermum*	26.8
T9	*Pythium* Group HS	23.5
T10	*Pythium coloratum*	24.4

To compare 2 different *Pythium* species

Significance	***
SED (23 df)	4.18

To compare 2 different treatments other than T2[#]

Significance	NS
SED (23 df)	4.19

[#] Due to the extreme treatment effect of T2 (*Pythium aphanidermatum*) from all other treatments, an ANOVA was carried out between the remaining treatments to examine for significant treatment differences.

4. Discussion and Conclusions

Fungicide screening

- Artificial inoculation of cucumber in this experiment with two *Pythium* spp. (demonstrated in earlier experiments to be pathogenic on both cucumber and tomato seedlings) was partially successful and yielded a valuable insight into the biology of individual *Pythium* spp. In this experiment, conducted under commercial conditions during November 2000 - January 2001, *Pythium sylvaticum* caused a significant reduction in overall plant growth following artificial inoculation and was regarded as a weak pathogen causing reduced plant vigour. In contrast, P. *aphanidermatum*, surprisingly, failed to cause a significant reduction in growth compared to the uninoculated control in this particular study – and yet it is normally regarded as a severe root pathogen of cucumber.

- *P.aphanidermatum* has a high optimum temperature for growth and as this experiment was undertaken during the winter period it is speculated that conditions were not ideal for pathogen development. This is of significance and potential practical benefit as manipulation of solution temperature at other times (ie summer replant period) could assist in minimising infection by this aggressive pathogen.

- It is also considered that the presence of *P. aphanidermatum* may be masked during low temperature conditions. A 'latent' dissemination of the pathogen may occur without obvious symptoms being expressed during the first crop. This may help explain the rapid collapse of young plants following contact with the rockwool slabs during the replanting period. Responsibility for this rapid infection of young plants during re-planting in the summer is usually attributed to propagators delivering infected plants. However, earlier work in this project has clearly demonstrated that *P. aphanidermatum* is uncommon on young plants at propagation nurseries. Improved routine monitoring of this pathogen in commercial crops may be of considerable help in pre-empting establishment of the summer re-plant crop. Growers and their advisers may wish to review current strategies aimed at *Pythium* control on the nursery with a view to eliminating this latent infection. In later studies conducted during the autumn (see below) when temperatures were higher the full pathogenicity of *P. aphanidermatum* was amply demonstrated.

- Of a range of novel fungicides screened on cucumber plants in propagation to assess their potential to control *Pythium* spp. several products were found to provide effective control.

- Treatment with the standard commercial treatment propamocarb-HCl (Filex/Proplant) was effective and resulted in a low level of root discoloration at the termination of the trial. No evidence was found to suggest a reduced sensitivity or resistance to this fungicide in the work reported though naturally the situation will need regular monitoring to detect any future shifts in pathogen sensitivity.

- Two novel fungicides, azoxystrobin (Amistar) and metalaxyl-M (SL567A), neither of which are currently approved for use on the root environment in hydroponic crops, both

maintained plant health and vigour during this trial following artificial inoculation with *Pythium* spp. Discussions are to be encouraged with the manufacturers to determine whether it would be worthwhile pursuing On- or Off-Label approval for this use in the future.

- Aliette, although controlling *Pythium* effectively, caused a significant reduction in plant growth and this was considered to be due to a phytotoxic response from the treatment.

- A number of other novel fungicides are known to have activity towards oomycetes but were not included in this study as they are unfortunately not currently approved in this country for protected crops use and there are no safety data packages available for use under protection.

- The differential pathogenicity expressed by each *Pythium* sp. requires further investigation as it makes it difficult to draw conclusions from the work on the various *Pythium* species carried out here. For example, whilst *P. sylvaticum* was weakly pathogenic in this study under different environmental parameters it may have been highly pathogenic or, conversely, non-pathogenic. The impact of mixed *Pythium* populations in crops has not been investigated either and yet this is likely to be relatively common under commercial conditions and could potentially account for on-going problems with *Pythium* infection in the industry. It is possible for example that different species are debilitating plants at different times of year (when environmental conditions are favourable) but alone their significance may be relatively small. Cumulatively, they may assume much greater significance.

