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The results and conclusions in this report are based on a series of experiments conducted over one year. 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

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

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GROWER SUMMARY

1. Headline

Fusarium wilt affected many crops of stocks in 2004 particularly in areas of glasshouses where the disease was severe in 2003. High temperatures are not essential for disease development. The fungus was shown to be capable of carrying over in the soil between crops at levels sufficient to cause wilt in stocks. Methyl bromide was more effective than steaming, Basamid and Formalin at eliminating the fungus from infested woody stem pieces buried in soil. A low level of seed-borne infection (1%) was detected in one seed lot. In a short-duration trial, Bavistin DF, Amistar and Stroby WG reduced development of fusarium wilt.

2. Background and expected deliverables

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

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

The specific objectives of the project are:

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

3. Summary of the project and main conclusions

Occurrence of fusarium wilt in 2004

Fusarium wilt affected many crops in East Anglia for a second year in succession, occurring from April through to September 2004. Most of the nurseries affected were also affected in 2003, suggesting persistence in soil between years. However, at least two nurseries affected in 2004 had not observed the disease in 2003. Areas badly affected in 2003 often developed symptoms when planted in 2004. Occasional severe outbreaks were noted in crops planted after treatment with methyl bromide or steam sterilisation of the soil. Strips of affected plants beneath gutters may have resulted from wetter soil that was not adequately sterilised.

Testing of soil samples

Soil samples were collected in December 2003 from six nurseries where fusarium wilt had occurred that summer. Samples were stored at 4ºC for seven months and then tested for viable fusarium by growing stocks plants in the soils. Fusarium wilt developed in plants grown in all 10 soil samples collected from untreated soil, affecting usually over 50 % and up to 88 % of the test plants. It was also detected in three out of six soil samples following methyl bromide treatment, affecting 7, 13 and 30 % of test plants. One glasshouse was steamed following a severe outbreak and in this soil, fusarium wilt affected 50 % of test plants. These results indicate that there is a risk of fusarium wilt persisting in soil for at least ten months after an outbreak, at sufficient levels to cause wilt in stocks. Also, that methyl bromide can eliminate or substantially reduce the inoculum of *F. oxysporum* in soil.

Survival of *Fusarium oxysporum* in soil

Stocks debris naturally infested with *F. oxysporum* was mixed with soil and placed in an unheated polythene tunnel. Samples recovered at intervals were tested for viable *F. oxysporum* by planting with stocks and then assessing plants after 4-6 weeks for fusarium wilt. Viable *F. oxysporum* sufficient to cause infection remained in soil that had been stored for 3 months. The incidence of wilt in test plants declined over this period from 91 to 58 %.

Occurrence of *F. oxysporum* on seed

Two seed lots were examined by plating onto agar. In the first lot (400 seeds), no fusarium was isolated. In the second lot, *F. oxysporum* was recovered from 7 out of 4400 seeds tested; the greatest level of infection recorded on a single variety/colour was 1 %. Fusarium was still recovered from seeds of this lot after surface sterilisation in sodium hypochlorite. The *F. oxysporum* isolated from seed 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

Occasional plug stock plants, cv Centum, potted into soil containing a very low inoculum level (0.3 spores / gram of soil) of *F. oxysporum* f. sp.

mathioli developed internal stem browning characteristic of fusarium wilt. Infection was more consistent (> 25% of plants) at inoculum levels of 1000 spores / g of 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 (around 50% of plants).

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.

Cross-infection risk

Isolates of *F. oxysporum* from stock, lisianthus and aster were tested for their ability to infect and cause wilt in these species and in radish. Typical and severe symptoms of fusarium wilt were only seen when a plant was inoculated with *F. oxysporum* isolated from the same species. When *F. oxysporum* isolated from one plant species was inoculated onto a different plant species, symptoms were generally nil or very slight. However, the fusarium from stock was able to survive on roots of aster and lisianthus for at least 8 weeks. This result suggests that root debris from an aster or lisianthus crop, following an infected stocks crop, could serve as a source of inoculum for subsequent stocks crops.

Fungicides

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. This work will be extended in 2005, testing a greater range of fungicides in a crop trial.

Soil sterilisation

A series of experiments was conducted 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 the same test procedure was used throughout.

Methyl bromide

In November 2004, methyl bromide applied at 50g / m² 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.

Basamid

In September 2004, Basamid was applied at 70 g/m² to a sandy loam soil in a glasshouse and incorporated to 20-25 cm using a spading machine. Soil temperature was 18.5ºC and the moisture level was 50.1% of field capacity at the time of treatment. The plastic covering sheet was removed after 8 days and stem pieces tested for fusarium. Treatment resulted in 78 % and 12 % kill at 0 and 15 cm depth respectively. As expected, there was very little effect (5 % kill) at 30 cm, which was below the depth to which Basamid was incorporated. Allowing the woody stems to start decaying for 2 weeks before applying Basamid increased the % kill to 82 and 54 % at 0 and 15 cm respectively.

Formalin

A Formalin drench to soil at 0.5 L / m² in 2.5 L water / m² resulted in 100, 100 and 67 % kill of fusarium in stem pieces buried at 0, 5 and 15 cm depth respectively. Application in a larger water volume (5 L / m²) resulted in slightly reduced control at 5 and 15 cm.

Sheet steaming

Sheet steaming of a silt loam soil raised the temperature to over 60ºC for 14 h at 0 cm depth and 8.7 h at 15 cm depth; 60ºC was not achieved at 30 or 45 cm depth. The percentage kill of fusarium in woody stem pieces was 72, 66, 44 and 0 at 0, 15, 30 and 45 cm depths respectively. Similar levels of control were achieved in a repeat experiment. In laboratory experiments, neither boiling stem pieces for 60 minutes nor heating in an oven at 100ºC for 60 minutes completely eliminated fusarium. The fusarium that survived the heat treatments was shown to be capable of infecting stocks and causing wilt. Inadequate penetration of heat into the woody stems may explain the ability of fusarium to survive such treatments.

Elimination of *Fusarium oxysporum* (% kill) from naturally infected woody stem pieces of stocks by various soil treatments

Soil depth (cm)	Treatment				
	Methyl Bromide	Basamid	Formalin drench	Steaming exp 1	Steaming exp 2
0	100	78	100	70	78
5	-	-	100	-	-
15	92	12	67	76	66
30	100	5	-	32	44
45	90	-	-	12	0

Soil and glasshouse air temperature during stocks production

The initial outbreaks of fusarium wilt in 2003 occurred in a year with high summer temperatures (especially August), prompting the suggestion that the problem was triggered by the weather. Soil temperature at 10-15 cm depth and crop canopy air temperature were measured in stocks crops from May to September 2004. Several outbreaks occurred in May 2004 when the mean daily soil temperature was around 14ºC and the mean daily air temperature around 12-13ºC indicating that high temperatures are not an essential requirement for occurrence of fusarium wilt.

Laboratory tests showed that some growth of *F. oxysporum* occurs as low as 5ºC, although the maximum rate of growth occurs around 25ºC.

4. Financial benefits

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

5. 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 in stocks can be easily confused with *Pythium* root rot and *Sclerotinia* stem base rot, unless you are familiar with the disease.
- Check within the stem base for dark brown staining which is usually a reliable indicator of fusarium wilt.

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 3 months and there is a high risk that the disease will re-occur if stocks are planted in an area where the disease was severe in the previous crop of stocks.
- If only a few plants are affected by wilt, and they are accessible, carefully remove them from the crop (bag them *in situ*) as soon as possible, before fusarium sporulation occurs on the lower leaves and stem.
- At the end of a crop, take care to remove as much crop debris as possible, and as soon as possible, before preparing the land for the next crop. Woody stem bases in particular pose a high risk and fusarium may survive in these despite soil sterilisation treatment.

Control of fusarium wilt: soil treatments

- If methyl bromide is available to you, treatment at 50g / m² under VIF gives good control of *F. oxysporum* to 45 cm depth.
- Basamid, Formalin drench 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 is used, conduct a cress seed germination test to ensure all fumes have dissipated before replanting.
- Where Formalin is used, ensure to follow the Commodity Substance Approval (maximum rate of 0.5 L / m² at a minimum dilution of 1:4 in water). Note that the cress seed germination test does not work for Formalin. The rate of Formalin breakdown depends on soil temperature. Persistence is reported to be 5-6 weeks at 0-5ºC soil temperature, and 7-8 days at 25º soil temperature.

Control of fusarium wilt : fungicides

- As a precaution against fusarium wilt, consider applying a carbendazim drench treatment to plug plants before and/or within a few days of planting. Treatment early in the life of a crop is likely to give better control than later treatment. Bavistin DF (SOLA 0009/99) where available, Cleancrop Curve (SOLA 1213/04) and Delsene 50 Flo (SOLA 1004/04) can all be applied as a drench treatment to stocks at growers' own risk.

SCIENCE SECTION

1. Introduction

In June 2003, a wilt disease occurred in Lincolnshire in a glasshouse crop of column stocks (*Matthiola incana*). Plants showed a one-sided wilt that progressed from the base upwards. Leaves subsequently became bleached, growth was stunted and plants died. Roots appeared healthy but the vascular tissue was stained dark brown. White fungal growth developed extensively on damp incubation of leaves and stems. Although stocks had been grown in the glasshouse for over 12 years, the disease had not been observed previously. *Fusarium* was consistently recovered, following surface sterilisation, from within stems and roots and from the fungal growth on leaves. Culture characteristics and morphology were typical of *Fusarium oxysporum* (Booth, 1971). Colonies on potato dextrose agar (PDA) produced a violet pigmentation in reverse and dark, purple sterile stromatic pustules. Ellipsoid microconidia, produced in slimy heads from short monophialides on sucrose nutrient agar, were 2–5(–8) μm long; chlamydospores and 3-septate macroconidia were produced sparsely.

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

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

The aim of this project is to devise a reliable and cost-effective strategy for managing fusarium wilt through studies on: survival of the fungus in soil; the influence of inoculum level and soil temperature on infection; efficacy of soil sterilisation, chemical disinfectants and fungicide drench treatments; the potential for cross-infection to and from other cut flowers grown in rotation with stocks.

2. Occurrence of fusarium wilt in 2004

Symptoms of fusarium wilt on stock were reported by growers from April 2004. The disease was first confirmed on 12 May 2004 on a sample from Norfolk, and was subsequently identified on over 10 nurseries in Cambridgeshire, Lincolnshire and Norfolk during May – September (Table 2.1). A range of varieties and colours were affected including Centum Blue, Debra Blue, Opera White, Opera Purple and Opera Red. Outbreaks occurred on areas of land treated with methyl bromide and steam in autumn 2003. One nursery unaffected in 2003 remained unaffected in 2004. The disease remained at a low level on some nurseries whilst on others the problem was severe with widespread patches of wilting and dead plants. Losses of 25-50 % were reported in some crops and one grower reported 80 % losses. Severe losses were often associated with areas badly affected by fusarium wilt the previous year. On two nurseries, the disease occurred predominantly in strips under the gutters. 'Singles' (unmarketable flowers) within a group of doubles affected by fusarium wilt showed no obvious symptoms. Most but not all of the nurseries affected by fusarium wilt in 2004 had been affected in 2003 – at least two did not record the disease in 2003. Sporulating *F. oxysporum* was confirmed on white fungal growth at the base of green stems (similar in appearance to *Sclerotinia sclerotiorum*) and as orange fungal growth at the base of dead, dried stems inadvertently left after harvesting.

Table 2.1 Outbreaks of fusarium wilt on stocks in 2004

Nursery	Fusarium wilt present in 2003	Sterilisation prior to planting	Fusarium wilt in 2004	Date confirmed by ADAS
1	Yes?	Methyl bromide	Yes	12 May*
2	Yes	Methyl bromide	Yes	12 May
3	Yes	Methyl bromide	Yes	19 May
4	No?	Methyl bromide	Yes	19 May
5	Yes	Methyl bromide	Yes	21 May
6	Yes	Steam	Yes	28 May
7	Yes?	Steam	Yes	14 June
8	Yes	Methyl bromide	Yes	15 June
9	No	Methyl bromide	No?	-
10	No?	?	Yes	-
11	No	?	Yes	-

*Symptoms first seen in late April

3. Testing soils from stocks nurseries for the presence of *F. oxysporum* pathogenic on stocks, using plant baiting

Introduction

In this experiment, soil baiting using stocks plug plants was used as a technique to detect in soil the presence of *F. oxysporum* pathogenic to stocks.

Methods

Soil samples were collected or sent from six different stocks nurseries. At each nursery, samples were collected from different houses or areas where stocks fusarium wilt had been observed. Where possible, soils were collected pre- and post-sterilisation treatments.

Soil samples were collected from the nurseries and stored at 4ºC for seven months. For each soil sample, nursery address, house, position in house, history of fusarium wilt and any soil disinfestation treatment was recorded. For each soil sample a seed tray was filled and 10-15 stocks plants per tray were planted (cv Centum Red). The trays were placed in a polytunnel and watered regularly.

