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Protected stocks: aspects of the biology and control of fusarium wilt, a new disease problem

Annual Report 2006

PC 213

Cut flowers: evaluation of drip-applied chloropicrin for control of soil-borne *Fusarium oxysporum*, *Rhizoctonia* species*, Sclerotinia sclerotiorum* and weed seeds

PC 249

Final Report

March 2006

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Report: Annual report, March 2006
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Date project commenced: 1 April 2004
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AUTHENTICATION

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

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1 GROWER SUMMARY

1.1 Headline

- For the third successive year, outbreaks of stocks fusarium wilt occurred on UK nurseries, with low levels of the disease on several nurseries and severe outbreaks on at least two sites.
- Chloropicrin (applied via drip-line irrigation) and Basamid were the most effective soil disinfestation treatments tested, resulting in a significant reduction in the survival of fusarium in woody stocks stem pieces, down to 30 cm depth.
- Treatment of stocks fusarium wilt using fungicides alone is unlikely to be fully effective due to the soil-borne nature of the fungus and infection of the vascular system within the roots and stem.

1.2 Background and expected deliverables

1.2.1 PC 213. Protected stocks: aspects of the biology and control of fusarium wilt, a new disease problem

In summer 2003, a wilt disease seriously affected production of stocks on several nurseries in Cambridgeshire, Lincolnshire, Norfolk and West Sussex, causing crop losses valued well in excess of £200,000. The disease was identified as fusarium wilt, a disease that has been confirmed in the UK only once previously. Both seed-raised and bought-in plug plants were affected; the source of the disease was unknown. This research project was commissioned to investigate the biology and control of this potentially devastating problem.

The overall aim of the project is to devise a reliable and cost-effective strategy for managing fusarium wilt of stock through an increased understanding of the biology of the disease.

The specific objectives of the project are:

- To determine the longevity of survival of *F. oxysporum ex* stocks on crop debris in soil and the risk of disease when cropping with stocks on land in the season after an outbreak
- To determine the inoculum level of *F. oxysporum* required to produce wilt in stocks
- To monitor the efficacy of soil sterilisation treatments for control of fusarium wilt on commercial nurseries that have experienced the disease
- To investigate the effect of temperature on infection and development of fusarium wilt.
- To evaluate a range of potential fungicide treatments for control of the disease and for their safety to stocks
- To evaluate a range of disinfectants against *F. oxysporum*

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- To investigate the ability of *F. oxysporum* isolated from wilting stocks to cause disease in other cut flowers (e.g. aster, lisianthus and chrysanthemum) and vice-versa
- To devise and write a Factsheet with illustrations of the disease symptoms and with recommendations for its control.

1.2.2 PC 249. Cut flowers: evaluation of drip-applied chloropicrin for control of soil-borne **Fusarium oxysporum,** **Rhizoctonia** ***species*****, Sclerotinia sclerotiorum** ***and weed seeds***

This short-term project utilises a trial of a new chloropicrin application method being undertaken by K&S Fumigation Ltd, in order to gain information on soil-borne pathogen and weed control in a cost-effective manner. Treatments will be applied by K&S Fumigation Ltd in a glasshouse on a commercial flower nursery in November 2005, prior to planting stocks in 2006.

The objective of the experiment is to determine the effectiveness of chloropicrin applied via drip-irrigation lines in controlling *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and soil-borne *Rhizoctonia*. The level of weed control will also be determined.

1.3 Summary of the projects and main conclusions

1.3.1 Occurrence of fusarium wilt in 2005

In 2005 fusarium wilt affected crops on at least eight nurseries in Lincolnshire and west Norfolk and one in Northern Ireland, occurring from May through to August. This was the third year in succession that the disease was noticeable in crops. It remained at a low incidence on many nurseries but was widespread and damaging on at least three; two of the badly affected crops followed methyl bromide soil treatment and one followed steaming. Red and lavender colours were affected worse.

1.3.2 Developments in pre-plant soil disinfestation

A prototype machine (`Agratron`) that heats soil by electro-magnetic waves (wavelength undisclosed) has been developed in the Netherlands as a labour-saving alternative to steaming. The treatment is reported to be effective against fusarium and has been demonstrated on freesia nurseries (a crop where fusarium is a major hazard). The weight of the machine and the requirement for a diesel generator to supply sufficient electricity may be drawbacks, although fuel costs are reported to be less than for steaming. It is not yet available commercially.

Another development is the `VDL Cultivit`. This is a rotary spading machine that blows hot air (800°C) into the tumbling soil as it is spaded. It is marketed as a soil steriliser in the Netherlands. Similar equipment has been used in Israel for several years. There is very little evidence of efficacy against soil-borne fungi and nematodes, but in both countries there are reports of improved crop growth following soil treatment. Further information is available on www.vdlcultivit.com.

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1.3.3 Survival of **Fusarium oxysporum** *in soil*

Stocks debris naturally infested with *F. oxysporum* was mixed with soil and placed in a glasshouse. Samples recovered at intervals were tested for viable *F. oxysporum* by planting with stocks and then assessing plants after approximately 6 weeks for fusarium wilt. Viable *F. oxysporum* sufficient to cause infection in 85% of test plants remained in soil that had been stored for 7 months.

1.3.4 Cross-infection risk

Due to the severe nature of fusarium wilt on stocks, there is concern that the same pathogen could affect other crop hosts, or that *Fusarium* species from other crops and weeds could affect stocks. Cross-pathogenicity studies commenced in 2004 and continued in 2005 to gain information on the host range affected.

The results are summarised in Table 1.1. Typical and severe symptoms of fusarium wilt were only seen when a host plant was inoculated with *F. oxysporum* previously isolated from the same host plant (i.e. stocks inoculated with *F. oxysporum* from stocks or lisianthus inoculated with *F. oxysporum* from lisianthus). However, there was some survival of *F. oxysporum* on roots of non-host crops that could subsequently act as a source of inoculum for subsequent stocks or lisianthus crops. These results agree with findings from project year 1.

Table 1.1. Summary of results from cross-infection studies in 2005

	Source of *F. oxysporum* inoculum	
Crop	Stock	Lisianthus
Stock	+++	+
Oilseed rape	+	+
Aubretia	++	+
Lisianthus	+	+++

\+ No symptoms but *F. oxysporum* isolated
++ No symptoms, trace of internal stem discolouration and *F. oxysporum* isolated
+++ Severe external wilt symptoms, internal stem discolouration and *F. oxysporum* isolated

1.3.5 Disinfectants

Six disinfectant products (Jet 5, sodium hypochlorite, Mossicide, Unifect-G, Vitafect and Iodel FD) were evaluated for their efficacy against spores and mycelium of *Fusarium oxysporum ex* stocks in laboratory bioassays. The products were tested at the recommended rate and half the recommended rate in comparison with an untreated control, both with and without peat contamination. For each rate of each disinfectant product, treatment durations of 5 and 30 minutes were tested.

All of the disinfectants were fully effective against spores of *F. oxysporum* even after 5 minutes exposure when used at the recommended rate. The efficacy of Jet 5 was reduced at half the recommended rate but the other products still gave full control. Peat contamination at 0.1% w/v did not reduce the efficacy of the disinfectants against spores.

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The disinfectant products were less effective against mycelium of *F. oxysporum* due perhaps to the development of survival structures that were observed in colonies growing on the filter paper pieces. However, Unifect G gave complete control of mycelial growth both with and without peat contamination, when the full rate was used for a 30 minute treatment.

1.3.6 Fungicides

1.3.6.1 Efficacy trial

Ten fungicide programmes (a drench to plug plants followed by sprays at 1, 14 and 28 days after planting) were evaluated for their ability to control fusarium wilt in an artificially infested crop of stocks. At harvest (8 weeks after planting), there was no significant effect of treatment on the proportion of plants showing wilt symptoms, with more than 42% plants affected for all treatments. However, there was a trend for a lower incidence of internal stem symptoms for plants treated with a Delsene 50 Flo (carbendazim) drench followed by Delsene 50 Flo, Amistar, Octave or Experimental product 1, with a reduction from 62.5% to 45% or less (Figure 1.1). Carbendazim (as Bavistin DF) and azoxystrobin (Amistar) were also the most effective products for reducing fusarium wilt in stocks plug plants in project year 1. Phytotoxic effects were observed following applications with Swing Gold, Folicur, Biosept Gold and an experimental product. The experiment demonstrated that a soil-borne disease such as stocks fusarium wilt, that infects the vascular system within the roots and stem, can be difficult to control effectively using fungicides alone.

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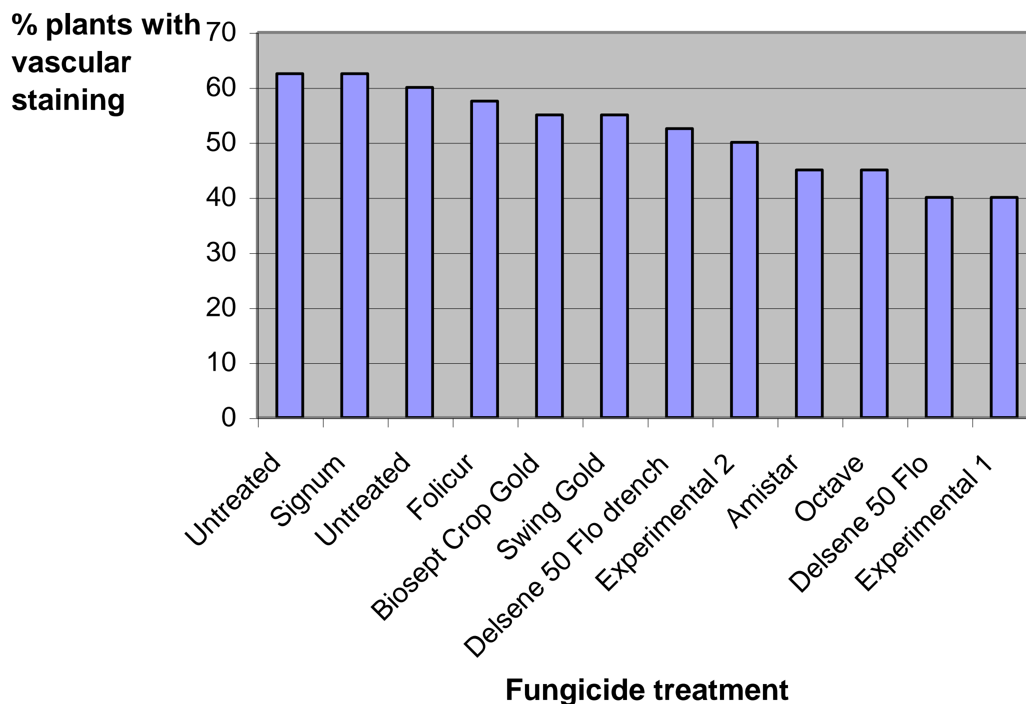

Figure 1.1. Effect of fungicide treatments on the incidence of internal stem symptoms in stocks, 8 weeks after planting

1.3.6.2 MBC resistance

An experiment was done to determine whether the failure of carbendazim to give greater control of stocks fusarium wilt in fungicide trials in this project and on commercial nurseries, was due to fungicide resistance. Six fusarium isolates (from stocks and other hosts) were tested for resistance to the fungicide Delsene 50 Flo at three concentrations of the active ingredient carbendazim. All of the isolates of fusarium, including those from stocks, were sensitive to carbendazim with mycelial growth strongly though not completely inhibited by the fungicide, even at a very low concentration (2 ppm). These results suggest that failure of carbendazim to give effective control of fusarium wilt on stocks is not due to fungicide resistance.

1.3.7 Soil sterilisation

1.3.7.1 Methyl bromide

A pot experiment was done using soil collected pre- and post-methyl bromide treatment from a nursery with severe fusarium wilt. Methyl bromide led to a substantial reduction in the incidence of fusarium wilt (with the proportion of wilted plants reduced from 86% to 10%) but, for this nursery, did not completely eliminate *F. oxysporum*.

1.3.7.2 Alternative treatments

Following on from experiments in 2004, six methods of soil disinfestation were examined in 2005 – Basamid, Chloropicrin, Formalin, Discovery, biological soil disinfestation (BSD) and Microgran (as well as sheet-steaming in 2004). All are permitted as pre-plant soil treatments for cut flower crops. Although experiments were done on different sites, a similar method was used throughout, in which

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naturally-infested stem pieces were buried at various depths, recovered after treatment and tested in the laboratory for viable *F. oxysporum*. A percentage kill was calculated. Stocks stem pieces were used so that the test was severe and the fungus was in a natural form. In some experiments the stem pieces were buried in soil for several weeks beforehand to encourage partial decay, to try and improve treatment efficacy. There was clear evidence from these experiments that fusarium survival in woody stem pieces is significantly reduced following burial in damp soil for approximately 4 weeks.

A summary of results for experiments in 2004 and 2005 is presented in Table 1.2. Basamid gave 80 to 100% control at one site but only 78% control (at the soil surface) at a second site. Formalin, applied as a drench at 0.5 L/m^2 according to the Commodity Substance Approval, gave 96 to 100% control at the soil surface but results at depth were variable. Both Discovery and sheet steaming gave moderate (70 to 86%) control at the soil surface but were less effective at depth. BSD and Microgran (calcium cyanamide) were less effective in eliminating *F. oxysporum* from stocks stem pieces (data not shown).

Chloropicrin was applied by injecting into drip irrigation lines laid on the soil surface and covered with polythene. This technique showed potential for broad-spectrum soil disinfestation, significantly reducing inoculum levels of the soil-borne pathogens *F. oxysporum* and *S. sclerotiorum,* and providing effective control of both dicotyledon and grass weeds. Lack of phytotoxic effects on plants placed 5 m from the treated area, suggests that turn-around time with this treatment could be rapid. Improvements in treatment efficacy could potentially be achieved using the full approved rate, by increasing soil moisture content prior to application, by reducing the distance between T-tapes and increasing the duration for which the soil is covered.

