ADAS

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CONTRACT REPORT

Black Root Rot of Pansy and Other Bedding/Pot Plants
PC 38
Final Report

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COMMERCIAL IN CONFIDENCE

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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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Contract Manager

Date 29 July 1992

Report authorised by ....

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SUMMARY

Black root rot which has caused considerable losses in pansies and violas grown for autumn bedding was well controlled by benomyl applied at sowing in composts inoculated with the fungus. There was no indication that this standard material was failing to control the disease due to development of strains of Thielaviopsis basicola resistant to benomyl. Failure to control is likely to be due to late application of benomyl which gave only limited control if applied two weeks after sowing. Octave showed promise as an alternative fungicide.

There was no evidence to support the circumstantial experience that the use of metalaxyl exacerbated BRR. In contrast, the use of Aaterra, Fongarid or Filex tended to reduce disease levels.

Growing seedlings in clean plug modules and isolating them on open mesh benching resulted in least disease under nursery conditions.

BACKGROUND

Black root rot (Thielaviopsis basicola) was recorded as a pathogen in the UK in 1850. It has a world wide distribution affecting over 15 families, particularly species of leguminosae,solanaceae and cucurbitaceae. Among popular bedding plants it has been found in the UK on antirrhinum, aster, begonias, cyclamen, nemesia, poinsettia, primula, stocks, verbena and viola. It has been particularly conspicuous in pansies and violas sown for autumn bedding during the last two years.

Control measures currently employed depend on strict nursery hygiene, skilled management of nutrients and watering and the use of carbendazim-based fungicides. Despite strict attention to the control measures recommended, black root rot has not been effectively controlled on many nurseries.

LITERATURE REVIEW AND INTRODUCTION

Literature Review

Much of the research on this disease has been concerned with its effect on the tobacco crop in the USA (Anderson et al 1926, Gayed 1972 and Lucas 1975) and in citrus (Tsao 1962, Timmer 1988). Studies concentrated on field factors which influenced disease, eg temperature and pH (Doran 1929 and 1931, Lucas 1955) and more recently, inoculum levels (Anderson et al 1988, Specht and Griffin 1988). Races of the pathogen received attention from several workers including Gayed (1972) and Corbaz (1985). Huber et al (1982) Meyer et al (1991) and Reddy et al (1992) studied naturally occurring suppressive agents in field soils. Fungicides received little attention, being an expensive method of controlling this soil borne disease. Allen and Hine (1969) demonstrated control of Thielaviopsis in citrus by benomyl and similar results were obtained by Hartil and Campbell (1972) in tobacco. More recently Delon and Pululu (1989) and Prinsloo et al (1989) found benefits from triazole fungicide against Thielaviopsis in tobacco seed beeds.

Until recently, Poinsettia was the main pot and bedding plant to receive attention for the control of black root rot. Manning and colleagues (1970) and Raabe et al (1971) obtained good control of the disease using benomyl and this fungicide has been the standard material for combating black root rot in pot plants for the last 20 years.

Powell and his colleagues at Ohio State University published a series of articles in their Florists Association Bulletins (eg No. 712 February 1989) and in locally distributed notes to growers on the control of black root rot in vinca and violas. The disease had appeared as a major problem in plug-raised plants during the 1980s. The consensus, on the basis of grower experience was to reduce stress, use benomyl (or related material) regularly and avoid composts with a high pH and ammonium sources of nitrogen. Powell (1989) and Koranski (pers communication).

The studies detailed in this report examine:

The activity of currently available fungicides for activity against black root rot.

Likely candidate fungicides tested under controlled conditions using artifically inoculated compost.

Likely candidate fungicides tested under natural conditions on growers nurseries.

A range of isolates of Thielaviopsis for resistance to Benomyl.

The alleged detrimental effects of fungicides used to control Pythium.

Fungicide screening

Materials and methods

Using a selective medium (appendix 1) growth from plugs of 5 different isolates of *Thielaviopsis basicola* were studied for their capacity to grow on agar amended with either benomyl, chlorothalonil, prochloraz, thiabendazole or flusilazole. Concentrations of fungicide were either 2ppm or 20ppm active ingredient. The plates, including unamended controls, were incubated at 22^{o}C for 14 days. There were 3 replicates.

Results

Details are given in Table 1.

Benomyl at either concentration prevented growth of all isolates. Thiabendazole and flusilazole prevented growth on the amended agar at both concentrations but the fungus remained viable on some of the inoculation plugs at the lower concentrations.

Prochloraz inhibited growth at 20ppm, although growth on plugs survived on 2 of the isolates. Two isolates of *Thielaviopsis* grew on agar amended with 2ppm prochloraz.

Chlorothalonil had little effect on fungal growth at 2ppm and caused only a partial growth reduction at 20ppm.

