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GROWER SUMMARY

Headline

Fusarium oxysporum (the cause of fusarium wilt of hebe) is now causing financial losses in the UK and can persist in sand beds, once-used pots and as symptom-less infection in stock plants.

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 in 2006. 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 year, they 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, 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 as the inoculum (spore concentration) increased. 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.

Effect of temperature and moisture on infection

Plug plants of 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 18°C.

Distribution of F. oxysporum within plants

In order to provide information on the extent of systemic infection within plants, isolation of *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 the roots of some plants (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 in 2006 on cvs. Autumn Glory, Blue Star, Caledonia, Pascal, Pink 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 (Caledonia, Pascal, Pink Paradise, Pink Pixie, Purple Pixie and Rosie) is in progress.

Sources of F. oxysporum on a nursery

In July 2006, samples of sand were collected from three sand beds where batches of affected hebe plants had stood. The samples were examined in the laboratory for *Fusarium* sp. by plating onto agar. Isolates of *Fusarium* sp. were recovered from all the samples tested.

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 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.

- At the end of the experiment, examination of apparently healthy plants revealed additional, symptom-less infection in plants grown in medium mixed with sand from one of the sand beds on the nursery.

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.

- 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).

- Consider checking 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.

SCIENCE SECTION

1. Symptoms and cause of fusarium wilt

The following symptoms were observed on several cultivars of container-grown hebe plants at a nursery in September 2005 and June 2006. Plants were being grown in a peat-based compost in a glasshouse on capillary sand beds:

- pale growth and wilting (crooked tips) affecting some but rarely all young shoots on a plant;
- brown patches on leaves, progressing to cause premature leaf death;
- shoot death, usually from the tip downwards;
- dark brown vascular staining in the stem base and in wilted shoots;
- pale pink fungal pustules on dead shoots.

Fusarium oxysporum was consistently isolated in pure culture from surface-sterilised samples of stem base, affected shoots and leaves. The above symptoms were observed when hebe cv. Pink Pixie was inoculated with a spore suspension of *F. oxysporum* obtained from affected plants; *F. oxysporum* was re-isolated from affected plants in pure culture. These results confirm that *F. oxysporum* is a cause of a vascular wilt in hebe. A fusarium wilt of hebe has previously been described in the USA and the causal fungus was named *F. oxysporum* f. sp. *hebae* (Raabe, 1957). More recently the disease was reported in Italy (Garibaldi *et al.,* 2000). Isolates obtained from affected hebe in the UK were identified as *F. oxysporum* based on fungal morphology and colony appearance. An isolate (AR05/175) from hebe Pink Pixie was subsequently examined by the Central Science Laboratory (CSL) and confirmed as *F. oxysporum* both on morphological features and by DNA sequencing, where a 100% match was found (CSL2051875). Host-specificity tests are in progress as part of this project to determine if the UK fungus is specific to hebe or if it has a wider host-range.

2. Occurrence of fusarium wilt on nurseries and garden centres in the UK

In 2006, the disease was confirmed on hebe on two nurseries in the UK. The varieties that were affected are listed in Section 5, varietal susceptibility. Hebe plants on several other nurseries and garden centres (in Cambs, Essex, Hants, Hereford, Kent, Notts, Warwick, Worcs and Yorks) were examined by ADAS Horticultural consultants in August – September 2006 and no symptoms of the disease were reported. On the nursery where the disease was first confirmed, the problem was first noticed in week 35 in 2004, in week 28 in 2005 and in week 24 in 2006. The disease continued to occur on new plants each year until November. On some varieties (e.g. Pink Pixie), losses averaged around 40% of plants potted.

The UK Plant Health and Seeds Inspectorate (PHSI) determined that the fungus isolated (*F. oxysporum*) from affected hebe was a non-quarantine organism and therefore not subject to any statutory controls.

3. Effect of inoculum level and infection point on disease development

Introduction

An experiment was devised to test the pathogenicity of *F. oxysporum* to hebe using three inoculum levels (10^2, 10^4, and 10^6 conidia/mL) and three inoculation points on plug-grown hebe plants (roots, stem base and cut shoot tips). Untreated controls were also included. The aim was to provide information on potential infection points, the latent period to symptom development and the virulence of *F. oxysporum* to hebe.

Materials and methods

Micro-propagated plants of cv. Pink Pixie were used in order to minimise the risk of using plants already infected with *F. oxysporum.* A preliminary test for *F. oxysporum* by isolation from the stem base and roots of 30 plants before inoculation was negative. Plants were potted into 9 cm diameter pots in Levington M3 compost and grown on capillary matting in gravel trays in a glasshouse. The growing medium was

kept wet for the first week after potting and subsequently plants were irrigated as required by hand-watering. The experiment was a randomised block design with 16 treatments, four-fold replication and 10 plants per plot (contained in one gravel tray). Plants were inoculated with an isolate (AR05/195) of *F. oxysporum* obtained from hebe.

