Project title:	Black root rot in containerised subjects – chemical and biological options for control
Project number:	PO 014
Project leader:	Erika F. Wedgwood, ADAS
Report:	Scoping study
Previous report:	none
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Location of project:	ADAS Boxworth, Boxworth, Cambridge, CB23 4NN
Date project commenced:	1 April 2013
Date project completed (or expected completion date):	30 April 2013

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Scoping Study: Black root rot in containerised subjects – chemical and biological options for control

Host range and symptoms

The fungus *Thielaviopsis basicola* (syn. *Chalara elegans*) is the causal agent of black root rot. The fungus infects a wide range of hosts, including plants from at least 15 families, and can be found in all parts of the world. In addition to crops such as cotton, tobacco, strawberry, tomato, *Phaseolus* beans, peas and clover, a wide range of ornamental plants are susceptible to *T. basicola* (Walker, 2008) including those shown in Table 1. *T. basicola* is a serious threat to pansies, petunias, and vinca. It may also infect cyclamen, calibrachoa, poinsettia, primula, impatiens, snapdragon, verbena, phlox, begonia, and nicotiana (Harlan and Hausbeck, 2012). *T. basicola* has also been implicated as the probable causal agent of the specific replant diseases of certain *Prunus* species (Sewell and Wilson, 2008).

Latin name	Common name
Antirrhinum majus	Snapdragon
Astilbe spp.	Astilbe
Begonia semperflorens	Begonia
Buxus sempervirens	Boxwood
Camellia japonica	Camellia
Clematis spp.	Clematis
Cyclamen persicum	Cyclamen
<i>Euphorbia</i> spp.	Spurges
<i>Fuchsia</i> x hybrid	Fuchias
Gerbera jamesonii	Gerbera
Hypericum calycinum	St. Johns Wort
<i>llex</i> spp.	Holly
Kalanchoe spp.	Kalanchoe
Lathyrus spp.	Sweet Pea
<i>Paeonia</i> spp.	Peony
Pelargonium spp.	Geranium
Penstemon spp.	Penstemon
Petunia spp.	Petunia
Poinsettia spp.	Poinsettia
Syringa vulgaris	Lilac
Verbena x hybrida	Verbena
<i>Viola</i> spp.	Violet / Pansy
Zantedeschia spp.	Arum / Calla Lilies

Table 1. Some ornamental plants susceptible to infection by T. basicola

The fungus belongs to the Phylum Ascomycota, Class Sordariomycetes, Subclass Hypocreomycetidae, Order Microascales, Family Ceratocystidaceae, Genus Thielaviopsis

(www.fieldmycology.net). Close relatives, in the Ceratocystidaceae, are the tree pathogens *Ceratocystis* spp. including Dutch Elm disease, oak and sycamore wilts. Fungicides active against other Ascomycetes (rather than the Basidiomycetes such as rusts, *Rhizoctonia* spp. and *Armillaria* spp.) may have efficacy against *T. basicola*. Fungicides directed at Phycomycetes (water-moulds) are less likely to be effective. The Ascomycete *Chalara fraxinea* (which causes ash dieback) has the same anamorph (asexual stage), the Chalara state, as black root rot and so fungicides with potential efficacy for *C. fraxinea* may also be effective for *C. elegans*. However, the sexual state of ash dieback, *Hymenoscyphus pseudoalbidus* is not closely related to *T. basicola*.

The root symptoms on ornamentals range from grey and brownish discolouration and little obvious rotting through to black discolouration and extensive loss of root mass. Above ground there is a reduction in growth and increasingly obvious foliage discolouration, followed by wilting and death (Trebilco *et. al.*, 1999). Plants are stunted and below ground black, rough, longitudinal cracks can develop (Raabe *et. al.*, 2005). Symptoms of black root rot are often mistaken for nutrient deficiencies. Leaves may turn yellow and the youngest leaves become stunted and tinged with red. In mild infections, older leaves are yellow-green with the veins retaining their green colour (Harlan and Hausbeck, 2012). The speed and severity of infection and symptoms depend on a wide range of factors including the susceptibility of the host species and cultivar and the strain of the fungus present (Trebilco *et. al.*, 1999). In pansy, infections usually start at root tips and through root hairs and the roots of infected plant plugs often fail to grow beyond the original plug after transplanting (Pscheidt & Copes, 2008).

