FINAL REPORT

Project title:	Evaluation of per-acetic acid (Jet 5) and fosetyl-al (Aliette) for the control of *Pythium* spp. in replanted cucumber
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The results and conclusions in this report are based on a single experiment. The conditions under which the experiment was carried out and the results obtained 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

Objectives and Background

Pythium spp, particularly *Pythium aphanidermatum*, are a persistent cause of root decay in hydroponically-grown cucumbers in the UK. In the cooler temperature conditions during spring disease expression is usually poor because of the relatively high temperature optimum of the fungus. Often, a latent infection exists within the rockwool rooting medium of the crop. As the temperature rises during May-June the pathogen develops more rapidly though by this growth stage the crop is mature with a well established root system and symptom expression is not always clear. However, during this period it is considered that transmission of the fungus occurs by mechanisms not fully elucidated though sciarid flies have been implicated.

Cucumber crops are usually replanted during June-July. The combination of high summer temperatures (usually!) and young plants with undeveloped root systems makes them very susceptible to infection by *P. aphanidermatum*, and possibly other species. Reports of plant losses due to *Pythium* spp. in the region of 5-10% are not unusual during the replanting period. Fungicides applied to the young plants provide some protection from infection but when disease pressure is high are generally unable to prevent plant losses.

The aim of this investigation was to determine whether an application of the disinfectant per-acetic acid (as Jet 5) either applied alone or in combination with *Trichoderma* or the novel fungicide fosetyl-aluminium (Aliette), applied to the rockwool substrate between crops, could effectively reduce the inoculum pressure from *P. aphanidermatum* and allow effective establishment of the young plants.

Summary of Results

Over a two year period the performance of per-acetic acid, as Jet 5, was evaluated alongside the standard fungicide propamocarb hydrochloride (Filex) and the experimental fungicide fosetyl-aluminium (Aliette) for the control of *Pythium* root-rot in replanted cucumber.

In year 1 of the investigation naturally infested slabs sourced from commercial nurseries were used. Following treatment application improved establishment was recorded though the relatively low incidence of *Pythium* infection and the slab to slab variation prevented effective interpretation of the results. Treatment with Aliette at the rate tested caused a marked phytotoxicity.

In the second year of the investigation, a cucumber crop at HRI Stockbridge House was inoculated with *P. aphanidermatum* to generate slabs 'naturally' infested with the root pathogen. Following removal of the first crop treatments were applied to the infested slabs *in situ* and young susceptible plants re-planted.

The various *in-situ* treatments with per-acetic acid (Jet 5) between crops was ineffective in preventing root disease and many plants failed to establish. Treatment with Filex, the standard commercial treatment, provided a slight suppression of the disease but the high infection pressure caused many plants to succumb and fail to establish successfully. Treatment with Aliette at a reduced rate alleviated the earlier phytotoxicity though the fungicide treatment failed to prevent Pythium infection effectively.

In summary none of the applied treatments were effective in preventing losses due to *Pythium* during replanting.

Action Points for Growers

- In the absence of effective treatments for the control of *Pythium* in replanted cucumbers growers should concentrate on hygiene to minimise epidemic development during the first crop, when symptoms of the disease are often masked.

- Rockwool slabs should be laid out ensuring occasional spaces between them to avoid hydroponic run-off (and the spores it may contain) 'tracking' between the polythene layers beneath the growing slabs.

- Where possible measures should be taken to minimise the numbers of sciarid/shore flies as these are reported to disseminate *Pythium* spp. between plants.

- Run-off solution should be drained away effectively and not allowed to build up in the gutters as this provides a source of both the pathogen and an additional breeding ground for sciarid flies.

- Filex should be used in propagation and immediately post-planting to encourage rapid plant establishment.

- It should be noted that *P. aphanidermatum* has a high temperature optimum (ca. 35°C) for growth and therefore will be most active under high summer temperature conditions. If the weather is conducive to *Pythium* infection at the time of planting consideration should be given to measures which reduce the temperature within the root environment as this may delay pathogen development.