Treatments

	Inoculation site	Inoculum level (spores/ml)
1.	Root dip (unwounded)	Nil
2.	Root dip (unwounded)	10^2
3.	Root dip (unwounded)	10^4
4.	Root dip (unwounded)	10^6
5.	Root dip (wounded)	Nil
6.	Root dip (wounded)	10^2
7.	Root dip (wounded)	10^4
8.	Root dip (wounded)	10^6
9.	Stem base drench	Nil
10.	Stem base drench	10^2
11.	Stem base drench	10^4
12.	Stem base drench	10^6
13.	Trimmed shoot dip	Nil
14.	Trimmed shoot dip	10^2
15.	Trimmed shoot dip	10^4
16.	Trimmed shoot dip	10^6

Treatments were applied and plants potted on 5 June 2006.

Treatments 1-8 were stood upright in the relevant spore suspension or water for 15 minutes. For treatments 5-8, roots were wounded by cutting off c. 0.5 cm of root tips. Treatments 9-12 were applied in 20 ml of water poured around the stem base immediately after potting. Treatments 13-16 were dipped into the spore suspension or water to a depth of c. 2 cm for 15 minutes after removing the tips of 3-5 shoots to create a flat top.

Statistical analysis

Data were analysed using generalised linear models (GLMs) for binomially distributed data in Genstat. While ANOVA makes the assumption that data are normally distributed, GLMs allow analyses of data that do not follow a normal distribution, or where a transformation needs to be applied before normality can be assumed. Data that are proportions will tend to follow a binomial distribution. One way of analysing these data would be to transform them, in order to normalize the data, and analyse the transformed data using ANOVA. The best transformation for binomial data is the logit function, which GLM processes internally to produce an accumulated analysis of deviance. This can be interpreted in much the same way as an analysis of variance. The PREDICT command in Genstat can then be used to get the estimated means and standard errors for each treatment level, back transformed so that they are produced on the original scale.

Results and discussion

A low incidence of general wilting and plant death developed in all treatments, including uninoculated controls, from soon after potting. *Pythium* root rot was confirmed in affected plants and a drench of Subdue (metalaxyl-M) at the recommended rate (0.0625 ml/L) was applied to control this infection. By five weeks after potting, plants had rooted well into the new compost. At this time there was a relatively high incidence of wilting and plant death following the two root dip treatments and the trimmed shoot dip treatment at the highest inoculum level (Table 3.1). A sample of dying plants was examined and *F. oxysporum* was recovered from stem-base vascular tissue. Although generally the incidence of visibly affected plants increased with time, there was a slight reduction in some treatments between 3 and 5 weeks after inoculation. This may have occurred due to some plants recovering from fusarium wilt, at least temporarily; or it may have been due to some of the initial wilting being caused by *Pythium*, from which plants recovered after fungicide treatment. At 15 weeks after inoculation treatments differed greatly in the incidence of dead and wilting plants (Table 3.1). There was a significant effect of inoculum level but not of inoculation site (Table 3.2). At the highest inoculum level, the percentage dead and wilting plants at 15 weeks after inoculation ranged from 43% (stem base drench) to 88% (root dip of unwounded plants). Wounding the roots did

not increase the incidence of affected plants. The incidence of wilting or dead plants in the uninoculated control treatments at this time ranged from nil (root dip, wounded) to 13% of plants (stem base drench with water). The incidence of wilt symptoms following inoculation with 10^4 spores/ml was generally less than at 10^6 spores/ml and greater than the relevant uninoculated control. The incidence of wilt symptoms following inoculation with 10^2 spores/mL was generally similar to that of the relevant uninoculated control.

At the end of the experiment, all apparently healthy plants were examined for vascular browning in the stem base (Table 3.3). There was a highly significant effect of inoculum level and a significant effect of inoculation site (Table 3.4). A high incidence of stem base vascular browning, indicative of infection by *F. oxysporum*, was found following stem base drench inoculation with 10^6 spores/ml (63% of plants). These results indicate that the development of external fusarium wilt symptoms can be relatively slow, and that apparently healthy plants may be infected by the fungus. The mean daily glasshouse temperature during the period of the experiment ranged from 21.3 to 26.9°C, with a minimum of 16.9°C and a maximum of 37.0°C (Appendix 1). Subsequent work (section 4), found that mycelial growth of *F. oxysporum* obtained from hebe was greatest at 25°C.

A sample of six visibly healthy plants selected from four treatments was examined for *F. oxysporum* by isolation from the stem base. *F. oxysporum* was recovered from the inoculated plants (root dip unwounded, root dip wounded, stem base drench, trimmed shoot dip) and not from the control plant treated with water (Table 3.5).

An estimated total number of plants affected by fusarium wilt at the end of the experiment is shown in Table 3.6. These values are the sum of dead or wilting plants combined with plants affected by obvious stem base vascular browning. They are probably an over-estimate by around 10% due to the occurrence of some initial plant death from pythium root rot. The mean effects of inoculum level and inoculation site on the estimated total numbers of plants affected at 15 weeks after planting are shown in Table 3.7. The incidence of infected plants is clearly seen to increase with inoculum level and is largely unaffected by inoculation site.

The occurrence of infection (including vascular staining) following trimmed shoot-tip inoculation is surprising for a vascular pathogen that is generally considered to infect via roots. Possible explanations are: a) spores attached to the leaves and shoots after the dip inoculation were subsequently washed down onto the roots by overhead watering; b) the combination of pythium root rot, shoot-trimming and fusarium inoculation of wounded shoots resulted in shoot die-back and plant death. It is noticeable that, in contrast to the root dip and stem base drench inoculation treatments, the incidence of dead and wilting plants did not increase after the initial 3-week assessment for this treatment. Also, the incidence of symptomless infected plants at the end of the experiment was relatively low (0-12%) and did not increase consistently with inoculum level. These observations suggest that wilting was not the result of infection of roots and development of a vascular wilt.

