Project title:	Hebe: aspects of the biology and control of Fusarium wilt
Project number:	HNS 146
Report:	Year 2 Annual Report
Previous reports:	Annual Report, April 2007
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Date project commenced:	1 April 2006
Date completion due:	31 March 2009
Key words:	Hebe, *Fusarium oxysporum*, wilt, sources, fungicides, biological treatments

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GROWER SUMMARY

Headline

Fusarium wilt of Hebe in the UK is caused by a host-specific strain of *Fusarium oxysporum*. Varieties differ in susceptibility to the disease so the disease can be managed by varietal choice and also by maintaining stock plants free of *F. oxysporum* and fungicide drenches to the growing medium. Incidental control may be afforded by the use of disinfectants for non-disease specific general hygiene purposes.

Background and expected deliverables

In 2005, *Fusarium oxysporum* was consistently isolated from stained vascular tissue of container-grown hebe plants affected by wilt and dieback. A vascular wilt disease of Hebe caused by *F. oxysporum* was first described in Europe in 2000 (in Italy) and it was considered that this might be the same problem. Hebe is a very popular garden plant and the occurrence of a new wilt disease could severely damage sales. By the start of this project, the problem had been recognised on one nursery, where it had been a continuing problem for several years. In 2005 it caused losses of over 15,000 plants.

The objectives of this project are:

1. To determine whether *F. oxysporum* is a cause of Hebe wilt in the UK;
2. To investigate aspects of the disease biology and spread;
3. To devise an effective control strategy.

Summary of the project and main conclusions (year 2)

Specificity of F. oxysporum in hebe

Young plug plants of Hebe cvs Pink Pixie and Purple Pixie and stock cv. Carmen were each inoculated with two strains of *F. oxysporum* obtained from Hebe plants affected by Fusarium wilt, and a strain obtained from stock (*Matthiola incana*) plants affected by Fusarium wilt. The plants were inoculated by dipping roots in a standardised spore concentration and then potted into new 9 cm plastic plant pots and grown in a heated glasshouse at around 25° C. Both strains of *F. oxysporum* obtained from Hebe only caused Fusarium wilt symptoms in Hebe, and the *F.*

oxysporum strain obtained from stock only caused Fusarium wilt symptoms in stock. These results suggest that the strain of *F. oxysporum* causing wilt in Hebe plants in the UK is a host-specific pathogen. It is unlikely that the fungus will readily cause a vascular wilt in herbaceous or nursery stock species unrelated to Hebe. It is also unlikely that *F. oxysporum* isolates causing vascular wilt diseases in other hosts, such as stock, will readily cause a vascular wilt in Hebe. In this experiment the latent period between inoculation and symptom development was 9 weeks.

Varietal susceptibility

All of six Hebe varieties inoculated with *F. oxysporum* from Hebe developed symptoms of Fusarium wilt. At 16 weeks after inoculation, the incidence of Fusarium wilt was significantly greater in Pink Pixie (35% of plants affected) than in Caledonia (5%), Rosie (8%) and Pascal (18%); levels were relatively high in Purple Pixie (25%) and Pink Paradise (30%).

Evaluation of fungicide and biological treatments

Four fungicides including 'Amistar' (asoxystrobin), 'Delsene 50 Flo' (carbendazim), 'Scotts Octave' (prochloraz) and an experimental material, as well as six biological treatments (composted pine bark incorporated into the growing medium, 'Trianum P 'drench, two experimental biocontrol agents, 'Mycoplex' granules incorporated at potting and 'Turf Vigour Special' applied as a drench) were evaluated for control of Fusarium wilt in container-grown hebe in a replicated experiment in a heated glasshouse. Fusarium wilt was first observed 10 weeks after inoculation and at the end of the experiment 23% of untreated plants were wilted or dead. 'Scotts Octave' significantly increased the number of surviving plants (i.e. not wilted or dead) (See Fig. 1). None of the other treatments had a significant effect. 'Amistar' drench treatment resulted in stunted growth.

Disinfection of sand

Three disinfectants ('Jet 5' at 2%, 'Horticide' at 0.08% and 'Unifect G' at 4%) were tested for their ability to eliminate *F. oxysporum* from sand. Sand was infested by inoculation with a suspension of *F. oxysporum* spores two weeks before drench treatment with the disinfectants. After fumes had dissipated, sand was tested in the laboratory by plating onto agar to determine levels of *F. oxysporum* infestation. All three disinfectants significantly reduced levels of *F. oxysporum*. 'Unifect G' at 4%

was the most effective and no *F. oxysporum* was recovered from sand treated with this product. Treated sand was mixed with a peat-based growing medium and used to grow Hebe plants for 12 weeks. Only a very low incidence of Fusarium wilt occurred and there were no significant differences between treatments.

