Project title:	Hebe: aspects of the biology and control of fusarium wilt
Project number:	HNS 146
Report:	Final Report (February 2009)
Previous reports:	April 2007, April 2008
Project leader:	Dr Tim O'Neill ADAS UK Ltd Boxworth Cambs, CB23 4NN
Key workers:	Mrs Helen Greenleaves, ADAS Ms Luci Kirkpatrick, ADAS Dr John Buxton, ADAS
Location of project:	ADAS Arthur Rickwood and ADAS Boxworth Commercial Nursery, Worcs
Project coordinators:	Mr John Adlam, Dove Associates Mr Geoff Caesar, Bransford-Webb Plants Company
Date project commenced:	1 April 2006
Date completion due:	31 March 2009
Key words:	Hebe, *Fusarium oxysporum*, wilt, sources, fungicides, biological treatments, disinfectant

Disclaimers

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

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.

All information provided to the HDC by ADAS in this report is provided in good faith. As ADAS shall have no control over the use made of such information by the HDC (or any third party who receives information from the HDC) ADAS accept no responsibility for any such use (except to the extent that ADAS can be shown to have been negligent in supplying such information) and the HDC shall indemnify ADAS against any and all claims arising out of use made of the HDC of such information.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use except where the crop or situation is the subject of an off-label extension of use. Before using all pesticides and herbicides check the approval status and conditions of use. Read the label before use: use pesticides safely.

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.

Dr T M O'Neill
Principal Research Scientist
ADAS UK Ltd

Signature …… Date ……..

Report authorised by:

Dr W E Parker
Horticulture Sector Manager
ADAS UK Ltd

Signature …… Date …….

TABLE OF CONTENTS

GROWER SUMMARY

Headline

Fusarium wilt of hebe in the UK is caused by *Fusarium oxysporum*; the disease can be managed by varietal choice, maintaining stock plants free of *F. oxysporum*, fungicide drenches to the growing medium and disinfection of re-used containers and standing beds.

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

Symptoms and cause of fusarium wilt in hebe

A *Fusarium* species consistently isolated from the vascular tissue of container-grown hebe plants exhibiting symptoms of a vascular wilt disease was identified as *F. oxysporum.* Identification of the species was confirmed by DNA sequencing at CSL.

Fusarium wilt of hebe caused by *Fusarium oxysporum* commonly appears as:

- wilting of one or more, but rarely all, shoot tips (Figure 1);
- loss of leaf colour;

- brown patches on leaves progressing to leaf, shoot and eventually plant death (Figure 1);
- dark brown staining of vascular tissue in the stem base and in affected shoots;
- occasionally, pale pink fungal pustules of *F. oxysporum* develop at the stem and/or on affected shoots.

Figure 1: Shoot-tip wilting (left) is an early symptom of fusarium wilt; leaf and shoot death (right) usually follow.

Occurrence of hebe fusarium wilt in the UK

Although reported to be widespread in the Netherlands, the disease was only identified on two UK nurseries. The UK Plant Health and Seeds Inspectorate (PHSI) determined that the fungus isolated (*F. oxysporum*) was a non-quarantine organism and therefore not subject to any statutory controls. ADAS horticulture consultants examined hebe plants on several other nurseries and garden centres during the project and found no evidence of fusarium wilt.

Infection and disease development

Micropropagated hebe plug plants, cv. Pink Pixie, were inoculated with spores of an *F. oxysporum* isolate obtained from wilted hebe. The fungus was applied as a root dip pre-potting. Symptoms of fusarium wilt developed after three weeks. *F. oxysporum* was consistently recovered from affected plants in pure culture. *F. oxysporum* was therefore confirmed as a cause of fusarium wilt of hebe in the UK according to Koch's postulates.

The incidence of infected plants increased with spore concentration. Root wounding (by cutting-off root tips) did not increase the incidence of infected plants. Other inoculation techniques were examined. Drenching potted plants around the stem base with *F. oxysporum* resulted in fusarium wilt symptoms, as did dip-inoculation of freshly cut shoot tips, but these methods of inoculation were generally less successful than the root-dip method.

Some plants that were still visibly healthy at 15 weeks after inoculation were found to have dark, stained vascular tissue at the stem base, and *F. oxysporum* was recovered from such tissue. These results indicate that the development of fusarium wilt can be relatively slow, taking more than 15 weeks.

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 plastic plant pots and grown in a heated glasshouse. 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.

Effect of temperature and moisture on infection

Plug plants of hebe cv. Pascal were inoculated with *F. oxysporum* by dipping roots in a spore suspension for 15 minutes. Plants were potted in a peat-based medium and held for seven days in controlled environment cabinets maintained at 18 and 25°C

with the growing medium maintained damp or wet. Plants were then placed in a warm glasshouse for 7 weeks and watered as required. Symptoms of fusarium wilt first appeared 4 weeks after inoculation. At 8 weeks after inoculation there was a significantly greater incidence of infected plants following an initial incubation period at 25°C, compared with at 18ºC.

Distribution of F. oxysporum within plants

In order to provide information on the extent of systemic infection within plants, isolation for *F. oxysporum* was made from different parts of apparently healthy cuttings and pot-grown plants. The plants tested were obtained from a nursery with a history of the disease. *F. oxysporum* was recovered at a low incidence from the stem base of rooted cuttings cv. Purple Pixie (3/20) and Rosie (1/20). It was also recovered from the stem base of 9 cm potted plants (3/30 plants), and from roots (1/30 plants). When older plants in 3 L pots were tested, *Fusarium* sp. was recovered from 3/6 shoots on one branch and from none of 19 shoots on eight other branches.