Table 3.1: Effect of inoculum level and inoculation site on occurrence of fusarium wilt at intervals after inoculation

Treatment		Inoculum level	Mean cumulative percentage dead and wilting plants at intervals after inoculation			
		Spores/mL	3 weeks	5 weeks	8 weeks	15 weeks
1.	Root dip (unwounded)	Nil	5	3	10	8
2.	Root dip (unwounded)	10^2	3	3	8	8
3.	Root dip (unwounded)	10^4	18	8	20	30
4.	Root dip (unwounded)	10^6	20	65	90	88
5.	Root dip (wounded)	Nil	0	0	3	0
6.	Root dip (wounded)	10^2	18	20	28	25
7.	Root dip (wounded)	10^4	15	20	28	28
8.	Root dip (wounded)	10^6	45	65	75	75
9.	Stem base drench	Nil	8	3	8	13
10.	Stem base drench	10^2	10	3	5	8
11.	Stem base drench	10^4	3	0	23	25
12.	Stem base drench	10^6	3	5	33	43
13.	Trimmed shoot dip	Nil	3	3	5	5
14.	Trimmed shoot dip	10^2	30	15	15	18
15.	Trimmed shoot dip	10^4	28	15	23	28
16.	Trimmed shoot dip	10^6	63	60	60	58

Table 3.2: Accumulated analysis of deviance for the effect of inoculum level and inoculation site on occurrence of dead and wilting plants at 15 weeks after inoculation

Change	df	Deviance	Mean deviance	Deviance ratio	F probability
Block	3	5.231	1.744	0.84	0.478
Inoculation site (IS)	3	6.400	2.133	1.03	0.389
Inoculum level (IL)	3	167.582	55.861	26.95	<0.001
IS x IL	9	28.268	3.141	1.52	0.172
Residual	45	93.260	2.072		
Total	63	300.740	4.774		

Table 3.3: Effect of inoculum level and inoculation site on incidence of stem base vascular browning in visibly healthy hebe at 15 weeks after inoculation

Treatment		Inoculum level	Total No. of plants assessed	% affected
1	Root dip (unwounded)	Nil	38	3
2	Root dip (unwounded)	10^2	32	8
3	Root dip (unwounded)	10^4	31	21
4	Root dip (unwounded)	10^6	1	0
5	Root dip (wounded)	Nil	35	0
6	Root dip (wounded)	10^2	17	0
7	Root dip (wounded)	10^4	23	4
8	Root dip (wounded)	10^6	12	15
9	Stem base drench	Nil	31	12
10	Stem base drench	10^2	38	5
11	Stem base drench	10^4	30	9
12	Stem base drench	10^6	25	63
13	Trimmed shoot dip	Nil	33	0
14	Trimmed shoot dip	10^2	31	6
15	Trimmed shoot dip	10^4	25	10
16	Trimmed shoot dip	10^6	14	8

Table 3.4: Accumulated analysis of deviance for the effect of inoculum level and inoculation site on occurrence of stem base vascular browning at 15 weeks after inoculation

Change	Df	Deviance	Mean deviance	Deviance ratio	F probability
Block	3	0.218	0.073	0.06	0.979
Inoculation site (IS)	3	12.569	4.190	3.62	0.021
Inoculum level (IL)	3	31.933	10.644	9.20	<0.001
IS x IL	9	14.946	1.661	1.43	0.205
Residual	42	48.608	1.157		
Total	60	108.274	1.805		

Table 3.5: Recovery of *F. oxysporum* from within the stem base of visibly healthy hebe plants at 15 weeks after inoculation

T	Inoculation site	Inoculum level	Fusarium recovered	No of isolates with fusarium growth (out of 5)
1	Root dip (unwounded)	Nil	No	0
3	Root dip (unwounded)	10^4	Yes	4
8	Root dip (wounded)	10^6	Yes	4
9	Stem base drench	Nil	No	0
12	Stem base drench	10^6	Yes	5
15	Trimmed shoot dip	10^4	Yes	5

T - treatment number sampled.

Table 3.6: Estimated incidence of fusarium wilt at 15 weeks after inoculation

Treatment	Inoculum level (spores/ml)	% plants affected		
		Dead & wilting	Stem base browning	Total
1. Root dip – unwounded	Nil	8	3	11
	10^2	8	6	14
	10^4	30	23	53
	10^6	88	0	88
2. Root dip – wounded	Nil	0	0	0
	10^2	25	0	25
	10^4	28	4	32
	10^6	75	25	100
3. Stem base drench	Nil	13	13	26
	10^2	8	5	13
	10^4	25	10	33
	10^6	43	52	95
4. Trimmed shoot dip	Nil	5	0	5
	10^2	18	6	24
	10^4	28	12	40
	10^6	58	7	65

Table 3.7: Mean effect of inoculum level and inoculation site on estimated incidence of fusarium wilt at 15 weeks after planting

Factor		% plants affected		
		Dead & wilting	Stem base browning	Total
Inoculum level (spores/mL)	Nil	6.5	4.0	10.5
	10^2	14.8	4.3	19.0
	10^4	27.8	12.3	39.5
	10^6	66.0	21.0	87.0
Inoculation site	Root dip - unwounded	33.0	8.0	41.5
	Root dip - wounded	32.0	7.3	39.3
	Stem base drench	22.3	20.0	41.8
	Trimmed shoot dip	27.3	6.3	33.5

4. Effect of temperature on growth of *Fusarium oxysporum*

Introduction

Previous studies on this disease, and observation on an affected nursery in the UK, suggest that hebe fusarium wilt is favoured by warm temperatures. The effect of temperature on the mycelial growth rate of *F. oxysporum* was therefore investigated.