A decline problem in *Choisya* spp. has been part attributed to Thielaviopsis root rot. Examination of plant clinic records for *Choisya* spp. over nearly ten years up to 2008 at CSL York (now Fera) and The Royal Horticultural Society (RHS), Wisley was made as part of HDC Project HNS 169 (Talbot and Wedgwood, 2009). Among root problems, *T. basicola* was as commonly recorded as *Phytophthora* spp. at CSL (as commonly as stress / damage). The RHS mainly received garden plants rather than nursery samples, and found *Phytophthora*, and in particular *Armillaria* spp. root rots. At both clinics the causes of plant decline were frequently not able to be diagnosed. Where diagnosis of root rotting had been sought, *Phytophthora, Thielaviopsis, Fusarium* and *Pythium* species had been identified on *Choisya* spp. on some of the 21 UK nurseries surveyed (Talbot and Wedgwood, 2009).

Black root rot has been identified as a major disease problem in Australian nurseries since 1993 where it has affected a wide range of crops. The disease has very frequently been

misdiagnosed as being due to fertiliser injury, underfeeding, heat and cold injury, overwatering, under-watering, *Rhizoctonia, Pythium* or *Cylindrocladium,* fungus gnats and nematodes. Although symptoms can be seen within a week in pansies, in perennial crops symptoms may take months or years to become obvious (Trebilco *et. al.*, 1999).

Control measures

Information on the control of black root rot, downy mildew and *Ramularia* leaf spot diseases on pansy and viola was given in HDC Factsheet 19/02 (Jackson and McPherson, 2002).

Cultural controls

T. basicola produces two types of conidia; brown macroconidia (aleuriospores) which break up into resting spores (chlamydospores), produced by the Thielaviopsis state; non-pigmented microconidia (endoconidia) produced by the Chalara state. The chlamydospores can survive in the air, dust and debris, whereas microconidia are likely to be easily spread in water (Powell, 1989). The fungus is capable of prolonged saprophytic survival in soils. Soil at between 17 to 23°C, 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) (Raabe *et. al.*, 2005). Walker (2008) confirmed that favourable conditions for the fungus include a pH between 5 and 8.5 and also a soil temperature between 12 and 21°C (55 and 70 °F).

Based on observations of crops with *T. basicola*, the following recommendations were given to pansy and vinca growers in Iowa to prevent the occurrence of black root rot (Koranski, 1989);

- Use new plug trays and pots
- Maintain pH of growing medium at 5.5 to inhibit disease growth and development
- Keep soluble salts low
- Regulate ammonium concentrations to be less than 15 ppm
- Do not use fertilisers and fungicides after transplanting plugs until after roots have emerged from the rootball
- Bark or sludge incorporation in the growing medium usually decreases *Thielaviopsis* incidence

When four growing media compositions (peat, peat/fine bark, peat/fine woodfibre, peat/Fi-Pro straw) were compared with pansy inoculated by drenching with *T. basicola* no difference was seen between them in the level of root rot. However, it is possible that the plants would have produced more black roots and allowed differences to be seen if they had been under greater stress (O'Neill, 1992). Stress produced by too low or high temperatures, nutrient imbalance (in particular in pansies excess ammonia nitrogen) or growing media pH above 5.5 can cause disease expression (Powell, 1989). Oregon State University advice is to check electrolyte concentrations of growing media regularly and they noted that pansies developing good root systems before winter often tolerate black root rot (Anon, 2013).

T. basicola was one of the causes of root rot in *Choisya* sp. recorded from 21 UK nurseries surveyed as part of HDC Project HNS 169. *Choisya* sp. liner losses to root rotting were equally divided between nurseries which had 5% or less, 6-15% or 16-50% of plants affected. Growers most commonly reported 5% or less lost at the final pot size. The three growers using the highest percentage of bark (30 or 33%) or wood fibre (30%) in the final potting mix, reported significantly lower average losses (of less than 5%) (Talbot and Wedgwood, 2009).

Crop hygiene (such as clean trays) is emphasised as being important to reduce inoculum spread by debris and air borne contamination. In addition, fungus gnats and shore flies can move *T. basicola* around a greenhouse by eating the spores and excreting them into nearby pots (Harlan and Hausbeck, 2012). It was shown in replicated trials that the proportion of pansy tray plants with *T. basicola* is significantly lower when plants are grown raised on mesh raised about 300 mm from the ground rather than on the floor (T. O'Neill, pers. comm.)

Resistance to T. basicola

Resistant nicotiana cultivars have been developed (Subramanian, 1968). A cultivar comparison of *Calibrachoa* (Daughtrey, 2005 and 2006b) indicated that Cabaret varieties Red, Cherry Rose, White Scarlet, Apricot and Purple; Superbell varieties Blue, Pink Kiss, Trailing Rose and Blue; and Million Bells varieties Cherry Pink and Crackling Fire were highly resistant to Thielaviopsis root rot, while Superbell White was slightly susceptible. All cultivars tested were much more resistant than Million Bells Terra Cotta.