Table 1.2. Effectiveness of soil disinfestation methods for elimination of *F. oxysporum* f. sp. *mathioli* from woody stem pieces of stock buried in soil

	% kill of fusarium by different treatments							
	Basamid		Chloropicrin	Formalin		Discovery	Steaming	
Soil depth (cm)	Exp 1	Exp 2		Exp 1	Exp 2		Exp 1	Exp 2
0	78	100	86	100	96	86	70	72
5	-	90	-	100	0	84	-	-
15	12	96	68	67	2	42	76	66
30	5	80	66	-	8	54	32	44
45	-	-	-	-	-	-	12	0

1.3.7.3 Laboratory experiment on boiling and microwaving

Results from soil steaming experiments in 2004 indicated that steaming was relatively ineffective in eliminating *F. oxysporum* from stocks stem pieces, even at the soil surface where 60°C was exceeded. Therefore, a laboratory experiment was done to determine the effect of different heating processes on the survival of *Fusarium oxysporum* in naturally infected stocks stem pieces.

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A microwave treatment (10 minutes) on dry and soaked stem pieces was effective in eliminating *F. oxysporum* from woody stocks stem pieces. Boiling in water (30 min or 60 min) was most effective when used on soaked stem pieces, with fusarium eliminated from 98.5% of stem pieces or more. Boiling of dry stem pieces was less effective, perhaps due to reduced heat penetration into the stems. This result may in part explain the limited efficacy of steaming against fusarium wilt observed in year 1 experiments (Annual Report, project year 1).

1.4 Financial benefits

Annual production of stock in the UK is estimated to be around 18 million stems, representing around 23 ha of crop. Assuming a return of 17p per stem, the annual UK crop production is worth around £3.1 million. The benefit to the industry from this project would be continued profitable production of stock despite the threat of fusarium from the soil, young plants or seed.

1.5 Action points for growers

1.5.1 Identification of fusarium wilt

- Take care to ensure that the cause of any wilting or plant death in a crop of stocks is correctly identified. Fusarium wilt of stocks can be easily confused with pythium root rot and sclerotinia stem base rot, unless you are familiar with the disease.
- Check within the stem base for dark brown staining which is usually a reliable indicator of fusarium wilt.

1.5.2 Reducing the risk of persistence between crops and disease spread

- Take measures to reduce disease risk after an outbreak of fusarium wilt (Section 1.5.3). Fusarium can persist in plant debris buried in soil for at least 7 months and there is a high risk that the disease will re-occur if stocks are planted in an area where the disease was severe in the previous crop of stocks.
- If only a few plants are affected by wilt, and they are accessible, carefully remove them from the crop (bag them *in situ*) as soon as possible, before fusarium sporulation occurs on the lower leaves and stem.
- At the end of a crop, take care to remove as much crop debris as possible, and as soon as possible, before preparing the land for the next crop. Woody stem bases in particular pose a high risk and fusarium may survive in these despite soil sterilisation treatment.
- Disinfectants can be used to eliminate fusarium from pathways, glasshouse structures and machinery. Unifect G used at the recommended rate for a 30 minute treatment is effective both against spores and mycelium of *F. oxysporum* from stocks. Information on the use of disinfectants can be found in HDC Factsheet 15/05.

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1.5.3 Control of fusarium wilt: soil treatment

- Basamid, Formalin drench, Discovery, Chloropicrin (applied in drip-line irrigation) and soil steaming all give some control of fusarium in woody stem pieces. Efficiency of these treatments is likely to be improved by reducing the inoculum of fusarium in soil (Section 1.5.2), and by encouraging rapid breakdown of any crop debris incorporated.
- Where Basamid, Discovery or Chloropicrin are used, conduct a cress seed germination test to ensure all fumes have dissipated before re-planting.
- Where Formalin is used, follow the Commodity Substance Approval (maximum rate of 0.5 L/m^2 at a minimum dilution of 1:4 in water). Note that the cress seed germination test does not work for Formalin. The rate of Formalin breakdown depends on soil temperature. Persistence is reported to be 5 to 6 weeks at 0 to 5^oC soil temperature, and 7 to 8 days at 25^oC soil temperature.

1.5.4 Control of fusarium wilt: fungicides

- Control of fusarium wilt using fungicides alone is unlikely to be effective since it is a soil-borne disease that colonises the vascular tissue within roots and stems, making it a difficult target. Fungicides should be used as part of an integrated disease management strategy.
- As a precaution against fusarium wilt, consider applying a carbendazim drench treatment to plug plants before planting. Cleancrop Curve (SOLA 1213/04) and Delsene 50 Flo (SOLA 1004/04) can be applied as a drench treatment to stocks at growers' own risk.
- Fungicide applications to the crop may reduce the severity of fusarium wilt. Delsene 50 Flo (SOLA 1004/04), Amistar (SOLA 1684/01) and Octave (LTAEU) can be used at growers' own risk.

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2 SCIENCE SECTION

2.1 Introduction

2.1.1 PC 213. Protected stocks: aspects of the biology and control of fusarium wilt, a new disease problem

In June 2003, a wilt disease occurred in Lincolnshire in a glasshouse crop of column stocks (*Matthiola incana*). Plants showed a one-sided wilt that progressed from the base upwards. Leaves subsequently became bleached, growth was stunted and plants died. Roots appeared healthy but the vascular tissue was stained dark brown. White fungal growth developed extensively on damp incubation of leaves and stems. Although stocks had been grown in the glasshouse for over 12 years, the disease had not been observed previously. *Fusarium* was consistently recovered, following surface sterilisation, from within stems and roots and from the fungal growth on leaves. Culture characteristics and morphology were typical of *Fusarium oxysporum* (Booth, 1971). Colonies on potato dextrose agar (PDA) produced a violet pigmentation in reverse and dark, purple sterile stromatic pustules. Ellipsoid microconidia, produced in slimy heads from short monophialides on sucrose nutrient agar, were 2 to 8 µm long; chlamydospores and 3-septate macroconidia were produced sparsely.

The roots of 10 plug plants (cv. Centum White) were dipped in a spore suspension ($3x10^6$ conidia ml^{-1}) of the *F. oxysporum* isolated from the stocks and then potted in soil-less compost. Another 10 plants were grown in compost mixed with bleached leaves taken from wilted plants (*c.* 10 g pot $^{-1}$). After 3 weeks, both sets of plants wilted and collapsed and *F. oxysporum* was re-isolated; control plants remained healthy.

The disease was subsequently confirmed in several other stocks crops in England, sometimes with over 80% losses. A fusarium wilt of stocks caused by *F. oxysporum* f. sp. *mathioli* has previously been described in Arizona and California (Baker, 1948). In England, *F. oxysporum* was consistently isolated from the vascular tissue of wilting stocks with brown vascular staining on a nursery in Kent in 1971. The problem reoccurred in 1975 and 1987 in the same glasshouse (JT Fletcher, pers. comm.). Baker (1948) demonstrated that the fungus was seed-borne, similar to many other *F. oxysporum* diseases. The inoculation tests reported here indicate that infection may also arise from crop debris incorporated into the soil.

The aim of this project is to devise a reliable and cost-effective strategy for managing fusarium wilt. In project year 2, the focus has been on: survival of the fungus in soil, cross-pathogenicity tests, and the efficacy of chemical disinfectants, fungicides and soil sterilisation treatments against stocks fusarium wilt.

2.1.2 PC 249. Cut flowers: evaluation of drip-applied chloropicrin for control of soil-borne **Fusarium oxysporum***,* **Rhizoctonia** *species*, **Sclerotinia sclerotiorum** *and weed seeds*

With the loss of methyl bromide as a soil sterilant, alternative methods are urgently required for control of important soil-borne pathogens. Chloropicrin has recently

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received approval (SOLA 1579/2005) for use pre-planting of raspberry, both outdoors and under protection. The Pesticides Safety Directorate (PSD) have confirmed this treatment can also be applied pre-planting of ornamentals. In several countries (e.g. USA, Israel, Italy) where methyl bromide was previously widely used, there is increased interest in application of soil fumigants, including chloropicrin, through drip irrigation lines laid on or buried just beneath the soil surface. This method is considered to be safer to the operator and a more cost-effective method of application.

K&S Fumigation are seeking to offer chloropicrin in-line injection using the same equipment as that used in Italy. In Sicily, soil is treated with chloropicrin and/or Telone by in-line injection prior to planting tomatoes, for control of fusarium, nematodes and other soil-borne pests. K&S Fumigation propose to undertake an experimental application through drip irrigation lines in a glasshouse on a cut flower nursery in Hertfordshire in November 2005. Working in collaboration with K&S Fumigation in this short-term project, the aim was to test the efficacy of chloropicrin applied by drip irrigation on soil-borne *Fusarium oxysporum* f. sp. *mathioli, Sclerotinia sclerotiorum*, *Rhizoctonia solani* and weeds. Fusarium wilt of column stock, sclerotinia stem rot of many crops and rhizoctonia stem base rot remain serious disease problems that can significantly affect cut flower crop production.

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2.2 Occurrence of fusarium wilt in 2005

Symptoms of fusarium wilt on stocks were first reported by growers in mid-May and outbreaks continued sporadically through to August 2005; most were on crops in Lincolnshire or west Norfolk. The disease was confirmed by ADAS on samples from eight nurseries. On many of these sites the disease remained at a low incidence and did not affect the crop significantly. Possibly this reflects a greater awareness by growers of the initial symptoms and prompt removal of affected stems in order to reduce secondary spread. A range of varieties was affected including Opera Francesca (red), Debora Blue, Aida White, Figaro Lavender and Esmerelda; red and lavender appeared to be the worst affected colours. There were three nurseries where fusarium wilt caused widespread and substantial losses. These were on two nurseries in Lincolnshire in June and July and on a nursery in Northern Ireland in July. The two Lincolnshire sites had been treated pre-planting with methyl bromide; and the nursery soil in Northern Ireland had been steam sterilised. On at least one of the badly affected nurseries, symptoms were not observed until the crop was in flower and almost ready for harvest.

2.3 Developments in pre-plant soil disinfestation

2.3.1 Methyl bromide

It has been confirmed that the critical use exemption (CUE) application for methyl bromide use on cut flowers was not granted for 2006 and will not be available in future.

2.3.2 Electro-magnetic waves (`Agratron`)

A prototype machine that heats soil by electro-magnetic waves has been developed in the Netherlands as an alternative to steaming for use in cut-flower production (e.g. freesia). A multiple-wheeled tractor is used to pull a magnetron unit over the soil at speeds of up to one metre per minute. Within the unit are 150 tubes that direct the electro-magnetic waves into the soil; the wavelength is not disclosed. Treatment of sandy soils is easiest, with disinfestation up to 50 cm depth possible. The treatment is reported to be effective against fusarium, pythium nematodes and weeds. The potential advantage of the system over steaming is the saving in physical labour, and a saving in fuel costs. The magnetron is powered by electricity (prototype machine draws 200 amps) and requires a diesel generator. Further developments to the prototype are planned, including a lighter tractor. The equipment is being developed by Aad Middelburg and Leen van der Hoek in the Netherlands and is not currently available for commercial use. (These notes were made from a translation of an article published in Vakblad voor de Bloemisterij in 2005.)

2.3.3 Hot air

Another new development is a rotary spading machine that treats soil with very hot (800°C) dry air and uses only a tenth of the energy required for steaming (Vegter, 2005). "Cultivit" is a hot air steriliser marketed by VDL Cultivit of Eindhoven, the Netherlands (www.vdlcultivit.com). The price in January 2006 was Euro 300,000 and fuel usage for heating the air was around 1,000 L of heating oil per hectare. In trials

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with squash crops on sand and clay/loam soils, yield increases of 90 to 150% have been recorded. Treatment is said to give a reduction in nematode numbers, but not a complete kill. The effect of soil treatment on soil fungi, such as fusarium, appears not to have been studied. The Cultivit machine is relatively large and heavy at 4.9 m long, 2.1 m high, and 2.4 m wide and with a weight of 9,000 kg. It has a work-rate of 2.7 – 6.0 metres/minute outside, slower in a glasshouse.

The theory of hot air soil disinfestation is that the combination of blowing hot air at an optimal speed into the moving humid soil enables each soil particle to reach a temperature high enough to kill or weaken any pathogen or pest within it (Runia & Greenberger, 2005). The method has been used commercially in Israel for several years and improvements in crop growth (e.g. potato) noted. Research in the Netherlands using an Israeli hot air machine, with a hot air temperature of 720°C, found no reduction in nematode numbers (*Meloidogyne fallax*) following treatment; however, tomato plants grown on nematode-infested soil that had been treated with the hot air machine produced a higher mean fruit weight than plants grown on uninfested soil (Runia & Greenberger, 2005). Immediate reduction in numbers of *Fusarium* propagules in soil was also reported to be poor (Runia, pers. comm.) although a longer-term effect through weakening was not tested.

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2.4 Experiment to determine the longevity of *F. oxysporum* survival on stocks debris in soil using plant baiting

2.4.1 Introduction

Following on from an experiment in project year 1, the aim of this experiment was to determine whether soil containing stocks crop debris could act as a source of inoculum for fusarium wilt between crops in the same season and between seasons.

2.4.2 Methods

Stock plants with typical symptoms of fusarium wilt were chopped into pieces (approximately 5 cm stem lengths) and added to a sack of soil collected from ADAS Arthur Rickwood. The infected crop debris was incorporated thoroughly into the soil. The sack was stored on the ground in a glasshouse from 13 July 2005 onwards.

At each sampling time (0, 1, 2, 3 and 7 months after soil infestation), three seed trays were filled with infested soil and planted with 20 stocks plants per tray (4 rows of 5 plants). Three seed trays were also filled with non-infested soil and planted with 20 stocks plants per tray. The trays were placed in a heated glasshouse (20°C, ambient light) and watered as required.

The plants were observed for typical symptoms of fusarium wilt (wilting, leaf bleaching and vein yellowing). Approximately 6 weeks after planting, the plants were uprooted, the stems cut longitudinally (whole stem length) and the incidence and severity (0-3 index) of vascular staining assessed, where 0 = no staining, 1 = slight staining, 2 = staining part-way up the stem and 3 = whole stem length discoloured. Stem pieces were plated on potato dextrose agar amended with streptomycin (PDA+S) to confirm the presence of *F. oxysporum*.