Table 1 – Growth of Thielaviopsis on agar amended with fungicides 14 days after inoculation

Isolate	Control	Benomyl		Chlorothalonil		Prochloraz		Thiabendazole		Flusilazole	
		2ppm	20ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1	+++	-	-	++	+	(-)	(-)	(-)	-	(-)	-
2	+++	-	-	++	+	(-)	-	-	-	-	-
3	+++	-	-	+++	+	+	-	-	-	(-)	-
4	+++	-	-	+++	+	+	-	(-)	-	(-)	-
5	+++	-	-	+++	++	(-)	(-)	(-)	-	(-)	(-)

Key: - = No growth

(-) = Growth through inoculum plug only

\+ = Growth 80% unamended control

++ = Growth 80-90% unamended control

+++ = Growth >90% unamended control

Evaluation of fungicides under controlled conditions

Growth of pathogens on fungicide-amended agar gives an indication of fungicidal activity but this potential may not be realised in the growing plant. Preliminary studies suggested that thiabenolazole (as Hymush) whilst giving excellent control of _Thielaviopsis_ in vitro delayed emergence and caused death of seedlings. It was therefore not included in this next phase of the work.

Materials and methods

Peat based compost was inoculated with _Thielaviopsis basicola_ using spores collected from 7-10 day-old cultures of the pathogen. Plug trays (10ml per cell) were filled by hand and sown with pansy (Universal Beaconsfield - mixed). Fungicides were applied either immediately after sowing or 14 days later. (Details are given in Appendix 2).

Treatments

Water	)
Benlate (1g/litre	)
Octave (0.72g/litre)	) inoculated with _Thielaviopsis_
Sanction (0.8g/litre)	)
Bravo (1.1ml/litre)	)

Water alone - uninoculated

There were 4 replicates of 35 cells per treatment.

After 8 weeks the middle 15 cells from each tray were assessed for vigour, fresh weight and disease.

Disease Index

Plants were placed in categories according to disease levels:

C1 = slight (1-2% roots affected)

C2 = moderate (21-40% roots affected)

C3 = severe (more than 40% roots affected)

Index was calculated according to the following:

$$\text{numbers of plants in } \frac{(C1 \times 1) + C2 \times 2) + (C3 \times 3)}{30} \times 100$$

RESULTS

Tables of results including statistical analysis are given in Appendix 3. The following histogram, Figure 1, summarise the findings.

Disease Control:

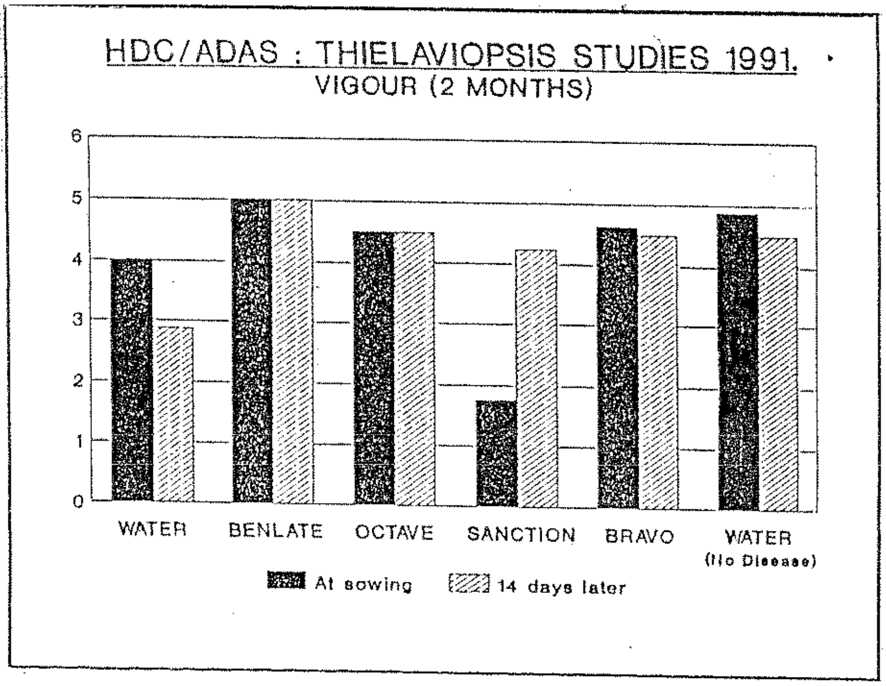
Benlate, Sanction and to a lesser extent Octave controlled black root rot when applied at sowing.