Pansy cultivars resistant to Thielaviopsis root rot reported by Oregon State University (Anon, 2013) include; Clear Sky Primros, Clear Sky blue, Clear Sky White, Crown Golden, Delta blue W/Blotch, Delta Pure Violet, Delta White W/Rose Wing, Fama Blue Angle, Fama Darkeyed White, Fama Love Me, Fama See Me, Fama Silver Blue and Happy Face Yl/Blotch.

Hollies vary in their resistance to *T. basicola*: *Ilex crenata* cultivars Helleri, Hoogendorn, Nigra, Green Cushion, Mobjack Supreme and Hetzzi are very susceptible. *Ilex vomitoria* and *Ilex opaca* are moderately resistant. *Ilex aquifolium* and *Ilex cornuta* are highly resistant (Moorman, 2012).

Breeding or selecting existing cultivars for resistance or tolerance should be possible, possibly using inoculated trials. Molecular methods involving identifying particular areas of the genetic make-up that may be contributing to resistance (as for example being done with strawberry breeding lines) could also be utilised.

Stimulants of plant defences

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 such as chitosan, laminarin, salicyclic acid and the salicylic acid derivative BTH benzo(1,2,3) thiadiazole-7-carbothioic acid S-methyl ester. BTH protects against a broad spectrum of pathogens (Rivière *et al.*, 2011).

Crop treatment with elicitors should not be considered to be replacements for fungicide application, but to be used as supplements that allow fungicide application to be reduced. Treatments with elicitors provide 20-85% disease control (Rivière *et al.*, 2011). The directions for use of the stimulant acibenzolar-S-methyl (1,2,3-Benzothiadiazole-7-carbothioic acid S-methyl ester) against bacterial pathogens of mangoes and tomatoes in South Africa state Bion 50 should be applied with suitable copper compounds. Recently Bion Plant Activator Seed Treatment (acibenzolar-S-methyl) product registration has been supported by Australian agricultural authorities (Anon, 2007). This is a cotton seed treatment which is an activator of systemic acquired resistance (SAR) and gives disease suppression of Fusarium wilt and black root rot. It should be used as a component of an integrated management strategy. Bion is recommended as a cotton seed treatment (Mondal *et al.*, 2005). Myclobutanil and acibenzolar-S-methyl seed dressings used in combination on cotton gave lower root discolouration than when used alone (Toksoz *et al.*, 2009). Bion would be available for testing for potential use on ornamentals in the UK, although a change of use would be required for a drench application (J. Ogborne, Syngenta, pers. comm.).

Cecropin-A a naturally derived antimicrobial peptide inhibits the growth of pathogens including *Phytophthora infestans*, *Fusarium* spp. and *T. basicola*, and there is potential for the utilisation of this chemical by application or via genetic incorporation of the peptide into plants (Cavallarin *et al.*, 1998).

Biocontrol products

Growing-medium incorporation of products containing *Trichoderma* species, such as DCM Bio-Fungus Instant and Trianum (carried out by the growing media supplier), were reported by UK nurseries to have reduced losses post-potting of *Choisya* sp. (Talbot and Wedgwood, 2009). Where root rot diagnosis was obtained by growers, via clinic samples or Lateral flow Devices (LFDs) then *Phytophthora* spp., *Pythium* spp. or *T. basicola* were present. Compost tea products were widely used bio-stimulants (eight out of 21 growers) with use as a foliar spray rather than through the irrigation supply during the winter when the root ball was sufficiently wet. Agralan Revive (*Bacillus subtilis*) was used by three of the surveyed growers. Potassium phosphite foliar fertiliser was used by four growers. Two growers were evaluating the benefits of Wormcast tea. The effects of these products on black root rot have not been reported.

Products containing Trichoderma harzianum strain T-22 produced by BioWorks Inc. are available in Canada for use on outdoor and protected ornamentals against root diseases. RootShield formulations as either granules for incorporated into growing media or as a wettable powder used during watering are available. Rootshield Plus also contains Trichoderma virens strain G-41 and has T. basicola in addition as a target pathogen on the label. The products are EPA-registered biofungicides for use in California where a wettable powder formulation PlantShield HC can also be used as foliar spray (Anon, 2008b). All the products provide preventative control of Thielaviopsis sp., Pythium sp., Rhizoctonia sp., Fusarium sp., Sclerotinia sp. and Cylindrocladium sp. by the Trichoderma sp. competing for rhizosphere space and food with pathogens and producing enzymes which dissolve the cell walls of many pathogens. Systemic acquired resistance, a mechanism whereby plants' own resistance mechanisms are triggered to function in response to the presence of particular Trichoderma, Pseudomonas or Bacillus species (Jacobsen, 2007), functions in addition to niche occupation and enzymatic action. Few fungicides tested in use on ornamentals were incompatible with the *Trichoderma* in PlantsShield HC and RootShield (www.bioworks.com) e.g. Terraguard SC (triflumizole) but they can still be used with an interval of 14 days. Products with thiophanate-methyl, azoxystrobin, fludioxonil, myclobutanil or phosphorous acid are compatible (Anon, 2008a). Disease and insect management information for poinsettias and integration of biological and chemical products is available (Anon, 2012).

