Contract report for the
Horticultural Development Council

Protected stock: aspects of the biology and control of *Fusarium* wilt, a new disease problem

PC 213 and PC 213a

January 2007

Project title:	Protected stock: aspects of the biology and control of *Fusarium* wilt, a new disease problem
Project number:	PC 213 and PC 213a
Project leader:	Dr Tim O'Neill, ADAS Arthur Rickwood
Report:	Final report
Previous reports	March 2005, March 2006
Key staff:	Kim Green, Amanda Shepherd, Martyn Watling
Location of project:	ADAS Arthur Rickwood and commercial nurseries in Norfolk and Suffolk
Project coordinator:	Mr Stuart West, Wests Nursery, Low Fulney, Spalding
Date project commenced:	1 April 2004
Date completion due:	31 January 2007
Key words:	Stock, fusarium wilt, soil disinfestation, fungicides

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our 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

Headline

- This project has demonstrated that *Fusarium oxysporum* can survive in soil at sufficient levels to cause wilt in stocks for at least 16 months but that soil disinfestation by steaming (either sheet steaming or a steam plough) can significantly reduce it to manageable levels provided the soil temperature reaches 80ºC for 30 minutes.
- Results from three fungicide efficacy experiments (2004-2006) demonstrated that treatment of stocks fusarium wilt using fungicides alone is unlikely to be fully effective.

Background and expected deliverables

In summer 2003, a vascular wilt disease seriously affected production of stocks (*Matthiola incana*) 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 was 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 were:

- 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 disinfestation 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 in stock
- 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*
- 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.

Summary of the project and main conclusions (2004-2006)

Occurrence of fusarium wilt

Since it was first identified in summer 2003, fusarium wilt has affected crops of stock on several nurseries each year. The disease has been confirmed in crops in Lincolnshire, Norfolk, West Sussex and Northern Ireland. Generally the disease was widespread and damaging on at least two or three nurseries each year and was present at a low incidence on several others. The nurseries with a severe disease problem have differed from year to year. Although various soil disinfestation treatments appear to have the potential to eliminate *F.* oxysporum from soil, occasional damaging attacks have occurred in crops following soil treatment with dazomet, methyl bromide and sheet steaming. The disease has occurred from late April to September, with reports of sudden symptom development as crops begin to flower. The disease is generally more obvious in hot weather and second crops, and tends to affect some colours more than others.

In 2006, fusarium wilt was first confirmed in late April, as stunted plants with dull leaves and inter-veinal chlorosis in a crop planted in early March. The house had grown two crops of stocks in 2005 and the soil was treated with Sistan 51 (metam sodium) in the winter, sealed in with a water cap. In May, the disease was confirmed on a second nursery, causing 15-20% plant loss as crops came to flower, with cvs Opera Red and Cream badly affected. The soil had been treated with Basamid (70g/m^2) before planting; it was thought that possibly the ground was not covered sufficiently

quickly and some of the sterilant gas (methyl isothiocyanate = MITC) escaped before it could be sealed in. There was also evidence on this nursery that the disease affected most of a tray of plants (e.g. 4 rows x ½ bed). Such a pattern of infection might occur if a tray of plants had been placed on a surface contaminated with fusarium, or the tray of plants was infected at planting. In June the disease was very damaging on another nursery, causing 90% loss of cvs Debora Blue and Centum Dark Blue; red colours were less affected. In this instance the soil was sheet-steamed before planting, although only for around 1 hour. Possibly the steam had not heated the soil to a sufficiently high temperature to adequate depth. On another nursery, fusarium wilt again developed after sheet steaming and caused moderate losses. In this instance it was suggested that planting before the soil had cooled sufficiently may have caused root damage and/or favoured disease development. Fusarium wilt was recorded at trace levels only on several other nurseries.

Survival of F. 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 all test plants remained in soil that had been stored for 16 months.

Occurrence of F. oxysporum *on seed*

Two seed lots were examined by plating on agar. In the first lot (400 seeds), no fusarium was isolated. In the second lot, *F. oxysporum* was recovered from 7 out of 4,400 seeds tested; the greatest level of infection recorded on a single variety/colour was 1%. *F. oxysporum* was still recovered from seeds of this lot after surface sterilisation in sodium hypochlorite. The isolate of *F. oxysporum* obtained from seed in this experiment caused wilting and plant death when used to inoculate young stock plants. This experiment confirmed that *F. oxysporum* pathogenic to stocks can survive on stocks seed.

Effect of F. oxysporum inoculum level on occurrence of wilt

Infection and symptom development due to *F. oxysporum* occurred on plug stock plants at inoculum levels as low as 0.3 spores/g soil, however symptom development was more consistent (more than 25% plants affected) at inoculum levels of 1,000 spores / g soil, or greater.

When plug plants were inoculated by dipping roots into a suspension of *F. oxysporum* spores for 5 minutes, all concentrations tested (from 10 to 1,000,000 spores/ml) resulted in fusarium wilt. The highest concentration gave more consistent infection (approximately 50% plants).

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 were done in 2004 and 2005 to gain information on the host range.

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) (Table 1). However, *F. oxysporum* was recovered from roots of non-host crops indicating that they could act as a source of inoculum for subsequent stocks or lisianthus crops.

Table 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

Effect of temperature on growth of F. oxysporum

The effect of temperature on the growth of three isolates of *F. oxysporum* obtained from stocks was examined. The highest rate of mycelial growth (8-10 mm / day) occurred at 23ºC for one isolate and 25ºC for the other two isolates. Growth was above 2 mm/day at temperatures between 10 and 30ºC; there was very little growth at 5 or 35ºC. These results supported grower observations that the development of fusarium wilt is favoured by high temperatures.

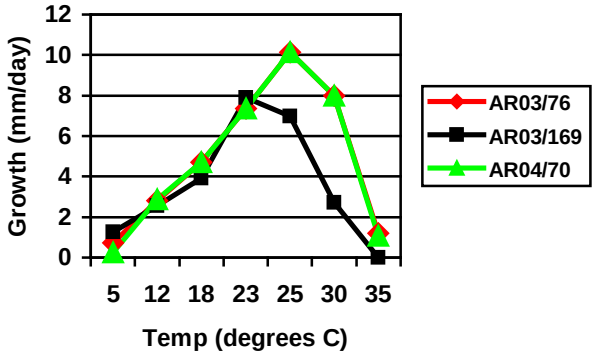


Figure 1. Effect of temperature on growth of three isolates of *F. oxysporum* from stock

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 just 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.

The disinfectant products were less effective against mycelium of *F. oxysporum* due perhaps to the development of survival structures (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.

Fungicides

In 2005, 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

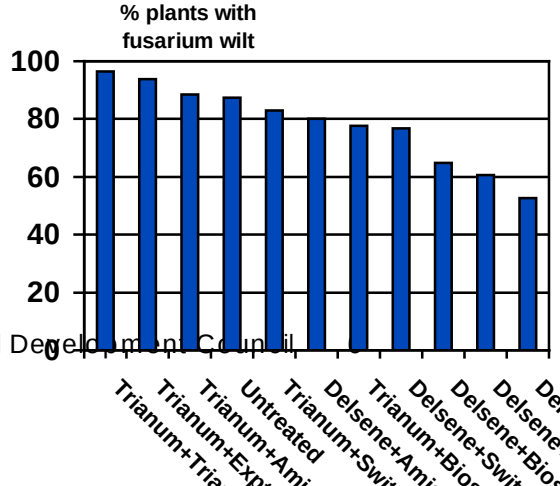
no significant effect of treatment on the proportion of plants showing wilt symptoms, with more than 42% plants affected for all treatments. However, there was a trend for a lower incidence of internal stem symptoms for plants treated with a Delsene 50 Flo (carbendazim) drench followed by Delsene 50 Flo, Amistar, Octave or Experimental product 1, with a reduction from 62.5% to 45% or less. Carbendazim (as Bavistin DF) and azoxystrobin (Amistar) were also the most effective products for reducing fusarium wilt in stocks plug plants in a preliminary experiment. Phytotoxic effects were observed following applications with Swing Gold, Folicur, Biosept Gold and an experimental product.

In 2006, a pre-planting drench using carbendazim (as Delsene 50 Flo) was more effective for reducing the incidence and severity of fusarium wilt, compared with a drench using Trianum-P (Fig 2).

These fungicide evaluation experiments demonstrated that stocks fusarium wilt is difficult to control effectively using fungicides alone.

In 2005, 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. Fusarium isolates from stocks were tested for resistance to the fungicide Delsene 50 Flo at three concentrations of the active ingredient carbendazim. All of the isolates of fusarium 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.

Figure 2. Effect of pre-planting drenches (first product) and foliar treatments (second product) on mean percentage of stock plants with fusarium wilt, 8 weeks after planting



Soil disinfestation

A series of experiments was conducted to determine the effectiveness of various soil treatments in eradicating *F. oxysporum* from naturally-infected woody stem pieces buried at different depths. Experiments were on different sites but a similar test procedure was used throughout.

In November 2004, methyl bromide applied at 50 g/m^2 beneath virtually impermeable film (VIF) resulted in over 80% kill of *F. oxysporum* in naturally infected woody stem pieces buried at 0, 15, 30 and 45 cm. There was no fall-off in efficiency with depth. Even at 45 cm depth, there were some replicate bags of stem pieces where no fusarium survived.

Alternative chemical treatments evaluated were Basamid (dazomet), K & S Chlorofume (chloropicrin) applied via drip-line irrigation (HDC project PC 249), Formalin (formaldehyde), and Discovery (metham sodium (MeNa)). The efficacy of these treatments against *F. oxysporum* in stock stem pieces at various soil depths is summarised in Table 1.2.

Steaming treatments (sheet steaming or injection of steam by steam plough) were also evaluated (Table 3). Excellent control of both *F. oxysporum* and *Sclerotinia sclerotiorum* were obtained using either sheet steaming or a steam plough in June 2006, but in other sheet steaming experiments treatment efficacy was reduced, due possibly to soil type, insufficient preparatory cultivation, sub-optimal soil moisture (too wet or too dry) or other factors. Temperatures achieved following sheet steam and steam plough treatments that effectively controlled *F. oxysporum* and *S. sclerotiorum* are illustrated in Figures 3 and 4.

On a site where conventional sheet steaming for 12 hours gave poor results, vacuum steaming for just 4 hours raised the soil temperature to above 90^{o}C at 23.5 cm depth

and to above 80°C at 33.5 cm depth (Figure 1.5). The cost for burying perforated plastic pipes to enable vacuum steaming is around £4.70/metre, or £27,000/ha at a pipe spacing of 1.6 m. Fuel consumption for vacuum steaming is estimated to be around 57% of that required for conventional sheet steaming.

The effect of incorporating soil amendments a few days after sheet steaming on the incidence of fusarium wilt in a crop of lisianthus was examined in September 2006. Fusarium wilt had been confirmed in lisianthus grown on the experimental area in early 2006. Sheet steaming failed to achieve soil temperatures above 80°C and the percentage kill of *F. oxysporum* in deliberately buried infested stem pieces at this site was relatively poor (30%). This result suggests the amendments were added to a soil that still contained *F. oxysporum*. Five soil amendments (Agralan Revive, Gliomix, Microgran, Trianum-P and a non-pathogenic fusarium) incorporated before planting were compared with drench treatments of Delsene 50 Flo, applied three times after planting, and a sheet steaming only control. Fusarium wilt was first confirmed in plants at 3 weeks after planting and increased to affect 26% of the steaming only control by harvest. None of the soil amendment treatments or post-planting fungicide treatments significantly reduced the cumulative incidence of fusarium wilt.

