Project title:	Evaluation of fungicides and novel treatments for the control of black root rot, *Thielaviopsis basicola*, in bedding and hardy nursery stock plants
Project number:	HNS-PO 190
Project leader:	Dr Erika F. Wedgwood, ADAS
Report:	Annual report, August 2016
Previous report:	Annual report, August 2015
Key staff:	Erika Wedgwood Jonny Kerley Sam Brown Chris Dyer
Location of project:	2014 Viola: ADAS Boxworth, Battlegate Rd., Boxworth, Cambridge, CB23 4NN. 2015 Choisya: Fletchers Lane site of New Place Nurseries Ltd, Sidlesham Common, West Sussex PO20 7QG
Industry representative:	Protected Ornamentals: Name: Ian Lavelle Address: Ivan Ambrose Co. Ltd., Rosemount Nursery, Pygons Hill Lane, Lydiate, Merseyside, L31 4JD Hardy Ornamental Nursery Stock: Name: Mike Norris Address: New Place Nurseries Ltd, London Road, Pulborough, West Sussex, RH20 1AT
Date project commenced:	1st September 2013
Date project completed (or expected completion date):	31st August 2017

DISCLAIMER

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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

AUTHENTICATION

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

Dr Erika F. Wedgwood
Research Scientist
ADAS UK Ltd

Signature  Date 29 July 2016.

Report authorised by:
Dr Barry Mulholland
Head of Horticulture
ADAS UK Ltd

Signature  Date 04 August 2016

CONTENTS

GROWER SUMMARY

Headline

Thielaviopsis basicola infection prevention by the use of either a thiophanate-methyl drench or application of novel products cannot be relied on. The extent of an infection can be underestimated while the fungus is feeding without producing typical blackened roots, and without plant stress, foliar vigour is maintained so supporting further *T. basicola* multiplication.

Background

Symptoms and prevalence

Black root rot (*Thielaviopsis basicola*, syn. *Chalara elegans*) affects plants from at least 15 families, causes root damage leading to reduced nutrient and water uptake, consequent leaf yellowing and potentially plant death. Losses to black root rot in *Viola* spp. production can be substantial, and some hardy nursery stock species such as *Choisya*, *Skimmia* and *Ilex* are particularly susceptible. Black root rot is a long-established problem for growers, and in the UK, it is likely that around 15% of ornamentals production on nurseries is susceptible to black root rot. It has been estimated that in any year 25% of susceptible crops on UK nurseries can be affected by black root rot, with a fifth of these failing to be sold because either the reduction in quality is too great or the infection has been so severe that the plants have died.

In bedding plants, such as *Viola* and *Primula* species, losses occur on nurseries within the couple of months preceding crop flowering. It is probable that around 2% of bedding and pot plants of *Viola* and *Primula* species become affected annually by black root rot, but as growers are aware of these plants' susceptibility, fungicide treatments can reduce this loss to around 0.5%. Losses tend to be greatest in July as the plants can suffer from heat stress and become more susceptible to infection. In nursery stock species, such as *Choisya* and *Skimmia*, losses are often seen during establishment shortly after crops are potted up, whereas losses tend to be seen in finished plants of herbaceous species such as geraniums. In addition to losses facilitated by stress from heat or root disturbance, early crops can succumb to black root rot when conditions are cold.

Existing control measures and potential novel products

Cultural control

Growers aim to employ cultural control measures such as reducing plant stress and taking care over crop hygiene to reduce the chance of plants becoming infected. However, the pathogen produces resting spores (chlamydospores) in roots which then survive in debris in matting, re-used containers and soil, and can be resistant to disinfectant treatments (as

reported in AHDB Project PC 38c and Factsheet 03/14 revision of 15/05). Details or various measures were given in the review of black root, PO 14 (Wedgwood, 2013).

Biological control

The biological products Prestop (*Gliocladium catenulatum*), Serenade ASO (*Bacillus subtilis*), T34 Biocontrol (*Trichoderma asperellum*), Trianum-G and Trianum-P (both *Trichoderma harzianum* T-22) can be used on ornamentals in the UK against root rots (principally targeting *Pythium* and *Phytophthora* spp.), although with various restrictions. Some growers have continued to only use chemical fungicides against root rots, in part dut to the relatively recent availability of biofungicides in the UK, and also due to uncertainty in their efficacy. Only Prestop is said in the product technical notes to have some activity against *Thielaviopsis* sp. and some significant reduction was achieved after preventative plus curative application to *Viola* sp. with a low infection severity in Year 1 of this project. The current project showed some benefit from incorporation of Trianum-G in the growing media of *Viola* sp., but no benefit from Serenade ASO. In projects such as SCEPTRE CP 077 some biofungicides have occasionally shown some reduction of pathogens at low levels and in general more work needs to be done on how growers can best integrate biofungicides into their management programmes.

Chemical control

Growers of crops susceptible to black root rot usually treat them with a protectant fungicide drench such as Cercobin WG (thiophanate-methyl), applied to container plants at sowing (bedding plants) or potting-on (nursery stock). The product also protects against *Cylindrocarpon, Rhizoctonia* and *Fusarium* species. Treatment with products such as Subdue (metalaxyl-M) or Fenomenal (fenamidone + fosetyl-aluminium) are used in addition against the oomycete pathogen species of *Pythium* and *Phytophthora,* and do not give control of *Thielaviopsis* sp..

Cercobin WG can only be applied once per crop, which must then be left for three weeks before planting into open ground (EAMU 2011 1887). The resistance risk of this benzimidazole fungicide is noted to be high, having the same FRAC 1 grouping as benomyl. A further issue for growers is that although one application can be adequate to maintain quality for short-production bedding plants, this does not maintain protection for nursery stock under long production cycles. Nursery stock usually starts under protection, often with plug plants potted-on as liners then around a year later potted-on to finals. Only container-grown ornamentals under permanent protection are allowed to be drenched with Cercobin WG,

although such crops are more likely to experience greater heat stress and consequently become more disease susceptible.

Research in AHDB project PC 38 showed good black root rot control by Benlate (benomyl), but resistance issues have since caused product withdrawal. Fungicide screening in AHDB project PC 143 against black root rot on pansy used foliar products as drenches. Amistar (azoxystrobin), Bavistin DF (carbendazim), experimental product F279, Scotts Octave (prochloraz), Plover (difenoconazole), Unix (cyprodinil) and Stroby WG (kresoxim-methyl) gave some control, without leaf scorch. Bavistin DF is now unavailable. Cyprodinil is available within Switch, but after use as a protectant and a curative foliar spray in the current project, the black root rot severity in *Viola* sp. did not differ significantly from that in untreated plants.

Amistar (EAMU 0443 of 2009) Scotts Octave (Full Approval) and Stroby WG (Full Approval) can currently be applied as foliar sprays in ornamental plant production. Scotts Octave (FRAC 3) can be applied as a growing media drench and this is the product currently used on susceptible ornamentals by many UK growers against black root rot in polytunnel crops. Scotts Octave does, however, cost about ten times that of Cercobin WG. There are other prochloraz products, such as Sporgon 50 WP, however in 2016, only limited volumes are still held by growers because of their impending withdrawal from use.

The current project aims to identify novel treatments, including non-conventional elicitors and microbial products, and to test their efficacy as preventative and curative drenches against black root rot. The products were tested individually (Experiments 1 & 2 on *Viola* sp., see 2014 Annual Report) and in programmes (Experiment 3 on *Viola* sp., see 2015 Annual Report). This current report is on *Choisya* sp. liners which received preventative and/or preventative + curative treatments found to be safe and effective on *Viola* sp. in 2014.