Materials and methods

The growth of *F. oxysporum* mycelium on plates of potato dextrose agar (PDA) was determined at a range of temperatures (5, 10, 15, 20, 25 and 30ºC) using three isolates of the fungus obtained from stained vascular tissue in the stem base of hebe cvs Rosie (AR05/195), Pascal (AR06/96) and Pink Pixie (AR06/98). There were three replicate plates per temperature. Growth was measured as radial growth from a 6 mm diameter plug over a period of 7 days and mean daily growth calculated. The different temperatures were achieved using a series of incubators and controlled environment (CE) cabinets; all plates were incubated in the dark.

Results and discussion

For all three isolates, the growth rate was greatest at 25ºC, with moderate growth at 15 and 20ºC, slight growth at 10 and 30ºC, and no growth after 7 days at 5ºC.

Table 4.1: Effect of temperature on mycelial growth of *F. oxysporum* isolates obtained from hebe

Isolates	Growth rate (mm/day) at incubation temperature (ºC):					
	5	10	15	20	25	30
AR05/195	0	0.5	2.6	3.4	5.1	1.3
AR06/96	0	0.3	2.3	3.2	4.7	1.4
AR06/98	0	0.5	2.6	4.0	5.4	1.4

5. Effect of temperature and moisture on infection of young hebe plants

Introduction

An experiment was devised to investigate the effect of two temperatures and three growing medium moisture levels on the infection of young hebe plants by *F. oxysporum.*

Materials and methods

Plug plants, cv. Pascal, obtained from a nursery with no history of fusarium wilt were inoculated by dipping roots in a spore suspension (1 x 10^6 spores/mL) of *F. oxysporum* obtained from hebe for 15 minutes. Plants were potted into Levington M3 compost in plastic thumb pots and stood on individual plant pot saucers. The potted plants were placed in illuminated (12 h daily) CE cabinets at 18 and 25°C for one week. The compost moisture was maintained dry (no watering for 1 week), damp (watered once, 3 days after inoculation) or wet (watered daily to maintain 1 cm depth of water at the base of pots). After one week all plants were transferred to a warm glasshouse (20-25°C) and watered as required. Uninoculated control plants were included for the damp moisture level. There were eight treatments with four-fold replication and five plants/plot. Results were examined by analysis of variance with the uninoculated control treatments excluded.

Results and discussion

Fusarium wilt was first observed at 4 weeks after inoculation, in plants maintained for 1 week after inoculation at 25°C and with wet compost. By 6 weeks after inoculation, fusarium wilt affected most plants in this treatment and over 50% of plants in other inoculated treatments (Table 5.1). None of the uninoculated control plants developed symptoms of fusarium wilt. Plants maintained without watering for one week after inoculation died, and it was not possible to determine if these plants had developed fusarium wilt.

These results indicate that *F. oxysporum* can infect hebe at both 18 and 25°C, and in both wet and damp growing media. At 5 weeks after inoculation the incidence of

fusarium wilt was significantly greater following an initial 1 week incubation at 25°C, compared with 18°C. There was no significant difference in the incidence of fusarium wilt according to growing medium moisture level. It may be possible to reduce the occurrence of fusarium wilt by growing in environments with more control over temperature (eg glasshouses). Recent studies on fusarium wilt of chickpea, caused by *F. oxysporum* f. sp. *ciceris* found that a temperature increase of just 3°C, from 24 to 27°C had a marked influence on disease development (Landa *et al*., 2006). Some varieties that were moderately or highly resistant at a constant 24°C were highly susceptible at 27°C.

Table 5.1: Effect of growing medium temperature and moisture for one week after inoculation with *F. oxysporum,* on subsequent development of fusarium wilt in hebe

Treatment				Mean number of plants (of 5) with fusarium wilt after:		
	Temperature (°C)	Moisture	Inoculated	4 weeks	5 weeks	6 weeks
1.	18	Wet	Yes	0.75	1.50	3.00
2.	18	Damp	Yes	0.25	1.50	3.75
3.	18	Dry	Yes	-[a]	-[a]	-[a]
4.	18	Damp	No	0.00	0.00	0.0
5.	25	Wet	Yes	1.75	3.75	4.50
6.	25	Damp	Yes	0.50	2.50	4.00
7.	25	Dry	Yes	-[a]	-[a]	-[a]
8.	25	Damp	No	0.00	0.00	0.00
Significance[b]			Temp:	NS	p= 0.007	NS
			Moisture:	NS	NS	NS
LSD			Temp:	0.889	1.076	1.037
			Moisture:	0.889	1.076	1.037

[a] All plants failed to recover after an initial 1 week without watering.