The plants were assessed after inoculation for incidence of fusarium leaf wilt, leaf bleaching and vein yellowing symptoms. Approximately 6 weeks after inoculation the plants were uprooted, the stems cut longitudinally (whole stem length) and incidence of vascular staining was recorded. Stem pieces were plated on potato dextrose agar amended with streptomycin (PDA+S) to confirm presence of *F. oxysporum*.

Results and discussion

Fusarium oxysporum pathogenic on stocks was detected in all of the soil samples collected from areas where stocks had previously been affected and soil left untreated (Table 3.1). Up to 88 % of plants grown in untreated soil samples developed typical symptoms of fusarium infection (brown/purple vascular staining). At nursery 3, the incidence of plants affected was reduced in steam sterilised soil compared with the untreated soil, although as confirmed in later experiments (Section 13), steaming failed to eliminate *F. oxysporum* from soil. The lowest incidence of fusarium symptoms was observed when plants were grown in soil that had been treated with methyl bromide. In half of the methyl bromide-treated soil samples, no wilt symptoms developed on bait plants, indicating that the soil treatment had been effective in controlling the pathogen.

Soil samples for this experiment were collected in winter 2003 from areas affected by fusarium wilt in summer 2003, and cold-stored until the following summer (July). The results show that *F. oxysporum* can survive in soil at sufficient levels to cause stocks wilt, for at least 10 months.

Table 3.1 Incidence of fusarium symptoms on stock plants grown in soil samples collected from nursery areas where stocks fusarium wilt was observed in 2003

Nursery	House	Soil fumigation treatment	% plants with vascular staining symptoms
1	1	Untreated	80
	4	Untreated	80
2	4	Untreated	79
3	-	Steamed (bad area)	50
	-	Untreated	88
4	1	Methyl bromide	13
	2	Methyl bromide	0
	3	Methyl bromide	7
	-	Untreated	40
5	1	Methyl bromide	30
	2	Methyl bromide	0
	3	Methyl bromide	0
6	1	Untreated	40
	2	Untreated	27
	3	Untreated	60
	4	Untreated	40
	5	Untreated	20
	6	Untreated	60
	7	Untreated	50

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

Introduction

The aim of the experiment was to determine whether soil containing stocks crop debris could act as a source of inoculum for fusarium wilt between crops in the same season and between seasons.

Methods

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

At each sampling time (0, 1.5, 3, 6, 9 and 12 months after soil infestation), three seed trays were filled with infested soil and planted with 15 stocks plants per tray (3 rows of 5 plants). Three seed trays were also filled with non-infested soil and planted with 15 stocks plants per tray. The trays were placed in a polytunnel 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 PDA+S to confirm the presence of *F. oxysporum*.

Results and discussion

For plants potted in soil immediately after infestation with infected stocks debris, typical symptoms of fusarium wilt were apparent after 4 weeks. The majority of plants had severe internal vascular discolouration (Table 4.1.) In the uninfested soil, all plants remained healthy except one dead plant which was not infected with fusarium wilt.

Plants potted 1.5 months after soil infestation also became infected with fusarium wilt, although the incidence of plants with medium or severe stem discolouration (scores 2 and 3) was greatly reduced. Plants potted 3 months after soil infestation generally remained visibly healthy, similar in appearance to the plants in non-infested soil. Approximately half of the plants showed a trace of internal stem discolouration indicating fusarium infection (*F. oxysporum* isolated). The incidence of infection was reduced from 91 % to 58 % after 3 months.

The results indicate that at summer polytunnel temperatures, *F. oxysporum* pathogenic on stocks can remain viable in soil for at least 3 months, although the incidence and severity of infection decreases with time. This experiment will be continued in 2005.

Table 4.1 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)	No. plants in score categories for internal stem discolouration (infested soil)*				No. plants in score categories for internal stem discolouration (non-infested soil)*			
	0	1	2	3	0	1	2	3
0	4	5	8	28	45	0	0	0
1.5	27	7	4	7	45	0	0	0
3	19	26	0	0	40	4	1	0

*out of 45

5. Survival and pathogenicity of *F. oxysporum* on stocks seed

Introduction

In this experiment, two lots of commercial stock seed were tested for the presence of *F. oxysporum* that was pathogenic on stock, to confirm whether the pathogen can be seed-borne.

Methods

Seed lot 1 contained 400 seeds of a single variety/colour. Seed lot 2 comprised 400 seeds of each of eleven varieties/colours. Stock seed was tested for the incidence of *Fusarium* spp. as follows. For each variety/colour, 200 seeds were plated directly on to PDA+S (25 seeds per plate). Another 200 seeds of each variety were surface sterilised (1 % sodium hypochlorite for 5 min), dried on absorbent paper towel and then plated onto PDA+S. The plates were incubated for 7 days at 20ºC in indirect light on a laboratory bench, then assessed for the incidence of colonies typical of *Fusarium* spp. Typical colonies of *Fusarium* spp. were subbed onto clean PDA+S plates and slopes and were demonstrated to have colony and spore morphology consistent with published descriptions of *F. oxysporum*

The pathogenicity of four of the isolates of *F. oxysporum* from stocks seeds in seed lot 2 was tested in comparison with an isolate of *F. oxysporum* known to be pathogenic on stocks (AR03/76) and an uninoculated control (sterile distilled water). Each treatment was applied to five stocks plug plants (var Opera Aida), and replicated four times in a randomised block design.

A spore suspension ($1x10^6$ spores/ml) was made of each isolate of *F. oxysporum* in SDW and filtered through one layer of muslin to remove fragments of agar and mycelium. For each treatment, the roots of 20 stock plug plants were dipped in the appropriate spore suspension or SDW for 1 min. The plants were potted in compost in 13 cm pots (5 plants per pot). The pots were placed on saucers, in a randomised block design in a glasshouse (20-24ºC, ambient light). Pots were sufficiently spaced (e.g. 30 cm), to avoid soil/water splash when watering in the saucers. The plants were kept relatively dry to provide conducive conditions for disease development and there was no overhead watering.

The plants were assessed approximately 3 weeks after inoculation for incidence of fusarium wilt symptoms (e.g. plant wilt, leaf bleaching, vein yellowing and stem discolouration). The severity of stem discolouration was scored on a 0-3 index where 0 = no staining, 1 = slight staining, 2 = staining part-way up the stem and 3 = whole stem length discoloured. For each treatment, a representative sample of stem staining symptoms thought to be due to *F. oxysporum* was plated onto PDA+S (following surface sterilisation in 90 % ethanol for 10 sec), to confirm the causal organism.

Results and discussion

No *F. oxysporum* was isolated from seeds in seed lot 1. From seed lot 2, colonies of *Fusarium* sp. were isolated from 7 out of 4400 seeds, from four stocks

varieties/colours (Table 5.1). Colony and spore morphology was consistent with published descriptions of *F. oxysporum*. The percentage incidence of fusarium for varieties/colours where it was detected ranged between 0.5 and 1 %, irrespective of surface sterilisation.

Table 5.1 Incidence of *F. oxysporum* on varieties of stocks seed

Variety code	No. of fusarium colonies (out of 200)	
	No surface sterilisation	Surface sterilised
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	1	0
8	1	2
9	0	0
10	0	1
11	0	2

In the pathogenicity study using isolates of *F. oxysporum* from seed lot 2, no symptoms of fusarium wilt developed on the uninoculated control plants. Typical symptoms of fusarium wilt were observed on all of the other treatments (Table 5.2) indicating that all of the *F. oxysporum* isolates tested were pathogenic on stocks. The incidence of wilting plants and plants showing internal stem discolouration in treatments inoculated with *F. oxysporum* from stocks seed was similar to that observed on plants inoculated with a known pathogenic isolate. For each of the inoculation treatments, typical colonies of *F. oxysporum* were isolated from discoloured stem tissue

The results of this experiment demonstrate that *F. oxysporum* pathogenic on stocks can survive on stocks seed. This is consistent with results from the USA indicating that *F. oxysporum* f. sp. *mathioli* could be seed-borne (Baker, 1948).

Table 5.2 Incidence of symptoms of fusarium wilt on stock plants inoculated with isolates of *F. oxysporum* isolates from stocks seed

Treatment	Plants wilted (out of 20)	Plants with internal stem discolouration (out of 20)	Mean stem discolouration index (0-3)	Vein yellowing observed	Leaf bleaching observed
SDW (control)	0	0	0	-	-
F. oxysporum (AR03/76)	5	6	0.6	+	+
F. oxysporum, seed isolate 1	7	8	1	+	+
F. oxysporum, seed isolate 2	4	4	0.3	+	+
F. oxysporum, seed isolate 3	5	5	0.35	+	+
F. oxysporum, seed isolate 4	3	5	0.25	-	+

6. Determining the inoculum level of *F. oxysporum* required to produce wilt in stocks

Introduction

A series of three experiments was carried out to determine the inoculum level of *F. oxysporum* required to produce wilt symptoms in stocks. In Experiments 1 and 2, inoculum (spore suspensions) was incorporated into soil into which stock plug plants were subsequently potted. In Experiment 3, plug plants were dipped in spore suspensions of different concentrations before potting in compost.

Methods

Experiment 1
Three rates of inoculum of *F. oxysporum* were tested:

	Inoculum	Concentration of spore suspension (spores/ml)	Volume added to each pot	No. spores added per pot (325 g soil)
1	SDW (control)	0	30 ml	0
2	*F. oxysporum*	10^2	1 ml in 29 ml SDW	10^2
3	*F. oxysporum*	10^4	1 ml in 29 ml SDW	10^4
4	*F. oxysporum*	10^6	1 ml in 29 ml SDW	10^6

Each treatment was applied to a 9 cm pot containing three stocks plants and replicated four times in a randomised block design. The stocks variety used was Opera, and each pot contained one plant of each of three colours of Opera (Figaro Lavender, Francesca and Carmen yellow).

To confirm that the stocks variety being used was susceptible to *F. oxysporum*, the roots of nine plug plants were dipped in a spore suspension (3 x 10^6 spores/ml) of *F. oxysporum ex* stocks. These plants were potted in soil-less compost in 9 cm pots (3 plants per pot, one of each colour). Three uninoculated plants were also potted as a control. The plants were maintained in the CE cabinet and glasshouse under the same conditions as the main experiment described below.

Sufficient silt loam soil (sieved) was placed in each of 16 labelled pots to ¾ fill them (325 g per pot). Spore suspensions of *F. oxysporum ex* stocks were made as detailed in the treatments list. 30 ml of inoculum (of the appropriate concentration for each treatment) was poured on to the soil surface of each pot and mixed thoroughly, ensuring there was no cross contamination of soil between pots of different treatments. Three plug plants (one of each colour) were planted per pot. The pots were placed on saucers, in a randomised block design in a controlled environment cabinet (23ºC, 16 h day) and watered daily with 75 – 100 ml water.

After 7 days the pots and saucers were transferred to a glasshouse (same experimental layout) (25ºC, ambient light). The pots were sufficiently spaced (30 cm), to avoid soil/water splash between pots when watering. Plants were kept relatively dry to provide conducive conditions for disease development. Water was applied to the saucer and not via overhead watering.

The plants were assessed weekly after inoculation for incidence of fusarium leaf wilt, leaf bleaching and vein yellowing symptoms. Approximately 5 weeks after inoculation the plants were uprooted, the stems cut longitudinally (whole stem length) and incidence of vascular staining was recorded (0-3 index as described in Section 5).

Experiment 2
Five rates of inoculum of *F. oxysporum* (*ex* stocks) were tested:

	Inoculum	No. spores/g soil
1	Sterile distilled water (control)	0
2	*F. oxysporum*	10
3	*F. oxysporum*	100
4	*F. oxysporum*	1,000
5	*F. oxysporum*	10,000
6	*F. oxysporum*	100,000

Each treatment was applied to a 9 cm pot containing three stocks plants var. Francesca, and replicated four times in a randomised block design.

The silt loam soil was sieved and thoroughly mixed. The weight of soil required to ¾ fill a 9 cm pot was determined and recorded (300 g). This weight of soil was placed in each of 24 labelled pots. Spore suspensions of *F. oxysporum* were made up and applied in 26 ml SDW per pot (poured onto the soil surface), to obtain the concentration of spores outlined in the treatment list. The inoculated soil was mixed thoroughly, ensuring there was no cross- contamination between the soils of different pots. Three plug plants were planted in each pot. The pots were placed on saucers, in a randomised block design in a controlled environment cabinet (23ºC, 16 h day) and watered daily with 75-100 ml water.

After 7 days the pots and saucers were transferred to a glasshouse (same experimental layout) (25ºC, ambient light). The pots were sufficiently spaced (30 cm), to avoid soil/water splash between pots when watering. Plants were kept relatively dry to provide conducive conditions for disease development and water was applied to the saucer and not via overhead watering.

The plants were assessed weekly after inoculation for incidence of fusarium leaf wilt, leaf bleaching and vein yellowing symptoms. Approximately 7 weeks after inoculation the plants were uprooted, the stems cut longitudinally (whole stem length) and incidence of vascular staining was recorded (0-3 index as described in Section 5). Leaves from each plot were placed into damp chambers and stem pieces plated onto PDA+S, to confirm that *F. oxysporum* was present.