Table 2. Efficacy of chemical soil disinfestation treatments for the control of *Fusarium oxysporum* in stock stem pieces (% kill of *F. oxysporum*)

Depth	Basamid		Chloropicrin		Formalin		MeNa
(cm)	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1
0	78	100	81	51	100	96	86
5	-	90	-	-	100	0	84
15	12	96	72	57	67	2	42
30	5	80	69	6	-	8	54
45	-	-	-	-	-	-	-

Table 3. Efficacy of soil steaming treatments for the control of *Fusarium oxysporum* in stock stem pieces (% kill of *F. oxysporum*)

Depth (cm)	Sheet steaming				Steam plough
	July 04	Sept 04	June 06	Sept 06*	June 06
0	70	72	94	30	94
5	76	66	92	67	90
15	-	-	94	23	98
30	32	44	92	2	98
45	12	0	-	-	-

*lisianthus stem pieces

Figure 3. Mean temperatures achieved at different depths below the soil surface during sheet steaming – Norfolk, June 2006

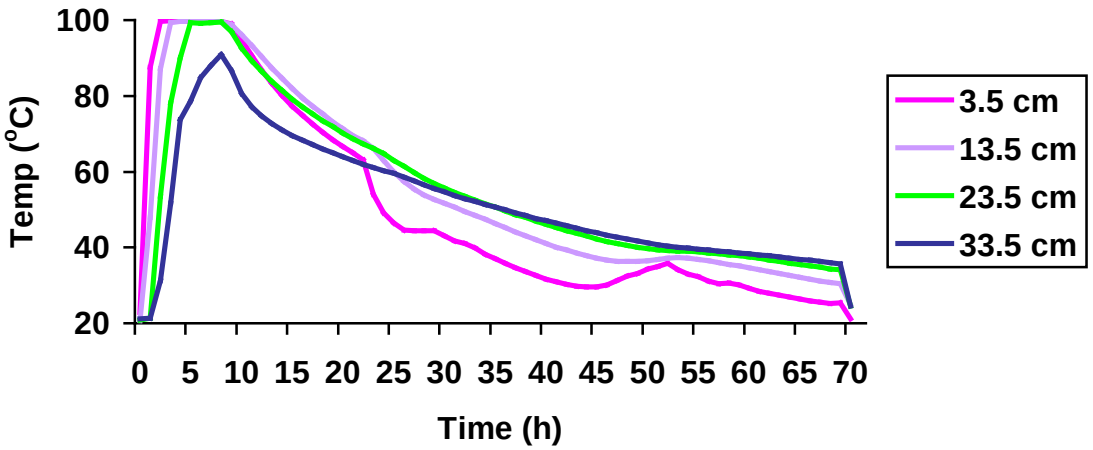


Figure 4. Mean temperatures achieved at different depths below the soil surface during injection of steam by steam plough – Norfolk, June 2006

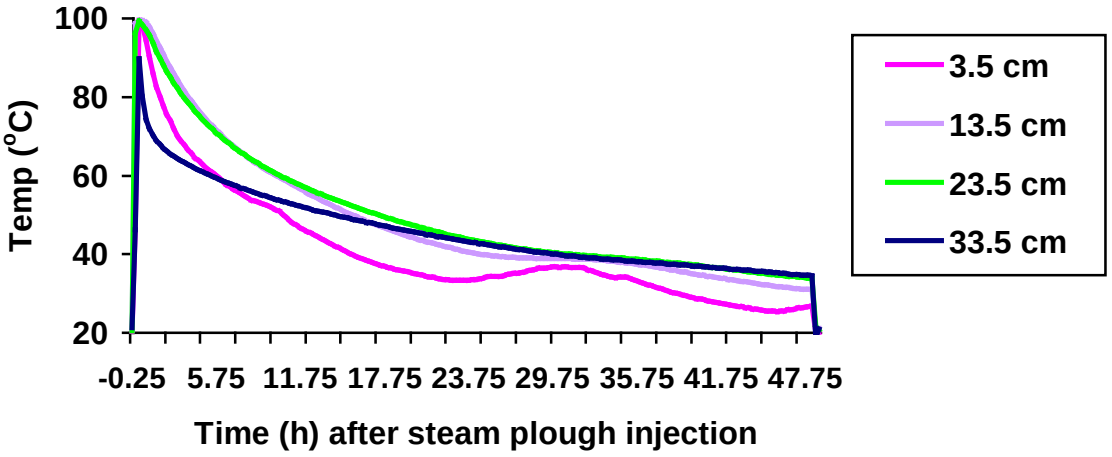
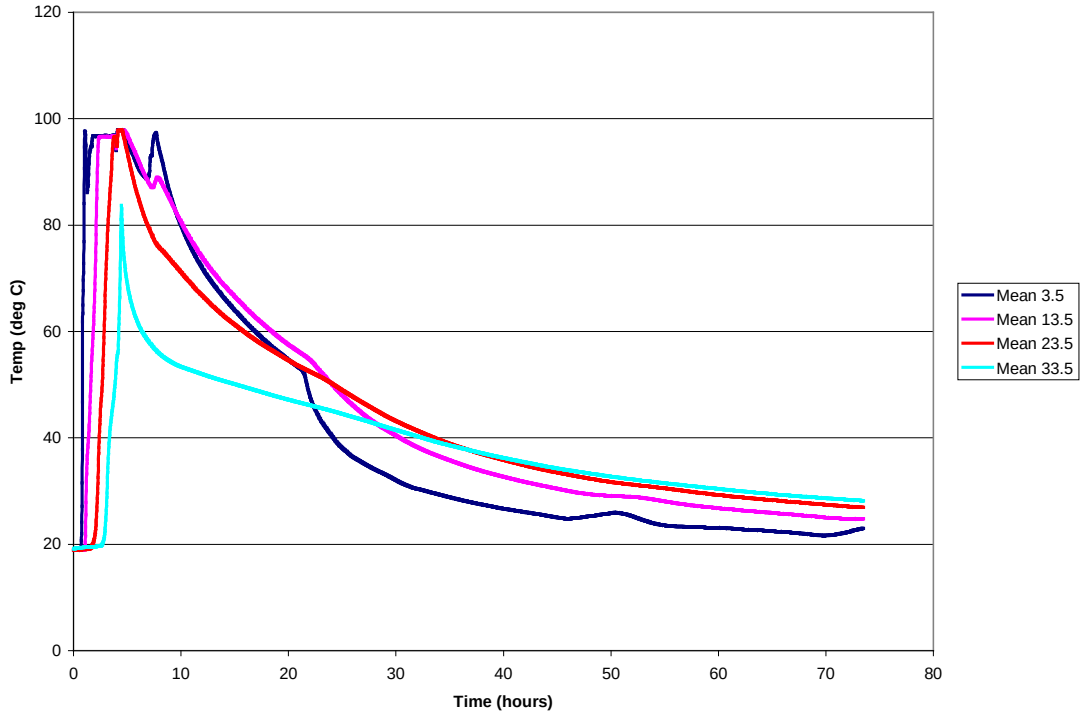


Figure 5 Mean temperatures achieved at different depths during vacuum steaming



(Suffolk, 2006)

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 is continued profitable production of stock despite the threat of fusarium wilt through a greater understanding of disease biology and identification of effective soil disinfestation treatments.

Action points for growers

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 (especially at the leaf nodes) which is usually a reliable indicator of fusarium wilt.

Reducing the risk of persistence between crops and disease spread

- Take measures to reduce disease risk after an outbreak of fusarium wilt (see below). Fusarium can persist in plant debris buried in soil for at least 16 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 as fusarium may survive in these despite soil disinfestation treatment.
- Disinfectants can be used to eliminate fusarium from pathways, glasshouse structures machinery and equipment (e.g. spikes used to push up plugs). Unifect G used at the recommended rate for a 30 minute treatment is effective against both spores and mycelium of *F. oxysporum* from stocks. Information on the use of disinfectants can be found in HDC Factsheet 15/05.
- Clean and disinfect surfaces where trays of plug trays are stood before planting out.

Control of fusarium wilt: soil treatment

- Basamid, Formalin drench, Discovery, K & S Chlorofume (applied in drip-line irrigation water) 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 (see above), and by encouraging rapid breakdown of any crop debris incorporated.
- Where Basamid, Discovery or K & S Chlorofume 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. Any Formalin remaining in the soil may damage the growth of the subsequent crop. The rate of Formalin

breakdown depends on soil temperature. Persistence is reported to be 5 to 6 weeks at 0 to 5°C soil temperature, and 7 to 8 days at 25°C soil temperature.

- Where sheet steaming is used, the soil should be fairly dry and cultivated to a coarse tilth (no lumps more than 5 cm diameter). Take care not to cultivate deeply after steaming, or to recontaminate the soil from dirty boots, equipment or unsteamed soil.
- The effectiveness of sheet steaming is profoundly influenced by soil type and requires attention to detail for good results. Consider vacuum steaming if steam penetration is poor.
- Steaming soil with a steam plough can also be effective against soil-borne fusarium. It is more time-consuming than sheet-steaming and requires constant attendance, but is also more fuel efficient; it is best on sandy loam soils.
- Use of soil amendments prior to planting may reduce the incidence of fusarium wilt, although application can be costly. This area of work warrants further research for the future.

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 the 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.

Integrated control

- There is no simple, single measure that will provide reliable control of fusarium wilt. However, by thorough application of a soil disinfestation treatment which is suitable for your soil, use of carbendazim fungicide treatment at planting, prompt

removal of affected plants (if only a few are affected), minimising crop debris incorporated into the soil, and general good hygiene, it should be possible to keep the disease at a very low level.

- Pay particular attention to control of fusarium in houses where the disease occurred the previous year, or where the ground was double-cropped.

Please note: Since the time of writing this report the approval status of K & S Chlorofume (chloropicrin) has changed and it is no longer approved for use under protection.

Regular changes occur in the approval status of pesticides, arising from changes in pesticide legislation or for other reasons. For the most up to date information, please check with your professional supplier, BASIS registered adviser or with the Information Section at the Pesticides Safety Directorate (PSD) (Telephone 01904 455775; email information@psd.gsi.gov.uk; website www.pesticides.gov.uk).

1.1 Introduction

In June 2003, a vascular 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. This initial experiment confirmed that *F. oxysporum* was the cause of the problem (O'Neill *et al.,* 2004).

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 re-occurred in 1975 and 1987 in the same glasshouse (J T 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 the final year of the project, the focus has been on survival of the fungus in soil, fungicide efficacy and a comparison of steaming methods for control of stocks fusarium wilt. In addition, sheet steaming and soil amendments were evaluated for control of fusarium wilt on lisianthus.

1.2 Occurrence of fusarium wilt in 2006

In 2006, fusarium wilt was first confirmed in late April, as stunted plants with dull leaves and inter-veinal chlorosis in a crop planted in early March. The house had grown two crops of stocks in 2005 and the soil was treated with Sistan 51 (metam sodium) in the winter, sealed in with a water cap. In May, the disease was confirmed on a second nursery, causing 15-20% plant loss as crops came to flower, with cvs Opera Red and Cream badly affected. The soil had been treated with Basamid (70g/m^2) before planting; it was thought that possibly the ground was not covered sufficiently quickly and some of the sterilant gas (methyl isothiocyanate = MITC) escaped before it could be sealed in. There was also evidence on this nursery that the disease affected most of a tray of plants (e.g. 4 rows x ½ bed). Such a pattern of infection might occur if a tray of plants had been placed on a surface contaminated with fusarium, or the tray of plants was infected at planting. In June the disease was very damaging on another nursery, causing 90% loss of cvs Debora Blue and Centum Dark Blue; red colours were less affected. In this instance the soil was sheet-steamed before planting, although only for around 1 hour. Possibly the steam had not heated the soil to a sufficiently high temperature to adequate depth. On another nursery, fusarium wilt again developed after sheet steaming and caused moderate losses. In this instance it was suggested that planting before the soil had cooled sufficiently may have caused root damage and/or favoured disease development. Fusarium wilt was recorded at trace levels only on several other nurseries.

Where fusarium wilt developed, it often appeared to start as one or two affected plants and then spread, resulting in a circle or oval of affected plants. One grower reported that disease spread was greater where overhead irrigation was used.

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

1.3.1 Introduction

Following on from an experiment in project year 1, the aim of this experiment done in project years 2 and 3, 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.

1.3.2 Methods

Stock plants with typical symptoms of fusarium wilt were chopped into pieces (approximately 5 cm stem lengths) and added to a plastic 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, 7, 9, 12 and 16 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 glasshouse 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*.

1.3.3 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 all of the sampling times. Plants in the uninfested soil remained healthy apart from symptoms of pythium rot at the 5th and 6th assessment time. For plants in infested soil, the incidence and severity of vascular staining was reduced at 2 months after infestation. At all other sampling times the

incidence of vascular staining due to *F. oxysporum* was 85% or more (Table 2.1). The experiment demonstrated that *F. oxysporum* can remain viable on debris in soil at sufficient levels to cause disease on stocks for at least 16 months. It is generally advised not to plant asters on the same ground more than one year in seven because of the risk of soil-borne aster fusarium wilt. This suggests that *F. oxysporum* can probably survive in soil for considerably more than 16 months.

