Grower Summary

PC 213 and PC 213a

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

Final report 2007

Contract report for the Horticultural Development Council

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

PC 213 and PC 213a

January 2007

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

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

AUTHENTICATION

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

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ADAS

Signature .... Date ....

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Report authorised by:

Dr W E Parker
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Grower Summary

Headline

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

Background and expected deliverables

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

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

The specific objectives of the project were:

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

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

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

Occurrence of fusarium wilt

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

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

Survival of F. oxysporum *in soil*

Stocks debris naturally infested with *F. oxysporum* was mixed with soil and placed in a glasshouse. Samples recovered at intervals were tested for viable *F. oxysporum* by planting with stocks and then assessing plants after approximately 6 weeks for fusarium wilt. Viable *F. oxysporum* sufficient to cause infection in all test plants remained in soil that had been stored for 16 months.

Occurrence of F. oxysporum *on seed*

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

Effect of F. oxysporum inoculum level on occurrence of wilt

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

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

Cross-infection risk

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

Typical and severe symptoms of fusarium wilt were only seen when a host plant was inoculated with *F. oxysporum* previously isolated from the same host plant (i.e. stocks inoculated with *F. oxysporum* from stocks, or lisianthus inoculated with *F. oxysporum* from lisianthus) (Table 1). However, *F. oxysporum* was recovered from roots of non-host crops indicating that they could act as a source of inoculum for subsequent stocks or lisianthus crops.

Table 1. Summary of results from cross-infection studies in 2005

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

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

Effect of temperature on growth of F. oxysporum

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

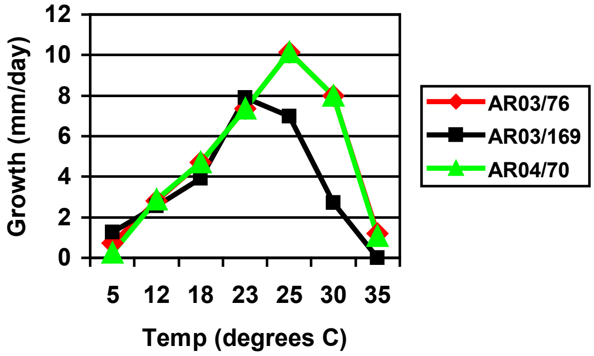


Figure 1. Effect of temperature on growth of three isolates of *F. oxysporum* from stock

Disinfectants

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

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

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

Fungicides

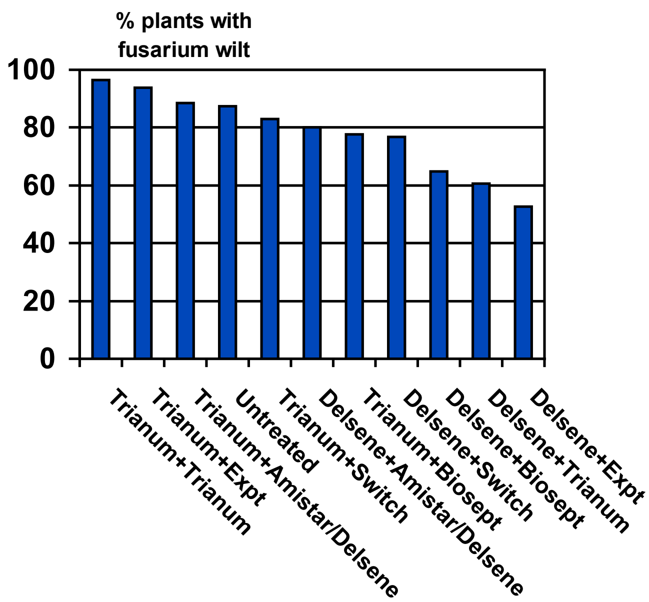
In 2005, ten fungicide programmes (a drench to plug plants followed by sprays at 1, 14 and 28 days after planting) were evaluated for their ability to control fusarium wilt in an artificially infested crop of stocks. At harvest (8 weeks after planting), there was no significant effect of treatment on the proportion of plants showing wilt symptoms, with more than 42% plants affected for all treatments. However, there was a trend for a lower incidence of internal stem symptoms for plants treated with a Delsene 50 Flo (carbendazim) drench followed by Delsene 50 Flo, Amistar, Octave or Experimental product 1, with a reduction from 62.5% to 45% or less. Carbendazim (as Bavistin DF) and azoxystrobin (Amistar) were also the most effective products for reducing fusarium wilt in stocks plug plants in a preliminary experiment. Phytotoxic effects were observed following applications with Swing Gold, Folicur, Biosept Gold and an experimental product.