[b] Analysis excludes uninoculated control treatments.

NS - not significant

6. Varietal susceptibility

Introduction

In the UK, fusarium wilt was observed on the following hebe varieties in 2006:

Variety	Type and origin
Autumn Glory	Described in 1912. Bushy habit. A spontaneous seedling, discovered in Northern Ireland.
Blue Star	An introduction from Leo Vergeer in Holland
Caledonia	Described in 1978. Dwarf shrub, origin unknown.
Pascal	No information found
Pink Paradise	An introduction from Joh van Nierkerk in Holland
Pink Pixie	A sport of Purple Pixie
Purple Pixie	From the same batch of seed as Rosie
Purple Shamrock	An introduction from New Place Nursery
Rosie	Described in 1991. Dwarf shrub, a spontaneous hybrid discovered in 1980s near Bransford Nursery.
Sapphire	Described in 1979. A `speciosa` hybrid.
Silver Dollar	A form of albicans probably a sport of Red Edge
Sutherlandii	Old New Zealand cultivar of unknown origin described by G Hutchins in 1997

Pink Pixie and Purple Pixie were more commonly affected than the other varieties. An experiment to test the relative susceptibility of six hebe varieties (Caledonia, Pascal, Pink Paradise, Pink Pixie, Purple Pixie and Rosie) is in progress. Results will be reported in the next annual report.

Although most of the hebe varieties observed affected by fusarium wilt in the UK are newly introduced dwarf forms, one (Autumn Glory) is a bush form grown for over 90 years. A further two (Caledonia and Sapphire) have been grown for over 25 years, and one (Rosie) has been grown for over 15 years (Metcalf, 2001). These four varieties were all identified as spontaneous seedlings, rather than originating from specific breeding lines. These observations indicate that susceptibility to fusarium wilt

in hebe has been present in some varieties for many years and is not a recently introduced trait.

7. Distribution of *F. oxysporum* within plants

Introduction

In order to provide information on the extent of systemic infection within plants, and possible infection points, isolations were made from different plant parts.

Materials and methods

Between November 2005 and June 2006, samples of rooted plugs and small potted plants supplied by a nursery with a history of the disease were tested for *F. oxysporum* by isolation onto agar. Micro-propagated plug plants supplied by Micropropagation Services Ltd., Leics. were also examined. Isolations were made onto PDA after surface sterilisation in sodium hypochlorite (1% for 1 minute). Plants with visible symptoms of fusarium wilt and apparently healthy plants were tested.

In October 2006, systematic isolations were made from three sets of large plants:

1. 2 x 3 L pots of cv. Pink Pixie standard stock from an affected nursery (25 shoots from each of two plants).
2. 2 x 3 L pots of cv. Pink Pixie micropropagated stocks from an affected nursery (25 shoots from each of two plants).
3. 5 x 1 L pots of cv. Pascal, standard stock from an unaffected nursery (10 shoots from each of five plants).

A total of 50 shoots were tested per sample, using 2 cm long shoots taken from around 5 cm from the tip. These semi-woody shoots were surface-sterilised in sodium hypochlorite (1% for 1 minute) and the central 1 cm length plated onto agar. The branch from which each shoot was taken was recorded. Isolations were made onto PDA + streptomycin and fungal growth was identified according to colony characteristics and microscopic examination of spores.

Results and discussion

A *Fusarium* species, most probably *F. oxysporum* was recovered at a low incidence from the stem base of apparently healthy rooted cuttings of cvs. Purple Pixie (1/20) and Rosie (3/20) in winter 2005 (Table 7.1). Dark brown staining was present in the stem base of most cuttings from which the fungus was recovered. In June 2006, dark brown vascular staining was observed in the stem base of potted plants of cv. Pink Pixie, and *F. oxysporum* was recovered from these tissues.

A *Fusarium* species, probably *F. oxysporum*, was also recovered from 3/50 shoots of visibly healthy stock plants of cv. Pink Pixie (Table 7.2). All of the infected shoots came from the same main branch, suggesting systemic infection. A *Fusarium* species was also recovered from three shoots on separate branches of cv. Pascal from a nursery with no history of fusarium wilt. Laboratory examination indicated that these isolates were probably a species other than *F. oxysporum.*

The recovery of *F. oxysporum* from within the shoot tips of large hebe plants used as a source of cuttings, together with recovery from within the stem base of apparently healthy young rooted cuttings, suggests that the fungus may inadvertently be maintained on a nursery in the propagation cycle.

Table 7.1: Recovery of fusarium from different tissues of small hebe plants

Date examined	Plant size	Variety	Fusarium wilt visible	Fusarium recovered from:	
				Stem base	Roots
16 Nov 2005	Rooted cutting	Rosie	No	3/20[a]	0/20[b]
12 Dec 2005	Rooted cutting	Purple Pixie	No	1/20	0/20
7 Feb 2006	Rooted cuttings	Pink Pixie	No	0/10	0/10[c]
	Overwintered cuttings	Pink Pixie	No	0/10	0/10[c]
	Overwintered cuttings	Rosie	No	0/10	0/10[c]
22 Feb 2006	Rooted cuttings	Pink Pixie	No	0/20	0/20[c]
15 Jun 2006	9 cm pot	Pascal	Yes	Yes	-
		Pink Pixie	Yes	Yes	-
20 Jun 2006	9 cm pot	Dark Angel	No	0/5	0/5[b]
		Pink Pixie	No	3/5[a]	1/5[b]
		Purple Pixie	No	0/10	0/10[b]
		Rosie	No	0/10	0/10

[a] Vascular browning present in the stem base.
[b] *Pythium* recovered from roots.
[c] *Trichoderma* recovered from roots.

