Project title:	Evaluation of fungicides and novel treatments for the control of black root rot, *Thielaviopsis basicola*, in bedding and hardy nursery stock plants
Project number:	HNS/PO 190
Project leader:	Dr Erika F. Wedgwood, ADAS
Report:	Annual report, August 2014
Previous reports:	None
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Date project commenced:	01 September 2013
Date project completed (or expected completion date):	31 August 2017

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 29 August 2014

Report authorised by:

Dr Barry Mulholland
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The results and conclusions in this report are based on an investigation conducted over 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.

CONTENTS

GROWER SUMMARY

Headline

- A number of novel chemical and microbial plant protection products have been shown to be effective in reducing the incidence and severity of black root rot, *Thielaviopsis basicola,* on *Viola cornuta*

Background

Black root rot (*Thielaviopsis basicola*, syn. *Chalara elegans*) causes root damage leading to reduced nutrient and water uptake, consequent leaf yellowing and potentially plant loss. Losses in *Viola* spp. 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*.

Growers of plants which are susceptible to black root rot often apply preventative treatments of thiophanate-methyl (Cercobin WG). Approval is for only one application per glasshouse grown crop and can provide adequate protection in bedding plants (which may only be on the nursery for eight weeks), but hardy nursery stock requires a longer programme of treatment. There is a need for products with alternative modes of action to reduce the probability of development of *T. basicola* resistance to thiophanate-methyl. Earlier projects investigated cultural controls and recorded the efficacy of products then available to growers (Scrace, 1993; Jackson, 2000). A review was carried out prior to this project to determine if there were active ingredients currently in use on other crops, or pre-registration as a plant protection product that might give effective control of black root rot (Wedgwood, 2013).

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 in inoculated plants. The workplan involves screening experiments using *Viola* sp., to select a number of products for testing in programmes. Subsequently, products safe and effective on *Viola* sp. will be tested on *Choisya* sp..

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

Scrace, J.M. (1993). The effect of pH, plug nutrition and fungicide timing on control of black root rot in autumn pansy. HDC Final Report for project PC38b.

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

Project aim:

To improve control of black root rot (*T. basicola*) and increase the quality of container-grown ornamentals through the use of plant protection products and plant stimulants.

Project objectives:

1. To determine the efficacy against black root rot and plant safety of some chemical plant protection products to *Viola* sp.
2. To determine the efficacy against black root rot and plant safety of some biological plant protection products and plant stimulants to *Viola* sp.
3. To utilise the results from work carried out under objectives 1 and 2 to select products for application to *Choisya* sp. to protect against black root rot
4. To communicate the research outputs in a form for immediate uptake by the industry

This report covers objectives 1, 2 on single product treatments, but does not report on the ongoing work on treatment programmes for *Viola* sp., nor on the work to be started on *Choisya* sp. for Objective 3.

Summary

Plant protection treatments were applied to *Viola cornuta* before and in most cases also after inoculation with black root rot, *T. basicola,* spores. Products tested were selected on the basis of their potential for control and their current or likely future availability for use on ornamentals under protection. Conventional and non-conventional products were compared in separate concurrent experiments arranged in replicate blocks with 15 plants assessed per plot. 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. Dose rates were as on the labels, or as advised as being most suitable. The products tested were Signum (boscalid + pyraclostrobin) and Switch (cyprodonil + fludioxonil) with authorisation for use on protected ornamentals plus novel products HDC codes F173, F174, F175 and F176. Seven non-conventional products were tested; Prestop (containing the fungus *Gliocladium catenulatum* strain J1446), Serenade ASO (containing the bacteria *Bacillus subtilis*, strain QST713), T34 (containing *Trichoderma asperellum* strain T34), HortiPhyte (a foliar feed containing potassium phosphite), microbial products Trianum G, HDC code F179 and a chemical, HDC F178. All except Cercobin WG, T34 and Trianum G were used curatively as well as preventatively in separate treatments.

No foliar phytotoxicity arose with any of the products, either from the preventative use of T34 or Trianum G at sowing, or from use of all other products at the two-leaf stage, or where in addition used curatively a fortnight later.

Roots were assessed nine weeks after sowing, four weeks after inoculation of the growing media with a spore suspension of *T. basicola*. Infection causing a pale brown discolouration of the roots was confirmed by microscopic examination and recording of condiospore production. Root staining was recorded in all treatments, including the uninoculated, but the incidence and severity was significantly greater in the inoculated untreated plots. No other pathogens were isolated from inside damaged roots. Some root desiccation was seen.

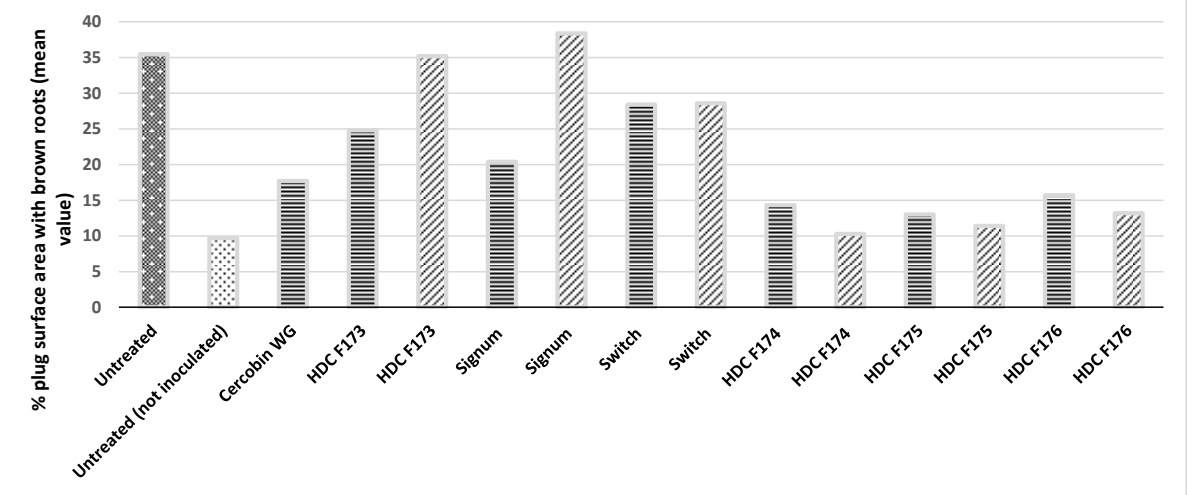

Figure 1: Experiment 1; conventional products. Mean % root area brown on the surface of *Viola* sp. plugs on 11 July 2014 nine weeks after sowing. (P<0.001, L.s.d. 13.964). All treatments with below 24% root browning differ significantly from the untreated inoculated.

Key:
Horizontal lines = product application before inoculation (preventative)
Diagonal lines = product applications before and after inoculation (preventative + curative)

In the conventional treatment experiment, although there was no foliar phytotoxicity to *Viola* sp., plants which received a double application of Signum had as high a proportion of brown roots as the untreated inoculated suggesting phytotoxicity. Root rot had caused a mean 36% rotting of the root plug surface area in untreated inoculated plants while Cercobin WG treated plants had 18% (**Figure 1**). Compared with the inoculated untreated plants, seven products gave highly significantly (P<0.001; **Figure 1**) less root damage, with a mean 14% area affected. After use of HDC F174, F175 and F176 as either preventative alone or with curative application the lower root damage was similar to the 9.7% of uninoculated plants. Signum preventative use also gave some benefit, but with 20% damage. Plants in five of the treatment

programmes were no healthier than untreated inoculated plants, with most plugs having rotted roots. Only preventative plus curative treatment by either HDC F175 or F176 significantly (P<0.002) increased the proportion of plants remaining healthy to a mean 26%; 58% of uninoculated plants remained healthy compared with 2.5% for inoculated untreated.

When non-conventional treatments were used, three products (HDC F178, Trianum G and Prestop) resulted in less root rot compared with 18% in the inoculated untreated plots (**Figure 2**), but the only significant (P<0.001) difference was seen following the use of the chemical HDC F178 in preventative plus curative drenches when only 6% root rot developed. Preventative use of HDC F178 alone also reduced mean damage severity, but two applications kept 37% plugs without any root damage (P<0.001) compared with 12% from the single application and 5% incidence in the untreated inoculated. Incorporation of Trianum G before sowing reduced root rotting by half to 9% rot, with 35% of plant plugs having no browning. Fungal product Prestop re-applied with a fortnight's interval after preventative application at the two leaf stage allowed 11% root rot, a slight reduction compared with the untreated, with significantly (P<0.001) more, 41% of plugs, having no rot. The other products had no benefit, with high volume sprays by two microbial products, Serenade ASO bacteria (preventative) and HDC F179 fungi (preventative plus curative) having more (although not significantly) of their root plug surfaces browned (a mean 24%) than the 18% in the untreated inoculated plots.

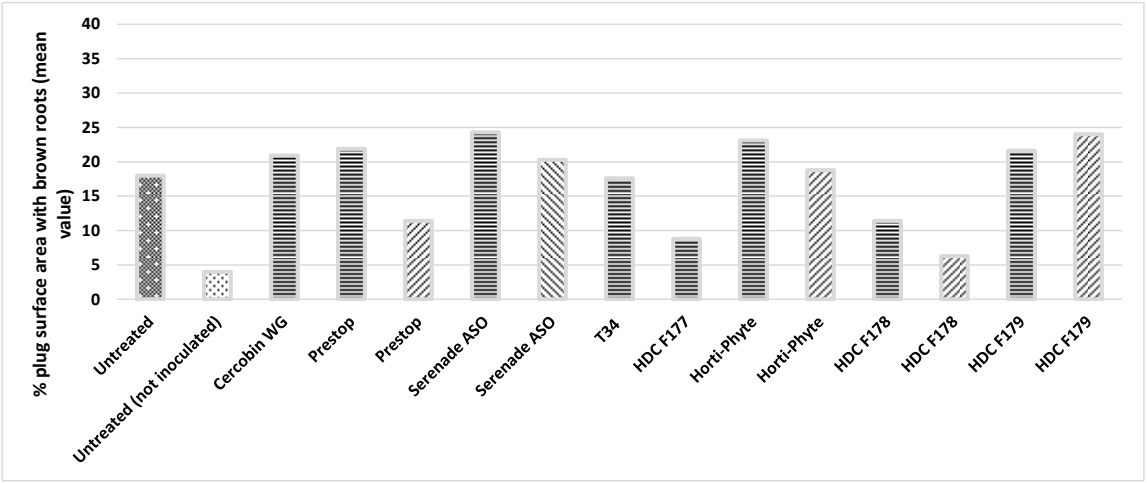

Figure 2: Experiment 2; non- conventional products. Mean % root area brown on the surface of *Viola* sp. plugs on 10 & 14 July 2014 nine weeks after sowing. (P<0.05, L.s.d. 5.84). Only treatments with 6.3% or less root browning (only HDC F178 applied twice) differ significantly from the untreated inoculated. NOTE: In this Figure, code HDC F177 is given to Trianum G

Key:
Horizontal lines = product application before inoculation (preventative)
Diagonal lines = product applications before and after inoculation (preventative + curative)

Financial Benefits

Effective treatments will improve crop quality through maintaining a healthy root system, improving crop establishment and reducing crop losses. Improved root system performance will be particularly important for hardy nursery stock where plants are sold by pot size.