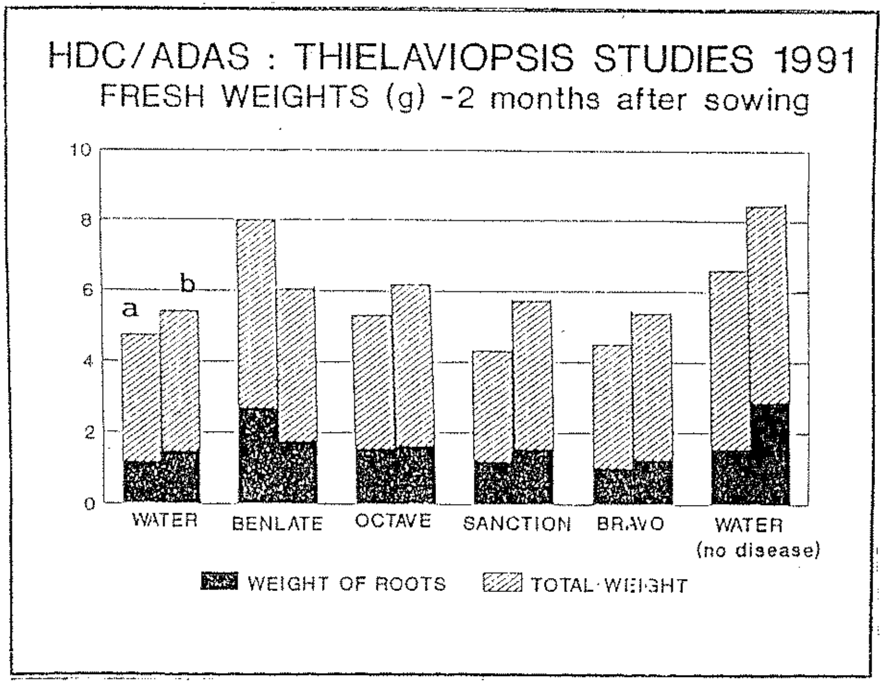
Benlate applied 14 days after sowing gave limited control of the disease.

Octave limited disease development when applied 14 days after sowing; Sanction was less effective.

Bravo gave no significant control of black root rot.

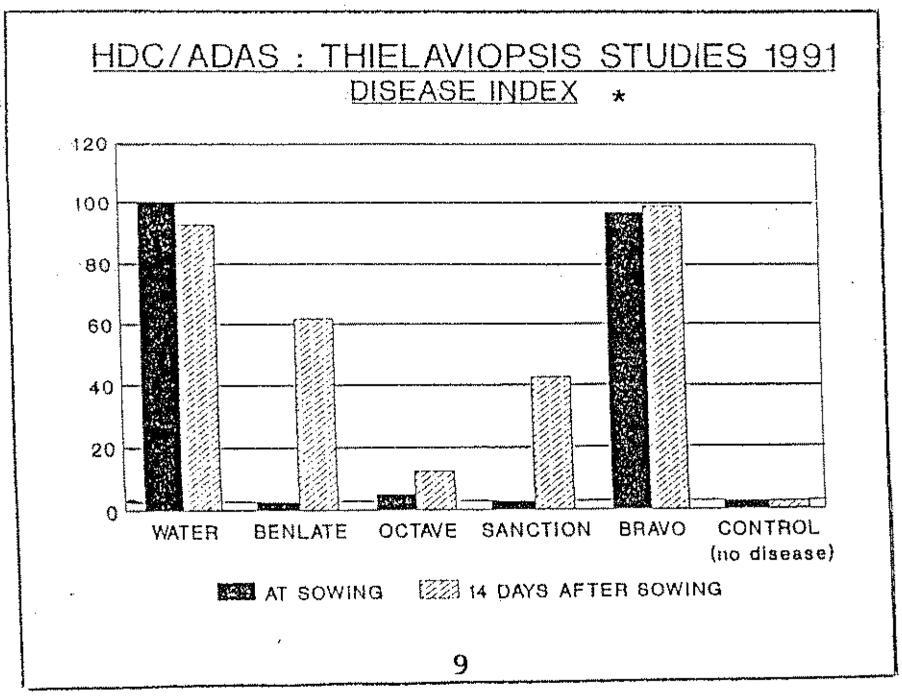
FIG.1





a=treatment applied at sowing

b=treatment applied 14 days after sowing



* See page 8

Vigour and plant weight:

Records of plant weights do not present a clear picture of treatment effects but Sanction at sowing substantially and significantly reduced vigour and plant weight.

The early application of Benlate had no adverse effect on plant weight; all other treatments applied at sowing reduced plant weight.

Later treatments with fungicide tended to have a less adverse effect on plant weight.

Conclusions:

Benlate applied at sowing gave good control of disease with no adverse effect on vigour or plant weight; delayed treatment greatly reduced control of BRR.