2.4.3 Results and discussion

Typical symptoms of fusarium wilt developed on all of the stocks plants transplanted into soil infested with *F. oxysporum ex* stocks, at 0, 1, 2 and 3 months after soil infestation. At 7 months after soil infestation, 85% of plants showed wilt symptoms. Plants in the uninfested soil remained healthy apart from symptoms of pythium rot at the last assessment time. For plants in infested soil, the incidence and severity of vascular staining was reduced at 2 months after infestation but increased again at 3 months after infestation (Table 2.1). The results confirm findings from year 1 that *F. oxysporum* pathogenic to stocks can remain viable in soil for at least 7 months. The experiment is continuing, in order to monitor fusarium survival for up to 15 months.

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Table 2.1. Incidence and severity of internal stem discolouration in stocks potted in soil at intervals after infestation with *F. oxysporum*

Time of planting after soil infestation (months)	% incidence of vascular staining		Mean vascular severity score (0-3)	
	Uninfested soil	Infested soil	Uninfested soil	Infested soil
0	-	100	-	3.0
1	0	96	0.0	2.2
2	2	25	0.1	0.8
3	0	100	0.0	3.0
7	17	85	0.3	2.4

- plants could not be assessed due to pest damage

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2.5 Testing the cross-pathogenicity of isolates of *Fusarium oxysporum* on stocks and other hosts

2.5.1 Introduction

Due to the severe nature of fusarium wilt on stocks, there is concern that the same pathogen could affect other crop hosts, or that *Fusarium* species from other crops and weeds could affect stocks. Cross-pathogenicity studies commenced in 2004 and continued in 2005 to gain information on the host range affected.

2.5.2 Methods

Each treatment was applied to a 13 cm pot containing five plants (either stock, aubretia, lisianthus) or three oil seed rape plants. Each treatment was replicated four times in a randomised block design.

Each host plant was inoculated with either an isolate of *F. oxysporum ex* lisianthus or *F. oxysporum ex* stocks, or left uninoculated. A spore suspension of each of the two isolates of *F. oxysporum* was prepared in sterile distilled water (SDW) (3×10^6 spores/ml). For each inoculation treatment, the roots of 20 host plants were dipped in the appropriate spore suspension for 5 minutes. The plants were potted in compost in 13 cm pots (5 plants per pot). Plants for uninoculated control treatments were dipped in SDW before potting. The pots were placed on saucers, in a randomised block design in an unheated polytunnel for three weeks and then into a heated glasshouse (20°C, ambient light) for the remaining three weeks.

The pots were sufficiently spaced (30 cm) to avoid soil/water splash between pots when watering. Plants were kept relatively dry to provide suitable conditions for disease development.

The plants were assessed regularly after inoculation for incidence of fusarium wilt symptoms (e.g. leaf wilt, leaf bleaching and vein yellowing). Approximately 6 weeks after inoculation the plants were uprooted, the stems cut longitudinally (whole stem length) and incidence and severity of vascular staining was recorded. For each treatment, a representative sample of symptoms thought to be due to fusarium was plated onto PDA+S to confirm the causal organism.

2.5.3 Results and discussion

Stock plants inoculated with *F. oxysporum ex* stocks consistently developed typical symptoms of fusarium wilt (wilting, vein yellowing and lower leaf bleaching). All of the plants in this treatment had severe vascular staining (Table 2.2). *F. oxyporum* was consistently isolated when affected stems and roots were plated on to agar media. Similar results were observed for lisianthus plants inoculated with *F. oxysporum ex* lisianthus.

Plants from the remaining host/pathogen treatments did not in general develop symptoms of disease that were visible prior to destructive sampling. Wilting symptoms observed on uninoculated stocks, lisianthus and oilseed rape plants were

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attributed to temporary dehydration. There was no vascular staining (except a trace in one aubretia plant inoculated with *F. oxysporum ex* stocks). When root material from these treatments and the uninoculated control treatments was plated on to agar media, *F. oxysporum* was isolated, but not consistently.

In summary, typical and severe symptoms of fusarium wilt were only seen when a host plant was inoculated with *F. oxysporum* previously isolated from the same host plant (Table 2.3). However, there was some survival of *F. oxysporum* on roots of non-host crops that could act as a source of inoculum for subsequent stocks or lisianthus crops. These results correspond with findings from project year 1.

Table 2.2. Incidence and severity of fusarium wilt symptoms on crops inoculated with isolates of *Fusarium oxysporum*, 6 weeks after inoculation

Treatment		% incidence of wilt symptoms	% incidence of vascular staining	Mean vascular severity score (0-3)
Host plant	Source of *F. oxysporum* inoculum			
Stocks	-	20*	0	0.0
Stocks	Lisianthus	0	0	0.0
Stocks	Stocks	100	100	3.0
Oil seed rape	-	0	0	0.0
Oil seed rape	Lisianthus	0	0	0.0
Oil seed rape	Stocks	25*	0	0.0
Aubretia	-	0	0	0.0
Aubretia	Lisianthus	0	0	0.0
Aubretia	Stocks	0	1	0.1
Lisianthus	-	25*	0	0.0
Lisianthus	Lisianthus	100	94	2.0
Lisianthus	Stocks	0	0	0.0

*wilting due to dehydration during a hot period when assessment was due

Table 2.3. Summary of results from cross-pathogenicity studies in 2005

	Source of *F. oxysporum* inoculum	
Crop	Stock	Lisianthus
Stock	+++	+
Oilseed rape	+	+
Aubretia	++	+
Lisianthus	+	+++

+ No symptoms but *F. oxysporum* isolated
++ No symptoms, trace of internal stem discolouration and *F. oxysporum* isolated
+++ Severe external wilt symptoms, internal stem discolouration and *F. oxysporum* isolated

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2.6 Evaluation of disinfectants for control of *Fusarium oxysporum ex* stocks

2.6.1 Introduction

A range of disinfectant products was evaluated for efficacy against spores and mycelium of *Fusarium oxysporum* in laboratory bioassays.

2.6.2 Methods

The disinfectant products listed in Table 2.4 were tested at the recommended rate and half the recommended rate in comparison with an untreated control. For each rate of each disinfectant product, treatment durations of 5 and 30 minutes were tested.

Table 2.4. Disinfectants tested in a laboratory assay for their efficacy against spores and mycelium of *F. oxysporum ex* stocks

Treatment	Notes	Recommended product rate	Volume (ul) to add to spore suspension (10 ml total): Full rate	Half rate
1. Untreated control	-	-	0	0
2. Jet 5	Peroxyacetic acid	1:125	80	40
3. Sodium hypochlorite	10-14% available chlorine	10% (=10,000 ppm OCl)	1000	500
4. Mossicide	30% dichlorophen	17 ml/L	170	85
5. Unifect-G	QAC + glutaraldehyde	4%	400	200
6. Vitafect	QAC +biguanidine salts	1%	100	50
7. Iodel FD	2% iodine	8 ml/L	80	40

2.6.2.1 Experiment 1: effect of disinfectants on spore germination

A culture of *Fusarium oxysporum ex* stocks (isolate code: AR03/76) was grown on PDA plates for 7 to 14 days. Spore suspensions were collected from these plates by washing with 2 ml SDW. The spore suspension was filtered through sterile muslin and adjusted to a concentration to 1 x 10^6 conidia/ml using a haemocytometer. Each disinfectant product was added to the spore suspension to give the recommended rate and half the recommended rate. Five minutes and 30 minutes after mixing with the test disinfectant at each rate, spores were spun down in a centrifuge (2000 rpm for 2 minutes) then re-suspended in 5 ml SDW. For each treatment combination, a 5 ul droplet of solution was placed in the middle of each of five wells of a 25-well plate (Sterilin Repli-plates) containing PDA+S. Spore suspension without the addition of disinfectant products was used as the control treatment. There were four replicate plates per disinfectant treatment. The plates were incubated at 20°C for 7 days. After this time, germination of spores was visible as growth of the fungus. For each treatment combination, the proportion of wells with fungal growth was recorded.

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2.6.2.2 Experiment 2: effect of peat contamination on the efficacy of disinfectants against spore germination

The method described in Experiment 1 was repeated but peat compost at 0.1% w/v was added to the spore suspension prior to treatment with disinfectant.

2.6.2.3 Experiment 3:effect of disinfectants on mycelial growth

Pieces of sterile filter paper (approximately 0.5 cm^2) were placed on the surface of an actively growing culture of *Fusarium oxysporum ex* stocks (isolate code: AR03/76) growing on PDA. The plates were incubated for 7 days at 20°C to allow fungal mycelium to penetrate the paper. The filter paper pieces were immersed in each of the disinfectant products at full or half rate, for 5 or 30 minutes. For the control treatment, filter paper pieces were immersed in SDW. Treated pieces of filter paper were drained, rinsed three times in SDW to remove disinfectant and allowed to dry in the air flow from a laminar flow hood. For each treatment combination, a filter paper piece was plated onto each of five wells of 25-well plates containing PDA. There were five replicate plates for each disinfectant. The plates were incubated for 7 days at 20°C and then for each treatment combination, the proportion of wells with fungal growth was recorded.

2.6.2.4 Experiment 4: effect of peat contamination on the efficacy of disinfectants against mycelial growth

The method described in Experiment 3 was repeated but 100 mg peat compost was placed in 100 ml of disinfectant at both the full rate and half rate, prior to treatment of the filter paper pieces with mycelial growth. SDW contaminated with peat (100 mg in 100 ml) was used as the untreated control treatment (i.e. peat added at 0.1% w/v).

2.6.3 Results and discussion

Mossicide results are presented for Experiments 1 and 3 only, as anomalous results were obtained in the other experiments. All of the disinfectants were fully effective against spores of *F. oxysporum* even after 5 minutes exposure when used at the recommended rate (Table 2.5). The efficacy of Jet 5 was reduced at half the recommended rate but the other products still gave full control. Peat contamination at 0.1% w/v did not reduce the efficacy of the disinfectants against spores (Table 2.6).

Table 2.5. Effect of disinfectants on germination of spores of *F. oxysporum*

Treatment	Mean % of wells with fungal growth*				
	Control	Full rate 5 min	Full rate 30 min	Half rate 5 min	Half rate 30 min
Jet 5	100.0	0.0	0.0	100.0	57.6
Sodium hypochlorite	100.0	0.0	0.0	0.0	0.0
Mossicide	100.0	0.0	0.0	0.0	0.0
Unifect G	100.0	0.0	0.0	0.0	0.0
Vitafect	100.0	0.0	0.0	0.0	0.0
Iodel	100.0	0.0	0.0	0.0	0.0

*4 reps of five wells

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Table 2.6. Effect of peat contamination on the efficacy of disinfectants against spore germination of *F. oxysporum*

Treatment	Mean % of wells with fungal growth*				
	Control	Full rate 5 min	Full rate 30 min	Half rate 5 min	Half rate 30 min
Jet 5	100.0	0.0	0.0	0.0	0.0
Sodium hypochlorite	100.0	0.0	0.0	0.0	0.0
Unifect G	100.0	0.0	0.0	0.0	0.0
Vitafect	100.0	0.0	0.0	0.0	0.0
Iodel	100.0	0.0	0.0	0.0	0.0

*4 reps of five wells

The disinfectant products were less effective against mycelium of *F. oxysporum* (Tables 2.7 and 2.8) due perhaps to the development of thick-walled resting spores (chlamydospores) that were observed in colonies growing on the filter paper pieces. However, Unifect G gave complete control of mycelial growth both with and without peat contamination, when the full rate was used for a 30 minute treatment.

Table 2.7. Effect of disinfectants on mycelial growth of *Fusarium oxysporum*

Treatment	Mean % of wells with fungal growth*				
	Control	Full rate 5 min	Full rate 30 min	Half rate 5 min	Half rate 30 min
Jet 5	100.0	79.0	89.9	77.8	88.5
Sodium hypochlorite	100.0	35.0	54.3	70.0	5.0
Mossicide	100.0	15.0	3.8	13.8	6.0
Unifect G	100.0	2.3	0.0	4.5	0.0
Vitafect	99.0	70.8	26.0	90.3	30.5
Iodel	95.5	61.3	59.5	93.3	64.8

*4 reps of five wells

Table 2.8. Effect of peat contamination on the efficacy of disinfectants against mycelium of *F. oxysporum*

Treatment	Mean % fungal growth				
	Control	Full rate 5 min	Full rate 30 min	Half rate 5 min	Half rate 30 min
Jet 5	98.5	97.8	97.8	96.3	98.0
Sodium hypochlorite	100.0	100.0	11.5	82.0	44.0
Unifect G	98.5	0.0	0.0	0.0	9.5
Vitafect	100.0	95.0	53.3	98.8	82.8
Iodel	97.0	95.5	10.5	96.5	80.5

*4 reps of five wells

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2.7 Evaluation of fungicides for control of fusarium wilt on stocks

2.7.1 Introduction

The objective of the experiment was to evaluate fungicides for controlling fusarium wilt on stocks grown in artificially infested soil.

2.7.2 Methods

Details of the fungicide drench and spray treatments applied during the experiment are shown in Table 2.9.