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

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

In 2005, an experiment was done to determine whether the failure of carbendazim to give greater control of stocks fusarium wilt in fungicide trials in this project, and on commercial nurseries, was due to fungicide resistance. Fusarium isolates from stocks were tested for resistance to the fungicide Delsene 50 Flo at three concentrations of the active ingredient carbendazim. All of the isolates of fusarium were sensitive to carbendazim with mycelial growth strongly though not completely inhibited by the fungicide, even at a very low concentration (2 ppm). These results suggest that failure of carbendazim to give effective control of fusarium wilt on stocks is not due to fungicide resistance.

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



Soil disinfestation

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

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

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

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

On a site where conventional sheet steaming for 12 hours gave poor results, vacuum steaming for just 4 hours raised the soil temperature to above 90^{o}C at 23.5 cm depth and to above 80^{o}C at 33.5 cm depth (Figure 1.5). The cost for burying perforated plastic pipes to enable vacuum steaming is around £4.70/metre, or £27,000/ha at a pipe spacing of 1.6 m. Fuel consumption for vacuum steaming is estimated to be around 57% of that required for conventional sheet steaming.

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

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

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

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

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

*lisianthus stem pieces

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

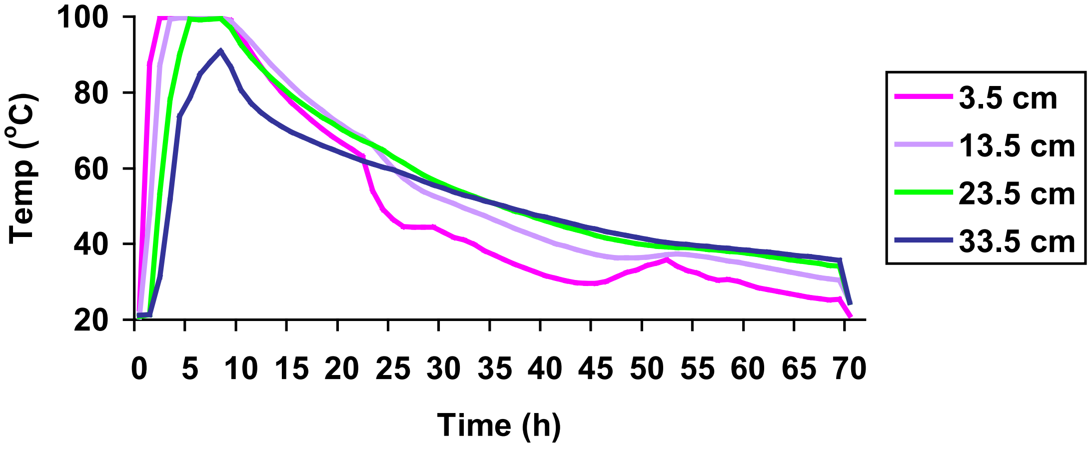


Figure 4. Mean temperatures achieved at different depths below the soil surface during injection of steam by steam plough – Norfolk, June 2006