Trichoderma harzianum strain Rifai T-22 is available in the UK to promote root health as the non-pesticide registered product Trianum (Gwynn, 2009). 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.

Prestop (32 % w/w *Gliocladium catenulatum* strain J1446) can be used on outdoor ornamentals as a spray at 6 kg/ha to soil, as a drench at 500g/100L and to growing media at 500g/m³. The product is approved for the control of *Botrytis cinerea*, and root diseases caused by species of *Pythium, Phytophthora, Rhizoctonia* and *Fusarium*. It is also approved for the moderate control of *Didymella*. The Fargro technical leaflet (Anon, 2011) states that Prestop can control *Thielaviopsis, Verticillium, Alternaria, Helminthosporium* and *Penicillium*.

T34 Biocontrol (10.3% 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 118 of 2012 allows use on other 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. The existing EAMU covers its use on protected and outdoor container grown ornamentals and could be used under this approval against black root rot (Paul Sopp, Fargro, pers. comm.).

Biocontrol products available to USA growers and their target pathogens are listed by Thomas (2009) and McGrath (no date). The only product with Thielaviopsis specifically listed as a target is Rootshield Plus, others referring to root pathogens generally. However, control of *T. basicola* on citrus seedlings in peat-based media was obtained post-infestation at high drench rates and volumes of Mycostop (*Streptomyces griseoviridis* K61) (Graham, 1994).

Biocontrols such as Rootshield Plus (also known as Plantshield) (*Trichoderma harzianum* T-22 + *T. virens* G-41), Companion (*Bacillus subtilis* GB03), and Actinovate (*Streptomyces lydicus* WYEC108) directly incorporated into the growing media as granular powders during the mixing process or as drenches soon after planting are recommended in California against root rots of ornamentals as well as monthly fungicide drenches (Styer, 2013).

Growing-media amendments such as chitin and biochar have been shown to enhance the establishment of biocontrol agents in growing media. These materials, biochar in particular, provide a good platform for establishment of beneficial microbes such as *Trichoderma* spp. and mycorrhizal fungi (Downie *et al.* 2009; Warnock *et al.* 2007). *Bacillus subtilis* seed treatment and root drenching of pepper plants with 0.5% chitin added became more effective against *Phytophthora capsici* and *Rhizoctonia solani* root rot. *Trichoderma harzianum* use as a drench was also more effective on *P. capsici* with chitin supplement (Ahmed *et al.*, 2003).

Disinfectants / soil sterilisers

Chlamydospores of *Thielaviopsis* are highly resistant to chemical sterilants and studies have been carried out in Australia (Trebilco *et. al.*, 1999) and the UK (O' Neill, 1995) to enable selection of the most effective products for disinfecting re-used pots, trays and general nursery hygiene. HDC project PC 38c compared the efficacy of disinfectants on plastic plug trays naturally contaminated with *T. basicola* and showed that although there was no complete control that Formalin (38% formaldehyde), lodel FD (2% iodine) and Jet 5 (hydrogen peroxide/peroxyacetic acid) were particularly effective, although others also reduced root rot incidence in the pansies grown in the disinfected trays (O'Neill, 1995). Treatment of glasshouse surfaces with sodium hypochlorite was effective against endoconidia and aleuriospores of *T. basicola*, but a quaternary ammonium (QAC) caused no reduction in propagule viability (Copes and Hendrix, 1996). However, it is known that the effectiveness of disinfectants is affected by the nature of the contaminated surface and that, while the germination of *T. basicola* chlamydospores can be prevented, on surfaces such as cement, wood and clay the variation in survival can be particularly great (Voss and Meier, 1987).