Experiment 3

Six rates of inoculum of *F. oxysporum* (*ex* stocks) were tested:

	Inoculum	Concentration of spore suspension (spores/ml)
1	Sterile distilled water (control)	0
2	*F. oxysporum*	1×10^1
3	*F. oxysporum*	1×10^2
4	*F. oxysporum*	1×10^3
5	*F. oxysporum*	1×10^4
6	*F. oxysporum*	1×10^5
7	*F. oxysporum*	1×10^6

Each treatment was applied as a root dip to three stocks plants (var. Carmen yellow) which were then planted into a 9 cm pot, and replicated four times in a randomised block design.

Spore suspensions of *F. oxysporum* were made up to the concentrations detailed in the treatment list. The spore suspensions were filtered through one layer of muslin to remove fragments of agar and mycelium.

For each of treatments 2-7, the roots of 12 stock plug plants were dipped in the appropriate spore suspension (approx 25 ml) for 5 min. The plants were potted (3 per pot) in compost in 9 cm pots. Plants for the uninoculated control treatment were dipped in SDW before potting. The pots were placed on saucers, in a randomised block design in a controlled environment cabinet (23ºC, 16 h day) and watered daily with 75-100 ml water to the saucer.

After 7 days, the pots and saucers were transferred to a polytunnel (same experimental layout) (25ºC, ambient light). The pots were sufficiently spaced (e.g. 30 cm) to avoid soil/water splash between pots when watering. The plants were kept relatively dry to provide conducive conditions for disease development.

Plants were assessed at regular intervals during the 8 weeks post inoculation for incidence of fusarium leaf wilt, leaf bleaching and vein yellowing symptoms. Approximately 8 weeks after inoculation the plants were uprooted, their stems cut longitudinally (whole stem length) and incidence of vascular staining was recorded (0-3 index as described in Section 5). Leaves from each plot were placed into damp chambers and stem pieces plated onto PDA+S, to confirm that *F. oxysporum* was present.

Results and discussion

Experiment 1

Slight plant wilting was observed 4 weeks after inoculation. Plants were sampled destructively 5 weeks after inoculation. On plants inoculated by root dipping, fusarium symptoms were observed on all three colours of var. Opera, indicating susceptibility of the variety to the disease. Seven out of nine plants had symptoms of vein yellowing, while all nine plants had internal stem discolouration. These symptoms were not observed in any of the uninoculated control plants.

On plants potted into inoculated soil, symptom development occurred but at a low incidence. Slight vein yellowing was observed on at least one plant in all of the treatments including the uninoculated control, and so was considered a less reliable indicator of fusarium infection. Internal stem discolouration was observed in a total of three plants in the trial, from pots inoculated with either 10^2 or 10^6 spores/ml (equivalent to 0.3 or 3000 spores/g soil, respectively).

Experiment 2

The trial was destructively assessed approximately 7 weeks after inoculation. At this time, no obvious external symptoms of fusarium infection were apparent for any treatments. *F. oxysporum* did not develop from leaves or stem pieces that were damp incubated. There was a significant effect of soil inoculum levels on the severity of internal stem discolouration, with more severe symptoms observed for treatments of 1000 spores/g soil and higher (Table 6.1). At lower inoculation levels (including the uninoculated control), only slight stem discolouration was observed (score = 1). When stem pieces were plated onto PDA+S, colonies of *F. oxysporum* did not develop from stem pieces scoring 1 for internal discolouration but developed consistently from stem pieces scoring 2 or 3.

Table 6.1 Effect of *F. oxysporum* inoculum levels in soil on the incidence and severity of internal stem discolouration in stock plants

Treatment	No. spores/g soil	No. plants in score categories for internal stem discolouration*				Median of stem discolouration scores**
		0	1	2	3	
1	0	11	1	0	0	0.01
2	10	11	1	0	0	0.01
3	100	12	0	0	0	-0.04
4	1,000	7	5	0	0	0.40
5	10,000	9	1	0	2	0.49
6	100,000	5	2	1	4	0.88
	d.f.					5
	p-value					0.023
	S					13.11

*Out of 12, **Analysed using Friedman's non-parametric analysis

Experiment 3

The trial was assessed destructively approximately 8 weeks after inoculation. There were no clear symptoms of fusarium wilt at this time. *F. oxysporum* was consistently isolated from stem pieces with internal discolouration scoring 2 or 3. There was no

significant effect of inoculum level on the severity of stem discolouration (Table 6.2), however the highest incidence and severity of stem discolouration occurred in plots that received the highest inoculum level (1 x 10^6 spores/ml) applied as a root dip.

Table 6.2 Effect of *F. oxysporum* inoculum levels (applied as a root dip) on the incidence and severity of internal stem discolouration in stock plants

Treatment	Concentration of spore suspension used as root dip (spores/ml)	No. plants in score categories for internal stem discolouration*				Median of stem discolouration scores**
		0	1	2	3	
1	0	9	3	0	0	0.00
2	1 x 10^1	4	6	2	0	0.69
3	1 x 10^2	10	1	0	1	0.00
4	1 x 10^3	10	1	1	0	0.00
5	1 x 10^4	7	5	0	0	0.02
6	1 x 10^5	9	2	1	0	0.00
7	1 x 10^6	6	2	2	2	0.95
	d.f.					6
	p-value					0.327
	S					6.94

*Out of 12
**Analysed using Friedman's non-parametric analysis

The results of Experiments 1 and 2 demonstrated that a very low inoculum level of *F. oxysporum* in soil can give rise to stock fusarium wilt (0.3 spores/g soil), however, more consistent infection occurs at levels of 1000 spores/g soil and higher. The results from Experiment 3, indicated that when inoculating plants by root dipping in a spore suspension, low levels of infection can arise from a spore concentration of 1 x 10 spores/ml. However, for situations where this technique is used for artificial inoculation of plants in experimental work, levels of 1 x 10^6 spores/ml or higher, give more consistent infection.

7. Effect of temperature on the mycelial growth of isolates of *Fusarium oxysporum ex* stocks

Introduction

A laboratory experiment was carried out to determine the optimum temperature for mycelial growth of *F. oxysporum* isolates pathogenic on stocks.

Methods

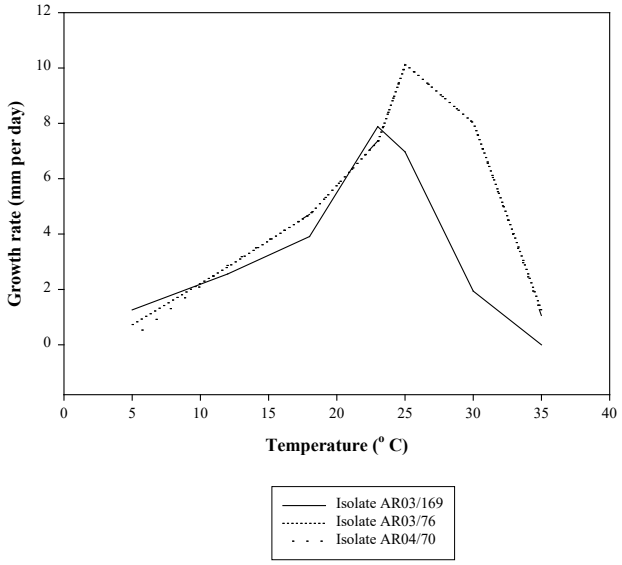
The effect of temperature on the mycelial growth of three isolates of *F. oxysporum ex* stocks was tested at the following temperatures: 5, 12, 18, 22, 25, 30 and 35ºC. The isolates used were AR03/76, AR03/169 and AR04/70. At each temperature, there were five replicate plates of each isolate placed in a randomised block design.

A Tiny Tag data logger was used to ensure that the required temperatures in available incubators and controlled environment cabinets were obtained. For each temperature, 5 mm discs were cut using a cork borer, from just within the leading edge of actively growing cultures of the three isolates of *F. oxysporum* on PDA+S. For each isolate, one agar disc was placed mycelial side down on the centre of each of five plates of PDA. The plates were inverted and incubated at the target temperature in the dark. Colony diameters were measured twice per week, following two perpendicular lines drawn on the back of each plate (passing through the agar disc) using a permanent marker pen.

Results and discussion

The effect of temperature was similar for all three isolates of *F. oxysporum ex* stocks, with the highest mycelial growth rates occurring at 23ºC for one isolate and 25ºC for the other two isolates (Figure 7.1). For the two isolates, with the higher temperature optimum, slow mycelial growth occurred at 35ºC, while for the other isolate there was nil growth at this temperature.

Figure 7.1 Effect of temperature on the mycelial growth rate of *F. oxysporum* isolates *ex* stocks



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

Introduction

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

Methods

A series of three experiments was carried out to test the cross-pathogenicity of isolates of *F. oxysporum* on stocks and other hosts. The treatment list for each experiment is shown below:

Experiment 1

	Host plant	Treatment
1	Stocks	Uninoculated control
2	Stocks	Inoculated with *F. oxysporum ex* lisianthus
3	Stocks	Inoculated with *F. oxysporum ex* stocks
4	Lisianthus	Uninoculated control
5	Lisianthus	Inoculated with *F. oxysporum ex* lisianthus
6	Lisianthus	Inoculated with *F. oxysporum ex* stocks

Experiment 2

	Host plant	Treatment
1	Stocks	Uninoculated control
2	Stocks	Inoculated with *F. oxysporum ex* aster
3	Stocks	Inoculated with *F. oxysporum ex* stocks
4	Aster	Uninoculated control
5	Aster	Inoculated with *F. oxysporum ex* aster
6	Aster	Inoculated with *F. oxysporum ex* stocks

Experiment 3

	Host plant	Treatment
1	Stocks	Uninoculated control
2	Stocks	Inoculated with *F. oxysporum ex* stocks
3	Radish	Uninoculated control
4	Radish	Inoculated with *F. oxysporum ex* stocks

For each experiment, each treatment was applied to a 9 cm pot containing three stock plants or three lisianthus/aster/radish plants, as required, and replicated four times in a randomised block design.

A spore suspension of each isolate of *F. oxysporum* was prepared in SDW (3×10^6 spores/ml). For each inoculation treatment, the roots of 12 host plants were dipped in the appropriate spore suspension for 30 sec. The plants were potted in compost in 9

cm pots (3 plants per pot). Plants for uninoculated control treatments were dipped in SDW before potting. The pots were placed on saucers, in a randomised block design in a controlled environment cabinet (23ºC, 16 h day) and watered daily with 75-100 ml water.

After 7 days the pots and saucers were transferred to a polytunnel (same experimental layout) (25ºC, ambient light) ensuring pots were sufficiently spaced (e.g. 30 cm), to avoid soil/water splash between pots when watering. Plants were kept relatively dry to provide conducive conditions for disease development.

The plants were assessed regularly after inoculation for incidence of fusarium wilt symptoms (e.g. leaf wilt, leaf bleaching and vein yellowing). Approximately 8 weeks after inoculation the plants were uprooted, the stems cut longitudinally (whole stem length) and incidence and severity of vascular staining was recorded. For each treatment, a representative sample of symptoms thought to be due to fusarium was plated onto PDA+S to confirm the causal organism.

Results and discussion

Stock/lisianthus

Uninoculated host plants remained healthy for the duration of the experiment. In stock plants inoculated with *F. oxysporum ex* stock, pronounced vein yellowing, wilting and stunted growth was observed for all plants, 3-4 weeks after inoculation. Medium to severe internal stem discolouration (score 2-3) was observed in all plants. When leaves from these plants were damp incubated, mycelial development and sporulation typical of *F. oxysporum* occurred for all but one plant. Wilting of all lisianthus plants inoculated with *F. oxysporum ex* lisianthus, was observed approximately 3 weeks after inoculation. Some leaf bleaching and reddish-orange root discolouration was also observed. *F. oxysporum* developed consistently from leaf and stem pieces that were damp incubated and plated onto PDA+S.

Approximately 5 weeks after inoculation, there were no visible symptoms of fusarium wilt on either of the cross-pathogenicity treatments, which were then sampled destructively. For both treatments, a low proportion of plants (less than a third) showed a trace of internal stem discolouration, which yielded *F. oxysporum* when plated onto PDA+S.

Stock/aster

The spore suspension of *F. oxysporum* from aster was applied at 1 x 10^6 spores/ml instead of 3 x 10^6 spores/ml, because the available isolate did not sporulate well. The stock plants did not thrive well in this experiment, irrespective of inoculum treatment. Severe internal stem discolouration was observed in stock plants inoculated with *F. oxysporum ex* stock and *ex* aster, although this was also observed in some of the uninoculated controls. The asters remained healthy irrespective of inoculation treatment. Some *F. oxysporum* was isolated when stem pieces were plated onto PDA+S.