Table 2.9. Details of fungicide drench and spray treatments applied to stocks

No.	Drench	Spray 1	Spray 2	Spray 3
1	Untreated	Untreated	Untreated	Untreated
2	Delsene 50 Flo	Untreated	Untreated	Untreated
3	Delsene 50 Flo	Delsene 50 Flo	Delsene 50 Flo	Delsene 50 Flo
4	Delsene 50 Flo	Amistar	Amistar	Amistar
5	Delsene 50 Flo	Biosept Gold	Biosept Gold	Biosept Gold
6	Delsene 50 Flo	Experimental 1	Experimental 1	Experimental 1
7	Delsene 50 Flo	Experimental 2	Experimental 2	Experimental 2
8	Delsene 50 Flo	Octave	Octave	Octave
9	Delsene 50 Flo	Signum	Signum	Signum
10	Delsene 50 Flo	Folicur	Folicur	Folicur
11	Delsene 50 Flo	Swing Gold	Swing Gold	Swing Gold
12	Untreated	Untreated	Untreated	Untreated

A plot comprised four rows of seven plants. There were two plots of untreated plants (Treatments 1 and 12) within each block. Visual assessments were initially done on the central 10 plants per plot and later on all plants per plot. Destructive assessments were taken only on the central 10 plants. There was a guard of 12.5 cm between plants and between rows, and a pathway of 1.0 m between plots in different blocks. There was a spacing of 0.5 m between plots within blocks which was planted with three rows of plants. Each treatment was replicated four times in a randomised block design. Data were analysed by analysis of variance or generalised linear models (GLMs) for binomially distributed data in Genstat. While ANOVA makes the assumption that data is normally distributed, GLMs allow analyses of data which do not follow a normal distribution, or where a transformation needs to be applied before normality can be assumed. Data which are proportions will tend to follow a binomial distribution. One way of analysing this data would be to do a transformation in order to normalize the data and analyse the transformed data using ANOVA. The best transformation for binomial data is the logit function, which GLM processes internally to produce an accumulated analysis of deviance. This can be interpreted in much the same way as an analysis of variance. The PREDICT command in Genstat can then be used to get the estimated means and standard errors for each treatment level back transformed so that they are produced on the original scale.

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Soil nutrition was determined on the basis of soil sampling to 15 cm depth. Soil samples were analysed for N, P, K, conductivity, pH and Mg. Based on results from analyses, two days prior to planting, sulphate of potash was applied at 102 g/m^2 and Nitraprill (34.5% N) was applied at 51 g/m^2 and rotavated into the top 200 mm of soil (D. Stokes, pers. comm.) Overhead irrigation was applied regularly from 2 weeks prior to planting to ensure that the soil was moist at the time of soil infestation and planting.

Stocks debris that was either naturally infested or that had been artificially infected by spraying with a spore suspension of *F. oxysporum ex* stocks was chopped into approximately 10 cm lengths. The debris was spread evenly over the soil surface of the whole trial area (approximately 65 g debris per m^2) and incorporated by raking. One day prior to planting, 2 L of a spore suspension of *F. oxysporum ex* stocks (1.6 x 10^6 spores/ml) was sprayed evenly over the soil surface of the whole trial area.

Plug plants of column stocks that had not already received a fungicide drench were obtained from a commercial nursery. Variety Centum purple was used for the experimental plots and Centum yellow was used for the guard rows between plots.

The fungicide drench treatment was applied to plug plants in trays, 1 day before planting at 1000 L/ha (100 ml/m^2). The fungicide drench was washed into the root zone by watering. Fungicide sprays 1, 2 and 3 were applied to the whole plot area of the appropriate plots at 1000 L/ha (100 ml/m^2) using an Oxford Precision sprayer with single 02F110 nozzle and guard. Sprays 1, 2 and 3 were applied 1, 14 and 28 d after planting, respectively. Details of sprays are shown in Table 2.10.

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Table 2.10. Details of fungicide applications applied to stocks

No.	Timing	Fungicide product	Active ingredient	Rate (in 1000 L/ha)
1	Drench Sprays 1-3	Untreated Untreated	- -	- -
2	Drench Spray 1-3	Delsene 50 Flo Untreated	Carbendazim -	1.0 ml/L (1L/ha) -
3	Drench Spray 1-3	Delsene 50 Flo Delsene 50 Flo	Carbendazim Carbendazim	1.0 ml/L (1 L/ha) 1.5 ml/L (1.5 L/ha)
4	Drench Spray 1-3	Delsene 50 Flo Amistar	Carbendazim Azoxystrobin	1.0 ml/L (1 L/ha) 1.0 ml/L (1 L/ha)
5	Drench Spray 1-3	Delsene 50 Flo Biosept Crop Gold	Carbendazim -	1.0 ml/L (1 L/ha) 4.0 ml/L (4 L/ha)
6	Drench Spray 1-3	Delsene 50 Flo Experimental 1	Carbendazim Not disclosed	1.0 ml/L (1 L/ha) 0.5 ml/L (0.5 L/ha)
7	Drench Spray 1-3	Delsene 50 Flo Experimental 2	Carbendazim Not disclosed	1.0 ml/L (1 L/ha) 2.0 ml/L (2 L/ha)
8	Drench Spray 1-3	Delsene 50 Flo Octave	Carbendazim Prochloraz	1.0 ml/L (1 L/ha) 1.0 g/L (1 kg/ha)
9	Drench Spray 1-3	Delsene 50 Flo Signum	Carbendazim Boscalid + pyraclostrobin	1.0 ml/L (1 L/ha) 1.2 g/L (1.2 kg/ha)
10	Drench Spray 1-3	Delsene 50 Flo Folicur	Carbendazim Tebuconazole	1.0 ml/L (1 L/ha) 1.0 ml/L (1 L/ha)
11	Drench Sprays 1-3	Delsene 50 Flo Swing Gold	Carbendazim Dimoxystrobin + epoxiconazole	1.0 ml/L (1 L/ha) 1.5 ml/L (1.5 L/ha)
12	Drench Sprays 1-3	Untreated Untreated	- -	- -

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The approval status of the fungicides and the basis for selecting the rates used were as follows:

Fungicide	Approval	Basis for rate selected
Delsene 50 Flo (drench)	LTAEU (SOLA 1004/04)	Carbendazim on protected ornamentals (container grown) (1 ml/L)
Delsene 50 Flo (spray)	LTAEU (SOLA 1004/04)	Carbendazim on protected ornamentals (soil grown) (1.5 ml/L)
Amistar	LTAEU (SOLA 1684/01)	Amistar on protected chrysanthemums (100 ml/100 L)
Biosept Crop Gold	-	Biosept Crop Gold: Max. label rate is 8 ml/L but there is grower experience with phytotoxicity on stocks at this rate. Used at half rate.
Experimental 1	AEA	Experimental 1: Max label rate is 0.5 L/ha for registered product on cereals (25 % ai). Used at 0.5 ml/L
Experimental 2	AEA	Experimental 2: Max planned label rate is 2 L/ha
Octave	LTAEU Full Approval	Octave for ornamental plant production. Use the lower rate of 1 g/L water, because of plant damage risk
Signum	LTAEU (SOLA 1673/04)	Signum on protected strawberries: Maximum individual dose = 1.8 kg/ha, Maximum total dose = 3.6 kg/ha. Use 3 applications of 1.2 kg/ha (1.2 g/L)
Folicur	LTAEU (SOLA 1874/03)	Folicur on protected broccoli:1 L/ha AEA for experimental conditions of use.
Swing Gold	AEA	Swing Gold on winter wheat: 1.5 L/ha

Overhead irrigation was provided from 2 weeks prior to planting and for the duration of the trial to obtain and maintain moist soil, without waterlogging. The trial was hand weeded as necessary.

Fubol gold (mancozeb + metalaxyl-M), Toppel 10 (cypermethrin) and Aphox (pirimicarb) were applied as routine sprays to all plots for control of downy mildew and insect pests (D. Stokes, pers. comm.). Fubol Gold + Aphox were applied in the first week after planting. Fubol Gold + Toppel 10 were applied together with spray 3.

Fubol Gold	Mancozeb + metalaxyl-M	190 g / 75 /1000 m^2
Toppel 10	Cypermethrin	25 ml /100 /1000 m^2
Aphox	Pirimicarb	50 g / 100 /1000 m^2

Two weeks after planting, the experiment was assessed weekly (assessments 1 to 3) and then fortnightly (assessments 4 to 6) for the incidence of plants in each plot out of 10 (assessments 1 to 3) and out of 28 (assessments 4 to 6) with typical symptoms of fusarium wilt (e.g. wilting, pronounced vein yellowing, leaf bleaching). At each assessment, the incidence of phytotoxic symptoms was also recorded. Nine weeks

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after planting, the height of the 10 central plants per plot was recorded. They were then uprooted and the lower 15 cm of the stems cut longitudinally. The severity of vascular staining was scored using a 0-3 index where 0 = no vascular staining, 1 = a trace of vascular staining, 2 = vascular staining up to 50 % of stem length, 3 = vascular staining affecting 50 % or more of the stem length. For each plot, typical stem symptoms were plated onto PDA+S (after surface sterilising for 10 sec in 90 % ethanol), to confirm the causal organism.

2.7.3 Results and discussion

2.7.3.1 Disease progression

Approximately 2 weeks after planting, sporulation of *F. oxysporum* was confirmed on stem and leaf debris remaining from artificial soil infestation, indicating high inoculum pressure.

At three weeks after planting, symptoms of fusarium wilt were confirmed in the untreated control treatments and not on fungicide-treated plants. Plants with typical symptoms of wilt (wilting, lower leaf bleaching and vein yellowing) were subsequently observed for all treatments. At five weeks after planting, there was a significant effect of fungicide treatment on the proportion of plants with vein yellowing and lower leaf bleaching (Table 2.11). The proportion of plants showing these symptoms was reduced in plots treated with Biosept Gold or Swing Gold. At the time of the last non-destructive disease assessment (8 weeks after planting), there were no significant effects of treatment on the proportion of plants showing wilt symptoms, with >42% plants affected for all treatments (Table 2.11).

At the time of harvest (8 weeks after planting), there was no significant effect of treatment on the incidence of vascular staining in uprooted plants (Table 2.12). However, there was a trend for a lower incidence of staining with a Delsene 50 Flo drench followed by Delsene 50 Flo, Amistar, Octave or Experimental product 1, with a reduction from 62.5% to 45% or less. Carbendazim (as Bavistin DF) and azoxystrobin (Amistar) were also the most effective products at reducing fusarium wilt in stocks plug plants in project year 1.

In recent studies in the USA using inoculated corms (Elmer, 2003), Amistar and an MBC fungicide (thiophanate methyl) applied as a corm soak followed by a post-planting drench both gave partial control of fusarium basal rot in gladiolus. Two newer fungicides (fludioxonil and triflumizole) were slightly more effective, reducing disease by around 50%. In Italy (Gullino *et al.*, 2002), Amistar, Benlate and Octave incorporated into the growing medium reduced fusarium wilt in pot-grown *Agryanthemum frutescens* (florist's chrysanthemum) from 100% to 40-70%; the strobilurins Stroby and Twist were ineffective. These results confirm those of our studies on inoculated stocks: i) the most effective products available at present include Amistar, Octave and MBC fungicides; ii) none of the fungicides gave outstanding control in experiments with fusarium-inoculated plants.

2.7.3.2 Phytotoxic effects

There was a significant effect of spray treatments on plant height at harvest, with applications of Swing Gold and Folicur resulting in stunted growth compared to the untreated control (P<0.05) (Table 2.13). Leaf scorch was observed on plants treated

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with Swing Gold and Experimental product 1, while plants treated with Biosept Gold showed lower leaf yellowing.

Table 2.11. Effect of fungicide treatments on development of fusarium wilt symptoms

Drench treatment	Spray treatment	5 weeks after planting: % plants with leaf bleaching	5 weeks after planting: % plants with vein yellowing	8 weeks after planting: % plants with wilt symptoms
Untreated	Untreated	12.0	29.2	53.2
Delsene 50 Flo	Untreated	11.7	30.9	54.7
Delsene 50 Flo	Delsene 50 Flo	19.7	17.3	42.6
Delsene 50 Flo	Amistar	17.9	23.2	54.2
Delsene 50 Flo	Biosept Crop Gold	6.9	9.9	49.5
Delsene 50 Flo	Experimental 1	13.9	26.9	44.4
Delsene 50 Flo	Experimental 2	23.5	23.3	52.0
Delsene 50 Flo	Octave	11.7	20.7	45.2
Delsene 50 Flo	Signum	13.8	27.5	54.3
Delsene 50 Flo	Folicur	13.2	15.1	54.5
Delsene 50 Flo	Swing Gold	4.5	3.6	57.8
Untreated	Untreated	17.2	30.8	64.1
	Df	33	33	33
	*Approx. F. pr.	0.039	0.005	0.924

*Analysed using generalised linear models

Table 2.12. Effect of fungicide treatments on the incidence of vascular staining in stocks plants, 8 weeks after planting

Drench treatment	Spray treatment	% stems with vascular staining
Untreated	Untreated	62.5
Delsene 50 Flo	Untreated	52.5
Delsene 50 Flo	Delsene 50 Flo	40.0
Delsene 50 Flo	Amistar	45.0
Delsene 50 Flo	Biosept Crop Gold	55.0
Delsene 50 Flo	Experimental 1	40.0
Delsene 50 Flo	Experimental 2	51.7
Delsene 50 Flo	Octave	45.0
Delsene 50 Flo	Signum	62.5
Delsene 50 Flo	Folicur	57.5
Delsene 50 Flo	Swing Gold	55.0
Untreated	Untreated	60.0
	D.f.	33
	*Approx. F. pr	0.925

*Analysed using generalised linear models

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Table 2.13. Effect of fungicide treatments on the height of stocks at harvest

Drench treatment	Spray treatment	Mean plant height (cm)
Untreated	Untreated	42.4
Delsene 50 Flo	Untreated	46.4
Delsene 50 Flo	Delsene 50 Flo	45.1
Delsene 50 Flo	Amistar	44.4
Delsene 50 Flo	Biosept Crop Gold	41.7
Delsene 50 Flo	Experimental 1	41.4
Delsene 50 Flo	Experimental 2	42.5
Delsene 50 Flo	Octave	45.2
Delsene 50 Flo	Signum	41.5
Delsene 50 Flo	Folicur	28.1
Delsene 50 Flo	Swing Gold	35.2
Untreated	Untreated	41.4
D.f.		33
SED		4.67

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2.8 MBC resistance tests on fusarium isolates

2.8.1 Introduction

This experiment was done to determine whether the failure of carbendazim to give greater control of fusarium wilt in fungicide trials in this project and on commercial nurseries was due to fungicide resistance.