TerraClean (<u>www.enviroselects.com</u>) is an EPA registered bactericide/fungicide available in the USA for use against soil pathogens such as *Thielaviopsis*. It can either be mixed with bed-setting water at planting time using banded or broadcast application, or used in drip irrigation systems. The active ingredients are peroxyacetic acid and hydrogen peroxide. According to the manufacturers, the oxidation reaction penetrates the soil profile, killing and

suppressing pathogens on contact, with trials results showing suppression of *Pythium* and *Phytophthora* in tomato and *Rhizoctonia* on lettuce.

In Australia in 2007 there were no fungicides registered or effective against black root rot (*T. basicola*) in cotton and work on biofumigation of soil with marigolds and onions is being carried out (Cross, 2007).

Fungicides

Details of products, active ingredients, rates and target pathogens in addition to the information given below are available from the websites given in the References section of this report.

The effectiveness of benomyl against black root rot was shown in several pieces of work (Manning *et al.*, 1970), but resistance issues caused the withdrawal of the product. Drenches of Bavistin DF (carbendazim) (50 ml/pansy) gave a significant reduction in the proportion of roots affected by black root rot (from 16% down to 1%) in an inoculated experiment (O'Neill, 1992). Bavistin DF is not now available in the UK and Cercobin WG (thiophanate-methyl) under SOLAs 165 of 2011, 1827 of 2011 and 1987 of 2011 is now used as the standard drench or granule incorporation into growing media against black root rot in protected ornamentals. However, there is concern that product overuse will result in resistance.

HDC project PC 143 (Jackson, 2000) screened a number of fungicides against black root rot on pansy bedding plants. The greatest reductions in infection severity (0-100) from the untreated at 58% to 23% were with Folicur (tebuconazole) and 33% with Genie (flusilazole), but these both caused leaf scorch. Neither product has UK approval for use on ornamentals. Other products used as drench treatments reduced root rot severity without any leaf or root damage, these were Amistar (azoxystrobin), Bavistin DF (carbendazim), F279 (trifloxystrobin), Scotts Octave (prochloraz), , Plover (difenoconazole), Unix (cyprodinil) and Stroby WG (kresoxim-methyl). Another three products were ineffective. Cyprodinil is now only available in the UK mixed with fludioxonil as Switch.

UK Choisya growers commonly utilised chemical treatments against root rotting (Talbot and Wedgwood, 2009) including Subdue (metalaxyl-M) which the manufacturers (Fargro) say is not effective against *T. basicola*, Aliette 80 WG (fosetyl-Al) (now withdrawn from the UK), which would be expected to control Oomycetes but not *T. basicola*, and Scotts Octave (prochloraz) for which the manufacturers claim a reduction of the fungal pathogen complex which includes *T. basicola* as well as the control of *Cylindrocarpon* spp., *Fusarium* spp. and

Phomopsis spp. root rots and wilts. Surveys of pansy growers in the USA, backed up by research, indicated that where Subdue (metalaxyl-M) was being used for *Pythium* and *Phytophthora* control that black root rot was worse (Powell, 1989).

In a fungicide trial at Cornell University, New York, with Thielaviopsis-inoculated Calibrachoas (Daughtrey, 2005), only those treated with thiophanate-methyl (Cleary 3336 WP, 16 oz per 100 gal) showed no symptoms, dry weight reduction, or sporulation on the roots. Drenches with triflumizole (Terraguard, 4 oz per 100 gal), phosphorous acid salts (Alude, 12.5 oz per 100 gal), and Trichoderma harzianum T-22 (Plant Shield, 5 oz per 100 gal) all reduced symptoms, whereas azoxystrobin (Heritage, 0.9 oz per 100 gal) and fludioxonil (Medallion, 2 oz per 100 gal) gave no benefit; azoxystrobin appeared to exaggerate symptoms (Daughtrey, 2005). Oregon State University advice is that Medallion WG (at 1 to 2 oz per 100 gal) should be used on pansies as a protectant before symptoms occur since it is ineffective once plants are infected (and also note that to avoid plant damage it should not be used with oils or adjuvants (Anon, 2013). Other fungicides to be used in combination with cultural controls were listed as polyoxin-D zinc salts (Affirm, 0.25 to 0.5 lb per 100 gal), thiophanate methyl + etridiazole (Banrot 40 WP, 4 to 8 oz/100 gal water per 800 sq ft of bed area), Terraguard SC (2 to 8 fl oz per 100 gal), thiophanate-methyl products Cavalier 50 WSB (12 to 16 oz per 100 gal), Cleary 3336 WP (12 to 16 oz per 100 gal), or OHP 6672 (7.5 to 20 fl oz per 100 gal), and polyoxin-D zinc salts (Veranda O, 0.5 lb per 100 gal water used as a drench (Anon, 2013).

