Project title:	Evaluation of fungicides and novel treatments for the control of black root rot, *Thielaviopsis basicola*, in bedding and hardy nursery stock plants
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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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GROWER SUMMARY

Headline

Root growth reduction and damage by *Thielaviopsis basicola* can be reduced, not stopped, by certain preventative fungicide programmes. Nursery hygiene to remove inoculum should be the primary control measure. Roots of susceptible host plants should be inspected regularly, as foliar vigour decline may only occur when the black root rot disease is well advanced.

Background

Symptoms and prevalence

Black root rot (*Thielaviopsis basicola*, syn. *Chalara elegans*) causes root damage (Figure 1) leading to reduced nutrient and water uptake, consequent leaf yellowing and potentially plant loss. Losses in pansy and viola production can be substantial and black root rot is frequently implicated in losses of susceptible hardy nursery stock species such as *Choisya*, *Skimmia* and *Ilex*. A massive peak of plant loss can occur in summer as temperatures rise and plants are put under stress. Early crops can also succumb when it is cold. Losses are greatest in propagation and at the liner stage. Growers producing container ornamental crops under glass can give a single preventative drench of Cercobin WG (thiophanate-methyl), while for those grown under polythene, Scotts Octave (prochloraz) is permitted for use to achieve disease control chemically

Figure 1. *Viola* sp. roots infected by *T. basicola* (left hand plant) causing reduced root growth, tissue browning and root hair loss (Experiment 3, September 2014).

Overview of the current project

In the review PO 14 carried out prior to this project, it was determined that there were active ingredients currently in use on other crops, or being used pre-registration as a plant protection product that might give effective control of black root rot (Wedgwood, 2013), which given approval, 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. The current project seeks to identify novel treatments, including non-conventional elicitors and microbial products, and to test their efficacy as preventative and curative drenches against black root rot. Initially, the species *Viola* and *Choisya* were used as example crops in which to test products individually, and then evaluate them in a range of programmes giving consideration to fungicide resistance management. In 2015, products that were found in 2014 to be safe and effective on *Viola* species (sp.) at various timings were tested on *Choisya* sp.

Summary

Three inoculated glasshouse experiments were carried out on *Viola* sp. in 2014, testing the protectant and curative activity of plant protection products against *Thielaviopsis basicola*. In each case *Viola cornuta* cv. Sorbet XP White Jump Up were grown in 24-cell module trays in glasshouse compartments at ADAS Boxworth. Some plant protection products were applied at sowing, but the majority were applied either a week before or after inoculation, or at both times. Inoculation was carried out by drenching the peat-based growing medium with a spore suspension of *T. basicola* four weeks after sowing. Experiment 1 and 2 were reported on in 2014. This report details the work carried out on Experiment 3, which used information gained in the earlier two experiments to determine the products tested. Experiment 4 started in May 2015 with *Choisya ternata* plugs potted into 90 mm pots set out within a commercial crop on a nursery where black root rot has previously occurred. This trial will not be completed until November 2015 and so it will be reported in full in the next report.

Experiment 3 – Simple programmes on *Viola* spp.

Four of the products which reduced black root rot in *Viola* spp. in the earlier experiments were selected for use in this experiment investigating two-product programmes. Ten programmes were tested. Treatment timings were at sowing, and three and five weeks later: treatments consisted of either one or two applications of test products (Table 1). The products selected for use on *Viola* sp. in comparison with Cercobin WG as a standard were:

- T34 Biocontrol (microbial, EAMU 1118 of 2012 as drench on ornamentals)
- F174 (conventional, registered for spray application on sugar beet)
- F175 (conventional, for spray application but not yet registered on any crop)
- F178 (non-conventional, a stimulant now registered for use on chrysanthemums)

Table 1. Experiment 3 – Programme of one or two products applied at different timings for control of black root rot. All except T1 were inoculated with *T. basicola* four weeks after sowing

	Treatment programme											
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 5				F174	F175							

There were two untreated controls: one left uninoculated (T1), the other (T2) inoculated with *T. basicola* at the same time as all the treated plots, four weeks after sowing. The number of spores used was was double that of the earlier experiments to seek to obtain a greater severity of black root rot that might then cause some foliar symptoms (not seen previously).

Cercobin WG alone (T3) was applied preventatively three weeks after sowing, and in two other programmes (T4 and T5) this was followed by a curative application of coded products a week after inoculation. All three coded products were tested preventatively, either as the only treatments (in T6, T7 and T8) or preceded by T34 as a drench to the trays straight after sowing (in T10, 11 and T12). T34 Biocontrol was also used only at sowing (in T9) (Table 1).

No difference in foliar vigour was seen between any of the plots (Figure 2), nor any phytotoxicity. Roots were assessed 10 weeks after sowing. In the uninoculated untreated plots (T1) the roots covered a mean 74% of the plug surface; in the inoculated untreated plants (T2) this was reduced to 42%. T10 (T34 Biocontrol folllowed by F174) was the only treatment with increased percentage root cover compared with untreated inoculated plants. It was noted that, although not significantly lower than the untreated inoculated, plants which received F174 application a week before inoculation (T6) had the smallest root area – only 39% (Figure 2). T10 also resulted in the least area of rotted root, reducing it from 67% to 39%.