Bravo offered no control of disease.

Octave applied at sowing gave good disease control but tended to reduce vigour and plant weight. This effect was reduced when treatment was delayed for 14 days but disease control obtained from this later application was still good.

Sanction applied at sowing gave good disease control but caused an unacceptable check to growth. When applied 14 days later, disease control was poor but growth was less affected.

EVALUATION OF FUNGICIDES ON GROWERS HOLDINGS

Benlate (the current standard for control of black root rot on pot plants), Bravo, Hymush (thiabendazole) and Octave were tested on 2 nurseries for control of Thielaviopsis under natural conditions. Sanction (fluzilazole) which has no clearance for use on commercial nurseries under glass was omitted.

Materials and Methods (Trial 1, Battlesbridge, Essex)

Treatments:

Compost and water only	Compost and Fongarid(2g/101)
Benlate	Benlate
Hymush	Hymush
Bravo 500	Bravo 500
Octave	Octave
Water alone	Water alone

Layout

Plots consisted of 1 x 45 -plug modules in trays with infested debris to encourage natural infection. There were 4 replicates arranged in randomised blocks. Treatments were applied at sowing, then twice more at monthly intervals (details are given in Appendix 4).

Results

Ten days after sowing all treatments except Octave showed 50% emergence. Two weeks later, 50-60% of Octave treated plants had emerged but 90% of those drenched with Hymush had collapsed. Other treatments gave 90-95% emergence. Pythium and Thielaviopsis were recorded in plants from an early stage but levels of disease at the end of the trial period were very low. Details of growth and disease incidence are given in Table 2.

Table 2 - Harvest data from Battlesbridge trial 1991

	Root browning (0-4)	Weight of leaves per plant	% plants Thiel-aviopsis	Root scorch score (0-5)
Water	1.08	0.39	3.7	1.46
Benlate	0.96	0.71	3.1	1.26
Bravo	1.07	0.50	5.4	1.71
Octave	1.09	0.56	1.2	1.39
SED	0.07	0.08	2.76	0.15

A second trial set up in Cheshire during April 1992 failed to germinate evenly when temperature control on the nursery broke down. The trial was abandoned.

RESISTANCE TESTING

Bavistin and Benomyl are the only fungicides available for use against black root rot in pot and bedding plants. Both materials are from the same chemical grouping and it is possible that failure to control the disease could have been associated with the development of resistance to MBC fungicides by the fungus.

The aim of this part of the study was to test 100 isolates for their capacity to grow on agar amended with either 2ppm or 20ppm a.i. benomyl.

Materials and Methods

Isolates of Thielaviopsis were obtained on V8 based selective medium. Plugs of actively growing fungus were transferred to the amended agars and growth measured after 5 days. Controls of Botrytis cinerea either sensitive to benomyl or resistant to benomyl at 20ppm were included for comparison.

Results

None of the isolates of Thielaviopsis tested grew on the amended agar. Control isolates of Botrytis grew as expected. Details of the isolates of Thielaviopsis tested are given in Appendix 5.

CONCLUSIONS

There is no evidence that resistance to benomyl among UK isolates of <u>Thielaviopsis basicola</u> is responsible for poor control of the disease.

EFFECT OF PYTHIUM - FUNGICIDES ON CONTROL OF BLACK ROOT ROT

Reports from USA suggested that fungicides based on metalaxyl used against Pythium could exacerbate damage by Thielaviopsis (Powell, 1989). This part of the project examined the effects of the three main fungicides used against Pythium when added to compost inoculated with the black root rot fungus. The influence of Benomyl on the activity of these fungicides was also studied.

Materials and Methods

Peat based compost was inoculated with Thielaviopsis basicola using spores collected from 7-10 day old cultures of the fungus (see Appendix 1 for details).

Plug trays (10ml/cell) were sown with pansy Universal Beaconsfield Fl. seed. Pythium fungicides were applied the day before sowing when benomyl treatments were added.