Summary

Experiment 4 investigated simple programmes on HNS liners, andwas set up in a commercial glasshouse with 640 *Choisya ternata* plug plants potted up on 29 April 2015. There were ten 90 mm diameter pots per treatment in each of four replicate blocks. As shown in Table 1, five treatments comprising four biofungicides (available for use on ornamentals) and a non-conventional coded product were applied the day after potting (timing P1). Six weeks later, when new roots had established, these plants were re-treated with the same products (timing P2). In addition, three experimental conventional chemical products and a standard containing thiophanate-methyl, were also applied over the foliage and growing media surface in separate treatments. After a further week, plants (except one untreated treatment) were

inoculated on 11 June 2015 using an isolate of *T. basicola* from *C. temata*. Three chemicals with potential curative activity were applied to several plots a week after inoculation (timing C). No products were applied to plots of two of the treatments at any of the three timings.

Table 1. Experiment 4 on Choisya sp. in 2015. Products, application timings and rates and approval status in ornamentals. (Protectant timings: P1 at potting on 30 April, and P2 five weeks after potting, one week before inoculation was done on 11 June. Curative timing: C 10 weeks after potting and four weeks after inoculation)

T	Product & code & [MAPP No.]	Active ingredient	Application timing			Dose & water volume	Comments & UK approval status
1	Un-treated	none	-	-	-	water alone	Inoculated with black root rot spores
2	Un-treated	none	-	-	-		
3	Un-treated	none	-	-	-	water alone	NOT inoculated
4	Topsin* [13988]	thiophanate-methyl	-	**P2**	-	1.1 g per 10 L water using 1000 L/ha	Standard rate for BRR. One Cercobin WG drench permitted
5	Signum [11450]	boscalid + pyraclo-strobin	-	**P2**	-	1.35 kg / ha in 400 L/ha water	EAMU 1107 of 2009 for ornamentals
6	HDC F174	chemical mixture	-	**P2**	-	Spray using 400 L/ha water	Agrochemical company rate
7			-	**P2**	**C**		
8	HDC F175	chemical	-	**P2**	-	Spray using 400 L/ha water	Agrochemical company test rate
9			-	**P2**	**C**		
10	HDC F176	chemical mixture	-	**P2**	-	Spray using 400 L/ha water	Agrochemical company test rate
11			-	**P2**	**C**		
12	HDC F178	chemical	**P1**	**P2**	-	**P1**: half rate in 400 L/ha water **P2**: full rate in 400 L/ha water	Agrochemical company rate
13	T34 Bio-control [15603]	*Trichoderma asperellum* T34	**P1**	**P2**	-	2 hours before use spore suspension made up 0.5 g in 10 L water per 100 L growing media.	EAMU 1118 of 2012 for outdoor container ornamentals and any protected ornamentals Irrigation rate at 10% of pot volume.
14	Trianum-P [16741]	*Trichoderma harzianum* T-22	**P1**	**P2**	-	**P1**: 0.3g /10 L growing media using 1 L water **P2**: 0.15 g /10 L growing media using 1 L water	"Lower crop density" rate. 10% of pot volume. Approved on protected ornamentals
15	Prestop [17223]	*Gliocladium catenulatum* J1446	**P1**	**P2**	-	5.0 g Prestop in 1 L water. Used at 10 % pot volume over foliage	Approved on ornamentals
16	Serenade ASO [16139]	*Bacillus subtilis* QST 713	**P1**	**P2**	-	10 L Serenade per ha (10,000 m sq) in 1000 L water / ha	EAMUs 0706 and 0708 of 2013 for ornamentals.

* Topsin WG was supplied in place of Cercobin WG, both have an identical formulation.

In July 2015, all of the treated inoculated *Choisya* sp. had foliar vigour equivalent to that of untreated uninoculated plants. By August nine treatments had become significantly more vigorous. By October, vigour was equally good in all treatments, but still significantly better after preventative F175 use as a foliar spray followed by irrigation. Unfortunately, product F175 is not now going to be brought to the UK. At destructive assessment in December 2015 all treatments (including the untreated) had become equally affected by black root rot, and there had also been natural infection by *Pythium* spp., leaving (on average) only 30% of the root surface area healthy across all the treatments. Biofungicides and novel chemical products tested on *Choisya* sp. liners inoculated with black root rot, were thus unable to prevent root rot any more than a standard thiophanate-methyl drench, but foliage vigour was improved most consistently by one novel chemical treatment. It was noted that the extent of root infection by *Thielaviopsis* sp. had the potential to be underestimated on nurseries as the pathogen invaded root tissue beyond the area of blackening caused by the resting spores.

Experiment 5 commenced in April 2016. A fresh batch of *Choisya* spp. liners were potted as finals and treated with products selected from earlier trials, using them in alternating programmes. The plants' foliage will be assessed until destructive assessment after a year. The results will be reported in the Final Report in August 2017.

In this project, growers using available products for use in these crops have proved to be effective against black root rot. Any chemical products that can be made available through the EAMU approval system will enable different modes of action to be employed, therefore reducing the chance of fungicide resistance developing. Biological products containing *Trichoderma* spp. with activity already known against other root pathogens such as *Pythium* spp. were shown to provide wider protection at no additional cost.

The use of biological products to suppress black root rot will help the industry to comply with the EU Sustainable Use Directive for reduced pesticide use, by using integrated crop management. This will ensure that suppliers can secure the business of clients anxious to source plants grown with minimal adverse environmental impact.

Financial Benefits

It is difficult to quantify plant losses due to black root rot for several reasons: the intermittent symptom expression usually associated with periods of heat stress, unrecorded incidence (particularly if symptoms develop no further than causing lower quality plants), and roots affected by *T. basicola* can also be infected by other root rotting pathogens without losses being quantified. Fungicides are used in preventative programmes by most growers. On the nursery hosting this project the *Choisya* sp. are grown under protection and so Prestop can be used at potting against the whole spectrum of root rot pathogens. It costs around £1.50 to

protect 1000 rooted plugs (selling at £280-£350) and £15 per 1000 liners (selling at £900-£1150). Cercobin WG is also applied in autumn to liners against fungal (not oomycete) root rots, costing around 8.3 pence per 1000 pots. A biofungicide application is currently more costly than Cercobin WG use, but Scotts Octave use would cost £3.33 per 1000 liners.

Nationally, in England and Wales, it is probable that 1% of pansies are killed by black root rot, equating to an annual loss of £21,000, but this would rise to £105,000 if fungicides were not used. Around 5% overall of Choisya and Skimmia are probably lost to black root rot. These are the main HNS subjects affected and they represent about 2% of the container plant range. This would equate to annual losses to the disease in the UK from these plants alone of £346,000. Losses however, can be around 20% on some nurseries in years where controls fail. Effective treatments will improve crop quality by maintaining a healthy root system, improving crop establishment and reducing crop losses. Providing a range of products that can be applied at intervals during production to improve root system performance will be particularly important for hardy nursery stock where plants are sold by pot size.

Action Points

- Pay close attention to nursery hygiene and avoid plant stress to prevent introducing and encouraging black root rot. See the PO 14 review for further guidance.
- Early infection stages may cause the roots to become pale brown and typical dark brown speckling will not be seen until resting spores are formed. Do not wait to see reduced vigour, regularly inspect roots.
- Treat or destroy affected plants promptly (do not compost) otherwise other plants can be infected and produce further spread within weeks.
- Clear off root debris on matting or trays, as the mycelium and resting spores can survive for several years.
- Consider preventative use of microbial products to increase resistance to a number of pathogens. Prestop (*Gliocladium catenulatum*) and Trianum-G (*Trichoderma harzianum*) are permitted as drenches to ornamentals and significantly reduced black root rot in the tests with inoculated *Viola* sp., with some beneficial effects from T34 Biocontrol (*Trichoderma asperellum*) applied at sowing.
- Check AHDB Horticulture e-mail alerts for EAMUS. One is being sought for F174.
- As and when new products become available, select a range of chemical plant protection products with different modes of action to avoid build-up of resistance to active ingredients. Be prepared to test the crop safety and efficacy of products with EAMUs before inclusion in nursery-wide programmes.