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
9	12	100	0.4	3.0
12	2	100	0.0	3.0
16	0	100	0.0	3.0

* plants could not be assessed due to pest damage

1.4 Evaluation of fungicides and alternative products for control of fusarium wilt on stocks

The objective of the experiment was to evaluate conventional fungicides and other novel products, both as pre-planting drenches and as foliar applications, for controlling fusarium wilt on stocks grown in soil where there had been a severe outbreak of fusarium wilt in the previous season.

1.4.1 Methods

Details of the drench and spray treatments applied during the experiment are shown in Table 2.2. In addition to conventional fungicides, two other products were included in the experiment, that are occasionally used by stock growers. Trianum-P contains the antagonistic fungus *Trichoderma harzianum*. Biosept Crop Gold contains a combination of plant extracts (details not disclosed).

Table 2.2. Details of drench and spray treatments applied to stocks

		Sprays						
	Drench	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
		W/c 05/06/06	W/c 12/06/06	W/c 19/06/06	W/c 26/06/06	W/c 03/07/06	W/c 10/07/06	W/c 17/07/06
1	-	-	-	-	-	-	-	-
2	Delsene 50 Flo	Expt 1	-	Expt 1	-	-	-	-
3	Delsene 50 Flo	Switch	-	Switch	-	-	-	-
4	Delsene 50 Flo	Biosept	Biosept	Biosept	-	Biosept	-	Biosept
5	Delsene 50 Flo	Trianum	-	-	-	Trianum	-	-
6	Delsene 50 Flo	Amistar	Delsene	Amistar	-	Delsene	-	Amistar
7	Trianum-P	Expt 1	-	Expt 1	-	-	-	-
8	Trianum-P	Switch	-	Switch	-	-	-	-
9	Trianum-P	Biosept	Biosept	Biosept	-	Biosept	-	Biosept
10	Trianum-P	Trianum	-	-	-	Trianum	-	-
11	Trianum-P	Amistar	Delsene	Amistar	-	Delsene	-	Amistar
12	-	-	-	-	-	-	-	-

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 done on all plants per plot. Destructive assessments were made on the central 10 plants only. 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 complete block design. Data were analysed by analysis of variance (ANOVA) in Genstat.

Based on results from soil analyses in 2005, 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 planting.

The stocks were planted in polytunnel 1 at ADAS Arthur Rickwood. The soil in the tunnel had been artificially infested with *F. oxysporum ex* stocks in June 2005, and a crop of stocks planted in 2005 developed widespread and severe symptoms of fusarium wilt (Project Annual Report, Year 2). Prior to setting up the experiment in 2006, the soil was rotovated and the pathogenicity of *F. oxysporum* in the soil to stocks was tested as follows:

Plug plants of column stocks (var. Centum Deep Blue) that had not received a fungicide drench were obtained from a commercial nursery. Soil was collected from polytunnel 1 at ten points along the sides, 1.5 m in from the edge. The soil was mixed thoroughly and divided between four seed trays. Each seed tray was planted with 20 stock plug plants. Plug plants were also sown in soil collected from another location on site (four trays of 20 plants) as an uninfested control. The trays of plants were maintained in a glasshouse and assessed typical symptoms of fusarium wilt after approximately 4 weeks.

For the main experiment, plug plants of column stocks (var. Fedora Deep Rose) that had not already received a fungicide drench were obtained from a commercial nursery. The drench treatments were applied to plug plants in trays, 1 day before planting. Delsene 50 Flo (carbendazim) was applied to plants for treatments 2-6 using an Oxford Precision Sprayer at a rate of 1.0 ml/L in a water volume of 100 ml/m^2 (based on LTAEU from SOLA 1004/04 for use of carbendazim on container-grown protected ornamentals). Trianum-P (*Trichoderma harzianum*) was applied to plants for treatments 7-11 using a watering can at a rate of 1.5 g/m^2 in 2.5 L water (rate recommendation from product leaflet).

Foliar sprays apart from Trianum-P 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. Trianum-P was applied using a watering can with a fine rose, following mixing recommendations in the product leaflet. Sprays were

applied at the timings shown in Table 2.2. Details of spray treatments are shown in Tables 2.3 and Table 2.4.

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. A data logger was used to record air temperature and soil temperature (10 cm depth) for the duration of the trial.

Table 2.3. Details of spray treatments applied to stocks

	Product	Active ingredient	Rate (in 1000 L/ha)
1	-	-	-
2	Experimental 1	-	0.5 ml/L (0.5 L/ha)
3	Switch	Cyprodonil + fludioxonil	1.0 g/L (1.0 kg/ha)
4	Biosept Crop Gold	-	2.0 ml/L (2.0 L/ha)
5	Trianum-P	*Trichoderma harzianum*	1.5 g/m^{2*}
6	Amistar	Azoxystrobin	1.0 ml/L (1.0 L/ha)
	Delsene 50 Flo	Carbendazim	1.5 ml/L (1.5 L/ha)
7	Experimental 1	Cyflufenamid	0.5 ml/L (0.5 L/ha)
8	Switch	Cyprodonil + fludioxonil	1.0 g/L (1.0 kg/ha)
9	Biosept Crop Gold	-	2.0 ml/L (2.0 L/ha)
10	Trianum-P	*Trichoderma harzianum*	1.5 g/m^{2*}
11	Amistar	Azoxystrobin	1.0 ml/L (1.0 L/ha)
	Delsene 50 Flo	Carbendazim	1.5 ml/L (1.5 L/ha)
12	-	-	-

*in 10 L water/m^2

Table 2.4. Approval status of the products applied and the basis for selecting the rates used

Fungicide	Approval	Basis for rate selected
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
Switch	AEA	Label rate for protected strawberries will be 1.0 kg product/ha, maximum of 2 sprays per crop (B. Hall, Syngenta, pers. comm.)
Biosept Crop Gold	Not marketed as a fungicidal product	Maximum label rate is 8 ml/L but there is grower and trial experience with phytotoxicity on stocks at this rate and ½ rate. Used at ¼ rate.
Trianum-P	Not marketed as a fungicidal product	Product leaflet recommends 1.5 g/m^2 for densely spaced ornamental crops and if drench has already been used in propagation
Delsene 50 Flo	LTAEU (SOLA 1004/04)	Carbendazim on protected ornamentals (soil grown) (1.5 ml/L)

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 together with spray 2 (10 days after planting. Fubol Gold + Toppel 10 were applied together with spray 4 (1 month after planting). The rates of product used are given below:

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

From two weeks after planting, the experiment was assessed weekly for the incidence of plants in each plot out of 28 with typical symptoms of fusarium wilt (e.g. wilting, pronounced vein yellowing, leaf bleaching). At assessments 3 and 6, disease severity was also assessed using the following index: 0 = no disease, 1 = up to 1/3 of plant affected, 2 = up to 2/3 of plant affected, 3 = more than 2/3 plant affected. At each assessment, the incidence of phytotoxic symptoms was also recorded. Eight weeks 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. Typical stem symptoms were plated onto PDA+S (after surface sterilising for 10 sec in 90 % ethanol), to confirm the causal organism.

1.4.2 Results and discussion

Of stock plug plants sown in soil collected from polytunnel 1, 56% developed symptoms typical of fusarium wilt, either stunting, wilting, leaf bleaching/veining, vascular staining or a combination of symptoms. None of the plants in the control soil developed these symptoms. It was concluded that the inoculum level of *F. oxysporum* in polytunnel 1 soil was sufficient to cause fusarium wilt without requiring artificial re-infestation of the soil.

Air and soil temperatures (10 cm depth) for the duration of the experiment are summarised in Appendix 2. There were no symptoms of disease except for those of fusarium wilt. Slight pest damage (leaf holes and distortion) occurred in three plots due to earwigs.

The first symptoms of fusarium wilt developed on plants in a range of treatments just 15 days after planting. At 3 and 6 weeks after planting, disease incidence and severity was significantly higher (P<0.001) in the central two blocks compared with the outer two blocks of the experiment, suggesting uneven distribution of fungal inoculum (Table 2.5). The block difference was also reflected in a significantly higher incidence of vascular staining for blocks 2 and 3 (P<0.001) (Table 2.6). There was no significant effect of foliar applications on the incidence or severity of fusarium wilt (at 3 and 6 weeks after planting). A pre-planting drench with Delsene 50 Flo gave significantly lower disease incidence (3 and 6 weeks after planting) and lower disease severity (6 weeks after planting) compared with a drench of Trianum-P, but did not improve disease control compared with the untreated controls. There was no significant effect of drenches or foliar applications on the incidence or severity of vascular staining (8 weeks after planting).

There were no beneficial or phytotoxic effects of the drench treatments or foliar applications. None of the treatments affected the height of the crop (at 8 weeks after planting) in comparison with the untreated control (data not presented).

Table 2.5. Effect of fungicide drenches and foliar application on the incidence and severity of fusarium wilt on stocks, 3 and 6 weeks after planting

	Drench	Foliar applications	Mean % plants with fusarium wilt		Mean disease severity score	
			3 weeks	6 weeks	3 weeks	6 weeks
1.	Untreated#	Untreated#	42.0	77.1	0.7	1.7
2.	Delsene 50 Flo	Expt 1	25.0	52.7	0.5	1.3
3.	Delsene 50 Flo	Switch	46.4	76.8	0.8	1.8
4.	Delsene 50 Flo	Biosept	33.2	65.0	0.6	1.4
5.	Delsene 50 Flo	Trianum	28.6	60.7	0.4	1.4
6.	Delsene 50 Flo	Amistar/Delsene 50 Flo	50.0	79.9	0.9	1.8
	Delsene 50 Flo	Mean	36.6	67.0	0.7	1.6
7.	Trianum-P	Expt 1	55.4	93.8	0.8	2.3
8.	Trianum-P	Switch	50.9	83.0	0.9	2.1
9.	Trianum-P	Biosept	50.0	77.7	0.9	1.7

10.	Trianum-P	Trianum	58.0	95.4	0.7	2.2
11.	Trianum-P	Amistar/Delsene 50 Flo	65.2	88.4	1.2	2.1
	Trianum-P	Mean	55.9	87.7	0.9	2.1
	D.f.		34	34	34	34
	S.e.d (Block)		11.1***	9.7**	0.23 ***	0.28***
	S.e.d (Drench)		8.6*	7.5**	0.18 ns	0.22*
	S.e.d (Foliar applications)		13.6 ns	11.9 ns	0.28 ns	0.34 ns

#Mean of treatments 1 and 12

* significant at *P*<0.05; ** significant at *P*<0.01; *** significant at *P*<0.001; ns non-significant

Table 2.6. Effect of fungicide drenches and foliar applications on the incidence and severity of vascular staining in stocks, 8 weeks after planting

	Drench	Foliar applications	Mean % plants with vascular staining	Mean vascular staining severity score
1.	Untreated#	Untreated#	71.3	2.1
2.	Delsene 50 Flo	Expt 1	52.5	1.5
3.	Delsene 50 Flo	Switch	65.0	1.9
4.	Delsene 50 Flo	Biosept	52.5	1.6
5.	Delsene 50 Flo	Trianum	65.0	2.0
6.	Delsene 50 Flo	Amistar/Delsene 50 Flo	67.5	1.9
	Delsene 50 Flo	Mean	60.5	1.8
7.	Trianum-P	Expt 1	82.5	2.4
8.	Trianum-P	Switch	70.0	2.0
9.	Trianum-P	Biosept	65.0	1.9
10.	Trianum-P	Trianum	75.0	2.1
11.	Trianum-P	Amistar/Delsene 50 Flo	70.0	2.1
	Trianum-P	Mean	72.5	2.1
	D.f.		34	34
	S.e.d (Block)		12.5**	0.38**
	S.e.d (Drench)		9.7 ns	0.30 ns
	S.e.d (Foliar applications)		15.3 ns	0.47 ns

#Mean of treatments 1 and 12

* significant at $P<0.05$; ** significant at $P<0.01$; *** significant at $P<0.001$; ns non-significant

The fungicide evaluation experiment was set up in a polytunnel where there had been a severe outbreak of stocks fusarium wilt in the previous season, such that inoculum levels would have been representative of a commercial situation. Due to uneven inoculum distribution, there were significant block effects on disease incidence and severity. As in previous fungicide evaluation experiments (Year 1 and Year 2 annual reports), use of fungicides either as pre-planting drenches or as foliar applications did not provide effective control of fusarium wilt on stocks. Overall, a pre-planting drench with Delsene 50 Flo gave better control of fusarium wilt than with Trianum-P but was not significantly better than the untreated control (possibly due to inoculum positional effects).