These results indicate that cuttings taken from apparently healthy container-grown plants, used as stock plants, may be infected with *F. oxysporum*. The disease could therefore be maintained on a nursery through the propagation cycle.

Varietal susceptibility

Fusarium wilt was observed in the UK on cvs. Autumn Glory, Blue Star, Caledonia, First Light, Pascal, Pink Paradise, Pearl of Paradise, Pink Pixie, Purple Pixie, Purple Shamrock, Rosie, Sapphire, Silver Dollar and Sutherlandii. Pink Pixie and Purple Pixie were more commonly affected than other varieties. An inoculation experiment comparing the relative susceptibility of six varieties showed that cv. Pink Pixie (35% or plants affected) was significantly more susceptible than Caledonia (5%), Rosie (8%) and Pascal (18%) after 16 weeks; Purple Pixie (25%) and Pink Paradise (30%) were also highly susceptible.

Sources of F. oxysporum on a nursery

In October 2006, samples of sand from three sand beds and once-used hebe pots were collected from a nursery with a history of fusarium wilt and tested for contamination with *F. oxysporum* by a growing-on test. The sand was mixed with a peat-based growing medium and used to fill new plastic plant pots; the once-used pots were filled with new growing medium. Both sets of pots were potted with plants of hebe cv. Pink Pixie.

The first symptoms of fusarium wilt in any of the media amended with nursery sand were observed after six weeks; all of the inoculated control plants were showing symptoms at this time. After 18 weeks, 25% of plants grown in medium amended with sand from one of the nursery beds, and 15% of plants in the once-used pots, had developed symptoms of fusarium wilt. None of the uninoculated control plants, or the plants in two of the sand-amended media, developed symptoms. Examination of apparently healthy plants revealed additional, symptomless infection in plants grown in medium mixed with sand from one of the sand beds on the nursery.

Evaluation of fungicide and biological treatments

In 2007, the fungicides Amistar (azoxystrobin), Delsene 50 Flo (carbendazim), Scotts Octave (prochloraz) and an experimental material, and six biological treatments (matured 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). None of the other treatments had a significant effect. Amistar drench treatment resulted in stunted growth.

In 2008, the fungicides Cercobin WG (thiophanate methyl) and Scotts Octave (prochloraz), an alternating programme of Cercobin WG and Octave, matured pine bark incorporated into the growing medium (30% v/v), and added lime (as ground chalk) incorporated into the growing medium, were evaluated for control of fusarium

wilt in container-grown hebe in a replicated experiment. The alternating fungicide programme was tested on plants in a peat-based growing medium and in the same medium amended with matured pine bark. Fusarium wilt was first observed after 2 weeks, and after 23 weeks 25% of plants in untreated plots were wilted or dead. The incidence of plants dead or showing symptoms of fusarium wilt was significantly reduced by Octave drenches and by the Cercobin WG/Octave programme applied to plants in the bark-amended growing medium (Figure 2). The latter treatment reduced the disease to 2.5% plants dead or wilted. Most other treatments (except for the added lime treatment) appeared to reduce the disease. None of the control uninoculated plants developed fusarium wilt. The added lime did not raise the pH which may explain the failure of this treatment to affect fusarium wilt.

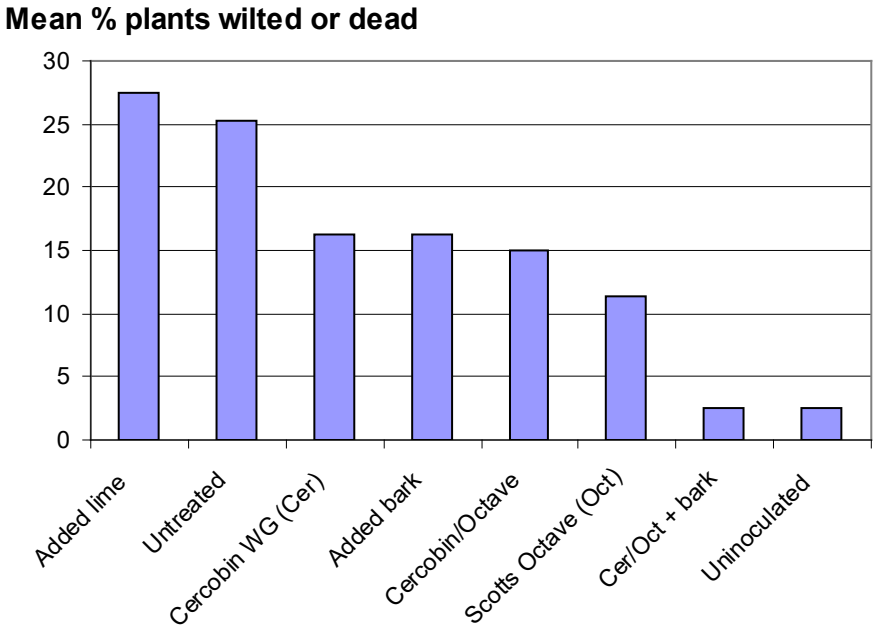


Figure 2: The effect of fungicides and growing medium amendments on fusarium wilt in hebe 23 weeks after inoculation – 2008

Spread of F. oxysporum in a sandbed

An experiment was devised to determine the effect of flood irrigation on the extent of movement of *F. oxysporum* through sand at levels sufficient to cause fusarium wilt in hebe cv. Pink Pixie. Miniature sandbeds were infested with *F. oxysporum* directly beneath, or approximately 3 cm to one side, of pots of hebe cv. Pink Pixie.

