Contract Report
for the Horticultural Development Council

Chemical disinfectants for treatment of plastic plug trays contaminated with *Thielaviopsis basicola*
1995
(PC 38c)

TIMO 44a

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FINAL REPORT (JULY 1995)

Project number	PC 38c
Title:	Comparison of chemical disinfectants for treatment of plastic plug trays contaminated with *Thielaviopsis basicola.*
Project Leader:	Dr T M O'Neill ADAS Horticulture Brooklands Avenue Cambridge CB2 2BL
Project Co-ordinator:	Mr J Overvoorde Delfland Nurseries Benwick Road Doddington, March Cambs PE15 0TZ
Location of project:	ADAS Arthur Rickwood
Date project commenced:	August 1994
Date project completed:	July 1995
Key words:	Disinfectant *Thielaviopsis basicola* Black root rot Plastic plug trays

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APPLICATION

The objective of the project was to provide information on the efficacy of selected chemical treatments in disinfecting used plastic plug trays contaminated with *Thielaviopsis basicola*. Chemicals were applied as specified on product labels or at the greatest concentration listed where no specific recommendation for tray or pot dipping existed. Nine disinfectant treatments were identified which reduced black root rot when pansies were grown in treated trays.

SUMMARY OF RESULTS

The objective of the work was to compare the efficacy of selected chemical treatments in disinfecting used plastic plug trays contaminated with *Thielaviopsis basicola*. Disinfectants were evaluated *in vivo* by growing pansy seedlings in plastic plug trays naturally contaminated with the fungus in adhering peat and root debris and assessing the incidence of plants developing black root rot. No fungicides were applied for control of black root rot. Trays were rinsed by dipping in water after treating with disinfectant and before sowing pansy seed. None of the plants developed phytotoxic symptoms. Although none of the treatments reduced the incidence of black root rot to zero, nine treatments (Formalin, Glu-Cid, Iodel FD, Jet 5, Opticide H, Panacide M, Purogene, Ter-Spezial and sodium hypochlorite) reduced the mean number of plants developing the disease compared with the incidence in plants grown in untreated trays. Formalin, Iodel FD and Jet 5 were particularly effective. No black root rot developed on plants grown in new plastic plug trays.

ACTION POINTS FOR GROWERS

1. On nurseries where black root rot has occurred in young pansy plants, consider treating plastic plug trays with Formalin, Glu-Cid, Iodel, Jet 5, Opticide H, Panacide M, Purogene, Ter-Spezial or sodium hypochlorite before growing another susceptible crop in the trays. Some disinfectants, particularly Formalin, Glu-Cid and Opticide H, should only be used in an area where there are no plants present because of the risk of fumes from these products causing crop damage.

2. Disinfection alone is unlikely to provide complete control of black root rot where there is a high inoculum of the fungus. Consider treating susceptible species with an appropriate fungicide treatment (e.g. an MBC product or Octave) to supplement tray disinfection.

3. Disinfectants in most areas of use are exempt from the 1986 Control of Pesticide Regulations. Formalin is an exception and is classed as a commodity chemical (0159/91) within the Control of Pesticide Regulations, with specific restrictions on fields of use and maximum concentration, and instructions on operator protection. Also, the Control of Pesticide Regulations do apply to any disinfectant if it is applied to a crop or a growing medium.

INTRODUCTION

Although there is a large body of published information on disinfectant efficacy against micro-organisms causing human or animal diseases, information on the effectiveness of different chemical disinfectants for use in commercial horticulture is relatively scarce. Moreover, the majority of economically important plant diseases are caused by fungi, whilst in medicine and veterinary science bacterial and viral diseases are more common and hence the usual target in disinfectant trials.

Black root rot caused by the fungus *T. basicola* can be a serious problem affecting production of bedding and pot/bedding plants. The disease causes leaf yellowing and uneven growth. Species commonly affected in recent years include impatiens, pansy and verbena. There is a risk of infection arising from plug trays when they are re-used and especially if they are re-used several times in the same season. Once-used plug trays were found to be a source of *T. basicola* in a recent ADAS-funded project on the source of this disease in winter pansies. With the increasing use of plastic plug trays in many sectors of horticulture, and the increasing practice of re-using them many times, identification of chemical disinfectants which are effective under commercial treatment conditions are urgently needed. The commercial objective of the work described here was to identify disinfectant products which will disinfect plastic plug trays contaminated with *T. basicola* and thereby allow growers to eliminate plug trays as a possible source of this disease.

