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:	Final report, August 2017
Previous report:	August 2014, 2015 and 2016
Key staff:	Erika Wedgwood Jonny Kerley Sam Brown Chris Dyer
Location of project:	2014 Viola: ADAS Boxworth, Battlegate Rd., Boxworth, Cambridge, CB23 4NN. 2015 to 2017 Choisya: Fletchers Lane site of New Place Nurseries Ltd, Sidlesham Common, West Sussex, PO20 7QG
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Date project commenced:	1st September 2013
Date project completed (or expected completion date):	31st October 2017

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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.

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Signature  Date 27 October 2017

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GROWER SUMMARY

Headline

- Black root rot severity in *Viola* sp. was reduced by Cercobin WG, Signum, three coded fungicides and one coded biofungicide; it appeared to be lessened by Prestop and another coded biopesticide.
- Neither a single application of Cercobin WG nor three applications of biofungicides (various products and sequences) reduced black root rot or increased plant vigour of Choisya.
- Infection by *Thielaviopsis basicola* may be underestimated; symptoms are fully described and illustrated in this report.
- *Fusarium oxysporum* was isolated from roots of wilted and dying Choisya; further work is required to determine if this a cause of Choisya wilt.

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. Pigmented resting spores (chlamydospores) form in roots and when abundant cause blackening of sections of root (Figure 1). 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 the disease, 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.

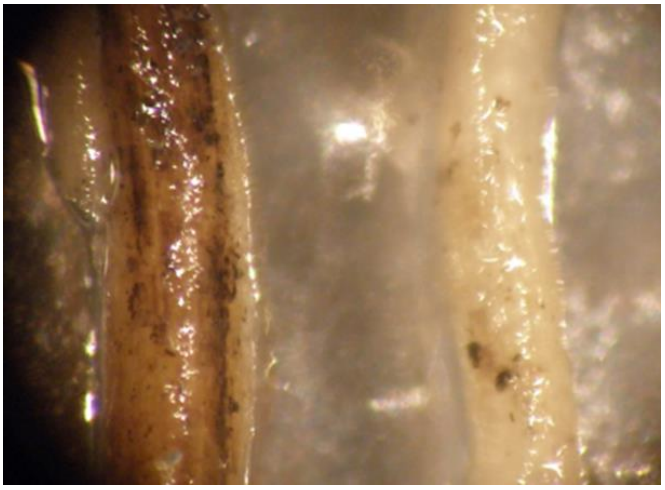


Figure 1:
Low magnification of natural infection of Viola sp. roots by *T. basicola*.

Brown chlamydospore clusters inside the outer cells of roots causing lengths of root to appear blackened, or in lower numbers producing dark specks on whiter roots.

In bedding plants, such as *Viola* and *Primula* species, losses occur on nurseries within the couple of months in which the crops are being grown up to flowering. It is probable that around 2% of bedding and pot plants of these 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% of total plants being unmarketable. 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.

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 15/05). Details of 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 Prestop product information also lists *T. basicola*. The effect of either Serenade ASO, T34 Biocontrol, Trianum-G or Trianum-P against black root rot is unclear.

Chemical control

Growers of crops susceptible to black root rot usually treat them with a protectant fungicide drench. Cercobin WG (thiophanate-methyl) is often applied to container plants at sowing (bedding plants) or potting-on (nursery stock). The resistance risk with this benzimidazole fungicide is high. This 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* but do not control *T. basicola* sp.. Cercobin WG can only be applied to container grown ornamentals under permanent protection, once per crop, and plants must be left for three weeks before planting into open ground (EAMU 2011 1887).

One application can be inadequate to maintain protection for nursery stock under long production seasons.

Summary

This project has sought to identify novel drench treatments with potential preventative or curative efficacy against black root rot, in order to seek use to supplement the small number of products available for use on pot and bedding plants and hardy nursery stock. The treatments (**Table 1**) included conventional chemicals and chemical elicitors and microbial products. The elicitors were withdrawn from testing after the second year due to registration issues.

Table 1. Products tested in the current project on either *Viola* sp. and/or *Choisya* sp..

Product or experimental code	Active ingredient	Mode of Action	Fungicide group or product type	FRAC code	Permitted use in UK on ornamentals		Approval status and additional comments
					Outdoor	Protected	
Conventional chemical fungicides							
Cercobin WG	thiophanate-methyl	Systemic	MBC or benzimid-azole	1	Yes	Yes	Container ornamental EAMU 1887 of 2011. One application
HDC F173	Confidential	-	SDHI	-	No	No	Experimental product
Signum	boscalid + pyraclo-strobin	Systemic & protectant	SDHI + QoI	7 + 11	Yes	Yes	EAMU 2141/12 (expires 31/07/2019)
Switch	cyprodinil + fludioxonil	Systemic & protectant	Anilino-pyrimidine + phenyl-pyrrole	9 + 12	Yes	Yes	On-label approval (Final use date 01/11/2014)
HDC F174	Confidential	Protectant (some curative)	DMI QoI	-	No	No	Approved product only on other crops
HDC F175	Confidential	-	Experi-mental	-	No	No	Experimental product
HDC F176	Confidential	Systemic & protectant	DMI QoI	3 + 11	No	No	Approved product only on other crops
Biofungicides and other biological products							
Prestop	*Gliocladium catenulatum* J1446	Protectant microbial	fungus	-	No	Yes	Approved on protected ornamentals
Serenade ASO	*Bacillus subtilis* QST 713	Protectant microbial	bacteria	44	Yes	Yes	EAMU 0708 of 2013 for ornamentals
T34 Biocontrol	*Trichoderma asperellum* T34	Protectant microbial	fungus	-	No	Yes	EAMU full protection 1118 of 2012
Trianum -G HDC F177	*Trichoderma harzianum* T22	Protectant microbial	fungus	-	No	Yes	Full Approval for fully protected plants
Trianum-P HDC F190	*Trichoderma harzianum* T22	Protectant microbial	fungus	-	Yes	Yes	Full Approval for fully protected plants
Horti-Phyte	Potassium phosphite	Nutrient & stimulant	chemical	-	Yes	Yes	Liquid fertiliser
HDC F178	Confidential	Plant activator	chemical	-	No	No	Not approved in the UK
HDC F179	Confidential	Protectant microbial	fungi	-	No	No	Not approved in the UK

Dose rates and water volumes were as on the product labels, or as advised by their product technical managers as being most suitable in order to test what might be permitted in an Extension of Authorisation for Minor Use (EAMU). The standard was a Cercobin WG drench, with untreated uninoculated and untreated inoculated plants as controls.

Objective 1: To determine the efficacy against black root rot and plant safety of some chemical plant protection products to *Viola* sp. (Experiment 1; 2014).

Objective 2: To determine the efficacy against black root rot and plant safety of some biological plant protection products and plant stimulants to *Viola* sp. (Experiment 2; 2014).

Viola sp. were sown in 2014 in multicell trays at ADAS Boxworth. Drench or overhead spray treatments were made before and after spore drenches of *T. basicola* four weeks after sowing. Roots were examined microscopically at termination in order to confirm infestation. Three out of the four experimental products (HDC F174, F175 and F176) very significantly reduced root rot severity on *Viola* sp. whether applied preventatively alone or followed by curative application. Preventative application of Signum (but not Switch) was also effective. Control by these products was equivalent to that given by Cercobin WG where 17.7% of the root surface was brown in contrast to 35.5% in the untreated inoculated plants. The plant activator HDC F178 applied both preventatively and curatively significantly reduced root rot severity in *Viola* sp. to 6.3% compared with 18.0% in the untreated inoculated. Both Trianum-G incorporation pre-sowing and Prestop applied preventatively plus curatively very significantly increased the proportion of *Viola* sp. plugs without root rot, with a mean 38% healthy in contrast to 0% following Cercobin WG application.

Objective 3: To determine the efficacy of simple programmes of products found to be effective in controlling black root rot in *Viola* spp. in Experiments 1 and 2. (Experiment 3; 2014)

Product selection for Experiment 3 was based on the results of foliar vigour at intervals thoughout the nine or 10 weeks of Experiments 1 and 2. When re-tested, HDC F174, F175 and F178 did not reduce root rot severity in the *Viola* sp. plants when applied either preventatively or curatively following Cercobin WG application. However, T34 Biocontrol at sowing before either HDC F174 or F175 significantly reduced the root area rotted from 67.2% in the untreated inoculated to 42.7%.

Objective 4: To utilise the results from work carried out under Objectives 1 to 3 to select products for application to *Choisya* sp. liners to protect against black root rot (Experiment 4; 2015)

In April 2015 *Choisya* sp. were potted up as liners at a nursery site. Drenches of HDC F178, T34 Biocontrol, Trianum-P, Prestop or Serenade ASO were applied the day after potting and repeated after five weeks at the same time as chemical drenches were applied to other plots. Cercobin WG, Signum, HDC F174, F175 and F178 were applied only preventatively and HDC F174, F175 and F178 were also re-applied 10 weeks after potting, following inoculation six weeks after potting. In August, foliar vigour was significantly better after treatment with F175 a week before inoculation than in untreated uninoculated plants. By December 2015 there was no significant difference in root rot (mean 30%) between these treatments and untreated plants.

Objective 5: To utilise the results from work carried out under Objectives 1 to 4 to select products for application to *Choisya* sp. finals to protect against black root rot (Experiment 5; 2016/17)

In April 2016 products were applied to *Choisya* sp. finals in the same nursery glasshouse as the liners that were destructively assessed in December 2015 (**Figure 2**) with protectant and curative treatments as shown in **Table 2**. Foliar vigour did not differ between treatments at any of the eight assessments. From August some *Choisya* sp. pots were yellowing and by the destructive assessment in May 2017 a number had wilted and desiccated and had fewer roots, however there were no significant differences between treatments. *Fusarium oxysporum* was isolated from a wilted plant's roots, suggesting that this fungus, not *T. basicola*, was the cause of wilting. All treatments developed a similar level of root rot leaving on average 26% healthy root area. *T. basicola* resting spores were confirmed in dark brown patches on roots and microscopic examination showed that cells containing less-pigmented mycelial growth of the fungus spread beyond these, with the dispersal spores being released from the roots.

Table 2. Fungicides and biofungicide programmes examined on *Choisya* sp. finals in 2016.

Treatment	At potting protectant drench or spray	At 5 weeks protectant drench	Inoculation *T. basicola* at 6 weeks	At 10 weeks curative drench or spray
1	Untreated	Untreated	Y	Untreated
2	Untreated	Untreated	N	Untreated
3	Cercobin	-	Y	-
4	T34 Biocontrol	Prestop	Y	Serenade ASO
5	Trianum-P	Prestop	Y	Serenade ASO
6	Serenade ASO	Prestop	Y	-
7	Prestop	Prestop	Y	Serenade ASO

Figure 2: *Choisya* sp. finals at the nursery trial site directly after potting on 27 April 2016.

Two rows of four 10-pot plots per replicate, (viewed from replicate four towards replicate one).

Financial Benefits

A review of Choisya root rots (HNS 169; Talbot & Wedgwood, 2009) listed *Fusarium* spp., *Phytophthora* spp. and *Pythium* spp. as well as *T. basicola* as common causal agents. Fungicides are thus used in preventative programmes by most growers. The cost of treatment is not great, but there are currently limited chemical options (risking resistance development) and only limited information on the efficacy of recently approved biofungicides. A Cercobin WG drench to *Viola* sp. could cost around £200 for a batch of 2500 plug trays (8 p per plug tray). With *Choisya* sp. the cost of Prestop treatment against a range of pathogens on 1000 plugs could be around £1.50 (1000 plugs selling at £280-£350), increasing to £15 per 1000 liners (1000 liners selling at £900-£1150). A Cercobin WG drench to *Choisya* sp. liners against fungal (not oomycete) root rots, could cost around 8.3 pence per 1000 pots.

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* sp. and *Skimmia* sp. are probably lost to black root rot. These are the main HNS subjects affected and they represent about 2% of the container plant range and so this would equate to annual losses to black root rot in the UK from these plants alone of £346,000. Losses can however be around 20% on some nurseries in years where controls fail. 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 and where greater losses arise when older plants are lost to disease.

Action Points

- Check plugs and liners have visibly healthy roots on delivery and before potting-on.
- Do not rely on the use of fungicides to maintain plant health as they will not be able to cure established root diseases, they only help to prevent new pathogen colonisation.

