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DISCLAIMER:

Authentication

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

Signature ………………………………………………………………………………………

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Official recognition certificate (HRI Stockbridge House)

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PRACTICAL SECTION FOR GROWERS

Commercial benefits of the project

This project has identified a number of novel and experimental fungicides for the control of black root rot (*Thielaviopsis basicola*) and downy mildew (*Peronospora violae*) on Pansy. Some of the novel fungicides identified could play an important part in integrated spray programmes to maintain control of these diseases, whilst at the same time minimising the development of fungicide resistance. Preliminary discussions are underway with agrochemical companies to seek more information regarding their plans for specific products. Further efforts will need to be made to secure legal use of specific products via the On- or Off-Label route for use under protection.

Background and Objectives

Black root rot caused by *Thielaviopsis basicola* and downy mildews (eg *Peronospora viola* on pansy), are two of the most common and insidious disease problems facing growers of ornamental and bedding plants in the UK. These pathogens are common, especially on subjects such as pansy. They cause a reduction in plant quality and, when severe, extensive plant losses can occur. Results from research on the use of chemical disinfectants on contaminated plug trays, pots and standing areas against *T. basicola* have provided an appreciation of the problems associated with this particular pathogen and demonstrated the efficiency of chemical disinfectants for reducing inoculum levels in these areas (O'Neill, 1995 & 1996; HDC project PC 38c).

Previously Bavistin (carbendazim) and Octave (prochloraz) have given good control of black root rot (Scrace, 1993; HDC Project PC 38b). However, more recently there have been reports of reduced performance of products containing carbendazim, possibly due to a build up of resistant strains of *T. basicola*. Several fungicides with potential activity against both *T. basicola* and downy mildews have been approved on broad acre (eg cereals) crops in the UK in recent years though little or no work has been conducted recently to identify those most effective for use on bedding plants. Several fungicides, but predominantly Fongarid (furalaxyl), have been used widely for downy mildew control in recent years. However, once again there is growing evidence that resistant strains may now be present in pathogen populations; though this remains largely unsubstantiated.

This project was commissioned following concerns regarding the relatively poor level of control attained using currently approved fungicides against black root rot (*T. basicola*) and downy mildew (*P. violae*) among bedding plant growers in the UK.

The objectives of this project were to:

1. determine whether isolates of both pathogens retained sensitivity to the commonly used and approved fungicides or whether resistant strains predominated in the population.
2. evaluate a range of novel fungicides for their efficacy against *T. basicola* (black root rot) and downy mildew in bedding plants.
3. determine the relative crop safety of the experimental fungicides on a range of bedding plant subjects.

Pansy was selected as a typical bedding plant species prone to attack by both *T. basicola* and downy mildew (*P. violae*). Information produced from this work relating to Pansy should have immediate application to similar disease problems in other bedding plant species providing potential differential phytotoxic reactions on different host species are taken account of.

Summary of results and conclusions

Years 1 and 2 (1997-1999)

Samples of Pansy (*Viola* spp.) plant material, exhibiting characteristic symptoms of downy mildew (*P. violae*) and black root rot (*T. basicola*) were collected from a range of UK nurseries. The fungal isolates used in this study were recovered from this plant material, as described in the first Annual Report for 1997.

Screening tests carried out *in vitro* for resistance of *T. basicola* towards carbendazim indicated that resistant strains were not common in the fungal population sampled during this study. No evidence of metalaxyl resistant strains of *P. violae* was recorded during the screening trials on Pansy plants.

In the first two years of this project a range of novel and experimental fungicides were screened initially *in vitro* against the root pathogen *T. basicola*. Their relative performance was determined by measuring inhibition of radial growth of mycelium of the fungus. Following this laboratory work the most effective fungicides were assessed on plant material to measure potential phytotoxicity towards bedding plants. Nineteen fungicides, including 2 approved (standard) and 17 novel (experimental) fungicides, were applied to 10 different bedding plant species. Symptoms assessed were foliage scorch, plant stunting, inhibition of flower development and, in some cases, death of the growing point. Assessment of plant quality, 14 days after fungicide application identified a number of fungicides that did not affect the quality or 'saleability' of the bedding plant material. These included Amistar (azoxystrobin), Stroby WG (kresoxim-methyl), Fytospore (cymoxanil), Bavistin (carbendazim), Aliette (fosetyl-aluminium), Fongarid (furalaxyl), Octave (prochloraz), Plover (difenoconazole), Invader (dimethomorph + mancozeb), Unix (cyprodinil), SL567 (metalaxyl), Bion (‘plant activator’), Aura (fenpropimorph), Alto 240 (cyproconazole) and Shirlan (fluazinam). Three fungicides, Folicur (tebuconazole), Torch (spiroxamine) and Topas (penconazole), caused severe scorching and stunting, over the range of bedding species tested.

In addition, in the second year of this project a replicated efficacy screen on Pansy plants inoculated with a spore suspension of *T. basicola* was used to assess the performance of ten experimental fungicides. Application of the fungicides Amistar and Octave resulted in a significant reduction in disease symptoms of black root rot (*T. basicola*) similar to those obtained by the currently approved fungicide Bavistin.

<u>Year 3 (2000)</u>

In the third and final year of this project further efficacy screens were carried out to identify novel chemicals with the potential to control one or both of these two important pathogens of pansy. Seven fungicides, Octave, Bavistin, Plover, Stroby WG, F279, Unix and Amistar all reduced levels of black root rot on Pansy after inoculation with a suspension of *T. basicola* chlamydospores. Although application of Folicur led to the greatest reduction of black root rot infection, plant quality was severely reduced through scorching and stunting of the treated plants.