SCIENCE SECTION

Introduction

Black root rot (*Thielaviopsis basicola*, syn. *Chalara elegans*) affects plants from at least 15 families (Table 2).

Table 2. Ornamental plants particularly susceptible to infection by *T. basicola*

Antirrhinum majus	*Ilex* spp.
Astilbe spp.	*Kalanchoe* spp.
Begonia semperflorens	*Lathyrus* spp.
Buxus sempervirens	*Paeonia* spp.
Camellia japonica	*Pelargonium* spp.
Clematis spp.	*Penstemon* spp.
Choisya ternata	*Petunia* spp.
Cyclamen persicum	*Poinsettia* spp.
Euphorbia spp.	*Syringa vulgaris*
Fuchsia x hybrid	*Verbena x hybrida*
Gerbera jamesonii	*Viola* spp.
Hypericum calycinum	*Zantedeschia* spp.

Black root rot infection by *T. basicola* leads to loss of plant vigour through root rot, wilt and eventually plant death. Bedding plants such as *Viola* spp. can decline rapidly, with an estimated 5% of the UK crop affected, although with early chemical treatments most are usually still marketable. Hardy nursery stock such as *Choisya* sp. affected at potting-on can fail to establish and older plants fail to thrive. In some years, perhaps one in four, poor cultural control in *Choisya* sp. can lead to heavy losses of 20 to 30%.

Infection typically becomes visible on the roots as dark brown flecks, caused by the resting spores (chlamydospores) of the fungus, developing at infection points in the outer cells of roots (Figure 1). The infection is not vascular, but damage to the surface of the root at the root hairs reduces the nutrient uptake capability of the plant, leading to purpling of the foliage and stunted growth.

Figure 1. Infection of *Choisya* sp. roots by *T. basicola* in the current project showing the “black” areas of roots caused by chains of resting spores forming inside the cells.

Existing control measures

Cultural control

Growers employ measures such as reducing plant stress and being careful with crop hygiene to reduce the chance of plants becoming infected. However, the pathogen produces resting spores (chlamydospores) in roots which then survive in debris in matting, re-used containers and soil, and can be resistant to disinfectant treatments (as reported in AHDB Project PC 38c and Factsheet 03/14 revision of 15/05). Further details of are given in the review of black root, PO 14 (Wedgwood, 2013). Once some plants become infected then abundant dispersal spores (endoconidia) are released from infected roots into the growing-media and can be flushed out by irrigation to infect neighbouring plants. The pathogen also produces resting spores (chlamydospores) in roots which then survive in debris in matting, in re-used containers or in soil, and can be resistant to disinfectant treatments (Factsheet 03/14).

Biological control

Products with potential against black root rot were reviewed in project PO 14. The biofungicides that can be used in the UK against root rots are: Prestop (*Gliocladium catenulatum* strain J1446) for protected ornamentals; Serenade ASO (*Bacillus subtilis* strain QST 713) spray under the EAMU 0706 from 2013 ex Bayer (previously ex BASF 0708 from 2013) for protected and outdoor ornamentals; T34 Biocontrol (*Trichoderma asperellum* strain T34) under the EAMU 1118 for 2012 for protected ornamentals and outdoor containerised

ornamentals; Trianum-G and Trianum-P (*Trichoderma harzianum* strain Rifai T-22) Authorised under 2769 and 2771 of 2014, respectively, for use on protected ornamentals.

Application methods and frequency of any re-application differ between biofungicide products, with a common recommendation being use at sowing or potting-on stages by incorporation into, or drenching of, the growing media. Only Prestop is reported in the Fargro product technical notes to have some activity against *T. basicola*. Some significant reduction was achieved in Experiment 2 of this project after preventative plus curative application to *Viola* spp. inoculated with black root rot. Some benefit from incorporation of Trianum-G into the growing media (several plots having low root rot) was also shown, but there was no benefit from Serenade ASO. In projects, such as CP 077, several biofungicides have shown some reduction in pathogens at low levels (inconsistently). There are practical considerations for their use (Factsheet 18/14), and more work is needed on how growers can get the best from biofungicides and integrate them into their management programmes.

Chemical control

Growers of plants susceptible to black root rot, such as *Viola* spp., often apply preventative treatments at sowing (bedding plants) or potting-on (nursery stock). This is in addition to treatment with products such as Subdue (metalaxyl-M) or Fenomenal (fenamidone + fosetyl-aluminium) against the oomycete pathogens *Pythium* spp. and *Phytophthora* spp. as these products are unable to control fungal species such as *T. basicola*.

Only container-grown ornamentals under permanent protection are allowed a drench with Cercobin WG (thiophanate-methyl). Cercobin WG can only be applied once per crop and three weeks before any planting into open ground (EAMU 2011 1887). The resistance risk of this benzimidazole fungicide is noted to be high, having the same FRAC 1 grouping as benomyl. One application can provide adequate protection for bedding plants on the nursery (which may only be on the nursery for eight weeks), however this does not maintain protection in nursery stock as it has a long production cycle in which the crop can be moved outdoors.

Scotts Octave (prochloraz) (FRAC Group 3) can be applied as a drench to the growing media and this product is currently used on susceptible ornamentals by UK growers to combat black root rot in polytunnel crops. Currently there is no suggestion of resistance of *T. basicola* to either Scotts Octave or Cercobin WG, but for resistance management, another product with a different mode of action would be advantageous. If resistance did occur, due to the pathogen being widespread across nurseries and there being regular movement of nursery stock produce between nurseries, resistant strains could spread and it could become impossible to produce marketable plants of susceptible species.

In PO 14 (Wedgwood 2013), active ingredients that might give effective control of black root rot were identified; actives either currently in use on other crops, or being used pre-registration as a plant protection product. If given approval, they might widen the number of products available to reduce the risk of fungicide resistance developing. Several products which reduced black root rot in earlier work in PC 143 (Jackson, 2000) are now unavailable to UK ornamentals growers. For the current project, chemical manufacturers were able to provide a further set of products for testing (some experimental and some in use on other crops).

Results on the efficacy of plant protection products in Experiment 1 (six conventional test chemicals) and Experiment 2 (seven non-conventional test products) were used to select products with different modes of action that could be used in programmes with or without the industry standard active, thiophanate-methyl (Cercobin WG) (see first Annual Report).

Experiment 3 took forward products from Experiments 1 and 2 that had reduced root browning, had a likelihood of full approval or an EAMU on ornamentals, and could be placed in alternating programmes with different modes of action. It was not possible to investigate all potential treatment programme combinations with all potential products, so four products were finally selected for testing, together with Cercobin WG, based on their effectiveness in previous trials. The plant protection products selected for use on *Viola* spp. in Experiment 3 were T34 Biocontrol, F174, F175 and F178. The objective of the Experiment 4 was to determine the efficacy of simple programmes on *Choisya* sp., based on products previously found to be effective in controlling black root rot in *Viola* spp.

Experiments in 2014 and 2015 were programmed to run during the summer months when plant stress was likely to increase losses to black root rot. *T. basicola* tends to be an opportunistic pathogen, taking advantage of susceptible plants under stressful growing conditions (Leahy, 1998). The fungus is capable of prolonged saprophytic survival in soils. Soil between 17 to 23ºC, soil pH about 5.7-5.9, high soil moisture content and inadequate aeration favour disease development (Subramanian, 1968). Disease can be reduced by maintaining compost moisture levels at 36% or lower (Trebilco *et. al.*, 1999) and acid conditions (with prevention at pH 4.8 and reduction at pH5.5, subject to plant tolerance) (Koike *et. al.*, 2005).