Treatments

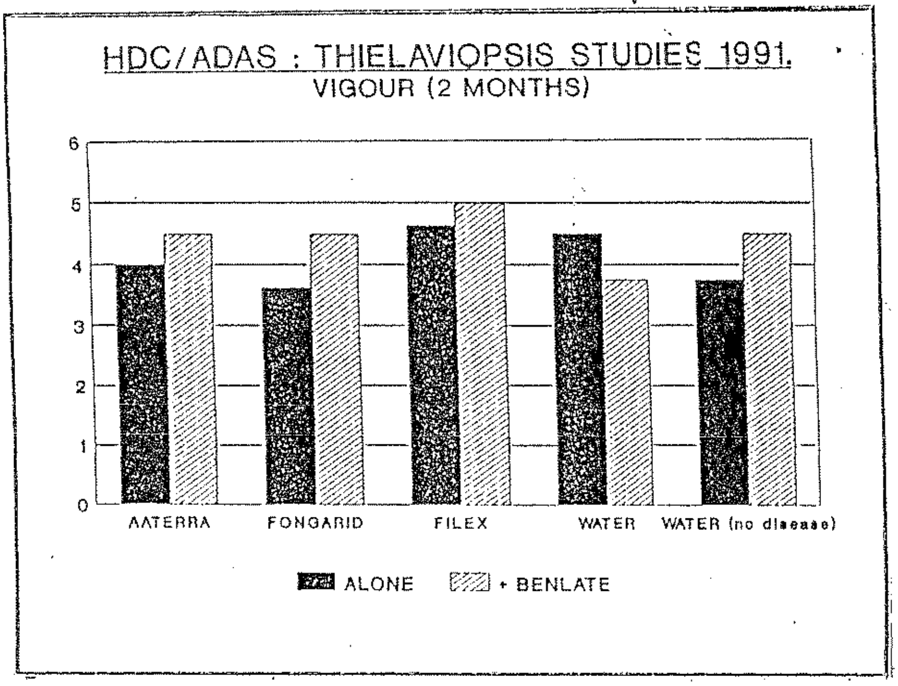
	(Aaterra (0.4g/10 litres compost)
	(Fongarid (4.0g/10 litres compost)
Infested	(Filex (2.5ml/litre; 125ml/tray of 35 cells)
compost	(Water only
	(Aaterra + Benlate (1g/litre; 125ml/tray)
	(Fongarid + Benlate
	(Filex + Benlate
	(Water + Benlate
Uninfested	(Water only
compost	(Water + Benlate

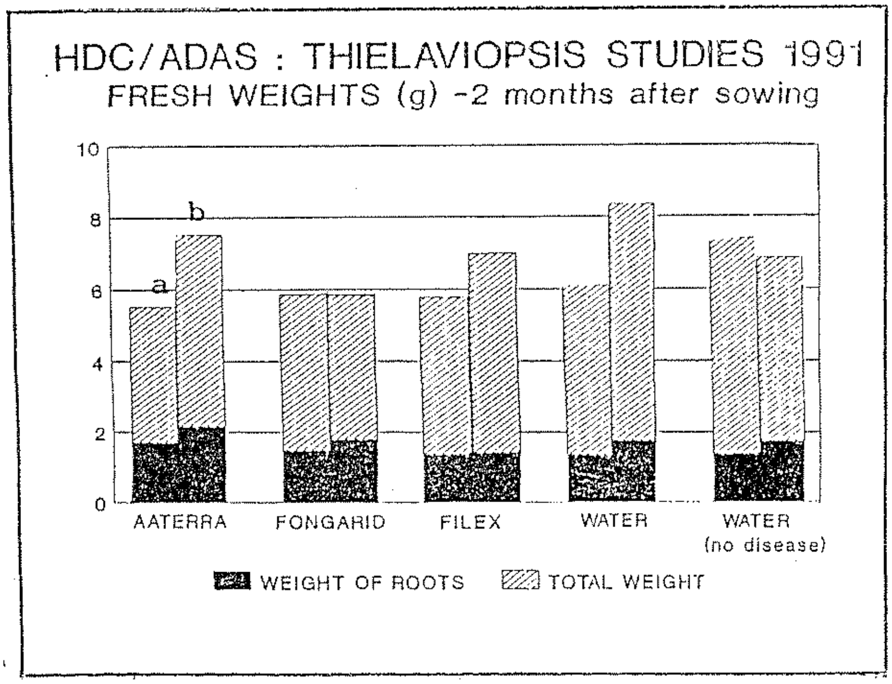
There were 4 replicates of each treatment. Eight weeks after sowing 15 cells from each tray were assessed for vigour, fresh weight and disease levels.

Results

Tables of results including statistical analysis, are given in Appendix 6. The histogram (Figure 2) summarises the findings.