Increasing the range of products available to growers which are potentially effective in the control of black root rot using the EAMU approval system would increase the range of active ingredients available and reduce the chance of fungicide resistance developing. The cost of disease management could be reduced by the use of some of the products tested, because when used for black root rot control they will also be effective against other pathogens such as *Pythium* spp. so saving separate applications.

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.

Action Points

- Ensure that propagation trays for re-use have as much growing-media and root debris removed from them as possible before disinfection so that most material potentially infected with resting spores is removed and the disinfectant in the washing water can gain contact with the infested tray surface
- Be aware that water-splash and run-off between plants can contain conidiospores of black root rot that infect to produce further spores within a few weeks
- Try to avoid stressing plants by e.g. inadequate ventilation in hot weather as this can reduce their resistance to pathogens such as black root rot
- Consider the preventative use of biopesticides as these can produce a root-zone environment which has increased resistance to infection by a number of pathogens and potentially a systemic, protectant, benefit for the whole plant
- To avoid the build-up of resistance to active ingredients select a range of products with different modes of action when using conventional products on the nursery.

SCIENCE SECTION

Introduction

Black root rot infection by *Thielaviopsis 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 resting spores (chlamydospores) of the fungus which develop at infection points in the outer cells of roots. The infection is not vascular, but damage to the surface of the root at the location of 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, but hardy nursery stock such as *Choisya* spp. affected at potting-on can fail to establish.

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 23 ºC, 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 pH 5.5, subject to plant tolerance) (Koike *et. al.*, 2005).

Chemical control products

Cercobin WG (thiophanate-methyl) can be used as a single fungicide drench application to glasshouse grown crops (under permanent protection) in the UK up to 3 weeks before planting in the ground, but there are concerns about reliance on one product and resistance developing. A recent AHDB Horticulture review (Wedgwood, 2013) examined options for the control of black root rot. Most work has been carried out by the USA States' Extension Services to provide grower recommendations for products to use on ornamental crops in alternation with thiophanate-methyl products including; azoxystrobin, fludioxonil + cyprodinil, myclobutanil, trifloxystrobin, phosphorous acid and a biopesticide containing *Trichoderma harzianum* T22 and *Trichoderma virens* G-41. In 2013, 15 UK registered products from a range of fungicide groups were tested for Defra to determine their potential activity against *Chalara fraxinea* (the cause of ash dieback). Signum (boscalid + pyraclostrobin) and some azole group products found to give some control of *C. fraxinea* on saplings were available for testing against black root rot (its asexual state is also in the Chalara family). There was also evidence for the activity against *T. basicola* by two experimental products and their use in

efficacy trials on ornamentals would be supported by the suppliers.

A range of fungicides for control of black root rot on pansy by drenching was screened in AHDB Horticulture project PC 143 fourteen years ago (Jackson, 2000). Infection of 58% in the untreated was reduced to 23% with Folicur (tebuconazole) and 33% with Genie 25 (flusilazole), but both caused leaf scorch. Some reduction was also seen from drenches of Amistar (azoxystrobin), Bavistin DF (carbendazim), F279 (trifloxystrobin), Scotts Octave (prochloraz), Plover (difenoconazole), Unix (cyprodinil) and Stroby WG (kresoxim-methyl). Cyprodinil is available in the UK mixed with fludioxonil as Switch.

Few plant protection products in the UK are approved as drenches and any tests must ensure the maximum dose per hectare approved as a spray is not exceeded. As azoles are systemic, effective protectively and curatively against a wide range of fungi, then foliar application may give efficacy against *T. basicola*, in addition to any uptake from run off into the growing medium. For reasons of resistance management, many recent products contain two active ingredients with different modes of action. Chemicals belonging to the succinate dehydrogenase inhibitor (SDHI) fungicide group have been developed relatively recently and are used in the control of cereal diseases for their persistence and protectant features (Impey, 2014). Their efficacy against *T. basicola* is not known.

Microbial control products

Novel biological products incorporated into the growing medium have potential to provide cost-effective control of black root rot. Two growing-media treatment biopesticides Prestop (*Gliocladium catenulatum* strain J1446), and T34 Biocontrol (*Trichoderma asperellum* strain T34), and the plant growth promoter Trianum G (*Trichoderma harzianum* T22) are available in the UK for root rot control. The biopesticide Serenade ASO (*Bacillus subtilis* QST 713) has an off-label approval for drench use on amenity vegetation in the UK.

Prestop (32 % w/w *Gliocladium catenulatum* strain J1446) can be used on ornamentals as a spray at 6 kg/ha to soil, as a drench at 500g/100L and to growing media at 500g/m^3. The product is approved for the control of *Botrytis cinerea*, and root diseases caused by species of *Pythium, Phytophthora, Rhizoctonia* and *Fusarium,* although technical information includes the control of *Thielaviopsis, Verticillium, Alternaria, Helminthosporium* and *Penicillium.* It works by competing for space with the pathogenic fungi and utilising enzymes to break down fungal cell walls (Fargro Technical Notes for Prestop www.fargro.co.uk).

T34 Biocontrol (10.8 % w/w *Trichoderma asperellum* strain T34) is a biopesticide registered in the UK for the reduction of *Fusarium oxysporum* f. sp. *dianthi* on carnations by incorporation, spraying or irrigating into growing media or as a root dip (www.fargro.co.uk). EAMU 1118 of 2012 allows use on protected and outdoor container grown ornamentals. The producers Bicontrol Technology have no specific data for T34 activity against *Thielaviopsis*, but they would expect it to have some effect as Trichoderma as a genus is known to have *Thielaviopsis* activity (Paul Sopp, Fargro, pers. comm.).

Trichoderma harzianum strain Rifai T-22 is available in the UK for protected ornamental production as the biofungicide Trianum G (2015/1292). Biofungicides containing this isolate are recommended at planting time for the control of *T. basicola* in landscape planting by Cornell University (Walker, 2008) together with fungicides containing thiophanate-methyl.

Serenade ASO (1.34 % *Bacillus subtilis* strain QST 713) contains a naturally-occurring beneficial soil bacterium. It was originally registered in the UK for foliar application against *Botrytis cinerea* on protected strawberries. In 2012, it gained a SOLA (2012 00140) for use against *Phytophthora* spp. on amenity vegetation and forest nurseries. Under EAMU 0708 of 2013 it can be used at 10 L/ha on ornamental plants. Serenade Soil is sold as a biopesticide in the USA to protect young root vegetable, fruiting vegetables and cucurbits against soil diseases like *Pythium, Rhizoctonia, Fusarium* and *Phytophthora*. Applied at planting, the beneficial bacteria multiply in the rhizosphere and attach to roots to give them protection (www.serenadesoil.com.). *T. basicola* is not mentioned as being controlled.

Plant health promoters and elicitors

Plants use physical and chemical barriers to prevent infection. In addition, they use their innate responses to ward off pathogens (Rivière *et al.*, 2011), having evolved the ability to detect microbes. Pathogens can suppress this by secreting effectors. Plants can respond by effector-triggered immunity (ETI). ETI is associated with both localised hypersensitive response (HR) and the whole plant systemic acquired resistance (SAR) that is long lasting and effective against a broad spectrum of pathogens. Natural compounds which activate various plant defence responses include chitosan, laminarin, salicylic acid and the salicylic acid derivative acibenzolar-S-methyl (also known as BTH; benzo(1,2,3) thiadiazole-7-carbothioic acid S-methyl ester). BTH protects against a broad spectrum of pathogens (Rivière *et al.*, 2011). Elicitors are recommended as supplements that allow fungicide application to be reduced.

Acibenzolar-S-methyl as Bion 50 is sprayed against bacterial pathogens of mangoes and tomatoes in South Africa, and registered in Australia as Bion Plant Activator Seed Treatment for cotton to suppress Fusarium wilt and black root rot. Syngenta are pursuing UK registration of acibenzolar-S-methyl as a foliar spray treatment in ornamentals (J. Ogborne, Syngenta, pers. comm.).

HortiPhyte contains phosphite and this is believed to prime plant cells to defend themselves against fungi. Phosphite is transported in the xylem and phloem. Phosphite is then slowly oxidized in growing media to become phosphate salts which act as a fertiliser.

Methods

Identification of candidate products for efficacy testing against black root rot

Information sources on active ingredients available overseas against root rots, in particular in the USA, as considered in the AHDB Horticulture review PO14 (Wedgwood 2013), and those being tested in the UK against the related fungus *Chalara fraxinea* were consulted. Products used in other crop sectors and experimental products were evaluated for use in the current project based on their known activities and a short-list of products was drawn up. Information was sourced from chemical companies and AHDB Horticulture experts on the likelihood of each product becoming registered and/or being supported in any application for an Extension of Authorisation for Minor Use (EAMU) for ornamentals. The products selected for the current project are given in **Table 1**.

Table 1. Information, where available, on the products tested on *Viola* sp. in Experiments 1 (conventional plant protection products) and 2 (non-conventional)

Product & code	Active	Mode of Action if declared	Fungicide group* or product type	FRAC code	Use in UK on ornamentals		Approval status and additional comments
					Outdoor	Protect-ion	
Cercobin WG	thiophanate-methyl	Systemic	MBC or benzimid-azole	1	Yes	Yes	Ornamentals EAMU 1887 of 2011. One application
HDC F173	Confidential	-	SDHI	-	No	No	Experimental product
Signum	boscalid + pyraclo-strobin	Systemic & protectant	SDHI + Qol	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 30/10/2019)
HDC F174	Confidential	Protectant (some curative)	DMI Qol	-	No	No	Approved product on other crops
HDC F175	Confidential	-	chemicals	-	No	No	Experimental product
HDC F176	Tebucon-azole + trifloxy-strobin	Systemic & protectant	DMI Qol	3 + 11	No	No	Approved product only on other crops
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	*Trichoderma asperellum* T34	Protectant microbial	fungus	-	Container grown	Yes	EAMU for protected + container grown ornamentals 1118 of 2012
Trianum G	*Trichoderma harzianum* strain T22	Protectant microbial	fungus	-	No	Yes	Approved on protected ornamentals
HortiPhyte	Potassium phosphite	Nutrient & stimulant	chemical	-	Yes	Yes	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

Information on ingredients from the UK Pesticide Guide 2014. *Key to Groups in Glossary

Conventional and non-conventional products were initially screened in this project in separate simultaneous glasshouse experiments (Experiments 1 and 2) with *Viola* sp.. The efficacy data generated from these experiments was used to set up a third experiment with *Viola* sp. and programmes drawn up in consultation with the industrial representative and AHDB Horticulture project manager. Preventative treatment options of *Viola* sp. at sowing and/or with two leaves and curative application (following inoculation) were proposed to include both conventional and non-conventional plant protection products in programmes for the control of black root rot.