Related projects on chemical disinfectants are:

Evaluation of disinfectants for treatments of sand contaminated with *Phytophthora* (PC 107).

Evaluation of disinfectants for treatment of capillary matting contaminated with *Fusarium oxysporum* f.sp. *dianthi* (HNS 63).

MATERIAL AND METHODS

Plants

Pansy plants (F1 Universal mixed) were sown in Fisons F2 compost in new plastic plug trays (first crop), or once-used trays (second crop), and germinated on a Danish trolley in a humid chamber at ADAS Arthur Rickwood. After germination, trays of seedlings were transferred to an unheated greenhouse and grown on a concrete floor (first crop) or wire mesh benches (second crop). The first and second crops were grown in different greenhouses. Trays were placed tray-tight on the floor and spaced 50 cm apart on benches. No fungicides were incorporated into the compost or subsequently applied to plants as drenches. Plants were watered by overhead irrigation.

Production of naturally contaminated plug trays

Pansy plants grown in new plastic plug trays were inoculated with a standard inoculum of *T. basicola* and allowed to develop black root rot so that the plug trays became naturally contaminated.

Treatments

Product	Active ingredient	Rate product used (ml/litre)
1. Untreated	(water dip)	-
2. Clearsol	40% tar acids	10
3. Cryptonol	14% hydroxyquinolene sulphate	3
4. Formalin	38% formaldehyde	20 (max. dip concentration)
5. Glu-Cid	20% glutaraldehyde	100
6. Iodel FD	2% iodine	8
7. Jet 5	hydrogen peroxide/PAA	11
8. Opticide-H 200	20% glutaraldehyde + 20% QAC	20
9. Panacide M	30% diclorophen	17
10. Purogene	chlorine dioxide	25
11. Ter-Spezial	quaternary ammonium compounds (QAC)	10
12. Sodium hypochlorite	10-14 % available chlorine	100
13. Virkon	organic acids and salts	10
14. Water (new trays)	negative control	-

Disinfectants were applied as a 15 minute, ambient temperature (13 C) dip apart from Jet 5 (1 hour dip) and Ter Spezial (2 hour dip) where the labels specified a longer dip time. The rates of use chosen for testing were as specified on product labels, wherever a specific recommendation for tray or pot dipping existed. In other cases, the minimum dilution (maximum concentration) stated on the label was used. The statutory maximum concentration of formalin in water for use as a dip treatment (1:50) was used. Loose peat was shaken from trays before treatment but they were not soaked in water or otherwise cleaned. After treatment, trays were dipped once in water to rinse. They were then allowed to dry, refilled with Fisons F2 compost and re-sown with F1 Universal pansy seed.

Experiment design

The experiment was arranged in randomised blocks with 4 replicates and double replication of the untreated control. Each plot consisted of one 35-cell plug tray.

Assessments

Plants were examined for black root rot symptoms at 3 and 16 weeks after germination. A final destructive assessment of the incidence of black root rot was made when more than 40% of plugs in the untreated trays were found to be infected with black root rot. At the final assessment the following attributes were assessed:

1. Number of surviving plants per tray.
2. Number of plants with black root rot.
3. Number of plants stunted and with severe yellowing or purpling of leaves
4. Plant quality (on 10 plants selected at random; 0-5 index, described below)

The following plant quality scale was used:

0 - Plant dead
1 - Severe leaf yellowing and/or severe black root rot; very poor plant.
2 - Some leaf yellowing (greater than three leaves) and/or obvious black roots; small plant.
3 - Slight leaf yellowing (2-3 leaves) and/or some root rot; moderate plant.
4 - Slight leaf yellowing (1 leaf) or slight root rot
5 - Excellent plug plant; no leaf yellowing and no root rot.

The cause of root discoloration and rotting was confirmed by appropriate laboratory tests. Trays were also examined for evidence of discoloration or brittleness.