This report details the fourth of five experiments within HNS-PO 190 on the efficacy of novel plant protection products against black root rot of pot and bedding plants and hardy ornamentals. Experiments 1 to 3 on Viola were completed in 2014, and the work on Experiment 4 in 2015 further investigated the timing and application frequency of products and their comparative efficacy on *Choisya* sp.

Materials and methods

Experiment 4 was set up in on 30 April 2015 with *Choisya ternata* plugs to investigate the efficacy and crop safety of individual products on these plants, as a test host for other nursery stock. The plugs had not received any fungicide drenches. The plugs were potted into 350 ml pots by the nursery staff on 29 April 2015. The nursery's standard peat-based mix was used containing 25% Irish 7-15 mm and 40% Baltic peat, 20% coir fibre and 15% bark, with 8 - 9 and 12 - 14 month slow release Osmocote Exact Std (15 N + 9 P + 11 K + 2 MgO) and PG Multi-Mix 12+14+24 + 2 +Trace Elements) plus a Starter feed for the first weeks.

Four replicate blocks of ten 90 mm diameter (350 ml) pots per plot were stood in disinfectant-sterilised open-bottom carry-trays on woven ground cover matting, which had been herbicide treated in a commercial glasshouse with programmed overhead irrigation (Figures 2 and 3). Disinfection of the matting was not done in case this reduced the inoculation success of the pots. Vacant holes in the carry-trays were used as guard strips between plots.

Uninoculated plots were randomised within the layout with pots stood inside a second (empty) pot to keep them from contact with the potentially naturally infested woven ground cover. The carry-trays used to hold the ten plants in a plot were disinfected and rinsed by the nursery to remove any resting spores. Two temperature loggers were placed at plant canopy height.

Figure 2. Four replicate blocks of *Choisya ternata* at the nursery site directly after the applications made to plants at potting-on from plugs to liners on 30 April 2015

Treatment applications

Products selected from the earlier experiments on *Viola* sp. were tested with one or two applications per product, timed at three stages in the crop depending on their potential activity from earlier work (Table 2). Thirteen treatment programmes plus three untreated (one uninoculated) were set up (Table 3).

PLOT	1	2	3	4	5	6	7	8
BLOCK	1	1	1	1	1	1	1	1
TREATMENT	15	2	5	10	8	13	9	16
PLOT	9	10	11	12	13	14	15	16
BLOCK	1	1	1	1	1	1	1	1
TREATMENT	11	1	12	3	7	6	14	4
PLOT	17	18	19	20	21	22	23	24
BLOCK	2	2	2	2	2	2	2	2
TREATMENT	10	16	6	9	14	12	2	5
PLOT	25	26	27	28	29	30	31	32
BLOCK	2	2	2	2	2	2	2	2
TREATMENT	15	3	8	13	1	7	4	11
PLOT	33	34	35	36	37	38	39	40
BLOCK	3	3	3	3	3	3	3	3
TREATMENT	11	15	5	2	8	3	6	7
PLOT	41	42	43	44	45	46	47	48
BLOCK	3	3	3	3	3	3	3	3
TREATMENT	16	12	14	1	4	10	9	13
PLOT	49	50	51	52	53	54	55	56
BLOCK	4	4	4	4	4	4	4	4
TREATMENT	14	7	2	6	13	8	11	3
PLOT	57	58	59	60	61	62	63	64
BLOCK	4	4	4	4	4	4	4	4
TREATMENT	5	1	16	4	9	15	10	12

Figure 3. Arrangement of *Choisya* sp. treatment plots in 2015

Table 3. Application timings in Experiment 4 on *Choisya* sp. in 2015

Day 0 Potting	Day 7	Day 14	Day 21	Day 28	Day 35 5 wks from potting	Day 42 6 wks from potting	Day 49	Day 56	Day 63	Day 70 10 wks from potting
30.04.15					04.06.15	11.06.15				09.07.15
P1					**P2**	**Inoculation**				**C**

P – preventative applications; C – curative

Four biological products (T13 - T16) and a non-conventional chemical product (F178) were applied straight after potting. Thiophanate-methyl was used as the standard active ingredient (as in previous experiments) with a single preventative application of this or of four conventional chemical fungicides (once roots had established), one week before the plants were artificially inoculated. Inoculation with a *T. basicola* spore suspension was made six weeks after potting. Three of the conventional chemical fungicides were coded products and these were also applied curatively a week after inoculation (Tables 3 & 4).

Table 4. Experiment 4 on Choisya sp. in 2015. Products, application timings and rates and product approval status for ornamentals. (Protectant timings: P1 at potting, and P2 five weeks after potting. Curative timing: C four weeks after inoculation 10 weeks after potting

T	Product & [MAPP No.]	Active ingredient	Application timing			Dose & water volume	Comments & UK approval status
1	Un-treated	none	-	-	-	water alone	Inoculated with black root rot spores
2	Un-treated	none	-	-	-		
3	Un-treated	none	-	-	-	water alone	NOT inoculated
4	Topsin* [13988]	thiophanate-methyl	-	**P2**	-	1.1 g per 10 L water using 1000 L/ha	Standard rate for BRR. One Cercobin WG drench permitted
5	Signum [11450]	boscalid + pyraclo-strobin	-	**P2**	-	1.35 kg / ha in 400 L/ha water	EAMU 1107 of 2009 for ornamentals
6	HDC F174	chemical mixture	-	**P2**	-	Spray using 400 L/ha water	Agrochemical company rate
7			-	**P2**	**C**		
8	HDC F175	chemical	-	**P2**	-	Spray using 400 L/ha water	Agrochemical company test rate
9			-	**P2**	**C**		
10	HDC F176	chemical mixture	-	**P2**	-	Spray using 400 L/ha water	Agrochemical company test rate
11			-	**P2**	**C**		
12	HDC F178	chemical	**P1**	**P2**	-	**P1**: half rate in 400 L/ha water **P2**: full rate in 400 L/ha water	Agrochemical company rate
13	T34 Bio-control [15603]	*Trichoderma asperellum* T34	**P1**	**P2**	-	Spore suspension made up 2 hours before use. 0.5 g in 10 L water per 100 L growing media.	EAMU 1118 of 2012 for outdoor container ornamentals & any protected ornamentals Irrigation rate at 10% of pot volume.
14	Trianum-P [16741]	*Trichoderma harzianum* T-22	**P1**	**P2**	-	**P1**: 0.3g /10 L growing media using 1 L water **P2**: 0.15 g /10 L growing media using 1 L water	"Lower crop density" rate. 10% of pot volume. Approved on protected ornamentals
15	Prestop [17223]	*Gliocladium catenulatum* J1446	**P1**	**P2**	-	5.0 g Prestop in 1 L water.	Approved on ornamentals. 10 % pot volume
16	Serenade ASO [16139]	*Bacillus subtilis* QST 713	**P1**	**P2**	-	10 L Serenade per ha (10,000 m sq) in 1000 L water / ha	EAMUs 0706 and 0708 of 2013 for ornamentals.

* Topsin WG was supplied in place of Cercobin WG, both have an identical formulation.

All products were applied at their label rates, or at rates agreed with the agrochemical companies agreeing product use. The standard thiophanate-methyl product (T4) was used as a drench at 1000 L/ha. Topsin WG was used instead of Cercobin (both contain 70% w/w thiophanate-methyl and are identical formulations) as a sample could not be supplied of the latter. Application followed guidance in the Cercobin WG EAMU 1887 of 2011 giving the

drench rate of 11 g per 100 L for the control of root diseases (*Thielaviopsis*, *Cylindrocarpon* and *Rhizoctonia* spp. etc. not the higher label rate.