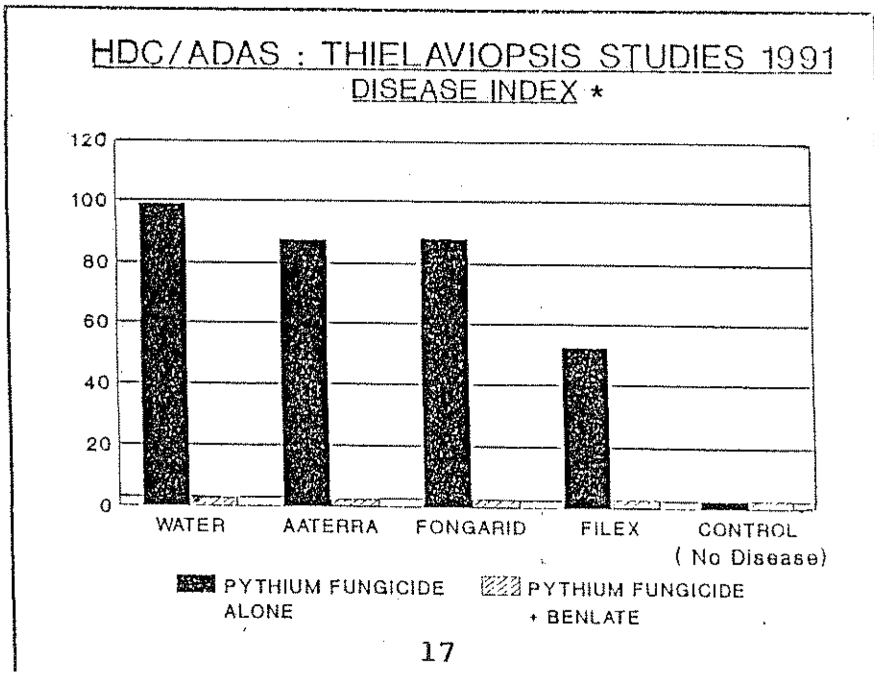
FIG.2





a= "Pythium fungicide alone

b= "Pythium fungicide + Benlate.



* See Page 8

Disease Control

Control of Thielaviopsis by Benlate was not adversely affected by the addition of any of the fungicides used to control Pythium.

Aaterra and Fongarid alone reduced the incidence of black root rot but Filex halved disease levels.

Plant vigour was usually enhanced by the addition of Benlate; plant weights showed less consistent changes.

CONCLUSIONS

There is no indication that the addition of any of the fungicides used to control Pythium have any detrimental effect on plant growth.

Black root rot is not exacerbated by the use of Aaterra, Fongarid or Filex.

OTHER STUDIES

Opportunities to examine factors other than those listed in this contract arose during the course of the work.

Effect of debris on source of disease

Sweepings collected from floor areas in 3 nurseries were mixed with potting compost (Fisons M3) and sown with seeds of Pansy Universal Beaconsfield. Seedling roots were examined 2 months later.

Results

Percent plants affected by black root rot ranged from 20-80% at each nursery.

Effect of re-used trays and standing area on incidence of disease

At the Battlesbridge site (see page 11) in addition to the major trial, the significance of used trays and different standing areas as potential sources for disease were examined.

Treatments

New/well washed trays	Used trays
1. Set out on Wire Bench	3. Set out on Wire Bench
2. Set out on matting floor	4. Set out on matting floor

Four large module trays for each treatment (Centre 40 plugs seeded). Trays were seeded on the 20 September 1991. Final assessments of root condition, <u>Thielaviopsis</u> infection and plant weight were made on the 11 December.