2.8.2 Methods

Six fusarium isolates (two from stocks, one from soil and three from other hosts) were tested for resistance to the fungicide Delsene 50 Flo at three concentrations of the active ingredient carbendazim: 2 ppm, 20 ppm and 100 ppm.

A 5 mm plug was taken from the edge of an actively growing colony of each fusarium isolate using a cork borer number 2. The single plug was placed in the centre of a plate of potato dextrose agar using a sterile scalpel. Four replicate plates were set up for each isolate at each concentration of carbendazim and a control containing no carbendazim. The plates were placed into a dark incubator at approximately 20°C. Growth of the fusarium isolates was measured at 2 and 14 days before the colonies reached the edge of the dish. The colony diameters were measured in two directions at right angles to each other. The percentage inhibition of mycelial growth was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{growth on control agar} - \text{growth on fungicide agar})}{\text{Growth on control agar}} \times 100$$

2.8.3 Results and discussion

The results in Table 2.14 show that all of the isolates of fusarium, including those from stocks, were sensitive to carbendazim and that mycelial growth was strongly though not completely inhibited by the fungicide, even at a very low concentration (2 ppm). These results suggest that failure of carbendazim to give effective control of fusarium wilt on stocks is not due to fungicide resistance.

Table 2.14. Inhibition of fusarium mycelial growth on agar plates amended with carbendazim, after 14 days at 20°C

			% inhibition at carbendazim concentration			
Identification	Isolated from	Year isolated	0 ppm	2 ppm	20 ppm	100 ppm
F. oxysporum	Stocks	2003	0	88.0	86.6	84.7
F. oxysporum	Stocks	2004	0	80.9	100.0	100.0
F. oxysporum	Infested soil	2005	0	98.3	100.0	100.0
Fusarium sp.	Hebe	2005	0	85.6	99.8	99.2
Fusarium sp.	Hebe	2004	0	82.7	100.0	100.0
Fusarium sp.	Lisianthus	2004	0	83.8	100.0	100.0

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2.9 Efficacy of methyl bromide against *Fusarium oxysporum ex* stocks (growing-on test)

2.9.1 Introduction

The aim of the experiment was to determine the efficacy of methyl bromide against *F. oxysporum ex* stocks, using a growing-on test.

2.9.2 Methods

Soil was collected from beds at a commercial nursery where stocks with severe fusarium wilt had been grown in 2005. Soil samples were collected pre- and post-methyl bromide treatment. At each sampling time, the soil sample was used to fill ten 13 cm pots which were each planted with stocks plants var. Opera Aida (50 plants pre-treatment and 40 plants post-treatment). Stocks were also planted into soil from ADAS Arthur Rickwood, as a control treatment.

The plants were assessed for incidence of fusarium wilt symptoms (e.g. leaf wilt, leaf bleaching and vein yellowing) at 4 and 9 weeks after planting (pre-soil treatment sample) and at 16 and 18 weeks after planting (post-soil treatment sample). Plants were left for longer in the post-soil treatment sample before assessment, because of cooler conditions. For the destructive assessment, the plants were uprooted, the stems cut longitudinally (whole stem length) and incidence and severity of vascular staining was recorded. For each treatment, a representative sample of symptoms thought to be due to fusarium was plated onto PDA+S to confirm the causal organism.

2.9.3 Results and discussion

Methyl bromide treatment led to a substantial reduction in the incidence and severity of fusarium wilt in plants grown in treated soil but, for the nursery sampled, did not completely eliminate *F. oxysporum* from the soil (Table 2.15). This is in agreement with results from year 1 when the survival of *F. oxysporum* was determined in stocks stem pieces following methyl bromide treatment.

Table 2.15. Effect of methyl bromide soil treatment on development of stocks fusarium wilt

Treatment	% plants with wilt symptoms	% plants with vascular staining	Mean vascular staining severity score (0-3)
Pre-methyl bromide	86.0	86.0	2.4
Post-methyl bromide	10.0	7.5	0.2
Control soil (uninfested)	7.5	0.0	0.0

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2.10 Efficacy of biological soil disinfestation against *Fusarium oxysporum* in stems of column stocks

2.10.1 Introduction

The efficacy of biological soil disinfestation (BSD) against verticillium wilt in trees is being investigated as part of HDC Project HNS 137. This experiment made use of the existing trial for the tree wilt project, to determine the efficacy of BSD against *F. oxysporum* from stocks.

2.10.2 Methods

The experiment was located at Andlers Ash Nursery, Liss, Hants in a loamy sand pH 7.2 with 1.9% organic matter.

Rye grass was sown on 30/05/05 at a rate of 50 kg/ha in a large plot area of 11 m x 18 m. One herbicide application of Starane (0.5 L/ha) was applied on 27/06/05. *Chenopodium album* (fat hen) was not controlled by the herbicide.

Biomass assessments were taken prior to incorporation of the ryegrass. Four 0.25 m^2 quadrats were taken across the plot and the ryegrass and green weeds cut and weighed. 3.3 kg/m^2 fresh green matter was present on plots. To increase the amount of green matter incorporated, a separate swath of ryegrass was cut and spread onto the plots to increase the fresh green matter incorporated to 5.7 kg/m^2. Three loads of water from a 1000 gallon bowser were used to flood the whole plot area. The whole area was then spaded with an Imants spader to depth of 30 cm. Once spaded the area was covered with VIF (virtually impermeable film) with the edges dug in to a depth of one spade blade.

Nylon gauze bags were prepared containing ten 1 cm long stem sections cut from stocks with symptoms of fusarium wilt (collected in 2005), mixed with moist silver sand. Sufficient sand was placed in each bag to separate the stem pieces. Each bag was placed in a nylon onion sack and labelled with a depth and location.

Treatments were as follows with five replicate bags of ten stem pieces per treatment:

No.	BSD treatment	Depth (cm)
1	Untreated – laboratory	-
2	Untreated – field	0
3	Treated	0
4	Treated	5
5	Treated	15
6	Treated	30
7	Treated	45

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Bags of stem pieces were buried once the plot had been spaded, prior to covering with VIF. Five holes were dug 1.5 m in from the edge of the plot, 0.5 m apart, to a depth of 45 cm. Samples were then placed at the required depth in each of the holes. All smeared soil was loosened with a spade before back filling each of the holes. Soil temperature was taken before the samples were buried and during the time they were buried and compared to untreated areas. Seven weeks after burial the plastic was cut and the samples retrieved.

The day after sample recovery, the stem pieces were sifted from the sand and the remaining sand washed away. The stem pieces were then dried before surface sterilising in 90% ethanol for 10 seconds. Sterilised pieces were then plated onto PDA+S. The number of fusarium colonies present after 7 and 14 days was counted.

2.10.3 Results and discussion

There was no effect of the BSD treatment on the viability of *F. oxysporum* in woody stocks stem pieces (Table 2.16). During the BSD process, pathogen kill is achieved under anaerobic conditions, through the build up of acetic acid and low molecular weight volatile fatty acids. It is probable that in this experiment, these compounds did not accumulate in the soil in sufficient amounts, or penetrate the woody stem tissue sufficiently to control *F. oxysporum*.

Table 2.16. Effect of BSD on the viability of *F. oxysporum* in woody stocks stem pieces.

No.	Treatment	Depth (cm)	Mean % stem pieces with growth of *F. oxysporum* after 14 d	% kill
1	Untreated – laboratory	-	90.0	10.0
2	Untreated – field	0	95.0	5.0
3	BSD	0	97.5	2.5
4	BSD	5	97.5	2.5
5	BSD	15	97.9	2.1
6	BSD	30	98.0	2.0
7	BSD	45	86.0	14.0

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2.11 Efficacy of Basamid treatment against *Fusarium oxysporum* in stems of column stocks

2.11.1 Introduction

Basamid (97% dazomet) is approved as a soil fumigant for field and protected crops. This experiment was a continuation of work started in project year 1 to evaluate the efficacy of Basamid for fusarium control.

2.11.2 Methods

The experiment was sited at a nursery near Boston, Lincolnshire, (light silt with low organic matter content).

Nylon gauze bags were prepared using ten 1 cm long stem sections cut from stocks with symptoms of fusarium wilt (collected in 2005), and mixed with silver sand. Sufficient sand was placed in each bag to separate the stem pieces. Half of the bags were moistened and then buried in soil that was regularly watered for 4 weeks at ADAS Arthur Rickwood, to partially rot the stem pieces. The remaining bags of stem pieces were stored dry in the laboratory and then dampened immediately prior to use. Two bags, one with partially rotted stem pieces and one with un-rotted stem pieces, were placed in an onion sack and labelled with a depth and location.

Treatments were as follows with five replicates bags of ten stem pieces per treatment:

No.	Treatment	Stem-piece type	Burial depth (cm)
1	Untreated	Fresh	0
2	Untreated	Rotted	0
3	Basamid	Fresh	0
4	Basamid	Rotted	0
5	Basamid	Fresh	5
6	Basamid	Rotted	5
7	Basamid	Fresh	15
8	Basamid	Rotted	15
9	Basamid	Fresh	30
10	Basamid	Rotted	30

Soil temperature was recorded before the samples were buried. Before the soil treatment was applied, 10 soil cores from 0-15 cm depth were bulked for determination of determination of water content as a percentage of field capacity (%FC).

Prior to Basamid treatment, the soil was broken up using a sub-soiler, rotavated to a fine tilth then watered. Basamid was applied by the grower at 760 kg/ha to the whole glasshouse. This was rotavated in to a depth of approximately 25 cm and covered with LDPE film once the samples had been buried.

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An area of 1 m^2 was marked out and five holes (in a dice pattern) dug to a depth of 30 cm. The samples were then placed at the required depth in each of the holes. All smeared soil was loosened with a spade before back filling each of the holes. Once buried, the area was covered with LDPE film. The untreated control samples were left on the soil surface in another glasshouse. Two weeks after burial the samples were retrieved.

After recovery the stem pieces were sifted from the sand and the remaining sand washed away. The stem pieces were then dried before surface sterilising in 90% ethanol for 10 seconds. Sterilised pieces were then plated onto PDA+S and incubated at 20ºC. The number of fusarium colonies present after 7 and 14 days was counted. Statistical analysis was by generalised linear models in Genstat.

2.11.3 Results and discussion

Soil temperature at the time of treatment was approximately 12ºC. Soil moisture content was measured at 45% FC (although grower observations suggest a higher moisture content).

There was a significant effect of soil treatment/depth on fusarium survival (Tables 2.17 and 2.18). The percentage kill of fusarium in Basamid-treated soil exceeded 60% even at 30 cm depth, with complete control of fusarium at the soil surface (in fresh stem pieces). This result suggests better pathogen control at depth compared with results from Year 1 when Basamid treatment was tested at a different nursery using a similar method (see Section 1.3.7.2 and Year 1 Annual Report).

There was no effect of stem material type on fusarium survival but there was a significant interaction effect, because of reduced survival of fusarium in rotted stem pieces from the untreated control at the soil surface.

Table 2.17. Effect of Basamid treatment on the viability of *F. oxysporum* in woody stocks stem pieces

No.	Treatment	Stem-piece type	Burial depth (cm)	Mean no. stem pieces with growth of *F. oxysporum* after 14 d (out of 10)	% kill
1	Untreated	Fresh	0	9.2	8
2	Untreated	Rotted	0	6.6	34
3	Treated	Fresh	0	0.0	100
4	Treated	Rotted	0	1.8	82
5	Treated	Fresh	5	1.0	90
6	Treated	Rotted	5	2.4	76
7	Treated	Fresh	15	0.4	96
8	Treated	Rotted	15	0.8	92
9	Treated	Fresh	30	2.0	80
10	Treated	Rotted	30	4.0	60

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Table 2.18. Accumulated analysis of deviance for effect of Basamid treatment on the viability of *F. oxysporum* in woody stocks stem pieces

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Rep	4	25.139	6.285	3.38	0.013
+ Stems	1	2.345	2.345	1.26	0.264
+ Treatment/Depth	4	189.280	47.320	25.47	<.001
+ Stems.Treatment/Depth	4	32.247	8.062	4.34	0.003
Residual	86	159.801	1.858		
Total	99	408.812	4.129		

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2.12 Efficacy of Formalin drench against *Fusarium oxysporum* in stems of column stocks

2.12.1 Introduction

Formalin has a Commodity Substance Approval as a soil sterilant in glasshouses. It can be used at a rate of up to 0.5 L/m^2 in a dilution of at least 1:4 parts water. This experiment was a continuation of work started in project year 1 to evaluate the efficacy of Formalin for fusarium control.

2.12.2 Methods

The experiment was done at West's Nursery, Spalding, Lincolnshire on a silt soil with low organic matter content.

Nylon gauze bags were prepared containing 10 x 1cm long stem sections cut from stocks with symptoms of fusarium wilt (collected in 2005), mixed with silver sand. Sufficient sand was placed in each bag to separate the stem pieces. Half the bags were moistened and then buried in the soil at ADAS Arthur Rickwood for approximately 4 weeks to partially rot the stem pieces. The remainder was stored dry and then dampened immediately before use. Two bags, one rotted one un-rotted, were placed in an onion sack and labelled with a depth and location.

Treatments were as follows with five replicate bags of ten stem pieces per treatment:

No.	Treatment	Stem-piece type	Burial depth (cm)
1	Untreated	Fresh	0
2	Untreated	Rotted	0
3	Treated	Fresh	0
4	Treated	Rotted	0
5	Treated	Fresh	5
6	Treated	Rotted	5
7	Treated	Fresh	15
8	Treated	Rotted	15
9	Treated	Fresh	30
10	Treated	Rotted	30

Soil temperature was recorded before the samples were buried. Before the soil treatment was applied, 10 soil cores from 0-15 cm depth were bulked for determination of determination of water content as a percentage of field capacity (%FC).

An area of 1 m^2 was marked out and five holes dug in a dice shape to a depth of 30 cm. The samples were then placed at the required depth in each of the holes. All smeared soil was loosened with a spade before back filling each of the holes.