Chemical products with label recommendations for application as a spray (T5 – T12 in Table 4) were sprayed at a standard 400 L/ha water volume. Treatment was followed straight away by a spray of water only to make up the total volume to 1000 L/ha to aid penetration into the growing media. Biological products T13 - T15 were applied to 10% of pot volume. Serenade ASO (T16) can be applied as a single drench under EAMU 0705 of 2013, but only to outdoor crops and so the product was instead applied as a high volume spray in the glasshouse under EAMUs 0706 and 0708 of 2013. Biopesticide candidates were products currently approved for use on ornamentals either on label or under off-label approval as detailed in Table 4. Chemical experimental products F174, F175, F176 and F178 not approved for use on ornamentals were used under Administrative Experimental Approvals (AEA) 2013/00644 and 2014/00736.

Fungicide drench applications were made to the growing media of individual pots using a measuring cylinder. The drenches were poured over the foliage and around the plant across the whole pot surface. The products would be applied commercially by spray application and thus any phytotoxicity to the leaves could be evaluated by including foliar contact. The 350 ml pots were drenched to 10% of pot volume (35 ml / pot). Spray applications were made with an Oxford gas-assisted sprayer operating at 2 bar pressure and using a single 02F110 nozzle giving a 0.5 m spray width.

Re-application (at timing P2) of T34 Biocontrol was done after a 4 week interval to keep in line with other products and the suggestion for repeat applications at two to three month intervals in the Fargro 05.15 Technical Notes was not adopted. The further suggestion of pre-soaking the product for a few hours prior to application was followed in order to aid faster establishment of the beneficial fungus on the plant roots (although said not to be essential). Instructions for “lower crop density” dose rates of Trianum-P were followed. Two applications were given to keep in line with other microbial products and not repeated (rather than follow the EAMU which allows application at four week intervals at half rate).Two applications of Prestop were given to keep in line with other microbial products in this experiment, and not repeated (rather than follow the label which allows re-application at 4-6 week intervals).

No routine fungicides were used, including no Subdue (metalaxyl-M) or other product on the plugs in propagation against oomycete root rots, to avoid any possible adverse effects on the beneficial microbe treatments. There was one application of Chess WG (pymetrozine) against aphids in June, July and August 2015.

Inoculation

This nursery had problems with black roo*t rot on species such as Skimmia, Convolvulus, Senico, Ceanot*hus and Cornus as well as Choisya. Historic plant clinic reports for the nursery had also shown Phytophthora, Pythium and Fusarium sp. had been frequently detected from Choisya root rots. A *Choisya* sp. cv. White Dazzler plant from this site in February 2015 was used to obtain an isolate of *T. basicola* for use as inoculum in 2015 (in place of an isolate from carrot used in 2014).

A technique to "bait" *T. basicola* (see Appendix 1 for details) from around plants was also used at this time following interest from the grower (Stuart Mills). Carrot root pieces were placed around the growing area in order to detect the presence of living tissue of this pathogen (spores or mycelium) that could gain contact with plant roots and infect them. The baits were then returned to ADAS Boxworth for inspection and potentially the isolation of the pathogen.

Agar plates were prepared using the isolate BX15/22 of *T. basicola* taken from *Choisya* sp. cv. White Dazzler roots. On the morning of the inoculation on 11 June 2015 the spores were removed from the plates to create a spore suspension in distilled water. Haemocytometer counts recorded an average 3388 chlamydospore chains together with approximately 2.5 x 10^5 endoconidia per ml.

The black root rot inoculum was applied to the *Choisya* sp. five weeks after potting, once plants had established new roots and after some plants had received at least one protectant treatment. The spore suspension was applied at 5% of the pot volume. Inoculation of 18 ml per pot was carried out using a 20 ml syringe, drenching the growing media around each plant stem and ensuring also that the new growing media beyond the plug root ball was inoculated. Treatment 3 was left uninoculated.

Assessments

At each assessment date, records were taken for plants in the same order so that developments of any symptoms could be tracked over time in individual plants. Crop vigour was recorded per plant as a combination of factors including leaf size, leaf greenness and plant size using a 0-9 vigour index (0 = dead, 9 = very good). At the first pre-treatment assessment a baseline record was taken of the separate vigour of both the leaves present when the cuttings were taken, and the subsequent new growth (Figure 4). Next, assessments were made looking mainly at the new growth as this formed the bulk of the plant. Standard husbandry of plant trimming when it became necessary was not carried out until after each set of scientific observations, but because of trimming the individual plant heights were not recorded after the first assessment at potting.

Old leaf	**0**	**3**	**7**
New leaf	**3**	**3**	**7**

Figure 4. Examples of indices for plant vigour 0-9 (poor – very good) for old and new growth on Choisya on 30 April 2015

The early symptoms of black root rot can be subtle (while water and nutrient uptake is gradually reduced by root infection) leading to loss of vigour. At the time of destructive assessment seven months from potting there was some wilting and leaf drop that could have been caused by root rot, and this was assessed on a 0 (healthy) to 9 (dead) index.

Phytotoxicity such as scorching or distortion of the foliage was also looked for at each of the assessments and assessed on a 0 (zero) to 9 (severe) index.

On 19 November 2015, plants were removed to a glasshouse at ADAS Boxworth in preparation for destructive assessment. Some roots were removed for isolation of pathogen/s onto agar and for microscope examination for *T. basicola* spores to assist identification of the cause of the various root damage symptoms seen. Assessments of damage severity started on 8 December 2015 when each plant was knocked out of its pot and the surface of the root ball assessed for rotting. The majority of the roots were around the surface of the root ball and the surface area covered by roots (rather than with the growing media visible), was recorded as a guide to root vigour. Roots were assessed by their colour, with pale yellowy brown roots (which under magnification had *Thielaviopsis* sp. endospores and mycelium), brown-blotchy roots (Figure 1) (with *Thielaviopsis* sp. chlamydospores viable under magnification as shown in Figure 5), and white, sometimes dry, roots (often with speckles of black root rot chlamydospores and/or softened (from which *Pythium* spp. was isolated)). Roots could be affected by more than one pathogen. A separate surface area of white healthy roots was recorded.

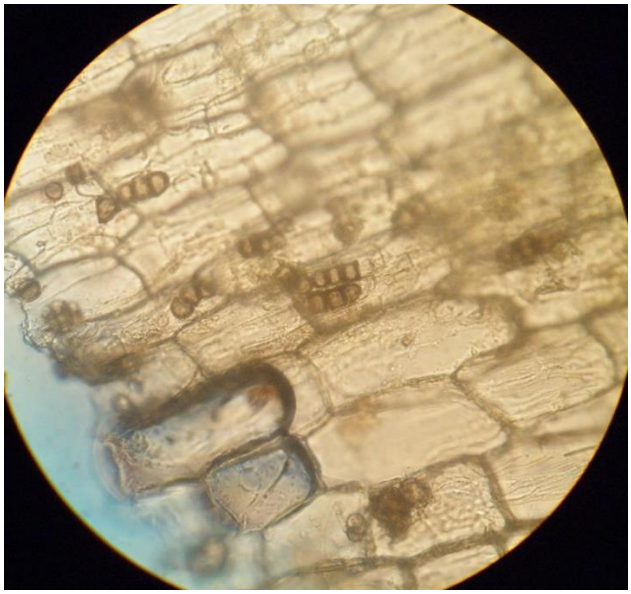

Figure 4. Microscope photograph of *Thielaviopsis sp.* chlamydospores in dark brown short chains within cells of a root taken from Plot 48 (treatment 13) in November 2015. Colourless fungal mycelium of *T. basicola* also fills some of the root cells.