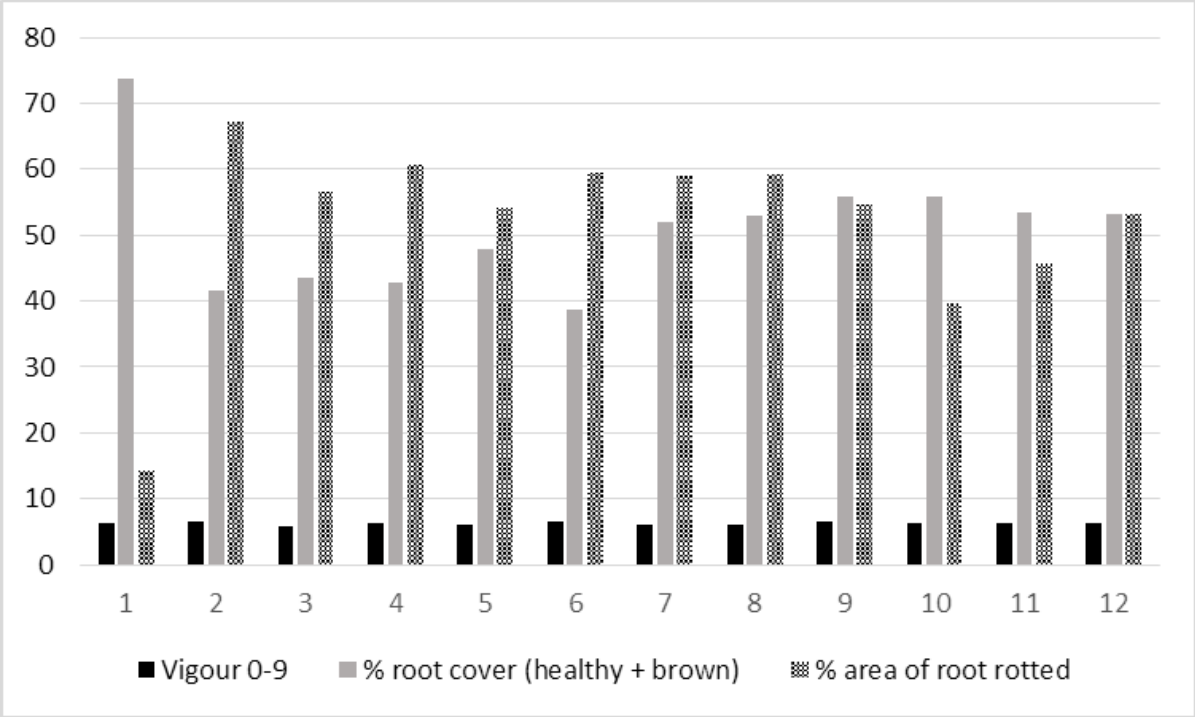

Figure 2. Effect of plant protection product programmes on plant vigour, root extent and root rot of *Viola* sp. in Experiment 3. Treatments assessed on 25 September 2014, 10 weeks after sowing, for above-ground vigour (0 = dead to 9 = very good – no significant differences), the coverage of the surface of the plug by roots (P=0.034, LSD 17.717), and the proportion of these roots that were browned (P<0.001, LSD 16.549).

Some pale brown roots were seen in all treatments, (including uninoculated), however microscope examination and isolation onto agar of uninoculated damaged roots showed no evidence of any root rot pathogens and it was likely that the 14% of the root ball surface affected was probably from desiccation; plants were checked for *Pythium* root rot and none was found. Evidence of *T. basicola* was confirmed in samples of rotted roots from inoculated plants by high-power microscope examination of root squashes, with mycelium and both endospore and chlamydospore production being visible inside roots.

On average, approximately half of the root area was discoloured for most of the inoculated treatments including those with Cercobin WG (T3, T4 and T5), significantly more than the 14% in the uninoculated. This was largely due to black root rot. Only two of the ten treatments reduced black root rot compared with the 67% incidence of it in the untreated inoculated treatments (Figure 2). These were the preventative treatments F174 (T10) with 40% rot, and F175 (T11) with 46% rot; both received T34 Biocontrol at sowing. Use of any of these three product alone (in T6, T7 and T9, respectively) did not reduce root rotting.

Experiment 4 – Simple programmes on a Choisya sp.

The experiment was set up in a commercial glasshouse with 640 *Choisya ternata* plug plants potted up on 29 April 2015. Four biofungicides available for use on ornamentals and a non-conventional coded product were applied the next day as five treatments. Six weeks later, when new roots had established, these and eight other products, all conventional chemicals, were applied over the foliage and peat surface. After a further week, plants were inoculated on 11 June 2015 using an isolate of *T. basicola* from *C. temata*. Three chemicals with potential curative activity were applied to some plots a week after inoculation. The plants are under observation to record phytotoxicity, vigour and disease, with root assessment due in November 2015. Results will be presented in the next Annual report.

Experiment 5 - Alternating programmes on *Choisya* spp.
This experiment will commence in spring 2016 using a fresh batch of *Choisya* spp. liners that will be potted into their final pot size. Products will be selected from the current experiment and six programmes developed with product alternation to reduce the risk of the pathogen developing resistance.

Financial Benefits

Effective treatments will improve crop quality by maintaining a healthy root system, improving crop establishment and reducing crop losses. Providing a range of products that can be applied at intervals during production to maintain a healthy root system will be particularly important for hardy nursery stock where plants are sold in a range of pot sizes.

Grower utilisation of the limited number of products already available for use in these crops has been shown to be effective against black root rot, and any that can be made available through the EAMU approval system will ensure different modes of action are employed, therefore reducing the chance of fungicide resistance developing. Products with activity already known against other root pathogens such as *Pythium* spp. will provide wider protection at no additional cost.

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

ADAS crop consultants have estimated that up to a quarter of some crops on UK nurseries can be affected and that a fifth of crops may not be sold either as the quality loss is too great or the infection has been severe enough to cause plant death.

Action Points

- The range of effective conventional plant protection products permitted as drench treatments to ornamentals is very limited. Consequently there is increased need for close attention to be paid to nursery hygiene (disinfection) and crop husbandry (avoiding plant stress) to avoid introducing and encouraging black root rot in crops.
- Examine sickly plants for black root rot infestation early, do not wait to see reduced vigour. Treat or destroy promptly, if necessary, otherwise endoconidia (dispersal spores of black root rot) can infect other plants and produce further sporulation within weeks.
- Prestop and Trianum G are permitted as drench treatments to ornamentals and significantly reduced black root rot in the inoculated treatment in the original trial; there was also evidence of some effect from T34 Biocontrol applied at sowing. Consider preventative use of these microbial products as they can produce a root-zone environment which has increased resistance to infection from a number of pathogens and potentially a systemic, protective benefit for the whole plant.
- AHDB Horticulture is seeking EAMUS for the use of some of the products within this project. As and when new products become available, select a range of chemical plant protection products with different modes of action to avoid the build-up of resistance to active ingredients. Be prepared to test the crop safety and efficacy of products with EAMUs before inclusion in nursery-wide programmes.