1.5 Comparison of sheet steaming and a steam plough for control of *Fusarium oxysporum* in stems and roots of stocks, and *Sclerotinia sclerotiorum*

1.5.1 Introduction

The objectives of the experiment were:

i) To determine the efficacy of steam applied to soil by two different methods (steam plough and sheet steaming) against *Fusarium oxysporum* in stems and roots of column stocks, and sclerotia of *Sclerotinia sclerotiorum*, and;

ii) To monitor soil temperature at four depths during steam treatment.

1.5.2 Methods

Sites and land preparation

The steaming methods were evaluated in glasshouses at two separate commercial cut flower nurseries in Norfolk in June 2006. Sheet steaming was done on a sandy clay loam and the steam plough treatment was done on a silt loam. For the sheet steaming, the soil was spaded to 35 cm and then steamed for 10 h (with a thermal fleece), left covered overnight then planted after 2-3 days. For the steam plough, the soil was initially spaded to between 30-35 cm. Steam was then injected at 31 cm depth and the soil covered by a 4 m sheet trailing behind the plough (giving soil coverage for approximately 20 min at any one point). Details of the general procedures used for the two steaming methods are described in Appendix 4.

Sample bag preparation

The steam treatments were tested against fusarium using woody stem pieces and roots of stocks naturally infected with *F. oxysporum* (collected from an infected crop in May 2006). Nylon gauze bags were prepared each containing ten 2-cm long stem sections mixed with sufficient moist silver sand to separate the stem pieces in the bag. Ten 2-cm root pieces were also placed in the bag, enclosed in muslin to enable easy retrieval. The gauze at the top of the bags was trimmed to give a height of approximately 5 cm. The sand in the bags was moistened prior to sample burial and soil treatment.

The steam treatments were tested against *Sclerotinia sclerotiorum* using sclerotia obtained from a flask culture of an isolate originally from lettuce. Nylon gauze bags were prepared (as for stocks stem pieces) using 20 sclerotia per bag. Bags were moistened prior to sample burial and soil treatment.

Treatments were as shown in Table 2.7, with five replicate plots per treatment. A plot comprised one nylon gauze bag containing 10 stocks stem pieces and 10 stocks root pieces (naturally infected with *F. oxysporum*) and one nylon gauze bag containing 20 sclerotia of *Sclerotinia sclerotiorum.*

Table 2.7. Steaming treatments applied to stocks stem and root pieces infected with *F. oxysporum*, and sclerotia of *S. sclerotiorum*

Treatment	Site	Soil treatment	Depth below the surface (cm)
1	1	Untreated	0-5*
2	1	Steam plough	0-5
3	1	Steam plough	10-15
4	1	Steam plough	20-25
5	1	Steam plough	30-35**
6	2	Untreated	0-5
7	2	Sheet steam	0-5
8	2	Sheet steam	10-15
9	2	Sheet steam	20-25
10	2	Sheet steam	30-35

*top of bag at 0 cm and base of bag at 5 cm
**below level of steam plough injection

For each steam treatment, a wooden 'T-frame' was used at each of five burial positions for attachment of sample bags and temperature sensors (three positions only). Pairs of labelled sample bags (one with stock stems/roots, one with sclerotia) and temperature sensors with probes horizontal) were attached to a wooden T-frame at the selected positions for burial (Appendix 3). Temperature was logged using a Delta-T DL2e field data logger and twelve ST1 thermistor soil temperature probes. Both the logger and probes were calibrated prior to use.

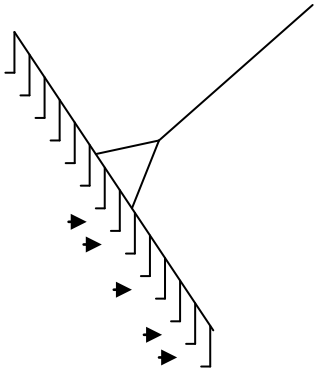
Sample burial and steam treatments

Before each steaming treatment, ten soil cores were collected per site from 0-15 cm depth to enable water content as a percent of field capacity (FC) to be determined.

For the steam plough treatment, the five replicate wooden T-frames with sample bags and temperature sensors were positioned evenly across one half of the bed width to be treated, corresponding with one half of the steam plough 'boom' and positioned mid-way between the tines of the steam plough. Temperature was

monitored at three of the five positions; just off-centre of the plough boom (rep 1), quarter way along the boom (rep 3) and close to the edge of the boom just inside the roller (rep 5). The T-frames (with bags and sensors attached) were buried in a trench prior to treatment (parallel to the direction of plough movement), approximately 10 m in front of the plough. Sensor cables were also positioned parallel to the direction of plough movement. At burial, soil was replaced firmly, but not compacted, around the T-frames. The control sample bags were buried in untreated soil (0-5 cm depth) at five separate positions in an area of 2 m^2, away from the bed being treated by steam plough.

Figure 2.1. Approximate position of five replicate sample bags in relation to steam plough



For the sheet steaming treatment, the five T-frames (with bags and sensors attached) were buried prior to treatment. At burial, the soil was replaced firmly but not compacted around the T-frames. The five replicates were positioned along the bed length at approximately 1 m (rep 1), 5 m (rep 2), 10 m (rep 3), 15 m (rep 4) and 20 m (rep 5) from the inlet pipes. Temperature sensors were used at reps 1, 3 and 5. All the replicates were positioned mid-way across the bed, between the two steam inlets. The control bags were buried in untreated soil (0-5 cm depth) in an area of 2 m^2, away from the bed being treated by sheet steaming.

For each steaming treatment, the samples were left in the ground for at least 48 h after treatment and soil temperature was monitored for the whole duration at 1 minute intervals. At the time of sample bag recovery, the position of each T-frame in

relation to the soil surface was recorded, to measure whether there had been upward or downward movement of sample bags and sensors.

Assessment of pathogen viability

Stocks stem and root pieces: After recovery from soil, the stem and root pieces were sifted from the sand and the remaining sand washed away. The stem sections were cut in half (transversely) then surface sterilised in 90% ethanol for 10 seconds. Root pieces were surface sterilised in the same way. Surface sterilised stem and root pieces were then plated onto potato dextrose agar amended with streptomycin (PDA+S) (five stem or root pieces per plate) and incubated at 20°C. The number of fusarium colonies (out of 10) present after 6-7 and 14-15 days was counted.

Sclerotia: after the samples have 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 minute 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 20) from which sclerotinia colonies developed after 4-5 and 8 days was recorded.

Statistical analyses were by generalised linear models in Genstat.

1.5.3 Results and discussion

Prior to sheet steaming, soil moisture was 37% of field capacity. The work rate was 272 m^2/10 h. For the steam plough, soil moistures was 35% and the work rate was 80 m^2/7 h.

There was no upward or downward movement of the buried wooden T-frames at either site.

For sheet steaming, temperatures increased rapidly after the start of steaming with 90°C achieved after only 0.5 h at the surface, 1 m from the inlet. The time taken to reach maximum temperature increased with both depth and distance from the inlet pipe (Figure 2.2, and Table 2.8). The only position at which the maximum

temperature did not exceed 90°C was at 33.5 cm, 20 m from the inlet. Once steaming was completed, the rate of temperature decrease, was more rapid once the covers were removed (after 22 h), particularly at the surface (Figure 2.2).

For the steam plough, maximum temperatures achieved at different depths were comparable to those obtained during sheet steaming, although the soil heating process was more rapid. Temperatures exceeded 90°C almost immediately at 23.5 cm (just above the level of steam injection) and 13.5 cm depth, and after 15 min at the soil surface. Below the level of steam injection, the soil reached a maximum temperature of 89.7°C after 15 min, but cooled rapidly (Figure 2.3). Rapid cooling also occurred at the soil surface.

Figure 2.2. Mean temperatures achieved at different depths below the soil surface during sheet steaming – Norfolk, June 2006

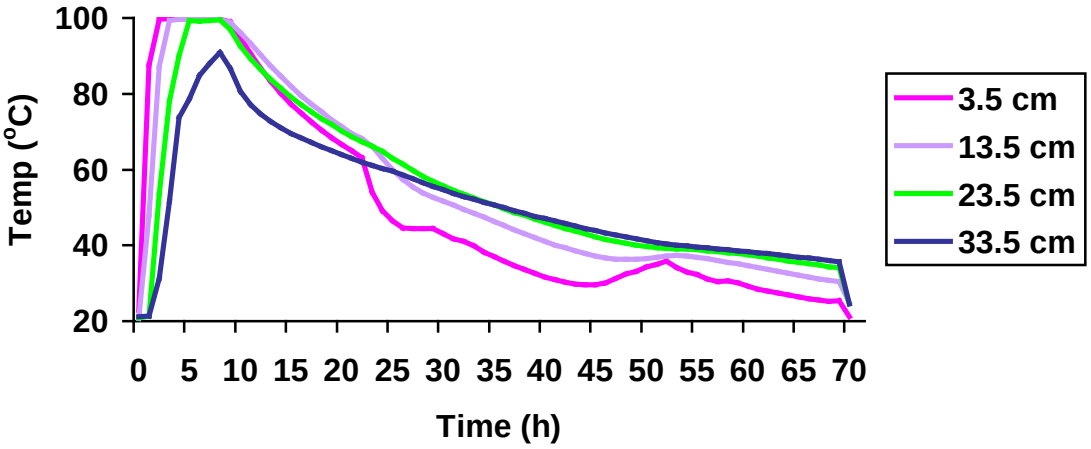
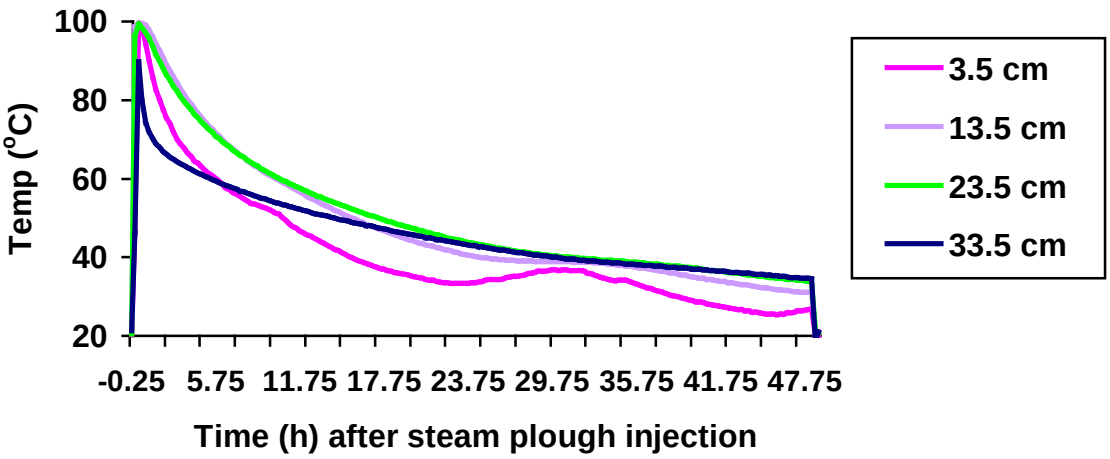


Table 2.8. Time taken to reach 90°C (after start of steaming) and maximum temperatures achieved at different depths and distances from the steam inlet

Depth (cm)	1 m from inlet pipe		10 m from inlet pipe		20 m from inlet pipe	
	Time (h) to reach 90°C	Max. temp (°C)	Time (h) to reach 90°C	Max. temp (°C)	Time (h) to reach 90°C	Max. temp (°C)
3.5	0.5	99.8	1.25	99.9	1.25	99.6
13.5	1.0	99.8	1.75	99.8	2.75	99.6
23.5	1.5	99.7	2.25	99.4	4.5	99.3
33.5	3.25	99.3	3.75	99.5	-	77.5

Figure 2.3. Mean temperature achieved at different depths below the soil surface during injection of steam by steam plough – Norfolk, June 2006.



A measure of the soil heating likely to be detrimental to survival of *F. oxysporum* was made by calculating the product of the extent and duration of temperature above 60ºC (degree-hours >60ºC). The thermal death point of many fungi in soil is reported to be around 60ºC. As expected, soil heating varied with depth and sheet steaming resulted in considerably greater soil heating (3-8 x greater) than the steam plough (Table 2.9). Heating for 31 degree – hours >60ºC (e.g. 46 min at 100ºC) was sufficient to result in 98% kill of *F. oxysporum* in stem pieces using the steam plough.