Results

The experiment ran at the nursery between the end of April and late November 2015. There were some days when the temperature reached 35°C in May (Appendix 1). Subsequently the temperature hovered at an average of 20°C between the start of June (when inoculation took place) and the end of August 2015. Although there were rises at times to around 32°C that may have caused plant stress and encouraged black root rot, the summer was not exceptionally hot.

Foliar vigour

There was no significant difference in either plant height or the vigour of old and new growth between plots before the application of the first preventative treatments on 30 April 2015 (data not shown). Later in the trial, there was a tendency for the uninoculated untreated plants to have a lower foliage vigour than the inoculated during the summer (Table 5). It was seen at destructive assessment that the root balls of the uninoculated plants were drier, probably because they had been prevented from absorbing water back up from the woven-ground cover, due to each pot being sat in an empty pot to prevent contamination from the matting.

Table 5. Vigour indices (0 dead - 9 very vigorous) for *Choisya ternata* foliage in 2015 with plants except those in T3 inoculated on 4 June. Not all assessment dates are shown.

	Dates of treatment applications			**Dates of assessments of 0-9 foliar vigour**					
T	**30.04.14**	**04.06.15**	**09.07.15**	**11.06**	**09.07**	**16.07**	**27.08**	**08.10.**	**08.12.**
1*	-	-	-	4.79	5.07	5.79	6.07	4.89	5.23
3	-	-	-	4.78	3.85	4.13	4.99	4.92	5.03
4	-	Topsin	-	4.72	5.19	5.75	5.94	5.13	5.07
5	-	Signum	-	4.99	4.99	5.59	5.82	4.97	5.84
6	-	F174	-	5.13	5.34	5.94	6.55	5.08	5.31
7	-	F174	F174	5.03	5.10	5.68	6.05	5.08	5.74
8	-	F175	-	5.20	5.55	6.15	6.50	5.55	5.58
9	-	F175	F175	4.98	5.10	5.70	5.70	5.03	5.14
10	-	F176	-	5.26	5.11	5.36	6.04	5.00	5.15
11	-	F176	F176	5.04	5.38	5.52	5.44	5.14	5.52
12	F178	F178	-	5.05	4.98	5.83	6.07	5.00	5.43
13	T34 Biocontrol	T34 Biocontrol	-	5.10	4.49	5.21	5.66	5.16	5.24
14	Trianum-P	Trianum-P	-	5.13	5.69	6.00	6.23	4.95	5.43
15	Prestop	Prestop	-	5.10	5.21	5.87	5.67	5.02	5.28
16	Serenade ASO	Serenade ASO	-	5.11	4.86	5.40	5.79	5.04	4.99
		Mean		5.012	5.059	5.607	5.912	5.053	5.325
		L.s.d. min.rep		0.436	1.033	0.933	0.799	0.266	0.820
		L.s.d. Max-min		0.378	0.894	0.808	0.692	0.230	0.710
		D.f.		46	46	46	46	46	46
		F. Pr		0.336	0.162	**0.026**	**0.038**	**0.002**	0.679

*Mean of T 1 and 2 (eight plots untreated inoculated). T3 four plots untreated uninoculated.

Plant vigour was very variable even within plots, initially because of variation in the cutting sizes and later contributed to by the variable production of new shoots following trimming. The vast majority of plants grew vigorously despite inoculation on 11 June with a high concentration of *T. basicola* inoculum (Table 5). By November there were only a few plants with slightly wilted foliage that might have been as a result of black root rot.

There were significant differences in vigour between mid-July and early October 2015 (Table 5). On 16 July the use of F175 a week before inoculation gave a mean vigour index of 6.15, above that of 4.13 for the untreated uninoculated plants, and this product then also resulted in significantly greater ($P<0.05$) vigour on the 27 August (index 6.5) and 8 October (index 5.5). Plots with most other products were not significantly different in vigour to F175, with an index of 5 representing average health and growing well. After T34 Biocontrol was applied at

potting and then after five weeks this had the lowest (but still acceptable) vigour index of 5.21 on 16 July.

By 27 August, in addition to greater vigour than the uninoculated untreated plants with F175 application pre-inoculation, plants which received seven other preventative treatments (Topsin, Signum, F174, F176, F178, Trianum P and Serenade ASO) were also significantly more vigorous (with a mean index above 5.7, L.s.d. of 0.799). Plants with F174 also applied curatively were also more vigorous, but not those with F175 and F176 applied before and after inoculation which, like after T34 Biocontrol and Prestop preventative use, had vigour equivalent to the untreated uninoculated plants. All treatments had vigour equivalent to the still symptomless untreated inoculated plants (0.692 L.s.d. for max-min).

By the time destructive assessment was carried out on 8 December 2015, there were no vigour differences between any treatments; on average, all were growing well. Throughout the assessment period, no product treatments showed any indication of phytotoxicity; the mean vigour scores between treated and untreated plants were not significantly different.

Root rotting

In August 2015, roots from some plants with yellowing and slight wilting were taken for laboratory examination. *T. basicola* spores were seen in the roots, but isolations onto agar showed that *Pythium* spp. were also present in roots that were greyer and softer and this could have contributed to the loss of foliar vigour. At the destructive assessment on 8 December, there was some paleness and/or wilting of foliage in a few pots and an occasional dead plant. There was no significant difference between treatments, with a low mean foliar disease of 0.6 including the inoculated plants left untreated (Table 6).

Root coverage scores were also made on the 8 December when the pot was removed and the root/pot ball assessed on its exposed surface for the proportion covered by roots (Table 6). There was no significant difference between treatments; on average, roots covered around half the root/pot ball. The results were extremely variable between plants in the same plot. At this stage black root rot did not cause the roots to disintegrate and so the roots produced were a reflection of plant vigour, not any loss to the pathogen.

Table 6. Final assessment of *Choisya* sp. commencing 8 December 2015 including foliar symptoms (index 9 severe), root cover around root ball and root rot discolouration and health.

Treatment programmes Product & timing of applications (protectants *P1, P2* , curative *C*)		Foliar disease index 0-9	% of root-ball faced by roots	% of root surface discolouration & healthy**			
				Yellowy brown (Black root rot)	Brown (Black root rot)	White dry or soft (Black root rot and Pythium)	White healthy
1*	UT inoculated	0.16	47.59	47.74	8.34	14.89	24.33
3	UT not inoculated	0.45	48.14	44.10	11.64	15.03	34.61
4	Topsin (*P2)*	0.92	49.49	47.72	4.97	8.30	26.29
5	Signum (*P2)*	0.30	47.64	46.42	5.81	3.38	42.32
6	F174 *(P2)*	0.23	46.49	44.22	6.18	5.69	32.03
7	F174 *(P2, C)*	0.12	44.40	55.68	4.40	4.73	27.33
8	F175 *(P2)*	0.08	56.62	40.25	6.45	8.00	39.50
9	F175 *(P2, C)*	0.72	51.81	39.99	4.77	14.56	25.90
10	F176 *(P2)*	0.31	42.69	45.92	5.89	6.34	34.05
11	F176 *(P2, C)*	0.67	45.83	45.51	5.87	19.92	25.17
12	F178 *(P1, P2)*	0.41	49.38	40.44	8.77	8.36	39.51
13	T34 *(P1, P2)*	0.68	45.61	46.33	9.43	12.19	27.75
14	Trianum-P *(P1, P2)*	0.51	47.68	45.58	5.86	10.83	19.35
15	Prestop *(P1, P2)*	2.73	47.95	46.47	12.14	10.11	23.81
16	Serenade *(P1, P2)*	1.12	49.19	38.79	5.65	18.09	30.91
	Mean	0.60	48.01	45.18	7.16	10.96	29.83
	L.s.d. min.rep	2.012	11.459	12.262	7.193	14.754	18.179
	L.s.d. Max-min	1.742	9.924	10.619	6.229	12.778	15.744
	D.f.	46	46	46	46	46	46
	F. Pr	0.579	0.803	0.498	0.525	0.505	0.344

*Mean of treatments 1 and 2 (both untreated inoculated with black root rot, BBR). Use the max-min L.s.d. to compare between this and the other treatments with only four plots each.