Crop diary

11 August	first pansy crop sown and placed on Danish trolleys.
16 August	trays removed from trolleys and placed in greenhouse
22 August	plants inoculated with a spore suspension of *T. basicola* (100 ml/tray)
2 September	black root rot first confirmed (on 4 of 5 plants sampled)
9 September	first appearance of yellowing leaves on inoculated plants
13 September	at least 5 plants with yellowing leaves present in all inoculated trays
21 September	black root rot evident on roots of most plants
5 October	first crop of pansies removed from trays; trays treated with disinfectants
6 October	70 plants selected at random from first crop assessed and all found to be infected with *T. basicola*
6 October	trays re-seeded
25 October	trays removed from Danish trolleys and placed on benches in greenhouse
9 November	assessment of plant growth
22 December	assessment of incidence and severity of black root rot in second crop

RESULTS

Contamination of trays

Inoculation of pansy seedlings with a spore suspension of *T. basicola* resulted in a high incidence of plants developing black root rot. Seventy plants selected at random 7 weeks after inoculation were all found to be infected. After removal of the plants, and before the trays were dipped, small pieces of peat were visible on the sides of many cells, with occasional short lengths of root adhering to the bases.

Effect of tray disinfection on black root rot

Plants were assessed for incidence of black root rot 11 weeks after re-seeding in contaminated trays. At this time, 46% of plants grown in trays dipped in water had developed symptoms of black root rot. This figure was reduced to less than 20% by Formalin, Glu-Cid, Iodel FD, Jet 5, Opticide H, Panacide M, Purogene, Ter-Spezial and sodium hypochlorite (Fig 1). Clearsol, Cryptonol and Virkon were relatively ineffective. No symptoms of black root rot developed in the control plants grown in new trays. The most effective treatments were Iodel FD, Formalin and Jet 5 which reduced the incidence of plants with black root rot to 3.5 %, 5.5 % and 8.7 % respectively. The mean number of plants with severe black root rot ranged from 0.7 on plants grown in trays treated with Iodel FD or Purogene to 4.7 on plants grown in untreated trays. Full results are shown in Table 1.

Plant quality

There were no significant differences between treatments when plant growth was assessed on 9 November, one month after seeding in contaminated trays (Table 2). At the final disease assessment on 22 December, a high plant quality index was recorded in plants grown in new trays (negative control), and in trays treated with Formalin, Glu-Cid, Iodel FD, Jet 5, Opticide H, Panacide M, Purogene, Ter-Spezial, and sodium hypochlorite (Fig. 2). These were the treatments most effective in reducing the incidence of black root rot.

Phytotoxicity

No phytotoxicity symptoms were observed in any of the plants grown in trays treated with disinfectant.

Effect of treatment on trays

None of the disinfectant treatments resulted in obvious change in colour of trays or increased brittleness.