A trial of materials for *Thielaviopsis* control on pansy indicated that plants were best protected against symptom development by an experimental product with an undeclared active ingredient (A9219B, 1 oz per 100 gal) as well as by a thiophanate methyl drench (Cleary 3336, 16 oz per 100 gal); only thiophanate methyl treated plants showed no *Thielaviopsis* sporulation on the roots. Further studies on *Thielaviopsis* root rot of calibrachoa and pansy (Daughtrey, 2006a) showed that only thiophanate-methyl treatment eliminated root colonization by *T. basicola*. Plants given other chemical treatments that suppress symptoms still had chlamydospores on the root system that were detectable by microscopic observation.

The advice for the control of *T. basicola* in ornamentals by the various University extension services of the different USA states is to drench monthly using one of the products containing thiophanate-methyl alternated with either a chemical or a biological product for resistance management (Daughtrey, 2006a; Chase, 2013; Walker, 2008 and Anon, 2013). A summary of products recommended in the USA is given at the end of this report in Table 5.

Styler (2013) recommends a tank mix of products so that water-moulds *Pythium / Phytophthora* and the fungi *Thielaviopsis, Fusarium* and *Rhizoctonia* are controlled. Five products were recommended against water-moulds and three other products, Medallion, Cleary 3336 or OHP 6672 against *Thielaviopsis, Fusarium* and *Rhizoctonia*. Banrot (thiophanate methyl + etridiazole) and Hurricane (mefenoxam + fludioxonil) were said to be good ready-to-use tank mixes. Vegetable and flower seeds treated with myclobutanil are effective for control of *T. basicola* (Olsen, 2011). Myclobutanil spray application (Nu-Flow M-HF) is recommended in California (Davis, 2012) for the control of Thielaviopsis on cotton seedlings as well as triadimenol (Baytan 30). Post *T. basicola* infestation drenches of benomyl, myclobutanil and thiophanate methyl were more effective in reducing citrus seedling root rot than pH adjustment in peat-based media (Graham, 1994).

Based on Michigan State University (MSU) studies, fungicides that have thiophanate-methyl as the primary active ingredient (e.g. Cleary 3336 F) should be used frequently. Good rotational products include Terraguard SC (triflumizole) and Medallion WG (fludioxonil) since they have a different mode of action and were shown to be effective in MSU studies against black root rot. It was recommended to use the highest labelled rate of each treatment with close reapplication intervals (Harlan, and Hausbeck, 2012). Trials in California on chemical rotations for ornamentals showed thiophanate-methyl rotation with Veranda O gave the best results (Chase, 2013, Chase, no date). Veranda O is not registered for use in the UK but checks are in progress to determine if it is registered in Europe as this will make UK registration easier. The products used in Pennsylvania shown in Table 2 illustrate that products have been selected from different FRAC groups to reduce the chance of resistance to thiophanate-methyl. Phosphorous acid is registered as a pesticide in the USA (Table 3), but only available as a fertiliser in the UK in the products Hortiphyte and FarmFos 44. It is widely known to have activity as a drench against *Phytophthora* spp., but the work by Daughtrey (2005) suggests it also has efficacy against T. basicola. Table 4 summarises the products recommended for black root rot control in ornamentals by USA extension services.

Table 2. Active ingredients and trade names of plant protection products in use in Pennsylvania (USA) as soil and peat growing media drenches against *T. basicola* on woody and herbaceous ornamentals (Mooreman, 2012)

FRAC Group No.*	Risk Leve	LIASS	Active ingredient	REI Restricted Entry Interval	Trade names (EPA Reg. no.)
1	3	Benzimidazole	thiophanate methyl	12h	Cleary 3336 (1001-69), OHP 6672 (51036-329- 59807)
					Fungo Flo (51036-329-59807)
					Systec 1998 (48234-12)
3	2	Imidazole	triflumizole	12h	Terraguard (400-433)
3	2	Triazole	myclobutanil	24h	Systhane (707-253) Eagle (62719-417)
12	2	Phenylpyrol	fludioxonil	12h	Medallion (100-769)
1 + M			thiophanate methyl + etridiazole	12	Banrot (58185-10)

Table 3. Phosphorus acid USA pesticide registration details in the USA. A chemical also shown to have activity against black root rot (Daughtrey, 2006)

FRAC Group No.	Risk Leve	l ^{Class}	Active ingredient	REI Restricted Entry Interval	Trade names (EPA Reg. no.)
U	1	Phosphite	phosphorus acid salts	4h	Agri-Fos (71962-1-54705)

* **Fungicides and Fungicide Resistance Management** – Certain fungicides, usually systemic fungicides, are said to be 'at risk' to the development of resistance if they are used repeatedly. See the Risk Level in the above table (1 = low risk; 3 = high risk). The Fungicide Resistance Action Committee has developed a numbering system in which chemicals with the same FRAC Group number have the same mode of action (See

<u>http://www.frac.info/frac/index.htm</u>). It is recommended that chemicals at high risk be used sparingly and in rotation or mixed with chemicals with different modes of actions (different FRAC number).