**% areas do not total 100 as other root discolourations were noted in small areas and some root surfaces had e.g. yellowing and speckling and so were scored in more than one category.

By December 2015, a large proportion of plots were found to have yellowing roots (a stained appearance) covering nearly half of the area of roots adjacent to the pot (Table 6). These were shown under microscope examination to include roots infested by *T. basicola* but without the formation yet of chlamydospores. White roots that were slightly grey were also seen, appearing to have dried out leaving a “husk”. Under microscope examination the roots were found to be speckled with the chlamydospores of *T. basicola* (which caused the

greyness). Other white, grey-tinged, unhealthy roots that were also softened, were potentially infested by *Pythium* sp.. All unhealthy white roots were scored together in one category as it was not possible to confidently divide them. There was no treatment difference, with around 10% of root surface area affected.

Dark brown/black mottled roots more traditionally characteristic of black root rot were also seen (such as shown in the photograph of roots of a plant from plot 48 in Figure 1). Most of the roots of this colour were towards the base of the pot, the speckling only covered on average 7% of the root surface area visible on the root ball when knocked out of the pots. The dark speckling was confirmed by microscope examination to be caused by black root rot chlamydospores inside the outer cells of root (Figure 4). Staining techniques, showed that the *T. basicola* fungal mycelium (which would have been feeding on the root cell nutrients) was widespread within the roots without causing cell necrosis. Similar observations were made of *Viola* sp. roots in this project in 2014 when tube-like colourless phialides (conidiophores) were seen protruding from the root surfaces releasing colourless transparent spores (endoconidia).

There were no significant differences between treatments for any root damage symptoms, and overall the proportion of healthy roots was around 30% of the root surface (Table 6). There was a range across the treatments of from 19% to 42% of root surface area remaining healthy by 8 December 2015 and, although differences were not significant, plots given either a single preventative Signum, F174, F175 or F176 treatment or two preventative treatments of either F178 or Serenade ASO, had above average healthy root surface area (30%).

Uninoculated plants had become infected by *T. basicola* and the pathogen had caused the same range of symptoms on the roots as on the inoculated plants. “Natural” infection by *T. basicola* of the plant roots (either as plugs or liners) was possible because this pathogen was confirmed in the carrot slice baits returned to ADAS in February 2015 before any inoculations were carried out. Although it was not possible to obtain a clean isolate from these baits because they had been colonised by bacteria and nematodes as well as the *T. basicola*, the dark brown chlamydospore chains were visible under a low-power microscope.

Discussion

Plant vigour above-ground was not visibly affected by the root loss and root browning seen in inoculated plants. The plants were kept well fed and watered and it is possible that given greater stresses the plants would have been less able to cope with fewer roots. Unlike root rotting pathogen species such as *Pythium*, *Fusarium* and *Cylindrocarpon,* the roots attacked by *Thielaviopsis* remain more intact (taking on a stained yellow appearance) and probably continue to function to some extent. This means that potentially the black root rot fungus can

continue extracting nutrition from the plant and releasing endoconidia. It may be that only once the plant becomes stressed the balance is lost, roots are killed and the *T. basicola* produces abundant survival/resting spores in the dead root tissue. These observations emphasise the need for growers to be checking the roots of samples of their susceptible species of plants in good light and potentially with a magnifying glass to see early subtle symptoms of black root rot, either treating or disposing of the plants to stop endospore spread well before foliar symptoms appear. Hygiene measures such as stopping the introduction of resting spores into new crops by using disinfected or clean propagation trays are needed. Endoconidia dispersal from infested to healthy plants may be avoided by standing trays so that they drain free and do not take up water from benches. It seems likely that some of the plants in the current work that were not inoculated became infested by cross-contamination.

Some effective preventative treatments were found in the *Viola* sp. experiments, but not in the *Choisya* sp. It is possible that the longer period of growth in the Choisya sp. allowed any residue of treated infection to build up again “naturally”. In a commercial situation with a history of black root rot on site, an ongoing treatment programme of susceptible HNS species would probably be worthwhile, and the biofungicides have recommendations for repeat application intervals. In the current project the products were applied only up to three times, using standardised intervals to aid efficacy comparison. Biofungicides can be re-applied with minimal risk of pathogen resistance developing because each organism has multiple modes of action (plant defence stimulation, competition for nutrients, hyper-parasitism and/or enzymatic action). Further work is needed with individual products in monitored environmental conditions, using a range of growing media compositions, to determine the extent of survival of beneficial organisms and provide information on re-application needs.

The amount of inoculum used was high and it is possible that better control could have been achieved with less of a challenge to the plants. The spore concentration used followed recommendations in projects prior to this one; lower concentrations do not cause damage.

It is clear that black root offers a real challenge to growers and that the chemical control measures that are currently still available should be used with the possibility of resistance in mind. The coded product F174 in this project has been put forward for an EAMU and if approved it is hoped that this could be used in programmes by growers of protected ornamental crops. It is now unlikely, however, that F176 will be able to be supported for an EAMU. Unfortunately, also, the product F175 (found effective on *Viola* sp.) is not now coming to the UK, and F178 although now registered in the UK it is not currently being marketed. This, therefore, leaves the biofungicides, and work in 2016 will be looking to see if their performance can be enhanced by combining biofungicides together in programmes.

Conclusions

- There were no significant differences in root rot of the *Choisya* sp. between any of the treatments after six months despite inoculation with a high level of *T. basicola*. All plots had become became infected (including the uninoculated), with a mean 5.6% of their root surfaces showing the characteristic dark brown markings caused by chlamydospores in the cells, with further infection which had not progressed to the production of dark brown chlamydospores.
- Around 45% of the root surfaces were yellowed and this was attributed to *T. basicola* mycelium and the early stages of brown chlamydospore formation. Infection occurred without any significant differences between treatments.
- *Pythium* spp. infected the plants naturally, but symptoms from these pathogens were not clearly separable from those of black root rot where roots had been destroyed without the formation of chlamydospores.
- Although by December only 30% of root surface area on average was healthy across all treatments, there was very little impact on foliar vigour and most of the *Choisya* sp. liners would have been marketable. Providing good growing conditions for the plants is likely to compensate for root loss.
- The standard single preventative application of a thiophanate-methyl high volume spray was ineffective against the high level of inoculation given a week later.
- There are indications that the chemical fungicide F175 used a week before *T. basicola* inoculation increased the vigour of *Choisya* sp. foliage slightly, but significantly so, later on between August and October 2015. In 2014, F175 was effective at the same timing in reducing root rot severity on *Viola* sp. if preceded by T34 Biocontrol at sowing.
- There were no problems with phytotoxicity from any of the experimental chemicals or biofungicides.

Knowledge and Technology Transfer

Powerpoint presentations on *Viola* sp. Experiments 1 to 3 given by Erika Wedgwood:

- 9 January 2015. Stoneleigh. Meeting with AHDB research managers and project industrial representatives.
- 21 January 2015. Oxford. British Protected Ornamentals Organisation (BPOA) conference.
- 4 February 2015. Boxworth. Protected Ornamentals and Bulbs and Outdoor Flowers panel members visit.
- 10 February 2015. London. Herbaceous perennials technical discussion group meeting.