Practical and Financial Benefits from the Study

The identification of an effective solution to the ever-increasing problem of *Pythium* root-rot in replanted cucumbers is becoming more important. The loss of young plants or delayed establishment of others, reduces yield and affects the commercial viability of the crop.

It is difficult to estimate the financial losses caused by *Pythium* root-rot to the industry. However, if 7% of all propagated plants are lost annually, the cost of their replacement would be approximately £83,475 (based on 157,500 plants lost at 53 p/plant). In addition, delayed establishment will cause a loss in yield estimated at £420,000 (based on 3 weeks lost production, equivalent to 1,653,750 cucumbers at £3.20/box). Additional labour/material costs in replacing affected plants, growing media and fungicide application may effect an annual loss of up to £0.5 million per annum.

The use of an *in situ* disinfection treatment of the rockwool slabs prior to re-planting in the summer in theory at least, provides an opportunity of reducing the inoculum pressure of the pathogenic fungus without risking damage to the young plants. Per-acetic acid (as Jet 5) is an effective biocide with proven activity against several of the primary root pathogens in the crop. Its breakdown products are environmentally acceptable. Fosetyl-aluminium (Aliette), a fungicide with an alternative mode of action to Filex, may additionally minimise the risk of resistance development.

The primary aim of this investigation therefore was to evaluate both per-acetic acid (Jet 5) and fosetyl-aluminium (Aliette) for their performance in enhancing the establishment of replanted cucumbers onto slabs, previously contaminated with *Pythium aphanidermatum.*

Results from the study have been disappointing as alternative chemical means of preventing *Pythium* have not been identified.

Growers who regularly experience establishment problems during the re-planting phase will have to rely on improved hygiene precautions to minimise dissemination of the pathogen during the first crop until, that is, other alternative strategies can be sought.

EXPERIMENTAL SECTION

Introduction

Most cucumber growers replant their crops mid-season in order to maximise both yield and quality of harvested fruit. Problems of poor plant establishment in replanted crops are becoming more common, with reports of individual growers losing 10% or more of their young plants within 14 days of planting out. In many cases, *Pythium* spp. particularly *P. aphanidermatum*, can be recovered from the root systems of affected plants and this fungus is considered to be the primary cause of the problem in many cases.

Interestingly, older established plants with large root systems appear more able to sustain *Pythium* infection of the root system, compared to weakly rooted replants.

There is an opportunity to treat *Pythium* infested slabs with a fungicide or disinfectant, during the short period between old crop removal and replanting the new crop. Providing a treatment can be identified which is (a) not phytotoxic to the young replanted crop, (b) is fungitoxic to oomycete fungi such as *Pythium* spp., and (c) is relatively inexpensive to use, establishment problems in replanted crops could be largely avoided.

Per-acetic acid, eg Jet 5 from Hortichem Ltd, is an effective biocide with proven activity against several cucumber root pathogens including *Pythium* spp. (McPherson pers. comm.). The product breaks down quickly to acetic acid, water and oxygen and, as such, has a short persistence and low risk to the environment. Applied as a drench to the substrate prior to replanting it has the potential to reduce disease inoculum within the slab and allow rapid establishment of new plants. Additionally, a combined treatment of per-acetic acid and Trichoderma inoculation was evaluated as the disinfectant has been demonstrated to support the growth of this biocontrol fungus (McPherson, pers. comm.) in other studies.

Fosetyl-aluminium, as Aliette (Rhone Poulenc), is an oomycete fungicide with activity against *Phytophthora* and *Pythium* spp., although currently it does not have approval for use in hydroponic cucumber crops. If Aliette could be demonstrated to be effective against *Pythium* root-rot in this short-term study, it would be worthwhile pursuing an On-Label Approval, or alternatively a Specific Off-Label Approval (SOLA) application.