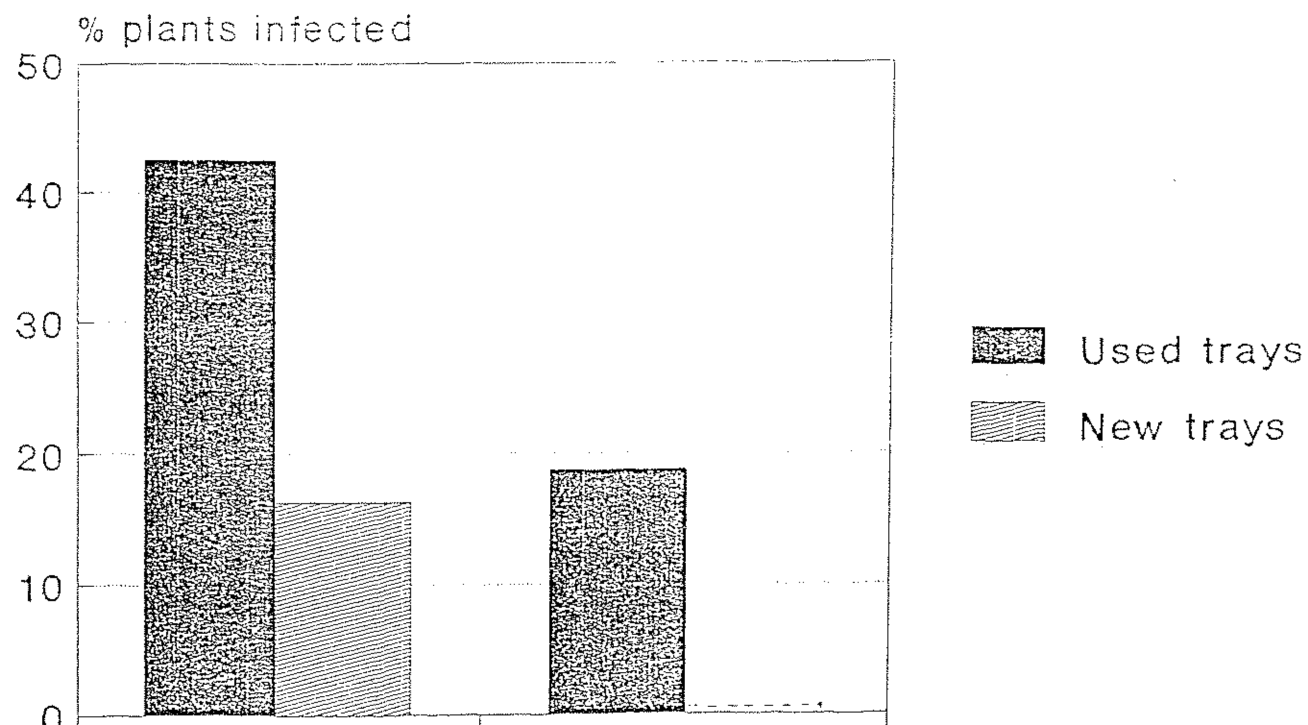
Results

Full details will be published elsewhere but a summary of the findings are that <u>Thielaviopsis</u> was found in the roots of up to 30% of the plants in the old trays compared with 8% in the new trays. Similarly, floor standing trays carried a significantly greater infection of 29%, compared to the wire bench trays where 9% of plants were infected.

Figure 3 shows the breakdown in better detail; where new trays had been placed on the mesh bench, disease was absent.

FIG.3

Battlesbridge pansies
Floor v' Mesh
Thielaviopsis - % plants



	Floor	Mesh
Used trays	42.5	18.8
New trays	16.3	0

CONCLUSIONS

Among the currently available fungicides tested (chlorothalonil, thiabendazole and prochloraz), only prochloraz (as Octave) shows promise as an alternative to benomyl for the control of Thielaviopsis. It did, however, check growth if applied at sowing, but when used 14 days later there was little or no adverse effect on growth and only a slight reduction in disease control using inoculated compost.

Checks on 100 isolates of Thielaviopsis failed to find any evidence of resistance to the fungicide benomyl. This fungicide remains the best material so far tested for control of this disease in seedling pansies.

The use of metalaxyl to control Pythium did not increase damage by Thielaviopsis. On the contrary, the use of Fongarid, Aaterra and particularly Filex, tended to reduce the incidence of BRR in plants sown in inoculated compost.

In the current work here reported, the effects of BRR on seedlings grown in compost inoculated with a massive spore load was surprisingly modest. This work was done at temperatures ranging from 18°C to 22°C. Later work using the same inoculum type and density but at temperatures of 24-28°C resulted in seedling collapse and death within 2 months of sowing. This effect tends to confirm field observations that the disease is particularly serious during the hotter summer months.

RECOMMENDATIONS

1. These trials confirm that benomyl remains an effective fungicide for the control of BRR in seedling pansies but the removal of label recommendations for its use on ornamentals indicates that alternative forms of carbendazim and thiabendazole should be tested.

2. The benefits of Filex in limiting BRR deserves a closer study.

3. Work on the influences of compost make-up and pH is under way but the effects of temperature need closer attention.

4. The effects of isolating plug modules from contaminated bench or floor surfaces needs more study.

5. The effects of disinfectants for cleaning equipment and for general nursery hygiene should be investigated.

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APPENDIX 1

Selective medium for <u>Thielaviopsis</u>

V8 Juice	=	100ml
Distilled water	=	400ml
Calcium Carbonate ($CaCO_3$)	=	0.5G
Tech Agar No.3	=	10g
Glucose	=	1g
Yeast Extract	=	1g
PCNB	=	0.5 g (added after autoclaving)

After the mixture had been autoclaved,antibiotic was added when the temperature had fallen to around 55^{o}C (5ml needed).

The antibiotic consisted of:

Sterile distilled water	=	100ml
Nystatin	=	0.6g
Streptomycin Sulphate	=	2.0g
Chlortetra cycline	=	0.04g

Final pH is adjusted to 5.2 using hydrochloric acid (HCI).

EVALUATION OF FUNGICIDES TESTED UNDER CONTROLLED CONDITIONS

Details of inoculation and fungicide treatments.

Compost: Fisons M2

Inoculation: Washings from 7-10 day old cultures of *Thielaviopsis* on V8 Agar containing approximately 3,500 whole chlamydospores/ml were used at the rate of 500mls of spore suspension to 10 litres of compost. (Delivering approximately 1,750 spores per 10ml cell).