- Pay close attention to nursery hygiene such as the disinfection of trays and matting as the mycelium and resting spores (chlamydospores) can survive for several years.
- Avoid plant stress to prevent facilitating the entry of pathogens and encouraging black root rot and other rots to develop. See the HDC PO 14 review for further guidance.
- Be aware that early infection stages may cause the roots to become pale brown and typical dark brown speckling will not be seen until resting spores (chlamydospores) are formed. By the time reduced foliar vigour is seen root death could be advanced.
- Treat or destroy (do not compost) affected plants promptly otherwise endoconidia (dispersal spores of black root rot) can infect other plants and produce further sporulation within weeks.
- Seek confirmation of the cause of any plant losses so that the most appropriate control measures can be applied and avoidance sought for future batches.
- 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.
- Look to select a range of chemical plant protection products with different modes of action to avoid the build-up of resistance to active ingredients and integrate with biofungicides alongside cultural controls to reduce disease susceptibility.

SCIENCE SECTION

Introduction

Black root rot (*Thielaviopsis basicola*, syn. *Chalara elegans*) affects plants from at least 15 families (**Table 3**).

Table 3. Ornamental plants particularly susceptible to infection by *T. basicola*

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

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 resting spores (chlamydospores) of the fungus which develop at infection points in the outer cell layer of roots (**Figure 3**). 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. In some species (e.g. *Fuchsia*, *Viola*), plants infected with *T. basicola* are often found to be infected with Pythium sp. or other root pathogen.

Figure 3:

Infection of *Choisya* sp. liner roots by *T. basicola* in the current project.

"Black" areas of roots are caused by dense agglomerations of resting-spore (chlamydospore) chains forming inside the outer cells of the roots. The paler-brown areas of root contain less-pigmented mycelium with scattered chains of these brown thick-walled chlamydospores.

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). Details of various measures were given in the review of black root, PO 14 (Wedgwood, 2013). Once 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.

T. basicola tends to be an opportunistic pathogen, taking advantage of susceptible plants under stressful growing conditions such as experienced during summer (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 low compost moisture of 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).

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) with Full Authorisation 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 Year 1 (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 no benefit from Serenade ASO. In other 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 latter products are unable to control fungal species such as *T. basicola*.

Only container grown ornamentals under permanent protection are permitted a drench with Cercobin WG (thiophanate-methyl) and it 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 (FRAC Group 1) is high. One application can provide adequate protection for bedding plants on the nursery, put not for nursery stock with a longer production cycle in which the crop can be moved outdoors. Until 2017 “use-up” Scotts Octave (prochloraz) (FRAC Group 3) was applied as a growing-media drench to susceptible ornamentals to combat black root rot in polytunnel crops. No resistance of *T. basicola* to either Scotts Octave or Cercobin WG has been reported, but due to the pathogen being widespread across nurseries and there being regular movement of plants between nurseries then resistant strains could spread making it impossible to produce marketable plants of plant species susceptible to *T. basicola*. More products with different modes of action are required.

Research in AHDB project PC 38 showed good black root rot control by Benlate (benomyl), but the product was subsequently withdrawn. Fungicide screening on pansy in AHDB project PC 143 showed drenches of the foliar fungicides Amistar (azoxystrobin), Scotts Octave (prochloraz), Plover (difenoconazole), Unix (cyprodinil) and Stroby WG (kresoxim-methyl) gave some control of black root rot. Cyprodinil is available within Switch. Amistar (EAMU 0443 of 2009) and Stroby WG (Full Approval for MAPP 17316) can be applied as foliar sprays in ornamental plant production. Sales of Scotts Octave ceased in 2016.

In review PO 14 (Wedgwood 2013), active ingredients that might give effective control of black root rot were identified that were either currently in use on other crops, or experimental. 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.

Work in Years 1 and 2 leading to product selection for Year 3 2016

Details of the work done and results obtained in Years 1 and 2 is summarised below in order to provide a comprehensive final report. A range of products with the potential to control *T. basicola* further to the range of products tested in PC 143, (some experimental and others in use on other crops), including a number of biofungicides were tested. The treatments applied in the first four experiments were detailed in annual reports and are summarised in Tables 4 to 7. Work commenced with *Viola* sp., a susceptible host with a short growing season that allowed a quick evaluation of products' efficacy both in "straight" applications and then as protectant and curative treatment combinations with different modes of action. To see how the products performed over a longer cropping season and with another susceptible host, *Choisya* sp., selected products were used in programmes both when plugs were potted into liners and when another set of liners from the same batch were potted into finals.

Table 4. Experiment 1: Programmes of one or two chemical plant protection products applied to *Viola* sp. at different timings for control of black root rot after Wk 0 sowing on 9 May 2014. All except T3 were inoculated with *T. basicola* four weeks after sowing.

	Experiment 1 Viola Treatment Programmes T1 to T8							
Timing	T1	T2	T3	T4	T5	T6	T7	T8
Wk 0	-	-	-	-	-	-	-	-
Wk 3	-	-	-	Cercobin WG	F173	F173	Signum	Signum
Wk 4	Inoculated		-	Inoculated				
Wk 5	-	-	-	-	-	F173	-	Signum

	Experiment 1 Viola Treatment Programmes contd. T9 to T16							
Timing	T9	T10	T11	T12	T13	T14	T15	T16
Wk 0	-	-	-	-	-	-	-	-
Wk 3	Switch	Switch	F174	F174	F175	F175	F176	F176
Wk 4	Inoculation							
Wk 5	-	Switch	-	F174	-	F175	-	F176

Table 5. Experiment 2: Programmes of one or two products including biofungicides applied to *Viola* sp. at different timings for control of black root rot after Wk 0 sowing on 9 May 2014. All except T3 were inoculated with *T. basicola* four weeks after sowing.

	Experiment 2 Viola Treatment Programmes T1 to T8							
Timing	T1	T2	T3	T4	T5	T6	T7	T8
Wk 0	-	-	-	-	-	-	-	-
Wk 3	-	-	-	Cercobin WG	Prestop	Prestop	Serenade ASO	Serenade ASO
Wk 4	Inoculation		-	Inoculation				
Wk 5								Serenade ASO

	Experiment 2 Viola Treatment Programmes contd. T9 to T16							
Timing	T9	T10	T11	T12	T13	T14	T15	T16
Wk 0	T34 Biocontrol	Trianum -G (F177)						
Wk 3			Horti-Phyte	Horti-Phyte	F178	F178	F179	F179
Wk 4	Inoculation							
Wk 5				Horti-Phyte		F178		F179

Table 6. Experiment 3: Programme of one or two chemical or biofungicide products applied to *Viola* sp. at different timings for control of black root rot from Wk 0 sowing on 17 July 2014. All except T1 were inoculated with *T. basicola* four weeks after sowing.

	Experiment 3 Viola Treatment Programmes T1 to T12											
Timing	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Wk 0									T34 Biocontrol			
Wk 3			Cercobin WG			F174	F175	F178		F174	F175	F178
Wk 4		Inoculation										
Wk 5				F174	F175							

The first two experiments on *Viola cornuta* were sown on 9 May 2014 and conventional (Experiment 1) and non-conventional products (Experiment 2) were compared in separate concurrent glasshouse experiments arranged in replicate blocks with 15 plants assessed per plot (Figures 4 and 5). In Experiment 1, six conventional products were compared against the standard Cercobin WG (thiophanate-methyl) applied over the plants at the two leaf stage a week before inoculation. Each of the test products was used preventatively, but in some cases a second batch of plants were also treated again a week after inoculation of the growing media. Seven non-conventional products were tested in Experiment 2. All except the standard Cercobin WG, T34 Biocontrol and HDC code F177 (Trianum-G, since approved as a fungicide) were used curatively as well as preventatively in separate treatments. As soon as the first two experiments had been assessed and analysed then products were selected to use in Experiment 3 in treatment programmes for *Viola* sp. which were sown on 17 July 2014. The microbial pesticide T34 Biocontrol was applied to four treatments at sowing. Preventative and/or curative products were then applied to some treatments a week before or after *T. basicola* inoculation. Inoculation timing was the same as in the first two experiments, but the inoculation volume was doubled to give around 30,000 chlamydospores per cell to acheive a higher root infestation level. Final applications were made two weeks after inoculation. Destructive assessment of the *Viola* sp. was made towards the end of flowering, nine to 10 weeks after sowing.

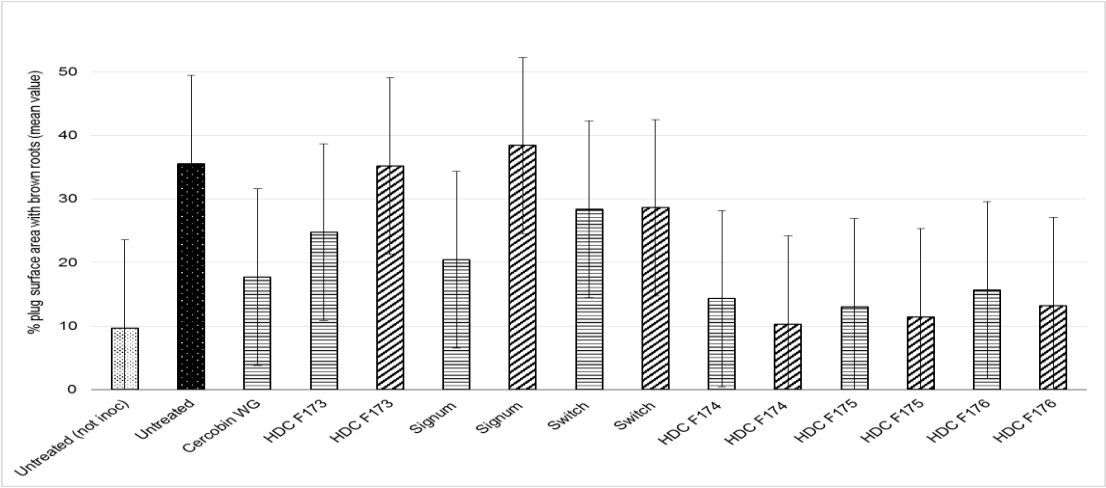
Figure 4.

Figure 5.

Figure 4: Multi-cells of Viola sp in Experiment 2 on 18 June 2014 five weeks from sowing.
Figure 5: Flowering trays of Experiment 1 in the foreground, with Experiment 2 in the next glasshouse compartment at ADAS Boxworth on 3 July 2014. Four plots of multicells per tray.

No foliar phytotoxicity arose with any of the products in any of the experiments on *Viola* sp. or *Choisya* sp.. No visible residue deposits were left on the leaves. The only potential issue was a greater area of root browning following two applications of Signum to *Viola* sp. plugs.

Infection of the *Viola* sp. roots caused a pale brown discolouration of the roots and microscopic examination showed mycelial growth in the cells and *T. basicola* endospore release from the root surface. This browning also dominated in the *Choisya* sp. and it was seen that the dark brown patches that give the disease the name ‘black root rot‘ result from clusters of chlamydospore chains. Less dense areas of production of these resting spores or where pigmentation is incomplete caused a paler discolouration of the outer cortex. This can be distinguished from *Pythium* sp. as the tissue does not soften. In both hosts, even when the majority of the roots where affected the foliar vigour remained good.

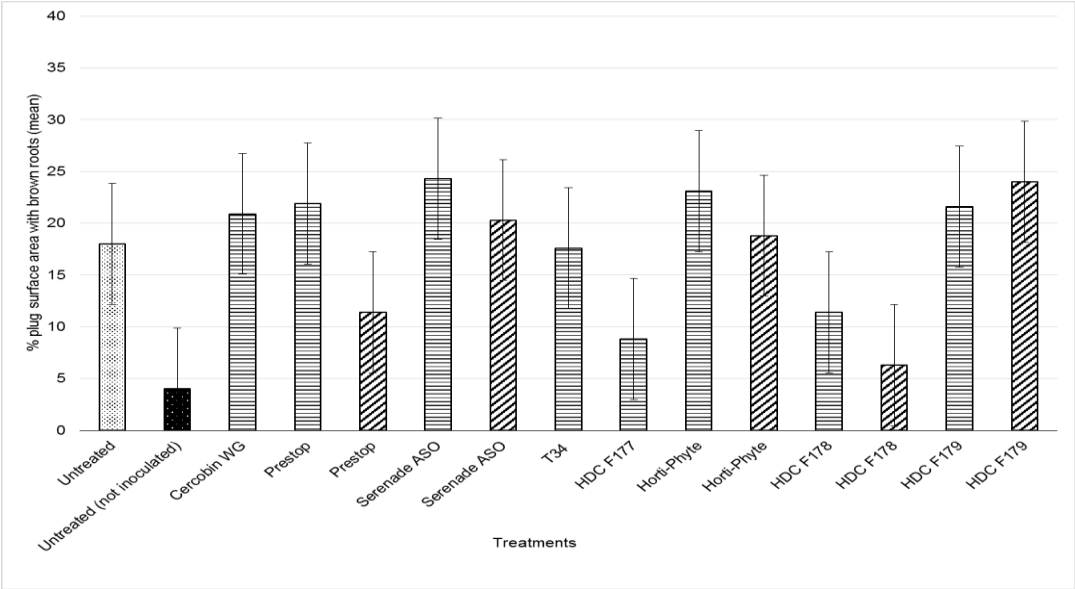


Key to bars: horizontal hatching Preventative, diagonal hatching Preventative + Curative
Figure 6: Experiment 1; Control of black root rot by conventional products. Mean % root area brown on *Viola* sp. plugs nine weeks after sowing. (P<0.001, L.s.d. 13.9). All treatments with below 24% root browning differ significantly from the untreated inoculated.