Materials and Methods

Site

Multi-factorial Unit (Zone 15)
Horticulture Research International
Stockbridge House
Cawood
Selby
North Yorkshire
YO8 3TZ

Duration

June - July 1997

Crop and Cultivar

Mildew tolerant cucumber cv. Enigma.

Trial Design

There were eight treatments (including inoculated and uninoculated controls) with four replicate blocks arranged in a randomised block design. Each plot consisted of six plants in three rockwool growing slabs. Single guard rows were provided in the East and West elevations of the glasshouse, and guard plots included on the southern edge of the glasshouse.

Treatments

Initially (during March-July 1997) a cucumber crop was grown in new rockwool slabs. Each slab (with the exception of the uninoculated control) was inoculated with a reference culture of *Pythium aphanidermatum* and allowed to develop from mid March to the early July. In early July, all plants were removed from the rockwool slabs avoiding accidental contamination of the uninoculated control. The slabs remained *in situ* where the following treatments were subsequently imposed:

1. Uninoculated control.

2. Inoculated control.

3. Propamocarb hydrochloride (Filex) applied as a drench during propagation (one week prior to transplanting) and repeated 1 week after planting out, at a rate of 200 ml/plant of a solution containing 2.5 ml product in 10 l.

4. Per-acetic acid (Jet 5) applied via the irrigation network at a rate of 1000 ppm (1 litre product in 50 litres water) at a volume of 5 litres per slab using nozzles designed to deliver 4 litres/hour. Slabs were left to stand for 24 hours and subsequently flushed with fresh nutrient solution until PAA and pH were reduced to acceptable levels.

5. Per-acetic acid (Jet 5) applied by hand at a rate of 1000 ppm (1 litre product:50 litres water) at a volume of 5 litres per slab by pouring 2.5 litres onto each planting hole ensuring an even cover over the surface. Slabs were then left to stand for 24 hours then flushed with fresh nutrient solution until PAA and pH reduced to acceptable levels.

6. Per-acetic acid (Jet 5) applied by hand at a rate of 1000 ppm (1 litre product/50 litres water) at a volume of 5 litres per slab by pouring 2.5 litres onto each planting hole ensuring an even coverage over the surface. Prior to the Jet 5 application, the drainage slit in each slab was sealed with tape. Slabs were left 24 hours before re-opening the drainage slits to flush with fresh nutrient solution until PAA and pH were reduced to acceptable levels.

7. Per-acetic acid (Jet 5) applied by hand as in Treatment 5. After flushing the slabs, a *Trichoderma* sp. was inoculated by hand as a 500 ml/plant spore suspension containing 10^5 spores/ml.

8. Fosetyl-aluminium (Aliette) applied as a drench during propagation (1 week prior to transplanting) and repeated one week after planting out at a rate of 100 g product/100 litres water (½ rate) applying 200 ml/plant.

With the exception of T8, all slabs were 'wetted up' according to normal commercial practice prior to treatment. Advice from the manufacturers recommended that the slabs in T8 should be relatively dry prior to Aliette treatment.

Young disease free plants were then planted onto the treated slabs according to normal commercial practice.

Inoculum

A virulent culture of *Pythium aphanidermatum* (ref. PC021/97) was isolated from a cucumber crop at Stockbridge House onto potato dextrose agar. Cultures were bulked and grown at 20°C for five days to provide actively growing mycelial colonies.

Inoculation

Cores (2.5 cm diameter) were aseptically removed from a position adjacent to each plant (two cores per slab, excluding the uninoculated treatment). Agar discs (1 cm diameter) containing *P. aphanidermatum* were placed in each hole before replacing the rockwool core and sealing each inoculation site with tape. Prior to inoculation, the uninoculated control plots were sealed around the blocks using tape in an attempt to reduce breeding opportunities for sciarid flies and the possible contamination of *P. aphanidermatum* which they have been demonstrated to transmit.