The sheet steaming treatment significantly reduced the viability of *F. oxysporum* in stocks stem and root pieces, and of sclerotia of *S. sclerotiorum* compared to the untreated control, with mean pathogen kill exceeding 93% (Table 2.10). Full control of *S. sclerotiorum* was achieved to a depth of 23.5 cm. For the sheet steam treatments, there was no significant effect of depth on treatment efficacy. The effect of distance from the steam inlet pipe could not be determined because, there was no replication at each of the five positions along the bed.

The steam plough treatment also significantly reduced the viability of the pathogens tested compared to the untreated control, with mean kill of both pathogens exceeding 95% (Table 2.11). For *F. oxysporum* in stem and root pieces, there was no effect of depth on treatment viability with pathogen kill exceeding 94% even at the surface and just below the level of steam injection. The steam plough was slightly

less effective against sclerotia of *S. sclerotiorum* at the soil surface than those buried at 13.5 cm or greater depth.

Table 2.9. Comparison of soil heating (degree x hours >60°C) using different steaming methods

Depth (cm)	Sheet steam	Steam plough
3.5	552	74
13.5	579	166
23.5	496	155
33.5	266	31

Table 2.10. Effect of sheet steaming on mean survival of *Fusarium oxysporum* in stocks stem and root pieces, and sclerotia of *Sclerotinia sclerotiorum* at different depths in soil

Soil treatment	Depth (cm)	*F. oxysporum* in stocks stem pieces		*F. oxysporum* in stocks root pieces		Sclerotia of *S. sclerotiorum*	
		% viable	% kill	% viable	% kill	% viable	% kill
Untreated	3.5	100.0	0.0	100.0	0.0	84.9	15.1
Sheet steam	3.5	6.0	94.0	6.0	94.0	0.0	100.0
Sheet steam	13.5	8.0	92.0	2.0	98.0	0.0	100.0
Sheet steam	23.5	5.5	94.5	0.0	100.0	0.0	100.0
Sheet steam	33.5	8.0	92.0	12.0	88.0	1.0	99.0
Sheet steam mean		6.9	93.1	5.0	95.0	0.25	99.75
D.f.		20		20		20	
F. prob (soil treatment)		<0.001		<0.001		<0.001	
F. prob (depth)		0.127		0.975		0.081	

Table 2.11. Effect of steam plough treatment on mean survival of *Fusarium oxysporum* in stocks stem and root pieces, and sclerotia of *Sclerotinia sclerotiorum* at different depths in soil

Soil treatment	Depth (cm)	*F. oxysporum* in stocks stem pieces		*F. oxysporum* in stocks root pieces		Sclerotia of *S. sclerotiorum*	
		% viable	% kill	% viable	% kill	% viable	% kill
Untreated	3.5	100.0	0.0	96.2	3.8	92.7	7.3
Steam plough	3.5	6.0	94.0	5.5	94.5	3.2	96.8
Steam	13.5	10.0	90.0	8.0	92.0	0.0	100.0

plough							
Steam plough	23.5	2.0	98.0	2.0	98.0	0.0	100.0
Steam plough	33.5	2.0	98.0	0.0	100.0	0.0	100.0
Steam plough mean		5.0	95.0	3.9	96.1	0.8	99.2
D.f.		20		20		20	
F. prob (soil treatment)		<0.001		<0.001		<0.001	
F. prob (depth)		0.403		0.264		0.028	

1.6 Evaluation of sheet steaming and soil amendments for control of fusarium wilt of lisianthus

1.6.1 Introduction

The objectives of the experiment were:

i) To monitor soil temperature at four depths during steam treatment, applied by sheet steaming

ii) To determine the efficacy of sheet steaming against *Fusarium oxysporum* in stems and roots of lisianthus

iii) To determine the effect of sheet steaming followed by soil amendments on the incidence of fusarium wilt in a crop of lisianthus planted in soil where fusarium wilt had been confirmed in 2006

1.6.2 Methods

Site and land preparation

The experiment was done at a commercial nursery in Suffolk in September 2006 on a medium sandy loam soil. Prior to steaming for objectives i) and ii), the soil was spaded to 35 cm depth without a crumbler bar.

Sample bag preparation

The efficacy of sheet steaming was tested against fusarium using woody stem pieces and roots of lisianthus naturally infected with *F. oxysporum* (collected from an infected crop in 2005). The viability of *F. oxysporum* in the stem and root pieces was confirmed in a preliminary experiment (data not presented). Nylon gauze bags were prepared, each containing ten 2-cm long stem sections mixed with sufficient moist silver sand to separate the stem pieces in the bag. Ten 2-cm root pieces were also

placed in the bag, enclosed in muslin to enable easy retrieval. The gauze at the top of the bags was trimmed to give a height of approximately 5 cm. The sand in the bags was moistened prior to sample burial and soil treatment.

Treatments were as shown in Table 2.12, with six replicate plots per treatment. A plot comprised one nylon gauze bag containing 10 lisianthus stem pieces and 10 lisianthus root pieces (naturally infected with *F. oxysporum*).

Table 2.12. Steaming treatments applied to lisianthus stem pieces and roots buried at different depths

	Soil treatment	Depth below the surface (cm)
1	Untreated	0-5*
2	Sheet steam	0-5
3	Sheet steam	10-15
4	Sheet steam	20-25
5	Sheet steam	30-35

*top of bag at 0 cm and base of bag at 5 cm

For the steam treatment on each of two beds, a wooden 'T-frame' was used at each of three burial positions for attachment of labelled sample bags and temperature sensors (probes horizontal) at the selected positions (see Appendix 3). Temperature was logged using a Delta-T DL2e field data logger and twelve ST1 thermistor soil temperature probes. Both the logger and probes were calibrated prior to use.

Sample burial and steam treatments

Before each steaming treatment, ten soil cores were collected from 0-15 cm depth to enable water content as a percent of field capacity (FC) to be determined.

For sheet steaming, the T-frames (with bags and sensors attached) were buried prior to treatment. At burial, soil was replaced firmly (but not compacted) around the T-frames. Three replicate plots were positioned at 1 m, 10 m and 20 m from the steam inlets on one bed. The remaining three replicate plots were positioned at 20 m, 30 m and 38.5 m from the steam inlet on an adjacent bed. The two separate beds were steamed on separate occasions and temperature sensors were used at each of the six positions. Replicate plots were split across the two beds because of limited cable length on the data logger. The bags and temperature sensors were positioned mid-

way across each bed, between the two steam inlets. The soil was steamed for 12 h then left covered overnight; a thermal fleece was used over the steaming sheet.

For the steaming treatments on beds 1 and 2, the samples were left in the ground for at least 48 h after treatment. On bed 1, the samples were left in the ground and temperatures logged (at 1 minute intervals) for 46 hours. On bed 2, the logging period was stopped after 26 hours because the required temperatures were not being reached during the steaming process.

Assessment of pathogen viability

After recovery from soil, the lisianthus stem and root pieces were sifted from the sand and the remaining sand washed away. The stem sections were cut in half (transversely) then surface sterilised in 90% ethanol for 10 seconds. Root pieces were surface sterilised in the same way. Surface sterilised stem and root pieces were then plated onto potato dextrose agar amended with streptomycin (PDA+S) (five stem or root pieces per plate) and incubated at 20^{o}C. The number of fusarium colonies (out of 10) present after 7 and 14 days was counted.

Statistical analyses were by generalised linear models in Genstat.

Soil amendments

For objective iii), each of the treatments listed in Table 2.13 was evaluated in an area of the nursery known to have had lisianthus fusarium in 2006, and which was to be steamed and replanted with a lisianthus variety known to be susceptible to fusarium (Palermo yellow). The standard nursery practice was to drench plants with Trianum-P prior to planting followed by carbendazim (up to six applications at full rate) in overhead spray lines. However, plants used for this experiment were not drenched or sprayed.

After a 12 h steam, the covers were left on overnight. The grower applied overhead irrigation to moisten the soil and cultivated with a Roterra to a depth of approximately 10 cm, followed by soil flattening with a roller. After cultivation, the experiment was marked out in a randomised block design, using the four central beds (in a bay of six beds) as the four blocks. Each plot was 2 m x 1.2 m . Treatments

1-6 were applied as in Table 2.13, 2 days prior to planting. Each treatment was incorporated to 5 cm depth using a rake. Trickle irrigation tapes were laid (three per bed) followed by planting mesh. Each gap in the mesh was planted with three plants in a triangle formation giving approximately 90 plants per m^2. For each plot, the mesh was 7 gaps wide by 10 gaps long. Only plants in the central 8 x 5 gaps were assessed (approximately 120 plants), with plants in the outer gaps used as guards.

To prepare inoculum of Fo47 (isolate of non-pathogenic *F. oxysporum*) for treatment 6, an isolate was sub-cultured by streaking onto 20 plates of PDA+S. Irish moss peat was autoclaved in four bags (2.5 kg in each) sealed with masking tape (2 x 55 min). The bags of compost were left on a laboratory bench to cool overnight. Sporulating plates of Fo47 on PDA+S were used to make a spore suspension in SDW. Each bag of peat was inoculated with 100 ml spore suspension (1 x 10^7 spores/ml). A further 900 ml of SDW was added to each bag. The bags were resealed with masking tape and shaken to mix the soil, then incubated at 18-25^{o}C. The bags were shaken after 4 days. The peat was used as a soil amendment 14 days after inoculation.

As *F. oxysporum* from lisianthus is known to be seed-borne, 20 plug plants from the same batch that was being planted were tested in the laboratory. For each plant, five stem base pieces and five root pieces were plated on PDA+S (after 10 sec surface sterilisation in 90% ethanol). The plates were incubated at 20^{o}C and checked for the development of *F. oxysporum* after 7 and 14 days.

Table 2.13. Soil amendment treatments incorporated post-steaming

	Treatment	Product rate	Time of application
1	Steaming only	-	-
2	Steaming followed by incorporation of Gliomix (*Gliocladium catenulatum*)	20 g per m^2 (suspended in 2 L water)	Pre-planting
3	Steaming followed by incorporation of Trianum-P (*Trichoderma harzianum*)	3 g per m^2 (suspended in 1 L water)	Pre-planting
4	Steaming followed by incorporation of Agralan Revive	0.5 ml (in 100 ml/m^2)	Pre-planting

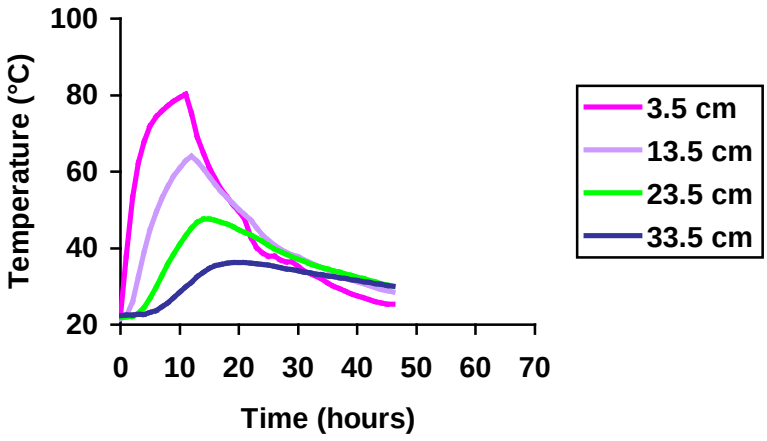
5	Steaming followed by incorporation of Microgran (calcium cyanamide)	100 g/m^2	Pre-planting
6	Steaming followed by incorporation of Fo47 (non-pathogenic *F. oxysporum*)	See text	Pre-planting
7	Steaming followed by drenches with carbendazim (Delsene 50 Flo)	1.5 ml/L (in 100 ml/m^2)	• 3 days after planting • 2 weeks after planting • 6 weeks after planting

The number of plants per plot with symptoms of fusarium wilt and other diseases was assessed at five sampling times. Representative symptoms on the stem base were plated onto PDA+S (after 10 sec surface sterilisation in 90% ethanol) to determine the causal organism.

1.6.3 Results and discussion

Prior to sheet steaming, soil moisture was 44% of field capacity. During steaming, the maximum temperature achieved near the soil surface (3.5 cm depth) on bed 1 was approximately 80°C and this decreased rapidly after 12 h when steaming was complete (Figure 2.4). At 13.5 cm depth, temperature only just exceeded 60°C. Comparable results were obtained for bed 2 (data not shown).