Additionally, sand in small open top plastic containers was infested with *F. oxysporum* and placed at the side of hebe plants on sandbeds; this treatment served to check for movement of *F. oxysporum* in ways other than through movement in water at flood-irrigation. After 27 weeks, fusarium wilt was confirmed at a very low incidence in plants where the causal fungus was placed on sand directly beneath pots or adjacent to pots. The disease was not confirmed in the uninoculated control. The plants had rooted considerably into the sandbeds and it is possible that infection of plants arose by root growth coming into contact with the inoculum rather than movement of inoculum through the sand in water. This experiment confirms that hebe fusarium wilt can arise from sandbeds infested with *F. oxysporum* but found no evidence to support the hypothesis that the fungus is readily spread through the sand by flood-irrigation.

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 2 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 plants grown in inoculated and uninoculated growing media.

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 progressed, increased understanding of the disease was gained, and new control measures were devised and implemented. The disease occurred at a lower incidence in 2008, affecting <1% of plants.

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

Recognition

- Growers should familiarise themselves with the symptoms of hebe fusarium wilt (see Fig. 1)
- 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.
- Note that hebe fusarium wilt may be confused with downy mildew. If in doubt, contact a plant pathologist or submit a sample to a Plant Clinic.

Disinfection

- If hebe fusarium wilt is known or suspected on your nursery, disinfect sand beds, other standing areas, pots and containers before re-using them for hebe (see HDC Factsheet 15/05). Disinfectants with good activity against *F. oxysporum* in sand are Jet 5, Horticide and Unifect G following manufacturers recommended rates.
- It is important to disinfect standing areas thoroughly, not just wet the surface.
- Do not re-use pots from plants visibly affected by hebe fusarium wilt.

Stock plant health

- Check the health of stock plants before taking cuttings; be aware that in addition to visible symptoms, symptomless, systemic infection by *F. oxysporum* can occur within shoot tips. Check for vascular staining on a few plants per batch.

- Fusarium wilt of hebe is known to occur in mainland Europe; carefully examine a sample of any plants contract-grown in mainland Europe for your nursery and consider testing for fusarium wilt.

Growing environment

- Where feasible, maintain growing temperatures below 20°C; there is evidence that fusarium wilt is favoured by temperatures around 25°C.
- Amendment of Levington M3 growing medium with 40% (v/v) fine matured bark significantly reduced hebe fusarium wilt.

Fungicide treatment

- The fungicides Cercobin WG (SOLA 1382/08) and Scotts Octave (label approval) applied in an alternating programme, and Scotts Octave alone, significantly reduced incidence of hebe fusarium wilt when applied as drench treatments.
- In an experiment on hebe cv. Pink Pixie where plants were inoculated with *F. oxysporum* soon after potting, the use of a matured pine bark amendment combined with alternating drenches of Cercobin WG (1.4 g/L) and Scotts Octave (1.0 g/L) at 2-week intervals (4 drenches in total) gave the most effective control of the disease.

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.

In year 2 of this project, inoculation of hebe and stock (*Matthiola incana*) with strains of *F. oxysporum* obtained from each host indicated that the strain of *F. oxysporum* causing wilt in hebe plants in the UK is a host-specific strain. All of six hebe cultivars inoculated with *F. oxysporum* from hebe developed symptoms of fusarium wilt; the variety Pink Pixie was highly susceptible, Purple Pixie and Pink Paradise were intermediate and Caledonia, Pascal and Rosie were less susceptible. Four fungicides and six biological treatments were evaluated for control of hebe fusarium wilt in a replicated inoculated experiment. Scotts Octave (prochloraz-Mn) applied as a drench at monthly intervals (x 4) was the only treatment that significantly reduced the disease. Three disinfectants (Jet 5, Horticide and Unifect G) all significantly reduced levels of *F. oxysporum* in infested sand; Unifect G was the most effective.

The objectives of work in year 3 were:

- To investigate some fungicides and growing medium amendments for control of the disease;
- To investigate the extent of spread of *F. oxysporum* in a flood-irrigated sandbed at levels sufficient to cause fusarium wilt in hebe cv. Pink Pixie.

1. Evaluation of fungicides and growing medium amendments

Introduction

An experiment was devised to test two fungicides, two growing medium amendments and some combinations of these treatments for control of hebe fusarium wilt. Previous studies in this project (HNS 146, Annual Report 2008), and elsewhere (O'Neill, 1992), have shown that Cercobin WG (thiophanate methyl), Scotts Octave (prochloraz-Mn), matured pine bark added on the growing medium and an increased pH of the growing medium can all reduce fusarium wilt in hebe or other hosts.

Materials and methods:

Crop details

Plug plants, cv. Pink Pixie obtained from a commercial nursery, were potted on 21 April 2008 into 9 cm diameter pots in Levington M3 compost and grown on capillary matting in gravel trays in a heated glasshouse (see Appendix 1 for trial diary). The growing medium was kept moist for one week after potting and then subsequently watered by hand when required. Daily maximum and minimum temperatures (air and growing medium) were recorded throughout using data loggers.

Inoculation

Plants were inoculated with spores obtained from four strains of *F. oxysporum* isolated from affected UK hebe plants and cultured on potato dextrose agar amended with streptomycin (PDA+S). A 50 mL aliquot of spore suspension was applied as a drench to each pot, apart from the uninoculated control, on 28 May 2008 to give a total application of 2×10^6 spores per pot. The spore suspension was poured over the growing medium surface around the full circumference of the pot.

Experimental design and statistical analysis

The experiment was a randomised block design with four-fold replication of treatments except for the inoculated control where there was eight-fold replication. Each plot contained 20 plants, with 10 in each of two gravel trays. The original T8 (an uninoculated control) was inoculated in error. This treatment was therefore equivalent to T1 resulting in twelvefold replication of the inoculated control. Spare plants were added to the end of each block as the new uninoculated control (T8). The data were binomially distributed and unsuitable for analysis of variance. Results were therefore examined in regression analysis using the Logit function.