Formalin (0.5 L) was diluted in 2 L water to give a total volume of 2.5 L. This volume was applied to the 1 m^2 plot using a clean watering can and rose.

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The samples were retrieved 2 weeks after burial. After recovery the stem pieces were sifted from the sand and the remaining sand washed away. The stem pieces were then dried before surface sterilising in 90% ethanol for 10 seconds. Sterilised pieces were then plated onto PDA+S and incubated at 20°C. The number of fusarium colonies present after 7 and 14 days was counted.

2.12.3 Results and discussion

The soil temperature at the time of treatment was 15.5°C with soil moisture at 53% of field capacity.

There was a significant effect of stem-piece type on fusarium viability (Tables 2.19 and 2.20) with greater percentage kill in the rotted stem pieces. Formalin was effective at the soil surface giving >95% kill of fusarium in both types of stem piece but was less effective below the soil surface (<40% kill). This result is in contrast to a similar experiment using Formalin in 2004, where 100% and 67% kill of fusarium was achieved at 5 and 15 cm depth, respectively, using the same Formalin rate and at the same nursery. Overall, the results indicate that Formalin may be useful as a soil surface fumigant but with insufficient soil penetration to give fusarium control at depth.

Table 2.19. Effect of Formalin treatment on the viability of *F. oxysporum* in woody stocks stem pieces

No.	Treatment	Stem-piece type	Burial depth (cm)	Mean no. stem pieces with growth of *F. oxysporum* after 14 d (out of 10)	% kill
1	Untreated	Fresh	0	8.4	16
2	Untreated	Rotted	0	5.8	42
3	Treated	Fresh	0	0.4	96
4	Treated	Rotted	0	0.2	98
5	Treated	Fresh	5	10.0	0
6	Treated	Rotted	5	6.4	36
7	Treated	Fresh	15	9.8	2
8	Treated	Rotted	15	8.0	20
9	Treated	Fresh	30	9.2	8
10	Treated	Rotted	30	6.8	32

Table 2.20. Accumulated analysis of deviance for effect of Formalin treatment on the viability of *F. oxysporum* in woody stocks stem pieces.

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Rep	4	20.435	5.109	4.16	0.004
+ Stems	1	26.104	26.104	21.24	<.001
+ Treatment/Depth	4	279.310	69.827	56.82	<.001
+ Stems.Treatment/Depth	4	9.866	2.467	2.01	0.101
Residual	86	105.696	1.229		
Total	99	441.410	4.459		

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2.13 Efficacy of Microgran incorporation against *Fusarium oxysporum* in stems of column stocks

2.13.1 Introduction

Microgran is a fine grade formulation of a fertiliser based on calcium cyanamide (>40%). The product is reported to have soil disinfection properties.

2.13.2 Methods

The experiment was done at West's Nursery, Spalding, Lincolnshire on a silt soil with low organic matter content.

Nylon gauze bags were prepared containing 10 x 1cm long stem sections cut from stocks with symptoms of fusarium wilt (collected in 2005), mixed with silver sand. Sufficient sand was placed in each bag to separate the stem pieces. Half the bags were moistened and then buried in the soil at ADAS Arthur Rickwood for approximately 4 weeks to partially rot the stem pieces. The remainder was stored dry and then dampened immediately before use. Two bags, one rotted one un-rotted, were placed in an onion sack and labelled with a depth and location.

Treatments were as follows with five replicate bags of ten stem pieces per treatment:

No.	Treatment	Stem-piece type	Burial depth (cm)
1	Untreated	Fresh	0
2	Untreated	Rotted	0
3	Treated	Fresh	0
4	Treated	Rotted	0
5	Treated	Fresh	5
6	Treated	Rotted	5
7	Treated	Fresh	15
8	Treated	Rotted	15
9	Treated	Fresh	30
10	Treated	Rotted	30

Soil temperature was recorded before the samples were buried. Before the soil treatment was applied, 10 soil cores from 0-15 cm depth were bulked for determination of determination of water content as a percentage of field capacity (%FC).

An area 1 m^2 was marked out and five holes dug in a dice pattern to a depth of 30 cm. The samples were then placed at the required depth in each of the holes. All smeared soil was loosened with a spade before back filling each of the holes.

Microgran (100 g /m) was applied evenly to the plot and raked in to a depth of 5 cm. Bags labelled '0 cm' were then placed on the soil surface. Control bags were placed on the soil surface in an area of the glasshouse away from the treated area.

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The samples were retrieved two weeks after burial. After recovery the stem pieces were sifted from the sand and the remaining sand washed away. The stem pieces were then dried before surface sterilising in 90% ethanol for 10 seconds. Sterilised pieces were then plated onto PDA+S and incubated at 20°C. The number of fusarium colonies present after 7 and 14 days was counted.

2.13.3 Results and discussion

The soil temperature at the time of treatment was 15.5°C with soil moisture at 53% of field capacity.

Given the relatively low recovery of fusarium from untreated stem pieces in this experiment, it is not possible to make conclusions on the efficacy of Microgran against the fungus. There was a significant effect of stem material type on the viability of fusarium, with higher percentage kill in the rotted stem pieces (Tables 2.21 and 2.22). This was particularly evident in the untreated control. This product may be more effective over a longer time period, and will be tested in 2006 for its efficacy against fusarium in a stocks crop, when incorporated into soil prior to planting.

Table 2.21. Effect of Microgran treatment on the viability of *F. oxysporum* in woody stocks stem pieces

No.	Treatment	Stem-piece type	Burial depth (cm)	Mean no. stem pieces with growth of *F. oxysporum* after 14 d (out of 10)	% kill
1	Untreated	Fresh	0	8.4	16
2	Untreated	Rotted	0	2.4	76
3	Treated	Fresh	0	8.4	16
4	Treated	Rotted	0	5.8	42
5	Treated	Fresh	5	9.0	10
6	Treated	Rotted	5	7.0	30
7	Treated	Fresh	15	9.6	4
8	Treated	Rotted	15	6.2	38
9	Treated	Fresh	30	9.2	8
10	Treated	Rotted	30	9.0	10

Table 2.22. Accumulated analysis of deviance for effect of Microgran treatment on the viability of *F. oxysporum* in woody stocks stem pieces

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Rep	4	21.716	5.429	3.46	0.011
+ Stems	1	58.889	58.889	37.57	<.001
+ Treatment/Depth	4	48.901	12.225	7.80	<.001
+ Stems.Depth	4	13.306	3.326	2.12	0.085
Residual	86	134.801	1.567		
Total	99	277.612	2.804		

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2.14 Efficacy of metam sodium treatment against *Fusarium oxysporum* in stems of column stocks

2.14.1 Introduction

Discovery (510 g/L metam sodium) is approved as a sterilant for glasshouse, nursery and outdoor soils.

2.14.2 Methods

The experiment was done at West's Nursery, Spalding, Lincolnshire on a silt soil with low organic matter content.

Nylon gauze bags were prepared containing 10 x 1cm long stem sections cut from stocks with symptoms of fusarium wilt (collected in 2005), mixed with silver sand. Sufficient sand was placed in each bag to separate the stem pieces. Half the bags were moistened and then buried in the soil at ADAS Arthur Rickwood for approximately 4 weeks to partially rot the stem pieces. The remainder was stored dry and then dampened immediately before use. Two bags, one rotted one un-rotted, were placed in an onion sack and labelled with a depth and location.

Treatments were as follows with five replicates bags of ten stem pieces per treatment:

No.	Treatment	Stem-piece type	Burial depth (cm)
1	Untreated	Fresh	0
2	Untreated	Rotted	0
3	Treated	Fresh	0
4	Treated	Rotted	0
5	Treated	Fresh	5
6	Treated	Rotted	5
7	Treated	Fresh	15
8	Treated	Rotted	15
9	Treated	Fresh	30
10	Treated	Rotted	30

Soil temperature was recorded before the samples were buried. Before the soil treatment was applied, 10 soil cores from 0-15 cm depth were bulked for determination of water content as a percentage of field capacity (%FC).

An area 1 m^2 was marked out and five holes dug in a dice pattern to a depth of 30 cm. The samples were then placed at the required depth in each of the holes. All smeared soil was loosened with a spade before back filling each of the holes.

Discovery (90 ml) was diluted in 15 L water. A watering can and rose were used to apply 5 L of the diluted product evenly across the 1 m^2 plot (30 ml product/m^2). This was allowed to drain through, ensuring the product did not drain out of the plot area on the surface. A second 5 L application was made and allowed to drain through before the final 5 L was applied, resulting in a total application of 90 ml/m^2 (900

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L/ha). The plot was immediately covered with LDPE film (approx. 1.2 m^2) and the edges were dug in. The untreated control samples were left on the soil surface in another area of the glasshouse (where land had also been prepared by rotavation and watering).

The samples were retrieved two weeks after burial. After recovery the stem pieces were sifted from the sand and the remaining sand washed away. The stem pieces were then dried before surface sterilising in 90% ethanol for 10 seconds. Sterilised pieces were then plated onto PDA+S and incubated at 20ºC. The number of fusarium colonies present after 7 and 14 days was counted.

2.14.3 Results and discussion

The soil temperature at the time of treatment was 15.5ºC with soil moisture at 53% of field capacity.

There was a significant effect of soil treatment/depth on fusarium survival, with >80% kill at the soil surface and >60% kill at 5 cm depth. There was some pathogen kill at 15 and 30 cm depth, but no better than in untreated rotted stem pieces (Table 2.23). There was a significant interaction effect due to reduced survival of fusarium in rotted stem pieces compared with fresh stem pieces at the soil surface (Table 2.24).

Table 2.23. Effect of metam sodium treatment on the viability of *F. oxysporum* in woody stocks stem pieces

No.	Treatment	Stem-piece type	Burial depth (cm)	Mean no. stem pieces with growth of *F. oxysporum* after 14 d (out of 10)	% kill
1	Untreated	Fresh	0	9.4	6
2	Untreated	Rotted	0	5.6	44
3	Treated	Fresh	0	1.4	86
4	Treated	Rotted	0	1.8	82
5	Treated	Fresh	5	1.6	84
6	Treated	Rotted	5	4.0	60
7	Treated	Fresh	15	5.8	42
8	Treated	Rotted	15	5.6	44
9	Treated	Fresh	30	4.6	54
10	Treated	Rotted	30	4.6	54

Table 2.24. Accumulated analysis of deviance on effect of metam sodium treatment on the viability of *F. oxysporum* in woody stocks stem pieces

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Rep	4	1.103	0.276	0.11	0.978
+ Stems	1	0.292	0.292	0.12	0.732
+ Treatment/depth	4	93.517	23.379	9.44	<.001
+ Stems.Treatment/depth	4	28.555	7.139	2.88	0.027
Residual	86	212.922	2.476		
Total	99	336.390	3.398		

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2.15 PC 249 Cut flowers: evaluation of drip-applied chloropicrin for control of soil-borne *Fusarium oxysporum*, *Rhizoctonia* species, *Sclerotinia sclerotiorum* and weed seeds

2.15.1 Introduction

The recent approval of chloropicrin for use pre-planting of raspberry, including protected raspberry, means that the product can be used pre-planting of protected ornamentals at growers' own risk. K&S Fumigation Ltd are seeking to develop an in-line application method for use in protected ornamental cropping.

2.15.2 Methods

2.15.2.1 Site

The experiment was done on a sandy loam soil in a glasshouse at Bury Lane Fruit Farm, Royston, Hertfordshire where the previous crop was lilies and the first crop in 2006 will be stocks. The beds were sub-soiled and rotavated a few weeks prior to treatment, then watered 2 days prior to chloropicrin treatment and re-rotavated.

2.15.2.2 Chloropicrin application

Chloropicrin was applied by a contractor (K&S Fumigation) to one bay (3.2 m wide) in the glasshouse at 200 L/ha (half the maximum permitted rate) using a maximum concentration of 0.87 ml chloropicrin in 1 L water. The treated bay was covered with polythene (LDPE). Nine drip tapes were set up along the treated bay at 35 cm apart, with 30 cm between drippers. The polythene covers were removed 14 days after treatment.