Stock/radish

Uninoculated stocks showed either nil or a trace of internal stem discolouration and yielded no *F. oxysporum* when plated out. Approximately half of the stock plants

inoculated with *F. oxysporum ex* stock, developed severe internal stem discolouration which consistently gave *F. oxysporum* when plated onto PDA+S. Results were not so clear cut for radish because of pest damage to plants. There were no apparent differences in root health between inoculated and uninoculated treatments however root pieces from inoculated plants consistently yielded *F. oxysporum* when plated onto PDA+S.

A summary of results from the cross-pathogenicity experiments is shown in Table 8.1 Typical and severe symptoms of fusarium wilt were only seen when a host plant was inoculated with *F. oxysporum* previously isolated from the same host plant. In some cases, *F. oxysporum* from one host plant caused either a trace of symptoms on another host plant or could survive on another host plant without causing symptoms. For example, fusarium from stocks may cause a symptomless infection on aster, such that debris from the aster crop could potentially serve as a source of inoculum on subsequent stock crops. This aspect of work will be continued in 2005-6, to include host plants such as ornamental brassicas and aubretia.

Table 8.1 Summary of results from cross-pathogenicity studies in 2004

	Source of *F. oxysporum* inoculum			
Host plant	Stock	Lisianthus	Aster	Radish
Stock	+++	+	++?	
Lisianthus	+	+++		
Aster	-		-	
Radish	-			

Shaded areas represent combinations not tested

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

9. Evaluation of fungicides for control of fusarium wilt on plug plants of stocks

Introduction

A range of fungicides including currently approved products and other products/experimental products were tested in two experiments for their efficacy against fusarium wilt on artificially inoculated stocks plug plants.

Methods

Experiment 1
The fungicides listed for treatments 3-7 were applied one week before and after plug plant inoculation with *F. oxysporum:*

Treatment	Fungicide	Active ingredient	Rate
1	Untreated uninoculated control	-	-
2	Untreated inoculated control	-	-
3	Bavistin DF	Carbendazim	1 g/litre*
4	Octave	Prochloraz Mn	1 g/litre**
5	Amistar	Azoxystrobin	1 ml/litre***
6	Stroby WG	Kresoxim-methyl	0.3 kg/ha (0.3 g/litre)#
7	Certis product code: CERF011 (NF 149)	-	0.5 ml/litre##

Notes:
* SOLA 009/99
** Full label approval
*** SOLA 1533/02

\# Extrapolated from protected strawberry label
[assume 1000 L/ha (100 ml/m2)
then 1 g/L = 1 kg/ha
So 0.3 g/L = 0.3 kg/ha]

CERF011
500 ml/ha (provisional maximum label rate)
so use 0.5 ml/L
(500 ml = 125 ppm i.e. 25 % a.i.)

Each treatment was applied to ten stocks plug plants, and replicated four times in a randomised block design.

Strips/groups of 10 plug plants (stock cv Carmen Yellow) were cut from the plant tray, placed in saucers, and labelled with treatment/plot number. The saucers were placed on mypex matting in a polytunnel in a randomised block design, with a spacing of at least 0.5 m between plots, to avoid spray drift between treatments.

Fungicide treatments 3-7 were applied to the appropriate plots, using a spray guard to ensure there was no drift between plots. All fungicides were applied at 1000 L/ha

(100 ml/m^2) using an Oxford precision sprayer (with single 02F110 nozzle) and washed into the root zone with a watering can.

One week after the first fungicide spray, a spore suspension of *F. oxysporum ex* stocks amended to 1 x 10^6 spores/ml was prepared. For each of treatments 2-7, 1 ml of spore suspension was applied to the compost of each plug plant. Treatment 1 remained uninoculated. Plants were kept relatively dry to provide conducive conditions for disease development, with water placed in individual saucers rather than from overhead. A second fungicide application was applied one week after inoculation.

Plants were assessed weekly after inoculation for incidence of fusarium leaf wilt, leaf bleaching and vein yellowing symptoms. Approximately 8 weeks after inoculation the plants were uprooted, the stems cut longitudinally (whole stem length) and incidence and severity of vascular staining was recorded (0-3 index as described in Section 5). Samples from all plots were placed into damp dishes. Stem pieces from plots showing symptoms of fusarium in initial assessments were plated onto PDA+S.

Experiment 2

The methods described above were repeated using a different selection of fungicides applied one week before and after plant inoculation with *F. oxysporum:*

Treatment	Fungicide	Active ingredient	Rate
1	Untreated uninoculated control	-	-
2	Untreated inoculated control	-	-
3	Bavistin DF	Carbendazim	1 g/litre*
4	Amistar	Azoxystrobin	1 ml/litre**
5	Stroby WG	Kresoxim-methyl	0.3 g/litre (0.3 kg/ha)***
6	Swing Gold	Dimoxystrobin + epoxiconazole	1.5 ml/litre (1.5 L/ha) #
7	Caramba	Metconazole	1.2 ml/litre (1.2 L/ha) ##
8	Folicur	Tebuconazole	1 ml/litre (1 L/ha)###
9	Twist	Trifloxystrobin	2 ml/litre $

Notes:

* SOLA 009/99

** SOLA 1533/02

*** Extrapolated from protected strawberry label

\# Under Administrative Experimental Approval (AEA), using label rate

Under AEA, using label rate

LTAEU from SOLA 1874/03

$ Under AEA, using rates described in HDC Factsheet 12/04 for rose diseases

Each treatment was applied to ten stocks plug plants, and replicated four times in a randomised block design. Methods were the same as in Experiment 1 except that the stock variety used was Centum Purple and the inoculum concentration used was 1.25 x 10^6 spores/ml

Strips/groups of 10 plug plants (stocks var. Centum Purple) were cut from the plant tray and placed in saucers, then labelled with treatment/plot number. Each saucer was placed on mypex matting in polytunnel 3 in a randomised block design ensuring that there was a spacing of at least 0.5 m between plots, to avoid spray drift between treatments. Destructive assessments were carried out approximately 6 weeks after inoculation.

Results and discussion

Experiment 1

Because of the small volume of soil in each plug, plants in this trial were prone to desiccation and poor nutrition, so external symptoms of fusarium wilt were difficult to detect. However, once stems were cut longitudinally, there was found to be a significant effect of fungicide treatments on the severity of stem internal discolouration (Table 9.1). Lowest and highest scores were recorded for the uninoculated and inoculated control treatments respectively. The fungicides Bavistin DF, Amistar and Stroby WG were the most effective in reducing symptoms of fusarium infection.

Table 9.1 Effect of fungicides applied to stock plug plants inoculated with *F. oxysporum* on the severity of stem internal discolouration

	Fungicide	Median of stem discolouration scores*
1	Untreated uninoculated control	0.07
2	Untreated inoculated control	1.63
3	Bavistin DF	0.66
4	Octave	0.91
5	Amistar	0.61
6	Stroby WG	0.46
7	Certis product code: CERF011 (NF 149)	0.85
	d.f. p-value S	6 0.007 17.89

*Analysed using Friedman's non-parametric analysis

Experiment 2

As in Experiment 1, it was difficult to maintain plug plants in trays for sufficiently long to observe treatment differences. In this experiment, no obvious symptoms of fusarium wilt developed on the plants, and the incidence and severity of internal stem discolouration was very low irrespective of treatment (data not presented).

10. Efficacy of methyl bromide treatment against *Fusarium oxysporum* in stems of column stocks

Introduction

A series of experiments was conducted on commercial nurseries in 2004 to determine the efficacy of different soil disinfection treatments for eliminating *F. oxysporum* from soil (Sections 10 – 13). In all of the experiments, the survival of *F. oxysporum* in naturally infected stock stem pieces, following burial in soil at a range of depths during soil treatment, was investigated. In some of the experiments, partially rotted stem pieces were used in addition to woody stem pieces, to determine whether this affected the efficacy of soil treatment.

Although there is no Critical Use Exemption (CUE) for use of methyl bromide on cut flowers in 2005, any methyl bromide already present in the UK on 31 December 2004 and available to growers can be used on stocks, following the label conditions. Once any methyl bromide present in the UK on 31 December 2004 is used up, the chemical can only be imported and used for agreed Critical Use Exemptions. An application for a CUE for use in cut flower production for 2006 is being evaluated by the EC.

Methods

A silt soil in Lincolnshire at a commercial nursery (glasshouse) was treated with methyl bromide at 50 g/m^2 in early November 2004 (soil temperature 14^{o}C) and left covered with virtually impermeable film (VIF) for 12 days. The survival of *F. oxysporum* in fresh and part-decayed (2 and 4 week) naturally infected stem pieces, buried prior to methyl bromide treatment, was determined at 0, 15, 30 and 45 cm depth.

Sample preparation

Nylon (180 um gauge) bags containing ten 1-cm long stem sections cut from stocks with symptoms of fusarium wilt, mixed with moist silver sand were prepared. Rotted stem pieces were prepared by burial of stem pieces in warm, moist soil for the duration shown in the treatments table. After recovery, soil was rinsed off and pieces were mixed with moist silica sand in nylon bags. Sufficient sand to separate pieces was used. The bags were marked with coloured string to distinguish between the dry woody pieces and the part rotted stem pieces. Three bags, one of each type of stem pieces, were then placed in onion bags

Treatments

MeBr treatment	Stem pieces	Depth (cm)
1. Untreated	Fresh	0
2. Untreated	Rotted (2 week burial)	0
3. Untreated	Rotted (4 week burial)	0
4. Treated	Fresh	0
5. Treated	Rotted (2 week burial)	0
6. Treated	Rotted (4 week burial)	0
7. Treated	Fresh	15
8. Treated	Rotted (2 week burial)	15

9. Treated	Rotted (4 week burial)	15
10. Treated	Fresh	30
11. Treated	Rotted (2 week burial)	30
12. Treated	Rotted (4 week burial)	30
13. Treated	Fresh	45
14. Treated	Rotted (2 week burial)	45
15. Treated	Rotted (4 week burial)	45

Sample burial

The bags were buried on the day prior to methyl bromide treatment. A bay opposite the main door, about half-way into the glasshouse was chosen. For each of the required depths, five replicate samples of each stem piece treatment were buried, alternately 1 and 2 m in from the pathway, and 1 m apart. Depth was measured to the top of the bag. Any smeared soil was loosened with a fork and the hole was then back-filled. The burial points were marked by the surface treatment sample with the other depths all below one another.

Control bags were left on the soil surface in a neighbouring glasshouse where soil remained untreated.

Methyl bromide application

The methyl bromide was applied by contractors at a rate of 50 g/m^2 and then covered with a VIF plastic covers.

Recovery of bags

The bags were recovered 12 days after burial, once polythene sheeting had been removed.

Assessment of viability

Within 7 days of recovery the stem pieces were sifted from the sand and the excess sand was washed away. The stem pieces were then dried, before surface sterilisation in 90 % ethanol (10 sec). Sterilised pieces were then plated onto PDA+S and incubated at approximately 20ºC. The number of fusarium colonies present after 7 and 14 days was counted.

Statistical analyses

Results were analysed using Generalised linear models in Genstat.

Results and discussion

Methyl bromide treatment resulted in over 80 % kill of fusarium at all depths. In some treatments, the fungus was eliminated (Table 10.1). Even at 45 cm depth, there were some replicates where nil fusarium developed on PDA+S plates, following methyl bromide treatment. Survival of fusarium was slightly higher at each depth for one of the five replicates (data not presented) resulting in a highly significant replicate effect, perhaps due to positioning of these bags. For the methyl bromide-treated plots and the untreated plots (analysed separately), there were no significant effects of burial depth or degree of stem piece decay on the survival of fusarium (Tables 10.2 and 10.3). These results demonstrate the consistent efficacy of methyl bromide even at depth.

Table 10.1 Effect of methyl bromide soil treatment on survival of *F. oxysporum* in stock stem pieces (14 d assessment)

Treatment	Bag burial depth (cm)	No. weeks stem pieces rotted					
		0		2		4	
		No. fusarium colonies*	% kill	No. fusarium Colonies	% kill	No. fusarium colonies	% kill
Nil	0	9.8	2	8.8	12	9.2	8.0
MeBr	0	0.0	100	0.0	100	1.2	88.0
MeBr	15	0.8	92	0.8	92	0.6	94.0
MeBr	30	0.0	100	0.6	94	1.2	88.0
MeBr	45	1.0	90	2.0	80	0.6	94.0

*out of 10, mean for five replicates

Table 10.2 Regression analysis on effect of methyl bromide on survival of *F. oxysporum* in stock stem pieces

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx F. probability
+ Rep	4	46.020	11.505	6.79	<0.001
+ Depth	3	8.175	2.725	1.61	0.201
+ Rotting	2	4.328	2.164	1.28	0.289
+ Depth.Rotting	6	25.269	4.211	2.49	0.037
Residual	44	74.566	1.695		
Total	59	158.358	2.684		

Table 10.3 Regression analysis on survival of *F. oxysporum* in stock stem pieces, in untreated plots

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx F. probability
+ Rep	4	10.521	2.630	1.77	0.229
+ Rotting	2	4.527	2.264	1.52	0.276
Residual	8	11.921	1.490		
Total	14	26.969	1.926		

11. Efficacy of Basamid treatment against *Fusarium oxysporum* f. sp. *mathioli* in stems of column stocks

Methods

Site:
Nursery in Cambridgeshire with sandy loam soil (7.4 % organic matter content)

Sample preparation
Nylon (180 um gauge) bags containing ten 1-cm long stem sections cut from stocks with symptoms of fusarium wilt, mixed with moist silver sand were prepared. Rotted stem pieces were prepared by burial of stem pieces in warm, moist soil for 2 weeks. After recovery, soil was rinsed off and pieces were mixed with moist silica sand in nylon bags. Sufficient sand to separate pieces was used. The bags were marked with coloured string to distinguish between the dry woody pieces and the part rotted stem pieces. Three bags, one of each type of stem pieces, were then placed in onion bags.