SCIENCE SECTION

Introduction

This report details the third experiment, on *Viola* spp. in 2014. It also mentions the fourth experiment, being carried out on a *Choisya* sp. in 2015, to determine the efficacy of novel plant protection products against *Thielaviopsis basicola*, the cause of black root rot. Black root rot infection by *T. basicola* leads to loss of plant vigour through root rot, wilt and eventually plant death. Infection typically becomes visible on the roots as dark brown flecks caused by the presence of the resting spores (chlamydospores) of the fungus which develop at infection points in the outer cells 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. Bedding plants such as *Viola* spp. can decline rapidly, and hardy nursery stock such as a *Choisya* spp. affected at potting-on can fail to establish.

Figure 3. Natural infection of *Viola* sp. by *T. basicola* showing the "black" areas of roots caused by chains of resting spores forming inside

Existing control measures

i) *Cultural control*

Growers employ measures such as reducing plant stress and being careful with crop hygiene to reduce the chance of plants becoming infected. Once some plants become infected then abundant dispersal spores (endoconidia) are released from infected roots into the growing-media and can be flushed out by irrigation to infect neighbouring plants. The pathogen also produces resting spores (chlamydospores) in roots which then survive in debris in matting, in

re-used containers or in soil, and can be resistant to disinfectant treatments (as noted in Factsheet 03/14).

ii) *Biological control*

Products with potential against black root rot were reviewed in HDC project PO 14 and in the first annual report of the current project. The biofungicides that can be used in the UK against root rots are: 1) Prestop (*Gliocladium catenulatum* strain J1446) for protected ornamentals; 2) Serenade ASO (*Bacillus subtilis* strain QST 713) under the EAMU 0706 from 2013 or previously 0708 from 2013 for protected and outdoor ornamentals; 3) T34 Biocontrol (*Trichoderma asperellum* strain T34) under the EAMU 1118 for 2012 for protected or outdoor containerised ornamentals; and 4) Trianum-G (*Trichoderma harzianum* strain Rifai T-22) now Authorised under 2769 from 2014 as a plant protection product for use on protected ornamentals.

Application methods and frequency of any re-application differ between products, with use at sowing or potting-on stages by incorporation into or drenching of the growing media being a common recommendation. Growers using them still find it necessary to use chemical pesticides against black root rot. Only Prestop is reported in the product technical notes to have some activity against *T. basicola*. Some significant reduction was achieved in Experiment 2 of this project after preventative plus curative application to *Viola* spp. inoculated with black root rot. This experiment also showed some benefit from incorporation of Trianum G into the growing media (several plots having low root rot), but no benefit from Serenade ASO. In projects, such as CP 077, several biofungicides have shown some reduction in pathogens at low levels (though not consistently), but there are practical considerations in their use (some are given in Factsheet 18/14), and more work needs to be done on how growers can get the best from biofungicides and integrate them into their management programmes.

iii) *Chemical control*

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

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

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

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

Experiment 3 took forward products from Experiments 1 and 2 that had reduced root browning, had a likelihood of full approval or an EAMU on ornamentals, and could be placed in alternating programmes with different modes of action. It was not possible to investigate all potential treatment programme combinations with all potential products and so four products were finally selected for testing, together with Cercobin WG. The plant protection products selected for use on *Viola* spp. in Experiment 3 were T34 Biocontrol, F174, F175 and F178.

All three experiments in 2014 were programmed to run during the summer months when plant stress was likely to increase losses to black root rot. *T. basicola* tends to be an opportunistic pathogen, taking advantage of susceptible plants under stressful growing conditions (Leahy, 1998). The fungus is capable of prolonged saprophytic survival in soils. Soil at 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).

The objective of the work reported here was to determine the efficacy of simple programmes, based on products previously found to be effective in controlling black root rot in *Viola* spp. A similar experiment to Experiments 1 and 2 was established on a *Choisya* sp. (Experiment 4) with both conventional and non-conventional plant protection products. This will be reported on in the next Annual report.

Materials and methods

Experiment 3

Experiment 3 used the same sized multicell (module) trays as in experiments 1 and 2. The trays are 30 mm x 30 mm wide, and have 39 mm deep cells filled with low nutrient peat-based growing media for modules. Seeds of *Viola cornuta* cv. Sorbet XP White Jump Up (Ball Colegrave Ltd) were sown (24 cells per plot) on 17 July 2014 and covered in polythene until germinated before being transferred to the glasshouse compartment at ADAS Boxworth (Figure 4). The plants were then grown on until 10 weeks after sowing, stood on individual pieces of capillary matting for each plot within slatted trays to raise them off the ground. The experiment received natural summer daylight, and fan ventilation set at 25°C. A digital temperature and humidity data logger recording at 30 minute intervals was placed within the foliage. Overhead watering was carried out by hand as required, using a hose with a rose, with liquid feed (Miracle Grow at 15 ml in 4.5 L water) given weekly after four weeks using a watering can.

Figure 4. Experiment 3. *Viola* sp. seedlings in module trays, showing one 24-cell tray per plot, with 12 trays per replicate block, on 7 August 2015 three weeks after sowing

All test products were applied at the same rates as in Experiments 1 and 2 (Table 2) using a calibrated automatic moving-boom pot sprayer in a polytunnel at ADAS Boxworth over the top of the plants in the module trays. All replicates of the same product treatments were treated in the same spray pass. The trays were then returned to their randomised positions in the glasshouse (Figure 5). Label instructions on dose and water volume, or supplier advice were followed. Products registered for spray application at a water volume of 400 L/ha were then sprayed with water (to mimic irrigation) to give the total equivalent of 1000 L/ha. The volumes used for all products gave penetration into the growing media to reach the roots without any running-out from the tray drainage holes.

Table 2. Products used on *Viola* sp. Experiment 3 and the programmes in which they were used, with application timings. Dose and water rates of approved products.