Objectives 1 and 2 – Evaluation of chemical and biological products for control of *T. basicola* in *Viola* sp.

Experiment set-up

Advice on pansy growing and the conditions in which black root rot was more likely to develop was sought from the industrial representative. For the purposes of these experiments, however, the plants were sown into multi-cell trays with cells of 22 ml volume. This allowed the plants to grow for eight weeks (the commercial marketing age) in one container and matched the procedure used in previous AHDB Horticulture projects on black root rot (Scrace, 1993, Jackson, 2000). Seeds were sown on 9 May 2014.

Seeds of Horned Violet, *Viola cornuta*, cv. Sorbet XP White Jump Up (Ball Colegrave Ltd) were sown singly in each 30 mm x 30 mm and 39 mm deep cell within a tray of 24 (quarter sections of 104-cell TEKU trays). Peat-based growing media (Bulrush seedling recipe) was used. One treatment required incorporation in the growing media. All trays were lightly watered to moisten the growing media before sowing. The seeds were lightly covered with a layer of fine vermiculite. Each tray was stood on its own rectangle of wetted capillary matting in a lattice-sided vegetable tray, watered and stacked up. One treatment was drenched over the vermiculite in place of watering. The stacks were covered tightly with transparent polythene to keep the humidity high and kept in a room at about 20 °C lit by daylight but out of direct sunlight to germinate. Once the shoot had begun to emerge from the seed, the trays were placed in the growing area. In Experiment 1 and 2 the trays were temporarily placed in a polytunnel where a greater air-flow could be given as the glasshouse compartment was very hot. Once they had produced two true leaves any treatment spray applications were given and all trays were moved to the glasshouse compartments.

After treatment, the trays of each experiment were placed in separate, adjacent, heated

glasshouse compartments at ADAS Boxworth. Temperatures were allowed to rise to the optimum for infection at over 25 °C. When temperatures inside were around 30 °C, cooling was achieved by the use of the side fan and roof vents and opening the door . The multi-cell trays were placed four to a vegetable tray, on a stack of vegetable trays so that they were at bench height. Treatments were randomised within replicate blocks which were arranged south to north across each compartment to take account of spatial and temporal variations for the experiment (**Figures 3** and **4**). Details of the treatments for each code are provided in **Tables 3** and **4**. Watering done by hand over the top of the trays with a sprinkler-head hose, with surplus water draining down (not flowing between trays). The aim was to subject plants to heat stress during peak day time temperatures and so increase their susceptibility to infection, combined with wet compost to aid pathogen survival and multiplication.

The experiments were carried out in cleaned, insect screened, glasshouse compartments at ADAS Boxworth in isolation from other crops so no plant protection products to control pests such as aphids or thrips were required. Liquid feeding (Miracle Grow at 15 ml in 4.5 L water) was given weekly after inoculation. After the root assessments in early July, nine weeks after sowing (when suppliers' plants would normally have been dispatched), the plants were kept to allow foliar symptoms to develop. They were set out in a polytunnel, under overhead irrigation, as the glasshouse was needed for Experiment 3.

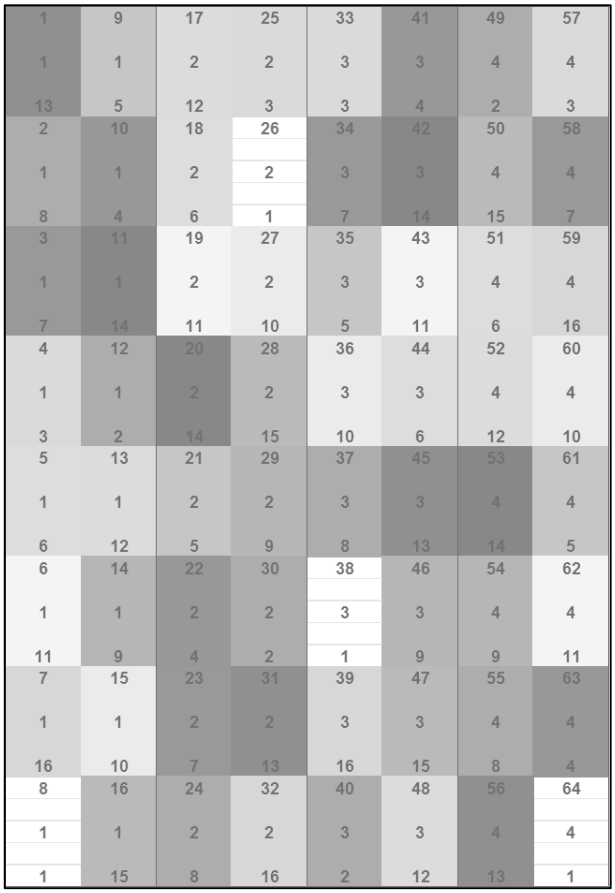

Figure 3:
Layout of conventional treatments (Experiment 1) within four replicate blocks

Plot number

Replicate

Treatment code

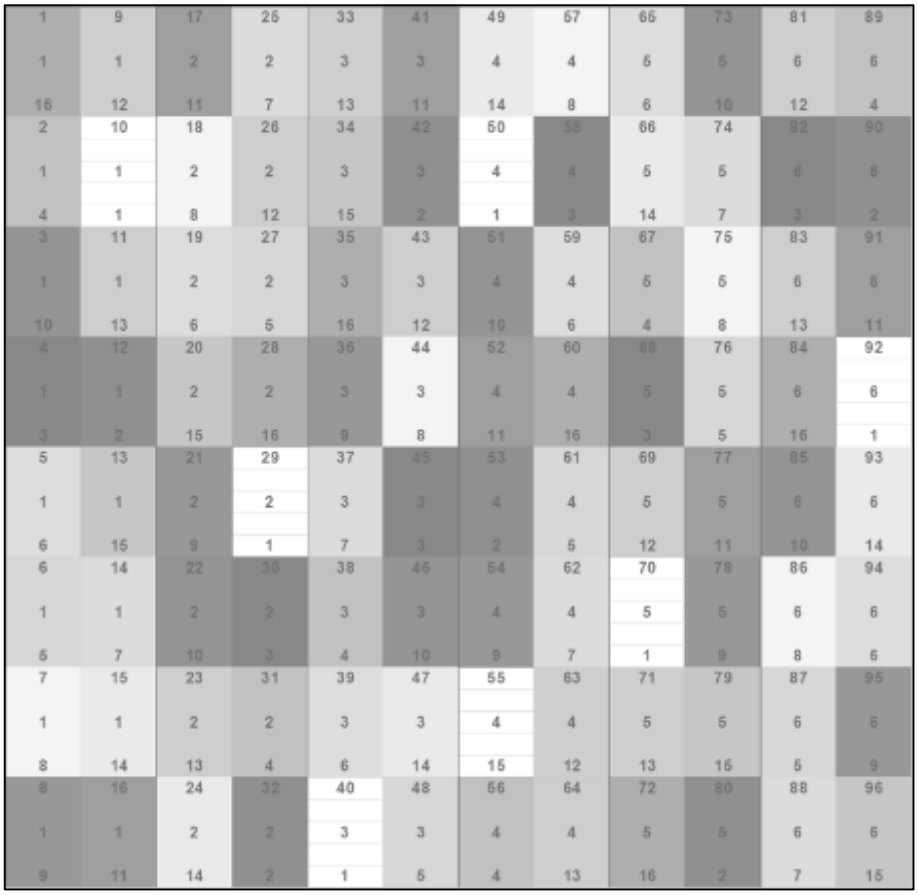

Figure 4: Layout of non-conventional treatment plots (Experiment 2) within six replicate blocks.

Figure 5: Multi-cell trays of *Viola* sp. plants in Experiment 2 on 18 June 2014.

One replicate of four vegetable trays with four randomised treatments per multi-cell tray.

24 cells sown per multi-cell for each treatment plot.

Figure 6: Southern half of Experiment 1 (conventional products) at the start of flowering on 3 July 2014. Experiment 2 (non-conventional products) is visible in the adjacent compartment

Efficacy against black root rot

Plant protection products were applied at intervals before and/or after inoculation at the timing intervals shown in **Table 2**. Inoculation of Experiments 1 and 2 with *T. basicola* spore suspension containing both chlamydospore (resting spore) chains and conidiospores was done by syringing the growing media around the plant. Each plant received around 5000 chlamydospore chains per ml (the chains form in tight clumps and so actual concentration will have varied) and 10,000 conidiospores per ml using 1 ml per 22 ml volume cell. The inoculum

used was isolated in November 2013 from a carrot root (BX13/114). The isolate was bulked up on potato dextrose agar on 1 May 2014 with plates used after five weeks of incubation in the dark. It had not been possible to produce or purchase an isolate from *Viola* sp..

Table 2. Application intervals of plant protection products and *T. basicola* inoculation

Procedure	Timing of procedure
Treatment incorporation or drench*	Just before or directly after sowing
Treatment spray	3 weeks after sowing, at two leaf stage
Inoculation drench	1 week after treatment sprays at two leaf stage
Treatment spray**	1 week after inoculation

Key: * Products used to treat the growing media before plant growth.

** Products without label restrictions for single use also received a curative application.

The products used in the two concurrently run experiments are shown in **Tables 3** and **4**. The thiophanate-methyl supplied for this project was the identical formulation product Topsin WG to Cercobin WG (Alan Horgan, Certis, pers. comm.). All procedures and assessments were carried out on the same days for both experiments (always on the same day of the week). All treatments except T3 in each experiment were inoculated with black root rot spores, with inoculation on Friday 6 June 2014.