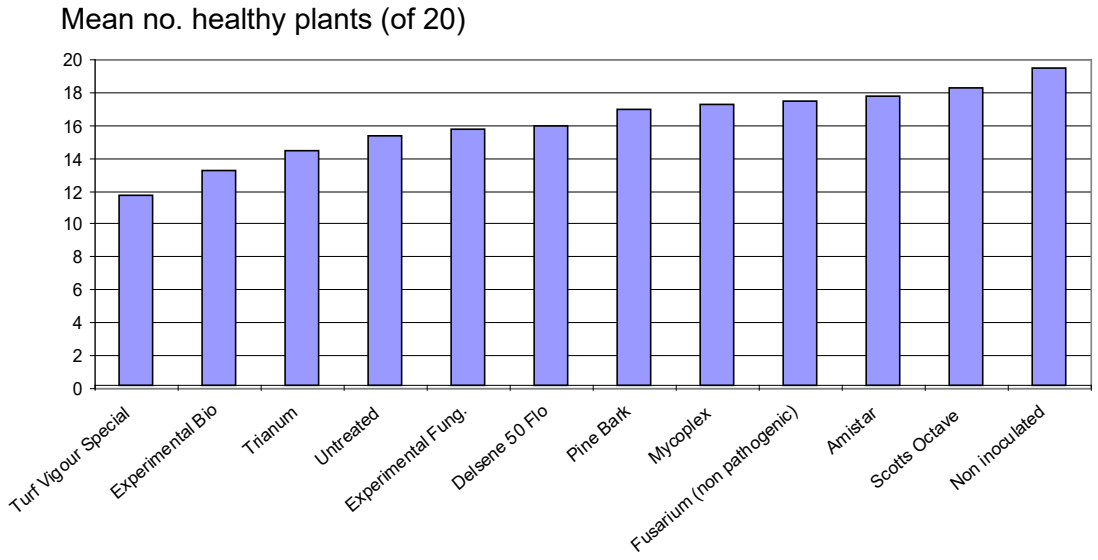

Fig. 1: The effect of fungicides and biological treatments on Fusarium wilt in Hebe - 2007

Financial benefits

Losses due to Fusarium wilt of Hebe on one nursery were at least £30,000 in 2005 and further substantial losses occurred in 2006. As the project progresses it is anticipated that an increased understanding of the disease will allow a reliable control strategy to be devised.

This disease is new to the UK and appears at present to be restricted in occurrence. If it can be controlled in the near future, the potential financial benefit is huge because widespread Fusarium wilt in garden centres or home gardens could severely damage the image of Hebe and subsequent sales.

Action points for growers

- Growers should familiarise themselves with the symptoms of Hebe Fusarium wilt.

- Note that Hebe Fusarium wilt could initially be confused with downy mildew. If in doubt, contact a plant pathologist or submit a sample to a Plant Clinic.
- When selecting a disinfectant for use as part of your general nursery hygiene/clean up programme, it makes sense to select a product which is also known to have activity against plant pathogens of concern.
- For effective hygiene it is important to disinfect standing areas thoroughly and not just wet the surface.
- With the transfer of responsibility of the Biocides Directive to the HSE, biocides for use in crop production must be registered as Plant Protection Products.
- Although thebiocides 'Unifect G', 'Horticide' and 'Jet 5' are known to have activity against Fusarium, they are unfortunately not approved as Plant Protection Products and can therefore not be used for this purpose.
- Check the health of stock plants before taking cuttings; symptomless, systemic infection by *F. oxysporum* can occur within plants.
- Where feasible, maintain growing temperatures below 20°C; there is evidence that Fusarium wilt is favoured by temperatures around 25°C.
- Many varieties of both large leaf and dwarf forms of Hebe are susceptible to Fusarium wilt. The varieties Pink Paradise, Pink Pixie and Purple Pixie are more susceptible than Caledonia or Rosie.

SCIENCE SECTION

Introduction

In year 1 of this project, *Fusarium oxysporum* was confirmed as the cause of Hebe Fusarium wilt in the UK. The disease was found in a wide range of varieties on one nursery and in a single variety on a second nursery. Young plug plants of Hebe developed symptoms 3 – 15 weeks after dipping roots in a spore suspension of the fungus; it was not necessary to artificially wound roots in order for infection to occur. Growth of *F. oxysporum* in culture was greatest at 25°C and there was some evidence that infection of plants was also greatest at this temperature. *F. oxysporum* was recovered from roots, stem bases and shoot tips of visibly healthy plants indicating a symptomless stage of systemic infection; the use of cuttings from such plants could perennate the disease on a nursery. The fungus was detected in sand taken from a sand bed where infected plants had been and also in once-used pots. In both, the pathogen was present at levels sufficient to cause Hebe Fusarium wilt.

The objectives of work in year 2 were:

- To investigate the specificity of *F. oxysporum* in Hebe;
- To determine the relative susceptibility of some commonly grown varieties;
- To investigate a range of fungicide and biological treatment with potential for control of the disease;
- To evaluate three disinfectants for elimination of *F. oxysporum* from sand.

Specificity of *Fusarium oxysporum* to Hebe

Introduction

Many strains of *F. oxysporum*, notably those responsible for vascular wilt diseases, are host-specific, i.e. they cause disease in only one or a few closely-related plant species. Such strains are generally given the host species name as a specific epithet. For example, Fusarium wilt of stock (*Matthiola incana*) is caused by *F. oxysporum* f. sp. *mathiolae* and fusarium wilt of tomato is caused by *F. oxysporum* f. sp. *lycopersici*. An experiment was devised to test the host-specificity of *F. oxysporum* isolated from hebe.