Programme	Product	Active ingredient	Treatment timing*	Dose & water volume
T1	Untreated, not inoculated	none	0	water alone (1000 L/ha)
T2	Untreated Inoculated	none	0	water alone (1000 L/ha)
T9, T10, T11, T12	T34	*Trichoderma asperellum*, strain T34	PA	0.5 g T34 in 1 L water / m² of growing media straight after sowing
T3, T4, T5	Cercobin WG	thiophanate-methyl	PB	1.1 g per 10 L water, using 1000 L diluted product / ha
T6, T10	F174	confidential	PB	-
T7, T11	F175	confidential	PB	-
T8, T12	F178	confidential	PB	-
T4	F174	confidential	CC	-
T5	F175	confidential	CC	-

* Treatment timings key:
PA = protectant at sowing, timing A, PB = protectant pre-inoculation, timing B
CC = curative post-inoculation, timing C

Plot No.	Treatment	Plot No.	Treatment	Plot No.	Treatment	Plot No.	Treatment	Plot No.	Treatment	Plot No.	Treatment	Plot No.	Treatment	Plot No.	Treatment
1	T12	7	T2	13	T11	19	T5	25	T9	31	T7	37	T1	43	T10
2	T11	8	T5	14	T2	20	T1	26	T2	32	T12	38	T7	44	T12
3	T3	9	T6	15	T9	21	T10	27	T5	33	T8	39	T3	45	T6
4	T1	10	T4	16	T4	22	T12	28	T3	34	T10	40	T11	46	T8
5	T9	11	T7	17	T3	23	T8	29	T6	35	T11	41	T4	47	T2
6	T10	12	T8	18	T6	24	T7	30	T1	36	T4	42	T5	48	T9

Figure 5. Experiment 3 showing arrangement of *Viola* sp. module trays in the glasshouse at ADAS Boxworth, with four replicate blocks of the twelve treatment programmes.

There were three possible treatment timings; at sowing (Timing A), at 1-2 true leaf (Timing B) and a curative treatment one week after inoculation (Timing C). Treatments were not given to all plots at each timing (Table 3). The standard programme used for comparison was a single drench of thiophanate-methyl at Timing B. Seedlings ranged from cotyledon stage to 2-leaf at Timing B sprays on 7 August 2014 as germination had not been very even. Four test products were selected which had shown efficacy in the earlier experiments; these were T34 Biocontrol (a microbial fungicide for preventative application), two conventional chemical fungicides (F174 and F175) and a plant stimulant (F178). There were 12 treatments arranged in four randomised blocks, including untreated *Viola* sp. with and without black root rot inoculum (Figure 5). Whether preventative and/or curative application was carried out was based on product efficacy at these timings in the earlier experiments.

Table 3. Experiment 3. *Viola* sp. treatment programmes, with details of timings in 2014

Treatment	Treatment Timing A directly after sowing	Treatment Timing B Plants at 1-2 leaf (3 wks after sowing)	Black root rot INOCULATION 1 wk after Timing B (4 wks after sowing)	Treatment Timing C 2 wks after Timing B (5 wks after sowing)
	17 July	**7 August**	**14 August**	**21 August**
T1 not inoculated UT	No treatment	No treatment*	NO spore drench	No treatment*
T2 UT	No treatment	No treatment*	Spore drench	No treatment*
T3	No treatment	Cercobin WG	Spore drench	No treatment*
T4	No treatment	Cercobin WG	Spore drench	F174
T5	No treatment	Cercobin WG	Spore drench	F175
T6	No treatment	F174	Spore drench	No treatment*
T7	No treatment	F175	Spore drench	No treatment*
T8	No treatment	F178	Spore drench	No treatment*
T9	T34	No treatment*	Spore drench	No treatment*
T10	T34	F174	Spore drench	No treatment*
T11	T34	F175	Spore drench	No treatment*
T12	T34	F178	Spore drench	No treatment*

* where no product was to be applied to the growing plants a tap water spray drench equivalent to 1000 L/ha was given over the foliage

Inoculation with *T. basicola* was carried out in all trays except T1 four weeks after sowing. The same isolate as in the earlier experiments was used (isolate BX 13/114 obtained from a carrot root). This was cultured on potato dextrose agar and the spores removed into a suspension to give a final concentration of approximately 5000 chlamydospore chains per ml (plus an unquantified number of endoconidia). 2 ml of the spore suspension was dispensed by syringe across the surface of the moist growing media of each 22 ml plant cell (approximately 10% of cell volume) to be inoculated. The volume of inoculum and thus the number of chlamydospores added per cell was therefore doubled from that used in Experiments 1 and 2, as the plants in these had not shown any reduction in vigour and Experiment 2 had achieved only 18% browning of the root plug surface. Inoculation was done on 14 August 2014, one week after the application of preventative treatments to all except T9, and curative treatments were applied in T4 and T5 a week later.

Assessment of the foliage for any phytotoxicity such as scorching or stunting was made throughout the experiment. Vigour was assessed per plant on a 0-9 index of ‘dead’ to ‘very good’ based on factors including leaf size and colour, plant height and branching, and flower production. As the initial above-ground symptoms of black root rot can appear as poor vigour

(in particular slower growth and purplish or yellow discoloured leaves) no separate score was done for black root rot above ground. However, a separate assessment would have been carried out had definitive symptoms been seen when comparing the inoculated untreated with the uninoculated untreated plots.

The final assessment was carried out 10 weeks and five days after sowing commencing on 24 September 2015. This is beyond the time at the start of flowering (at around eight weeks) that the plants would commercially leave the nursery. This timing was to allow the development of black root rot symptoms and followed the procedures used in the earlier experiments. At the final assessment, 15 plants from plot were assessed for foliage and root vigour and disease. Any phytotoxicity was also noted. The plants were removed from the trays and the total percentage of the plug root ball surface covered by roots assessed as a measure of root vigour. The percentage of the root area made up of brown rotted roots was recorded separately. Statistical analysis of the results was carried out using GENSTAT to perform Analysis of Variance, without any transformations.