Figure 2.4. Mean temperatures achieved at different depths below the soil surface during soil steaming (lisianthus), bed 1



Although sheet steaming significantly reduced the viability of *F. oxysporum* in stem and root pieces, the percentage pathogen kill was low (30% in stems, 69% in roots) compared with the previous sheet steaming experiment (Table 2.14). There was also a significant effect of sample burial depth on steaming efficacy with the highest pathogen kill occurring at 13.5 cm depth. Results at 33.5 cm depth were comparable to the untreated control. The poor results for pathogen kill corresponded with the fact that high temperatures were not sustained during the steaming process.

Table 2.14. Effect of sheet steaming on mean survival of *F. oxysporum* in lisianthus stem and root pieces at different depths in soil

Soil treatment	Depth (cm)	*F. oxysporum* in stem pieces		*F. oxysporum* in root pieces	
		% viable	% kill	% viable	% kill
Untreated	3.5	100.0	0.0	80.0	20.0
Sheet steam	3.5	70.0	30.0	15.0	85.0
Sheet steam	13.5	33.3	66.7	3.3	96.7
Sheet steam	23.5	76.7	23.3	33.3	66.7
Sheet steam	33.5	98.3	1.7	73.8	26.2
Sheet steam mean		69.6	30.4	31.3	68.7
D.f.		25		25	
F. prob (soil treatment)		0.001		0.003	
F. prob (depth)		<0.001		0.002	

The failure of the soil temperature to rise above 80ºC even at 3.5 cm depth and after steaming for 12 h indicates poor penetration of steam into the soil. The reason for this was not identified but possible factors include:

- Steaming over too large an area relative to the steam capacity (i.e. too low a pressure) (400 m^2 was treated using a Wilkie S950 steam generator with a steam capacity of 1,000 kg/h). The grower reported that if he steamed a smaller area, steam production had to be reduced or there were frequent 'blow-outs' at the edge of the steaming sheet (which was weighed down with bags of stone). In the sheet steaming experiment in Norfolk in June 2006, where a soil temperature of 100ºC was achieved to 23.5 cm depth, the steam generator was capable of producing 2,000 kg of steam/h at 4 Bar pressure, and an area of 272 m^2 was treated.
- The soil tilth was too fine, or had a high silt content, and consequently the soil surface 'capped' shortly after steaming started, preventing effective downward movement of the stem. Although the soil was observed to have been cultivated to a relatively fine tilth prior to steaming, no cap was observed after steaming.
- The soil was not at the optimum moisture content for efficient energy transfer. Italian research indicates that a sandy loam soil (10% clay, 57% sand, 33% loam)

transfers steam heat most efficiently at 33-50% FC (8-12% water on a dry soil), and a sandy soil at 50-60% FC. The soil at our experimental site was described as a sandy loam (percentage composition not known) and was at 44% FC when steamed. It was therefore probably close to the optimum moisture content for efficient sheet steaming.

- A soil pan may have prevented steam penetration. No pan was found when holes were dug to 35 cm depth in order to bury samples and temperature sensors. This seems unlikely to be the explanation.

In the soil amendment experiment, lisianthus plants in plots treated with Microgran had severe symptoms of phytotoxicity at 12 days after planting. The supplier confirmed that this would have been due to toxicity from product breakdown. These plots were re-planted with lisianthus 2 weeks after soil treatment.

No *F. oxysporum* was isolated from the roots or stems of lisianthus plants sampled from the plant batch used for the soil amendment experiment. New plants used to re-plant the Microgran plots were also tested and no *F. oxysporum* was recovered. This result indicates seed-borne infection, if present in these plant batches, was at an incidence less than 5%.

The first symptoms of fusarium wilt were observed by the grower 3 weeks after planting. From a disease assessment in December (approximately 3 months after planting), there were no significant effects of treatment on the cumulative total of plants with fusarium wilt (Table 2.15). In the last four weeks of production, the incidence of fusarium wilt increased at least three-fold for all treatments. Just prior to harvest, there were no significant treatment effects on the incidence of fusarium wilt. Despite phytotoxicity symptoms, there was a trend for a lower disease incidence following Microgran treatment. However, plants assessed in these plots were two weeks younger than those in other plots (because of replanting), such that direct comparison may not be appropriate. None of the treatments increased the number of marketable plants at harvest, in comparison with the steam only control (Table 2.16).

Table 2.15. Effect of steaming and soil amendment treatments on the cumulative incidence of lisianthus fusarium wilt

Soil treatment	December 2006		January 2007	
	Mean number plants with fusarium wilt	Approx. % incidence fusarium wilt*	Mean number plants with fusarium wilt	Approx. % incidence fusarium wilt*
Steaming only	9.5	7.9	30.8	25.6
Steaming + Gliomix	7.3	6.0	22.3	18.5
Steaming + Trianum-P	5.8	4.8	27.0	22.5
Steaming + Agralan Revive	5.5	4.6	29.8	24.8
Steaming + Microgran	6.3	5.3	20.0	16.7
Steaming + Fo47	11.5	9.6	30.5	25.4
Steaming + carbendazim	6.7	5.6	26.7	22.2
D.f.	16		16	
P	0.125		0.193	
S.e.d	5.0		13.5	

*Based on 120 plants per plot (excluding guard area)

Table 2.16. Effect of steaming and soil amendment treatments on the number of unmarketable lisianthus plants at harvest

Soil treatment	Mean number of unmarketable plants	Approx. % marketable plants*
Steaming only	25.2	79.0
Steaming + Gliomix	25.7	78.6
Steaming + Trianum-P	22.5	81.3
Steaming + Agralan Revive	21.5	82.1
Steaming + Microgran	48.6	59.5
Steaming + Fo47	31.2	74.0
Steaming + carbendazim	17.7	85.3
D.f.	15	
P	0.011	
S.e.d	7.0	

*Based on 120 plants per plot (excluding guard area)

1.7 Evaluation of vacuum steaming

1.7.1 Introduction

In an attempt to increase the effectiveness of sheet steaming in raising soil temperatures, the grower at the Suffolk site (Section 2.6) installed equipment for 'vacuum steaming' in one bay of a glasshouse. In this technique, also known as negative pressure steaming, steam is forced under a steaming sheet as normal and pulled down into the soil by a negative pressure created in the soil by a pump which sucks air out of the soil through buried perforated plastic pipes. The tubes have a porosity of around 12 cm^2 per m length of tube and are wrapped in fibre to prevent blockage of the perforations by soil. This system was introduced in the Netherlands in 1981 and is reported to be used there widely (Runia, 2000). It was found to be considerably more effective than conventional sheet steaming in raising soil temperatures (Runia, 2000). It was also suitable for all soil types whereas sheet steaming worked best on soils with a high clay content.

1.7.2 Methods

Slit-perforated plastic pipes (60 mm diameter), covered with woven plastic mesh, were buried 1.6 m apart at 60 cm depth along the length of beds with the open ends emerging just outside the glasshouse. A suction was applied to the open ends using a small pump (100W Martin Lishman Model FTDS).

The soil temperature was monitored at four depths (3.5, 13.5, 23.5 and 33.5 cm) using sensors attached to a wooden frame and connected to a Delta-T logger as described previously (Section 2.6). Two sets of sensors were inserted directly over a buried plastic suction pipe, at 5 and 12 m distance from the steam inlet at the end of the beds. A third set of sensors was inserted at 12 m distance from the steam inlet and midway between two buried suction pipes. An area of 125 m^2 was steamed for 4 h using a mobile steam generator (Wilkie S950 with a steaming capacity of 1,000 kg/h). The steaming sheet and thermal fleece were left on for 24 h. The steam generator was turned off after 4 h.

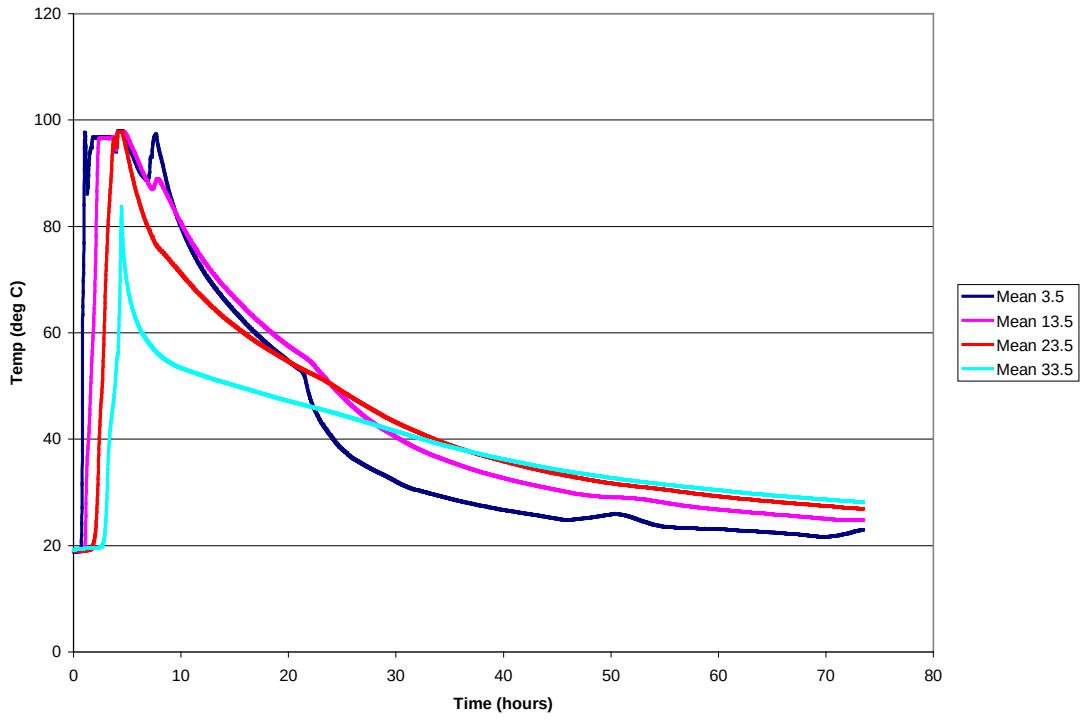
1.7.3 Results and discussion

With the vacuum applied, sheet steaming resulted in soil temperatures in excess of 95ºC to 33.5 cm depth in less that 4 hours (Fig 2.5). This was using the same steam generator and was on the same soil type as the earlier experiment (Section 2.6)

where the maximum temperature achieved, after 12 h, was 80°C at 3.5 cm depth. These data are consistent with the findings of Runia (2000) in The Netherlands, who reported that vacuum steaming was more effective at heating soil than conventional sheet steaming. The vacuum experiment differed from the earlier experiment in that a smaller area was steamed (125 m^2 rather than 400 m^2), and the soil was slightly drier (37% FC at vacuum steaming, 44% FC at conventional steaming). This result suggests that in the initial experiment, using conventional sheet steaming, there was insufficient steam pressure to force the steam through the soil.

The cost for installation of perforated plastic pipe for vacuum steaming is around £4.70/m length, or £27,000/ha assuming a pipe spacing of 1.6 m. The cost of a suitable suction pump is around £100. These costs need to be set against the reduction in time required to achieve a soil temperature >95ºC to 33 cm depth compared with conventional sheet steaming (i.e. a fuel saving), and the increased output of marketable flowers due to greater control of soil-borne fungal pathogens such as *F. oxysporum*. In The Netherlands it was estimated that the fuel consumption for vacuum steaming was around 57% of that used in sheet steaming (Runia, 2000).

Figure 2.5 Mean temperatures achieved at different depths during vacuum steaming (Suffolk, 2006)



1.8 Overall project conclusions

1.8.1 Occurrence of fusarium wilt

Fusarium wilt has severely affected several crops of stocks each year since it was first identified in 2003 and the disease remains a threat to profitable crop production. Various pre-plant soil disinfestation treatments will significantly reduce the risk of infection from the soil, but soil treatment does not guarantee freedom from the disease.

1.8.2 Survival of F. oxysporum *in soil*

In soil artificially infested with stocks debris, *F. oxysporum* survived for at least 16 months at levels sufficient to cause typical wilt symptoms on young stocks plants.

1.8.3 Occurrence of F. oxysporum on seed

F. oxysporum was recovered from 7 out of 4,400 seeds tested; the greatest level of infection recorded on a single variety/colour was 1%. The isolate of *F. oxysporum* obtained from seed in this experiment caused wilting and plant death when used to inoculate young stock plants. This experiment confirmed that *F. oxysporum* pathogenic to stocks can survive on stocks seed.

1.8.4 Effect of F. oxysporum inoculum level on occurrence of wilt

Infection and symptom development due to *F. oxysporum* occurred on plug stock plants at inoculum levels as low as 0.3 spores/g soil, however symptom development was more consistent at inoculum levels of 1000 spores/g soil, or greater.