Table 3. Experiment 1. List of treatments using six chemical plant protection products plus Cercobin WG as standard

Products used curatively (C) only (one week after inoculation), or also preventatively (P) (one week before inoculation) have different treatment codes

T	Product & code	Active	Type	Dose & water volume	Comments
1	Untreated	none	n.a.	water alone at 1000 L/ha	
2	Untreated	none	n.a.	water alone at 1000 L/ha	
3	Untreated	none	n.a.	water alone at 1000 L/ha	NOT inoculated
4	Cercobin WG	thiophanate-methyl	P	1.1 g per 10 L water using 1000 L/ha water	Standard. One application permitted. Ornamentals EAMU 1887/2011. Label rate *Thielaviopsis* drench
5	HDC F173	confidential	P	0.3 L/ha in 400 L/ha water	Recommended by BASF
6	HDC F173	confidential	P+C	0.3 L/ha in 400 L/ha water	Recommended by BASF
7	Signum	boscalid + pyraclostrobin	P	1.35 kg/ha in 400 L/ha water	Label spray rate. EAMU 1107/2009

8	Signum	boscalid + pyraclostrobin	P+C	1.35 kg/ha in 400 L/ha water	Label spray rate. EAMU 1107/2009
9	Switch	cyprodinil + fludioxonil	P	0.8 kg/ha using 1000 L/ha water	Approved on ornamentals. 80 g/100 L maximum dose rate for protected crop
10	Switch	cyprodinil + fludioxonil	P+C	0.8 kg/ha using 1000 L/ha water	Approved on ornamentals. 80 g/100 L maximum dose rate for protected crop
11	HDC F174	confidential	P	0.35 L/ha using 400 L/ha water	Rate agreed with manufacturer
12	HDC F174	confidential	P+C	0.35 L/ha using 400 L/ha water	Rate agreed with manufacturer
13	HDC F175	confidential	P	0.75 L/ha using 400 L/ha water	Recommended by manufacturer
14	HDC F175	confidential	P+C	0.75 L/ha using 400 L/ha water	Recommended by manufacturer
15	HDC F176	confidential	P	0.4 kg/ha using 400 L/ha water	Rate agreed with manufacturer
16	HDC F176	confidential	P+C	0.4 kg/ha using 400 L/ha water	Rate agreed with manufacturer

Table 4. Experiment 2. List of treatments using seven biological plant protection products/activators, plus Cercobin WG as standard

Products used curatively (C) only or also preventatively (P) have different treatment codes.

T	Product	Active	Ty-pe	Dose & water volume	Comments
1	Untreated	none	n.a.	water alone at 1000 L/ha	
2	Untreated	none	n.a.	water alone at 1000 L/ha	
3	Untreated	none	n.a.	water alone at 1000 L/ha	NOT inoculated
4	Cercobin WG	thiophanate-methyl	P	1.1 g per 10 L water using 1000 L/ha water	Standard. One application permitted. Ornamentals EAMU 1887/2011. Label rate *Thielaviopsis* drench
5	Prestop	*Gliocladium catenulatum*	P	5.0 g in 1 L water using 1 L diluted product per m²	Approved on ornamentals. Label rate for seedling drench. *Thielaviopsis* control (Fargro tech. notes)
6	Prestop	*Gliocladium catenulatum*	P+C	5.0 g in 1 L water using 1 L diluted product per m²	Approved on ornamentals. Label rate for seedling drench. *Thielaviopsis* control (Fargro tech. notes)
7	Serenade ASO	*Bacillus subtilis*	P	10 L product per ha (10,000 m²) in 1000 L water / ha	EAMU 0708/2013 for ornamentals

8	Serenade ASO	*Bacillus subtilis*	P+C	10 L product per ha (10,000 m²) in 1000 L water / ha	EAMU 0708/2013 for ornamentals
9	T34	*Trichoderma asperellum*	P	0.5 g in 1 L water /m² growing media straight after sowing	Water at 10 % container volume (Fargro tech. notes). EAMU for ornamentals 1118/2012
10	Trianum G	*Trichoderma harzianum* strain T22	P	750 g/m cubed of growing media before tray filling	Approved on protected ornamentals. Label rate for propagation in trays/modules
11	HortiPhyte	Potassium phosphite	P	15.0 ml/L water. Use 1 L diluted product / m²	Fertigation rate for ornamental seedlings and cuttings.
12	HortiPhyte	Potassium phosphite	P+C	15.0 ml/L water. Use 1 L diluted product / m²	Fertigation rate for ornamental seedlings and cuttings
13	HDC F178	Confidential (chemical)	P	25 g/ha using 1000 L water /ha	Experimental rate for young plants from manufacturer
14	HDC F178	Confidential (chemical)	P+C	25 g/ha using 1000 L water /ha	Experimental rate for young plants from manufacturer
15	HDC F179	Confidential (microbial)	P	1 kg/ha using 1000 L water/ha	Rate given by manufacturer
16	HDC F179	Confidential (microbial)	P+C	1 kg/ha using 1000 L water/ha	Rate given by manufacturer

All untreated water controls and plant protection product drenches and sprays were carried out using a pot spraying machine. Only T34 was applied to trays before foliage was present. Trianum G was the only product incorporated as granules pre-sowing.

Assessments

The plants were examined for foliar phytotoxicity symptoms such as yellowing, distortion, stunting or necrosis after treatment applications and records of this and plant vigour recorded. Plant vigour was recorded using a 1 to 9 index, with 9 being the best vigour.

Root assessment was carried out starting nine weeks after sowing. The plugs were pushed out of each cell and after examination replaced to allow further assessment. Fifteen plants were scored per plot. Most of the root systems grew out to encapsulate the growing media plug and so the proportion of the surface area on the four sides of the plug affected by browning was recorded as a measure of % root rot.

At the end of the experiment in mid-August a total of eleven samples of roots were taken in order to determine if any other pathogens might have been causing root browning. Brown roots from one plant each across a range of treatment and the untreated inoculated and

uninoculated treatments were taken and isolations made onto potato dextrose agar with and without ethanol surface sterilisation of the roots.

Table 5. Dates of procedures carried out on *Viola* sp. in 2014

Date	**Description of trial task**
8 April	Bulked up inoculum of *T. basicola* for Experiments 1 & 2
9 May	Part 1; Experiments 1&2: Chemical & biological product efficacy tests Sowed Viola seeds in multi-cell trays and germinated in sealed stack Some compost treated pre-sowing or directly after sowing
14 May	Checked for germination and moved to polytunnel until after spraying
	Weekly records of vigour, phytotoxicity and disease commenced
19 May	Inoculum of *T. basicola* (both isolates) bulked up for Experiment 3
30 May	Treatment drenches applied three weeks after sowing
6 June	Inoculated with *T. basicola* spore solution four weeks after sowing
13 June	Curative treatment drenches applied five weeks after sowing
9 to 14 July	Experiments 1 & 2: Final assessments nine weeks after sowing
15 August	Final observations on plants from Experiments 1 & 2
17 July	Experiment 3 sown after examining the results of Experiments 1&2
22 July	Checked for germination and moved to glasshouse
	Weekly records of vigour, phytotoxicity and disease commenced
7 August	Treatment drenches applied three weeks after sowing
14 August	Inoculation with *T. basicola* spore solution four weeks after sowing
21 August	Curative treatment drenches applied five weeks after sowing
18 September	Experiment 3: Final assessment nine weeks from sowing

Results

Figures 9 and **10** show the % of root ball surface area that was brown for each replicate tray

of 15 plants. The data shows that in some instances the mean result was influenced by an unusually high scoring tray. Mean results are shown in **Tables 6** and **7**, with charts of root rot in the Grower Summary (**Figures 1** and **2**).

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

In the first four weeks after sowing on the 9 May there were no differences in vigour (Appendix 1). After this there was a gradual decline in vigour in the T1 replicates of untreated inoculated plots, becoming significantly (P<0.001) poorer (Index 6.0) on the 20 June. Plots where HDC F176 was applied twice also became similarly poor from this date. There were no treatment-related phytotoxicity symptoms on the foliage (data not presented). A few plants with severe crinkling and stunting were seen in six trays and recorded on 20 June, but this was discounted as a treatment effect, as two of the trays were untreated (data not presented).

On 10 July, by full-flowering, nine weeks from sowing, there was no difference in foliar vigour between the treatments, all being good. Replicate 4 had slightly poorer foliar vigour (principally yellowing of the younger leaves) than the other three replicates.

The severity of root rotting was similar across the replicates at nine weeks, a mean 22% of root surface area, but more (P<0.056) plugs (95%) had visible root damage in Replicate 4 than the average 84% of plants in the other replicates (data not shown).

Table 6. Experiment 1, conventional products nine weeks after sowing. Mean foliar vigour index, % surface area of root ball of plug with browning and percentage of plugs with zero browning. Six products plus Cercobin WG as standard (10 & 11 July 2014).

Treat-ment code	Product name or AHDB Horticulture code	Treat-ment timing	Foliar vigour 1-9 index	% of plug surface area with brown roots	% of plugs with zero root browning
1+2	Untreated	n.a.	8.6	35.5	2.5
3	Untreated (not inoculated)	n.a.	8.5	**9.7**	**58.3**
4	Cercobin WG	P	8.8	**17.7**	18.3
5	HDC F173	P	9.0	24.8	1.7
6	HDC F173	P+C	8.5	35.2	0.0
7	Signum	P	8.5	**20.4**	0.0
8	Signum	P+C	8.5	38.4	0.0
9	Switch	P	9.0	28.4	1.7
10	Switch	P+C	9.0	28.6	5.0
11	HDC F174	P	8.2	**14.3**	15.0

12	HDC F174	P+C	8.5	**10.3**	20.0
13	HDC F175	P	8.8	**13.0**	21.7
14	HDC F175	P+C	8.8	**11.4**	**26.7**
15	HDC F176	P	8.8	**15.7**	13.3
16	HDC F176	P+C	8.5	**13.2**	**25.0**
	Grand mean		8.6	22.0	13.2
	F value (treatments)		P=0.331 No sig. diff	P<0.001	P=0.002
	L.s.d.		0.51	13.96	22.17
	Residual d.f.		45	46	46
	F value (blocks)		P<0.001	P=0.880 No sig. diff	P=056

Treatments which differ significantly from the untreated inoculated are shown **shaded**

A mean 35% of the root area on the plug surface was affected in the inoculated untreated plots. Some browning was seen in the uninoculated (**Table 6**). Infection severity varied between plugs in each multi-cell tray, with for example a range between 6% and 100% root rot for the 15 untreated inoculated plants in Replicate 1 (data not shown).

The root rot was a pale, yellowish brown, colour with a slight softening of the roots. Where it was less severe it tended to be present in the roots in the upper 10 mm of the plug. When an inoculated root (**Figure 8**) was examined under a high power microscope (utilising a blue stain to show fungal tissue) on 3 June fungal structures were seen inside root cells on the surface and projecting from them. These were phialides that produce short-lived conidiospores (endoconidia). There was a cluster of rectangular endoconidia around the structures (**Figure 7**). The phialides and spores are colourless and so were not producing the dark brown flecking that results from the presence of the pigmented resting spores.