Materials and methods

Young plug plants of Hebe cv. Pink Pixie and stock cv. Carmen were each inoculated with two strains of *F. oxysporum* obtained from hebe (AR05/195 from a plant in Worcs; AR06/136 from a plant in Yorks) and with a strain obtained from stock (AR04/70 from a plant in Lincs). Plants were inoculated by dipping roots for 5 minutes in a suspension containing 1 x 10^6 spores/ml of sterile distilled water (SDW). Plants were then potted into Levington M3 compost in 9 cm pots and grown in a heated glasshouse for 9 weeks. There were 10 plants per treatment. The plants were examined weekly for symptoms of Fusarium wilt. All plants were examined for brown staining of vascular tissue in the stem base, a symptom usually found in plants with Fusarium wilt, after 10 weeks. Samples of stained vascular tissue were plated onto potato dextrose agar (PDA) to determine the cause of staining.

Results and discussion

Fusarium wilt symptoms were first observed in stock after 3 weeks and in Hebe after 9 weeks. At 10 weeks after inoculation, shoot wilting symptoms were observed in Hebe plants inoculated with each of the strains of *F. oxysporum* isolated from Hebe, and not with the strain isolated from stock. Fusarium wilt symptoms in stock were observed in plants inoculated with the strain of *F. oxysporum* obtained from stock and not with either of the strains obtained from Hebe (Table 1.1).

Table 1.1: Ability of three strains of *F. oxysporum* to cause wilt symptoms in Hebe and stock (2007)

Treatments		Hebe (10 plants)			Stock (10 plants)		
		No. plants wilted	No. plants with stem browning	No. from which fusarium recovered	No. plants wilted	No. plants with stem browning	No. from which fusarium recovered
1.	Water (control)	0	0	0	0	0	0
2.	*F. oxysporum* ex Hebe (Worcs)	3	6	10	0	0	0
3.	*F. oxysporum* ex Hebe (Yorks)	3	2	3	0	0	0
4.	*F. oxysporum* ex Stock (Lincs)	0	0	0	7	0	10

These results suggest that the strain of *F. oxysporum* causing wilt in Hebe plants in the UK is specific to Hebe. It is unlikely that this strain will cause vascular wilt disease in other nursery stock or herbaceous species that are unrelated to Hebe. The results also suggest that other host-specific strains of *F. oxysporum*, such as *F. oxysporum* f. sp. *mathiolae,* are unlikely to cause wilt in Hebe. The original report of Fusarium wilt in Hebe in the USA named the species as *F. oxysporum* f. sp. *hebae*. It appears appropriate to use the same name for the strain causing Hebe wilt in the UK.

Varietal susceptibility

Introduction

Collation of plant numbers lost to Fusarium wilt on a commercial nursery indicated that many varieties of Hebe are susceptible to the disease, and that the variety Pink Pixie was particularly susceptible. An experiment was devised to determine if the observed greater losses in Pink Pixie were due to chance or reflect a greater inherent susceptibility of this variety.

Materials and methods

Crop details

Plug plants of six Hebe varieties (Caledonia, Pascal, Pink Paradise, Pink Pixie, Purple Pixie and Rosie) were obtained from a commercial nursery. Plants were either potted directly, or inoculated with an isolate of *F. oxysporum* obtained from Hebe, by dipping roots in a suspension of spores (5 x 10^5 spores/mL) for 15 minutes, and then potted on 13 February 2007. Plants were grown in Levington M3 compost in 9 cm pots on capillary matting in gravel trays in a heated glasshouse. Dead plants were removed as they occurred.

Assessments

Plants were examined for symptoms of Fusarium wilt (shoot tip yellowing and wilting) at regular intervals, and full assessments were done at 8, 12 and 16 weeks after inoculation. At the final assessment, all plants were also examined for vascular staining at the stem base.

Statistical design and analysis

The experiment was a factorial design arranged in four randomised blocks. Each plot contained 10 plants in a gravel tray. Results were examined by analysis of variance.

Results and discussion

Symptoms of Fusarium wilt first occurred after 5 weeks in one inoculated plant on cv. Pink Pixie. Inoculation with *F. oxysporum* had a significant effect on the incidence of affected plants after 8 and 12 weeks (Table 2.1). By 16 weeks after inoculation the disease was present in all varieties and there was a significant variety x inoculation interaction. At this time, the incidence of plants with wilt symptoms significantly in

Pink Pixie (35%), Pink Paradise (30%) and Purple Pixie (25%) than in Rosie (8%) or Caledonia (5%) (Table 2.1). The occurrence of vascular browning in the stem base closely matched the occurrence of Fusarium wilt symptoms (Table 2.1).

These results confirm nursery observations that varieties differ in susceptibility to Fusarium wilt.

During this project the disease has been confirmed on a wide range of varieties additional to those inoculated in this experiment (see Year 1 Annual Report).