When plug plants were inoculated by dipping roots into a suspension of *F. oxysporum* spores for 5 minutes, all concentrations tested (from 10 to 1,000,000 spores/ml) resulted in fusarium wilt. The highest concentration gave more consistent infection (approximately 50% plants).

1.8.5 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. From cross-pathogenicity studies in 2004 and 2005,

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.

1.8.6 Effect of temperature on growth of F. oxysporum

The effect of temperature on the growth of three isolates of *F. oxysporum* obtained from stocks was examined. The highest rate of mycelial growth (8-10 mm / day) occurred at 23ºC for one isolate and 25ºC for the other two isolates. Growth was above 2 mm/day at temperatures between 10 and 30ºC; there was very little growth at 5 or 35ºC. These results supported grower observations that the development of fusarium wilt is favoured by high temperatures.

1.8.7 Disinfectants

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.

1.8.8 Fungicides

In 2004, five fungicides were evaluated for their ability to control fusarium wilt in a short-term trial on plug plants. Internal stem browning was greatest in plants inoculated with *F. oxysporum* that remained untreated with fungicide. Bavistin DF, Amistar and Stroby WG were most effective at reducing fusarium wilt.

In 2005, ten fungicide programmes were evaluated for their ability to control fusarium wilt in a crop of stocks grown on artificially infested soil. There was a trend for a lower incidence of internal stem symptoms in plants treated with a Delsene 50 Flo (carbendazim) drench followed by Delsene 50 Flo, Amistar, Octave or an experimental product. In 2006, a pre-planting drench using carbendazim (as Delsene 50 Flo) was more effective for reducing the incidence and severity of

fusarium wilt, compared with a drench using Trianum-P (Fig 1.2). These fungicide evaluation experiments demonstrated that for a soil-borne vascular pathogen where the fungus infects the vascular system within the roots and stem, the disease 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.

1.8.9 Soil disinfestation - chemical

A series of experiments was done to determine the effectiveness of soil treatments in eradicating *F. oxysporum* from naturally infected woody stem pieces buried at different depths. Experiments were on different sites but a similar test procedure was used throughout.

In November 2004, methyl bromide applied at 50 g/m^2 beneath VIF resulted in over 80% kill of *F. oxysporum* in naturally infected woody stem pieces buried at 0, 15, 30 and 45 cm. There was no fall-off in efficiency with depth. Even at 45 cm depth, there were some replicate bags of stem pieces where no fusarium survived.

Alternative chemical treatments were evaluated. These were Basamid, Formalin, K & S Chlorofume applied via drip-line irrigation (HDC project PC 249) and Discovery. 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. Soil treatment with K & S Chlorofume applied via drip-line irrigation showed potential for broad spectrum soil disinfestation, significantly reducing inoculum levels of the soil-borne pathogens *F. oxysporum, R. solani* and *S. sclerotiorum*. Discovery gave moderate control at the soil surface but was less effective at depth. Other treatments evaluated were Microgran (calcium cyanamide) and biological soil disinfestation (see HNS137 for treatment details) neither of which reduced the incidence of *F. oxysporum* in stocks stem pieces.

1.8.10 Soil disinfestation - steaming

Steaming treatments (sheet steaming, vacuum steaming and injection of steam by a steam plough) were also evaluated. Excellent control of both *F. oxysporum* and *S.*

sclerotiorum were obtained using either sheet steaming or a steam plough in June 2006, but in other sheet steaming experiments treatment was less effective, due possibly to soil type, insufficient preparatory cultivation or soil moisture (too wet or too dry), or other factors. Vacuum steaming proved very effective in heating soil on a nursery when conventional sheet steaming for 12 h failed to raise soil temperature above 80°C.

1.8.11 Soil disinfestation – cultivation and amendments

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.

The effect of incorporating soil amendments after sheet steaming on the incidence of fusarium wilt in a crop of lisianthus was examined in September 2006. None of the soil amendment treatments (Agralan Revive, Gliomix, Microgran, Trianum-P and a non-pathogenic fusarium) or post-planting fungicide treatments (three drenches of Delsene 50 Flo) significantly reduced the cumulative incidence of fusarium wilt, compared with a sheet steaming only control.

1.9 Technology transfer (March – December 2006)

- Telephone advice to several growers on soil disinfestation methods (Tim O'Neill)
- 'Fusarium wilt of stocks – Research update 2006'. Presentation to South Holland Growers' Club, Spalding, 20 November 2006 (Tim O'Neill)
- O'Neill T & Green K. (2006). Fighting fusarium. *HDC News*, **122**: 28-30
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1.11 Acknowledgements

The assistance of the following is gratefully acknowledged:

- Growers who provided trial sites
- Ball Holland for plug plants
- Suppliers of fungicides and other products
- Claude Alabouvette (INRA, France) for isolate Fo47 of *F. oxysporum*
- Chris Dyer (ADAS) for assistance with statistical analyses
- David Stokes (Horticultural Consultant) for advice on crop production

Please note: Since the time of writing this report the approval status of K & S Chlorofume (chloropicrin) has changed and it is no longer approved for use under protection.

Regular changes occur in the approval status of pesticides, arising from changes in pesticide legislation or for other reasons. For the most up to date information, please check with your professional supplier, BASIS registered adviser or with the Information Section at the Pesticides Safety Directorate (PSD) (Telephone 01904 455775; email information@psd.gsi.gov.uk; website www.pesticides.gov.uk).

3. APPENDIX 1. EXPERIMENT DIARIES

Survival of *F. oxysporum* in soil

Date	Trial Diary
13 July 2005	The two soil treatments were prepared (approx. 50 kg of soil + fusarium infected stocks stem material and 50 kg of soil only)
13 July 2005	T0 was set up using the newly prepared soil treatments and set out in polytunnel 2.The two bags of soil treatments were stored in glasshouse 4.
26 July 2005	T0 trays of plants were moved into the glasshouse boiler room.
24 August 2005	Destructive harvest of T0 plants.
30 August 2005	T1 was set up using the two soil treatments. The trays of plants were put into glasshouse 1.
9 September 2005	First assessment of T1 plants.
15 September 2005	Second assessment of T1 plants.T2 was set up using the two soil treatments. The trays of plants were put into glasshouse 1.
23 September 2005	Third assessment of T1 plants. First assessment of T2 plants.
3 October 2005	Fourth assessment of T1 plants. Second assessment of T2 plants.
10 October 2005	T1 and T2 plants were put into glasshouse 3.
12 October 2005	T3 was set up using the two soil treatments. The trays of plants were put into glasshouse 3.
14 October 2005	Destructive harvest of T1 plants.
26 October 2005	First assessment of T3 plants.
27 October 2005	Destructive harvest of T2 plants.
11 November 2005	Second assessment of T3 plants.
01 December 2005	Destructive harvest of T3 plants.
07 February 2006	T4 was set up using the two soil treatments. The trays of plants were put into glasshouse 3.
21 February 2006	First assessment of T4 plants.
07 March 2006	Second assessment of T4 plants.
23 March 2006	Destructive harvest of T4 plants.
11 April 2006	T5 was set up using the two soil treatments. The trays of plants were put into glasshouse 3.
26 April 2006	First assessment of T5 plants.
10 May 2006	Second assessment of T5 plants.
23 May 2006	Destructive harvest of T5 plants.
14 July 2006	T6 was set up using the two soil treatments. The trays of plants were put into glasshouse 3.
28 July 2006	First assessment of T6 plants.
15 August 2006	Second assessment of T6 plants.
05 September 2006	Destructive harvest of T6 plants.
17 November 2006	T7 set up using the two soil treatments. The trays of plants were put into glasshouse 3.
28 November 2006	First assessment of T7 plants.
12 December 2006	Second assessment of T7 plants.
19 December 2006	Destructive harvest of T7 plants.

Fungicide experiment

Date	Trial Diary
02 May 2006	Polytunnel 1 soil tested for level of fusarium infection.
19 May 2006	Nitraprill (34.5% N) and sulphate of potash spread and rotovated into soil.
30 May 2006	Test plants planted in polytunnel 1 soil assessed for fusarium.
05 June 2006	All plants collected from Wests' Nursery. T2-T6 plants drenched with Delsene 50 Flo. T7-T11 plants drenched with Trianum P.
06 June 2006	Trial planted in polytunnel 1. Trial overhead irrigated and logger switched on.
08 June 2006	Fungicide spray 1 applied.
16 June 2006	Fungicide spray 2 applied. Fubol gold and aphox applied to whole trial area.
21 June 2006	First symptoms noted.
22 June 2006	Fungicide spray 3 applied.
23 June 2006	Disease assessment 1 completed.
28 June 2006	Visit by David Stokes.
29 June 2006	Disease assessment 2 completed.
06 July 2006	Fungicide spray 4 applied. Fubol gold and Toppel 10 applied to whole trial area.
07 July 2006	Disease assessment 3 completed.
13 July 2006	Disease assessment 4 completed.
21 July 2006	Disease assessment 5 completed. Fungicide spray 5 applied.
01 August 2006	Disease assessment 6 completed. Plant height assessment.
02 August 2006	Final harvest assessment.

Comparison of sheet steaming and steam plough

Date	Trial Diary
11 June 2006	Test sample bags of Fusarium-infected stock stems/roots and Sclerotinia sclerotia completed.
14 June 2006	Steam plough experiment: • First test run of steam plough on prepared glasshouse bay. • Soil prepared with a tractor-driven spader to 31 cm depth. • Test bags/soil temperature sensors buried on T-frames between steam injection tines just prior to steaming. • Soil sample taken to determine soil moisture. • Problems with temperature logging during steaming
16 June 2006	Test bags lifted.
17 June 2006	Checked new configuration and recalibrated sensors.
20 June 2006	• Re-run of steam plough. Test T-frames buried as before (minus test bags). • Steaming started at 08.25 am. Timed plough rate was 255 cm over 15 mins = 10.2 m/h or 33.5 ft/h.
21 June 2006	Steam Plough received into laboratory and rep 1-4 and control bags processed.
22 June 2006	• Steam Plough rep 5 bags processed. • Soil temperature sensor T-frames lifted and data downloaded. • Cleaned T-frames and cables. • Re-loaded T-frames with fresh sample bags ready for sheet steaming experiment.
23 June 2006	Sheet steaming experiment: • Soil prepared with a tractor-driven spader to 31 cm. depth. • Soil slightly damper than at Needhams. Soil sample taken to determine soil moisture. • Test bags/soil temperature sensors buried on T-frames, positioned down the centre of the prepared bay just prior to steaming. • T-frames positioned at 1m in front of steam inlets, 5m, 10m, 15m and 20m from steam inlets. • Steaming started at 10.30 am and finished at 18.30 pm
24 June 2006	Sheets removed at 09.00 am
26 June 2006	• Soil temperature sensor T-frames lifted and data downloaded. • Cleaned T-frames and cables. • Steam Plough 4 day assessment of sclerotia plates.
27 June 2006	Sheet steam bags received into laboratory.
28 June 2006	Steam Plough 6 day assessment of stocks stem and root plates.
29 June 2006	Sheet steam rep1-4 and control bags processed.
30 June 2006	• Steam Plough 8 day assessment of sclerotia plates. • Sheet steam rep 5 bags processed.
03 July 2006	Sheet steam 4 day assessment of sclerotia plates.
04 July 2006	Sheet steam 5 day assessment of sclerotia plates
06 July 2006	• Field capacity of soil samples calculated. • Steam Plough 14 day assessment of stocks stem and root plates. • Sheet steam 7 day assessment of stocks stem and root plates.
07 July 2006	Sheet steam 8 day assessment of sclerotia plates.