- 9 October 2015. Exeter. Research into Thielaviopsis and early work on HNS subjects. IPPS European Region Conference “Pursuing a Passion in Horticulture”.

Publications by Erika Wedgwood:

February 2015. HDC News. Firm foundations to protect the roots – Erika Wedgwood describes the first round of trials looking for new solutions to black root rot control in ornamentals. pp 26 & 27.

Glossary

Chlamydospore: a resting spore, usually dark-walled, formed within plant tissue. The thick walls allow long-term survival. In *Thielaviopsis basicola* the spores are almost square and form together in short stiff chains.

Endospore / endoconidia: the dispersal spores of *T. basicola* released from tube-like phialides which project from infected root tissue.

Stimulant / elicitor: a chemical product or a by-product of a biofungicide that may stimulate plant response to attack by various pathways so that the plant is already primed to produce defence reactions when the pathogen arrives.

Biofungicide: a suspension or powder containing the resting stages of a live bacterium or fungus registered as a plant protection product with efficacy shown against named plant pathogens. These act by various means including direct attack on the pathogen tissue by enzymatic digestion, or physical penetration, and competition for resources. In some products e.g. Serenade ASO the enzymes produced by the bacteria during the manufacturing/culturing of the product can be of more importance than the activity of the live microbe.

References

Jackson, A.J. (2000). Bedding plants: evaluation of fungicides for the control of black root rot and downy mildew. HDC report for project PC 143.

Koike, S.T., Wilen C.A., Raabe, R. D. McCain A.H. and Grebus, M. E. (2005). Floriculture and Ornamental Nurseries. University of California management guidelines for *Thielaviopsis* Root Rot, UC ANR Publication 3392. http://www.ipm.ucdavis.edu/PMG/r280100411.html

Leahy, R.M. (1998). Black Root Rot of Pansies. Plant Pathology Circular No. 387, January/February 1998. FDACS, Division of Plant Industry, Gainesville, Florida. Consumer Services

O’Neill, T. (2015). Factsheet 03/14. Use of chemical disinfectants in protected ornamental plant production.

O'Neill, T. (2014). Factsheet 18/14. Getting the best from biopesticides.

O'Neill, T. (project co-ordinator) (2015). Project CP 077. SCEPTRE – Sustainable Crop & Environment Protection – Targeted Research for Edibles. (Final Report)

Scrace, J.M. (1993). The effect of pH, plug nutrition and fungicide timing on control of black root rot in autumn pansy. HDC Final Report for project PC 38b.

Subramanian, C. V. (1968). *Thielaviopsis basicola*. CMI Descriptions of Pathogenic Fungi and Bacteria No. 170.

Trebilco, E., Howell, J., Forsberg, L. and Bodman, K. (1999). Beware of *Chalara elegans* Black root rot. The Nursery Papers, Issue Number 1999/13. Nursery Industry Association of Australia. www.ngia.com.au/publication_resources/NP_Pdf/NP_2001-07.pdf

Wedgwood, E. F. (2013). Black root rot in containerised subjects-chemical and biological options for control. HDC report for project PO 14.

Wedgwood, E. F. (2014). Evaluation of fungicides and novel treatments for the control of black root rot, *Thielaviopsis basicola*, in bedding and hardy nursery stock plants. HDC report for project HNS-PO 190.

Appendices

Appendix 1.

Methods for the deployment of baits to detect the presence of *Thielaviopsis basicola* in soil, growing media, plant debris or on nursery surfaces, and its isolation onto agar.

It is possible to use carrots to bait/attract the fungus out of soil (or out of the roots), but this will also pick up other organisms as well which can prematurely degrade the carrot and so success is not guaranteed.

There are at two potential baiting methods using carrot slices:

- For both methods a fresh carrot root with no external blemishes (such as *Pythium violae* cavity spot) should be placed in a solution of bleach (approximately 1 part bleach to 9 parts water) for about a minute to get rid of bacteria. Also sterilise a plate and knife.
- After sterilising, take the carrot out with clean hands and soak the carrot in clean tap water for a minute to remove the bleach and then blot dry with a clean paper towel.
- Chop into circular slices (approximately 4 mm thick) without peeling the carrot.
- Carrot slices can be used on material removed for testing, or used to test *in situ,* as detailed below.
- Carrot slices are effective put directly on the surface, but if they are likely to become desiccated, saturated, or dislodged then they can be held in “tea-bags” or tied-top bags made of horticultural fleece (or similar clean perforated or netted material) and fixed tight against the surface with e.g. wire bent into a U-shaped pin or a couple of cocktail sticks.

Removing potentially infested material for *T. basicola* testing

- Soil/growing media samples are taken in a clean plastic bag and moistened with a little water. A handful of soil is sufficient, but the more tested the greater the chance of detecting the pathogen. The pathogen is most likely to be baited by direct contact, and so flatten the soil sample to give a wide surface area (around 30 mm deep minimum).
- Carrot slices are laid on the surface and the bag sealed.
- Leave the bag out of the sun, at room temperature (the fungus grows well in the dark at 22 -24 °C), for four to nine days during which time a dusty grey growth should form on the carrot surface.

In-situ bait testing for *T. basicola* infestation

- Place the carrot slices directly on the soil or other surface to be tested. Baits can e.g. be put underneath woven ground cover. This could be done to assess the need for disinfection or to record the control of the pathogen by any such treatment.
- In both cases, after 9 days the pieces of carrot will need to be picked up gently (without rubbing the surface as this will displace the fungus).
- Use magnification to confirm the presence of dark brown speckles of *T. basicola* spores. The bait can also pick up other species such as *Pythium*.
- If to be sent to a laboratory, then roll up the slices in dry paper towel so that the slices do not touch each other, and so that any soil on them is not scattered about.
- Isolation of *T. basicola* onto agar requires incubation in the dark at 22 -24 °C for 10 days on a selective TB-CEN media (see table below). It is not readily isolated.
- Subsequent culturing of *T. basicola* isolates can be made on PDA, but faster growth is made on carrot agar (see instructions below).

Modified TB-CEN medium for the isolation of *Thielaviopsis basicola*

Materials per litre of distilled water	
• Agar	12 g
Autoclave and cool to 45°C, then add:	
• $CaCO_3$	1.0 g
• Streptomycin sulphate	0.50 g
• Penicillin G	60 mg
• Chlortetracycline HCl[y]	30 mg
• Nystatin[z]	250,000 units
• Etridiazole	400 mg a.i.
• Fresh carrot juice from strained liquidised carrots	80 ml
Adjust pH to 5.3 with H_2SO_4	

[y] Place chlortetracycline in 5 ml of water, add 5N NaOH (approx. 2 drops) until dissolved
[z] Dissolve nystatin in approximately 2 ml of ethanol

Carrot agar for the sub-culturing of *Thielaviopsis basicola*

- Macerate 200 g of fresh carrots (carrots sold with leaves will be very fresh) after washing. Use a blender with 500 ml of tap water added.
- Filter through muslin, squeezing the carrot mash to extract as much juice as possible. Make the juice up to one litre with tap water.
- Autoclave in 1 litre bottles, in batches of 500 ml adding 7.5 g of Technical Agar No.3 to each.
- Autoclave for 15 mins, at 121°C. Leave to set overnight if a 2nd autoclave is to be done to produce a clearer agar.
- To produce a clearer agar autoclave again the next day for 15 mins, at 121°C.
- Ensure carrot solids are kept in suspension while the agar is being poured.

Appendix 2.

Temperature logged at foliage height within *Choisya* sp. plots in Fletchers Lane glasshouse. Minimum (lowest line), maximum (top line) and mean (middle line) daily values are shown