In Experiment 1 root rot caused a mean 36% rottting of the root plug surface area in untreated inoculated plants while Cercobin WG treated plants had 18%. Seven treatments resulted in significantly (P<0.001) less root damage, with a mean 14% area affected (**Figure 6**). After use of HDC F174, F175 and F176 as either preventative alone or with curative application then root damage was similar to that of uninoculated plants (9.7%). Signum preventatively treated plants also had significantly less severe root rot than untreated inoculated plants, with 20% of the surface affected. Plants in five of the treatment programmes were no healthier than untreated inoculated plants, with most plugs having rotted roots.

The incidence (rather than severity) of root infection was only significantly (P<0.002) reduced by preventative plus curative treatment with either HDC F175 or F176. A mean 26% of plants remained healthy, in contrast to 2.5% for untreated inoculated plants (data not presented).

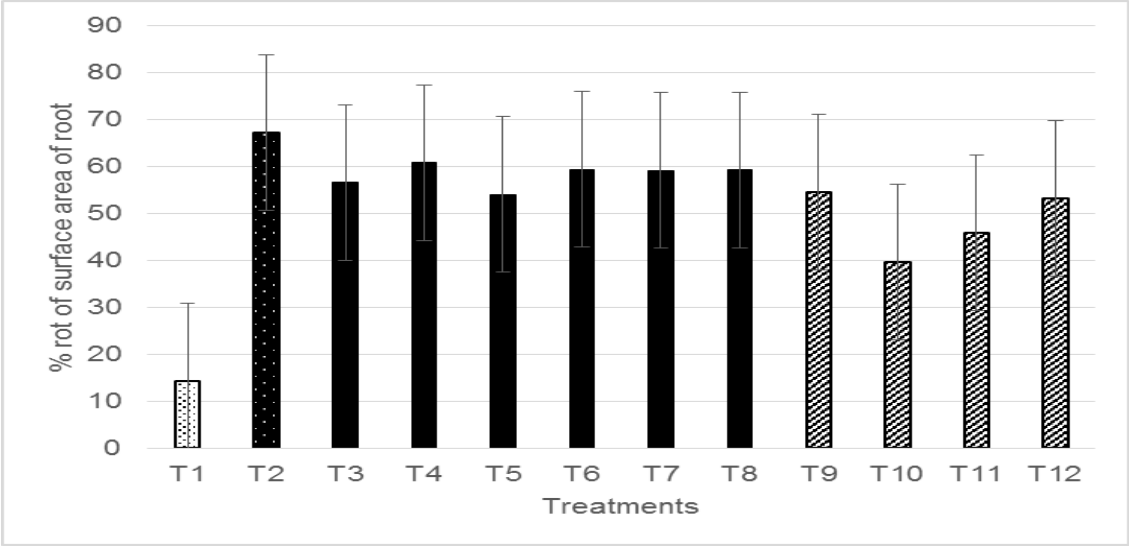
In Experiment 2, which examined non-conventional treatments in the adjacent glasshouse, root infestation severity was lower in the untreated inoculated plants (18.0%) than in the conventional treatments experiment. The reason for this is not known. Root rot was significantly (P<0.001) less severe than for the untreated plants following the use of HDC F178 preventatively plus curatively, with 6.3% root rot (**Figure 7**). There were no other significant differences in rot severity, but plants receiving preventative use of either HDC F177, HDC F178, or Prestop applied preventatively plus curatively had less than 12% root rot when a number of treatments had over 20% (**Figure 7**).



Key to bars: horizontal hatching Preventative, diagonal hatching Preventative + Curative.
Figure 7: Experiment 2: Non-conventional products. Mean % root area brown on the surface of *Viola* sp. plugs on 10 & 14 July 2014 nine weeks after sowing. Only HDC F178 applied twice differs significantly (P<0.05, L.s.d. 5.84) from the untreated inoculated.

In Experiment 2, preventative use of HDC F177, and preventative plus curative use of either HDC F178 or Prestop significantly (P<0.001) reduced the proportion of plugs with root rot (data not presented). Incorporation of the fungus in HDC F177 before sowing resulted in a mean 35% of plugs having no brown roots. Giving a curative application as well as a preventative significantly improved the performance of both HDC F178 and Prestop, to leave 37% and 41% of plants root rot free, respectively. Most other treatments had less than 15% of plugs with healthy roots, similar to the 5% remaining healthy in the untreated inoculated. There were no treatment differences in plant foliar vigour.

In Experiment 3, two preventative treatments resulted in significantly (P< 0.001) less rot than the untreated inoculated (67%). One was using HDC F174 (T10) having 40% rot, the other was HDC F175 (T11) with 46% rot, both having received T34 Biocontrol at sowing (**Figure 8**). Preventative use of any of these three products alone (T6, T7 and T9, respectively) did not significantly reduce root rotting.



T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
UTUn	**UT**	**-/C/-**	**/C/4**	**/C/5**	**/4/--**	**/5/-**	**/8/-**	**B/-/-**	**B/4/-**	**B/5/-**	**B/8/-**

UT-Untreated, Un-Uninoculated, C-Cercobin, B-T34 Biocontrol, 4-F174, 5-F175, 8-F178, **-** No application at first preventative, second preventative or curative (third) timings.

Figure 8: Experiment 3: Treatment programmes on *Viola* sp. plugs showing % root rot. Only T10 and T11 differ significantly (P<0.001, L.s.d. 16.55) from T2 untreated inoculated.

Experiment 4, to investigate simple programmes on HNS liners (**Table 7**), was set up in a commercial glasshouse with *Choisya ternata* plug plants potted up into 90 mm diameter pots on 29 April 2015 (**Figure 9**).

Figure 9: 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.

Replicates of five treatments comprising the four biofungicides T34 Biocontrol, Trianum- P, Prestop and Serenade ASO and a non-conventional coded product were applied the day after potting. Six weeks later, when new roots had established, these plants were re-treated with the same products. 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 in one untreated treatment) were inoculated on 11 June 2015 using an isolate of *T. basicola* from *C. temata*. Three experimental chemicals with potential curative activity were applied to some plots a week after inoculation. No products were applied at any of the three timings to plots of two untreated controls.

Table 7. Experiment 4: Programmes of one or two products including biofungicides applied to *Choisya* sp. liners at different timings for control of black root rot after plugs were potted-on in Wk 0 on 30 April 2015. All except T3 were inoculated with *T. basicola* six weeks after potting. F178 used at half rate at Wk 0 and Trianum-P used at half rate at Wk 5.

	Experiment 4 Choisya Liner Treatment Programmes T1 to T8							
Timing	T1	T2	T3	T4	T5	T6	T7	T8
Wk 0	-	-	-	-	-	-	-	-
Wk 5	-	-	-	Cercobin WG	Signum	F174	F174	F175
Wk 6	Inoculation		-	Inoculation				
Wk 10	-	-	-	-	-	-	F174	-

	Experiment 4 Choisya Liner Treatment Programmes contd. T9 to T16							
Timing	T9	T10	T11	T12	T13	T14	T15	T16
Wk 0	-	-	-	F178	T34 Biocontrol	Trianum -P	Prestop	Serenade ASO
Wk 3	F175	F176	F176	F178	T34 Biocontrol	Trianum -P	Prestop	Serenade ASO
Wk 4	Inoculation							
Wk 5	F175	-	F176	-	-	-	-	-

By August 2015, nine treatments to the *Choisya* sp. liners had resulted in significantly ($P<0.05$) more vigour than in the uninoculated untreated plants. These were single preventative treatments of Cercobin WG, Signum, F174, F175, F176 or double of F178, Trianum-P and Serenade ASO; and curative as well as preventative treatment with F174. After two preventative applications of T34 Biocontrol and Prestop, plants had vigour equivalent to the untreated uninoculated plants. All treatments had vigour equivalent to the still symptomless untreated inoculated plants. By October, vigour was equally good in all treatments, but still significantly better than the untreated uninoculated after preventative use of F175 as a foliar spray followed by irrigation. Unfortunately, product F175 is not now going to be brought to the UK and so was not selected for further work.

At the time of destructive assessment in December 2015, there were no vigour differences between any treatments or the untreated; on average, all were growing well even though they had become equally affected by black root rot plus some natural infection by *Pythium* spp., Overall the proportion of healthy roots was around 30% of the root surface area. 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.

In Year 3, the final project year, the Objective was to select and test the efficacy of some fungicides and biofungicides applied in programmes for the control of black root rot on *Choisya* sp.. With no significant difference in *Choisya* sp. root rotting shown by Experiment 4, products for testing on *Choisya* sp. finals were selected to fill particular treatment timings and for a selection of product alternations. F174 was the chemical determined to be most likely to be able to gain an EAMU and this had given greater plant vigour with two applications to liners and there was the possibility of curative activity. T34 Biocontrol use at sowing followed by other treatments had been beneficial to *Viola* sp. and so this and the biofungicides Trianum-P, Serenade ASO and Prestop were allocated to be the first treatment at potting. A Trianum- P drench was substituted for the Trianum-G used with *Viola* sp. because granules of the latter if incorporated into the new growing media of liners might not protect inside the existing root ball. Prestop was selected to be applied as a second preventative treatment

across all test programmes in order to see whether using two different products was more beneficial than re-application. Serenade ASO drenching is permitted only once in outdoor crops and as this product is known to have part of its efficacy through the action of metabolites it was included at a single, curative, timing.

Materials and Methods

Site and crop details

An experiment was set up in West Sussex on 27 April 2016 with *Choisya ternata* 3 L finals. The aim was to investigate the efficacy and crop safety of fungicide and biofungicide programme combinations on these plants, used as a test host for other nursery stock. The plants had not received any fungicide or biofungicide drenches as liners in 90 mm pots and were from the same stock as used in the 2015 experiment. The plants were potted into peat-based growing medium in 3 L pots by the nursery staff on 25 April 2016 and lightly watered. The mix was 45% Baltic coarse peat, 25% Irish peat, 20% bark, 10% pure coco fibre with 4.5 kg Osmocote Exact 8 to 9 month slow release fertiliser, 0.25 kg KCM starter feed and 0.5 kg PG Multi-Mix per m^3.

Four replicate blocks of ten pots were stood on woven ground cover matting, which had been herbicide treated in a commercial glasshouse with programmed overhead irrigation (**Figure 10**). Uninoculated plots were randomised within the layout (**Figure 11**). Two temperature and humidity loggers were placed at plant canopy height under white covers to shield them from direct sun and overhead irrigation.

Figure 10: Plot arrangement of Choisya finals with two rows of four 10-pot plots per replicate block, viewed from block four towards block one, 27 April 2016. Spare plants across front.

PLOT	1	2	3	4
TREATMENT	2	8	4	7
PLOT	5	6	7	8
TREATMENT	5	3	6	1
PLOT	9	10	11	12
TREATMENT	7	5	1	6
PLOT	13	14	15	16
TREATMENT	3	2	4	8
PLOT	17	18	19	20
TREATMENT	3	1	8	4
PLOT	21	22	23	24
TREATMENT	7	6	2	5
PLOT	25	26	27	28
TREATMENT	4	2	7	3
PLOT	29	30	31	32
TREATMENT	5	8	1	6

Figure 11: Treatment layout for *Choisya ternata* of at the nursery site between 25 April 2016 and 6 May 2017. Four replicate blocks of 3 L pots. Eight treatment programmes with 10 pots per plot in two rows of five plants. See Table 8 for treatment details.

Details of treatment applications

Products selected from the earlier experiments on Viola and Choisya were tested with one or two applications per product, timed at three stages in the crop, before and after inoculation with *T. basicola*, depending on their potential activity from earlier work. Five programmes plus two untreated (one uninoculated) were set up in comparison with the standard Cercobin WG drench (**Table 8**) and applied at the timings given in **Table 9**.

Table 8. Treatments and application methods using five plant products, plus Cercobin WG (Topsin) as standard, on *Choisya* sp. finals. Experiment 5 (2016-17).