Treatments

1. Untreated inoculated control
2. Cercobin WG (thiophanate methyl) at 1.4 g/L
3. Scotts Octave (prochloraz-Mn) at 1.0 g/L
4. Cercobin WG alternating with Scotts Octave (rates as above)
5. Melcourt Growbark Pine (a fine matured pine bark) incorporated into Levington M3 growing medium at 30% V/V
6. Cercobin WG alternating with Scotts Octave (rates as above) applied to fine matured bark amended growing medium
7. Added lime (ground chalk at 2.5 kg/m^3) to raise pH, no fungicides
8. Uninoculated control

The growing medium was Levington M3 compost. Mature pine bark (T5 and T6) and ground chalk (2.5kg/m^3) (T7) were incorporated three weeks before potting. Extra nitrogen was added to the growing medium in T5 and T6 to account for nitrogen immobilisation by the bark; ammonium nitrate was added at 100 g/m^3 for every 10% of bark added. Fungicides were applied as drench treatments in water at a volume equal to 10% of pot volume on four occasions at 14 day intervals. The first fungicide drench treatment was applied 1 week before inoculation with *F. oxysporum*.

The growing medium in T1 (Levington M3), T5 (added bark) and T7 (added lime) were analysed for pH, conductivity, bulk density, major nutrients and trace elements at the start of the experiment to determine the effect of added bark and lime on pH and nutrient levels.

Disease and plant quality assessment

The crop was assessed for wilt symptoms and occurrence of dead plants at 12, 15, 18 and 23 weeks after inoculation. *F. oxysporum* was confirmed as the cause of wilt by laboratory examination of the fungal sporulation around the stem base of dead plants and by isolation for fungi from stained vascular tissue at the stem base. Plants were thoroughly watered the day before disease assessments to ensure that wilt symptoms were not due to lack of water. At the final assessment on 6 November plants were assessed destructively for vascular staining at the stem base. The

occurrence of fusarium sporulation on the stem base on dead plants was also recorded.

Results

Effect of added bark and lime

Results of growing media analyses are shown in Appendix 2. In brief, the addition of matured pine bark reduced pH and bulk density. The addition of ground limestone increased calcium and nitrate levels and conductivity but did not affect pH. A sample of unused lime-amended medium was re-analysed near the end of the experiment and the pH remained unaltered.

Control of fusarium wilt

Symptoms of fusarium wilt were first observed 3 weeks after inoculation when a few plants died and were confirmed as infected by *F. oxysporum*. Results of interim disease assessments are given in Appendix 3. At 23 weeks after inoculation, 25% of untreated plants (T1) were dead or showing symptoms of fusarium wilt (Table 1.1). The incidence of dead plants was significantly reduced by Octave, the Cercobin WG/Octave programme and by Cercobin WG/Octave applied to bark-amended growing medium. The incidence of plants dead or wilting was significantly reduced by Octave and Cercobin WG/Octave applied to bark amended growing medium. All other treatments except for T7 (added lime) appeared to reduce the disease compared with the untreated control (T1). Around 3% of the uninoculated plants died during the experiment; none of these showed symptoms of fusarium wilt and no *F. oxysporum* was isolated from them.

Mean daily glasshouse air and growing medium temperature during the experiment ranged from 13°C to 25°C; the growing medium temperature was above 20°C for most of the experiment (Appendix 4).

Table 1.1: Effect of fungicides and growing medium amendments on hebe fusarium wilt – 6 November 2008

Treatment		Mean % plants (out of 20) affected 23 weeks after inoculation			
		Wilting	Dead	Wilting + Dead	Healthy
1.	Untreated	5.4	19.8 (4.1)	25.3 (4.6)	74.7 (4.6)
2.	Cercobin WG (Cer)	2.5	13.8 (5.9)	16.3 (6.6)	83.8 (6.6)
3.	Scotts Octave (Oct)	6.4	5.1 (3.8)	11.4 (5.7)	88.6 (5.7)
4.	Cercobin/Octave	5.0	10.0 (5.1)	15.0 (6.4)	85.0 (6.3)
5.	Added bark	3.8	12.5 (5.6)	16.3 (6.6)	83.8 (6.6)
6.	Cer/Oct + bark	0	2.5 (2.7)	2.5 (2.8)	97.5 (2.7)
7.	Added lime	7.5	20.0 (6.8)	27.5 (7.9)	72.5 (7.9)
8.	Uninoculated	2.5	0.0	2.5 (2.7)	97.5 (2.8)
Significance (24 df)		NS	0.019	0.022	0.022

( ) – standard error.

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

Treatment had a significant effect ($p<0.001$) on the incidence of stem base vascular browning in remaining live plants (Table 1.2). Stem base vascular browning is an early symptom of fusarium wilt. Browning was greatest in inoculated untreated plants (46%) and least in uninoculated plants (0%). Among the inoculated plants, the Cercobin WG/Octave treatment applied to bark-amended growing medium was most effective (11% plants with vascular staining). This reflects the efficacy as noted against other symptoms of fusarium wilt (Table 1.1). The bark-amended growing medium also significantly reduced vascular browning.

Fusarium sporulation had developed at the stem base of a majority of the dead plants (Table 1.2). Treatment had no significant effect on the proportion of dead plants with fusarium sporulation, as expected where fusarium wilt was the primary cause of plant death. No fusarium sporulation was observed on the stem bases of live plants.