Crop Schedule

	First Crop	Second Crop (replants)
Sowing date	29 December 1996	20 June 1997
Propagation treatments	-	6 July 1997
Slab treatments (PAA)	-	9 July 1997
Planting date	20 January 1997	11 July 1997
Pythium inoculation	13 March 1997 17 June 1997	-
Post-planting treatments	-	16 July 1997

Assessment dates	-	15 July 18 July 21 July 25 July
Crop termination	2 July 1997	6 August 1997

Monitoring of Residual PAA concentration in Slab and pH Levels

Following application of Jet 5, the PAA concentration and its distribution within each slab was monitored. 24 hours after treatment, solution samples were withdrawn from three sites in each slab:

a. Directly beneath the block.
b. Equidistant between the two plants.
c. In the top corner.

The PAA level in each sample was measured using a commercially available PAA dipstick as it was necessary to ensure that the residual PAA concentration in each slab was below 50 ppm to prevent potential phytotoxicity. Plants were flushed with nutrient solution until an acceptable PAA concentration was achieved. pH was also closely monitored similarly.

Disease Assessments

After slab contact the plants were monitored daily for signs of wilting. As soon as wilt symptoms became apparent assessments were undertaken using a 0-3 scale of severity where:

0 = No wilt apparent.

1 = Slight wilting in shoot apex only.

2 = More generalised but transient wilting.

3 = Generalised wilting, plant not recovering overnight.

Prior to termination of the experiment, root samples were aseptically removed from each slab and examined microscopically for oospores (resting spores) of *Pythium* spp. Not surprisingly perhaps, a positive identification of *P. aphanidermatum* was recorded in all slabs which were artificially inoculated. It should also be noted that however *Pythium* spp. was also found in several uninoculated slabs. Despite strict hygiene standards, employed throughout the trial, contamination occurred. It is possible that this can be largely accounted for by dispersal of the pathogen by sciarid flies which are difficult to control in hydroponic crops. Although contamination did not appear to significantly affect the 'uninoculated' controls, it should be noted when comparing the results.

Agronomic Assessments

Plant height was assessed on two occasions and leaf area measured once using young detached leaves of a similar age.

On two occasions (21 July and 25 July) plant vigour was subjectively assessed using a 0-3 scale where:

0 = No vigour, plant dead.

1 = Poor vigour, plant small and not thriving. Severe wilting.

2 = Moderate vigour but performance below best. Wilting.

3 = Good vigour plant thriving. No wilting.

Results

Results from the first experiment in 1996 were largely inconclusive, largely due to variability between rockwool slabs and poor *Pythium* development in cool weather conditions. They did however, suggest that there may be merit in treating slabs with PAA prior to re-use to minimise root infection and to aid plant establishment. Also, because phytotoxicity was observed in the Aliette treated plants, at the rate applied it was considered necessary to evaluate a reduced rate to determine whether efficacy could be retained without causing crop damage from the chemical treatment.

The aim of the second year study was to repeat the trial during the summer replant season using slabs of known provenance (ie generated by artificial inoculation), and also to determine efficacy and phytotoxicity of Aliette at a reduced (50%) rate compared to 1996.

Inoculation of the early cucumber crop with *P. aphanidermatum* was very successful. The pathogen was recovered consistently from sampled slabs and characteristic wilting and loss of vigour was evident in inoculated slabs. This provided an excellent base from which to conduct an experiment on replanted cucumbers and a change to warm sunny weather assisted the experiment considerably.

Immediately prior to replanting plants raised in the propagation were a) checked for the presence of root disease and b) examined for symptoms of damage following earlier application of Filex and Aliette. Neither root disease or plant damage was observed and replanting commenced.

Due to the warm sunny weather the young plants had considerable difficulty establishing and the presence of *P. aphanidermatum* in the inoculated control slabs prevented root development into the re-used slabs. Within 48-72 hours of replanting wilting was both apparent and severe in the inoculated control. In addition, plants were also wilting in several other treatments.