T	Product &/or code	Active ingredient	Application dose & water volume	Comments
1	Untreated	-	water alone (overhead irrigation)	*T. basicola* inoculation at 5 weeks from potting
2	Untreated	-	water alone (overhead irrigation)	NOT inoculated
3	Topsin WG* (Cercobin)	thiophanate-methyl	1.1 g per 10 L water using 1000 L/ha	Standard rate for black root rot. One application permitted.
4	T34 Biocontrol	*Trichoderma asperellum*	Spore suspension made up 2 hrs before use. 5g per 1000 L compost drenched at 10% pot volume	EAMU for ornamentals 1118 of 2012. Irrigation rate at 10% of pot volume.
5	Trianum-P HDC F190	*Trichoderma harzianum*	15g per 1000 plants and drench at 10% pot volume	EAMU 2771 of 2014.
6	Serenade ASO	*Bacillus subtilis*	10 L product per ha based on a pot surface area of 0.02835 m². Dilute to be able to drench at 10% pot volume	1 drench / crop under EAMU 0306 of 2015
7	Prestop	*Gliocladium catenulatum*	5.0 g Prestop in 1 L water. Applied at 10 % of pot volume over foliage	Approved on ornamentals.
8	HDC F174	confidential	Label rate using 400 L/ha water followed by irrigation of 600 L/ha	

* Topsin WG was supplied in place of Cercobin WG; the two products have an identical formulation.

Table 9. Experiment 5: Programmes of one or two products including biofungicides applied to *Choisya* sp. finals at different timings for control of black root rot after liners were potted-on on 25 April 2016. All except T2 were inoculated with *T. basicola* six weeks after potting.

	Experiment 5 Choisya Finals Treatment Programmes T1 to T8							
Timing	T1	T2	T3	T4	T5	T6	T7	T8
Wk 0	-	-	Cercobin WG	T34 Biocontrol	Trianum - P	Serenade ASO	Prestop	F174
Wk 5	-	-	-	Prestop	Prestop	Prestop	Prestop	Prestop
Wk 6		-	Inoculation					
Wk 10	-	-	-	Serenade ASO	Serenade ASO	-	Serenade ASO	F174

Dates of treatment timings in 2016: Wk 0 on 27 April; Wk 5 on 1 June; Wk 10 on 6 July.

Only one conventional chemical product (F174) was taken forward from the previous testing as this had the possibility of obtaining an EAMU soon whereas this was less likely for the other experimental product (F175) with earlier evidence of activity. Neither of the plant

activators tested on the *Viola* sp. were available for further testing. Four biological products were included which all have authorisation for use in protected ornamentals:

- Prestop (*Gliocladium catenulatum* strain J1446) as a drench under EAMU 2773 of 2015;
- Serenade ASO (*Bacillus subtilis* strain QST 713) as a drench under EAMU 0306 of 2015 (formerly EAMUs 0706 of 2013 and 0708 of 2013);
- T34 Biocontrol (*Trichoderma asperellum* strain T34) as a drench under EAMU 1118 of 2012 applied to plants when under permanent protection with full enclosure;
- Trianum-P (*Trichoderma harzianum* T-22) as a drench under Authorisations 2771 of 2014 and 2379 of 2015.

Thiophanate-methyl was used as the standard active ingredient (as in previous experiments) as this active is used by growers (in Cercobin WG) as a single preventative application. Topsin WG was used instead of Cercobin WG throughout this project (both products contain 70% w/w thiophanate-methyl and are identical formulations) as samples could not be supplied of the latter. Topsin does not have authorisation for use on ornamentals. Application to the crop followed guidance in the Cercobin WG EAMU 1887 of 2011 which gives the drench rate of 11 g per 100 L for the control of root diseases including *Thielaviopsis*, *Cylindrocarpon* and *Rhizoctonia* spp. etc. rather than the higher label rate. This and other preventative treatments were given on 27 April 2016, two days after potting and initial watering-in. T34 Biocontrol was applied only at this first timing; the label recommendation of soaking for two hours in the spray tank at the dilution to be used were carried out before application. Trianum-P was also only applied at the first timing; this was not soaked prior to use. Prestop was given, without soaking, to all but the standard treatment at the second timing five weeks after potting, on 1 June 2016. This was followed a week later on the 8 June 2016 by inoculation with *T. basicola.* At the third application timing, Serenade ASO was applied, on 6 July 2016 10 weeks from potting, to all the treated plots except those that had received a Serenade ASO drench at the first timing (to keep within the EAMU for drenching) and also not following Cercobin. All the biological products had spores in suspension and so were agitated repeatedly during application. All products were applied at their label or relevant EAMU rates.

Re-application of biofungicides beyond 10 weeks was not made in order to keep the programmes simple and so aid comparison; the EAMU for Trianum-P gives four week intervals, the label for Prestop gives re-application at half-rate at four to six week intervals whereas the T34 Biocontrol EAMU has two to three month intervals.

The biofungicides were applied as drenches to pots that had been watered by overhead irrigation two hours beforehand. A syringe was used with the liquid released over the foliage as well as into individual pots to be able to check for any subsequent foliar phytotoxicity. All dose rates and water volumes were the same *pro rata* as in previous experiments in this

project, with all biological products applied with a water volume that was 10% of pot volume (300 ml per 3 L pot) in order to fully wet the growing media and be retained in the pot. For Serenade ASO the standard dose rate of 10 L/ha (0.001 L / m²) was adhered to, so that each 190 mm diameter pot of 0.02835 m² received 2.84 x 10^{-5} L of product per pot and this was diluted in 300 ml (in excess of 1000 L water/ha) in order to allow as good penetration through the growing media as the other biofungicides.

The experimental chemical F174 was given at the dose rate supplied by the agrochemical company representative who agreed product use and applied as a spray in a water volume of 400 L/ha. It was followed by irrigation to aid product entry into the growing media. The Topsin WG application was made at the label water volume recommendation of 1000 L/ha. Both products were applied using an Oxford gas assisted sprayer operating at 2 bar pressure with a single 02F110 nozzle. This product and Serenade ASO were the only products to be applied curatively, on 6 July 2016. Plants were moved out of the plots and all replicates of a treatment sprayed together.

Inoculation

The host nursery had problems with black root rot on species such as *Skimmia*, *Convolvulus*, *Senecio*, *Ceanothus* and *Cornus* as well as *Choisya*. Baiting of ground cover matting (Appendix 1) in 2015 confirmed the presence of *T. basicola*. Historic plant clinic reports for the nursery had also shown *Phytophthora*, *Pythium* and *Fusarium* species had been frequently detected from Choisya roots. A *Choisya* sp. cv. White Dazzler plant from this site in February 2015 was used to obtain an isolate of *T. basicola* (stored under ADAS code BX15/22) for use as inoculum in 2015.

Agar plates made using fresh carrots (Appendix 1) were sub-cultured using the *T. basicola* isolate BX15/22. On the morning of the inoculation on 8 June 2016 the spores were removed from the 80 agar plates to create a spore suspension in distilled water. Following haemocytometer counts, the spore suspension was diluted to hold a calculated 1590 chlamydospore units/ml together with a calculated 113636 endospores per ml. The chlamydospore concentration used was half that used in 2015 in this project on Choisya liners in 350 ml pots because the liners received inoculum suspension at 5% of the liner pot volume, so received 17.5 ml delivering approximately 52,500 chlamydospore units per pot from a suspension concentration of 3000 chlamydospores / ml. To ensure penetration of the inoculum into the growing media also required 5% by volume for the Choisya finals, but increasing the volume of suspension without adjusting the concentration for 3 L pots would have given many more spores per plant. The 190 mm diameter 3 L pots had approximately x10 the pot volume of the 90 mm liner pots. In a nursery situation spores would be taken up from the matting and so infestation level will be affected by the pot base surface area making

contact. A 3 L pot has about x4.5 of the floor coverage of a 0.3 L pot, so 52,500 x 4.5 equals 236,250 spores for a 3 L pot which would be given by 150 ml of inoculum with 1,575 spore units / ml.

The black root rot inoculum was transported to the nursery glasshouse straight after creating the spore suspension and applied to the Choisya (except those in Treatment 2). Inoculation on 8 June 2016 was six weeks after potting on 25 April (a week longer interval than used for the liners in 2015), 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 150 ml per pot, drenching the growing media around each plant stem and ensuring also that the new growing media beyond the plant root ball was inoculated.

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). Standard husbandry of plant trimming was if necessary delayed until observations for that month were complete.

The early symptoms of black root rot when water and nutrient uptake is gradually reduced by root infection, leading to a slight loss of vigour, can be subtle. Initial symptoms would have been reflected in the vigour assessment. When yellowing and wilting started to show this was recorded as the % area of the plant that was yellowed or wilted. Samples of roots from wilted plants were returned to the laboratory for pathogen isolation in October in order to determine the cause of wilting prior to the final destructive assessment.

Phytotoxicity from the treatment applications 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.

The final assessment that included roots as well as the foliage was carried out on 4 and 5 May 2017 at the trial site. The plants at this time would normally have been marketed. Foliage vigour, wilting and yellowing was assessed and then each plant was knocked out of its pot and the surface of the root ball assessed for the % surface area covered by roots and the proportions that were either white (and so apparently healthy) or with potential black root rot. Inspection was carried out using a hand-magnifier examining all around the sides and the base of each root ball. Some plants' root balls were broken open and it was seen that the inside root density and proportion of healthy roots matched that visible on the outside and so only the outside was assessed. This root ball surface would be that used by a grower or purchaser to assess the health of the roots. Plant samples were returned to the laboratory for

examination of the roots under high powered magnification and for isolation onto agar in order to confirm the cause of any root browning and the foliar wilt.

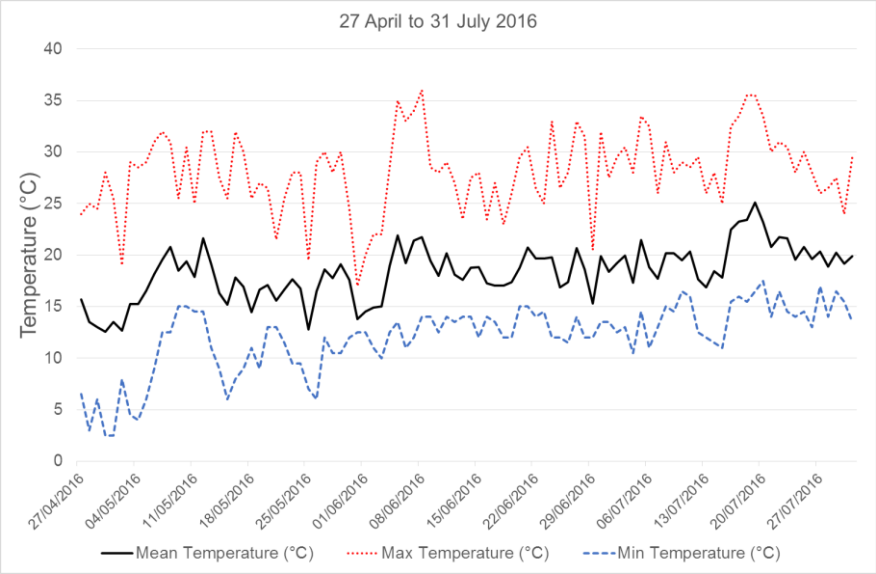
Data from the assessments were examined statistically by Analysis of Variance.