Treatments

Basamid Treatment	Stem Pieces	Burial Depth (cm)
1. Treated	Fresh	0
2. Treated	Rotted (2 week burial)	0
3. Treated	Fresh	15
4. Treated	Rotted (2 week burial)	15
5. Treated	Fresh	30
6. Treated	Rotted (2 week burial)	30
7. Untreated	Fresh	0
8. Untreated	Rotted (2 week burial)	0
9. Untreated	Fresh	15
10. Untreated	Rotted (2 week burial)	15
11. Untreated	Fresh	30
12. Untreated	Rotted (2 week burial)	30

Soil samples
Soil temperature at 15-cm depth was measured on the day of treatment (1 September 2004). Before Basamid was applied, 10 soil cores were taken from 0-15 cm depth, placed in a polythene bag, and weight was determined in the laboratory. Two further soil samples were taken and brought back to the laboratory. First their fresh weight was measured. Then, they were watered to field capacity (FC), allowing surplus water to drain off, and FC weight was taken. Then the samples were placed onto trays and dried in an oven for 24 h to determine dry weight. This information was used to calculate the water content of the fresh weights as a % of the FC.

Sample burial
A bay opposite the main door, about half-way into the glasshouse was chosen. Five samples were buried at 30 cm, alternately 1 and 2 m in from the pathway, and 1m apart. The burial points were marked on the adjacent pathway. Depth was measured to the top of the bag. Any smeared soil was loosened with a fork and the hole was then back-filled. The basamid was applied by contractor to the soil surface at 70 g/m^2 using a spinning disc, then incorporated to 20-25 cm using a spader. Before the polythene covers were put on, the remainder of the samples were buried. These were

buried in the same locations as the 30 cm samples but at 15 cm and on the surface. Treated areas were immediately sheeted over with 38 um polythene, which remained in place for 8 days. The burial procedure was repeated in an untreated glasshouse with bags at the same three depths, but placed 30 cm apart.

Recovery of bags

8 days after Basamid application, after the polythene film cover was removed, the bags were recovered.

Assessment of viability

Within 7 days of recovery the stem pieces were sifted from the sand and the excess sand was washed away. The stem pieces were then dried, before surface sterilisation in 90 % ethanol (10 sec). Sterilised pieces were then plated onto PDA+S. The number of fusarium colonies present after 7 and 14 days was counted.

Results and discussion

Soil temperature at the time of Basamid treatment was 18.5ºC, soil moisture was 50.1% (mean of 3 samples, 45.8-52.8%).

None of the treatments gave 100 % kill of fusarium inoculum in woody stem tissue. However, treatment with basamid significantly reduced fusarium survival compared with the untreated controls (Tables 11.1 and 11.2). Fusarium survival was significantly reduced in partially rotted stem pieces compared with unrotted stem pieces, particularly in the basamid-treated plots. There was a significant depth.treatment interaction, indicating that in untreated soil there was no difference in fusarium survival at different depths, while in basamid-treated soil, fusarium survival was greater at depth. This is reflected in the % kill values which decrease from 78-82 % at the soil surface to 5-12 % at 30 cm depth. The results demonstrate that while basamid can provide good control of *F. oxysporum* at the soil surface, control at depth may be difficult to achieve.

Table 11.1 Effect of Basamid soil treatment on survival of *F. oxysporum* in stock stem pieces (14 d assessment)

Treatment	Bag burial depth (cm)	No. weeks stem pieces rotted			
		0		2	
		No. fusarium colonies*	% kill	No. fusarium colonies	% kill
Nil	0	10.0	0	7.2	28
Nil	15	10.0	0	8.6	14
Nil	30	9.8	2	8.0	20
Basamid	0	2.2	78	1.8	82
Basamid	15	8.8	12	4.6	54
Basamid	30	9.5	5	8.8	12

*out of 10, mean for five replicates

Table 11.2 Regression analysis on effect of Basamid soil treatment on survival of *F. oxysporum* in stock stem pieces

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx F. probability
+ Rep	4	9.042	2.260	1.00	0.420
+ Depth	2	69.787	34.894	15.40	<0.001
+ Rotted	1	31.570	31.570	13.94	<0.001
+ Treat	1	99.594	99.594	43.97	<0.001
+ Depth.rotted	2	7.144	3.572	1.58	0.219
+ Depth.treat	2	31.242	15.621	6.90	0.003
+ Rotted.treat	1	12.195	12.195	5.38	0.026
+ Depth.rotted.treat	2	2.716	1.358	0.60	0.554
Residual	40	90.605	2.265		
Total	55	353.893	6.434		

12. Evaluation of Formalin drench for control of *Fusarium oxysporum* f. sp. *mathioli* in stems of column stocks

Methods

Site
The experiment was conducted in a heated glasshouse on a commercial nursery in Lincolnshire on land previously cropped with outdoor cut flowers with erection of a new glasshouse just completed. The soil was a silt and had to be broken up with a fork when samples were buried as it was wet and slightly cloddy.

Sample preparation
Nylon (180 um gauge) bags of ten 1-cm long stem sections from column stocks with symptoms of fusarium wilt, mixed with moist silver sand were prepared. Sufficient sand to separate pieces was used. Each bag was labelled with depth, treatment and replicate number.

Sample burial
Samples were buried in three blocks at the side of the glasshouse. The burial points were marked on the adjacent pathway. Depth was measured to the top of the bag. Any smeared soil was loosened with a fork and the hole was then back-filled. One block was left untreated and each of the other two had a formalin treatment applied.

Treatments
Formalin was applied to 3 x 1 m^2 squares at the maximum approved rate of 0.5 L/m^2 of soil, in the minimum dilution of water (2.5 L/m^2) (Commodity Substance Approval), and in twice that volume of water (5 L/m^2). The third plot was treated with water alone. For each of the formalin treatments, three replicate bags were buried at 0, 5 and 15 cm. For the water only treatment, two replicate bags were laid on the soil surface.

In addition, 6 bags were scattered on the soil surface and watered with Formalin at 0.17 L/m^2 and then irrigated with water via overhead sprinklers at a rate of 3.9 L/m^2. This land was sub-soiled, rotovated and planted with stocks 3 days after treatment.

Soil temperature was recorded at 15-cm depth using a mercury soil thermometer.

Assessment of viability
Bags were recovered 2 days after Formalin treatment. Within 7 days of recovery the stem pieces were sifted from the sand and the excess sand was washed away. The stem pieces were then dried, before surface sterilisation in 90 % ethanol (10 sec). Sterilised pieces were then plated onto PDA+S. The number of fusarium colonies present after 7 and 14 days were counted.

Results and discussion

There was a significant effect of the Formalin treatment on the survival of *F. oxysporum* in stock stem pieces (Tables 12.1 and 12.2). The treatment was most effective when applied in 2.5 L/m^2 water rather than 5.0 L/m^2, perhaps because the higher water volume washed the chemical too rapidly through the soil. Formalin

treatment resulted in elimination of *F. oxysporum* at the soil surface, but the efficacy of the chemical decreased with depth, particularly when applied in the higher water volume.

Complete control of *F. oxysporum* was obtained when Formalin was applied to the surface and watered in via overhead sprinklers.

Table 12.1 Effect of Formalin drench on survival of *F. oxysporum* f. sp. *mathioli* in stem pieces of column stocks laid on and buried in soil.

Treatment	Depth of stem piece burial (cm)	Application volume (L/m^2)	Mean number stem pieces with viable *F. oxysporum**	Mean % kill
1. Hand watered (no formalin)	0	5.0	10.0	0
2. Hand watered (no formalin)	5	5.0	10.0	0
3. Hand watered (no formalin)	15	5.0	10.0	0
4. Hand watered (formalin)	0	2.5	0.0	100
5. Hand watered (formalin)	5	2.5	0.0	100
6. Hand watered (formalin)	15	2.5	3.3	67
7. Hand watered (formalin)	0	5.0	0.0	100
8. Hand watered (formalin)	5	5.0	4.7	53
9. Hand watered (formalin)	15	5.0	8.3	17
10. Formalin drench at 0.15 L/m^2, watered in via overhead sprinklers at 3.9 L/m^2	0	-	0.0	100.0
11. No formalin drench	0	-	10.0	0.0

*out of 10

Table 12.2 Regression analysis on effect of Formalin soil drenches on survival of *F. oxysporum* in stock stem pieces

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx F. probability
+ Rep	2	5.178	2.589	1.95	0.175
+ Depth	2	55.758	27.879	20.97	<0.001
+ Treat	2	231.997	115.999	87.27	<0.001
+ Depth.treat	4	1.212	0.303	0.23	0.919
Residual	16	21.268	1.329		
Total	26	315.413	12.131		

13. Evaluation of soil steaming for control of *Fusarium oxysporum* f. sp. *mathioli*

Materials and methods

Site
The experiment was conducted in a heated glasshouse on a commercial nursery in Lincolnshire. The previous crop was stocks and the soil was a silt loam, which was sub-soiled to 42 cm and rotovated thoroughly prior to burial of samples.

Sample preparation
Nylon (180 um gauge) bags containing ten 1-cm long stem sections from column stocks with symptoms of fusarium wilt, mixed with moist silver sand were prepared. Sufficient sand to separate pieces was used. Each bag was labelled with depth, position along bed and replicate number.

Sample burial
Three replicates bags were buried at each of four depths (0, 15, 30 and 45 cm) at three locations along the area to be steamed; 1 m from the inlet, midway and 1 m from the end of the area. The burial points were marked on the adjacent pathway. Depth was measured to the top of the bag. Any smeared soil was loosened with a fork and the hole was then back-filled. The untreated control comprised six replicate bags on the surface of an adjacent unsteamed bed

Treatment
The soil was treated by sheet-steaming using a Wilkie boiler (model 250S) operating at 0.3 bar pressure. An area of approximately 48 m^2 (3 x 16 m) was steamed for around 3 h until the soil temperature had reached 100^{o}C at 15 cm depth. Steaming was maintained for 30 mins and then switched off. The maximum temperature recorded under the sheet was 102^{o}C. The cover was left on for a further 6 hours, and then removed and the soil allowed to cool. The soil temperature at 15-cm depth on removal of the sheet was around 70 ^{o}C. The next day, soil was shallow-rotovated (7-10 cm), using a tractor and rotovator, prior to planting.

Assessment of viability
The bags were recovered 2 days after steam treatment. Within 7 days of recovery the stem pieces were sifted from the sand and the excess sand was washed away. The stem pieces were then dried, before surface sterilisation in 90 % ethanol (10 sec). Sterilised pieces were then plated onto potato dextrose agar. The number of fusarium colonies present after 7 and 14 days were counted.

The experiment was repeated at the same site. In the second experiment, rotted stem pieces (2 week burial) were compared with unrotted stem pieces. The bags were buried at the same depths as in Experiment 1, with five replicate bags per depth, at a single position. The untreated control comprised a single bag of rotted or unrotted stem pieces on the surface of an adjacent unsteamed bed. Soil temperature at the different depths was logged for the duration of the steaming treatment.