Table 2.1: Relative susceptibility of six Hebe varieties to Fusarium wilt

Factor	Mean number plants (of 10) with Fusarium wilt at:			Mean N° plants (of 10) with vascular browning at 16 weeks
	8 weeks	12 weeks	16 weeks	
Inoculated				
Inoculated (I)	0.3	1.0	2.0	2.4
Uninoculated (U)	0.0	0.1	0.2	0.3
Significance	0.044	0.001	<0.001	<0.001
LSD (33 df)	0.24	0.53	0.59	0.60
Variety				
Caledonia	0.1	0.1	0.3	0.9
Pascal	0.1	0.1	0.9	1.3
Pink Paradise	0.0	0.6	1.9	2.1
Pink Pixie	0.4	1.1	1.8	1.9
Purple Pixie	0.4	0.9	1.4	1.4
Rosie	0.0	0.4	0.5	0.5
Significance (F test)	NS	NS	0.011	0.033
LSD	-	-	1.01	1.04
Inoculation x variety				
I x Caledonia	0.3	0.3	0.5	1.5
Pascal	0.3	0.3	1.8	2.3
Pink Paradise	0.0	1.0	3.0	3.5
Pink Pixie	0.8	2.3	3.5	3.8
Purple Pixie	0.5	1.5	2.5	2.5
Rosie	0.0	0.8	0.8	0.8
U x Caledonia	0.0	0.0	0.0	0.3
Pascal	0.0	0.0	0.0	0.3
Pink Paradise	0.0	0.3	0.8	0.8
Pink Pixie	0.0	0.0	0.0	0.0
Purple Pixie	0.3	0.3	0.3	0.3
Rosie	0.0	0.0	0.3	0.3
Significance (F test)	NS	NS	0.038	0.054
LSD	-	-	1.44	1.47

Evaluation of fungicides & biological treatments

Introduction

An experiment was devised to test a range of fungicide and biological treatments, shown to provide some control of Fusarium diseases in other crops or have activity against *F. oxysporum*, for control of Hebe Fusarium wilt.

Materials and methods:

Crop details

Plug plants, cv. Pink Pixie obtained from a commercial nursery, were potted on 24 July into 9 cm diameter pots in Levington M3 compost and grown on capillary matting in gravel trays in a heated glasshouse. The growing medium was kept moist for one week after potting and then subsequently watered by hand when required. Day and night temperatures were set on 11 September to 15° C and shade screens were programmed to come on during the night and off during the day. Day temperature was then increased on 20 September to 25° C to encourage *Fusarium* growth, and on 2 October night temperature was increased to the same level. Daily maximum and minimum temperatures were recorded throughout using data loggers.

Inoculation

Plants were inoculated with spores obtained from two strains of *F. oxysporum* isolated from affected UK Hebe plants in 2005 (AR05/195) and 2007 (AR07/92). A 50 mL aliquot spore suspension was applied as a drench to each pot, apart from the uninoculated control, on 1 August 2007, to give a total application of 1 x 10^6 spores per pot.

Experimental design and statistical analysis

The experiment was a randomised block design with four-fold replication. Each plot contained 20 plants, with 10 in each of two gravel trays. There was eight-fold replication of the inoculated control. Results were examined using analysis of variance.

Treatments

a) Fungicides

Treatment no.	Product
T1	Untreated inoculated control
T2	'Delsene 50 Flo' (carbendazim) at 1.0 mL/litre (SOLA 1004/2004)
T3	Experimental fungicide at 1.4 g/litre
T4	'Amistar' (azoxystrobin) at 1.0 mL/litre (SOLA 1684/01)
T5	Scotts Octave (prochloraz Mn) at 1.0 g/litre

b) Biological treatments

Treatment no.	Product
T6	30% composted pine bark (Melcourt) incorporated once at 3 weeks before potting
T7	Trianum P (*T. harzianum* T-22) applied as a drench at 0.3 g/litre of water, using a drench of 10% pot volume/pot. Compost treated 1 week before inoculation and again 10 weeks later
T8	Experimental biological product incorporated once at 250 g/m^3 of compost (0.25 g/litre)
T9	Turf Vigour Special applied at 5 mL/Litre every 14 days as a drench to pot capacity
T10	Mycoplex granules incorporated into compost at 2 g/litre at potting
T11	Non-pathogenic fusarium (F047) applied as a spore suspension mixed into the compost one week before potting
T12	Non-inoculated control

All fungicide drench treatments (T2, T3, T4, T5) were applied one day after potting with repeat applications after 4, 8, 12 and 16 weeks. On 20 July, 7.5 g of CF–140-07 (T8) was mixed with 30 L growing medium, and 3 L FO47 spore suspension was mixed into 30 L compost (T11). Melcourt Growbark Pine (a fine matured pine bark) for treatment 6 was incorporated three weeks before potting, on the 6 July, as a mixture of 30% composted pine bark (Melcourt Fine) with 70% M3 compost, with 9 g Nitram added to compensate for nitrogen immobilisation. T9 drench was applied at 14 day intervals (8 treatments in total).

Disease and plant quality assessment

The crop was assessed for wilt symptoms and occurrence of dead plants at 3, 7, 11, 16, 18 and 19 weeks after inoculation. On 6 December three plants were removed from each treatment, assessed destructively for vascular staining at the stem base and then tested in the laboratory by isolation on PDA+S to confirm presence of *F. oxysporum*.