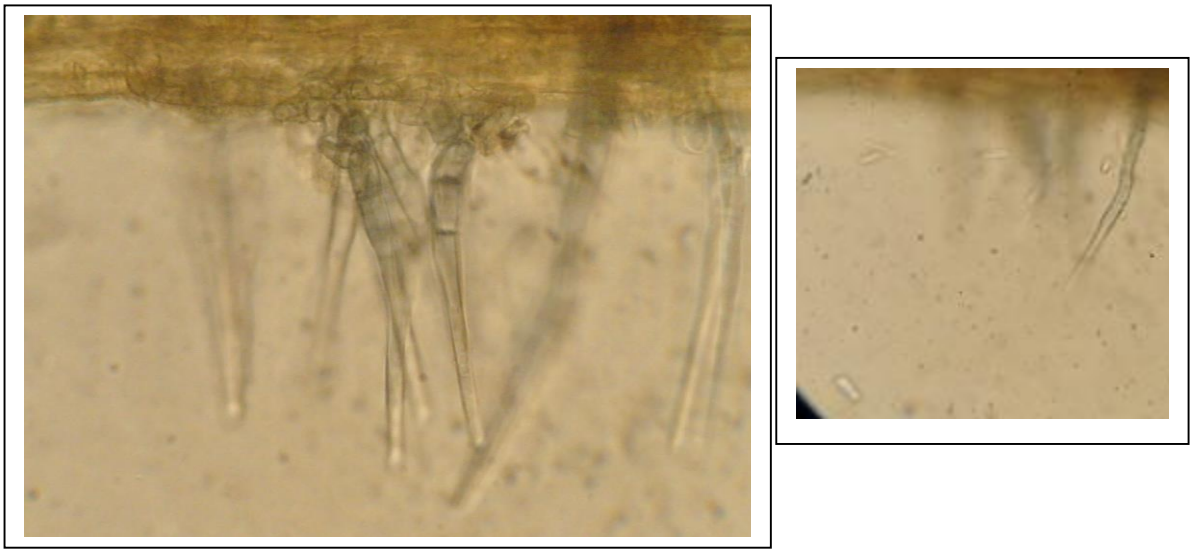

Figure 7: High powered microscope photograph of a browned root (root seen across the top of the picture). Phialides of *T. basicola* growing out of the root surface cells of a *Viola* sp. plant sampled from an untreated plot on 3 July a month after inoculation. Rectangular

endoconidia produced from out of the top of the phialide "tubes" are visible nearby on the right-hand inset photograph.

Figure 8: Small area of pale brown roots (0.5% of plug surface) shown encircled, a month after plug inoculation. These were shown by microscope examination to contain *Thielaviopsis* sp. mycelium and spore-producing phialides. Assessment of the surface area of plugs visibly affected commenced a week later after further infection spread.

Some browning was also seen in the uninoculated treatment, although not in Replicate 2 and there was only a little in Replicate 1 (**Figure 9**). Isolation from brown roots from the uninoculated cells did not show any other consistent fungal presence. *Botrytis* spp. (not usually a root invading fungus) was isolated from uninoculated and inoculated roots, but not any *Fusarium* or *Pythium* spp.. Isolation of black root rot from roots was not expected as in preparation for this work it had not been possible to isolate *T. basicola* from the roots of various ornamental species even when chlamydospores could be seen. Isolation onto agar was only successful after chlamydospores where collected from the surface of a carrot root.

Eight inoculated treatments had significantly less infection than the 36% of plug surface area in the inoculated untreated plots ($P<0.001$) and did not differ significantly in the severity of infection from uninoculated plants, thus indicating that the treatments had given control of black root rot. Both preventative and preventative plus curative treatments of HDC F174, F175 and F176 and preventative application of Signum gave equivalent control to preventative application of Cercobin WP (**Table 6**) with a mean 10% to 20% root rot. Some plugs had no staining; a quarter of plugs given preventative plus curative application of either

HDC F175 or HDC F176 had only white, apparently healthy, roots. 58% of uninoculated plugs had no root browning compared with only 2.5% of inoculated untreated plug plants.

HDC F173, Signum and Switch, whether or not applied also curatively did not differ significantly from the untreated inoculated plots. In plugs where Signum was applied curatively as well as preventively a mean 38% of the root area was stained (with damage high in all replicates) and it is possible that this was a phytotoxic effect causing either direct root damage or increasing plant susceptibility to pathogen entry and/or invasion within the root cells. All, or virtually all, plugs had root damage after the HDC F173, Signum or Switch treatments, this being the same as in the untreated inoculated plots (**Table 6**).

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.

There was no phytotoxicity, and vigour was good across the treatments in the first six weeks of the experiment (Appendix 2). After 20 June, plots had some yellowing; probably they required more feed at flowering, although the plots which received two treatment applications of the fertiliser HortiPhyte had significantly ($P<0.01$) poorer vigour with a mean Index 6.8 compared with Index 7.8 in the untreated inoculated plots. The same crinkling as seen in some plants in Experiment 1 was also seen in two trays. In June and July days with high outside temperatures (Appendix 4) caused the compartment on the sunny end of the glasshouse with the non-conventional products to rise close to 40°C. At destructive assessment it was seen that there had been damage to roots throughout Replicate 1 (which was close against the window) and so this replicate was removed from the analysis (results for this replicate are given in Appendix 3). There were still some differences between the replicates, with Replicates 4 and 5 averaging 22% root rot compared with 7% in Replicate 3 (**Figure 10**). The root browning in Replicates 3 and 6 was scored by a different person and this may account for the lower level e.g. if slight browning was not included.

Table 7. Experiment 2, non-conventional products nine weeks after sowing. Mean foliar vigour index, % surface area of root ball with browning and percentage of plugs with zero browning. Seven products plus Cercobin WG as standard (9, 10 & 14 July 2014).

Treat-ment code	Product name or AHDB Horticulture code	Treat-ment timing	Foliar vigour 1-9 index	% of plug surface area with brown roots*	% of plugs with zero root rot*
1+2	Untreated	n.a.	8.5	18.0	5.3
3	Untreated (not inoculated)	n.a.	8.7	4.0	**74.7**
4	Cercobin WG	P	8.5	20.9	0.0
5	Prestop	P	8.8	21.9	1.3
6	Prestop	P+C	9.0	11.4	**41.3**
7	Serenade ASO	P	8.2	24.3	4.0
8	Serenade ASO	P+C	8.2	20.3	8.0
9	T34	P	8.3	17.6	12.0
10	Trianum G	P	8.8	8.8	**34.7**
11	HortiPhyte	P	8.8	23.1	0.0
12	HortiPhyte	P+C	8.7	18.8	4.0
13	HDC F178	P	**9.0**	11.4	12.0
14	HDC F178	P+C	8.2	**6.3**	**37.3**
15	HDC F179	P	**9.0**	21.6	10.7
16	HDC F179	P+C	8.5	24.0	8.0
	Grand mean		8.6	16.91	16.2
	F value (treatments)		P=0.013	P=0.036	P<0.001
	L.s.d.		0.50	11.68	24.26
	Residual d.f.		76	61	61
	F value (blocks)		P<0.001	P<0.001	P=0.263

* These figures omit Replicate 1
Treatments which differ significantly from the untreated inoculated are shown **shaded**

Significantly (P<0.05) better vigour (Index 9 compared with 8.5 in the untreated inoculated) followed preventative treatment by either HDC F178 or F179. Slightly poorer vigour (mean Index 8.2) was recorded in both Serenade treatments, T34 and the twice applied HDC F178 treatments (**Table 7**). Replicate 6 had significantly (P<0.001) more leaf yellowing (although only a difference between Index 7.9 and Index 8.8) (data for replicates not shown).

The mean severity of root rot in Experiment 2 was 17% of root plug surface stained, compared with 22% in Experiment 1. However the inoculated untreated was 18% affected in Experiment 2 whereas in the conventional treatments experiment this was 35%. Most treatments in both experiments showed a similar range of plug surface area with browning. The majority of

treatment plots in both experiments did however fall within a similar range of root area damage, with grand means of 22% root rot for Experiment 1 and 17% for Experiment 2.

The standard, Cercobin WG, in contrast to Experiment 1, did not reduce the severity of browning from that in the untreated inoculated and most other treatments were equivalent to this. Only preventative plus curative use of HDC F178 with 6.3% root surface area brown was significantly different ($P<0.05$) from the untreated inoculated with 18% damage. Trianum G gave the next lowest severity (8.8%), but there was no significant difference. T34 gave good results in Replicates 3 and 6 (mean 3.7%) (**Figure 10**), but unusually higher results for plants in Replicate 5 thus raised the mean severity to show little overall control. Preventatively used Serenade ASO or HDC F179 had 24% browning which was above, but not significantly greater than, the untreated inoculated. Prestop applied preventatively and curatively and HDC F178 preventative application gave a slight reduction in browning.

A mean 4% browning occurred in the uninoculated plots and levels following Trianum G incorporated into the compost before sowing, or two drenches of Prestop or a single or double spray of HDC F178 did not differ significantly ($P<0.001$, L.s.d. 13.494) from this (**Table 7**).

More significant ($P<0.001$) damage reduction was seen in root browning incidence as shown by the mean proportion of plants in a multi-cell without any root browning (**Table 7**). Whereas 5% of inoculated untreated plants were unaffected 75% of uninoculated had only white roots. Most products had similar incidence to the untreated inoculated. Three products stood out as having a much lower root browning incidence than the inoculated untreated; Trianum G incorporated and the preventatively plus curatively applications of HDC F178 and Prestop with 35%, 37% and 41%, respectively, of plants without root rot.

The proportions of plants with totally healthy roots following the use of non-conventional products were higher than in the conventional treatments. With 58% healthy plants in the uninoculated, the best conventional treatments were the preventative plus curative applications of HDC F175, F176 and F174, which had 27%, 25% and 20% plants without root rot respectively.