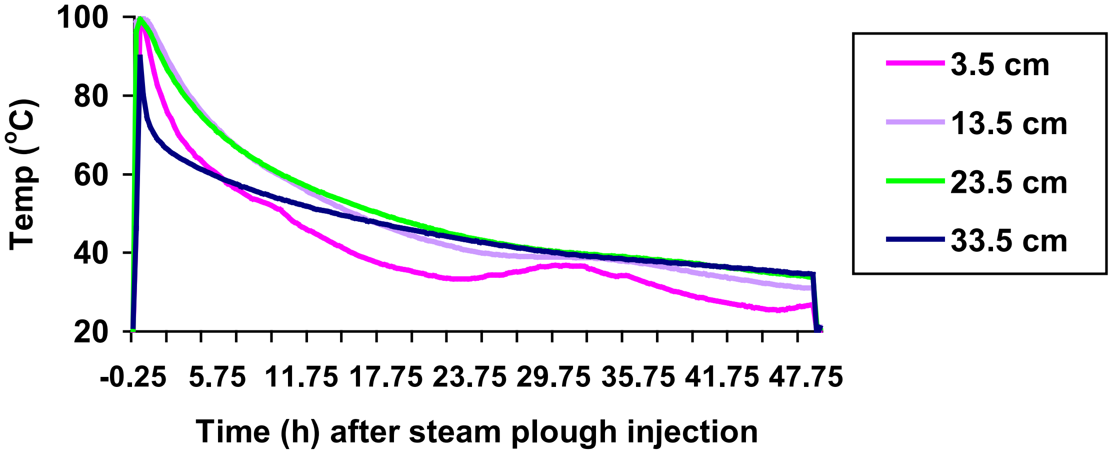
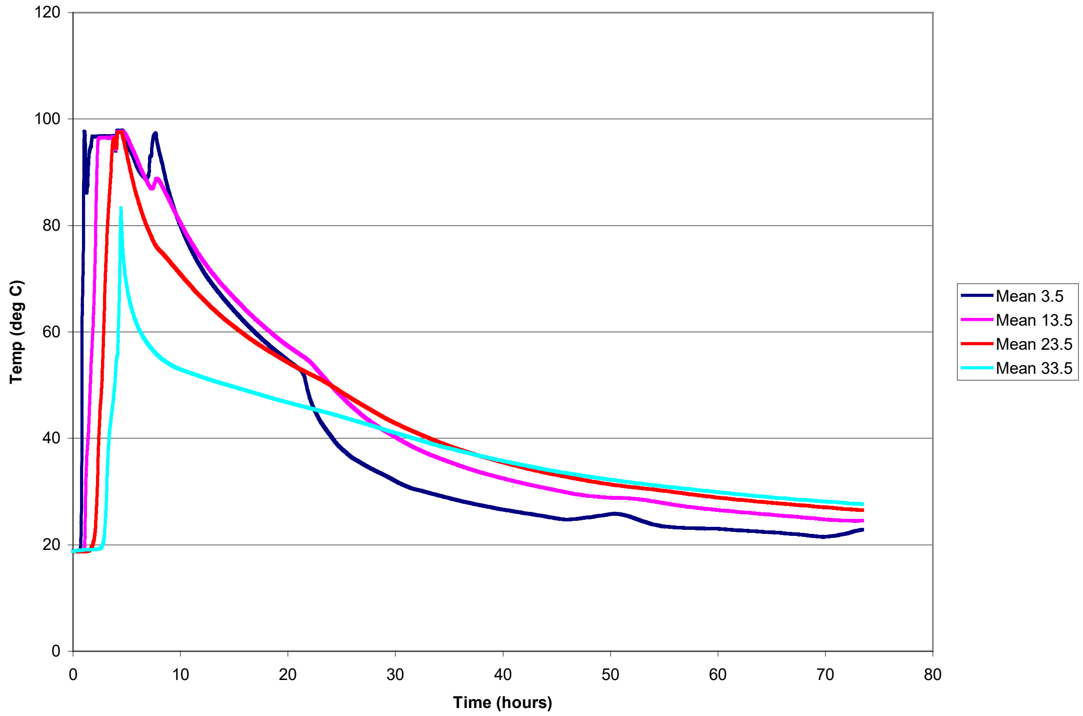


Figure 5 Mean temperatures achieved at different depths during vacuum steaming (Suffolk, 2006)



Financial benefits

Annual production of stock in the UK is estimated to be around 18 million stems, representing around 23 ha of crop. Assuming a return of 17p per stem, the annual UK crop production is worth around £3.1 million. The benefit to the industry from this project is continued profitable production of stock despite the threat of fusarium wilt through a greater understanding of disease biology and identification of effective soil disinfestation treatments.