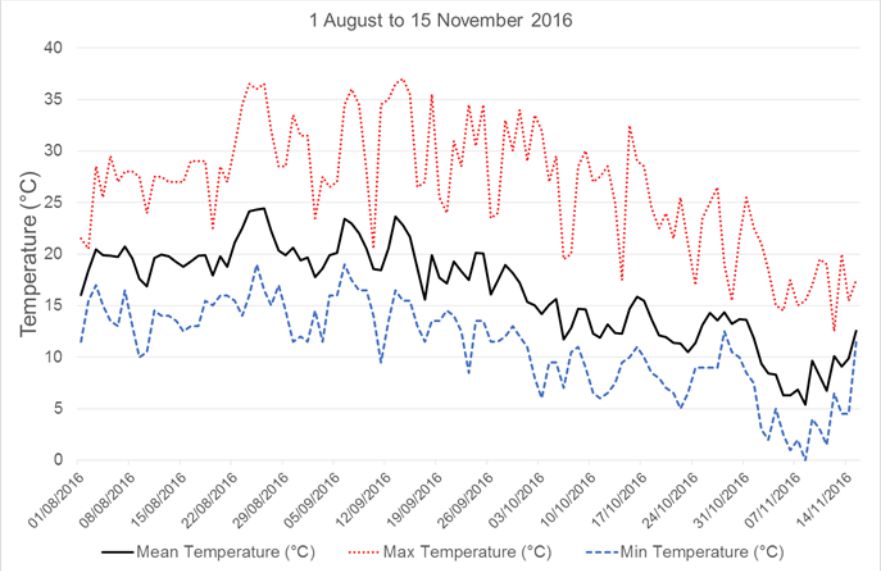
Results

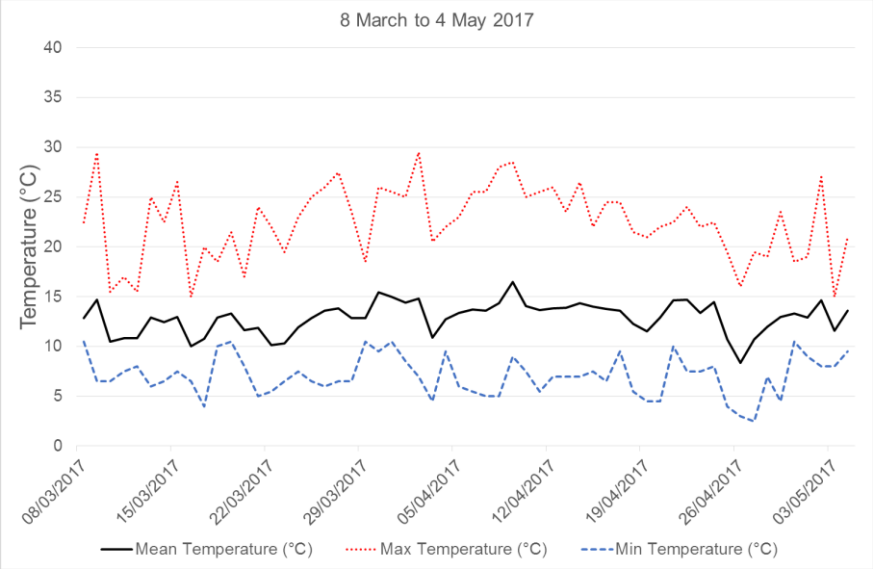
Environmental conditions

Products were applied in the glasshouse under sunny conditions. Applications on 26 April between 11:58 h and 13:25 h were made in air temperatures around 25°C, the drenches on 1 June between 10:00 h and 11:00 h around 19°C and applications on 6 July 2016 between 2:15 h and 3:31 h when temperatures rose from 21°C to 25°C.

Air temperatures in the glasshouse during summer 2016 and spring 2017 were very hot at times (**Figures 12a-c**), although white shading paint is applied to the glass by the nursery in early May to give some shade from the sun. The heat would have stressed the plants and grower experience has been that this increases the black root rot symptoms.







Figures 12a-c. Minimum, maximum and mean daily temperature readings in the Choisya canopy in the nursery glasshouse during the 2016/17 growing season

Foliar assessment: vigour and wilting

Eight vigour assessments were made between May 2016 and May 2017 (**Figure 13**), with interim inspections carried out by the nursery site manager (and reported to ADAS) to guide plant husbandry. The vigour index recorded the condition of the foliage, giving the highest scores to plants which had green foliage and a branched habit, with tall shoots that were usually indicative of active new growth. No significant differences ($P>0.05$, 21 d.f.) in vigour developed between any of the treatments (**Table 10**), with both treated and untreated showing similar vigour and no difference between the inoculated (T1) and uninoculated (T2) untreated plots. Treatments had a mean index of 6 (good vigour) throughout May and June 2016, with vigour increasing through July and August 2016 to reach a mean index of 7, before

decreasing in November 2016 to a mean index of 5 and remaining in this condition until early May 2017 (**Figure 13**).

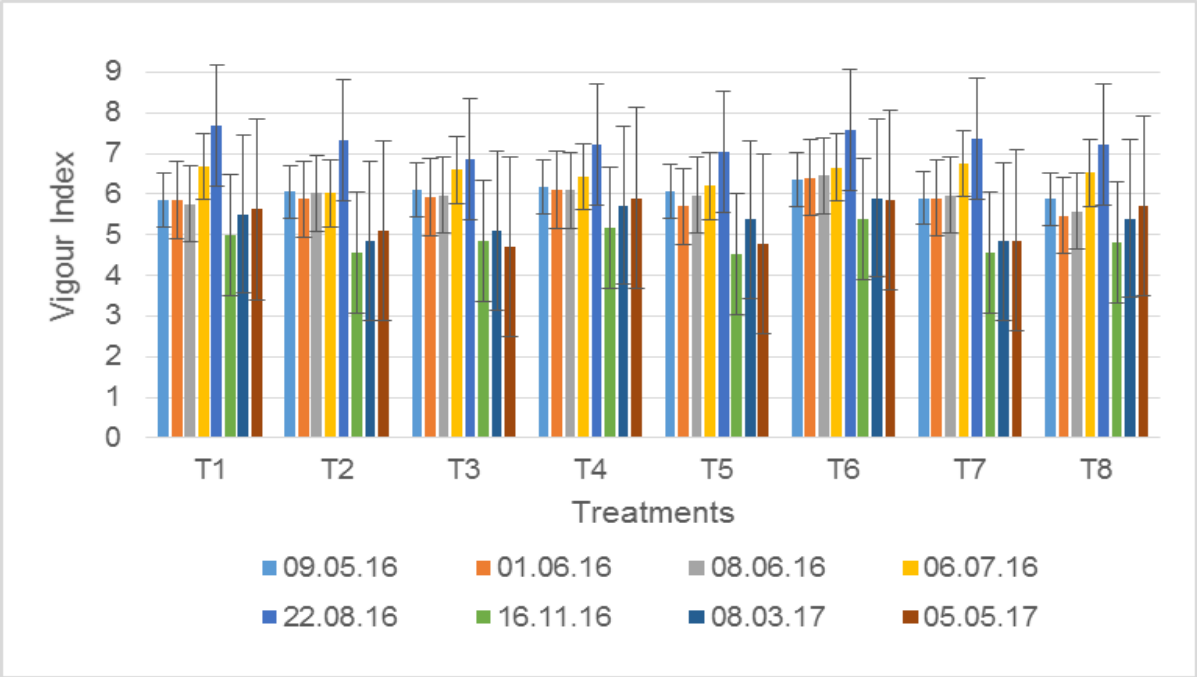


Figure 13: Mean 0-9 foliar vigour index of Choisya for Treatments T1 to T8 recorded at intervals between May 2016 and May 2017 after potting-on into 3 L containers. After peaking in August, by November 2016 new growth had ceased and some plants were wilting. Treatments application dates; 26 April, 1 June and 6 July 2016. *T. basicola* inoculation, except for T2, on 8 June 2016. No significant differences between treatments (see Table 10).

Table 10. Overall mean foliar vigour indices (0-9 dead-excellent) for Choisya finals across all treatments between 9 May 2016 to 5 May 2017 showing Least Significant Difference (L.s.d) and no significant treatment differences (F pr. > 0.05).

	Assessment Dates							
	2016						2017	
	9 May	1 June	8 June	6 July	22 Aug.	16 Nov.	8 April	5 May
Mean	6.05	5.90	5.98	6.48	7.29	4.86	5.33	5.31
L.s.d.	0.654	0.941	0.934	0.821	1.539	1.491	1.946	2.218
F pr.	0.763	0.658	0.716	0.585	0.967	0.902	0.924	0.858

From the start of the experiment in April 2016 there was some variation in plant vigour, but mostly within the range of indices 5 to 9 (acceptable to excellent vigour). All except T6 had 3 or 4 out of the 40 plants per treatment in the trial showing no new growth (Index 4) by 9 May 2016, a fortnight since potting (individual plant data not shown). The plants of T6, which had received Serenade ASO application at potting, all had new growth with vigour ranging between index 5 and 9. At the assessment on the 22 August 2016, 12 plants across the trial had interveinal chlorosis (**Figure 14**) and four of these were starting to wilt resulting in lower vigour indices (**Table 11**). By the final assessment on 5 May 2017 there were 47 dead or

severely wilted plants (Index 0), and 17 plants showing poor vigour (indices 2 to 4) (**Table 12**) out of 320 plants in the experiment, with no treatment unaffected. From the results of individual plants (**Tables 11 & 12**) it is noteworthy that by May 2017 nine out of 40 of the plants had excellent foliar vigour after receiving T8, in which F174 was followed by Prestop preventatively and then a further F174 curatively.

Figure 14. Ten Choisya pots in Plot 1 on 22 August 2016 at the nursery with some plants (left hand side) showing foliar chlorosis.

Table 11. Vigour index per plant assessed on 22 August 2016 (four weeks after final treatment applications) to show the number of plants out of 40 within each index category (0 = dead, 9 = excellent). Most plants showed new leaf and branching (Index 5 and above).

Treatment series*	Vigour Index								
	0	2	3	4	5	6	7	8	9
1 UT	0	0	0	1	1	4	11	10	12
2 UT UN	0	1	0	0	4	5	8	10	11
3 C/--/--	0	0	0	0	5	11	12	8	4
4 B/P/S	0	0	0	1	6	5	10	7	11
5 T/P/S	0	2	0	2	4	4	11	4	13
6 S/P/--	0	0	0	0	1	5	12	14	8
7 P/P/S	0	0	0	2	5	7	3	6	16
8 F/P/F	0	3	0	0	2	4	11	8	12

* UT-Untreated, UN-Uninoculated, B-T34 Biocontrol, C-Cercobin, F-F174, P-Prestop, S-Serenade ASO, --No application/s at second preventative or third (curative) timings

Table 12. Vigour per plant at final assessment on 5 May 2017 (10 months after treatment) to show the number of plants out of 40 within each index category including the number of plants that had died (index 0) or were starting to wilt (index 2) or yellow (index 3). Three dying plants removed for laboratory assessment in October 2016 are included in Index 0.

Treatment series*	Vigour Index								
	0	2	3	4	5	6	7	8	9
1 UT	4	0	1	2	7	14	9	2	1
2 UT UN	9	1	0	1	2	10	14	2	1
3 C/--/--	8	0	2	1	7	14	6	1	1
4 B/P/S	3	0	2	1	4	11	14	3	2
5 T/P/S	7	0	1	1	11	11	9	0	0
6 S/P/--	2	0	0	2	2	24	8	2	0
7 P/P/S	10	0	0	1	6	8	10	5	0
8 F/P/F	7	0	0	1	6	8	7	2	9

* UT-Untreated, UN-Uninoculated, B-T34 Biocontrol, C-Cercobin, F-F174, P-Prestop, S-Serenade ASO, -- No application/s at second preventative or third (curative) timings.

It was noted that some plants (**Figure 15**) had one branch that was dead and the other still green and one cause of this can be an infection of the vascular strands in the stem, and this one-sided symptom is commonly reported for infections by *Verticillium* spp.. It was thought unlikely that the sudden wilting leading to rapid stem death was a symptom of root destruction by Black Root Rot.

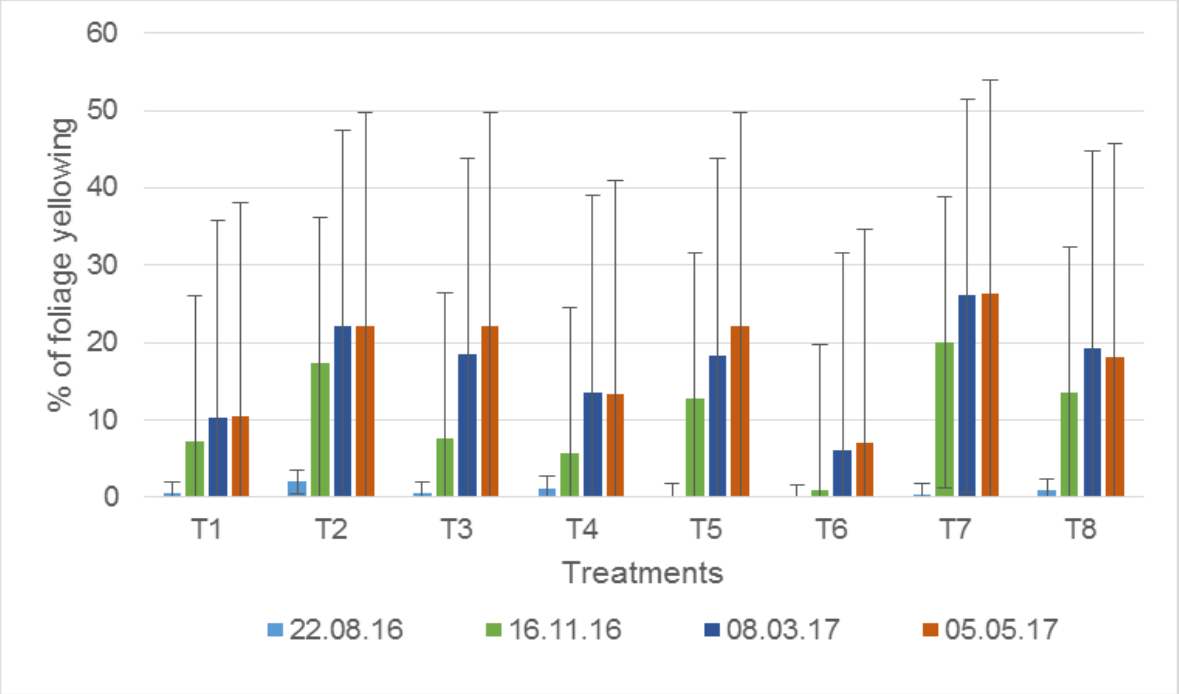
Figure 15:
Choisya plant in May 2017 with chlorotic and wilted leaves and dead stems, but also some apparently healthy stems.