An assessment conducted on 15 July, some 4 days after planting, showed that ca. 25% plants were wilting in the inoculated control (Table 1) compared to only 2-3% in the uninoculated control (some evidence of dissemination, possibly by sciarid flies, had been noted in the first crop). Wilting was also apparent to a greater or lesser extent in all other treatments. Plants receiving the fungicide treatments with Filex and Aliette exhibited the least wilting whereas plants treated with per-acetic acid wilted to a level similar to, or greater than, that of the inoculated control.

By 18 July wilt severity had increased (Table 1) in most treatments, including the uninoculated control. Again, the two most effective treatments were Filex and Aliette though plants in both treatments still exhibited appreciable disease levels. By this stage, the severity of wilting was greater in all the PAA treatments than the inoculated control. No phytotoxicity was noted following application of the reduced rate of Aliette in this experiment.

Measurements of plant height (Table 3) conducted on 18 and 25 July supported the wilt assessments. Where *Pythium* infection was most severe, plants became stunted compared to the uninoculated control plants. Disappointingly, none of the treatments allowed the plants to establish and achieve a plant height comparable to that of the uninoculated control. Whilst measurement of leaf area was also conducted on 25 July the results were largely inconclusive and few significant differences were noted.

Table 1: Assessments of wilt severity at intervals in a replanted cucumber crop grown in rockwool and artificially infested with *Pythium aphanidermatum*.

Treatment[+]	Wilt Index (0-100)	
	15 July	18 July
1. Uninoculated	2.8	12.2
2. Inoculated	24.9	32.2
3. Filex	12.2	9.2
4. PAA (via irrigation lines)	19.3	42.2
5. PAA (by hand)	23.6	40.0
6. PAA (by hand drainage sealed)	40.3	50.0
7. PAA + *Trichoderma*	26.1	66.7
8. Aliette	6.9	16.7
Significance between inoculated v. uninoculated treatments	*	*
Significance between T2, 3, 8 vs (456)	NS	***
Significance between treatments 4, 5, 6	NS	NS
SED (18 df) only where significant	10.30	11.47
LSD (5%)	21.64	24.09

[+] All artificially inoculated with *P. aphanidermatum* except T1.

Table 2: Assessments of plant vigour at intervals in a replanted cucumber crop grown in rockwool and artificially infested with *Pythium aphanidermatum*.

Treatment[+]	Vigour Index (0-100)	
	21 July	25 July
1. Uninoculated	63.3	57.0
2. Inoculated	65.3	47.3
3. Filex	66.7	61.0
4. PAA (via irrigation lines)	43.7	40.3
5. PAA (by hand)	42.7	30.3
6. PAA (by hand drainage sealed)	36.3	23.3
7. PAA + *Trichoderma*	23.7	22.0
8. Aliette	58.0	52.3
Significance between inoculated v. uninoculated treatments	NS	NS
Significance between T2, 3, 8 vs (456)	NS	*
Significance between treatments 4, 5, 6	NS	NS
SED (18 df) only where significant	-	15.47
LSD (5%)	-	32.50

[+] All artificially inoculated with *P. aphanidermatum* except T1.

Table 3: Assessments of plant height and leaf area in a replanted cucumber crop, grown in rockwool and infested with *Pythium aphanidermatum*.

Treatment[+]	Plant Height (cm)		Leaf Area (cm^2)
	18 July	25 July	25 July
1. Uninoculated	75.7	99.0	89.8
2. Inoculated	60.1	76.3	69.7
3. Filex	59.4	76.0	80.4
4. PAA (via irrigation lines)	55.3	67.3	100.3
5. PAA (by hand)	58.9	68.4	102.0
6. PAA (by hand drainage sealed)	54.3	59.9	94.1
7. PAA + *Trichoderma*	54.5	58.8	98.0
8. Aliette	55.4	76.4	83.6
Significance between inoculated v. uninoculated treatments	***	***	NS
Significance between T2, 3, 8 vs (456)	NS	NS	*
Significance between treatments 4, 5, 6	NS	NS	NS
SED (18 df) only where significant	4.27	10.59	12.59
LSD (5%)	8.97	22.25	26.45

[+] All artificially inoculated with *P. aphanidermatum* except T1.