Samples of brown roots were taken for isolation to confirm that rotting was not being caused by a pathogen other than *Thielaviopsis* (such as *Pythium* spp.). Two plants were sampled from plots in Replicate 2, taking three browned roots from each. Isolation was made onto potato dextrose agar with Streptomycin antibiotic added to reduce secondary bacterial growth (PDA +) with and without surface sterilisation of the roots in ethanol for 10 seconds.
The roots from an untreated and a treated inoculated plant were examined under a high power microscope after being stained with cotton blue. The roots were squashed to be able to see through the cells so that mycelium and any spore producing bodies could be seen and photographed.

Experiment 4

Experiment 4 was set up in spring 2015 with *Choisya ternata* plugs to investigate the efficacy and crop safety of individual products on these plants, as a test host for other nursery stock. Four replicate blocks of ten pots per plot were set up on 30th April 2015 in the glasshouse of a commercial grower stood in carry-trays on woven ground cover with programmed overhead irrigation (Figure 6). This nursery has had problems with black root rot on nursery stock and a *Choisya* sp. cv. White Dazzler plant obtained from this grower in February 2015 was used to obtain an isolate of *T. basicola* for use as inoculum in 2015 (instead of the isolate from carrot used in 2014).

Products selected from the earlier experiments on *Viola* sp. were tested with one or two applications per product depending on their potential activity, timed at three stages in the crop depending on their potential activity (Table 4). Thirteen treatment programmes and three untreated were set up (one uninoculated), which will be detailed in the next Annual Report. Four biological products and a coded non-conventional chemical product were applied straight after potting. Cercobin WG was again used as the standard product with a single preventative application of this and also of four conventional chemical fungicides once roots had established, one week before the plants were artificially inoculated by a *T. basicola* spore suspension drench six weeks after potting. Three of the conventional chemical fungicides were coded products and these were also applied curatively a week after inoculation.

Table 4. Experiment 4 application timings used on *Choisya* sp.

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

P – preventative; C – curative

Figure 6. Experiment 4. 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

Results

Experiment 3 – Viola sp.

Staggered emergence of the seedlings occurred so that some had only just produced true leaves when they were treated a week before inoculation (Figure 7). This would have meant that in some cases, root growth was less advanced when the plants were inoculated a week later, compared with the root establishment in the earlier experiments. Extra cells had been sown in case of low emergence and the required 15 plants grew in all the plots.

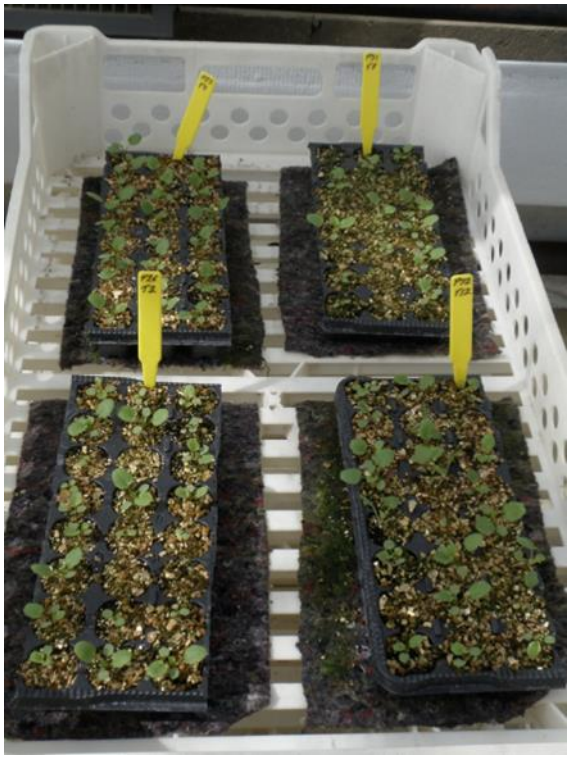

Figure 7. Experiment 3. Four plots of *Viola* sp. seedlings in the glasshouse at ADAS Boxworth on 7th August three weeks after sowing, with treatments applied a week before the seedlings were inoculated.

Phytotoxicity and vigour

As in earlier experiments, no phytotoxicity was seen following application of any of the treatments, nor any vigour differences such as leaf size or flower production (only final assessment data presented). The results for vigour at 10 weeks are shown in Table 5; the average vigour index of 6 (acceptable) reflecting the fact that the plants were beyond the stage at which they would commercially have been transplanted into a new growing position (Figure 8). By this time, a number of the Cercobin WG treated plants (T3) with more root rot than others were losing vigour, although on average, they were not statistically significantly poorer.

Photographs of five randomly selected plants from each plot of replicate 2 to show typical root and shoot growth ten weeks from sowing at the final assessment, are given in Appendix 1. The experiment was set up in July to obtain the heat stress that is known to generally precede

severe attacks of black root rot. Maximum temperatures were often above 35°C into mid-August, and then fluctuated between 25°C and 30°C into late September 2014 when the experiment was terminated (Appendix 2).

Table 5. Destructive assessment of *Viola* sp. in Experiment 3 starting on 25th September 2015 for each treatment programme showing plant vigour, total root coverage around growing-media plug and the proportion of the area of the root surface that was rotted.
* All except T1 inoculated with *T. basicola* four weeks after sowing.

Pro-gramme	Wk 0	Wk 3	Wk 5	Vigour index (0-9)	% Root cover of plug	% Area of roots rotted
T1*	-	-	-	6.2	73.6	14.4
T2	-	-	-	6.5	41.6	67.2
T3	-	Cercobin	-	5.9	43.5	56.5
T4	-	Cercobin	F174	6.2	42.8	60.8
T5	-	Cercobin	F175	6.1	47.8	54.1
T6	-	F174	-	6.5	38.7	59.4
T7	-	F175	-	6.1	52.0	59.1
T8	-	F178	-	6.1	53.0	59.2
T9	T34	-	-	6.5	55.9	54.6
T10	T34	F174	-	6.4	56.0	39.7
T11	T34	F175	-	6.2	53.4	45.8
T12	T34	F178	-	6.2	53.3	53.2
			Mean	6.25	50.95	51.98
			L.s.d.	0.633	17.72	16.55
			D.f.	33	33	33
			F. Pr	0.568 (no sig. diff.)	0.034	<0.001

Figure 8. Trays of *Viola* sp. replaced in trays after assessments of root growth and rot on the plug root balls on 25th September 2015. T1 uninoculated (plot 30 with a central white label) at bottom left of picture.
Picture shows no differences in plant vigour between treatments.