Fig 1. Disinfection of plug trays contaminated with *T.basicola* - 1994

Pansies grown in treated trays

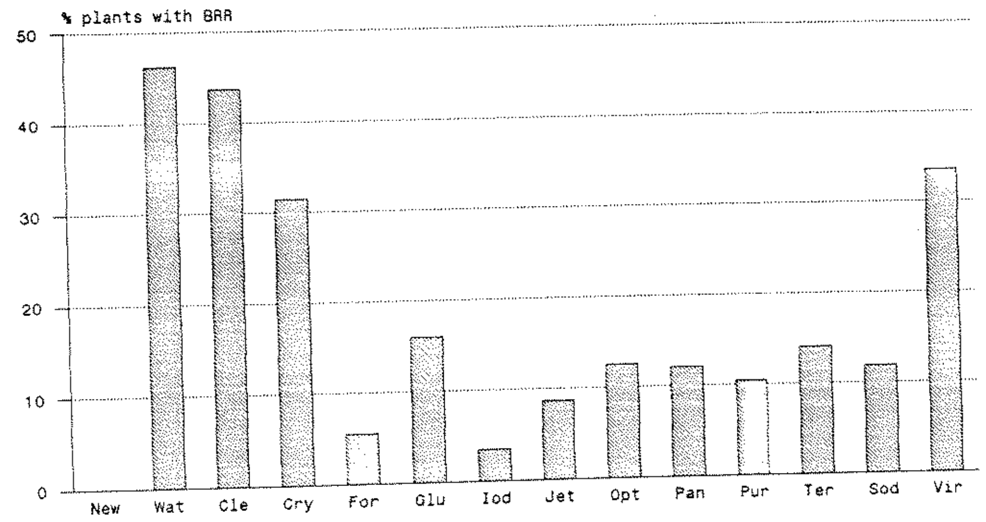

Fig 2. Effect of tray disinfection on plant quality

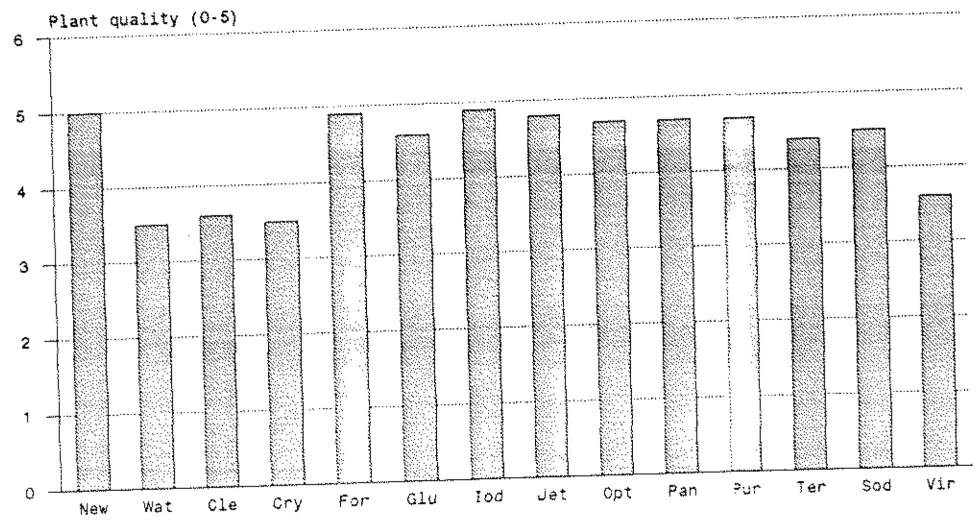

Table 1. Effect of disinfecting trays contaminated with *T. basicola* on incidence of black root rot

Treatment	Incidence of plants with black root rot		Incidence of unthrifty plants (severe black root rot)	
	Mean no. plants/tray	%	Mean no. plants/tray	%
1. Water	12.0	46.2	4.7	18.1
2. Clearsol	11.0	43.7	4.0	15.9
3. Cryptonol	8.5	31.5	4.5	16.7
4. Formalin	1.5	5.5	1.5	5.5
5. Glu-Cid	4.0	16.0	1.7	6.8
6. Iodel FD	1.0	3.5	0.7	2.5
7. Jet 5	2.2	8.7	2.5	9.9
8. Opticide H	3.7	12.5	2.0	6.8
9. Panacide M	3.7	12.1	1.7	5.6
10. Purogene	3.0	10.5	0.7	2.5
11. Ter-Spezial	4.2	14.1	3.2	10.8
12. Sodium hypochlorite	3.0	11.9	2.2	8.7
13. Virkon	10.0	33.6	4.5	15.2
14. Water (new trays)	0.0	0.0	1.0	3.1
Significance	***	-	NS	-
SED Min	1.96	-	1.37	-
Max-Min	1.66	-	1.19	-

*** Significant differences at P<0.001

NS- not significant

Table 2. Effect of disinfecting trays contaminated with *T. basicola* on growth of pansies

Treatment	9 November Plant growth (0-3)	22 December Mean no. plants surviving (of 35)	Plant quality (0-5)
1. Water	2.0	26.0	3.5
2. Clearsol	2.0	25.2	3.6
3. Cryptonol	1.5	27.0	3.5
4. Formalin	1.7	27.2	4.9
5. Glu-Cid	2.2	25.0	4.6
6. Iodel FD	2.0	28.2	4.9
7. Jet 5	1.7	25.2	4.8
8. Opticide H	2.0	29.5	4.7
9. Panacide M	2.0	30.5	4.7
10. Purogene	2.2	28.5	4.7
11. Ter-Spezial	2.0	29.7	4.4
12. Sodium hypochlorite	2.0	25.2	4.5
13. Virkon	2.5	29.7	3.6
14. Water (new trays)	2.0	31.7	5.0
Significance	NS	*	***
SED Min	0.28	2.08	0.28
Max-Min	0.24	1.80	0.24

NS- not significant

* Significant at $P<0.05$

*** Significant at $P<0.001$

DISCUSSION

A high incidence of black root rot occurred on plants grown in naturally contaminated trays while no black root rot was found on plants grown in new trays. These results indicate that contaminated trays were the source of *T. basicola* and also that there was no secondary spread between trays.