Table 1.2: Effect of fungicides and growing medium amendments on hebe fusarium wilt – 6 November 2008

Treatment		Mean % plants with	
		Vascular staining	Fusarium sporulation[a]
1.	Untreated	39.6 (5.4)	59.1 (8.3)
2.	Cercobin WG (Cer)	38.8 (8.8)	62.4 (17.8)
3.	Scotts Octave (Oct)	29.2 (8.3)	78.3 (23.4)
4.	Cercobin/Octave	22.5 (7.6)	59.3 (22.7)
5.	Added bark	25.0 (7.9)	24.3 (18.2)
6.	Cer/Oct + bark	11.3 (5.8)	60.0 (42.4)
7.	Added lime	35.0 (8.6)	69.0 (14.0)
8.	Uninoculated	0.0 (0.0)	-
Significance (24 df)		0.002	NS

( ) - standard error; [a]Assessed on dead plants only.

Discussion

As in most other experiments in this project, there was a long latent period (10+ weeks) between inoculation with *F. oxysporum* and a significant proportion of plants developing fusarium wilt symptoms. The incidence of dead and wilted plants in the inoculated control at the end of the experiment was over 25%, only slightly higher than that (23%) in a similar experiment in 2007, despite the greater level of inoculum of *F. oxysporum* spores used to inoculate plants in 2008.

Added lime (T7) had no effect on fusarium wilt. Analysis of growing media revealed that although the lime increased calcium levels (from 170 to 275 mg/L), there was no effect on pH which remained at 5.6. The quantity of ground chalk added (2.5Kg/m^3) should have increased pH by around one unit. No conclusions can be drawn on the effect of growing medium pH on fusarium wilt in hebe. The reason that the added chalk failed to raise pH is unknown. Unused growing medium was re-analysed in September and was still unaltered. Examination of records revealed no error in the type or amount of lime added; possibly the failure of the ground chalk to raise the pH may have been due to insufficient moisture in the growing medium prior to it being sent for analysis.

The Cercobin WG/ Scotts Octave fungicide programme applied to plants with added bark in the growing medium was notably better than either the fungicide programme

alone or added bark alone, suggesting a possible synergistic effect between these treatments.

2. Spread of fusarium wilt with flood-irrigation of a sandbed

Introduction

In previous work we demonstrated the occurrence of *F. oxysporum* in sand taken from a sandbed beneath hebe plants affected by fusarium wilt (HNS 146, Annual report 2007). Incorporation of infested sand into a peat-based growing medium resulted in development of fusarium wilt in hebe plants grown in the medium. It is possible that *F. oxysporum* within a sandbed may be spread by flood irrigation, such that a localised infestation of the sandbed may lead to widespread infection in a crop. An experiment was therefore devised to determine whether the spread of *F. oxysporum* in a sandbed using flood-irrigation occurred at levels sufficient to cause fusarium wilt in a susceptible variety of hebe.

Materials and methods

Crop details

Plug plants of cv. Pink Pixie obtained from a commercial nursery were potted into 9 cm pots in Levington M3 compost and grown in a heated glasshouse at ADAS Boxworth.

Treatments

1. Uninoculated control
2. Sandbed infested with *F. oxysporum* in a strip approx 3 cm away from potted hebe, sandbed flood-irrigated
3. Isolated container of sand infested with *F. oxysporum*, adjacent to potted hebe, sandbed flood-irrigated. This treatment served to check for movement of *F. oxysporum* in ways other than through movement in water at flood-irrigation.
4. Sandbed infested with *F. oxysporum* directly beneath potted hebe, sandbed flood-irrigated.

The experimental unit was a miniature sandbed on which eight potted hebe plants were placed in two rows of four (Fig 2.1). The sandbeds comprised seed trays with drainage holes lined with nylon mesh and filled with sand up to 1 cm from the top. Each tray of sand was placed within a plastic gravel tray. The plants were flood-irrigated, pouring water between the seed tray and the gravel tray until it was half-way up the depth of sand; the water moved to the top of the sand and into the base

of plant pots by capillary action. Plants were flood-irrigated as required throughout the experiment.

Figure 2.1: Miniature sandbed used for investigating spread of *F. oxysporum* in sand (left) and view of the experiment (right)

Inoculation

For treatment 2, 20 mL of a *F. oxysporum* spore suspension (10^6 spores/mL), a mixture of four isolates as described in the previous experiment, was carefully poured onto the sand at one side of the tray, adjacent to each of four plants. The plants close to the inoculation points were termed 'near plants', the plants at the opposite site of the tray were termed 'distant plants'.

For treatment 3, a plastic drinking cup half-filled with sand infested with *F. oxysporum* (20 mL of 10^6 spores/ml) was placed on the strip of sand at one side of the gravel tray, adjacent to each of four plants. No watering was applied to the cup only to the sand bed.

For treatment 4, sand directly beneath each of the eight pots in a tray was inoculated with *F. oxysporum* (20 mL of 10^6 spores/mL).

Experimental design and statistical analysis

Each treatment was replicated 10 times in a modified split-plot design. The split was between near plants and distant plants to assess near spread and distant spread by *F. oxysporum* through the sand. Statistical analysis was not appropriate due to the very low incidence of the disease (see below).

Disease assessment

Plants were examined for symptoms of fusarium wilt every two weeks. After 27 weeks, plants were destructively assessed for fusarium infection by examination for vascular staining at the stem base. All plants with stem base browning were tested for *F. oxysporum* infection by isolation from the stained stem base tissue onto agar (PDA+S).

Results and discussion

A single plant wilted and died from fusarium wilt in treatment 4 (sandbed infested below pots) at 8 weeks after inoculation. At the end of the experiment, the incidence of plants affected by vascular staining at the stem base was very low (Table 2.1). The staining was light brown in colour compared with the dark brown colour usually found with fusarium wilt in hebe. *F. oxysporum* was recovered from three out of 16 plants tested. The infected plants in treatments 2 and 3 (strip of inoculum and isolated source of inoculum, respectively) both occurred in the row of plants adjacent to inoculum.