Results and discussion

During sheet steaming, temperature exceeded 100ºC at the soil surface but not at lower depths. Temperature only exceeded 60ºC (usual thermal death point for fungal pathogens) at the soil surface and at a depth of 15 cm. (Table 13.1)

Steaming did not achieve 100 % kill of *F. oxysporum* even at the soil surface, in either Experiment 1 or 2, perhaps due to the inability of the heat to penetrate the woody stem tissue. In both experiments, efficacy of steaming decreased with soil depth, due to the poor penetration of heat below 15 cm. In Experiment 1, there was no effect of bag position in relation to the steam inlet, on fusarium survival. In Experiment 2, there was no effect of using partially rotted stem pieces, on fusarium survival. (Tables 13.2 – 13.5)

Table 13.1 Summary of temperatures recorded during soil steaming experiment 2

Soil depth (cm)	Period of time when temperature >60ºC (h)	Maximum temperature (ºC)
0	13.8	102
15	8.7	92
30	0	58
45	0	31

Table 13.2 Effect of soil steaming on survival of *F. oxysporum* in stem pieces of column stocks laid on and buried in soil (Experiment 1)

Treatment	Burial depth (cm)	Nº pieces with viable fusarium*			Mean % kill
		Inlet end	Mid-way	Far end	
Untreated	0	-	9.7	-	3.0
Steam	0	3.0	4.0	2.0	70.0
Steam	15	3.0	3.3	1.0	75.7
Steam	30	9.3	6.3	4.7	32.3
Steam	45	9.7	6.7	10.0	12.0

*out of 10

Table 13.3 Regression analysis for effect of soil steaming on survival of *F. oxysporum* in stem pieces of column stocks laid on and buried in soil (Experiment 1) (steam-treated plots only)

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx F. probability
+ Rep	2	7.108	3.554	0.39	0.679
+ Depth	3	79.172	26.391	2.93	0.056
+ Location	2	20.866	10.433	1.16	0.333
+ Depth.location	6	32.134	5.356	0.59	0.731
Residual	22	198.219	9.010		
Total	35	337.500	9.643		

Table 13.4 Effect of soil steaming on survival of *F. oxysporum* in stem pieces of column stocks laid on and buried in soil (Experiment 2)

Treatment	Bag burial depth (cm)	No. weeks stem pieces rotted			
		0		2	
		No. fusarium colonies*	% kill	No. fusarium colonies	% kill
Nil	0	9.0	10	8.0	20
Steam	0	2.8	72	0.8	92
Steam	15	3.4	66	3.0	70
Steam	30	5.6	44	6.6	34
Steam	45	10.0	0	9.6	4

*out of 10

Table 13.5 Regression analysis for effect of soil steaming on survival of *F. oxysporum* in stem pieces of column stocks laid on and buried in soil (Experiment 2)

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx F. probability
+ Rep	4	42.202	10.551	3.19	0.028
+ Depth	3	209.293	69.764	21.11	<0.001
+ Rotted	1	1.670	1.670	0.51	0.483
+ Depth.rotted	3	11.604	3.868	1.17	0.339
Residual	28	92.518	3.304		
Total	39	357.287	9.161		

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

Introduction

Results from the soil steaming experiments indicated that steaming was relatively ineffective in eliminating *F. oxysporum* from stocks stem pieces, even at the soil surface where 60°C was exceeded. An experiment was carried out to determine whether wet heat (steam) would be more effective than dry heat (in an oven) for pathogen kill.

Methods

Sample preparation

Stocks stems showing typical severe symptoms of fusarium infection (wilting and internal stem discolouration) were collected from a commercial nursery. The stems were cut into 1.5-2 cm lengths with sharp secateurs and were placed ten per muslin bag in either moist sand, or no sand. The two types of bag were exposed to wet heat by boiling (30 min and 60 min) or dry heat in an oven at 100°C (30 min and 60 min).

For hot water treatments, the water bath was allowed to reach the target temperature before bags were put in. After the appropriate time interval, bags were removed from the water bath.

The oven had reached 100°C before bags were treated for the specified time. After the required time the bags were removed

Assessment of viability

Within 7 days of treatment the stem pieces were sifted from the sand and the excess sand was washed away. The stem pieces were then dried, before surface sterilisation in 90 % ethanol (10 sec). Sterilised pieces were then plated onto potato dextrose agar. The number of fusarium colonies present after 7 and 14 days were counted.

The experiment was repeated once using the same methods.

Results and discussion

It was interesting to note that none of the boiling or dry heat treatments eliminated *F. oxysporum* from stock stems (Table 14.1). The heat treatments were significantly more effective when the stems were mixed with sand (Table 14.2), indicating that there was perhaps more efficient conductance of heat through the sand. In a repeat of the experiment (data not presented), the heat treatments again failed to eliminate *F. oxysporum* from stock stem pieces. This aspect of work will continue in 2005-2006.

Table 14.1 Effect of wet and dry heat treatments, and the presence of sand during treatment, on viability of *Fusarium oxysporum* within stem pieces of stocks (Run 1)

Treatment	Medium	Number of stem pieces (of 30) with growth of *Fusarium* after 14 days	% kill
1. Untreated	Nil	30	0
2. Untreated	Sand	29	1
3. Boil, 30 mins	Nil	30	0
4. Boil, 60 mins	Nil	30	0
5. Boil, 30 mins	Sand	9	70
6. Boil, 60 mins	Sand	8	73
7. Oven, 30 mins	Nil	30	0
8. Oven, 60 mins	Nil	25	17
9. Oven, 30 mins	Sand	2	93
10. Oven, 60 mins	Sand	6	80

Table 14.2 Regression analysis on effect of wet and dry heat treatments, and presence of sand during treatment, on viability of *Fusarium oxysporum* within stem pieces of stocks (Run 1)

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx F. probability
+ Medium	1	161.626	161.626	50.63	0.002
+ Boil/oven	1	8.246	8.246	2.58	0.183
+ Time	1	0.171	0.171	0.05	0.828
Residual	4	12.770	3.193		
Total	7	182.812	26.116		

15. Effect of heat on the pathogenicity of *F. oxysporum ex* stocks

Introduction

Previous work has reported that some isolates of *F. oxysporum* that are thermotolerant may be less pathogenic on the host plant. In Section 14, *F. oxysporum* was isolated from stocks stem pieces following high temperature treatments. The aim of this experiment was to determine whether isolates of *F. oxysporum* obtained from stock stem pieces exposed to either wet or dry heat treatments, were pathogenic to stock.

Method

Treatments

	Inoculum	Source of isolate	Concentration of spore suspension in SDW (spores/ml)
1	Sterile distilled water (control)	-	0
2	*F. oxysporum*	Stocks stem pieces boiled for 60 min – isolate 1	1 x 10^6
3	*F. oxysporum*	Stocks stem pieces boiled for 60 min – isolate 2	1 x 10^6
4	*F. oxysporum*	Stocks stem pieces boiled for 60 min – isolate 3	1 x 10^6
5	*F. oxysporum*	Stocks stem pieces oven heated for 60 min – isolate 1	1 x 10^6
6	*F. oxysporum*	Stocks stem pieces oven heated for 60 min – isolate 2	1 x 10^6
7	*F. oxysporum*	Stocks stem pieces oven heated for 60 min – isolate 3	1 x 10^6
8	*F. oxysporum*	Stocks stem pieces untreated – isolate 1	1 x 10^6
9	*F. oxysporum*	Stocks stem pieces untreated – isolate 2	1 x 10^6
10	*F. oxysporum*	Stocks stem pieces untreated – isolate 3	1 x 10^6

Each treatment was applied to five stocks plug plants, and replicated four times in a randomised block design.

Spore suspensions (1x10^6 spores/ml) of each isolate of *F. oxysporum* were made in SDW. For each of treatments 2-10, the roots of 20 stock plug plants were dipped in the appropriate spore suspension for 1 min. The plants were potted in compost in 13 cm pots (5 plants per pot). Plants for the uninoculated control treatments were dipped in SDW for 1 min before potting. The pots were placed on saucers, in a randomised block design in a glasshouse (20ºC, ambient light). The pots were sufficiently spaced (e.g. 30 cm), to avoid soil/water splash between pots when watering in the saucers.

Plants were kept relatively dry to provide conducive conditions for disease development (e.g. watering every 2-3 days) with no overhead watering.

The plants were assessed approximately 3 weeks after inoculation for severity of fusarium wilt symptoms (e.g. leaf wilt, leaf bleaching and vein yellowing) on an index of 0-3. Plants were uprooted, stems cut longitudinally (whole stem length) and the severity of vascular staining scored on an index of 0-3 where 0 = no staining, 1 = slight staining at stem base, 2 = staining part way up the stem, 3 = stem all stained. For each treatment, a representative sample of stem staining symptoms thought to be due to fusarium was plated onto PDA+S to confirm the causal organism. Stem pieces were sterilised in 90 % ethanol for 10 sec before plating.

Results and discussion

Plants in the uninoculated control treatment remained healthy. Plants with typical symptoms of fusarium wilt (wilting, vein yellowing, leaf bleaching and internal stem discolouration) were observed in each of the inoculation treatments. Typical colonies of *F. oxysporum* developed from stem pieces with vascular discolouration that were plated onto PDA+S. The experiment demonstrated that thermotolerant isolates of *F. oxysporum* can be pathogenic on stock.

16. Soil and air temperature during stocks production – 2004

Soil and canopy temperature were logged during stocks production in glasshouses at a commercial nursery in Lincolnshire from May to September 2004 (Appendix 2).

In 2003, the major outbreaks of fusarium wilt coincided with high summer temperatures in August, leading to suggestions that the crop was most prone to infection under hot dry conditions. Outbreaks of stock fusarium wilt in 2004 were reported from May onwards when the mean daily soil temperature was around 14ºC and the mean daily air temperature around 12-13ºC, indicating that high temperatures are not an essential requirement for occurrence of fusarium wilt. Results from the laboratory experiment on effect of temperature on mycelial growth (Section 7), support these observations in that colony growth still occurred at temperatures as low as 5^{o}C despite a temperature optimum of 25^{o}C.

17. Overall conclusions

- Severe outbreaks of stocks fusarium wilt occurred in the UK for a second year in succession. Many affected nurseries in 2004 had seen disease outbreaks the previous year, suggesting pathogen persistence in the soil between seasons. Occasional severe outbreaks were noted in crops planted after treatment with methyl bromide or steam sterilisation of the soil.
- Results from a baiting experiment using soil from six nurseries demonstrated that *F. oxysporum* could survive for at least ten months after an outbreak, at sufficient levels to cause wilt in stocks and that methyl bromide can eliminate or substantially reduce the inoculum of *F. oxysporum* in soil.
- In soil artificially infested with stocks debris, *F. oxysporum* survived for 3 months at levels sufficient to cause typical wilt symptoms on young stocks plants. However, the incidence of wilt in test plants declined over this period from 91 to 58 %.
- *F. oxysporum* was isolated from 7 out of 4400 seeds (from 11 varieties/colours). For a single variety/colour, the maximum incidence of infection was 1 %. *F. oxysporum* isolated from stock seed caused typical symptoms of fusarium wilt when used to inoculate young stock plants. The experiment confirmed that *F. oxysporum* pathogenic to seed can survive on stocks seed.
- Occasional plug stock plants, cv Centum, potted into soil containing a very low inoculum level (0.3 spores / gram of soil) of *F. oxysporum* f. sp. *mathioli* developed internal stem browning characteristic of fusarium wilt. Infection was more consistent (> 25% of plants) at inoculum levels of 1000 spores / g of 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 (around 50% of plants).
- 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.
- Isolates of *F. oxysporum* from stock, lisianthus and aster were tested for their ability to infect and cause wilt in these species and in radish. Typical and severe symptoms of fusarium wilt were only seen when a plant was inoculated with *F. oxysporum* isolated from the same species. When *F. oxysporum* isolated from one plant species was inoculated onto a different plant species, symptoms were generally nil or very slight. However, the Fusarium from stock was able to survive on roots of aster and lisianthus. This result suggests that debris from an aster or lisianthus crop, following an infected stocks crop, could serve as a source of inoculum for subsequent stocks crops.
- 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.
- Methyl bromide applied at 50g / m² 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.

- Basamid treatment (70g / m²) resulted in 78 % and 12 % kill of *F. oxysporum* in naturally infected stocks stem pieces at 0 and 15 cm depth respectively. There was very little effect (5 % kill) at 30 cm, which was below the depth to which Basamid was incorporated. Allowing the woody stems to start decaying for 2 weeks before applying Basamid increased the % kill to 82 and 54 % at 0 and 15 cm respectively.
- A Formalin drench to soil at the maximum permitted rate (0.5 L / m²) in 2.5 L water / m² resulted in 100, 100 and 67 % kill of fusarium in stem pieces buried at 0, 5 and 15 cm depth respectively. Application in a larger water volume (5 L / m²) resulted in slightly reduced control at 5 and 15 cm.
- Sheet steaming of a silt loam soil raised the temperature to over 60ºC for 14 h at 0 cm depth and 8.7 h at 15 cm depth; 60ºC was not achieved at 30 or 45 cm depth. The percentage kill of fusarium in woody stem pieces was 72, 66, 44 and 0 at 0, 15, 30 and 45 cm depths respectively. Similar levels of control were achieved in a repeat experiment.
- In laboratory experiments neither boiling stem pieces for 60 minutes nor heating in an oven at 100ºC for 60 minutes completely eliminated fusarium. The fusarium that survived the heat treatments was shown to be capable of infecting stocks and causing wilt. Inadequate penetration of heat into the woody stems may explain the ability of fusarium to survive such treatments.
- The initial outbreaks of fusarium wilt in 2003 occurred in a year with high summer temperatures (especially August), prompting the suggestion that the problem was triggered by the weather. Several outbreaks occurred in May 2004 when the mean daily soil temperature was around 14ºC and the mean daily air temperature was around 12-13ºC indicating that high temperatures are not an essential requirement for occurrence of fusarium wilt.

18. References

Baker KF. 1948. Fusarium wilt of garden stock (*Mathiola incana*). *Phytopathology* **38**: 399-403.

Booth C. 1971. *The Genus Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, UK. Pp130-154.