Table 4. Products recommended by various USA state extension services for use in alternation with thiophanate-methyl on ornamentals to prevent the build up of resistance

Products available in the USA	Active ingredient
Cleary 3336 or OHP-6672 or Fungo Flo or Cavalier	thiophanate-methyl (target <i>T. basicola</i> on label)
Alternated with Alude	phosphorous acid
Banrot 40 WP	thiophanate-methyl + etridiazole
Baytan 30 (on cotton)	(target <i>T. basicola</i> on label) triadimenol
Compass O	trifloxystrobin
Heritage	azoxystrobin
Hurricane	mefenoxam + fludioxonil
Medallion or Mozart TR (protectant use)	fludioxonil
Palladium	fludioxonil + cyprodinil
Terraguard SC	triflumizole
Veranda O or Affirm WDG	polyoxin-D zinc salts
PlantShield HC	Trichoderma harzianum T22
RootShield Plus (BW240)	<i>Trichoderma harzianum</i> T-22 + <i>T. virens</i> G-41 (target <i>T. basicola</i> on label)
Systhane, or on cotton Nu-Flow M-HF	myclobutanil (also as a seed dressing)

Several of the active ingredients shown by studies to have activity against *T. basicola* are not available in products approved in the UK for ornamentals (on-label or Extension of Authorisation for Minor Use (EAMU)), these include polyoxin-D zinc salts, fludioxonil (used in the UK as a cereal seed treatment), flusilazole and triflumizole. Tebuconazole was available as Folicur but is now only registered as Bezel (a wound paste). Tebuconazole (+ trifloxystrobin) is present in Nativo 75 WG, but this product has no approvals for ornamentals. Products with potential activity against black root rot which are available for spray application in the UK for use on ornamentals include Amistar (azoxystrobin), Scotts

Octave (prochloraz), Signum (boscalid + pyraclostrobin), Switch (cyprodinil + fludioxonil) and Stroby WG (kresoxim-methyl). Drenching could require a higher dose per ha of the product and this would require a change of use.

There are a number of strobilurins that have been reported to have activity against the root infecting pathogen *Rhizoctonia* and so might also be effective on roots against *T. basicola*, these include fluoxastrobin (Disarm), pyraclostrobin (in Signum, or alone in Insignia WDG or Empress) and trifloxystrobin (Compass O). Nativo could have activity against black root rot (Ian Cockram, Bayer CropScience pers. comm.) – tebuconazole and trifloxystrobin as separate active ingredients were effective as pansy drenches against *T. basicola* (Jackson, 2000). Chemical and biological plant protection products in the UK "pipeline" which would be worth testing against black root rot are HDC F139, HDC F140 and HDC F141 (pers. comm.).

Some of the plant protection products shortlisted as candidates for Defra funded field and laboratory testing against Chalara fraxinea (Table 4) may also have relevance for T. basicola (syn. Chalara elegans) control. Two of the most important criteria used for selecting products were 1) products that had shown activity against Chalara fraxinea or other species with a similar lifecycle such as apple scab (Venturia inaequalis) 2) products were already registered as plant protection products in the UK, elsewhere in the EU or were close to achieving registration. In addition, potassium phosphite will be tested as a stimulant of host resistance in field trials using ash saplings (Fera, 2013). The experimental product HDC F139 was also put forward by BASF for ash dieback control and might be suitable against black root rot (Simon Townsend, pers. comm.). Another active, chlorothalonil (e.g. Bravo 500), which has approval for used on outdoor ornamentals in the UK (or Folio Gold which also contains metalaxyl for use on protected ornamentals under EAMU 0032 of 2012), should also be considered (Peter Gladders, ADAS plant pathologist, pers. comm.). SDHI (succinate dehydrogenase inhibitor) actives such as boscalid (which is component of Signum and Bellis) are very effective chemicals against Ascomycetes (S. Townsend and P. Gladders pers. comm.). Both boscalid and pyraclostrobin can be taken up by roots when applied as a high volume spray, although an approved use as a drench might need to be developed for use against black root rot (Simon Townsend, BASF, pers. comm.). Prothioconazole is systemic and could be suitable for treating black root rot, but the addition of bixafen (as in Aviator 234 Xpro) might not be worthwhile as it is not systemic (lan Cockram, Bayer CropScience, pers. comm.) and so would not give movement into roots.