Plants were also assessed for plant quality:

1 – unsaleable, dead or dying
2 – unsaleable, very poor growth
3 – saleable, second class, some yellowing or stunted growth
4 – saleable, good plant
5 – saleable, excellent quality plant

On 13 December, all remaining plants were assessed destructively by cutting stems longitudinally on two sides at the stem base and recording occurrence of dark brown vascular staining (0 = no vascular staining, 1 = staining one side of stem, 2 = staining on two sides of stem). The number of surviving healthy plants (i.e. not wilted and not dead) was also recorded.

Results and discussion

Control of Fusarium wilt

Symptoms of Fusarium wilt were first observed on 11 October, 10 weeks after inoculation. At 19 weeks after inoculation, 23% of untreated plants were dead or showing symptoms of Fusarium wilt (Table 3.1). The incidence of plants with Fusarium wilt and vascular staining was significantly reduced by treatments 2-7 and 10-11. However, when other symptoms and dead plants were accounted for, only 'Scotts Octave' (T5) significantly increased the number of surviving healthy plants, from 15.4 to 18.3.

Mean weekly glasshouse temperature during the experiment ranged from 14.4 to 26.4°C; it was above 20°C for most of the experiment (Appendix 1). Results of interim disease assessments are given in Appendix 2.

Plant quality

Plant quality (measured on a 0-5 index) was greatest in plants grown with composted bark in the growing medium (T6) and in the uninoculated control (T12), and least following treatment with 'Amistar' (T4) (Table 3.2). Differences were not quite significant at the 5% level. 'Amistar' resulted in stunting of plants, first noted after the third drench treatment.

Occurrence of vascular browning and recovery of *F. oxysporum*

There was a high occurrence of stem base vascular browning in wilted plants, and a high level of recovery of *F. oxysporum* from wilted and dead plants (Table 3.3).

Table 3.1: Effect of fungicides and biological treatments on occurrence of Fusarium wilt, vascular staining and dead plants at 19 weeks after inoculation

No.	Treatment	Mean no. of plants (of 20)					Mean % healthy
		Wilted only	Vascular stain only	Wilt + vascular stain	Dead	Healthy	
T1	Untreated	0.0	1.5	1.8	1.4	15.4	77
T2	'Delsene 50 Flo'	0.3	1.0	0.3	2.5	16.0	80
T3	Expt. Fungicide	0.3	1.0	0.5	2.5	15.8	79
T4	'Amistar'	0.3	0.5	0.5	1.0	17.8	89
T5	'Scotts Octave'	0.3	0.5	0.3	0.8	18.3	92
T6	Pine bark	0.3	1.3	0.5	1.0	17.0	85
T7	'Trianum P'	0.3	1.3	0.8	3.3	14.5	73
T8	Expt. Biocontrol	0.3	1.3	2.0	3.0	13.3	67
T9	'Turf Vigour Special'	1.3	2.3	2.3	2.5	11.8	56
T10	'Mycoplex'	0.3	0.5	0.5	1.5	17.3	87
T11	Non-pathogenic Fusarium	0.3	1.0	0.3	1.0	17.5	88
T12	Uninoculated control	0.0	0.3	0.0	0.3	19.5	98
Significance		NS	NS	0.014	NS	0.001	
LSD between treatments		-	-	1.44	-	3.25	
LSD vs. control		-	-	1.24	-	2.82	

Table 3.2: Effect of fungicides and biological treatments on plant quality 18 weeks after inoculation– 2007

Treatment		Mean plant quality (0-5 index)	Number saleable plants (of 20) (quality score 3-5)
T1	Untreated	3.7	16.6
T2	'Delsene 50 Flo'	3.7	16.5
T3	Expt fungicide	3.6	16.5
T4	'Amistar'	2.8	14.0
T5	Scotts Octave'	3.6	16.3
T6	Pine bark	4.0	18.3
T7	'Trianum P'	3.3	15.0
T8	Experimental biocontrol	3.0	14.3
T9	'Turf Vigour Special'	3.2	13.8
T10	'Mycoplex'	3.7	17.8
T11	Non-pathogenic Fusarium	3.6	18.3
T12	Uninoculated control	4.0	19.5
Significance		0.059	0.068
LSD between treatments		0.77	3.83
LSD vs. control		0.67	3.32

Table 3.3: Recovery of *F. oxysporum* from the stem base of Hebe plants with different symptoms at 18 weeks after inoculation

Symptom	Occurrence of vascular browning (% plants)	Recovery of *F. oxysporum* (% plants)
No symptoms	0	23
Wilted	83	75
Dead	100	77

As in most other experiments in this project, there was a long latent period (10+ weeks) between inoculation with *F. oxysporum* and occurrence of Fusarium wilt symptoms. The incidence of dead and wilted plants at 19 weeks after inoculation was relatively low at 23%. Only one of the chemical and biological treatments ('Scotts Octave') had a statistically significant beneficial effect on control of the disease. Several other treatments appeared to reduce the disease. In future experiments evaluating treatments for control of this disease, it is suggested that a higher inoculum of *F. oxysporum* is used, with the aim of achieving a higher level of disease. Also that initial fungicide drench treatments are applied at intervals of two rather than four weeks, with the aim of achieving improved disease control.

A low incidence of Fusarium wilt (2%) occurred in the uninoculated control plants. In all other treatments the incidence of dead or wilted plants at the end of the experiment was 9% or greater. It seems probable therefore that most of the disease occurred as a result of inoculation and did not originate in the plug plants.