Fungicides: 125ml of diluted fungicide were applied to each tray (approx. 3.5mls/cell) either the day after compost preparation or 2 weeks later.

Husbandry: Trays were maintained at 18-22^{o}C with 12 hr minimum day length and fed (200ppm N?) at weekly intervals from full emergence.

Sown: 20.11.91

Fungicide Treatments: 20.11.91, 18.12.91

Assessed: 27. 1.92

APPENDIX 3

FUNGICIDES TESTED UNDER CONTROLLED CONDITIONS: DETAILS OF RESULTS

Table 3 Vigour and fresh weights at 2 months (mean values/plot)

Treatments (a = applied at sowing (b = applied 14 days later)		Vigour (1= poor) (5= good)	Fresh Weights (g) Total	Roots only
Water	a	4.0	4.7	1.1
	b	2.9	5.4	1.4
Benlate	a	5.0	8.0	2.6
	b	5.0	6.0	1.7
Octave	a	4.5	5.3	1.5
	b	4.5	6.1	1.6
Sanction	a	1.7	4.3	1.2
	b	4.2	5.7	1.5
Bravo	a	4.6	4.5	1.0
	b	4.5	5.4	1.2
Water (no disease)	a	4.9	6.6	1.5
	b	4.5	8.4	2.8
SED		0.17	0.53	0.22

Table 4 Disease levels at 2 months

Treatments		Number of plants/plot Disease free	Slight	Moderate	Severe
Water	a	0	0	0	10
	b	0	0	2.0	8
Benlate	a	10	0	0	0
	b	0	1.2	8.7	0
Octave	a	8.5	1.5	0	0
	b	6.2	3.7	0	0
Sanction	a	10.0	0	0	0
	b	0	7.2	2.7	0
Bravo	a	0	0	1.0	9
	b	0	0	0.2	9.7
Water	a	10	0	0	0
	b	10	0	0	0
SED		0.23	0.47	0.53	0.42

APPENDIX 4

EVALUATION OF FUNGICIDES ON GROWER HOLDINGS

Husbandry details

Site: Newlands Nursery, Battlesbridge, Essex

Cultivar: Winter flowering Fl Pansy, Universal Beaconsfield

Sown and first drench: 17. 9.91

Second drench: 25.10.91

Potted up: 6.11.91

Third drench: 22.11.91

Final assessment: 11.12.91

DETAILS OF THIELAVIOPSIS ISOLATES TESTED FOR RESISTANCE TO MBC FUNGICIDES

Reference	Host	Location	No. isolates
-	Tomato	Hants	2
B91/C5/517	Pansy	Glos	6
1653/A/91	Pansy	Lancs	15
91/CS/483	Poinsettia	Bristol	1
263/C/91	Pansy	Yorks	1
259/C/91	Pansy	Somerset	2
1547/A/91	Pansy	Worcs	8
289/C/91	Pansy	Devon	9
1524/A/91	Pansy	Worcs	7
1558/A/91	Pansy	Worcs	10
654/A/91	Antirrhinum	Cheshire	8
-	Pansy	Bristol	8
626/A/91	Verbena	Lancs	7
-	Verbena	Herts	8
971/A/91	Verbena	Cheshire	1
207/C/91	Pansy	Humberside	4
52/A/91	Fuchsia	Worcs	3

EFFECTS OF PYTHIUM-FUNGICIDES ON CONTROL OF BRR

DETAILS OF RESULTS

Table 5 Vigour and fresh weight at 2 months (mean values/plot)

Treatments (a=Pythium fungicide alone) (b=Benlate added)		Vigour 1=poor 5=good	Fresh weights (g) Total	Roots only
Water	a	4.5	6.1	1.3
	b	3.7	8.4	1.7
Aaterra	a	4.0	5.5	1.6
	b	4.5	7.5	2.1
Fongarid	a	3.6	5.9	1.4
	b	4.5	5.8	1.7
Filex	a	4.6	5.8	1.3
	b	5.0	7.0	1.4
Water (no disease)	a	3.7	7.4	1.3
	b	4.5	6.9	1.7
SED		0.17	0.53	0.22

Table 6 Disease levels at 2 months

Treatments (a=Pythium fungicide alone) (b=Benlate added)		Number of plants/plot			
		Disease free	Slight	Moderate	Severe
Water	a	0	0	0.3	9.7
	b	9.2	0.8	0	0
Aaterra	a	0	0	3.7	6.3
	b	10	0	0	0
Fongarid	a	0	0	3.5	6.5
	b	9.7	0.3	0	0
Filex	a	0	5.5	3.2	1.3
	b	10	0	0	0
Water (No disease)	a	10	0	0	0
	b	10	0	0	0
SED		0.23	0.47	0.53	0.42