Action points for growers

Identification of fusarium wilt

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

Reducing the risk of persistence between crops and disease spread

- Take measures to reduce disease risk after an outbreak of fusarium wilt (see below). Fusarium can persist in plant debris buried in soil for at least 16 months and there is a high risk that the disease will re-occur if stocks are planted in an area where the disease was severe in the previous crop of stocks.

- If only a few plants are affected by wilt, and they are accessible, carefully remove them from the crop (bag them *in situ*) as soon as possible, before fusarium sporulation occurs on the lower leaves and stem.
- At the end of a crop, take care to remove as much crop debris as possible, and as soon as possible, before preparing the land for the next crop. Woody stem bases in particular pose a high risk as fusarium may survive in these despite soil disinfestation treatment.
- Disinfectants can be used to eliminate fusarium from pathways, glasshouse structures machinery and equipment (e.g. spikes used to push up plugs). Unifect G used at the recommended rate for a 30 minute treatment is effective against both spores and mycelium of *F. oxysporum* from stocks. Information on the use of disinfectants can be found in HDC Factsheet 15/05.
- Clean and disinfect surfaces where trays of plug trays are stood before planting out.

Control of fusarium wilt: soil treatment

- Basamid, Formalin drench, Discovery, K & S Chlorofume (applied in drip-line irrigation water) and soil steaming all give some control of fusarium in woody stem pieces. Efficiency of these treatments is likely to be improved by reducing the inoculum of fusarium in soil (see above), and by encouraging rapid breakdown of any crop debris incorporated.
- Where Basamid, Discovery or K & S Chlorofume are used, conduct a cress seed germination test to ensure all fumes have dissipated before re-planting.
- Where Formalin is used, follow the Commodity Substance Approval (maximum rate of 0.5 L/m^2 at a minimum dilution of 1:4 in water). Note that the cress seed germination test does not work for Formalin. Any Formalin remaining in the soil may damage the growth of the subsequent crop. The rate of Formalin breakdown depends on soil temperature. Persistence is reported to be 5 to 6 weeks at 0 to 5^{o}C soil temperature, and 7 to 8 days at 25^{o}C soil temperature.
- Where sheet steaming is used, the soil should be fairly dry and cultivated to a coarse tilth (no lumps more than 5 cm diameter). Take care not to cultivate deeply after steaming, or to recontaminate the soil from dirty boots, equipment or unsteamed soil.
- The effectiveness of sheet steaming is profoundly influenced by soil type and requires attention to detail for good results. Consider vacuum steaming if steam penetration is poor.

- Steaming soil with a steam plough can also be effective against soil-borne fusarium. It is more time-consuming than sheet-steaming and requires constant attendance, but is also more fuel efficient; it is best on sandy loam soils.
- Use of soil amendments prior to planting may reduce the incidence of fusarium wilt, although application can be costly. This area of work warrants further research for the future.

Control of fusarium wilt: fungicides

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

Integrated control

- There is no simple, single measure that will provide reliable control of fusarium wilt. However, by thorough application of a soil disinfestation treatment which is suitable for your soil, use of carbendazim fungicide treatment at planting, prompt removal of affected plants (if only a few are affected), minimising crop debris incorporated into the soil, and general good hygiene, it should be possible to keep the disease at a very low level.
- Pay particular attention to control of fusarium in houses where the disease occurred the previous year, or where the ground was double-cropped.

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

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