Table 7.2: Recovery of *Fusarium* sp. from shoots of large hebe plants – October 2006

Sample	No. shoots with Fusarium	No. main branches carrying infected shoots
1. Pink Pixie, standard stock, affected nursery	3/20	1/17
2. Pink Pixie, microprop stock, affected nursery	0/50	0/21
3. Pascal, standard stock, unaffected nursery	4/50	3/14

8. Investigation of sand beds and once-used pots as sources of infection

Introduction

It is known that *formae speciales* of *F. oxysporum* affecting other hosts (e.g. aster, stock) can survive in soil for at least one and probably several years. In July 2006, roots found in a sand bed on which hebe plants were standing were tested for *Fusarium* by isolation onto nutrient agar. A *Fusarium* sp. was recovered from 13/16 root sections indicating that sand beds could act as a source of infection. Experiments were therefore devised to determine if the *F. oxysporum* causing wilt in hebe occurred in sand beds on which container-grown hebe plants affected by fusarium wilt had stood. Samples of sand were tested directly by plating out onto a selective medium and indirectly by a growing-on test. Additionally, plastic plant pots that had contained hebe plants affected by fusarium wilt were tested by a growing-on test to determine if they contained *F. oxysporum*.

Materials and methods

Once-used plastic plant pots and samples of sand from sand beds (below Mypex matting) were collected from a nursery with a history of hebe fusarium wilt in July and October 2006. All of the pots had contained hebe plants affected by fusarium wilt in 2005. All of the sand beds had been covered in hebe plants, some affected by fusarium wilt. The sand samples comprised around 1 kg and were taken from a minimum of 10 points per bed. The samples were taken from the following beds:

July 2006	October 2006
Misting house	Bed 23 (untreated)
Propagation house (below Pink Pixie liners)	Bed 14 (Jet 5 treated)
Main house, finishing (Bed 27)	Original propagation tunnel

Growing-on test

Samples collected in July 2006 were used in a growing-on test. Sand from the three beds was mixed with soil-less growing medium (Levington M3) in a 1:3 ratio (V/V) and used to fill new plastic plant pots (9 cm diameter). Unamended growing medium was used to fill the once-used plastic plant pots. In the positive control, plants were

inoculated at potting by a 15-minute root dip in a suspension of *F. oxysporum* spores (1 x 10^6 spores/mL) using an isolate obtained from hebe. Treatments are listed below:

Sand amendment to growing medium	Pots	Inoculated with *F. oxysporum*
Effect of sand		
1. Sterile sand (negative control)	New	No
2. Mist house sand	New	No
3. Propagation house sand	New	No
4. Main house sand	New	No
Effect of pots		
5. No sand (negative control)	New	No
6. No sand	Old	No
Positive control		
7. No sand (positive control)	New	Yes

The pots of growing medium were potted with rooted cuttings of hebe cv. Pink Pixie. The plants in each plot were placed on capillary matting in a gravel tray. Plants were hand-watered as required and grown for 18 weeks (September 2006 – January 2007) in a heated glasshouse.

Plants were inspected every 1-2 weeks and the incidence of plants with fusarium wilt symptoms was recorded. At the end of the experiment, plants were destructively assessed and any non-wilting plants found to have strong dark brown vascular staining in the stem base were considered to be infected by fusarium wilt. Isolations were made from stem base vascular tissue in a sample of plants to confirm infection by *F. oxysporum*.

Treatments were arranged in four randomised blocks and individual plots contained 10 plants. Results were examined by analysis of variance.

Direct testing of sand

Samples collected in October 2006 were used in this test.

1. Sand from bed 23 (high risk, untreated)
2. Sand from bed 14 (high risk, Jet 5 treated*)
3. Sand from original propagation tunnel
4. Sterilised silver sand (negative control)
5. Sterilised silver sand inoculated with *F. oxysporum* ex hebe AR05/195 (positive control)

*The bed was flooded with Jet 5, applied at a 1 in 100 dilution through the header tank to deliver 5 L/m^2 of the diluted material, and drained after 2 hours.

The positive control (treatment 5) was created by adding 1 mL of a 1 x 10^7 spore / mL spore suspension to 500 mL tap water containing 200 g of sterile silver sand.

200 g of sand from each sample was thoroughly mixed in 500 mL tap water. Most of the water was immediately decanted off into a clean beaker and then allowed to settle for 24 h.

After this time, all of the water was decanted off (and disposed of) leaving only the sediment. The sediment was re-suspended in 20 mL of sterile distilled water (SDW) and filtered through two layers of muslin. The remaining liquid was centrifuged at 2000 rpm for 15 minutes. The supernatant was removed and the pellet was re-suspended in 5 ml SDW. For each treatment, 1 ml of liquid and a 1 in 10 dilution were spread onto each of three plates of Komada's medium (Appendix 2), a medium selective for *F. oxysporum*. Plates were incubated in the dark at 20°C for 4-5 days. Where possible the number of fusarium colony forming units (cfus) were counted on each plate.