One of the biological treatments ('Turf Vigour Special' applied as a drench every 14 days) significantly reduced the proportion of healthy plants compared with the untreated control (tale 3.3). The reason for this effect is unknown.

Disinfection of sand infested with *F. oxysporum*

Introduction

In year 1 we confirmed that *F. oxysporum* was present in a sand bed, on which Hebe plants affected by Fusarium wilt had stood, at levels sufficient to cause Fusarium wilt in Hebe. An experiment was devised to compare the efficacy of three disinfectants in reducing the level of *F. oxysporum* in infested sand.

Materials and methods

Newly purchased horticultural sand was sterilised by autoclaving for 30 mins at 15 psi. The sterilised moist sand was then placed in new seed trays to a depth of around 3 cm and inoculated with a strain of *F. oxysporum* ex Hebe by pouring a suspension of spores (350 mL of 1 x 10^5 spores/mL) in sterile distilled water (SDW) over the surface of each tray. The trays of inoculated sand were mixed thoroughly and then allowed to dry in a polythene tunnel for 2 weeks. The trays of sand were then drenched (5 mL/m^2) with various disinfectants. The different treatments were:

1. Uninoculated sand (control)
2. Drench with sterile distilled water
3. 'Jet 5' (hydrogen peroxide + peroxyacetic acid) at 2%
4. 'Horticide' (quaternary ammonium compound + inorganic acids) at 0.08%
5. 'Unifect G' (glutaraldehyde + quaternary ammonium compounds) at 4%

After treatment, excess disinfectant was allowed to drain away; 1 day later the sand from each tray was tumbled to release residual fumes, and then re-laid in the trays.

After the fumes had dissipated (2 days), a 1g sample of sand was removed from

each tray, suspended in 10 mL SDW, and the number of viable *F. oxysporum* propagules determined by plating onto PDA + S at three dilutions (1, 1 in 10 and 1 in 100). 0.1 mL was spread across three replicate plates. The level of *F. oxysporum* in the sand was then calculated as number of propagules/g of sand.

Additionally, sand from each treatment was mixed with Levington M3 compost at 10% v/v. This growing medium was used to fill 9 cm plastic pots that were planted with plug plants of Hebe cv. Pink Pixie. The plants were grown for 12 weeks on capillary matting in gravel trays in a heated glasshouse. There were four replicates per treatment with 10 potted plants per plot arranged in a randomised block design. After 12 weeks, the plants were assessed for symptoms of Fusarium wilt and occurrence of vascular browning in the stem base.

Results and discussion

All three disinfectants significantly reduced levels of *F. oxysporum* in the sand (Table 4.1). 'Unifect G' at 4% was most effective and no *F. oxysporum* was recovered from sand treated with this product. Only a very low incidence of Fusarium wilt (2/160 plants) was observed after 12 weeks and there were no significant differences between treatments.

These results indicate that all three disinfectants at the rates and volumes used in this experiment are potentially useful for disinfestation of sand and other surfaces where the products can be safely used.

Table 4.1: Effect of three disinfectants in reducing levels of *F. oxysporum* in sand

Treatment		Mean levels of *F. oxysporum* after treatment	
		No. cfu/g*	Log 10 cfu/g
T1	Uninoculated sand	0	0.0
T2	Sterile distilled water	416	2.6
T3	'Jet 5' at 2%	8	0.9
T4	'Horticide' at 0.08%	0	0.0
T5	'Unifect G' at 4%	7	0.9
Significance (4 df)		-	0.002
LSD		-	1.3

*back transformed means

Effect of nursery treatments on occurrence of Fusarium wilt in Hebe cv. Pink Pixie

Introduction

The effect of cutting source (micropropagated or conventional cuttings), plant pot cleanliness and sand-bed irrigation on occurrence of Hebe Fusarium wilt were compared in an observation study on a nursery with a history of the disease.

Materials and methods

In spring 2007, blocks of 1,000 or more liners of Hebe cv. Pink Pixie were grown on three capillary sand beds in glasshouse where Hebe Fusarium wilt had occurred for several years. Subsequently the plants were potted and transferred to another glasshouse.

Losses to Fusarium wilt were recorded at both the liner and pot stage. Factors examined for evidence of an effect on Fusarium wilt were:

Factor	Method
Source of cuttings	Micropropagated Nursery stock plants
Age of pot	New Once-used for hebe
Disinfection of once used pots	None Treated with Jet5
Moisture level in sand bed	Dry Nursery standard Wet
Compost tea sprays	None Weekly

Results and discussion

Losses to Fusarium wilt between April and October 2007 are shown in Table 5.1. The proportion of plants affected was relatively low and ranged from nil to 1.2%. The greatest loss was recorded in the pot stage for nursery cuttings grown in new pots on a sand bed irrigated according to the nursery standard practice (T10). At the liner

stage, Fusarium wilt occurred at a low incidence in all blocks of plants except for treatments 1 (micropropagated cuttings, new pots), 3 (own cuttings, new pots) and 10 (own cuttings, new pots). The occurrence of Fusarium wilt at the pot stage in treatment 1 may have originated from the cuttings and developed slowly, or from the sand bed in either of the two glasshouses where the plants were grown.