This wilting may have been the result of infection by *Fusarium oxysporum* (see isolation results)

Wilted plants were also present amongst the plants that had been surplus to the trial and been kept to one side of the trial. As it was thought possible that wilted plants could have masked subtler effects of *T. basicola* on vigour, the data was analysed before and after removing plants from the data set that were recorded as wilting at each of the assessments.

No significant differences between treatments were seen in either the full or the restricted data sets in which wilted plants were removed, and so the results from the restricted set have not been presented. The number of wilted plants in a plot was presumed to be determined at random with invisibly affected plants allocated positions at random in the trial layout.

Foliage yellowing and foliage wilting (rather than just poor vigour) were recorded separately alongside vigour on 22 August 2016 and on 16 November, and then again in 2017 on 8 March and 5 and 6 May 2017 (**Figures 16 and 17**). Although there was consistently less yellowing in T6 (preventative Serenade ASO and Prestop) than in other treatments, this was not borne out in the statistics, as there were no significant differences between treatments in the mean % of leaf yellowing (P>0.05, 21 d.f.) (**Figure 16 and Table 13**) and the mean % of wilted foliage per plant (P>0.05, 21.d.f.) (**Figure 17 and Table 14**) at any of the five assessment dates following symptom expression.



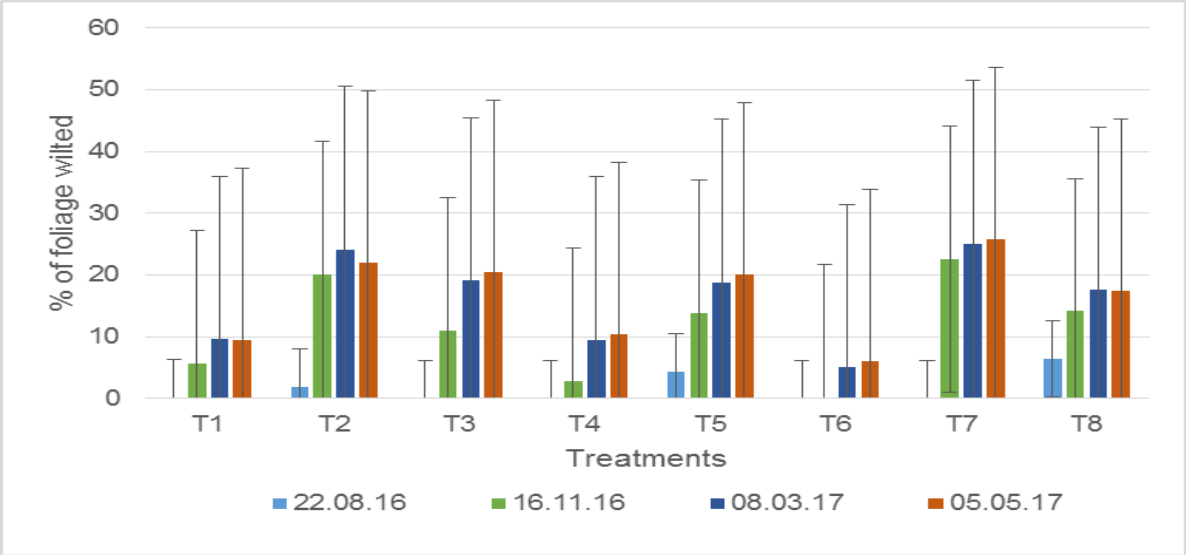
T1 UT **T2 UTUn** **T3 C/--/--** **T4 B/P/S** **T5 T/P/S** **T6 S/P/--** **T7 P/P/S** **T8 F/P/F**

UT-Untreated, Un-Uninoculated, B-T34 Biocontrol, C-Cercobin, F-F174, P-Prestop, S-Serenade ASO -- No application/s at second preventative or third (curative) timings.

Figure 16: Mean % of foliage per plant yellowing in each of the eight treatments once apparent in most plots (August 2016) and then in November 2016, and March and May 2017.

Table 13. Overall mean % foliage yellowing for each assessment date. There were no significant differences (F pr >0.05 at any of the assessments.

	2016		2017	
	22 Aug.	16 Nov.	8 April	5 May
Mean	0.71	10.60	16.80	17.80
L.s.d.	1.550	18.81	25.41	27.55
F pr.	0.224	0.455	0.776	0.810



T1 UT **T2 UTUn** **T3 C/--/--** **T4 B/P/S** **T5 T/P/S** **T6 S/P/--** **T7 P/P/S** **T8 F/P/F**

UT-Untreated, Un-Uninoculated, B-T34 Biocontrol, C-Cercobin, F-F174, P-Prestop, S-Serenade ASO -- No application/s at second preventative or third (curative) timings.

Figure 17: Mean percentage of foliage that was wilted in each of the eight treatments once apparent in some plots in August 2016 and recorded again in November 2016, and March and May 2017. N.B. for the majority of wilted plants, 100% of the foliage was wilted and the majority of plants had no wilted foliage so the means reflect the proportion of wilted plants.

Table 14. Overall mean % of foliage that was wilted for each assessment date. There were no significant differences (F pr >0.05) at any of the assessments.

	Assessment Dates			
	2016		2017	
	22 Aug.	16 Nov.	8 April	5 May
Mean	1.60	11.20	16.10	16.50
L.s.d.	6.193	21.52	26.36	27.76
F pr.	0.265	0.348	0.700	0.792

Further investigation is required to determine whether the fewer dead plants (5% and 7.5% in T6 (Serenade ASO then Prestop preventatively) and in T4 (T34 Biocontrol and Prestop preventatively then Serenade ASO after *T. basicola* inoculation), was by chance in contrast

to a range of 17.5% to 22.5% respectively of plants in other treatments by May 2017, particularly as the untreated *T. basicola* inoculated (T1) plots also contained fewer dead plants (as calculated from the Vigour index table). This “difference” is reflected in the mean % of foliage wilted (**Figure 17**).

By May 2017 there were more wilted plants across all the replicate blocks down one side of the experiment (the south side), with plant vigour tending to be better in the two lines of plots on the other side (**Figure 18**). It was probable that the wilting resulted from a disease as examination of where the plants were standing did not give any indication that there had been less water available to the wilted plants. It is possible that the overhead irrigation had washed spores out of originally infested pots into the matting from where neighbouring plants took it up, or there had been splash dispersal between the plants.

Figure 18: Choisya before starting destructive assessment on 5 May 2017 showing abundant foliar growth in most plots, but dying pots more common in the lines starting with plots 1 and 2 in the foreground on the right hand side to plots 25 and 26 at the rear.

No phytotoxicity was observed on any of the nine visits to the crop, nor any dried spray deposits following product applications.

Isolations for disease identification

On 22 August 2016, a wilting plant from the plants left surplus to the trial (and so uninoculated) was taken away for laboratory assessment. Isolation from the roots consistently produced a white floccose aerial mycelium with a yellowy underside to the colony producing the ovate spores. These were probably *Fusarium* sp. microconidia. Macroconidia (septate elongate curved spores) were not being produced, and as these are used to help to determine the *Fusarium* species no further identification was possible.

A severely wilting plant was taken out of the experiment in October 2017 from each of plots 1, 8 and 9 (different treatments) and isolations onto potato dextrose agar were made from the yellowy-grey roots. The cause of wilting was not determined. No *Verticillium* spp. (a potential cause of wilt) was isolated (although this is a difficult pathogen to isolate). Some thick-walled resting spores (oospores) of an oomycete (such as *Pythium* or *Phytophthora* spp.) and resting spores (chlamydospores) which were probably produced by a yellow-pigmented *Fusarium* sp. were detected. No *T. basicola* was isolated, but this is not readily cultured onto agar and could have been present in roots other than those sampled, as dark roots more likely to contain this pathogen were not selected.

The dead plant sample from Plot 1 (Treatment 2 untreated uninoculated) had little root growth beyond the root ball of the liner and one potential cause of the wilting isolated from the roots was a *Fusarium* sp. that was a pinky-mushroom colour on the underside of the colony with white floccose aerial mycelium (ADAS isolate code BX17/94A, **Figure 19**). Microconidia were seen together with a few elongate macroconidia. Another *Fusarium* sp. was isolated from the same plant, but from blackened root tips, and it was recognised from the brown and cherry-red on the underside of the colony on agar with felty yellow/white aerial mycelium as a common root rot pathogen, (ADAS isolate code BX17/94B, **Figure 20**). Macroconidia were present. To be able to confirm the identity, the species of Fusarium the isolates were sent for molecular diagnostic testing to Warwick Crop Centre. The results were:

1. BX17/94A – *F. oxysporum* – identical EF1 sequence to *F. oxysporum* f. sp. *radicis lycopersici* (pinky-mushroom coloured underside to colony, white floccose aerial)
2. BX17/94B – *F. solani* (brown and cherry-red underside, felty yellow-white aerial)

F. oxysporum f. sp. *radicis lycopersici* causes wilt of tomatoes and other Solanaceae only. The molecular test utilised the diagnostics available for this common *formae speciales*, as an *F. oxysporum* comparator is unavailable for a Choisya host, but molecular sequences other than EF1 could be expected to be different for the actual *formae speciales* isolated as Choisya is a member of the Rutaceae. Dark brown vascular staining in the roots and stem base is a typical symptom of Fusarium wilt disease, however it was not possible to confirm this in the wilted Choisya plant sampled as the stem had dried out and the roots had rotted through.

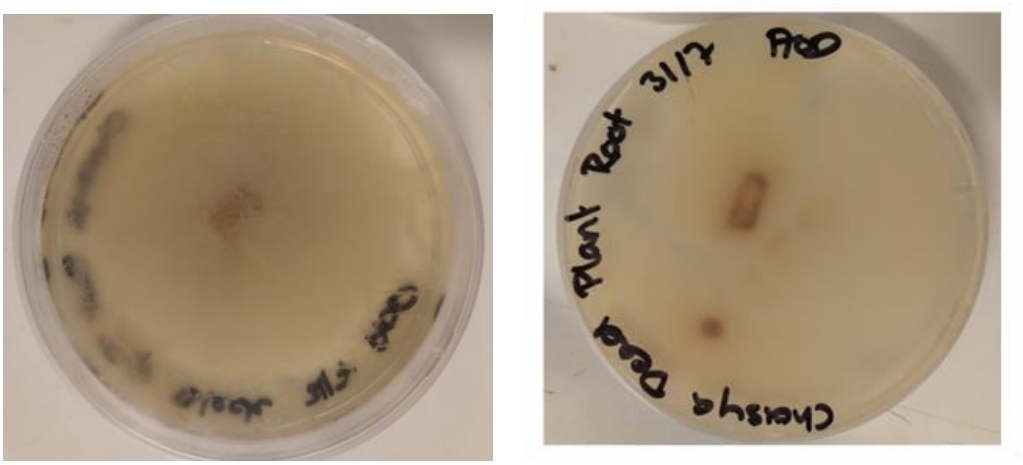


Figure 19. Sub-culture onto PDA of Isolate BX/94A from the roots of a dead Choisya showing aerial mycelium (left) and colony underside (right) of the pale coloured colony. Fusarium microconidia seen, and ***F. oxysporum*** identified by molecular test.