Selected isolates obtained from the sand beds were tested for their pathogenicity to hebe by root-dip inoculation of plug plants, cv. Pascal, in a spore suspension (10^6 spores/mL). There were six replicate plants per isolate, three of each variety. An untreated control and a positive control (i.e. an isolate of *F. oxysporum* known to be pathogenic to hebe) were included. Plants were grown on in a heated glasshouse.

This experiment is continuing and results will be reported in the second annual report.

Results and discussion

Growing-on test

The mean daily glasshouse temperature during this experiment ranged from 18 to 24°C. Symptoms of fusarium wilt first occurred at 3 weeks after inoculation. By 6 weeks after inoculation, all of the root-dip inoculated plants (positive control) had wilted and died; occasional plants in one of the sand-amended growing media were showing symptoms at this time (Table 8.1). At the end of the experiment, there were clear differences between treatments in the incidence of plants affected by fusarium wilt (Table 8.2). One of the three sand sources, and the once-used pots, were demonstrated to be infested with *F. oxysporum* at levels sufficient to cause fusarium wilt in hebe. The two negative control treatments (sterile sand admixed with new growing medium, and unamended growing medium) remained free of fusarium wilt symptoms. *F. oxysporum* was recovered from plants with obvious stem base vascular browning. Because of the large number of zero values, ANOVA was not valid and no statistical analysis is shown.

Direct testing of sand

Fusarium was recovered from all three sand beds, and the positive control, and not from the negative control (Table 8.3). The levels of *Fusarium* in the sand beds were similar (10-21 cfu/g). Isolates recovered from the sand have been cultured and their pathogenicity to hebe will be tested.

Table 8.1: Effect of sand from nursery sand beds and once-used plastic pots on development of fusarium wilt in hebe, cv. Pink Pixie

Treatment			Mean number plants (of 5) with fusarium wilt after:			
Sand	Pots	Inoculated	3 weeks	6 weeks	12 weeks	18 weeks
Effect of sand						
1. Sterile (negative control)	New	No	0	0	0	0
2. Mist house sand	New	No	0	0	0	0
3. Prop house sand	New	No	0	0.3	1.3	1.3
4. Main house sand	New	No	0	0	0	0
Effect of pots						
5. No sand (negative control)	New	No	0	0	0	0
6. No sand	Old	No	0	0	0	0.8
Positive control						
7. No sand	New	Yes	0.8	5.0	5.0	5.0

Table 8.2: Occurrence of plant wilting and stem base vascular browning in hebe, cv. Pink Pixie, at 18 weeks after potting

Treatment			Mean % plants affected		
	Pots	Inoculated	Plant wilting	Vascular staining in non-wilting plants	Total
Effect of sand					
1. Sterile (negative control)	New	No	0	0	0
2. Mist house sand	New	No	0	0	0
3. Prop house sand	New	No	25	15	40
4. Main house sand	New	No	0	0	0
Effect of pots					
5. No sand (negative control)	New	No	0	0	0
6. No sand	Old	No	15	0	15
Positive control					
7. No sand	New	Yes	100	0	100

Table 8.3: Recovery of *Fusarium* sp. from nursery sand beds and peat debris in once-used pots

Treatment	Mean number of propagules (cfu) per g of moist sand
1. Sand from bed 23 (high risk, untreated)	13.3
2. Sand from bed 14 (high risk, Jet 5 treated)	20.7
3. Sand from original propagation tunnel	9.8
4. Sterilised silver sand (negative control)	0.0
5. Sterilised silver sand inoculated with *F. oxysporum* (positive control)	Too numerous to count

9. Overall conclusions

1. Fusarium wilt of hebe caused by *F. oxysporum* occurs in the UK. Pathogenicity of the fungus to hebe was confirmed.

2. The disease was found on two nurseries. It affects a wide range of hebe varieties including Autumn Glory, Blue Star, Caledonia, Pascal, Pink Paradise, Pink Pixie, Purple Pixie, Purple Shamrock, Rosie, Sapphire, Silver Dollar and Sutherlandii.

3. 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.

4. Mycelial growth of *F. oxysporum* isolated from hebe occurs at 10-30°C and is optimal at 25°C.

5. There is some evidence that a high temperature (25°C) favours infection of roots by *F. oxysporum*.

6. *F. oxysporum* was recovered from roots, stem bases and shoots of some visibly healthy hebe plants indicating that symptomless, systemic infection can occur.

7. *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.

10. Technology transfer

- Project meetings at Bransford-Webb Nursery, 14 July and 13 October 2006.
- Project review meeting, London, 12 March 2007.

11. Acknowledgements

We are grateful to Ann Barnes of CSL for confirming the identification *of Fusarium oxysporum*. Also to Martin McPherson of Stockbridge Technology Centre (STC) for supply of an isolate of *F. oxysporum* from hebe.