Table 5. Initial products recently selected for testing for fungicidal activity against *Chalara fraxinea* in laboratory tests for the control of ash dieback

Active Ingredient	Product	Fungicide Group	Manufacturer
Myclobutanil	Systhane 20EW	Triazole	Dow Agrosciences Ltd
Cyproconazole	Alto 100 SL	Triazole	Syngenta Crop Protection Ltd
Prothioconazole	Proline	Triazole	Bayer CropScience AG
Fenbuconazole	Indar 5EW	Triazole	Dow Agrosciences Ltd
Flutriafol	Consul	Triazole	Cheminova
Azoxystrobin	Amistar	Strobilurin	Syngenta Crop Protection Ltd
Fluxapyroxad	Imtrex	SDHI	BASF plc
Bixafen + prothioconazole	Aviator 235 Xpro	SDHI/triazole	Bayer CropScience AG
Boscalid +pyraclostrobin	Signum	SDHI/strobilurin	BASF plc
Mancozeb	Dithane 945	Dithiocarbamate	InterFarm (UK) Ltd
Pyrimethanil	Scala	Anilinopyrimidine	BASF plc
Dithianon	Dithianon WG	Quinone	BASF plc
Garlic extract (allicin)	n/a	Organosulfur	To be confirmed
Copper oxychloride	Cuprokylt FL	Inorganic copper	Universal Crop Protection

Conclusions

- Thiophanate-methyl drenches are the mainstay of *Thielaviopsis* black root rot control programmes across the world. Resistance management measures for thiophanate-methyl include alternation with other chemical and biological pesticides (see Table 4).
- Alternative chemical products to Cercobin WG (thiophanate-methyl) with likely activity against *T. basicola* are available in the UK for use on ornamentals including Switch (cyprodinil + fludioxonil), Systhane 20EW (myclobutanil), Scotts Octave (prochloraz), Signum (boscalid + pyraclostrobin) and Amistar (azoxystrobin). Only Scotts octave is currently approved as a drench treatment.
- Laboratory and plant screening on both herbaceous and hardy ornamentals for the
 effectiveness of fungicides in use in the USA that are present in UK registered
 products (including those only registered for other crops) together with other products
 from the *Chalara fraxinea* testing programme should be carried out. Novel antifungal
 compounds such as the cecropin-derived peptides should be included.
- The biological products Prestop (*Gliocladium catenulatum*), Serenade ASO (*Bacillus subtilis*) and T34 (*Trichoderma asperellum*) and Trianum (*Trichoderma harzianum*) can be used on ornamentals in the UK and activity against *T. basicola* has been reported. Other biological control organisms, such as *Streptomyces griseoviridis* K61, could also be considered for experimental use.
- The use of chitin or biochar supplements to increase the effect of biological control organisms in growing media should be considered.
- Research in the USA has shown that potassium phosphite use can be beneficial on ornamentals with Thielaviopsis root rot. Its inclusion in the Chalara ash dieback programme in the UK also supports the suggestion that it should be tested against black root rot on ornamentals.
- There has been recent approval in Australia for the use of the bio-stimulant Bion (acibenzolar-S-methyl) in cotton as a seed treatment against *T. basicola* based on Australian government field trials and its use should be investigated for ornamentals as a seed treatment and as a spray or drench.
- Growing media physical and chemical characteristics have been known for some time to affect Thielaviopsis root rot incidence and severity in ornamentals, in particular using bark mixtures at pH 5.5. Black root rot infection increases with plant stress and so a combined approach with cultural controls with the use of plant protection products should be recommended.

- Where feasible, growers should plant resistant varieties and selection for resistance should be part of the breeding selection process.
- Up to date information is required on current range of disinfectant products to determine their efficacy against black root rot. Removal of inoculum at the start of the production process is key to the control of black root rot in ornamentals.

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A search of the United States Department of Agriculture Database (which links to the AGRICOLA database) gave 110 references to Thielaviopsis which included information on the pathogen epidemiology. A search engine enquiry on "Thielaviopsis fungicide" produced 19,900 results including multiple and duplicate references to USA Extension service advice on cotton, tobacco and ornamentals by a limited number of authors. A Scopus and a Google scholar search did not produce many refereed papers for *T. basicola. Thielaviopsis basicola* is the current name for black root rot, reverting in 2006 to this name after a period as *Chalara elegans* see; British Mycological Society record for *Thielaviopsis basicola*. http://www.indexfungorum.org/Names/SynSpecies.asp?RecordID=119974

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Plant protection product information

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