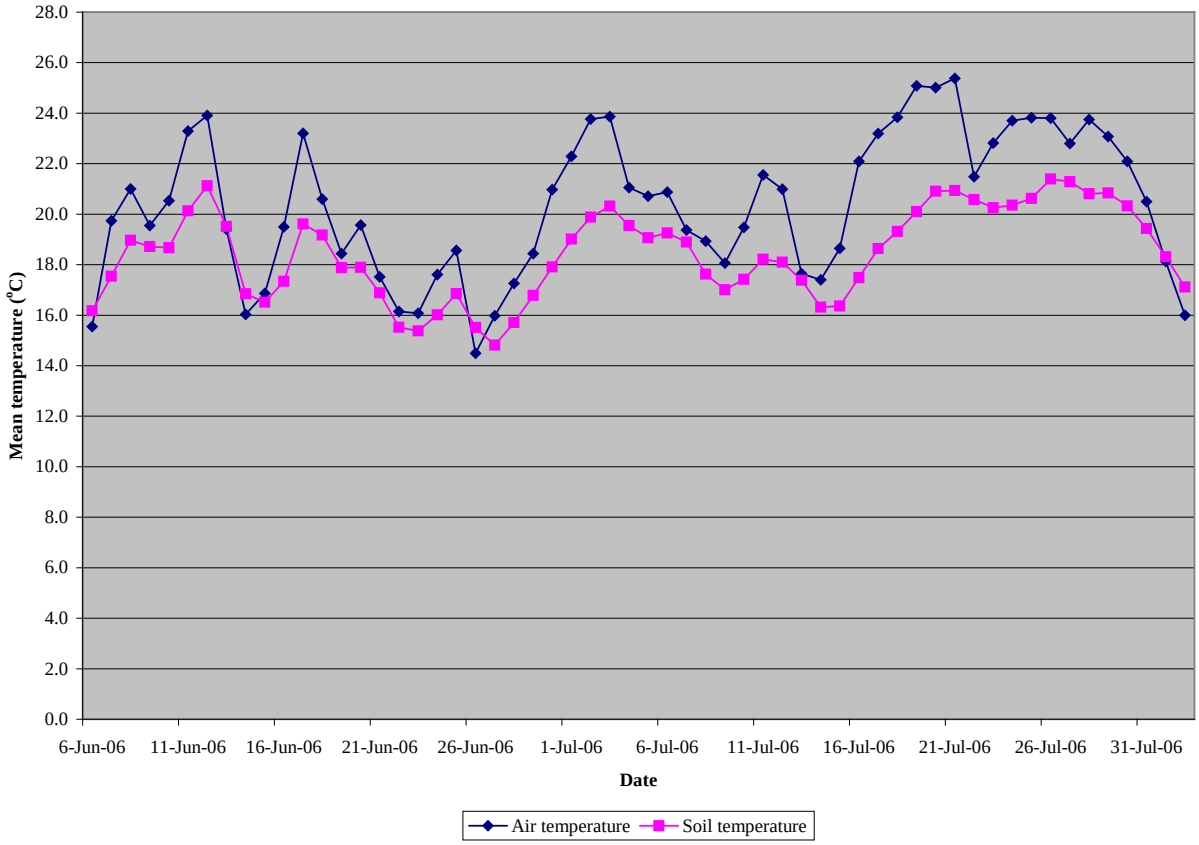
14 July 2006	Sheet steam 15 day assessment of stocks stem and root plates.

Evaluation of sheet steaming and soil amendments for control of fusarium wilt on lisianthus

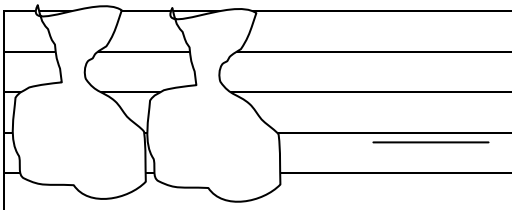
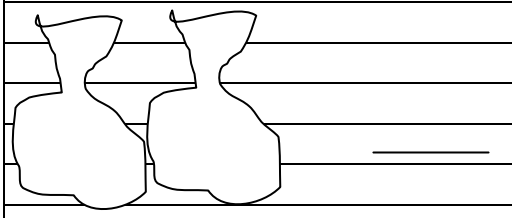
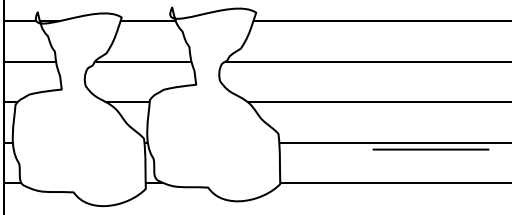
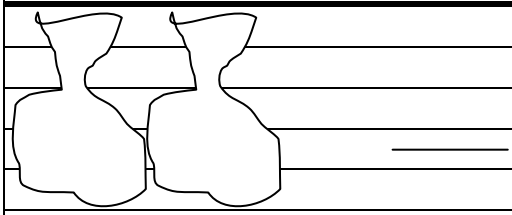
Date	Trial Diary
29 August 2006	Prior to sheet steaming, irrigation applied to Bay 1 and 2 for 1 h via overhead sprinklers
30 August 2006	Bags of Irish moss peat autoclaved.
31 August 2006	Bags of Irish moss peat inoculated with isolate Fo47-1 and Fo47-2.
10 September 2006	Bay 1 spaded to a depth of between 30-35 cm.
11 September 2006	• Sheet steaming in bay 1. • Test bags/soil temperature sensors buried on T-frames, positioned down the centre of the prepared bay just prior to steaming. Control samples (3) buried @ 5cm in adjacent bay. • Soil sample taken for FC determination. • T-frames positioned at 1 m in front of steam inlets, 10 m and 20 m from steam inlets. Bay 1 sheeted and fleeced. • Steam boiler switched on at 09.45. Total inflation of sheets and vents closed by 10.00. • Steam switched off at 21.00.
12 September 2006	Sheets removed. Bay 2 spaded to a depth of between 30-35 cm.
13 September 2006	• Logger data downloaded from sheet steaming in bay 1. T-frames lifted from bay 1 and samples bagged for testing. Control samples lifted from bay 1 and bagged for testing. • Fresh samples loaded onto T-frames and buried at 20m, 30m and 38.5m from the steam inlets in Bay 2. Bay 2 sheeted and fleeced. Control samples (3) buried @ 5cm in adjacent bay. • Steam boiler switched on at 09.50. Steam introduced at 10.10. Total inflation of sheets and vents closed by 10.30. Steam switched off at 21.30 approximately . • Trial area marked out in Bay 1 and all pre-planting treatments applied and incorporated as per protocol (later established that treatment 5 (Microgran) had been applied incorrectly to plot 5 and not plot 7 as per plan).
14 September 2006	• Fleeces and sheets removed from bay 2 at 07.30. Logger data downloaded from sheet steaming in bay 2 at 12.00 after discussion with Kim Green. • T-frames lifted from bay 2 and samples bagged for testing. Control samples lifted from bay 1 and bagged for testing. • Lisianthus stem and root pieces (Bay 1 plus controls) processed
15 September 2006	• Trial plots planted by grower with lisianthus plug seedlings cv. Palermo yellow • Lisianthus stem and root pieces (Bay 2 plus controls) processed
18 September 2006	20 lisianthus seedlings arrived in post (1st set)

19 September 2006	20 lisianthus seedlings processed (1st set)
21 September 2006	Lisianthus stem and root pieces assessed (at approx. 7 days).
25 September 2006	• 6 day assessment of lisianthus seedlings (1st set). • All microgran treated plots (including incorrectly treated plot 5) are showing plant death due to nitrogen scorch
26 September 2006	Lisianthus stem and root pieces assessed (at approx. 12 days).
Date	Trial Diary (continued)
27 September 2006	Grower replanted plots 5, 10, 20 and 23 (phytotoxic effects) with fresh plug plants after light raking. Grower to send a sample of fresh plants for testing. Confirmed with grower that only 3 of the 4 replicate plots will receive further Delsene Flo sprays (plot 5 omitted).
28 September 2006	Another 20 lisianthus seedlings arrive in the post and are processed (2nd set).
29 September 2006	10 day assessment of lisianthus seedlings (1st set).
05 October 2006	7 day assessment of lisianthus seedlings (2nd set).
06 October 2006	Fusarium symptoms observed by grower. Numbers affected per plot counted and removed.
11 October 2006	Field capacity of soil sample calculated.
19 October 2006	Trial assessed and plants with symptoms of fusarium wilt removed
20 October 2006	Plant tissue from lisianthus checked for fusarium.
11 November 2006	Grower recorded number of affected plants per plot and removed them.
07 December 2006	Trial assessed and plants with symptoms of fusarium wilt removed.
08 December 2006	All removed plants checked for fusarium and pythium.
03 January 2006	Pre-harvest assessment. Removed plants checked for fusarium and pythium

4. APPENDIX 2. TEMPERATURES FOR DURATION OF FUNGICIDE EXPERIMENT



5. APPENDIX 3. POSITION OF SAMPLE BAGS AND SENSORS IN SOIL PROFILE DURING STEAMING TREATMENTS

	1 cm below soil surface
	2
	3
	4 (sensor at 3.5 cm)
	5
	6
	7
	8
	9
	10
	11
	12
	13
	14 (sensor at 13.5 cm)
	15
	16
	17
	18
	19
	20
	21
	22
	23
	24 (sensor at 23.5 cm)
	25
	26
	27
DEPTH OF PLOUGH	28
	29
	30
	31
	32
	33
	34 (sensor at 33.5 cm)
	35

6. APPENDIX 4. DETAILS OF SOME SOIL STEAMING METHODS BEING USED IN THE UK

(notes by Tim O'Neill from South Holland Growers' Club visits, 3 June 2006)

Sheet steaming with a mobile boiler

MSD mobile boiler, *ex* Germany, generates 2 tonnes (2,000 L) of steam/hour at 4 Bar pressure and 200°C. This is sufficient to treat 480 m^2 at a time (75 m x 6.4 m wide bay). The steam is 'superheated' (i.e. > 100°C) and dry, so there is no condensation and wet areas at the edge of steaming sheets. Steaming between posts is done by pulling the sheet between the posts.

'Green' sheet (£1/m^2) is a better cover than 'black' (60p/m^2) as it is elastic and does not creep inwards with steaming, reducing the treated area.

Estimated cost to treat 1 bay (480 m^2):

Labour

5 people at 2 ½ h to move machine = 12 ½ h spread over 5 bays = 2 ½ h per bay

Labour to treat 1 bay = 7 ½ h

Total Labour: 10h @ £7/h = £70 or 15p/m^2 for labour

Fuel

1200 L/bay = 2.5 L/m^2

Fuel (gas oil) at 31p/L = 77.5p/m^2

Electricity = 2.5p/m^2

Total fuel = 80p/m^2

Depreciation

On boiler and sheet

Boiler and bowser and sheet cost £50,000

Over 5 years, cost is 25p/m^2/year

Total steaming cost: £1.20 /m^2 (£12,000/ha)

This compares with methyl bromide at 60 p/m^2 (£6,000/ha)

Procedure

Monitor the boiler and gradually turn back the steam output during the first 3 hours. Constantly check sand bags at the edges and stop leaks. Soil is prepared by rotovating to 15-20 cm, to keep any infestation in the upper layer. Although the steam output temperature is 200°C, it soon drops. Temperature achieved in the soil varies with depth and distance from the inlet point, being coldest midway between the two steam inlets.

Temperatures recorded after 8h steaming, with 15 cm soil thermometer were:

Near steam inlet (between injectors)	-	68°C
Next to steam injector	-	83°C
At edge of sheet	-	90°C (condensation occurs here)

After 24 h, soil temperature was 40°C at 15 cm deep. It is too hot to plant until 48h after steaming.

Steam plough

A small, geared motor winches the plough usually at 22-26 feet/h (6.7-8 m/h). The winch has been modified to speed the pull to 36 feet/h (11.1 m/h). It can pull a full bay length (75 m) in a day. The plough is around 3.2 m wide, so two pulls are required to treat a 6.4 m wide bay. At present it cannot treat between posts, but a drop-leg steam outlet could be added to achieve this. Steam outlets are 9 inches (23 cm) apart, arranged in two rows. Steam is injected at 13 inches (33 cm) deep. It is not possible to steam closer to the surface as the steam blows out. The steam leg creates a `mole-drain` and steam blows back along this.

Steam is generated by the nursery boiler, leaving at a pressure of 45 psi and a temperature of around 120°C (boiler pressure could be reduced to 20 psi if required).

Cost of fuel is estimated to be £6,000 for 9000 m^2 (67p/m^2). Additional costs are a man to steer it (all the time) plus a second person to help dig in the plough at the start of each pull. Assuming 1 man supervises 2 ploughs, a 75 m x 6.4 m bay can be treated in one day, or 480 m^2 for £56 (12p/m^2)

Additional costs would be incurred for boiler insurance, and depreciation on boiler and plough.

It is possible that a steam plough would not work well on a sandy soil, due to escape of steam.

The steam plough heats soil to 100°C at 15cm for around 30 mins. A 5 m steaming sheet pulled behind the plough would cover the steamed soil for around 30 mins if pulled at 11 m/h.

Temperatures recorded immediately under steam plough:
110°c at 15 cm (just behind sheet)
90°C at surface (beneath sheet)