Nine disinfection treatments markedly reduced the incidence of black root rot when applied to the contaminated trays before re-seeding with pansies. These represented six types of disinfectant: aldehydes, iodine, hydrogen peroxide, phenolics, chlorine, and quaternary ammonium compounds (QACs). Iodel FD, Formalin and Jet 5 were particularly effective.

In the same experiment, hydroxyquinoline sulphate (Cryptonol), one phenolic product (Clearsol) and an organic acid and salt (Virkon) were ineffective at the rates tested.

In previous experiments investigating chemical disinfectants for control of *T. basicola*, effective treatments were found to be formalin (O'Neill, 1995), an organic acid and a QAC (Powell, 1989; Pilgaard, 1990) and a phenolic (Voss & Meier, 1987). Hydroxyquinolene sulphate and hydrogen peroxide/peracetic acid were found to be less effective (Voss & Meier, 1987). The results of this project confirm the efficacy of formaldehyde and some phenolic products and the relatively poor control using hydroxyquinolene sulphate (Cryptonol). Interestingly, Voss and Meier (1987) found a hydrogen peroxide/ peracetic acid product to be relatively ineffective, whilst we found a similar product (Jet 5) to be effective. Possibly a long treatment time, as we used (1 hour), is necessary for good results. Bohmer & Rhineland (1990) reported good results with an organic acid (Venna Cycla 2), while in this study a product with a similar active ingredient (Virkon) was relatively ineffective at the rate and treatment time tested. Again, treatment time and concentration may explain these differences; Venna Cycla 2 was effective against *T. basicola* after 5 minutes when used at 1 % but only after 4 hours when used at 0.25 %.

None of the treatments tested resulted in complete disease control; this may indicate the difficulty of killing chlamydospores of *T. basicola* which are thick walled, resistant to decay and can survive on structures for long periods. Additionally, in this study disinfectants were tested with only a minimal pre-cleaning of trays before dip treatment. It is possible that better results would be obtained if dirty trays were soaked in water and cleaned to remove dried peat and root debris before treatment with a disinfectant.

In the absence of any fungicide treatment to plants or compost, a single application of Iodel FD to trays reduced the number of plants with black root rot from 46% to 3.5%. Several other products also resulted in similar large reductions in the number of diseased plants. These results emphasise the importance of tray cleaning and disinfection as a method of disease prevention. It is likely that, in the absence of applying a disinfectant to trays, one or more applications of fungicide would be needed to achieve a similar degree of disease control.

Further work is needed to determine if pre-cleaning of trays before disinfection, a longer treatment time, or application of an appropriate fungicide to supplement tray disinfection, would result in complete elimination of the disease.

CONCLUSIONS

1. Pansy seed grown in plug trays naturally contaminated with *T. basicola* developed a high incidence of black root rot. The disease did not spread to plants grown in new trays on the same wire mesh bench.

2. In the absence of any fungicide treatment, a single application of chemical disinfectant to contaminated trays before seeding markedly reduced the incidence of black root rot and increased plant quality.

3. Nine disinfectants (Formalin, Glu-Cid, Iodel FD, Jet 5, Opticide H, Panacide M, Purogene, Ter-Spezial and sodium hypochlorite) markedly reduced the incidence of black root rot. These represented six types of disinfectants.

4. Formalin, Iodel FD and Jet 5 were particularly effective.

5. A single dip in disinfectant at the rates tested had no apparent effect on tray colour or brittleness.

REFERENCES

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Contract between ADAS (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for research/development project.