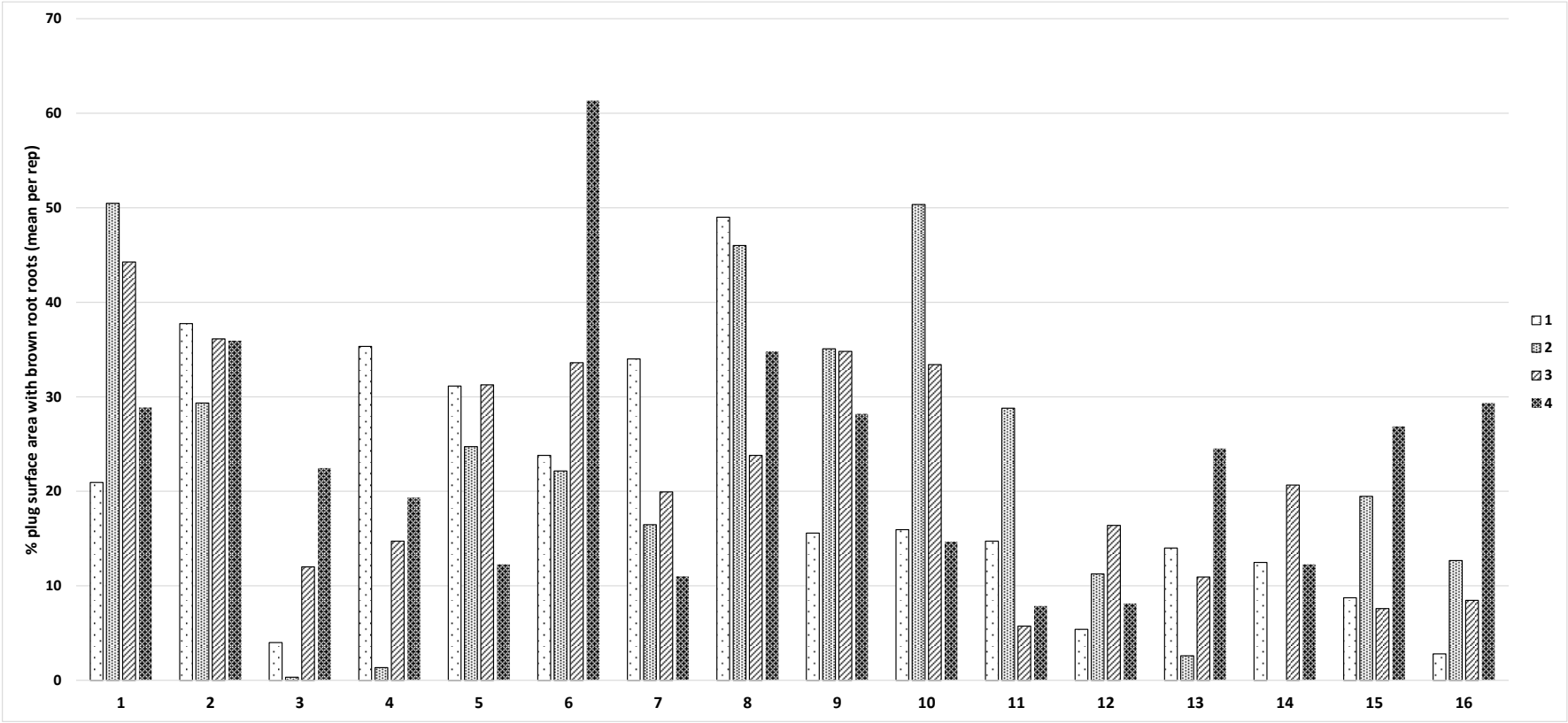

Figure 9: Experiment 1, untreated or treated with conventional products for treatment codes 1 to 16 as shown in table below. % of brown root surface area on plugs/replicate on 10-11 July 2014, 3 months after sowing (1 month after curative drenches)

Treatment code	Product name or AHDB Horticulture code (and timing)	Treatment code	Product name or AHDB Horticulture code (and timing)
1	Untreated	9	Switch (Preventative)
2	Untreated	10	Switch (Preventative + Curative)
3	Untreated not inoculated	11	HDC F174 (Preventative)
4	Cercobin WG (Preventative)	12	HDC F174 (Preventative + Curative)
5	HDC F173 (Preventative)	13	HDC F175 (Preventative)
6	HDC F173 (Preventative + Curative)	14	HDC F175 (Preventative + Curative)
7	Signum (Preventative)	15	HDC F176 (Preventative)
8	Signum (Preventative + Curative)	16	HDC F176 (Preventative + Curative)

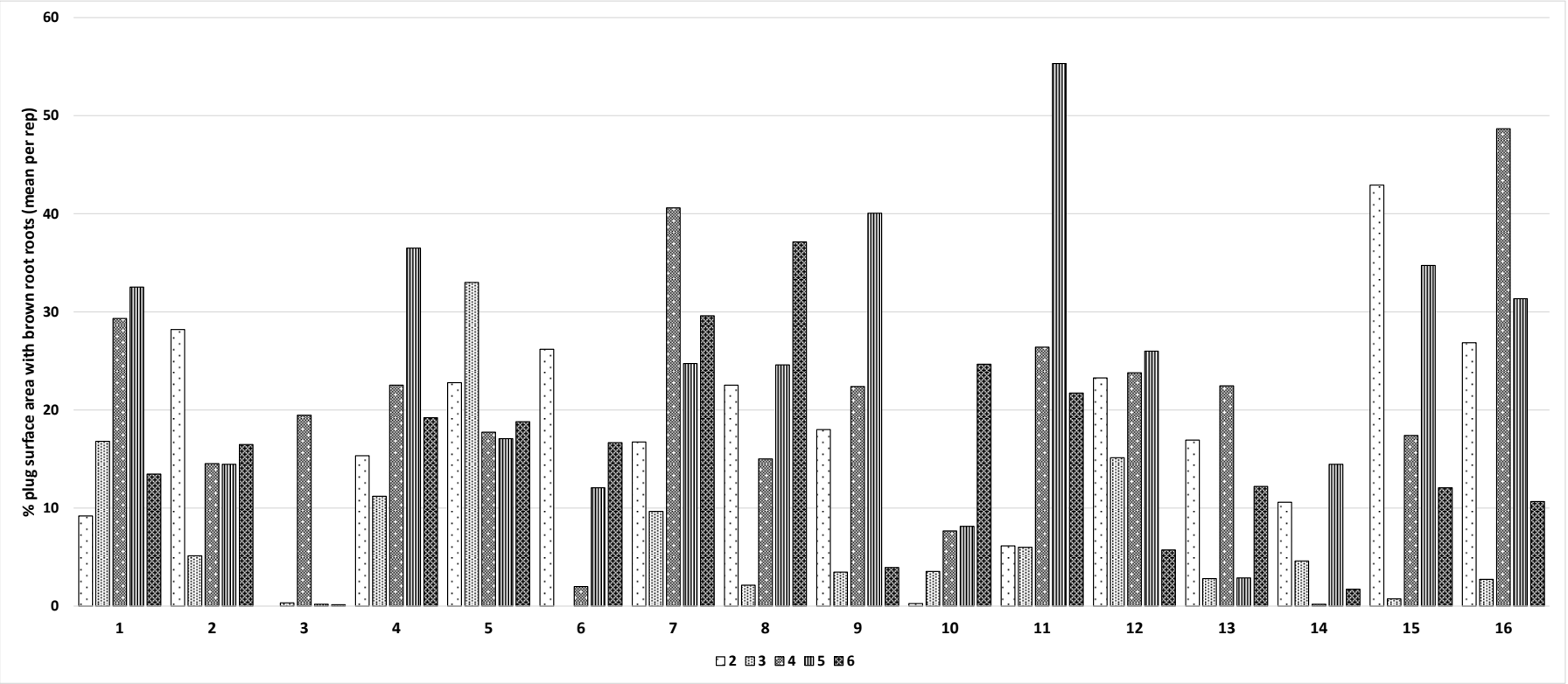

Figure 10: Experiment 2, untreated or treated with non-conventional products for treatment codes 1 to 16 as shown in table below. % of brown root surface area on plugs/replicate between 9-14 July 2014, 3 months after sowing (1 month after curative drenches)

Treatment code	Product name or AHDB Horticulture code (and timing)	Treatment code	Product name or AHDB Horticulture code (and timing)
1	Untreated	9	T34 (Preventative)
2	Untreated	10	Trianum G (Preventative)
3	Untreated (not inoculated)	11	Horti-Phyte (Preventative)
4	Cercobin WG (Preventative)	12	Horti-Phyte (Preventative + Curative)
5	Prestop (Preventative)	13	HDC F178 (Preventative)
6	Prestop (Preventative + Curative)	14	HDC F178 (Preventative + Curative)
7	Serenade ASO (Preventative)	15	HDC F179 (Preventative)
8	Serenade ASO (Preventative + Curative)	16	HDC F179 (Preventative + Curative)

There was no plant collapse in either experiment after leaving the trays for foliar symptoms to develop. Trays were left until mid-August, by which time the plants had outgrown the cells and were going to seed. No differences in plant vigour were seen between the multi-cell trays (data not presented). Sample plants were examined to note the progress of root loss from a range of treatments, but a full assessment was not carried out because of the time since the last treatments. Additional root loss had occurred in all treatments, with 30% to 100% of the plug surface areas covered by pale brown roots. It is possible that the regular overhead automatic watering allowed the plants to survive with few roots. Samples of root plugs taken for isolation on agar plates were examined after seven days; root browning following *T. basicola* inoculation had not been supplemented by root rots such as *Pythium* or *Rhizoctonia* spp.. Some *Fusarium* spp. was isolated from a few of the root pieces, principally those that had not been surface sterilised (indicating it was not inside the roots), and it was not prevalent enough to have been a widespread cause of root discolouration.

When examined on 14 August, as the last flowers were dying off across the trial, it was noted that plants which had received Treatment 12 (two applications of HDC F174) still had flowers (**Figure 11**). This product is known to have a greening effect on plants (Dorin Pop, Bayer, pers. comm.), although this had not been noticed in the earlier vigour assessments. New roots were being produced over the plug surface across most of the treatments.

Figure 11: Plants in plot 17 (arrowed) which received HDC F174 protective plus curative applications continuing to grow and flower on 15 August 2014.

Selection of products from Experiments 1 and 2 for Experiment 3

Experiment 3 was set up to test combinations of products with different modes of action that showed efficacy in Experiments 1 and 2. There was no duplication of a product within a programme in keeping with best practice for active ingredient resistance management. Originally six novel programmes had been proposed, but this was increased to nine with the inclusion of a fourth product.

One product was selected for use at sowing because in commercial use black root rot is likely to infest the growing media straight away from contaminated trays. The microbial product T34 was chosen instead of the incorporated granules of Trianum G (which had shown good efficacy) as it could be drenched.

The conventional products HDC F174 and F175 were selected for preventative and also curative use. This was based on their significantly lower severity and incidence of black root rot when applied as preventative and preventative plus curative treatments and the likely modes of action of the active ingredients. HDC F176 gave comparable efficacy, but this product had become less likely to soon be available to ornamental plant growers.

The non-conventional, chemical, product HDC F178 was selected for preventative use, although it had been more effective in Experiment 2 with a second application, as curative-only use recommendations are unlikely.