- Future studies to evaluate the efficacy of novel fungicides will need to be undertaken at different times of the year (ideally coinciding with the typical commercial propagating periods) to ensure both crop safety under low & high solar radiation and efficacy against the predominant *Pythium* spp. likely to cause root infection under low & high temperatures.

Bio-control screening

- The reduction in tomato plant growth following inoculation with *P. aphanidermatum* in this trial conducted during relatively high autumn temperature conditions (September to November 2000) supports the comments made above and provided a stern test for each of the bio-control agents under evaluation.

- In contrast, inoculation with *P. sylvaticum* led to little difference between the controls and the treatments under test, though again supports the view that this fungus is generally of less significance as compared with *P. aphanidermatum* in hydroponic production systems.

- Numerous bio-control agents with potential for use as disease control agents were accumulated from various worldwide sources for this work. Applied as directed, several products alleviated the root rot symptoms caused by *P. aphanidermatum* and provided a measurable growth response compared to the inoculated untreated control plants. QRD 713, Companion and BS-MBI-600 all gave a significant increase in stem diameter of tomato plants. Each of these products and, additionally, Gliomix and Pseudomonas 15,

also provided an increase in leaf area compared to the inoculated control.

- At trial termination, when a detailed assessment of root development and discoloration could be made, most of the biocontrol agents evaluated provided an increase in root development when compared to the inoculated control. However, none of the bio-control agents were able to protect the roots sufficiently to provide root development equivalent to the uninoculated control.

- Root discoloration in the *P. aphanidermatum* inoculated control plants was high and significantly greater than all of the bio-control treatments under test. Compared to the uninoculated control, where the incidence of root discoloration was very low (RdiI for *P. aph.* = 6.3), the bio-control treatments proved to be only partially effective in preventing root discoloration though many showed considerable promise and are worth further investigation to refine their efficacy. There was little difference in the level of root discoloration between the *P. sylvaticum* inoculated plants and the uninoculated controls.

- The effects of all of the biocontrol agents in this trial were less marked than the differences recorded where the pesticides were applied to control the *Pythium* infected root tissues.

- In conclusion, several of the bio-control agents evaluated showed considerable promise in this study though would require further development work with respect to dose rate, timing, frequency of application, formulation etc before they could compete effectively with fungicide treatment in terms of overall efficacy.

Epidemiological aspects

TOMATO

- In this trial, conducted during the period May-October 2000, the various *Pythium* spp. introduced into the root environment had a varied impact on crop development. During this period, conditions were ideal for *P. aphanidermatum,* and this root pathogen caused the greatest reduction in plant development. This was most evident at termination of the trial when root development and discoloration could be assessed and particularly when total cumulative yield was calculated.

- In comparison to *P. aphanidermatum*, none of the other *Pythium* spp. introduced into the crop had a significant impact on overall crop development, ie yield, in tomato.

- Some *Pythium* spp. eg *Pythium* HS, *P. coloratum* did appear to depress total yield slightly, though not significantly, compared to the uninoculated control. Other species eg *P. ultimum* appeared to reduce root development and cause an increased level of root discoloration, yet appeared to have no effect on overall crop yield at termination of the experiment.

- It should be considered, however, that depending on the optimum temperature requirements of each of the species evaluated, they may have had a greater or lesser impact at different times of the year.

- Considerable significant differences were recorded in the various agronomic measurements over the time course of the study, particularly with respect to *P. aphanidermatum*. Interestingly, these differences became less evident towards the later stages of the trial. It is considered that the plants severely affected by root disease (ie *P. aphanidermatum*) diverted limited resources from (non-functional) root systems to the shoot apex and this could account for the apparent improvement in the various plant growth parameters measured in Tables 19-21. However, at the same time, the nutrient resource to the fruit was restricted and there was a significant impact on the economic (marketable) yield from the crop.