This experiment confirms that hebe fusarium wilt can arise from sandbeds infested with *F. oxysporum* but found no evidence to support the hypothesis that the fungus is readily spread through the sand by flood-irrigation. The plants had rooted considerably into the sandbeds and it is possible that infection of plants in treatments 2 (strip of inoculation) and 4 (pots over infested sand) arose by root growth coming into contact with the inoculum rather than movement of inoculum through the sand. Infection of the plant in treatment 3 (isolated source of inoculum) may have arisen by transfer of infested sand from the open-topped pot adjacent to the plants (e.g. by water splash or insect-transmission).

The form in which *F. oxysporum* survived in sandbeds was not investigated. Conidia are considered to be short-lived (days to weeks) and it is more likely that survival spores (chlamydospores) developed from germinated conidia, or were present in the inoculum applied to the sand. Chlamydospores of *F. oxysporum* can survive in the absence of a host for months and subsequently germinate and cause infection when in contact with roots of a susceptible host.

Table 2.1: Effect of flood irrigation of sand infested with *F. oxysporum* on occurrence of fusarium wilt in hebe cv. Pink Pixie - 2008

Treatment	Total number plants affected (of 80):		
	Fusarium wilt*	Dead	Vascular staining
1. Uninoculated control	0	0	3
2. Sandbed infested to one side of plants	1	0	3
3. Isolated sand infested (negative control)	1	0	4
4. Sandbed infested beneath plants	1	1	6

*Confirmed by isolation from stained vascular tissue at the stem base.

Overall conclusions (Years 1-3)

1. Fusarium wilt of hebe in the UK is most probably caused by a host-specific strain of *F. oxysporum.*
2. Hebe fusarium wilt affects a wide range of both small leaf and large leaf varieties.
3. 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.
4. Young plug plants of hebe developed symptoms of fusarium wilt when inoculated with *F. oxysporum* applied to the roots; wounding of roots was unnecessary for infection to occur. The incidence of infection increased with inoculum level.
5. Mycelial growth of *F. oxysporum* isolated from hebe occurs at 10-30°C and is optimal at 25°C.
6. There is some evidence that a high temperature (25°C) favours infection of hebe roots by *F. oxysporum*.
7. *F. oxysporum* was recovered from roots, stem bases and shoots of some visibly healthy hebe plants indicating that symptomless, systemic infection can occur.
8. *F. oxysporum* was detected in sand taken from a sand bed where infected plants had been, and in once-used plant pots. The pathogen was present in both at levels sufficient to cause hebe fusarium wilt.
9. Infestation of a capillary sandbed with *F. oxysporum* can result in infection of potted hebe plants placed on the bed. There was no evidence to support the hypothesis that the fungus is readily spread through the sand by flood-irrigation at

levels sufficient to cause fusarium wilt in hebe.

10. Drench treatment of the growing medium at monthly intervals with Scotts Octave significantly reduced hebe fusarium wilt.
11. Drench treatment of growing medium with Scotts Octave at 14 day intervals (x4 in total), and of growing medium amended with matured pine bark at 14 day intervals alternately with Cercobin WG and Scotts Octave (x 4 in total) significantly reduced hebe fusarium wilt.
12. Drench treatment of hebe cv. Pink Pixie with Amistar at 1 mL/litre at monthly intervals resulted in stunted growth
13. 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.

References

O'Neill TM (1992). Methods of inoculating cyclamen with Fusarium oxysporum f. sp. cyclaminis. Proceedings of ANPP Conference on Artificial Contamination of Diseases, Arras, 5 November 1992, pp. 171-178.

Technology transfer

Meetings

- Project meetings at a nursery: 14 July and 13 October 2006, 21 May and 5 October 2007 and 16 September 2008.
- Project review meeting, London, 12 March 2007.

Articles

- Putting a stop to hebe wilt. *HDC News* **141,** 26-27.
- Likely control for hebe wilt. *HDC News* **144**, p. 4.
- Hebe fusarium wilt explained. *HDC News* (in press).

APPENDIX 1: Trial Diaries

1. Fungicides and growing medium amendments

Date:	Trial Diary Entry
1 April 2008	Four isolates of fusarium: AR04/07, AR05/195, AR06/136 and AR07/192 plated out
16 April 2008	Above isolates subbed on
17 April 2008	Further subbing-on of original plates.
21/22April 2008	Hebes potted up and placed pot thick in glasshouse 5 until large glasshouse free.
22 April 2008	Further subbing-on of fusarium isolates.
30 April 2008	Further subbing-on of fusarium isolates.
13 May 2008	Scotts and Cercobin WG located in the pesticide store.
	Trial set up on central and right hand bench in large greenhouse. Currently 20 pots per tray.
	Fungicides applied as per protocol for initial drenches.
28 May 2008	Hebes inoculated with spore suspension. Aphid biocontrol program advised by Jude Bennison.
3 June 2008	Fungicides applied as per protocol – Cercobin to T2, Octave to T3, 4 and 6.
7 June 2008	Watering at weekend observed that central bench of hebes had been pushed over, leading to watering by the overhead system
17 June 2008	Fungicides applied as per protocol.
19 June 2008	Severe wilt / dead plants noticed in T8 and T3.
	Hebes spaced out – moved into 2 trays of 10.
20 June 2008	Delta logger set up with probes in compost. 2 probes for channel 4 placed in block 2, treatment 1, plot 7; 1 probe for channel 3 placed in block 4, treatment 7, plot 3; 1 probe for channel 2 placed in block 4, treatment 5, plot 1. Other probes left open to air.
23 June 2008	Plants inspected – 3 plants removed from block 4 – T8 and 1 from block 3 – T3. Realised T8 may have been inoculated by mistake.
26 June 2008	Floats checked and fusarium confirmed on T3 and T8. 80 control plants split into 4 groups of 20 and placed on bench as new uninoculated control.
1 July 2008	Hebes drenched with final fungicides as per protocol.
2 July 2008	Hebes assessed for visible signs of wilt damage.
27 July 2008	Hebes checked – no change. Assessment pushed back.
14 August 2008	Whitefly discovered on hebes – control program initiated – Conserve.
16 August 2008	Thrips infestation on hebes – Control program initiated