Viola sp. cv. White Jump Up for Experiment 3 were sown on 17 July 2014, with destructive assessment anticipated after nine weeks (18 September) and results will be available in the 2015 report. Three alternative treatment timings are being investigated – at sowing (timing A), at 1-2 true leaf (timing B) and a curative treatment one week after inoculation (timing C). The standard programme was again a single drench of thiophanate-methyl at timing B (Table 8). All dose and water rates used in Experiment 3 were the same as used in Experiments 1 & 2 (Table 9). There are four replicate blocks with the same number of plants and arrangement of trays in the glasshouse as used in the earlier experiments.

Table 8. Experiment 3. Viola treatments using products selected from Experiments 1 & 2 with Cercobin WG (Topsin WG) as standard.

Inoculation and Timing C to follow at a 7 and 14 day interval, respectively from Timing B.

Treatment	Treatment Timing A (directly after sowing)	Treatment Timing B (3 wks after sowing)	Inoculation 1 wk after Timing B (4 wks after sowing)	Treatment Timing C 2 wks after Timing B (5 wks after sowing)
T1 not inoculated UT	No treatment	No treatment (water only)	NO spore drench	No treatment (water only)
T2 UT	No treatment	No treatment (water only)	Spore drench	No treatment (water only)
T3	No treatment	Thiophanate-methyl (standard)	Spore drench	No treatment (water only)
T4	No treatment	Thiophanate-methyl (standard)	Spore drench	HDC F174
T5	No treatment	Thiophanate-methyl (standard)	Spore drench	HDC F175
T6	No treatment	HDC F174	Spore drench	No treatment (water only)
T7	No treatment	HDC F175	Spore drench	No treatment (water only)
T8	No treatment	HDC F178	Spore drench	No treatment (water only)
T9	T34	No treatment (water only)	Spore drench	No treatment (water only)
T10	T34	HDC F174	Spore drench	No treatment (water only)
T11	T34	HDC F175	Spore drench	No treatment (water only)
T12	T34	HDC F178	Spore drench	No treatment (water only)

Table 9: Experiment 3. Product rates and timings for application to *Viola* sp.
PA=protectant at sowing timing A
PB=protectant pre-inoculation timing B
CC=curative post-inoculation timing C

Treatment codes	Product	Active ingredient	Time	Product dose & water volume	Comments
T1	Un-treated	none	n.a.	water alone (1000 L/ha)	Not inoculated with black root rot
T2	Un-treated	none	n.a.	water alone (1000 L/ha)	Inoculated with black root rot
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	Fargro Tech. Notes water at 10% container volume EAMU for ornamentals 1118 of 2012
T3, T4, T5	Topsin or Cercobin WG	thiophanate-methyl	PB	1.1 g per 10 L water, using 1000 L diluted product / ha	Standard. One application permitted. Ornamentals EAMU 1887 of 2011. Dose rate for *Thielaviopsis* sp.
T6, T10	HDC F174	confidential	PB	0.35 L / ha using 400 L/ha water	Rate agreed with manufacturer
T7, T11	HDC F175	confidential	PB	0.75 L per ha using 400 L/ha water	Recommended by manufacturer
T8, T12	HDC F178	confidential	PB	25 g/ha with 1000 L water /ha	Experimental rate for young plants given by manufacturer
T4	HDC F174	confidential	CC	0.35 L / ha with 400 L/ha water	Rate agreed with manufacturer
T5	HDC F175	confidential	CC	0.75 L per ha with 400 L/ha water	Recommended by manufacturer

When the work on Choisya sp. is started in 2015 products will be selected for testing from across the three *Viola* sp. experiments.

Discussion

This project showed that some of both the conventional chemical and non-conventional products selected as having potential efficacy against *Thielaviopsis basicola* were able to give a significant reduction in the proportion of the root surface area damaged by black root rot, and in some cases the number of plants without any root damage was significantly greater. The standard product, Cercobin WG as a drench, was unable to stop *T. basicola* infection totally from inoculation a week after application. Four of the chemicals (HDC F174, F175, F176 and Signum), used at spray treatment dose rates gave equivalent control to the 18% root rot seen for Cercobin WG (between 10% and 20% compared with 35.5% in the untreated inoculated). In the separate experiment with the non-conventional products Cercobin WG treated plants had root browning as severe (21%) as the untreated inoculated plants, but HDC F178 performed better, with a mean 6% root rot. Cercobin WG had 100% of plugs affected, but three non-conventional treatments (HDC F177, F178 and Prestop) had significantly fewer root-browned plants.

Trianum G (granule incorporation in growing media) was one of the products with the least root area affected by root rot, but T34 (drenched onto trays after sowing) was selected as the "at-sowing" preventative biological product for re-testing in Experiment 3. Two T34 replicates had minimal infection (Figure 10). The plants in Replicate 4 had exceptionally rotted roots, with several plants in the tray missing, raising the mean level of damage recorded. A protectant drench is the treatment application method also used by the industry standard product. As bedding plants can be sown into very small volume cells, mixing of the granules of Trianum G into the growing media on nurseries would need to be very thorough or some cells would not gain protection. Trianum G can be used in protected ornamental plant production. Prestop has a label recommendation for use against *Thielaviopsis* sp. and this beneficial fungus was confirmed to have reasonable efficacy but only as a two application treatment (which tallies with label instructions). All microbial products are best used before any pathogen is known to be present in order for the beneficial fungi or bacteria to colonise the location before the pathogen arrives and repeat application is often advised so that new plant growth is protected. T34 acts by hyper-parasitism, by producing antibiotic compounds, through competition and by priming the natural defence mechanisms of roots (Fargro Technical Notes www.fargro.co.uk).

There was variability in the severity of infection between plugs. One explanation of this could be that *T. basicola* generates chlamydospores in clusters in roots (**Figure 12**) and on culture plates. The chlamydospores are produced in segmented lengths and the segments then

break off as infection propagules. It is impossible to obtain a spore suspension with individually separated segments and so some plants could receive many more propagules than others. Artificial inoculation has been shown to be variable in success and dependent on the density of propagules in the inoculum with symptomless infection being seen in *Viola* sp. plants inoculated with 100 or 1000 spores (Entwistle, 1996).

Wet compost, heat stress or root damage, and poorly disinfected propagation trays can encourage the build-up of pathogens such as *Pythium* spp. as well as black root rot. To ensure that the appropriate cultural and conventional plant protection product control measures are used growers should confirm the cause of any root browning. Commercially available lateral flow devices can be used by growers to detect Pythium root rot but there is no diagnostic kit for black root rot and so root samples need to be examined in a laboratory. As microbial products and plant activators tend to be less specific to particular plant ailments than conventional products, probably because of their multiple modes of action, they should find a place in nursery plant management programmes. Their adoption should be investigated where insect biocontrol measures are employed, which can be affected by chemical plant protection products.

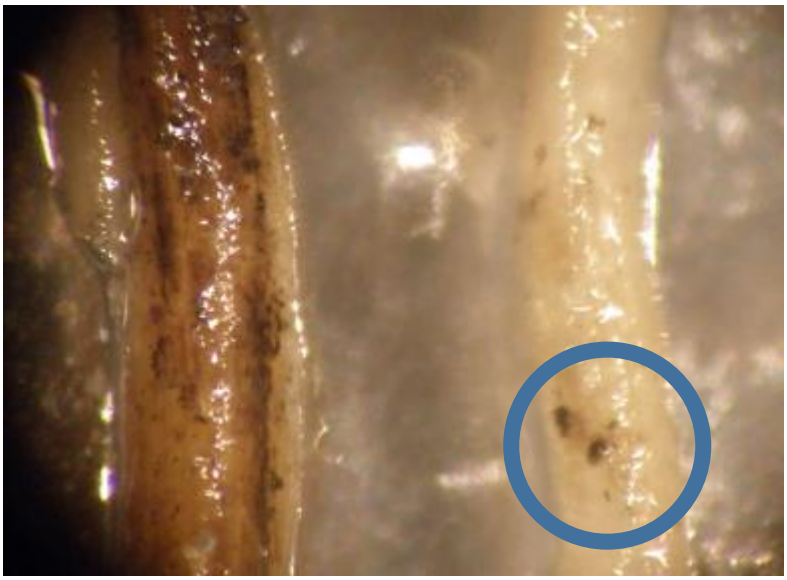

Figure 12:
Low magnification photograph of natural infection by black root rot. Clusters of chlamydospores on the surface of *Viola* sp. roots scattered inside epidermal cells throughout the brown root, and encircled on the whiter root.

Conclusions

- Seven products in addition to Cercobin WG reduced root browning in plug-grown *Viola* sp. inoculated with black root rot *Thielaviopsis basicola,* without phytotoxicity
- Three out of the six conventional (chemical) test products were effective; HDC F174, F175 and F176 used either preventatively alone or also curatively. Signum had benefit if only used preventatively
- Three out of seven non-conventional (microbial and stimulant) products tested were effective; Trianum G before sowing, and either Prestop or F178 applied before and after inoculation. T34 at sowing showed some good results
- Root rot of up to 36% in the untreated inoculated plants was seen nine weeks after sowing, but this did not cause poor plant vigour or any plant death
- After plants were left for another four weeks there was no obvious development of foliar wilting through root loss and there was some healthy root regrowth
- Three novel (HDC F174, F175 and F178) and one approved product, T34, were selected for further testing on *Viola* sp. in programmes on the basis of their activity against black root rot and their likely efficacy in preventative or curative use

Knowledge and Technology Transfer

Information on the project will be provided in AHDB Grower and to the BPOA Technical meeting in October 2014.