2.15.2.3 Fusarium oxysporum

Chloropicrin was tested against *F. oxysporum* using woody stem pieces of stocks naturally infected with fusarium (collected in 2005). Nylon gauze bags each containing ten 2-cm long stem sections were prepared. The stem pieces were mixed with sufficient silver sand to separate the stem pieces in the bags. For the fresh stem piece treatments, the sand in the bags were moistened prior to sample burial at the nursery and chloropicrin treatment. For the rotted stem piece treatments, the bags were buried in moist soil that was regularly watered for 2 weeks prior to sample burial at the nursery site and chloropicrin treatment. Treatments were as follows with three replicate bags of ten stem pieces per treatment:

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No.	Soil treatment	Stem material	Position	Depth (cm)
1	Untreated	Fresh	-	0
2	Untreated	Rotted	-	0
3	Untreated	Fresh	-	15
4	Untreated	Fresh	-	30
5	Chloropicrin	Fresh	1	0
6	Chloropicrin	Rotted	1	0
7	Chloropicrin	Fresh	1	15
8	Chloropicrin	Rotted	1	15
9	Chloropicrin	Fresh	1	30
10	Chloropicrin	Rotted	1	30
11	Chloropicrin	Fresh	2	0
12	Chloropicrin	Rotted	2	0
13	Chloropicrin	Fresh	2	15
14	Chloropicrin	Rotted	2	15
15	Chloropicrin	Fresh	2	30
16	Chloropicrin	Rotted	2	30
17	Chloropicrin	Fresh	3	0
18	Chloropicrin	Rotted	3	0
19	Chloropicrin	Fresh	3	15
20	Chloropicrin	Rotted	3	15
21	Chloropicrin	Fresh	3	30
22	Chloropicrin	Rotted	3	30

2.15.2.4 Sclerotinia sclerotiorum

Effects of chloropicrin were tested against *Sclerotinia sclerotiorum* using sclerotia obtained from an oilseed rape field. Nylon gauze bags were prepared (as for stocks stem pieces) using 20 sclerotia per bag. Bags were moistened for a period of 24 h prior to burial and chloropicrin treatment. Treatments were as follows with three replicate bags of 20 sclerotia:

No.	Soil treatment	Position	Depth (cm)
1	Untreated	-	0
2	Untreated	-	15
3	Untreated	-	30
4	Chloropicrin	1	0
5	Chloropicrin	1	15
6	Chloropicrin	1	30
7	Chloropicrin	2	0
8	Chloropicrin	2	15
9	Chloropicrin	2	30
10	Chloropicrin	3	0
11	Chloropicrin	3	15
12	Chloropicrin	3	30

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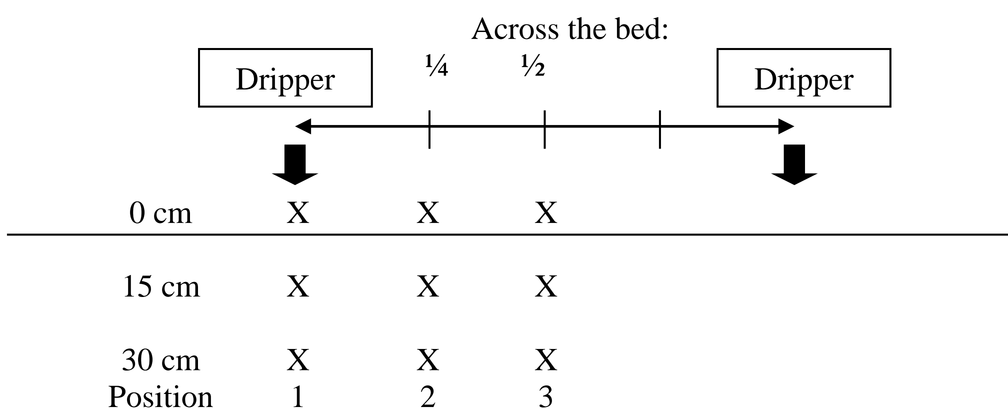
2.15.2.5 Sample burial

For each position/depth treatment, one bag of fresh stem pieces, one bag of rotted stem pieces and one bag of sclerotia were labelled and placed into a nylon mesh onion bag. Sample burial took place once the drip-line irrigation was in place but prior to sheeting and chloropicrin application. There were nine drip tapes across a bed at 35 cm apart. The central T-tape on the nine was selected for sample burial. The three replicates were positioned at approximately 1 m, 10 m and 20 m from the inlet end of the bed. For one replicate, bags were positioned adjacent to the drip tape (position 1), and at a quarter (position 2) and a half (position 3) the distance across the bed to the next drip tape (Figure 2.1). This was repeated for the other two replicates.

Figure 2.1. Position of samples buried prior to chloropicrin application.



X = sample position

At each selected position, samples were buried at 15 and 30 cm depth, with depth measured to the top of the bag. Smeared soil was loosened with a fork and back-filled. The bags for 0 cm depth were placed on the soil surface. The control bags were buried in untreated soil (0, 15 and 30 cm depth) at three separate positions away from the chloropicrin treated area.

Rhizoctonia solani

The effect of chloropicrin treatment on *Rhizoctonia solani* was determined using a semi-quantitative baiting technique based on the method of Paulitz & Schroeder (2005). Prior to soil treatment, 20 soil cores were collected from each of three areas (coinciding with the position of the three replicates for stocks fusarium/sclerotinia sample burial) of the bed to be treated, to a depth of 15 cm using a clean soil auger. The cores from each replicate were bulked to give three samples. The soil was air-dried and stored at ambient temperature until required. Each soil sample was mixed thoroughly and then sub-sampled to fill five clean 9-cm diameter pots. The pots were maintained at 16°C for 2 days and watered daily (15% wt/wt). Wooden toothpicks were inserted vertically into the soil so that they were completely immersed (five toothpicks per pot, evenly spaced). After 64 h, the toothpicks were removed and placed on plates of *Rhizoctonia*-selective medium (five toothpicks per plate). This medium was made using water agar amended with carbendazim at 1 ug/ml and chloramphenicol at 100 ug/ml (Paulitz & Schroeder, 2005). After 24 h, the plates were examined under a dissecting microscope using a 5 mm grid underneath the plate. For each toothpick, the number of squares adjacent to the toothpick containing a

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colony of *R. solani* was counted (out of 22). Colonies of *R. solani* were identified from their typical morphology, as described in Paulitz & Schroeder (2005).

The soil sampling procedure for *R. solani* was repeated 13 days after chloropicrin application, when the polythene covers had been removed.

2.15.2.6 Soil samples

Soil temperature was recorded at 15 cm depth on the day of treatment in the bay that was to be treated with chloropicrin. Before the chloropicrin treatment was applied, 10 soil cores from 0 to 15 cm depth were taken and bulked to determine soil moisture content as a percentage of field capacity (FC).

2.15.2.7 Phytotoxicity

Potted plants of rose and poinsettia were placed on the polythene laid over the treated area, at the edge of the treated area, and 5 m away from the treated area. The plants were observed at the time of sample recovery for symptoms of phytotoxicity.

2.15.2.8 Assessment of pathogen viability

Stocks stem pieces: after recovery the stem pieces were sifted from the sand and the remaining sand washed away. The stem pieces were then dried before surface sterilising in 90% ethanol for 10 seconds. Sterilised pieces were then plated onto PDA+S and incubated at 20°C. The number of fusarium colonies present after 7 and 14 days was counted.

Sclerotia: after the samples had been recovered from soil, the sclerotia were sifted from the sand and rinsed in water to remove excess debris. Sclerotia were surface sterilised for 3 min in a 50:50 v/v mixture of 90% ethanol and 10% sodium hypochlorite, followed by three 1 min rinses in sterile distilled water. The sclerotia were left to air dry on filter paper in a laminar flow cabinet. The sclerotia were cut in half using aseptic technique and then one half of each of the 20 sclerotia per bag was plated on to PDA+S (five halves per plate). The plates were incubated at 20°C. The number of sclerotial halves (out of ten) from which sclerotinia colonies developed after 7 and 14 days was recorded.

2.15.2.9 Weeds

Soil was collected from 0 to 5 cm, 10 to 15 cm and 20 to 25 cm before and after soil treatment from three replicate areas of the bed (20 cores bulked from each area). Each sample was thoroughly mixed, but not sieved. For each soil sample, a seed tray was lined with paper towel and filled with soil. The seed trays were maintained in the laboratory at ambient temperature and watered as necessary to prevent the surface from drying out. The trays were checked weekly and numbers of emerged weeds recorded. The total number of emerged weeds was recorded after 40 days and the main species identified.

~~2.15.2.10~~2.15.2.10 Statistical analyses

Data were analysed using generalised linear models (GLMs) for binomially distributed data in Genstat. While ANOVA makes the assumption that data is normally distributed, GLMs allow analyses of data which do not follow a normal distribution, or where a transformation needs to be applied before normality can be assumed. Data which are proportions will tend to follow a binomial distribution. One

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way of analysing this data would be to do a transformation in order to normalize the data and analyse the transformed data using ANOVA. The best transformation for binomial data is the logit function, which GLM processes internally to produce an accumulated analysis of deviance. This can be interpreted in much the same way as an analysis of variance. The PREDICT command in Genstat can then be used to get the estimated means and standard errors for each treatment level back transformed so that they are produced on the original scale.

2.15.3 Results and discussion

2.15.3.1 Treatment conditions

The soil moisture was 32% FC at the time of sampling, but further irrigation was applied prior to treatment. The soil appeared dry after the polythene was removed, although it was wetter in the area of the bed around replicate area A. The contractor noted that covers could have been left on for longer than the 14 day interval tested, since fumes had not fully dissipated when the polythene was removed. However, the chloropicrin was applied at a soil temperature of approximately 10°C and at higher temperatures, the treatment time could potentially be reduced. Prior to subsequent planting, a cress test would need to be done to ensure crop safety.

2.15.3.2 Crop safety

Plants placed centrally on the polythene-covered treated area were badly affected by the chloropicrin. The poinsettia had collapsed completely, and buds drooped and older leaves dried-up on the rose; new unaffected shoots were developing. Plants placed at the edge of the treatment area were similar in appearance. Plants positioned 5 m from the treated area were unaffected. Based on these observations, there is some evidence that beds could be treated in a house that still had plants growing, provided that they were not immediately adjacent to the treatment area.

2.15.3.3 Fusarium oxysporum

There was a significant effect of soil treatment on the survival of *F. oxysporum* with a mean percentage kill of 74% for chloropicrin treated samples compared with 4% in the untreated controls (Tables 2.25 and 2.26). The effects of position, depth and stem material type (fresh or rotted) were not significant.

2.15.3.4 Sclerotinia sclerotiorum

There was a significant effect of chloropicrin treatment on sclerotial viability, with treatment means of 75% kill for chloropicrin treated samples compared with 13% in the untreated controls (Tables 2.27 and 2.28). Considering treated samples only, there were significant effects of position ($P<0.05$) and depth ($P<0.001$), with percentage kill of sclerotia reducing with depth and distance from the T-tape (Figure 2.2).

There was also a significant block effect (data not shown) suggesting that percentage kill of sclerotia was higher at positions closer to the inlet pipe. Soil sampled from the block closest to the inlet pipe (replicate A) was wetter than the other two blocks further along the bed, which may have had an effect on treatment efficacy.

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Table 2.25. Effect of chloropicrin treatment on survival of fusarium in stocks stem pieces

Soil treatment	Position*	Depth (cm)	% stem pieces in which fusarium remained viable	% kill
Untreated	-	0	100.0	0.0
Untreated	-	15	93.3	6.7
Untreated	-	30	93.0	7.0
Untreated mean			95.4	4.1
Chloropicrin	1	0	18.3	81.7
Chloropicrin	1	15	23.3	76.7
Chloropicrin	1	30	20.2	79.8
Chloropicrin	2	0	28.3	71.7
Chloropicrin	2	15	29.3	70.7
Chloropicrin	2	30	41.9	58.1
Chloropicrin	3	0	11.7	88.3
Chloropicrin	3	15	30.6	69.4
Chloropicrin	3	30	30.0	70.0
Chloropicrin mean			26.0	74.0

*Bags of fusarium stem pieces adjacent to the drip tape (position 1), and at a quarter (position 2) or a half (position 3) the distance across the bed to the next drip tape.

Table 2.26. Accumulated analyses of deviance for effect of chloropicrin treatment on survival of fusarium in stocks stem pieces

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	2	9.410	4.705	1.31	0.280
+ Treatment	21	282.315	13.444	3.75	<.001
Residual	42	150.667	3.587		
Total	65	442.392	6.806		

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	2	12.990	6.495	1.51	0.234
+ Depth_cm	2	6.523	3.261	0.76	0.475
+ Position	2	7.583	3.792	0.88	0.422
+ Stem_material	1	0.306	0.306	0.07	0.791
+ Depth_cm.Position	4	5.983	1.496	0.35	0.843
+ Depth_cm.Stem_material	2	5.376	2.688	0.63	0.540
+ Position.Stem_material	2	11.671	5.835	1.36	0.270
+ Depth_cm.Position.Stem_material	4	9.634	2.408	0.56	0.692
Residual	34	145.841	4.289		
Total	53	205.907	3.885		

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Table 2.27. Effect of chloropicrin treatment on percentage of viable sclerotia of *Sclerotinia sclerotiorum*

Soil treatment	Position*	Depth (cm)	% viable sclerotia	% kill
Untreated	-	0	97.6	2.4
Untreated	-	15	84.2	15.8
Untreated	-	30	79.4	20.6
Untreated mean			87.1	12.9
Chloropicrin	1	0	0.0	100.0
Chloropicrin	1	15	16.6	83.4
Chloropicrin	1	30	33.3	66.7
Chloropicrin	2	0	9.3	90.7
Chloropicrin	2	15	23.9	76.1
Chloropicrin	2	30	33.3	66.7
Chloropicrin	3	0	4.9	95.1
Chloropicrin	3	15	30.2	69.8
Chloropicrin	3	30	76.3	23.7
Chloropicrin mean			25.3	74.7

*Bags of sclerotia adjacent to the drip tape (position 1), and at a quarter (position 2) or a half (position 3) the distance across the bed to the next drip tape.

Table 2.28. Accumulated analysis of deviance for effect of chloropicrin treatment on percentage of viable sclerotia of *Sclerotinia sclerotiorum*

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	2	61.847	30.924	6.03	0.008
+ Treatment	11	361.862	32.897	6.41	<.001
Residual	22	112.839	5.129		
Total	35	536.549	15.330		

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	2	75.678	37.839	7.59	0.005
+ Depth_cm	2	101.177	50.588	10.14	0.001
+ Position	2	37.921	18.961	3.80	0.045
+ Depth_cm.Position	4	17.804	4.451	0.89	0.491
Residual	16	79.793	4.987		
Total	26	312.373	12.014		

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Figure 2.2. Effect of position and depth from application point on percentage kill of sclerotia (*Sclerotinia sclerotiorum*) by chloropicrin

Depth (cm) \ Position	1 Dripper	2 8.75 cm	3 17.5 cm
0	100	91	95
15	83	76	70
30	67	67	24

2.15.3.5 Rhizoctonia solani

There was a significant effect of soil treatment (*P*<0.001) on rhizoctonia survival (Table 2.29) but giving limited control of the pathogen. It is possible that following chloropicrin application, any propagules of *R. solani* that survived the treatment could have rapidly re-colonised the soil prior to the baiting technique that was used.

Table 2.29. Effect of chloropicrin treatment on the survival of rhizoctonia in soil

Soil treatment	% toothpick sections from which rhizoctonia colonies developed
Untreated	53.2
Chloropicrin	41.0

2.15.3.6 Weeds

The majority of seedlings that grew in untreated soil were chickweed, although grass species were also present (Table 2.30). Chloropicrin gave excellent control of chickweed and grasses. The level of control did not vary with depth.