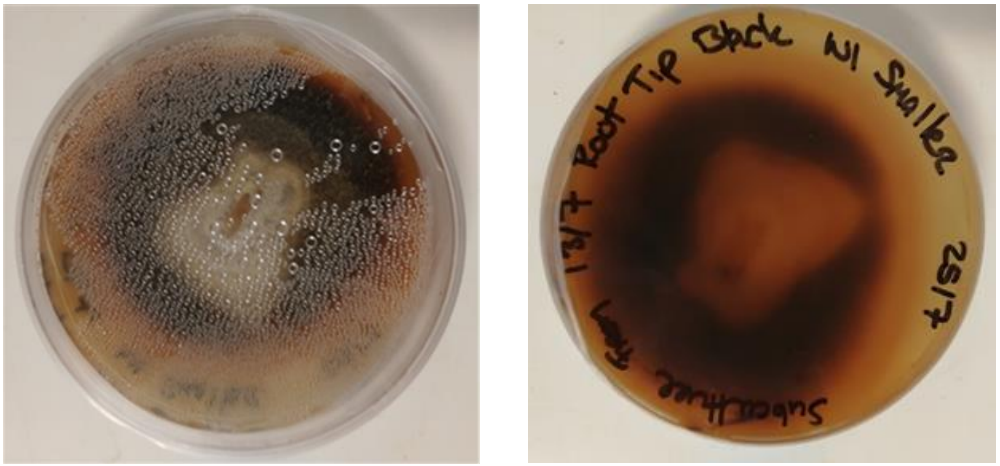


Figure 20. Sub-culture onto PDA of Isolate BX/94B from the blackened root tips of a dead Choisya showing aerial mycelium (left) and colony underside (right) of the brown coloured colony. Fusarium microconidia seen, and ***F. solani*** identified by molecular test.

Microscope examination of damaged roots

During destructive assessment in May 2017 five plants (from plots 1, 4, 10, 18 and 24) with various arrangements of root browning and one dead plant were collected for laboratory examination. Roots had a dark brown speckling along their lengths and these roots were also pale brown with other roots also pale brown but without the darker areas (**Figure 21**). This colouration contrasted with white roots also present and presumed to be healthy.

Figure 21: Choisya roots with dark brown elongate patches caused by pigmented chlamydospores inside outer cells of the root cortex. Root tip infection by *T. basicola* could have stunted the growth of the lateral roots resulting in the stubby appearance.

Under high power magnification, chains of dark brown *T. basicola* chlamydospores were seen to be present inside individual cells in the root epidermis; the outermost cortical cells. The darker the speckling visible to the naked eye, then the denser the clusters of the spores, to the point where sometimes chlamydospores filled the epidermal root cells (**Figures 22a and 22b**). Some spore chains had broken apart into separate chlamydospores. In some roots the development of lateral roots appeared to have been reduced, resulting in darkened warty/stubby roots that were totally crammed with chlamydospores (**Figure 21**). Where there was pale brown discolouration of the roots, chlamydospores were present, but they were only lightly pigmented and usually had not fragmented from the chain, probably being less mature than the spores in the clusters. Lightly pigmented mycelium was also visible filling root cells. Conidiophores that produced endoconidia were seen projecting from the root surface and were colourless. It was noted that although the dark and paler brown roots contained chlamydospores these were mainly confined to the outer cell layer of the roots and the pathogen did not usually cause a localised rot (collapse or softening) of the roots (such as would be caused by infestation of Pythium or Fusarium) although when in high density there was some fracturing of the root surface.

The non-wilted plants sampled on 6 May 2017 were retained in the laboratory and sparsely watered. When re-examined on 6 July 2017 there was a much greater prevalence of dark brown speckling on the roots (confirmed to be *T. basicola* chlamydospores) where previously the roots had mainly been pale brown. Drying of the root ball may have been a trigger for the production of the resting spores in affected tissue. The foliage had still not yellowed or died.

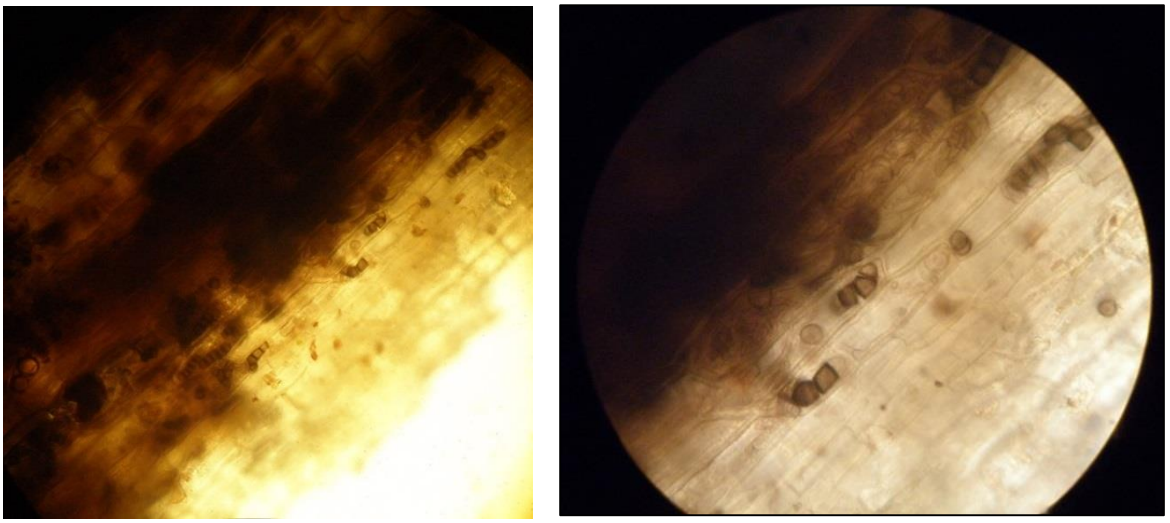


Figure 22 a & b: A side of a Choisya root at x100 and x400 magnification (9 May 2017). *T. basicola* mycelium growing inside the root epidermal cells and producing chains of drum-shaped chlamydospores with dome-shaped terminal spores. The darker areas result from denser spore clusters. Slightly pigmented mycelium fills the cells at the margin of the dark zone and colourless mycelial strands are in the tissue to the right side of the x400 view.

Root surface area

The roots were assessed over two days, the 4 and 5 May 2017. The proportion of the root ball face (sides and base) on removal of the pot comprised of roots rather than of growing media was recorded (**Figure 23**). When samples of root balls were broken open, the amount of rooting internally was confirmed to be reflected in the density of roots that were wrapped around the outside of the root ball.

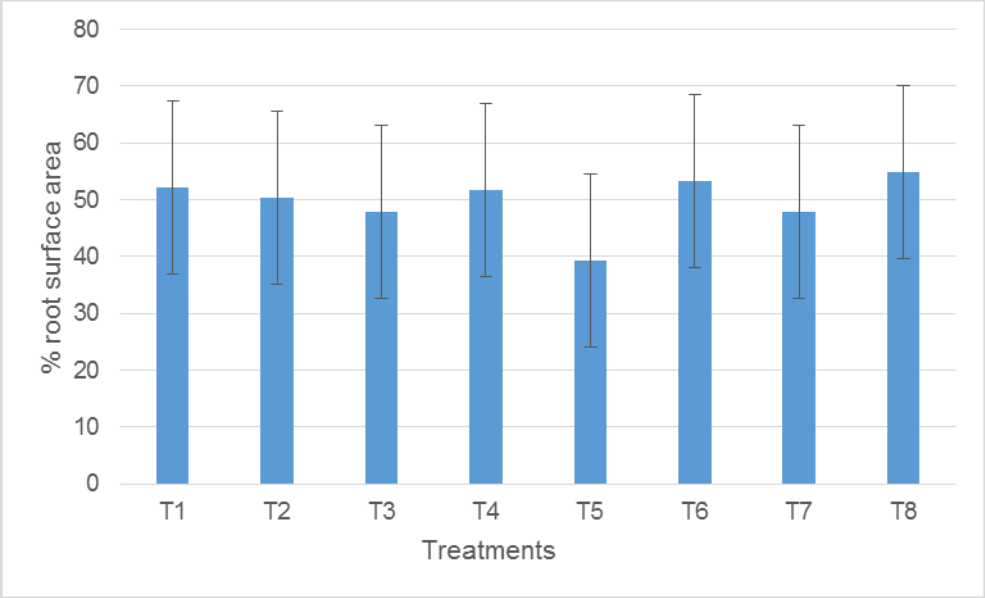
Root surface area tended to range between 40% and 60%, with plants at random across the treatments occasionally achieving 90% root cover. The minority of plants that had totally wilted had fewer roots, covering between 15% and 30% of the root ball. No differences were seen between treatments ($P>0.05$, 21 d.f.) in the percentage of the root ball face covered by roots, with a mean 49.7% coverage of roots around the outside of the root ball (**Figure 24**).

Figure 23: Root balls of two Choisya plants within the same plot on 5 May 2017. One plant had no foliar yellowing or wilting and 65% root cover over the root-ball surface of which 65% of the roots were healthy. The wilted plant had 15% root cover with 0% of the root area being healthy.

Infected roots

The proportion of apparently healthy white roots covering the surface area of the root ball was recorded together with that of two different damage symptoms (**Figure 25**). The majority of the root surface area in all treatments was a pale brown colour (**Table 15**). The remainder, a small proportion of the total root area, had dark brown speckles typical of black root rot. These speckled roots were often around the pot base. The different coloured roots were subsequently examined under magnification in the laboratory. The speckling was seen to be caused by dense clusters of brown *T. basicola* chlamydospore chains within the epidermal cells. Brown staining was seen to occur when these resting spores were either in ones or twos or had not matured and taken on their brown colouration. Non-pigmented mycelium was also only visible inside the cells under high power magnification. It was noted in all the root infections that the pathogen remained in the epidermis (outer cell layer) of the root and that it did not cause the root tissue to collapse. Root hairs growth was reduced by the damage to the root cortex. This was in contrast to infection that typically results from pathogens such as *Pythium* spp. where there is softening of the cortex of roots.

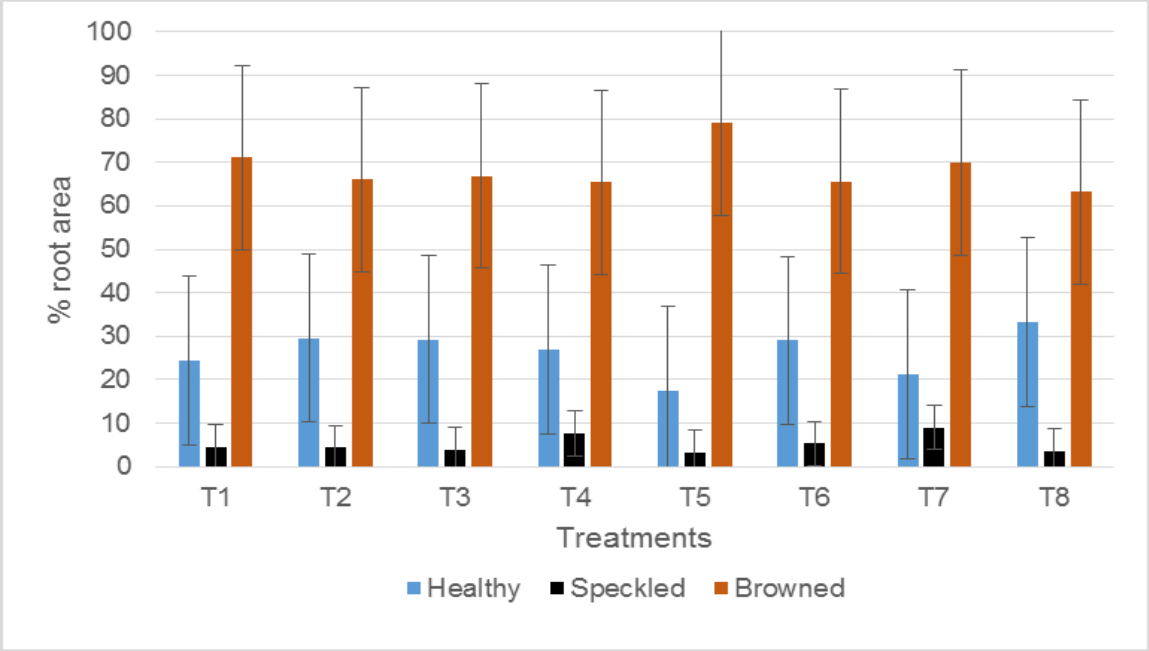
Some of the roots in some pots were brown and also dry and this root death was thought to have been because of desiccation due to the difficulty of watering pots through a dense foliage canopy, but these were not scored separately from roots that were browned and had not died.



T1 UT **T2 UTUn** **T3 C/--/--** **T4 B/P/S** **T5 T/P/S** **T6 S/P/--** **T7 P/P/S** **T8 F/P/F**

UT-Untreated, Un-Uninoculated, B-T34 Biocontrol, C-Cercobin, F-F174, P-Prestop, S-Serenade ASO, -- No application/s at second preventative or third (curative) timings.