19. Technology transfer

Publications

O'Neill TM & Green KR. 2005. Soil-borne fusarium: taking stock. *HDC News* **109**: 22-23.

O'Neill TM, Shepherd A, Inman AJ & Lane CR. 2004. Wilt of column stock (*Matthiola incana)* caused by *Fusarium oxysporum* in the United Kingdon. *Plant Pathology* **53**: 262.

O'Neill TM, Green KR & Ratcliffe T. 2005. Evaluation of soil steaming and formalin for control of fusarium wilt on column stocks. Proceedings VIth International Conference on Chemical and Non-chemical Soil and Substrate Disinfestation, Corfu, October 2004, p.63 (Abstract).

Presentations

- Diseases of stocks and their control. Presentation to South Holland Growers Club, Spalding, 19 January 2004 (Tim O'Neill).
- Fusarium wilt of stocks: research update. Presentation to South Holland Growers Club, Spalding, 29 November 2004 (Tim O'Neill).
- Alternatives to methyl bromide for cut flower growers. Presentations to growers at HDC Seminar, Arundel, 10 March 2005 (Tim O'Neill and Dan Drakes).

20. Acknowledgements

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APPENDIX 1

1. Experiment diaries

Testing soils from stocks nurseries for the presence of *F. oxysporum* pathogenic on stocks using plant baiting

Date	Activity
Dec 2003	Soils collected from 4 nurseries Soil Samples received from D. Davidson, Greenmont College, NI
19/07/04	Centum Red stocks planted into soil samples – 10 –15 plants per tray.
28/08/04	Plants destructively assessed for fusarium symptoms. Plants in some trays had been eaten by rabbits, so these were replanted.
18/10/04	Second batch of plants destructively assessed.

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

Date	Activity
02/06/04	Bag of fusarium infested soil prepared by mixing soil with *fusarium* infested debris. 3 trays of 15 stocks plants (Carmen Yellow) planted into clean soil and 3 trays of 15 stocks plants planted into infested soil were prepared (T0).
18/06/04	No symptoms on any plots
01/07/04	Suspected symptoms observed on plants growing in infested soil – symptoms assessed.
13/07/04	Control plants in clean soil assessed visually – all healthy. Second treatment (T1) set up 3 trays of 15 plants planted in infested soil prepared on 02/06/04. Var. Centum Red.
03/08/04	Assess second treatment (T1). Many plants completely brown and dead, most showed no discolouration – may have been rabbit eaten. Green plants showed some discolouration and severe bleaching.
02/09/04	Third treatment (T2) set up 3 trays of 15 plants in same infested soil and 3 trays of 15 plants in clean soil.
18/10/04	Assess third treatment (T2) – all plants were large and green. Some plants showed vein yellowing on lower half of plant. Stem discolouration barely noticeable. 5 Stem pieces per rep plated onto PDA+s.

Determining the inoculum level of *F. oxysporum* required to produce wilt in stocks – Experiment 1

Date	Activity
23/02/04	25 Plug plants of each of 'Opera' 1) Figaro Purple, 2) Francesca and 3) Carmen yellow collected from Stuart West's nursery.
24/02/04	Experiment set up in CE cabinet (23ºC, 16 h, 0% RH). 352g of sieved soil added to 9 cm pots, 1 ml of inoculum added in 29 ml SDW. One plant of each variety was planted in each pot. Pots

	were 15cm apart in CE cabinet.
26/02/04	Pots watered with 75 – 100 ml of distilled water
27/02/04	Pots watered with 75 ml water
01/03/04	Pots watered with 75 ml water (overhead)
02/03/04	All pots were moved to glasshouse 2 and watered. No disease seen. One plant (T1) dead – no vascular browning seen.
09/03/04	2nd disease assessment – no disease seen – most cotyledons were yellowing.
12/03/04	Signs of yellowing on cotyledons – including on uninoculated controls no signs of wilting.
01/04/04	Destructive harvest. Plants assessed then damp dishes set up of one leaf per plant per treatment. Stem pieces from inoculated control plated out onto PDA+s
05/04/04	*Fusarium oxysporum* reisolated from PDA+s plates
14/04/04	Assessed leaves in damp chambers.

Determining the inoculum level of *F. oxysporum* required to produce wilt in stocks – Experiment 2

Date	Activity
29/04/04	Experiment set up – 1 ml of each spore concentration 3.52 x10^{3,4,5,6,7} used was added with 25 ml SDW to 300g of soil. Three plugs (variety 'Opera' Francesca) were planted in each pot. Pots placed into CE cabinet.
06/05/04	Pots moved from CE cabinet to polytunnel 3 – no wilt symptoms seen.
13/08/04	2 week disease assessment – no symptoms seen.
19/05/04	3 week disease assessment – vein yellowing seen on lower leaves of all plots.
2/06/04	5 week disease assessment – no symptoms present, no wilting seen.
11/06/04	6 week disease assessment – Destructively checked plot 19 of treatment 6 – 1 plant of 3 showed vascular browning, no other symptoms present. One leaf of each plant from t6 taken and placed in a damp dish
18/06/04	7 week disease assessment – no visible symptoms. All plots fed miracle gro at recommended rate.
15/06/04	No fusarium seen on damp dished leaves
29/06/04	No fusarium seen on damp dished leaves
01/07/04	Final assessments on all plots, set up damp dishes
14/07/04	Damp dishes assessed – very little fusarium development. Stem pieces from 4 plants with discolouration scores of 2-3 were plated onto PDA+S to check for fusarium
19/07/04	Assessed plates

Determining the inoculum level of *F. oxysporum* required to produce wilt in stocks – Experiment 3

Date	Activity
7/05/04	Trial set up and placed in CE cabinet
10/05/04	Plants watered from base
11/05/04	Plant watered from base
13/05/04	Pots removed from CE cabinet and placed in polytunnel – no disease symptoms.
19/05/04	2 week disease assessment – no disease symptoms
28/05/04	3 week disease assessment – no disease symptoms
2/06/04	4 week disease assessment – no signs of wilt, yellowing present on lower leaves of all plots.
11/06/04	5 week disease assessment – no disease symptoms. Took 1 leaf per plant from T7 and placed in damp dishes.
15/06/04	No fusarium seen on damp dishes
18/06/04	6 week disease assessment – no symptoms, all plots fed miracle gro at recommended rate.
9/07/04	All plots destructively assessed, leaf and stem pieces taken and damp dished.
14/07/04	Damp dishes assessed – very little fusarium development. Stem pieces taken from 4 plants with stem discolouration scores of 2-3 and placed onto PDA+s
19/07/04	Plated stem pieces assessed.

Cross-pathogenicity 1

Date	Activity
06/05/04	Lisianthus cross pathogenicity trial set up and placed into CE cabinet
10/05/04	Plants watered from base
11/05/04	Plants watered from base
13/05/04	Plants removed from CE cabinet to polytunnel 1 week disease assessment – no symptoms
19/05/04	2 week disease assessment – no symptoms
24/05/04	3 week disease assessment – T5 (*F.o.* from lisianthus on lisianthus) all wilting, no symptoms in other treatments.
28/05/04	All T5 wilting some leaf bleaching and root discolouration samples taken for damp dishes and PDA+s. Lisianthus in other treatments fine. T3 (*F.o.* from stocks on stocks) showed pronounced vein yellowing and stunted growth. Stocks in other treatments fine.
1/06/04	Fusarium presence confirmed in damp dishes from T5
02/06/04	4 week disease assessment – T3 show yellow veining and wilting. T3 destructively sampled and stem discolouration found in all plants. Damp dishes set up for T3.
08/06/04	T3 damp dishes assessed
11/06/04	5 week disease assessment – no symptoms in remaining plots.
18/06/04	6 week disease assessment – no symptoms, all plots fed miracle gro at recommended rate.
9/07/04	Final destructive assessment – all plots damp dished.
14/07/04	Damp dishes assessed – no fusarium development, stem pieces

	scoring 1-3 for discolouration were plated onto PDA+s to check for fusarium.

Cross-pathogenicity 2

Date	Activity
19/07/04	Cross pathogenicity trial set up using stocks (purple Centum) and asters and stocks and radish with stocks fusarium. Placed into CE cabinet
25/07/04	Moved to polytunnel
08/04	Half trial eaten by rabbits
23/09/04	Final assessment

Cross-pathogenicity 3

Date	Activity
17/08/04	Aster cross pathogenicity trial set up using an aster *Fusarium* as well as the stocks *fusarium.* (Aster *fusarium* had very few spores therefore suspension only $1x10^6$ and a very small quantity. Stocks streated first, aster plugs large and therefore may not have been well treated.
24/08/04	Moved to polytunnel 2
24/09/04	Final destructive assessment – sections from each plant plated onto PDA+s
04/10/04	Plates assessed.

Fungicides 1

Date	Activity
13/05/04	28 blocks of 10 plugs prepared and placed in saucers in polytunnel Chemicals weighed out Single nozzle calibrated
14/05/04	First sprays applied to trial plots.
19/05/04	T2-7 inoculated with 1 ml spore suspension (1×10^6 spores/ml) No signs of *fusarium* wilt in any plot but lower leaves yellowing.
24/05/04	1 week disease assessment – No symptoms all plots yellowing. Watered with phosphogen.
28/05/04	Second fungicide treatment applied. No treatments effects visible, no symptoms present, lower leaf yellowing in all treatments.
2/06/04	No treatments effects visible, no symptoms present, lower leaf yellowing in all treatments.
11/06/04	No visible symptoms, leaf taken from each plant in T2 and placed in a damp dish to check for fusarium.
15/06/04	No *fusarium* on damp dished leaves
18/06/04	No visible symptoms of *fusarium*. All plants looking nutritionally challenged. Miracle gro applied to all plots at recommended rate.
12/07/04	Final destructive assessment – damp dishes set up.
03/08/04	Stem pieces removed from damp dishes and placed on PDA+s for those plots showing *fusarium* symptoms in initial assessments.
06/08/04	Stem pieces on PDA+s assessed for presence of *fusarium*.

Fungicides 2

Date	Activity
28/07/04	Chemicals weighed out
29/07/04	Trial set up and watered (Centum Purple)
30/07/04	Trial sprayed
06/08/04	Trial inoculated with *fusarium* concentration 1.25 x 10^6 spores/ml
13/08/04	Second fungicide spray applied All plants lower leaves were senescing but no visible difference between plots. No wilting.
9-10/09/04	Final destructive assessment
14-15/09/04	Stem pieces plated onto PDA+s
21/09/04	Plates assessed for *fusarium* presence

Methyl bromide

Date	**Activity**
04/10/04	Prepared and buries first 25 bags of stem pieces mixed with soil – dampened before burial.
18/10/04	Prepared and buries second 25 bags of stem pieces mixed with soil – dampened before burial.
1/11/04	Dug up buried samples and replaced soil with moist silica sand. Prepared 25 bags of fresh stem pieces in silica sand. Dampened all bags and placed three bags (one 4 week rotted, one 2 week rotted and one fresh) into each of 25 carrot sacks labels up.
3/11/04	Bags buried at commercial nursery – soil temp 14ºC, controls placed in neighbouring glasshouse
4/11/04	MeBr applied and covers put on
16/11/04	Covers removed and bags collected
17/11/04	Plating up onto PDA+s begun – completed on 19/11/04 – final rep sterilised with Sodium hypochlorite because no ethanol available.
25/11/04	First plate assessment
2/12/04	Second plate assessment – noticed more fusarium present on plates sterilised with sodium hypochlorite.