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Table 2.30. Effect of chloropicrin treatment on weed emergence

Treatment	Depth sampled (cm)	Mean no. of weeds emerged*			
		Chickweed	Other dicots	Grasses	Total
Untreated	0-5	60.5	2.0	8.5	71.0
Untreated	10-15	36.0	1.0	4.5	41.5
Untreated	20-25	1.5	0.0	1.5	3.0
Chloropicrin	0-5	0.0	0.0	1.0	1.0
Chloropicrin	10-15	0.0	0.0	1.0	1.0
Chloropicrin	20-25	1.0	0.0	1.0	2.0

*Based on 2 replicate samples for the untreated control and 3 replicate samples for chloropicrin treatment. Soil was collected from different areas before and after treatment.

2.15.4 Summary

Soil treatment with chloropicrin applied via drip-line irrigation showed potential for broad spectrum soil disinfestation, significantly reducing inoculum levels of the soil-borne pathogens *F. oxysporum* and *S. sclerotiorum,* and providing effective control of dicotyledon and grass weeds. Lack of phytotoxic effects on plants placed 5 m from the treated area suggests that it may be possible to treat beds when there is still a crop growing in the glasshouse. Improvements in treatment efficacy could potentially be achieved using the full approved rate, by increasing soil moisture content prior to application, by reducing the distance between T-tapes and increasing the duration for which the soil is covered.

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2.16 Investigation of possible factors resulting in failure of soil steaming to give 100 % kill of *Fusarium oxysporum* in naturally infected stocks stem pieces

2.16.1 Introduction

Results from soil steaming experiments in 2004 indicated that steaming was relatively ineffective in eliminating *F. oxysporum* from stocks stem pieces, even at the soil surface where 60°C was exceeded. This laboratory experiment was done to determine the effect of different heating processes on the viability of *Fusarium oxysporum* in naturally infected stocks stem pieces

2.16.2 Methods

Each treatment shown in Table 2.31 was applied to three replicates of 20 stem pieces.

Table 2.31. Treatments applied to stocks stem pieces naturally infested with *Fusarium oxysporum.*

No.	Moisture treatment prior to heating	Heat treatment
1.	Dry	Nil
2.	Soak for 7 d	Nil
3.	Dry	Boil for 30 min (100°C)
4.	Soak for 7 d	Boil for 30 min (100°C)
5.	Dry	Boil for 60 min (100°C)
6.	Soak for 7 d	Boil for 60 min (100°C)
7.	Dry	Microwave for 10 min
8.	Soak for 7 d	Microwave for 10 min

Stem pieces (2 cm length) were prepared from stocks debris collected from a crop infected with *F. oxysporum* in 2005 and stored at ambient temperature in the laboratory. Nylon gauze bags each containing 20 stem pieces and dry sand were prepared. For treatments, 2, 4, 6 and 8, the prepared bags were left to soak in tap water for 7 days. Bags for the remaining treatments were left dry.

For hot water treatments, the water bath was allowed to reach the target temperature before bags were put in, ensuring that they were completely immersed. After the appropriate time interval, bags were removed from the water bath. For the microwave treatments, bags to be treated were placed in an open plastic box.

Within 7 days of treatment the stem pieces were sifted from the sand and the excess sand was washed away. Each stem piece was cut transversely to give 1 cm stem lengths. The stem pieces were surface sterilised in 90 % ethanol (10 sec) and left to dry on filter paper in a laminar flow hood. Sterilised pieces were then plated onto PDA+S. The number of fusarium colonies present after incubation for 20°C for 7 and 14 days was counted.

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2.16.3 Results and discussion

The microwave treatment on dry and soaked stem pieces was effective in eliminating *F. oxysporum* from woody stocks stem pieces (Table 2.32). The boiling treatment was most effective when used on soaked stem pieces, with fusarium eliminated from >98.5% stem pieces. A reduction in percentage kill was observed when dry stem pieces were boiled, perhaps due to reduced heat penetration into the centre of the stem pieces. This result may in part explain the limited efficacy of steaming against fusarium wilt observed in year 1 experiments (Annual Report, project year 1).

Table 2.32. Effect of heat treatments on the survival of *Fusarium oxysporum* in woody stocks stem pieces.

No.	Moisture treatment prior to heating	Heat treatment	Mean no. stem pieces (of 60) with growth of *F. oxysporum* after 14 d	% kill
1.	Dry	Nil	19.7	1.5
2.	Soak for 7 d	Nil	19.7	1.5
3.	Dry	Boil for 30 min (100°C)	0.3	98.5
4.	Soak for 7 d	Boil for 30 min (100°C)	0.0	100.0
5.	Dry	Boil for 60 min (100°C)	3.0	85.0
6.	Soak for 7 d	Boil for 60 min (100°C)	0.3	98.5
7.	Dry	Microwave for 10 min	0.0	100.0
8.	Soak for 7 d	Microwave for 10 min	0.0	100.0

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2.17 Overall conclusions

- For the third successive year, outbreaks of stocks fusarium wilt were confirmed on UK nurseries.
- In soil artificially infested with infected stocks debris, *F. oxysporum* survived for 7 months at levels sufficient to cause typical wilt symptoms on most young stock plants grown in the soil.
- In cross-pathogenicity experiments, typical and severe symptoms of fusarium wilt were only seen when a host plant was inoculated with *F. oxysporum* previously isolated from the same host plant (i.e. stocks inoculated with *F. oxysporum* from stocks or lisianthus inoculated with *F. oxysporum* from lisianthus). However, there was some survival of *F. oxysporum* on roots of non-host crops that could subsequently act as a source of inoculum for subsequent stocks or lisianthus crops.
- A range of disinfectant products were fully effective against spores of *F. oxysporum* even after 5 minutes exposure when used at the recommended rate. Peat contamination at 0.1% w/v did not reduce the efficacy of the disinfectants against spores. The disinfectant products were less effective against mycelium of *F. oxysporum*. However, Unifect G gave complete control of mycelial growth both with and without peat contamination, when the full rate was used for a 30 minute treatment.
- Ten fungicide programmes were evaluated for their ability to control fusarium wilt in an artificially infested crop of stocks. There was a trend for a lower incidence of internal stem symptoms for plants treated with a Delsene 50 Flo (carbendazim) drench followed by Delsene 50 Flo, Amistar, Octave or an experimental product. The experiment demonstrated that a vascular wilt disease, such as stocks fusarium wilt, that colonises inside roots and stems can be difficult to control effectively using fungicides alone.
- Results from a laboratory resistance test suggested that failure of carbendazim to give more effective control of fusarium wilt on stocks is not due to fungicide resistance.
- Following on from experiments in 2004, six methods of soil disinfestation were examined in 2005 – Basamid, Chloropicrin, Formalin, Discovery, biological soil disinfestation (BSD) and Microgran (in addition to sheet-steaming in 2004). A summary of results for experiments in 2004 and 2005 is as follows: Basamid gave very good results at one site and moderately good results at a second site. Formalin, applied as a drench at 0.5 L/m^2 according to the Commodity Substance Approval, gave excellent control at the soil surface but results at depth were variable. Chloropicrin applied by injecting into drip irrigation lines laid on the soil surface and covered with polythene looks to be a promising new treatment, with scope to improve efficacy using an increased rate. Both Discovery and sheet steaming gave moderate control at the soil surface but were less effective at depth. Neither BSD nor Microgran (calcium cyanamide) were effective in significantly reducing *F. oxysporum* in stocks stem pieces.

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- There was clear evidence from the soil disinfestation trials, that fusarium survival in woody stem pieces is significantly reduced following burial in damp soil for approximately 4 weeks.
- In a laboratory experiment, microwave treatment of dry and soaked stem pieces was effective in eliminating *F. oxysporum* from woody stocks stem pieces. Boiling in water (30 min) eliminated fusarium from woody stem pieces when they had been thoroughly soaked but was less effective when dry stem pieces were used, perhaps due to reduced heat penetration into the centre of the stem pieces. This has implications for the efficacy of steam treatments to sterilise soil pre-planting.

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2.19 Technology transfer

2.19.1 Presentations

- Project update by Tim O'Neill at Ball Holland grower visit, West Nurseries, Spalding, 6 June 2005
- 'Alternatives to methyl bromide for cut flower growers'. Presentations to growers by Tim O'Neill and Dan Drakes at HDC Seminar, Holbeach, 5 October 2005
- 'Fusarium wilt of stocks – research update 2005'. Presentation to South Holland Growers' Club, Spalding, 7 November 2005 (Tim O'Neill)
- 'Control of fusarium wilt diseases in stocks and lisianthus'. Presentation at the Cut Flowers Annual Conference, Holbeach, 23 November 2005 (Kim Green).

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2.19.2 Publications

O'Neill TM, Green KR & Ratcliffe T. 2005. Evaluation of soil steaming and a formaldehyde drench for control of fusarium wilt in column stocks. *Acta Horticulturae* **698**, 129-133.

2.20 Acknowledgements

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3 APPENDIX 1. EXPERIMENT DIARIES

Fungicide trial

Date	Trial Diary
19/05/05	Soil sampled in ADAS Arthur Rickwood Polytunnel 1
03/06/05	Infected stocks debris spread over and into soil
07/06/05	Fertiliser spread and rotovated into soil
08/06/05	Fusarium spore suspension sprayed over soil surface
09/06/05	Trial planted into polytunnel 1. Fungicide drench applied where necessary. Logger started. Chopped infected stocks plants spread between planted rows.
10/06/05	Fungicide spray 1 applied
15/06/05	Fubol Gold and Aphox applied to whole trial area
17/06/05	Slug pellets spread around trial area
22/06/05	Fungicide spray 2 applied
23/06/05	Disease assessment 1
01/07/05	Disease assessment 2
07/07/05	Disease assessment 3. Fungicide spray 3. Toppel 10 and Fubol Gold applied to whole trial area.
14/07/05	Disease assessment 4
28/07/05	Disease assessment 5
09/08/05	Disease assessment 6
10/08/05	Plant height assessment. Final harvest assessment

Biological soil disinfestation

Date	Trial Diary
25/05/05	Trial area ploughed
30/05/05	Rye grass sown at 50 kg/ha
02/08/05	Quadrats taken of fresh weight of ryegrass/green matter = 3.3 kg/m^2
08/08/05	Gauze bags containing fusarium infected stocks stem pieces and sand prepared
09/08/05	Decision taken to add more ryegrass to plot from neighbouring area – total fresh weight of ryegrass = 5.7kg/ m^2 18m x 11m plot flooded with 3 loads of water from 1000Gal bowser Ryegrass spaded in to depth of 30cm with Imants spader Samples buried to required depths in holes 0.5m apart along length of plot. Controls placed on headland area at end of plot. Whole plot covered with VIF (Virtually impermeable film) buried to one spade blades depth around all edges and joins.
26/08/05	Soil temp in BSD 19.5°C (09:00 h), untreated 14°C Soil temp in BSD 27°C (13:45 h), untreated 20°C
27/09/05	Plastic cut and samples retrieved
28/09/05	Stem pieces extracted from gauze bags and plated up onto PDA+s
05/10/05	Assessed plates for fusarium
12/10/05	Assessed plates for fusarium

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Basamid soil treatment

Date	Trial Diary
	Trial area prepared – rotavated and watered
29/09/05	Stem pieces placed in bags and buried to produce partially rotted stem pieces
25/10/05	Buried bags recovered and placed with unrotted bags in onion sacs in preparation for treatments.
09/11/05	Basamid applied to soil surface and rotavated in. Onion bags containing stem pieces (rotted and un-rotted) buried to required depth at Jenny Whitehead's Nursery in Boston, Lincs. Whole area covered with LDPE film.
24/11/05	Samples retrieved from Boston Stem pieces removed from bags, washed, surface sterilised and plated onto PDA + S Plates assessed 7 days after plating Plates assessed 14 days after plating

Formalin soil treatment

Date	Trial Diary
	Trial area prepared – rotavated and watered
29/09/05	Stem pieces placed in bags and buried to produce partially rotted stem pieces
25/10/05	Buried bags recovered and placed with unrotted bags in onion sacs in preparation for treatments.
26/10/05	Onion bags containing stem pieces (rotted and un-rotted) buried to required depth at Stuart West's Nursery in Spalding The formalin was applied diluted 0.5 L Formalin in 2 L water to give a total volume of 2.5 L. This 2.5 L diluted Formalin was applied to the 1 m^2 plot using a clean watering can and rose.
09/11/05	Samples retrieved from Spalding
15/11/05	Stem pieces removed from bags, washed, surface sterilised and plated onto PDA + S
22/11/05	Plates assessed 7 days after plating

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Microgran soil treatment

Date	Trial Diary
	Trial area prepared – rotavated and watered
29/09/05	Stem pieces placed in bags and buried to produce partially rotted stem pieces
25/10/05	Buried bags recovered and placed with unrotted bags in onion sacs in preparation for treatments.
26/10/05	Onion bags containing stem pieces (rotted and un-rotted) buried to required depth at Stuart West's Nursery in Spalding
	Microgran applied (100 g /m) evenly to the plot and raked in to a depth of 5 cm.
09/11/05	Samples retrieved from Spalding
17/11/05	Stem pieces removed from bags, washed, surface sterilised and plated onto PDA + S
24/11/05	Plates assessed 7 days after plating

Metham sodium soil treatment

Date	Trial Diary
	Trial area prepared – rotavated and watered
29/09/05	Stem pieces placed in bags and buried to produce partially rotted stem pieces
25/10/05	Buried bags recovered and placed with unrotted bags in onion sacs in preparation for treatments.
26/10/05	Onion bags containing stem pieces (rotted and un-rotted) buried to required depth at Stuart West's Nursery in Spalding
	Diluted 90 ml Discovery (510 g/L metham sodium) in 15 L water and gradually applied to plot. Covered the plot with LDPE film (approx. 1.2 m^2 and ensured the edges were dug in.
09/11/05	Samples retrieved from Spalding
10/11/05	Stem pieces removed from bags, washed, surface sterilised and plated onto PDA + S
17/11/05	Plates assessed 7 days after plating
24/11/05	Plates assessed 14 days after plating

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