Date:	Trial Diary Entry
21 August 2008	Hebes assessed – wilt in several plants.
22 August 2008	Hebes moved to Rickwood. Uninoculated control plants placed at end of each row.
10 September 2008	Hebes assessed at Rickwood – 15 weeks. More disease found.
19 September 2008	Soil sample for increased pH sent to NRM
24 September 2008	Soil sample returned. pH slightly reduced / the same as M3
25 September 2008	Biocontrols renewed in glasshouse at Rickwood.
30 September 2008	Temp set to 25 c day, 15 c night.
1 October 2008	Visual assessment carried out
6 November 2008	Final assessment - visual condition, visible sporulation and vascular staining. Some examples removed and spores checked. Destructive assessment.

2. Disease spread in a capillary sandbed

Date:	Trial Diary Entry
01 April 2008	20 plates each of isolates AR04/07, AR05/195, AR06/136 and AR07/92 plated out.
16 April 2008	Above fusarium isolates sub cultured on.
17 April 2008	Further subbing on of above isolates from colonies from 1st April 2008.
21 April 2008	Hebes potted up into 9cm pots and placed pot thick in glasshouse 5.
22 April 2008	Further subbing on of fusarium isolates.
30 April 2008	Further subbing on of fusarium isolates.
24 June 2008	Hebes laid out as per trial plan in GH 5.
2 July 2008	Inoculate as per protocol. Spores from frozen solution and plate scrapings. Spore count lower than desired – 1 x 10^6. Spore reduction possibly due to freezing process.
7 July 2008	Watering method advised to site technician . Watering method essential.
7 August 2008	Western flower thrips noticed – treatment program advised and started.
14 August 2008	Whitefly infestation noticed. Treatment started.
10 Sept 2008	1 plant dead in T4 – positive control.
21 Oct 2008	Infestation of whitefly much reduced. All apart from 1 hebe healthy except sooty mould on the leaves – feeding on whitefly excreta. Should not overly affect growth at this stage.
8 Dec 2008	Hebes checked – growing well. No new signs of disease or wilt. Whitefly much reduced still. Trays dry and topped up – has been noticed sporadically over last few months. Have been checking every few days and topping up. Continue monitoring.
5 Jan 2009	Destructive assessment carried out. Few signs of disease and vascular staining faint. Samples of every plant with any

Date:	Trial Diary Entry
	staining plated out and placed in UV incubator.
9 Jan 2009	Plates checked. Fusarium confirmed from T4 plant and T3 plant. Another T3 plant and T2 plant look likely to be fusarium if left to grow,

APPENDIX 2: Analysis of growing media

Test	Levington M3	Added bark	Added lime
pH in compost extract	5.60	5.30	5.60
Conductivity in compost extract (µS/cm)	657	561	832
Compacted bulk density (g/L)	329	302	347
Major nutrients (mg/L)			
Phosphorus	75	60	76
Potassium	252	256	321
Magnesium	204	201	344
Nitrate – N	231	267	419
Ammonium – N	71	4	<1
Calcium	170	167	275
Sodium	29	34	44
Chloride	186	28	37
Sulphur	289	170	316
Trace elements (mg/L)			
Boron	0.15	0.20	0.15
Copper	<0.15	<0.15	<0.15
Manganese	0.49	1.47	0.83
Zinc	0.36	0.45	0.35
Iron	<0.50	<0.50	<0.50

Analyses by Eurofins, samples 400-2008-40015601 to 3.

APPENDIX 3: Full details of disease assessments - fungicide experiment

Table 1: Hebe: effect of fungicide treatments and growing medium amendments on fusarium wilt – 21 August 2008

Treatment		Mean % plants affected (out of 20) after 12 weeks		
		Wilting	Wilting or dead	Healthy
1.	Untreated	1.3	1.7	98.3
2.	Cercobin WG	1.3	1.3	98.8
3.	Octave	3.8	3.8	96.3
4.	Cercobin WG/Octave	1.3	1.3	98.3
5.	Added bark	0.0	0.0	100.0
6.	Cer/Oct + bark	0.0	0.0	100.0
7.	Added lime	0.0	0.0	100.0
8.	Uninoculated control	-	-	-
Significance (24 df)		NS	NS	NS

NS – Not significant

Table 2: Hebe: effect of fungicide treatments and growing medium amendments on fusarium wilt – 10 September 2008

Treatment		Mean % plants (out of 20) affected after 15 weeks			
		Wilting	Dead	Wilting or dead	Healthy
1.	Untreated	9.7	3.3	13.0	87.0
2.	Cercobin WG	2.5	1.3	3.7	96.3
3.	Octave	1.3	1.3	2.5	97.5
4.	Cercobin/Octave	7.5	1.3	8.7	91.2
5.	Added bark	6.2	0	6.2	93.8
6.	Cer/Oct + bark	1.2	0	1.3	98.8
7.	Added lime	8.7	0	8.7	91.2
8.	Uninoculated control	2.5	0	2.5	97.5
Significance (24 df)		NS	NS	NS	NS