Root growth

Reduced root growth measured by % root cover of the plug was shown. The amount of root growth around the plug principally showed whether root development had been stopped at an earlier growth stage by damage to the root tip growing points. Reduced growth could be from disease or phytotoxicity. It was unlikely that roots affected by black root rot would have been lost through disintegration in the relatively short period of the experiment. The uninoculated plants had roots which had grown to cover 74% of the plug surface, whereas the untreated inoculated plants had significantly less root area (P = 0.034) with only 42% coverage (Table 5 and Figure 9). All of the treated inoculated plants, including the standard preventative treatment of Cercobin WG, had significantly less root area (except T10 by a slight margin), with less than 55.9% of the surface area of plugs covered by roots, compared with the untreated uninoculated (Figure 10). T10 had received T34 followed by preventative use of F174. The reduced root growth in most of the inoculated treatments was probably as a result of *Thielaviopsis* rather than any phytotoxicity to the root area as it was similar to the untreated inoculated in most of the treatments. Root growth was only marginally greater in programmes without the standard Cercobin WG. Treatment T6 with F174 preventative use had the lowest (though not significantly) root coverage of 38.7%.

Figure 9. Roots of T2 (inoculated with *T. basicola* and untreated) showing reduced root coverage of growing-media plug and pale brown root roots with sparse root hairs in contrast to the white roots growing in T1 (not inoculated untreated). 10 weeks after sowing.

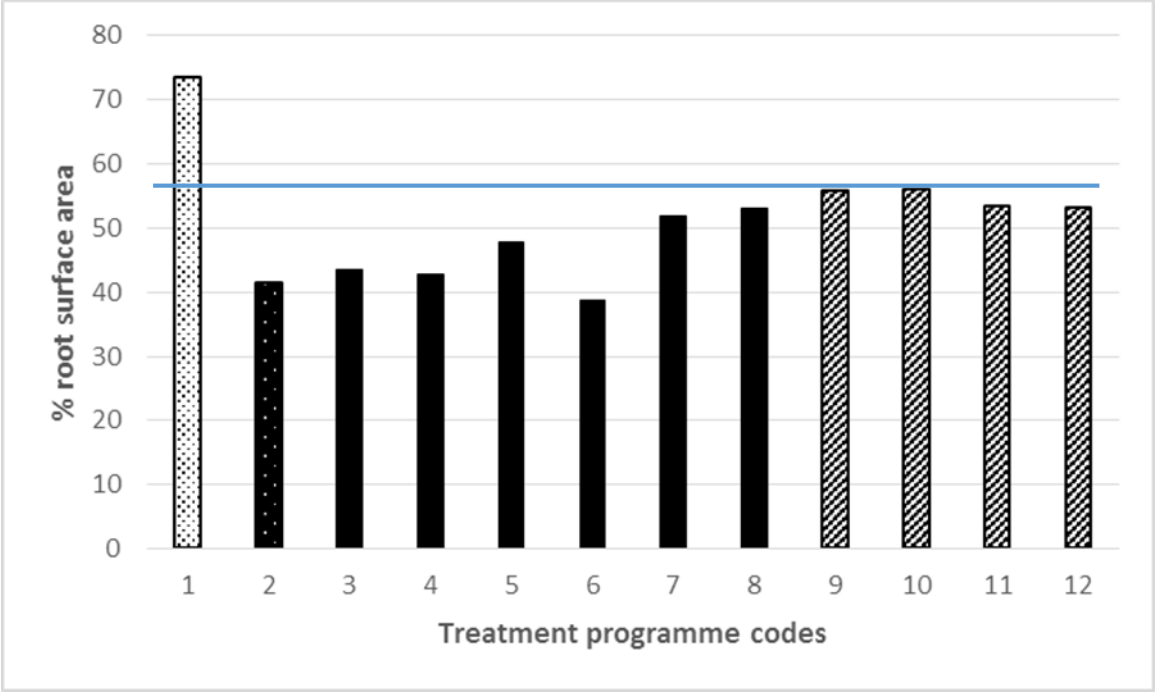

Figure 10. Effects of treatments on root growth in *Viola* sp. (Experiment 3). Final assessment after 10 weeks, showing the extent of root growth as seen by the % coverage of the surface area of the growing-media plug. See Table 3 for T1 – T12 codes. The line at 55.9% shows the minimum root surface area needed to not differ significantly from the uninoculated (T1); only T10 had a statistically similar root area to T1 (LSD 17.72). No differences in root area between inoculated treated (T3 – T12) and inoculated untreated (T2).

Root rot

Root rot was, as in Experiments 1 and 2, seen as a pale brown discolouration of the roots with few root hairs, rather than the dark brown / “black” patches along roots more often reported for black root rot (Figure 9). The dark brown banding seen with the naked eye that is often described for black root rot is where the chlamydospore chains form and agglomerate just under the root surface in and between the root cells (Figure 3); it is not caused by brown, necrotic, tissue (such as with other root rots). When root squashes from inoculated roots were examined under the microscope after adding blue stain, abundant colourless mycelium of black root rot could be seen in the discoloured tissue, and tube-like colourless phialides (conidiophores) were protruding from the root surface (Figure 11). The phialides were seen releasing colourless transparent endoconidia. Chlamydospores were seen being formed in chains within roots amongst the cells, but often only the more terminal spores were becoming dark brown as their walls became thicker. The immature chlamydospores were therefore not yet producing “black” speckled areas on the roots. Root hairs were confirmed as being sparser on the affected roots.