Basamid

Date	**Activity**
18/08/04	Stem pieces placed into bags – half in silica sand and stored on lab bench half in Rickwood soil and buried 5 cm below soil surface.
31/08/04	Stem pieces recovered from soil, soil sifted away and replaced with silica sand. All bags then dampened and placed into onion sacks. One rotted and one fresh in each sack.
1/09/04	30 cm samples buried and location marked. Basamid applied and spaded in. 15 cm and 0 cm samples buried after spading. Covers put on.
9/09/04	Covers removed and samples retrieved
14-15/09/04	Samples plated up on to PDA+s
27/09/04	First plate assessment
4/10/04	Second plate assessment

Formalin

Date	Activity
9/07/04	Fusarium infected stocks collected and cut into 2cm lengths
12/07/04	Gauze bags prepared each containing 10 stem pieces in moist silica sand – these were placed into carrot sacks.
14/07/04	Samples buried
15/07/04	Formalin treatment applied
16/07/04	Samples collected
22/07/04	Samples plated up
28/07/04	First plate assessment
03/08/04	Second plate assessment

Soil Steaming

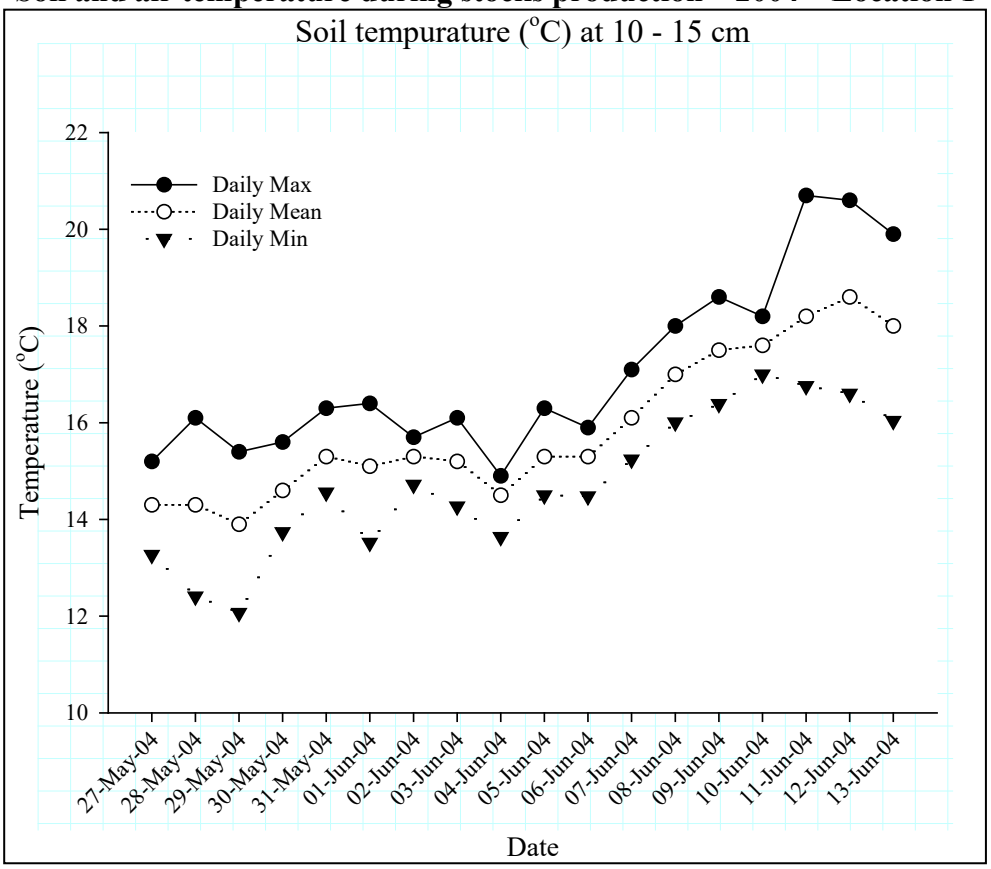
Date	Activity
9/07/04	Fusarium infected stocks collected and cut into 2cm lengths
12/07/04	Gauze bags prepared each containing 10 stem pieces in moist silica sand – these were placed into carrot sacks.
14/07/04	Steaming experiment 1 samples buried
15/07/04	Steam treatment applied
16/07/04	Samples collected
22/07/04	Samples plated up
28/07/04	First plate assessment
03/08/04	Second plate assessment
8/09/04	Set up second steam treatment experiment. Bed area spaded mechanically before trial set up. 2 x 4 depth temperature probes set up in different parts of the bed
12/09/04	Steam treatment applied
14/09/04	Remove probeds and sample bages
15/09/04	Plate up sterilised stem pieces on PDA+s and place in and incubator
22/09/04	First plate assessment
29/09/04	Second plate assessment

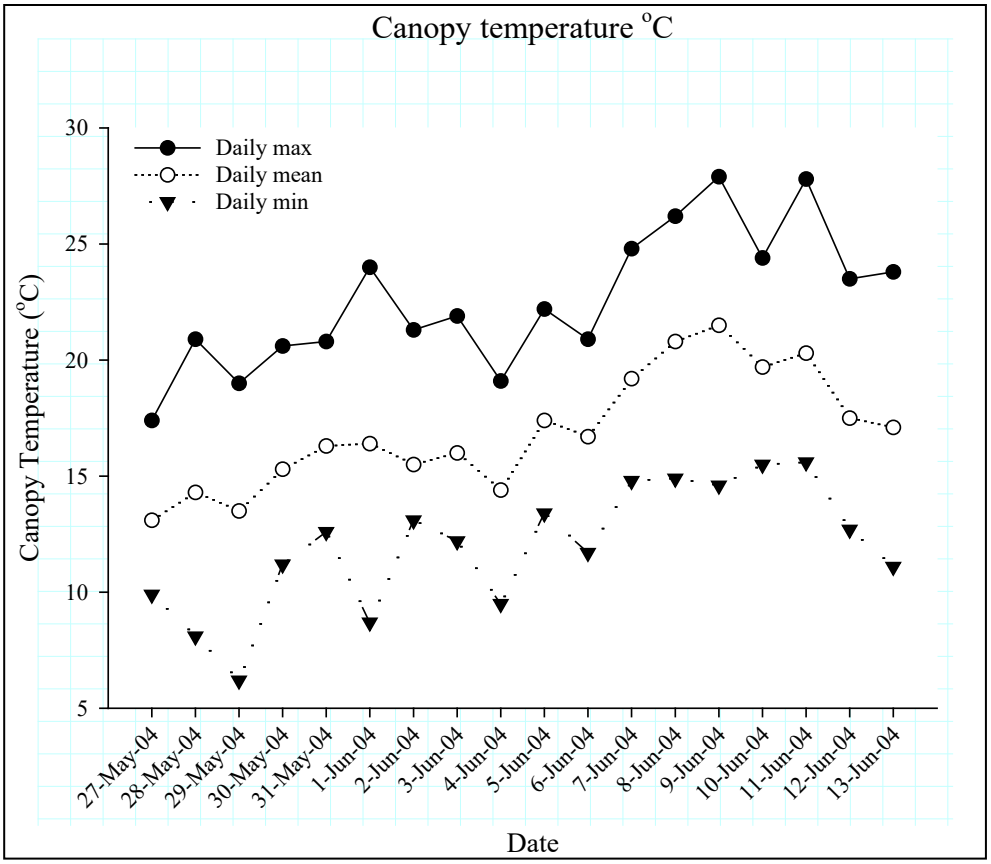
Boiling and oven

Date	Activity
10/08/04	Set up and treated all boiling and oven treatments
	Plated up immediately after removal from oven or water bath.
16/08/04	First plate assessment
24/08/04	Second plate assessment
1/10/04	Set up second identical boiling and oven experiment
	Plated up immediately
7/10/04	First plate assessment – expt 2

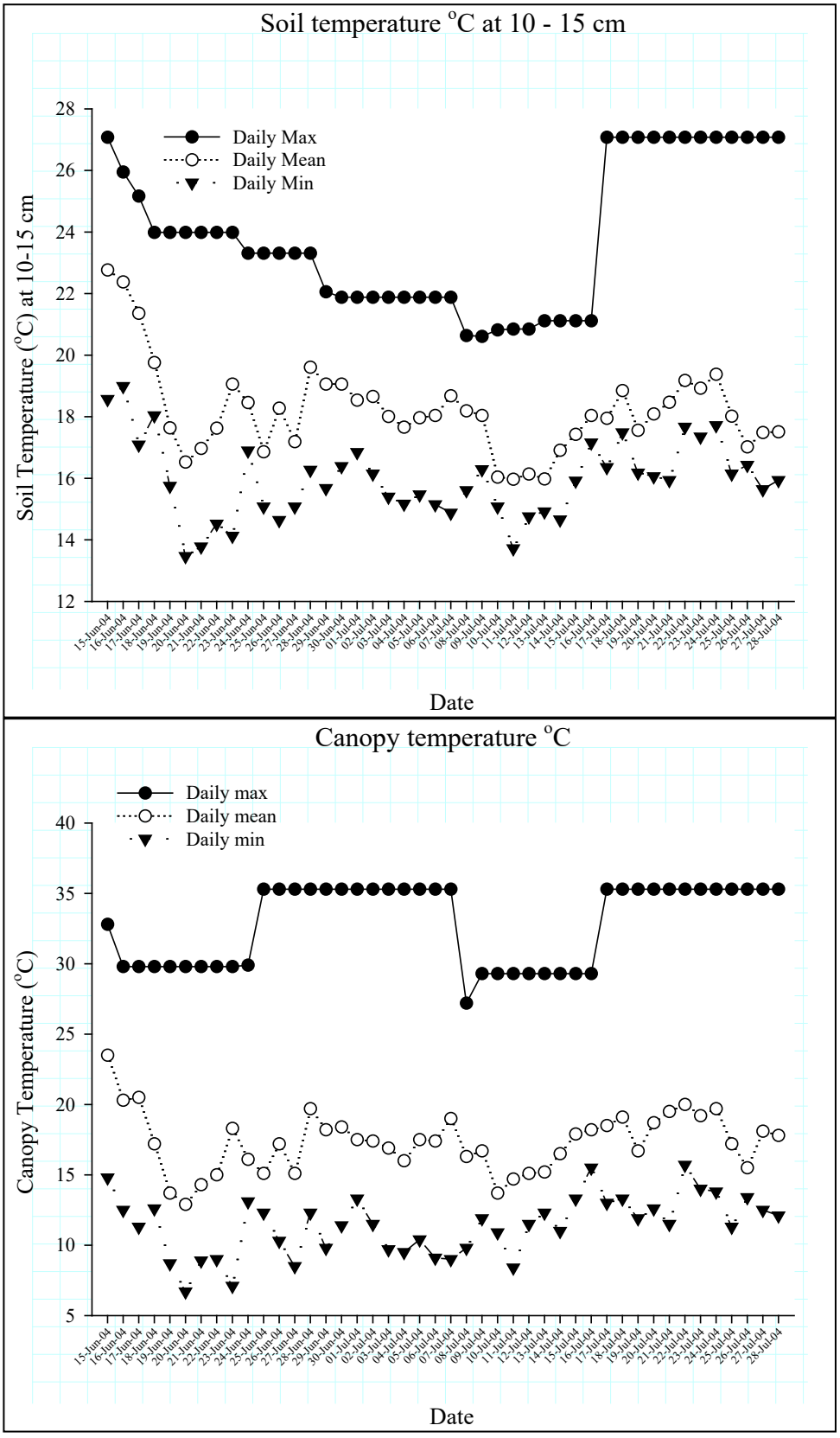
Appendix 2 Soil and air temperature during stocks production 2004

Soil and air temperature during stocks production – 2004 – Location 1

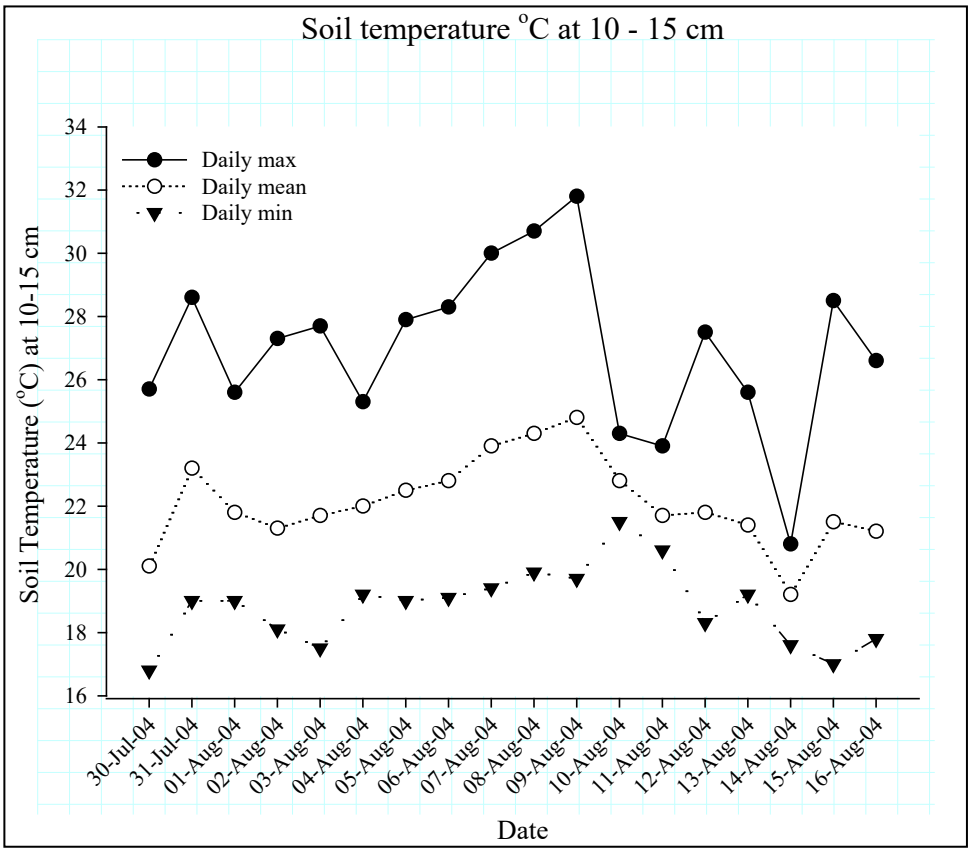




Soil and air temperature during stocks production – 2004 – Location 2



Soil and air temperature during stocks production – 2004 – Location 3



Soil and air temperature during stocks production – 2004 – Location 4