- In conclusion, relative to *P. aphanidermatum*, the other *Pythium* spp. recovered from commercial tomato crops and used in this trial were relatively unimportant as they failed to have a significant impact on crop development. We cannot, however, necessarily assume that their impact would not have been greater under different climatic and environmental conditions. It is also possible that their cumulative effect, assuming several species were present in a single crop, could be deleterious economically.

CUCUMBER

- During these trials, conducted during the high temperature summer-autumn period, significant differences were recorded in the relative pathogenicity of the various *Pythium* species inoculated when compared with the uninoculated control.

- Plants inoculated with *P. aphanidermatum* were stunted with a significantly reduced plant height, leaf area and leaf length.

- There was very little significant variation among the other *Pythium* isolates introduced when compared with the uninoculated control treatment, the only exception was *Pythium* Group HS which appeared to have a slight (usually non-significant) effect on the various growth parameters measured.

- At termination of the trial *P. aphanidermatum* had, by far, the greatest reduction in root development. One other species ie *P. coloratum* caused a slight reduction in root development though this was not significant.

- A moderate to high level of root discoloration was recorded across all treatments, including the uninoculated control. Some *Pythium* species, particularly *P. ultimum*, *P. monospermum*, *P.* Group HS and *P. middletonii* led to significant increase in root discoloration compared to the controls. Surprisingly, the *P. aphanidermatum* treatment recorded a relatively low level of root discoloration, but this is considered to be due to the fact that there was very little root remaining for assessment and that root visible was relatively new and healthy not yet succumbing to the *Pythium* infection.

- *P. aphanidermatum* was the only species introduced to cause a significant reduction in the total cumulative yield in the cucumber crop, and the yield loss was quite dramatic (ca. 95% reduction). Most of the other species, but particularly *Pythium* HS, *P. coloratum*, *P.' mycelium only'* (Isolate 2) and *P. ultimum* all gave a lower total yield compared to the uninoculated control but the difference was not significant.

- In conclusion therefore, relative to *P. aphanidermatum*, the other *Pythium* spp. introduced into cucumbers were relatively unimportant with respect to their overall impact on the crop. However, as for the tomato study reported above, we are unable to necessarily assume that their impact would not have been greater under different climatic and environmental conditions. It is also possible that their cumulative effect, assuming several species were present in a single crop, could be more economically damaging than is reported here with single species.

5. Technology transfer

Articles

- *Pythium is all around you.* HDC News **71**, pages 18-19, March 2001.

Presentations at grower meetings

- *Pythium in protected crops.* Vegetable Consultancy Association meeting at HRI Stockbridge House, 28 September 1999. (Andy Jackson and Martin McPherson).
- Pythium*: prevalence and diversity in cucumber crops.* HDC Cucumber Conference, Peterborough, 14 October 1999 (Andy Jackson).
- *Pythium problems in protected edible crops.* Southern Tomato Growers Association, HRI Stockbridge House, 15 September 1999 (Andy Jackson and Martin McPherson).

Review meetings

- HRI Wellesbourne, 30 September 1998.

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7. Acknowledgements

The authors would like to acknowledge all of the participating plant propagators and commercial cucumber and tomato nurseries for allowing extensive sampling on their nurseries during the course of this study. The identity of the sampling nurseries will remain anonymous in all HDC publications and presentations.

We would also like to thank the various companies producing the bio-control products and fungicides for the free samples that were provided for use in this project.

In addition, we would like to thank the project co-ordinators Mr Derek Hargreaves, Hargreaves Consultancy, Mr John Overvoorde, Delflands Nursery and Dr Nigel Dungey, Humber-VHB for their support and advice during this project.

Finally thanks are due to Mr Rodney Edmondson, Biometrics Department, HRI Wellesbourne for advice on trial design, randomisation of treatments and statistical analysis of data.