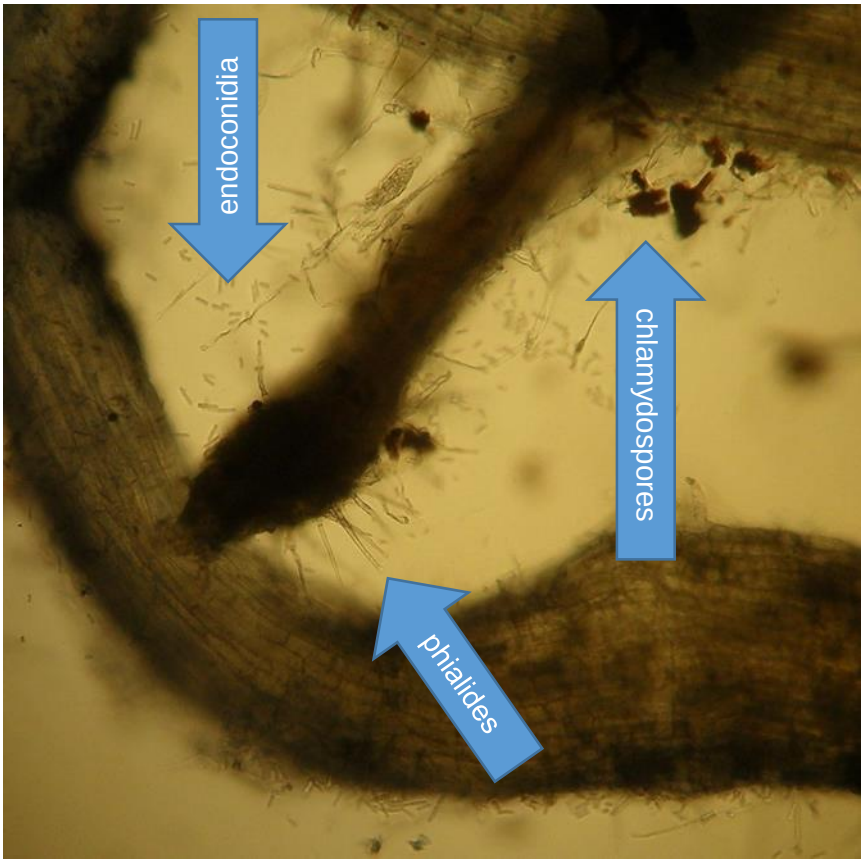

Figure 11. Microscope view of the end of a root infected with *Thielaviopsis basicola* bristling with tube-like phialides which have released rectangular endoconidia (spores). The dark

chlamydospore chains may be from the inoculum. Chlamydospore chains were also being produced within the roots (and are thus not clearly visible in the photograph).

The isolations made after the final assessment were examined on the 6th October for root browning by species other than *Thielaviopsis* sp. and no *Pythium* spp. were seen in any of the isolations with or without surface sterilisation. Some *Fusarium* spp. colonies grew from one of the uninoculated plants and from two Cercobin WG treated plants, but none of the other eleven plants had any of this fungus. *Trichoderma* spp. and *Penicillium* spp. were also present in some samples, and also secondary bacteria. No *T. basicola* was re-isolated, but this was not expected using the techniques, as agar was used for this check examination – isolation of this pathogen had been shown earlier in the project to require multiple attempts and a complex specialised agar (T. basicola-carrot-etridiazol-nystatin (TB-CEN)), as used in HDC Project PC 38b.

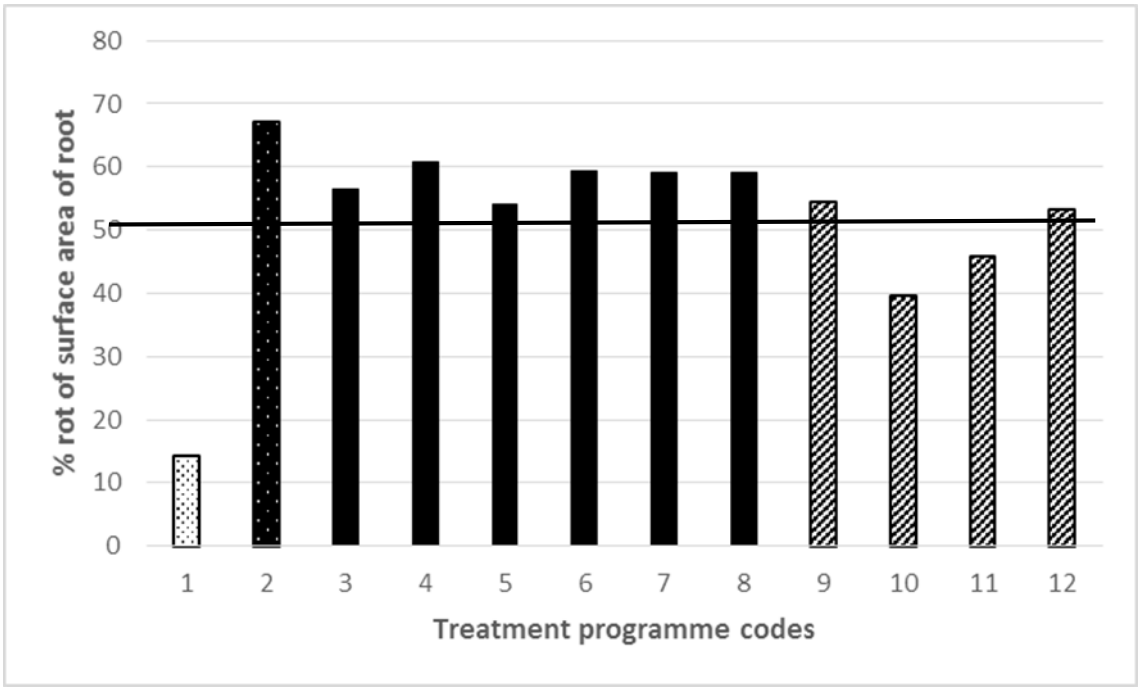

Figure 12. Effect of treatments on black root rot in *Viola* sp. (Experiment 3). Final assessment after 10 weeks, showing the proportion of the root surface area rotted. See Table 3 for T1 – T12 codes. The line at 51% shows the proportion of root surface area that could be rotted, or less, to be significantly lower than the untreated inoculated (T2); only T10 and T11 had less root rot than T2.