Figure 24: Mean proportion of the root ball surface covered by roots in each of the eight treatments on 5 May 2017. No significant differences (F pr. 0.539) with overall mean 49.7% of pot root ball covered by roots, with a least significant Difference of 15.23.



T1 UT **T2 UTUn** **T3 C/--/--** **T4 B/P/S** **T5 T/P/S** **T6 S/P/--** **T7 P/P/S** **T8 F/P/F**

UT-Untreated, Un-Uninoculated, B-T34 Biocontrol, C-Cercobin, F-F174, P-Prestop, S-Serenade ASO **--** indicates application/s were not made at second (preventative) or third (curative) timings.

Figure 25: Mean proportion of the root surface area that was white/healthy, speckled dark brown or pale brown on 5 May 2017.

Table 15. Mean overall proportions of root area around the root ball that were white and healthy, speckled by pigmented *T. basicola* chlamydospores, or pale brown usually as a result of infestation by *T. basicola* mycelium and immature spores. There were no significant (F Pr > 0.05)

	Division of root ball surface area into healthy or darkened diseased tissue		
	Healthy	Speckled	Browned
Mean	26.4	5.22	68.40
L.s.d.	19.41	5.111	21.25
F pr.	0.755	0.261	0.840

Plants that had wilted totally had no healthy white roots. These roots had often died and dried out and were pale brown; it was not possible to determine whether these roots had been brown before they died. No brown speckled roots were seen on these plants at the final assessment, potentially because the roots had died before the chlamydospores had formed.

Isolations of browned roots onto potato dextrose agar produced at least two different *Fusarium* species. One isolate produced pale, pinky-grey, colonies (resembling *F. oxysporum*) with microconidia and this was confirmed by molecular diagnosis to be this species. The other isolate had macroconidia in a cherry-red and brown colony which is more typical of *Fusarium* species that attack stem bases and roots and this was confirmed by molecular diagnosis to be *F. solani.* There was one isolation of *Rhizoctonia* sp.. *T. basicola* was isolated from dark speckled roots in addition to the many direct observations of the chlamydospores within root epidermal cells. Verticillium was not confirmed in any of the isolations, although it is known to be difficult to isolate. Neither *Pythium* spp. nor *Phytophthora* spp. were isolated from roots in either the October 2016 or the May 2017 isolations.

Analysis of the data of the proportion of healthy, speckled or browned roots was carried out both with (**Figure 25**) and without the roots of plants that had wilted (only full data set presented). This was in case wilted plants were not a result of *T. basicola* infection. Separating results might highlight differences between non-wilted plants. There were no significant differences (P>0.05, 21 d.f.) in the % of the root surface that was white and apparently healthy, comprising a mean 26.4% of the roots (**Table 15**). If wilted plants were taken out of the data (resulting in a mean 29.6% of roots surface being healthy) then there were still no treatment differences. There was no difference in the area of healthy roots between the inoculated plants and the uninoculated untreated, however the untreated uninoculated had the greatest improvement in mean root area healthy going from 29.6% to 35.9% healthy roots once wilted plants were excluded from the analysis. There were also no significant treatment differences (P>0.05, 21 d.f.) in either the area of dark speckled (chlamydospore cluster) roots (mean 5.2%) or area of browned roots (mean 68.4%) (**Table**

15). When wilted plants were removed from the analysis there were still no differences between treatments. Chlamydospores of *T. basicola* were confirmed in all the treatments, including the uninoculated and it is presumed that this infection arose from the inoculum in the matting. “Natural” infection by *T. basicola* of the plant roots (as plugs, liners or finals) was possible because this pathogen was confirmed in the carrot slice baits returned to ADAS in February 2015 from the nursery site. There was potential for endospore movement from inoculated to uninoculated pots in run-off, but the plots had been separated by 0.4 m wide pathways to reduce the chance of this occurring.

Discussion

Plant vigour above-ground in *Choisya* sp. finals was not visibly affected by the root browning in plants that had not wilted. It is possible that in the wilted plants scattered throughout the treatments that the roots died after the foliage wilted, rather than the other way round i.e. root death resulted from what resembled a vascular wilt rather than as a result of black root rot. The plants that did not wilt were kept well fed and watered and it is possible that given greater stresses the plants would have been less able to cope with the fewer healthy 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 as a result of taking up spore-infested water from the matting following overhead irrigation, however, the floor of the glasshouse was quite uneven and no connection was able to be made between plant vigour and whether pots were stood in a slight hollow or slightly raised.

The plants (excluding those scattered throughout the trial that had wilted) were all marketable based on foliage vigour. There was no benefit shown from the treatments given (the untreated pots were also marketable). None of the treatments caused any phytotoxicity. Treatment

effects were found in the *Viola* sp. experiments earlier in this project, but not in the *Choisya* sp. It is possible that the longer period of growth in the *Choisya* sp. required further product applications as any surviving pathogen mycelium or resting spores would have time to build up again. 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 treatments 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 generally 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 was put forward by AHDB for an EAMU, but this is still under consideration. It is now unlikely that other HDC coded products tested earlier in the project will come into use. The chemical F176 was not supported for an EAMU. Unfortunately, the product F175 (found effective on *Viola* sp.) is also now not coming to the UK, and F178 although now registered in the UK, is not currently being marketed. This, therefore, leaves the biofungicides as potential new treatment options, and work in projects such as CP 158 (AMBER) will be looking to see if their performance can be enhanced by understanding more about their conditions for growth and host colonisation or attack.

Choisya wilt caused by *Fusarium oxysporum* had not been recorded in UK clinic samples up to 2008 (HNS 169, Talbot & Wedgwood, 2009), and it has not been possible to find any other record of sudden death in *Choisya* spp. in the literature. The main problems reported are a number of root rots (principally *Pythium* and *Phytophthora* spp., *T. basicola* and *Fusarium* spp. in containerised plants), usually expected to cause a slow decrease in water uptake capability as the root hairs and cortex are destroyed. Wilting and death was also seen in a few of the liners potted-on from bought-in plug plants in 2015, but the cause of the wilting was not identified at this time (although *Pythium* sp. as well as *T. basicola* was isolated). Some of the same batch of plug plants were potted as liners and kept without fungicide treatments to

pot up as finals for the current experiment. In May 2017 it was seen that some of the wilted finals had not grown roots since potting up. 15% of the plants in the experiment had died and if these are all attributable to the *F. oxysporum* then this is a cause for serious concern for Choisya growers. Further work is required to determine how frequently *F. oxysporum* is present in the roots of wilted Choisya plants; to conduct pathogenicity tests to determine if the isolates are pathogenic to Choisya; and to fully describe the symptoms of Choisya Fusarium wilt. It will be important to determine whether spread of spores from infested plants to healthy plants via run-off does occur. It is possible that some growers have previously attributed a sudden decline in vigour to black root rot taking hold in stress conditions, rather than to Fusarium wilt. In the current work, none of the wilting, dying plants had any of the dark brown root speckling confirmed to be *T. basicola* in surviving plants (only uniformly brown, dead often dry, roots). It is possible that plants were killed before *T. basicola* was able to produce its chlamydospores.

Experimental treatment of the Choisya finals in May, June and July 2016 would not have been expected to be curative (for any pathogen) for the biofungicides, and chemical treatment of tissue containing pathogens is rarely effective and particularly difficult if the pathogen has caused blocking of the vascular system. The product T34 Biocontrol was originally registered specifically for protectant use against Fusarium wilt of *Dianthus* sp.. There was thus potential for at least T34 Biocontrol to protect plants from any *Fusarium* sp. spores washing from infested to healthy pots via the ground-cover matting in 2016/17, but no differences were seen between treatments.

Conclusions

Product efficacy and crop safety

- There were no problems with foliar phytotoxicity from any of the experimental chemicals or biofungicides, alone or in programmes, on either *Viola* sp. or *Choisya* sp. after applications were made over the foliage. There was however additional root browning on *Viola* sp. after two applications of Signum (boscalid + pyraclostrobin).
- In both *Viola* sp. and *Choisya* sp., root infection by *T. basicola* was confirmed by microscopic examination of the roots and it was noted that a high infection severity was able to be tolerated by both hosts without loss of foliar vigour.
- In the 2014 sown *Viola* sp., three out of the four conventional chemical plant protection products tested (HDC F174, F175 and F176; not currently approved for use on ornamentals) reduced the severity of black root rot whether applied preventatively alone or followed by curative application. Control was equivalent to that given by the standard preventative drench of Cercobin WG (thiophanate-methyl).

- Use of the non-conventional chemical product HDC F178 applied both preventatively and curatively reduced black root rot severity in *Viola* sp..
- Two microbial products, Prestop (*Gliocladium catenulatum* applied preventatively and curatively) and compost incorporated Trianum-G (*Trichoderma harzianum* HDC F177) reduced the number of *Viola* sp. plugs showing black root rot to significantly fewer than when Cercobin WG had been applied.
- In subsequent treatment programmes with *Viola* sp., when re-tested under higher inoculum pressure to younger plants the chemicals HDC F174 and F175 did not reduce root rot severity when applied either preventatively or curatively following Cercobin WG application. However, they were effective when preceded by T34 Biocontrol (*Trichoderma asperellum*) at sowing.

Control of black root rot in Choisya

- In the 2015 *Choisya* sp. liner crop there were no significant differences in root rot between any of the treatments after six months despite inoculation with a high level of *T. basicola*. All plots 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 *Choisya* sp. liner 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.
- There were indications that the chemical fungicide F175 used a week before *T. basicola* inoculation increased the vigour of *Choisya* sp. liners' foliage slightly, and significantly so later on between August and October 2015. In 2014, this product had been effective in reducing root rot severity on *Viola* sp. if applied as a protectant preceded by T34 Biocontrol at sowing.
- Although by December 2015 only 30% of Choisya 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 to *Choisya* sp. liners of a thiophanate-methyl was ineffective against the high level of *T. basicola* when inoculation followed a week after treatment.
- In the 2016/2017 crop of *Choisya* sp. finals there were no significant differences in the foliar vigour or root health between the five test protectant and curative treatment

programmes, and no difference between these and either the standard protectant Cercobin drench or when the pots were left untreated.

- There was an indication that application of Serenade ASO directly after potting *Choisya* sp. finals resulted in new foliar growth within a fortnight of application across all plants, whereas in all other treatments some plants had not grown new leaves.
- There was consistently less yellowing a wilting in the *Choisya* sp. finals that received Serenade ASO at potting and Prestop five weeks later, when followed by *T. basicola* inoculation six weeks from potting.
- None of the product programme combinations to *Choisya* sp. finals caused any phytotoxicity.
- All treatments to *Choisya* sp. finals, including the uninoculated, had roots affected by black root rot and this shows that infestation from spore spread via the ground-cover matting is important.

Root and foliage symptoms

- Pale brown roots were shown to be the commonest symptom of black root rot (a mean 68% of the root surface area) in *Choisya* sp. finals, with the dark brown patches resulting from the formation of clusters of chlamydospore chains (mean 5% of the root surface area) seen to develop further after drought stress.
- Black root rot disease was not apparent from either foliage discolouration or lack of shoot vigour even when around three quarters of the root area of *Choisya* sp. finals were browned.
- *T. basicola* infection did not cause roots of *Choisya* sp. finals to rot, but infection of the tips stunted root growth and loss of root hairs through damage to the outer cortex.
- All treatments had pots of Choisya finals with foliage that wilted in summer 2016 and then mainly died. *F. oxysporum* (a vascular wilt pathogen), although not known previously to affect *Choisya* sp., was isolated from roots and identified by molecular assay and believed to be responsible for the wilt. No treatment programme affected wilt incidence or severity.

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:

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. Plant extracts with fungicidal activity can also be termed biofungicides although there are no living beneficial organisms present.

References

Including useful publications on black root rot not referenced in the text.

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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. Two baiting methods using carrot slices were used in this project:

- For both methods a fresh carrot root with no external blemishes (such as *Pythium violae* cavity spot) should be bleached (approximately 1 part bleach to 9 parts water) for a minute to get rid of bacteria.
- After sterilising, 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.

Sampling 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, so flatten down the soil sample to give a wide surface area (minimum 30 mm deep).
- 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

- Carrot slices are effective if 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.
- Place the carrot slices or bait bags directly on the soil or other surface to be tested. Baits can 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*

Ingredients	**Quantity per litre 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*

- Blend 200 g of washed fresh carrots in 500 ml of tap water.
- Filter through muslin, squeezing the carrot mash to extract as much juice as possible.
- Make the juice up to one litre with tap water. Add 7.5g of Technical Agar No.3 to 500 ml of diluted juice.
- 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.