12. References

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13. Appendix 1. Trial diaries and temperature records

1. Inoculation site and inoculum level experiment

Date	Actions
24 May 06	*Fusarium oxysporum* Isolate No AR05/195 sub-cultured onto 20 PDA +s plates and incubated at 20 ^{0}C.
5 June 06	Spore suspensions prepared at different inoculum levels (10^2, 10^4 & 10^6) and treatments applied according to protocol. Post treatment: All plants potted using M3 compost and placed, in a randomised design, into an ADAS glasshouse.
28 June 06	Removed dead plants (*Pythium* affected). Dead plants were placed into damp chambers for fusarium growth identification. First wilt assessment. First symptoms of wilt in the experiment.
30 June 06	Applied Subdue drench treatment (0.0625 ml/L) to all pots.
7 July 06	Dead plants removed on 28 June assessed for fusarium growth.
12 July 06	Second wilt assessment.
2 August 06	Third wilt assessment.
7 September 06	Fourth wilt assessment. Spider mite damage to plants masked the wilt symptoms so this assessment was treated with caution.
26 September 06	Final assessment: stem base vascular staining.

	Weekly temperatures (^{0}C)		
Week Number	Min	Mean	Max
1	18.1	24.3	31.7
2	19.4	22.8	30.3
3	16.9	24.0	35.0
4	20.3	25.1	34.3
5	16.9	24.5	35.0
6	20.3	26.9	37.0
7	20.0	25.6	34.7
8	19.4	22.6	31.7
9	18.8	22.0	29.2
10	18.1	21.3	26.8
11	18.8	21.3	27.2
12	18.8	21.4	29.9
13	18.8	22.8	30.6
14	20.0	22.7	30.6
15	19.7	22.3	29.6

2. Temperature and growing medium moisture experiment

Date	Actions
21 August 06	*Fusarium oxysporum* Isolate No AR05/195 sub-cultured onto 20 PDA +s plates and incubated at 20 ^{0}C
6 September 06	Spore suspension prepared (10^6 spores per mL) and treatments applied according to protocol. Post treatment: All plants potted using M3 compost and placed, in a randomised design, into ADAS CE Cabinets, one set at 18°C and the other at 25°C. Both Cabinets were set at 70% relative humidity. Plants either watered (via saucer) or not, according to treatment requirement. Wet treatments watered to capacity.
8 – 12 September 06	Plants either watered or left dry according to treatment requirement. Wet treatment watered to capacity.
13 September 06	Removed all plants from CE cabinets and placed, in the same randomised design, into an ADAS glasshouse.
2 October 06	First Wilt assessment.
9 October 06	Second wilt assessment.
18 October 06	Third wilt assessment.

	Weekly temperatures (^{0}C)		
Week Number	Min	Mean	Max
2	20.0	22.7	30.6
3	19.7	22.3	29.6
4	18.8	22.0	31.7
5	18.5	22.4	32.1
6	20.0	22.5	29.9

3. Sand and once-used pots experiment

Date	Actions
21 August 06	*Fusarium oxysporum* Isolate No AR05/195 sub-cultured onto 20 PDA +s plates and incubated at 20°C
6 September 06	Spore suspension prepared (10^6 spores per ml) and treatments applied according to protocol. Post treatment: All plants potted using M3 compost and placed, in a randomised design, into an ADAS glasshouse.
2 October 06	First Wilt assessment. First wilted plant recorded in treatment 7 (inoculated control).
9 October 06	Second wilt assessment.
18 October 06	Third wilt assessment. First wilted plant recorded in Treatment 3 (propagation house sand).
8 November 06	Fourth wilt assessment.
20 November 06	Fifth wilt assessment.
11 December 06	Sixth wilt assessment.
18 December 06	First wilted plant recorded for treatment 6 (old pots).
8 January 07	Final wilt assessment.
19 January 07	Stem browning assessment.
26 January 07	Fusarium recovery assessment set up.

	Weekly temperatures (^{0}C)		
Week Number	Min	Mean	Max
5	13.2	20.3	37.8
6	13.5	19.2	38.2
7	11.3	18.0	38.2
8	8.3	20.9	41.1
9	19.4	24.0	39.4
10	18.8	23.4	38.2
11	17.8	22.9	33.9
12	8.3	21.5	29.2
13	7.0	19.6	36.2
14	4.2	18.8	38.2
15	16.9	21.3	34.7
16	9.8	21.2	31.0
17	7.3	21.4	36.2
18	9.5	20.8	35.0

14. Appendix 2: Details of Komada's medium

For isolation of *Fusarium oxysporum* from soil and plant tissue – not recommended for other *Fusarium* species.

	500 mL	1 litre
• Distilled water		
• $K_2 HPO_4$	0.5 g	1.0 g
• KCl	0.25 g	0.50 g
• $MgSO_4 7H_2O$	0.25 g	0.50 g
• FeNa EDTA	0.005 g	0.01 g
• L-asparagine	1.0 g	2.0 g
• D-galactose	10 g	20 g
• Agar	7.5 g	15 g

Autoclave for 15 minutes, cool to 50°C and add the following supplements, mixing thoroughly before pouring.

Supplements

	500 mL	1 litre
• Pentachloronitrobenzene (PCNB)	0.5 g	1.0 g
• Oxgall	0.25 g	0.50 g
• $Na_2B_4O_7 10H_2O$ (Borax)	0.5 g	1.0 g
• Streptomycin sulphate	0.15 g	0.30 g