Highly significant ($P < 0.001$) differences were shown between programmes in the % area of the roots that were rotted at 10 weeks after sowing (Table 5 and Figure 12). Some browning was scored in the uninoculated plots. No *Pythium* spp. were found in isolations from any of the roots (potentially from natural infection) and so it was likely that the browning was physiological and most likely as a result of some roots becoming dry. This resulted in 14% of

the root area being brown across untreated uninoculated plug surfaces that were nearly three quarters covered by roots. It is not possible to say if black root rot was the cause of any loss of roots in the inoculated plugs with nearer to 50% root cover. The larger root mass which developed in the uninoculated plugs in contrast to the inoculated may have had greater susceptibility to desiccation.

In the untreated inoculated plots, 65% of the root area rotted, and most of the other treatments did not differ significantly from this (Table 5 and Figure 12). There were two programmes with significantly less affected root systems ($P < 0.001$), both after two preventative treatments; T10 having 39.7% of roots rotted and T11 having 45.8%. Both had received a T34 Biocontrol drench directly after sowing and then a week before inoculation T10 received F174 and T11 received F175. The Cercobin WG standard treatment, T3, did not give a significant level of root rot reduction, with 56.5 % of roots rotted.

Experiment 4 – Choisya sp.

The results of the 2015 efficacy experiment will be reported in the next Annual report.

Discussion

Plant vigour above-ground was not visibly affected by the root loss and root browning seen in inoculated plants. The plants were kept well fed and watered and it is possible that given greater stresses the plants would have been less able to cope with fewer roots. It should also be noted that, unlike root rotting pathogens species such as *Pythium*, *Fusarium* and *Cylindrocarpon,* the roots attacked by *Thielaviopsis* tend to remain more intact. In this experiment, endoconidia were produced in high numbers and are capable of spreading in the irrigation water to infect more roots. Chlamydospores were produced to be released mainly on the breakdown of the root. These observations emphasise the need for growers to be checking the roots of their susceptible species of plants for black root rot and either treating or disposing of the plants to stop spore spread well before foliar symptoms appear. However, growers also should be aware that microscope examination of the tissue may be needed to see the earlier stages of infestation. 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.

Although two coded products applied as protectant sprays to plants gave a significant reduction in the severity of black root rot when preceded by T34 Biocontrol at potting-on, there was still substantial root rot. Effective curative treatments were not found in any of the *Viola* sp. experiments, a significant reduction in root rot only being seen when combined with a protectant application. Biological and elicitor products would not be expected to work curatively and work in CP 077 indicated that they can contribute best to control programmes when disease pressure builds up slowly. 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.

It is clear that black root offers a real challenge to growers and that the chemical control measures that are currently available should be used with the possibility of resistance in mind. One of the coded products in this project has been put forward for an EAMU and if approved it is hoped that this could be used in programmes by growers of protected ornamental crops.

Conclusions

- By 10 weeks after sowing, artificial inoculation of *Viola* sp. with *T. basicola* four weeks after sowing had reduced root coverage of the plant plug surface by over half, and resulted in two thirds of the root area rotted
- When tested in programmes under high inoculum pressure the chemicals F174 and F175 used a week before *T. basicola* inoculation were effective in reducing root rot severity on *Viola* sp. if preceded by T34 Biocontrol at sowing.
- There was no root rot reduction by any other treatment programmes, including curative use of either F174 or F175 following standard preventative application of Cercobin WG, and preventative use of T34 alone.
- There were no problems with phytotoxicity and plant vigour was similar throughout.
- The three experiments carried out on *Viola* sp. in 2014 allowed selection of a number of conventional and non-conventional plant protection products that are being tested on *Choisya ternata* in 2015, with product applications at one or two timings each.

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.

Publications by Erika Wedgwood:

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

Glossary

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

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

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

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

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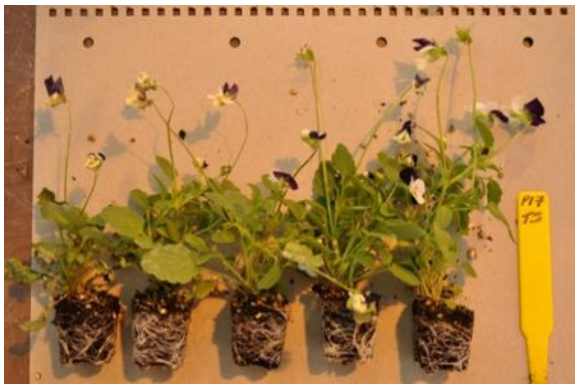
Appendices

Appendix 1. Random samples of four of the 15 plants per plot (replicate 2) on 24 September 2014 taken out of the module trays to assess root coverage of the plug and the proportion of their area browned by *T. basicola* after treatment programmes T1 to T12. All except T1 were inoculated six weeks before (showing products used at Week (Wk) 0 at sowing / Wk 3 a week before inoculation / Wk 5 curative).

T1 (untreated, not inoculated)

T2 (untreated)



T3 (- Cercobin -)

T4 (- Cercobin F174)

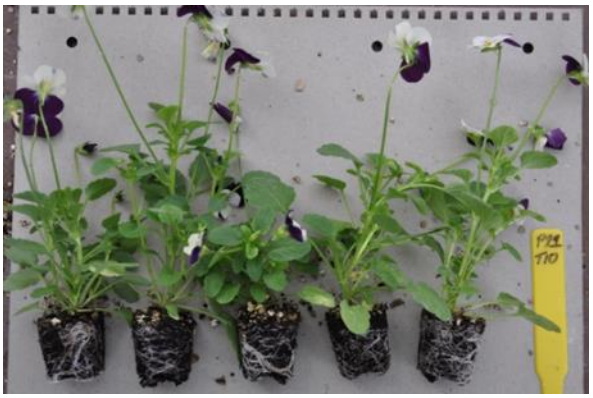
T5 (- Cercobin F175)

T6 (- F174 -)

T7 (- F175 -)

T8 (- F178 -)

T9 (T34 - -)



T10 (T34 F174 -)

T11 (T34 F175 -)

T12 (T34 F178 -)

Appendix 2. Temperature logged in the trays with *Viola* sp. plants of Experiment 3 and shown as minimum, maximum and mean daily values