1. TITLE OF PROJECT

Contract No: PC38c

BEDDING PLANTS: COMPARISON OF CHEMICAL DISINFECTANTS FOR TREATMENT OF PLASTIC PLUGS TRAYS CONTAMINATED WITH *THIELAVIOPSIS BASICOLA*

2. BACKGROUND AND COMMERCIAL OBJECTIVE

Black root rot caused by *T. basicola* can be a serious problem affecting production of bedding and pot-bedding plants. The disease causes leaf yellowing and uneven growth. Species commonly affected in recent years include impatiens, pansy and verbena. There is a risk of infection arising from plug trays when they are re-used and especially if they are re-used several times in the same season. Once-used plug trays were found to be a source of *T.basicola* in an ADAS-funded project on control of this disease in winter pansies. HDC Review CP4 identified the scarcity of reliable information on the effectiveness of chemical disinfectants and recommended evaluation of products against a range of important pathogens including *T. basicola* and on a range of surfaces (eg plastic, sand, glass). With the increasing use of plastic plug trays in many sectors of horticulture, and the increasing practice of re-using them many times, identification of chemical disinfectants which are effective under commercial treatment conditions is urgently needed. The commercial objective of the work proposed here is to identify disinfectant products which will disinfect plastic plug trays contaminated with *T. basicola* and thereby allow growers to eliminate plug trays as a possible source of disease.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

Financial benefit to the industry will accrue for the following reasons:

1. A reduction in uneven growth due to black root rot.

2. Assurance to customers that an effective disinfectant is being used when plug-trays are re-used.

3. Reduced risk of black root rot and hence a reduced need to apply preventative fungicide treatments to control the disease.

4. Reduced reliance on fungicides and consequently a reduced risk of fungicide resistance developing.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

To determine the effectiveness of selected chemical disinfectants applied at recommended rates in disinfecting plastic plug trays naturally contaminated with *T. basicola*.

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

1. HDC Review of chemical disinfectants (CP4) - completed 1992.

2. HDC-funded projects investigating the biology of *T. basicola* (PC38a, HRI Wellesbourne) and fungicides for control of *T. basicola* (PC38b). The work ongoing at HRI Wellesbourne (PC38a) does **not** involve studies on disinfectants.

3. HDC-funded work in progress at HRI Wellesbourne evaluating disinfectants for activity against *Olpidium brassicae*, the vector of lettuce big vein.

4. MAFF-funded work at CSL Harpenden evaluating disinfectants for activity against *Polymyxa betae* the vector of the virus which causes sugar beet rhizomania - completed in 1991.

5. An ADAS-funded experiment comparing disinfectants on a sand bed naturally contaminated with fungal pathogens - completed in 1993.

None of the above projects have identified a treatment for disinfecting plastic plug trays contaminated with *T. basicola*.

6. DESCRIPTION OF THE WORK

Pansy plants will be grown in new plug trays inoculated with a standard inoculum of *T. basicola* and allowed to develop black root rot so that the plug trays become naturally contaminated. Plants will then be removed and plug trays will be treated with the disinfectants under test. Plug trays will then be re-sown with pansy seed and grown on, and seedlings assessed for the incidence and severity of black root rot. The experiment will be of a randomised block design with four-fold replication. Treatments would include the following disinfectants applied according to label recommendations:

1. Untreated (water dip)
2. Cryptonol
3. Formalin
4. Glu-Cid.
5. Jet 5
6. Panacide M
7. Clearsol
8. Iodel
9. Opticide-H 200
10. Ter-Spezial
11. Sodium hypochlorite
12. Purogene
13. Virkon

Trays will be dipped in water to rinse after treatment. Plant growth would be examined to check for any effect on root growth or foliage development.

7. COMMENCEMENT DATE AND DURATION

01.09.94; duration 1 year. Results would be reported by a written report to be completed by September 1995. Results would also be presented at a suitable HDC/ADAS/HRI Conference.

8. STAFF RESPONSIBILITIES

Project Leader:	Dr T M O'Neill	ADAS Cambridge
Key staff:	D Pye	ADAS Cambridge

9. LOCATION

ADAS Arthur Rickwood - glasshouse
ADAS Cambridge - Laboratories.

Contract No: PC38c
Date: 21.9.94

TERMS AND CONDITIONS

The Council's standard terms and conditions of contract shall apply.

Signed for the Contractor(s)	Signature......M. C. Heath...............
	Position......ACCOUNT.....MANAGER
	Date...............1.10.94.................
Signed for the Contractor(s)	Signature..
	Position..
	Date..
Signed for the Council	Signature............ J Murray / E.J. Kennedy ....
	Position pp CHIEF EXECUTIVE ....
	Date.......... 21-9-94 ....