It is difficult to draw conclusions from this observation study due to the low level of the disease. This low disease incidence in 2007 was probably due partly to on-going nursery efforts to eliminate the problem (by disinfection of sand beds and careful selection of stock plants) and partly to the unusually cool and wet summer, conditions unfavourable to development of Fusarium wilt.

Table 5.1: Effect of nursery treatments on incidence of Fusarium wilt in Hebe (2007)

Treatment						Proportion of plants affected (and %)	
Cutting source		Pots	Pots treated	Bed irrigation	Compost tea-used	Liners	Pots
T1	Microprop	New	-	Std	No	0/6072	10/5417 (0.18)
T2	Nursery	New	-	Std	Yes	1/1000 (0.10)	1/990 (0.10)
T3	Nursery	New	-	Std	No	0/1000	0/1000
T4	Nursery	New	-	Std	Yes	Not grown	Not grown
T5	Nursery	New	-	Dry	No	20/5958 (0.33)	Not grown
T6	Nursery	New	-	Wet	No	8/5976 (0.13)	Not grown
T7	Nursery	New	-	Std	No	4/1000 (0.40	0/984
T8	Nursery	Used	No	Std	No	1/1000 (0.10)	0/989
T9	Nursery	Used	Yes	Std	No	2/1000 (0.20)	0/989
T10	Nursery	New	-	Std	No	0/7020	67/5574 (1.2)

Overall conclusions

1. Fusarium wilt of Hebe in the UK is caused by a host-specific strain of *F. oxysporum.*
2. Hebe varieties differ in susceptibility to Fusarium wilt. The varieties Pink Pixie, Pink Paradise and Purple Pixie are more susceptible than the varieties Caledonia and Rosie.
3. Drench treatment of the growing medium at monthly intervals with ‘Scotts Octave’ significantly reduced Hebe Fusarium wilt.
4. Drench treatment of hebe cv. Pink Pixie with ‘Amistar’ at 1 mL/litre at monthly intervals resulted in stunted growth.

6. Jet 5 at 2%, ‘Horticide’ at 0.08% and ‘Unifect G’ at 4% all significantly reduced infestation of sand by *F. oxysporum*; ‘Unifect G’ was the most effective.

Technology transfer

- Project meetings at Bransford-Webb Nursery, 21 May and 5 October 2007.
- Hebe Fusarium wilt. *HDC News* (in press)

Appendix 1. Trial diaries and temperature records

Experiment 2: Variety trial

13 February 07	Plants potted and trial set up in GH4.
	Plugs dipped for 15 mins in 100 mL inoculum
	Potted in 9 cm pots.
19 March 07	Wilting on 1 plant in plot 16 (inoculated Pink Pixie)
22 March 07	Wilting on 1 plant in plots 14, 16 and 26 (inoculated Pascal + Pink Pixie x 2)
2 April 02	Wilt confirmed in plot 22 (inoculated Caledonia)
5 April 07	First full disease assessment
16 April 07	Second full assessment
8 May 97	Third full assessment
5 June 07	Fourth and final disease assessment
7 June 07	Assessment of stem base browning

Experiment 3: control of Fusarium wilt with fungicides and growing medium amendments

Date	Actions
6 July 07	70% M3 compost mixed with 30% pine bark treatment. 30L of mix made up in clean cement mixer (20 mins mixing time). 9g of Nitram added
20 July 07	7.5g of Exp. biocide (T8) mixed in to 30L compost 3L of FO47 (T11) spore suspension mixed in to 30L of compost – see Doc 9 for spore calculations.
23 July 07	Fusarium ex Hebe subbed on to PDA+S plates. 30 plates from isolate AR05/195 and 30 plates from isolate AR07/92.
24 July 07	All Hebes potted in to 9cm pots.
25 July 07	Trial randomised in Glasshouse 3 and all drench treatments (T2, T3, T4, T5, T9) applied.
1 August 07	All pots except for T13 inoculated. 50ml of spore suspension applied to each pot = $1x10^6$ spores in total in to each pot. See Doc 13 for calculations of spore concentration.
9 August 07	T9 applied. 30ml to each pot, 5ml per L.
21 August 07	Assessment of all plants for symptoms of Fusarium wilt – none found. Soil drenches applied to T2, T3, T4, T5 and T9 at 30ml per pot.
4 September 07	T9 drench applied at 30ml per pot (plot 9 also treated by accident).
11 September 07	Day and night temperatures set to 15°C. Shade screens programmed to come on during the night and off during the day.
18 September 07	Soil drenches applied, 30ml per pot for T2, T3, T4, T5 and T9. All plants assessed.
20 September 07	Day temperature set to 25°C to encourage Fusarium growth. night temperatures left at 15°C.
2 October 07	Day and night temperatures set to 25°C. Vents closed.
3 October 07	T9 drench applied at 30ml per pot.
11 October 07	Crop walked – 1 plant dead, others look dead and have curling leaves = Fusarium wilt symptoms. Confirmed by Dr. Tim O'Neill.
17 October 07	Assessment of all plants for symptoms, lots more plants affected and dead than there were last Thursday. Drench treatments applied to T2, T3, T4, T5 and T9.
23 October 07	Photos taken of wilting plants.
31 October 07	T9 drench applied at 30ml per pot.
15 November 07	T9 drench applied at 30ml per pot.
20 November 07	Assessment of all plots for Fusarium wilt. Plants still dying and showing new symptoms. Fungicide treatments applied to T2, T3, T4 and T5.
28 November 07	T9 drench applied at 30ml per pot
6 December 07	Full assessment carried out.
13 December 07	Vascular staining destructive assessment carried out.