Discussion

The results obtained at the end of the first years investigation, conducted under relatively low disease pressure, provided an indication that *in situ* disinfection of rockwool slabs may be effective in preventing *Pythium* infection and allowing effective establishment of young cucumber plants.

Yet, in the second years study, under hot, sunny conditions, the *in situ* treatments were largely ineffective in preventing root infection by *P. aphanidermatum*. Clearly, under high disease pressure neither PAA, Aliette or the standard commercial treatment with Filex is sufficient to prevent significant plant losses.

The lateral spread of *P. aphanidermatum* to the uninoculated control plots in this trial was significant. During establishment of the first crop, when temperatures are generally lower, young plants are able to develop effectively and any *Pythium* present is likely to be masked because the pathogen is operating significantly below its optimum of 35°C. As temperatures increase during spring *P. aphanidermatum* undoubtedly becomes more active though, because plants by this stage have a well developed root system, they are usually able to survive infection. Yet, during this 'latent' period it is possible that the fungus is disseminated from slab to slab by a variety of mechanisms. Only when the crop is replanted does the full extent of the *Pythium* infection become evident. Therefore, until alternative effective treatments can be sought growers should concentrate on maintaining effective hygiene precautions in the first crop to minimise spread. Spaces should be retained between slabs to avoid movement of the fungus by capillary beneath the rockwool slabs and efforts should be made to minimise sciarid fly development and transmission.

It is recommended that further studies should concentrate on the mechanisms of spread of the fungus and on the identification of alternative effective fungicides to assist during crop establishment.

Conclusions (1996-1997)

- The use of Jet 5 (per-acetic acid) as an *in situ* disinfection treatment of rockwool slabs in order to aid plant establishment and prevent infection by *Pythium* showed some promise in preliminary studies conducted in 1996.

- Treatment of young cucumber plants with Aliette, at the rate used, was phytotoxic, in the 1996 evaluation.

- Variability between 'used' rockwool slabs collected from commercial nurseries hindered effective interpretation of the results.

- Weather conditions during the initial study were not particularly conducive to symptom expression by *Pythium* spp. and disease severity was low.

- A second experiment using artificially inoculated slabs known to be contaminated with *P. aphanidermatum* was conducted during June - July 1997.

- Artificial inoculation with *P. aphanidermatum* was effective and provided an extreme test to measure the potential of an *in situ* slab disinfection treatment alongside standard (Filex) and experimental (Aliette) fungicides.

- During the summer period the weather conditions were optimum for *Pythium* infection and under this severe disease pressure, Filex, Aliette (half rate) and various methods of application of PAA were not effective and did not adequately disinfect *Pythium* infested rockwool slabs.

- Treatment with Filex or Aliette (half rate) did not induce phytotoxicity in replanted cucumbers.

- Alternative methods must continue to be sought to control *Pythium* infections during the establishment phase in replant cucumbers.

- In the interim period every effort must be made to minimise dissemination of *Pythium* during the early crop when below optimum temperature conditions 'mask' symptoms of the disease.