Glossary

Key to fungicide groups and target site codes. Information from FRAC Guide 2014

Fungicide group abbreviation	**Full meaning of fungicide group code**	**Target site code**
Bacillus sp. QST713	Microbial disrupters of pathogen cell membranes	F6
DMI	De Methylation Inhibitors	G1
MBC	Methyl benzimidazole carbamates	B1
QoI	Quinone outside Inhibitors	C3
SDHI	Succinate dehydrogenase inhibitors	C2

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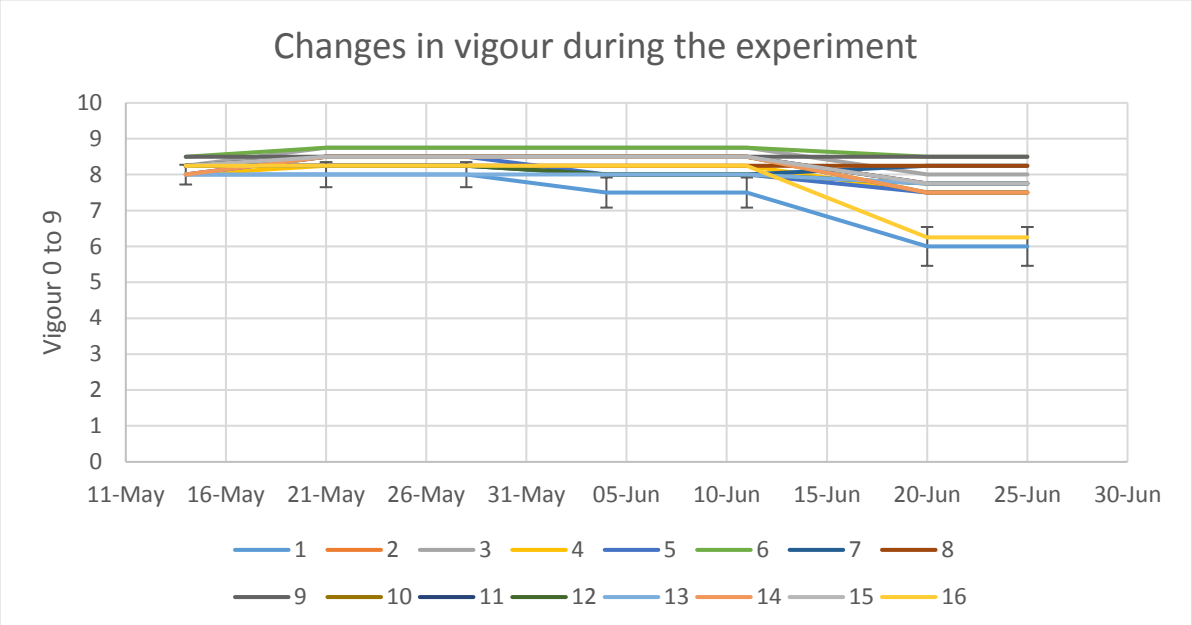
Appendices

Appendix 1

Experiment 1; conventional products. Mean 1-9 vigour index from germination to flowering. T1 lost vigour gradually from the beginning of the experiment and T1 and T16 were significantly different from all other treatments on 20 and 25 June

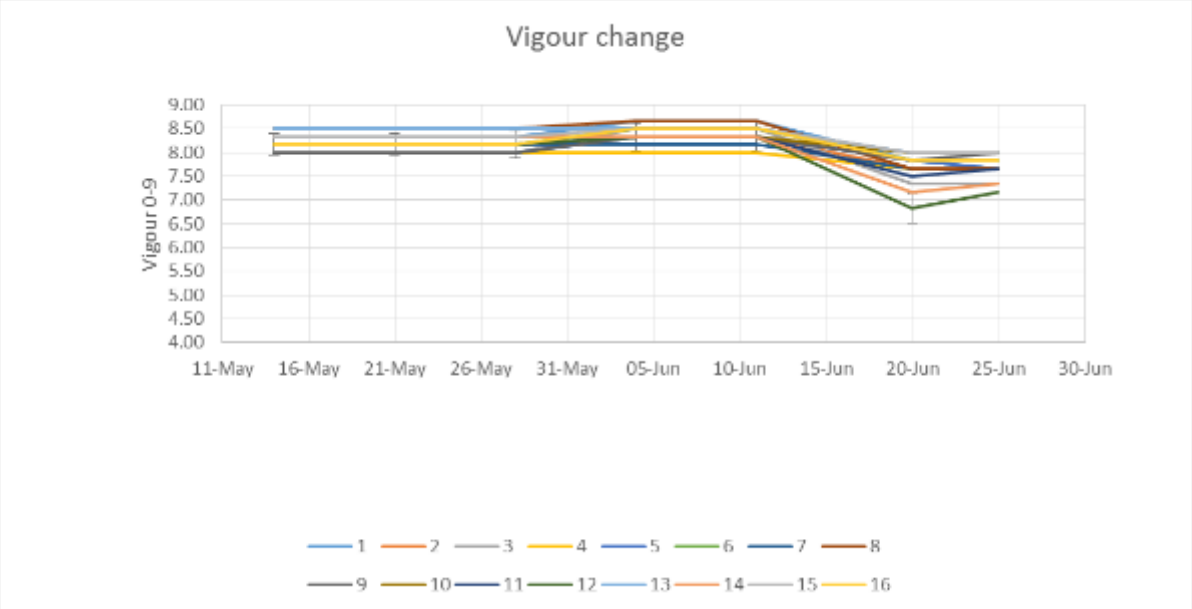
T	Product (Protectant +/- Curative	14 May	21 May	28 May	4 June	11 June	20 June	25 June
1	Untreated	8.0	8.0	8.0	7.5	7.5	6.0	6.0
2	Untreated	8.0	8.5	8.5	8.5	8.5	7.8	7.8
3	UT no inoculum	8.2	8.8	8.8	8.8	8.8	8.0	8.0
4	Cercobin WG (P)	8.0	8.3	8.2	8.2	8.2	7.5	7.5
5	HDC F173 (P)	8.0	8.5	8.5	8.0	8.0	7.5	7.5
6	HDC F173 (P+C)	8.5	8.8	8.8	8.8	8.8	8.5	8.5
7	Signum (P)	8.2	8.2	8.2	8.0	8.0	8.2	8.2
8	Signum (P+C)	8.2	8.2	8.2	8.2	8.2	8.2	8.2
9	Switch (P)	8.5	8.5	8.5	8.5	8.5	8.5	8.5
10	Switch (P+C)	8.2	8.5	8.5	8.5	8.5	7.5	7.5
11	HDC F174 (P)	8.2	8.5	8.5	8.5	8.5	7.8	7.8
12	HDC F174 (P+C)	8.2	8.2	8.2	8.0	8.0	7.8	7.8
13	HDC F175 (P)	8.0	8.0	8.0	8.0	8.0	7.8	7.8
14	HDC F175 (P+C)	8.0	8.5	8.5	8.5	8.5	7.5	7.5
15	HDC F176 (P)	8.2	8.5	8.5	8.5	8.5	7.8	7.8
16	HDC F176 (P+C)	8.2	8.2	8.2	8.2	8.25	6.3	6.3
	F value	0.679	0.654	0.654	0.265	0.265	0.001	0.001
	S.e.d.	0.2713	0.3489	0.3489	0.4177	0.4177	0.5445	0.5445
	L.s.d.	0.5465	0.7026	0.7026	0.8413	0.8413	1.0968	1.0968
	cv block	2.1	2	2	1.8	1.8	5.1	5.1
	cvblock unit	4.7	5.9	5.9	7.1	7.1	10.1	10.1



Appendix 2

Experiment 2; non-conventional products. Mean 1-9 vigour index from germination to flowering showing no difference between treatments except on 20 June when T12 is less vigorous than all treatments except T14.

T	Product (Protectant +/- Curative)	14 May	21 May	28 May	4 June	11 June	20 June	25 June
1	Untreated	8.3	8.3	8.3	8.7	8.7	7.8	7.7
2	Untreated	8.2	8.2	8.2	8.3	8.3	7.7	7.7
3	UT no inoculum	8.0	8.0	8.0	8.5	8.5	7.3	7.3
4	Cercobin WG (P)	8.0	8.0	8.0	8.0	8.0	7.7	7.7
5	Prestop (P)	8.0	8.0	8.0	8.3	8.3	7.8	7.7
6	Prestop (P+C)	8.2	8.2	8.2	8.5	8.5	7.8	7.8
7	Serenade ASO (P)	8.2	8.2	8.2	8.2	8.2	7.7	7.7
8	Serenade ASO (P+C)	8.5	8.5	8.5	8.7	8.7	7.7	7.7
9	T34 (P)	8.0	8.0	8.0	8.3	8.3	7.8	8.0
10	HDC F177 (P)	8.2	8.2	8.2	8.3	8.3	8.0	8.0
11	HortiPhyte (P)	8.2	8.2	8.2	8.3	8.3	7.5	7.7
12	HortiPhyte (P+C)	8.2	8.2	8.2	8.3	8.3	6.8	7.2
13	HDC F178 (P)	8.5	8.5	8.5	8.5	8.5	8.0	8.0
14	HDC F178 (P+C)	8.3	8.3	8.3	8.3	8.3	7.2	7.3
15	HDC F179 (P)	8.3	8.3	8.3	8.5	8.5	8.0	8.0
16	HDC F179 (P+C)	8.2	8.2	8.2	8.5	8.5	7.8	7.8
	F value	0.423	0.423	0.423	0.794	0.794	0.01	0.233
	S.e.d.	0.2264	0.2264	0.2264	0.2925	0.2925	0.3012	0.315
	L.s.d.	0.451	0.451	0.451	0.5827	0.5827	0.6001	0.6275
	cv block	1.6	1.6	1.6	1.4	1.4	2.8	2.1
	cvblock unit	4.8	4.8	4.8	6	6	6.8	7.1



Appendix 3

Experiment 2; non-conventional products. Mean % of root surface area browned showing results for all replicate blocks. Presenting results for replicate 1 which removed from analysis as the browning of plugs included damage in very hot weather

Treat-ment code	Product name or AHDB Horticulture code	Treat-ment timing	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
1	Untreated	n.a.	17.7	9.2	16.8	29.3	32.5	13.5
2	Untreated	n.a.	48.9	28.2	5.1	14.5	14.5	16.5
3	Untreated, Un-inoculated	n.a.	69.3	0.0	0.3	19.5	0.2	0.1
4	Cercobin WG	P	41.3	15.3	11.2	22.5	36.5	19.2
5	Prestop	P	49.1	22.8	33.0	17.7	17.1	18.8
6	Prestop	P+C	22.6	26.2	0.0	2.0	12.1	16.7
7	Serenade ASO	P	44.7	16.7	9.7	40.6	24.7	29.6
8	Serenade ASO	P+C	22.7	22.5	2.1	15.0	24.6	37.1
9	T34	P	17.7	18.0	3.5	22.4	40.1	3.9
10	HDC F177	P	23.2	0.3	3.5	7.7	8.1	24.7
11	HortiPhyte	P	45.8	6.1	6.0	26.4	55.3	21.7
12	HortiPhyte	P+C	27.5	23.3	15.1	23.8	26.0	5.7
13	HDC F178	P	50.8	16.9	2.8	22.5	2.9	12.2
14	HDC F178	P+C	3.0	10.6	4.6	0.2	14.5	1.7
15	HDC F179	P	37.6	42.9	0.7	17.4	34.7	12.1
16	HDC F179	P+C	27.5	26.9	2.7	48.7	31.3	10.7
	Mean % rot		**34.3**	**17.9**	**7.3**	**20.6**	**23.4**	**15.3**

Significant difference between blocks P<0.001 L.s.d. 8.44 with replicate 1, P<0.001 L.s.d. 7.54 without replicate 1.

Appendix 4

Maximum and minimum daily temperatures outside at ADAS Boxworth throughout May, June and July 2014 during Experiment 1 and 2. (Rainfall also shown)