Weekly temperatures in glasshouses as recorded by loggers (Experiment 3)

	weekly temperatures °C in glasshouse		
Week number	Min	Mean	Max
31.	11.6	22.3	52.7
32.	11.3	22.3	47.5
33.	11	19.2	36.2
34.	11.6	20.2	45.1
35.	11.6	20.6	33.5
36.	11.6	21.4	37.4
37.	13.2	21.0	39.4
38.	12.9	21.0	37.0
39.	13.2	20.8	34.7
40.	20.3	26.5	41.9
41.	21.0	26.4	39.4
42.	12.0	21.6	39.0
43.	12.6	14.4	39.0
44.	13.5	19.6	40.2
45.	13.2	18.9	36.6
46.	14.4	20.7	41.1

	weekly humidity		
Week number	Min	Mean	Max
31.	17.0	52.2	84.8
32.	17.8	56.5	93.0
33.	26.0	73.9	100.0
34.	20.7	71.5	100.0
35.	22.5	62.6	94.1
36.	22.5	63.9	94.1
37.	18.9	57.3	86.8
38.	18.9	59.9	96.8
39.	26.8	55.3	87.8
40.	19.9	45.8	60.9
41.	28.4	47.9	65.5
42.	20.7	48.4	78.1
43.	20.3	58.7	87.3
44.	22.5	57.9	88.3
45.	17.4	49.6	78.6
46.	18.1	41.4	54.9

Appendix 2: Full results of disease assessments – Experiment 3

Incidence of plants with Fusarium wilt symptoms – 2007

		Mean number of plants (of 20) with fusarium wilt symptom at intervals after inoculation:		
		12 wks	16 wks	18 wks
1.	Untreated inoculated control	1.0	1.5	2.0
2.	'Delsene 50 Flo'	0.5	0.2	0.5
3.	Exp. fungicide	0.7	1.2	0.7
4.	'Amistar'	0.0	0.2	0.7
5.	'Scotts Octave'	0.0	0.2	0.7
6.	Pine bark	0.5	0.7	0.7
7.	'Trianum'	0.5	1.7	0.7
8.	Exp. biocide	1.7	1.5	2.5
9.	'Turf Vigour Special'	2.2	1.7	3.0
10.	'Mycoplex'	0.5	0.5	0.7
11.	Fusarium (non pathogenic)	0.0	0.0	0.7
12.	Non inoculated control	0.0	0.0	0.0
Significance		0.032	0.082	0.012
LSD between treatments		1.387	1.520	1.623
LSD vs. control		1.201	1.317	1.406

Assessment for weeks 3 and 7 showed no discernable wilt symptoms on any plants

Mean number of dead plants – 2007

		Mean number of dead plants (of 20) at intervals after inoculation:		
		12 wks	16 wks	18 wks
1.	Untreated inoculated control	0.2	1.2	1.3
2.	'Delsene 50 Flo'	0.2	1.2	2.5
3.	Exp. fungicide	1.0	2.0	2.5
4.	'Amistar'	0.0	0.5	1.0
5.	'Scotts Octave'	0.0	0.5	0.7
6.	Pine bark	0.0	0.7	1.0
7.	'Trianum'	0.5	1.5	3.2
8.	Exp. biocide	0.2	2.5	3.0
9.	'Turf Vigour Special'	0.7	2.5	2.5
10.	'Mycoplex'	0.0	1.0	1.5
11.	Fusarium (non pathogenic)	0.2	0.2	1.0
12.	Non inoculated control	0.2	0.2	0.2
Significance		0.252	0.106	0.181
LSD between treatments		0.7907	1.758	2.328
LSD vs. control		0.6848	1.523	2.016

Effect of fungicides and biological treatments on mean number of healthy plants - 2007

Treatment		Mean number of healthy plants (of 20) at intervals			
		12 wks	16 wks	18 wks	19 wks
1.	Untreated inoculated control	18.7	17.2	16.6	15.4
2.	'Delsene 50 Flo'	19.2	18.5	17.0	16.0
3.	Exp. fungicide	18.2	16.7	16.7	15.8
4.	'Amistar'	20.0	19.2	18.2	17.8
5.	'Scotts Octave'	20.0	19.2	18.5	18.3
6.	Pine bark	19.5	18.5	18.2	17.0
7.	'Trianum'	19.0	16.7	16.0	14.5
8.	Exp. biocide	18.0	16.0	14.5	13.3
9.	'Turf Vigour Special'	17.0	15.7	14.5	11.8
10.	'Mycoplex'	19.5	18.5	17.7	17.3
11.	Fusarium (non pathogenic)	19.7	19.7	18.2	17.5
12.	Non inoculated control	19.7	19.7	19.7	19.5
Significance		0.008	0.014	0.024	0.001
LSD between tests		1.55	2.57	3.00	3.25
LSD vs. control		1.35	2.22	2.59	2.82