Acknowledgements

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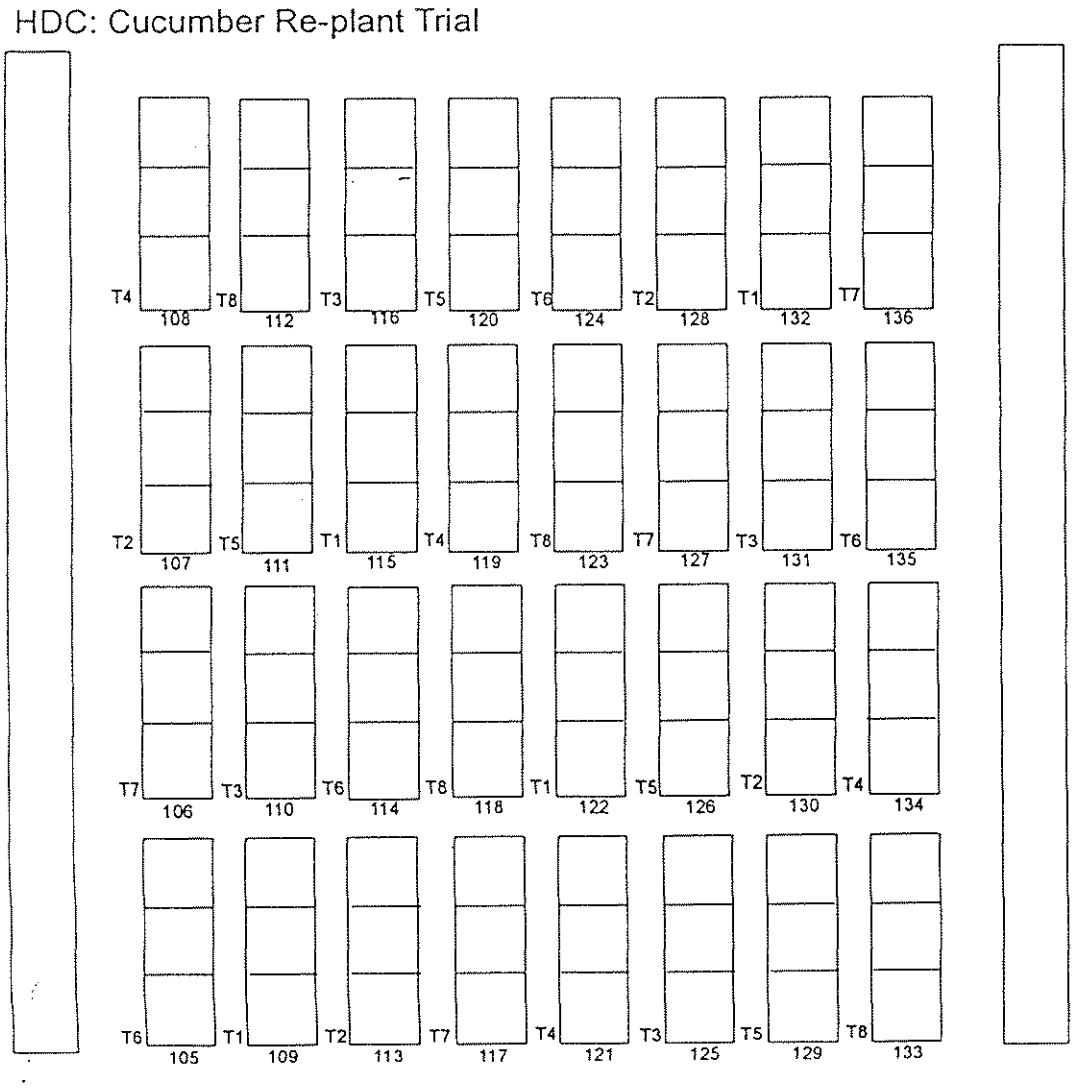
APPENDIX I: TRIAL PLAN (1997)



Treatments

1. Uninoculated control
2. Inoculated control
3. Filex (standard treatment)
4. Jet 5 via irrigation lines
5. Jet 5 by hand
6. Jet 5 by hand (drainage slits sealed)
7. Jet 5 followed by *Trichoderma*
8. Aliette drench

6 plants/plot
Trial inoculated with *P. aphanidermatum*