NS – Not significant

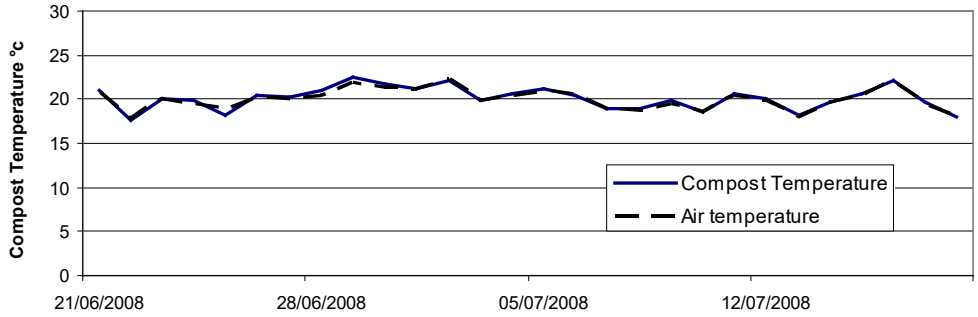
Table 3: Hebe: effect of fungicide treatments and growing medium amendments on fusarium wilt – 1 October 2008

Treatment		Mean % plants (out of 20) affected after 18 weeks			
		Wilting	Dead	Wilting or dead	Healthy
1.	Untreated	5 (2.0)	15 (3.5)	20 (4.0)	80 (4.1)
2.	Cercobin	8 (3.4)	6 (4.0)	14 (6.0)	86 (6.1)
3.	Octave	4 (2.5)	4 (3.2)	8 (4.6)	93 (4.7)
4.	Cercobin/Octave	0 (0.0)	10 (5.0)	10 (5.2)	90 (5.3)
5.	Added bark	5 (2.8)	8 (4.4)	13 (5.7)	88 (5.9)
6.	Cer/Oct + bark	0 (0.0)	1 (1.9)	1 (1.9)	99 (2.0)
7.	Added lime	13 (4.2)	11 (5.2)	24 (7.3)	76 (7.5)
8.	Uninoculated control	3 (2.0)	0 (0.0)	0 (0.0)	98 (2.8)
Significance (24 df)		0.022	0.043	0.013	0.028

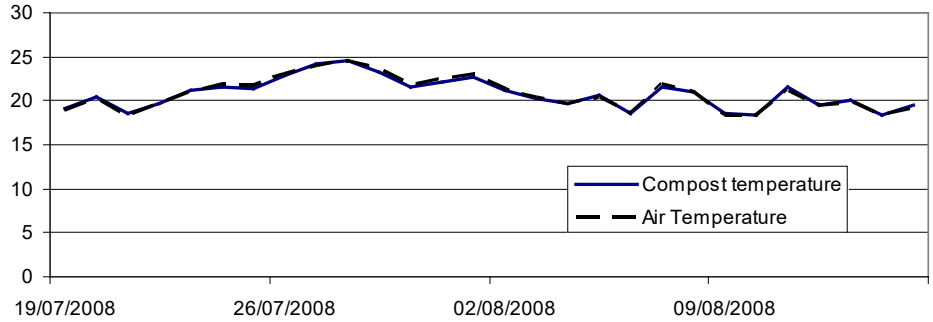
APPENDIX 4: Temperature records

Mean daily temperatures - fungicide experiment

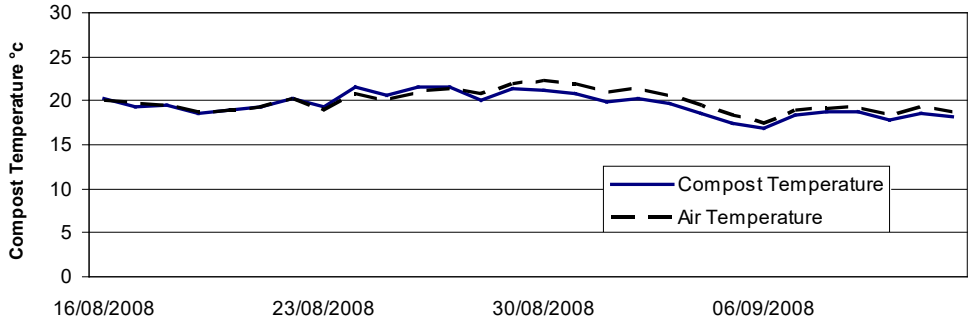
Weeks 1 – 4



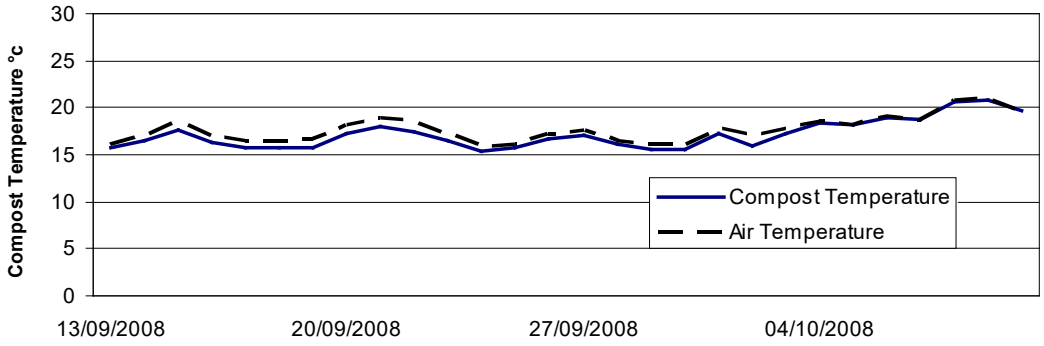
Weeks 5 – 8



Weeks 9 – 13



Weeks 13 - 16



Weeks 16 - 19