After continued technical difficulties in obtaining consistent reproducible infection of *P. violae* on host plant material during the first two years of this project, two efficacy experiments were successfully carried out to assess a range of novel fungicides against downy mildew. A number of chemicals significantly reduced the disease including Favour (metalaxyl + thiram) and Shirlan where downy mildew was eliminated completely. Application of SL567A (metalaxyl-M), Filex (propamocarb HCl), Trustan (cymoxanil + mancozeb + oxadixyl), Curzate M (cymoxanil + mancozeb), Amistar (azoxystrobin) Stroby WG (kresoxim-methyl), Fongarid (furalaxyl) and Aliette (fosetyl-aluminium) also significantly reduced downy mildew infection compared to the control.

During the experiments conducted in the third year of this project a natural but severe outbreak of leaf spotting symptoms caused by the fungal pathogen *Ramularia* occurred. Characteristic symptoms of this disease are leaf spotting which often develop on the leaf margins under high disease pressure and can quickly spread, particularly with overhead watering, across the leaves leading to defoliation. Amistar and Octave significantly reduced these leaf spotting symptoms. The currently approved fungicide Bavistin, whilst providing some control of *Ramularia* infection, was not as effective as Amistar or Octave in this respect.

Action points for growers

- Good hygiene practices are essential to reduce black root rot and downy mildew diseases on nurseries.

- Used trays should be cleaned with a disinfectant such as Jet 5 (Per acetic acid).

- Standing bedding plant trays on the glasshouse floor, particularly where there is contact with debris or soil, increases the risk of disease infection.

- The fungicide efficacy screens against black root rot disease (*T. basicola)* have demonstrated that the currently approved fungicide Bavistin can still be effective in controlling black root rot on pansy though it should not be used repeatedly, as there is a danger of fungicide resistance.

- The fungicides screened during this project and their efficacy for the control of black root rot, downy mildew and *Ramularia* diseases are listed in the Table overleaf together with their current approval status.

- Growers must note that several of the experimental fungicides evaluated in this work are **not** currently approved for use under protection and therefore cannot legally be applied to bedding plant species to control the relevant diseases.

- Growers need to be aware that approval will need to be sought for one or more of the experimental fungicides identified during this project. This should then allow more flexible disease control strategies that will limit the development of fungicide resistance within the pathogen populations. The implementation of anti-resistance strategies involves the alternation of products containing active ingredients with different modes of action.

Efficacy and approval status of the fungicides evaluated in this study

Fungicide Product	Active Ingredient	Products evaluated in this study			Approval Status [1]
		Black Root Rot	**Downy Mildew**	***Ramularia* leaf spot**	
Aliette	fosetyl-aluminium	-	**X**	-	Approved
Amistar	Azoxystrobin	4	4	4	Approved*
Bavistin	Carbendazim	4	-	**X**	Approved*
Curzate M	Cymoxanil + mancozeb	**X**	-	**X**	Not Approved
Dorado	Pyrifenox	**X**	-	**X**	Not Approved
Favour[2]	Metalaxyl + thiram	-	4	-	Approved[2]
Filex	Propamocarb hydrochloride	-	4	-	Approved
Folicur	Tebuconazole	**X**	-	X	Not Approved
Fongarid[3]	Furalaxyl	-	4	-	Approved[3]
F279	Trifloxystrobin	4	-	**X**	Not Approved
Genie	Flusilazole	**X**	-	**X**	Not Approved
Invader	Dimethomorph + mancozeb	-	4	-	Not Approved
Octave	Prochloraz	4	-	4	Approved
Plover	Difenoconazole	4	-	**X**	Approved*
Unix	Cyprodinil	4	-	**X**	Not Approved
Scala	Pyrimethanil	**X**	-	**X**	Approved*
Shirlan	Fluazinam	-	4	-	Not Approved
SL567A	Metalaxyl-M	-	4	-	Not Approved
Stroby WG	Kresoxim-methyl	4	4	**X**	Not Approved
Trustan	Cymoxanil + mancozeb + oxadixyl	-	4	-	Not Approved

Key:

4 = Efficacious and no phytotoxicity, X = Not efficacious, - = not tested.

Shaded area denotes Currently Approved products

[1] Where the approval is based on either the Long Term Arrangements or Specific Off-Label Approval they are signified by an asterisk (*).

[2] Metalaxyl based products to be replaced with metalaxyl-M, an entantiomeric form of the same molecule. Future for metalaxyl-M based products for use under protection to be determined by manufacturers.

[3] Product unsupported at Round 1 of EU Review Programme.

It is important for Growers to check the approval status of any fungicide, before application to the crop, with the product label recommendations or current edition of the Long-Term Arrangements for Extension of Use.

- The phytotoxicity screen provided an indication of the relative safety of the fungicides tested on a range of bedding plant species (see Table). The potential for phytotoxic damage has been demonstrated highlighting the importance of ascertaining the correct application rate to avoid phytotoxic symptoms whilst maintaining effective disease control. It should, however, be noted that different varietal reactions may occur and growers should test any new chemical on a small number of plants in each cultivar grown before applying to the whole crop.

- Growers should remember that where fungicides are approved on ornamentals either via the Long-Term Arrangements for Extension of Use (2000) or by a Specific Off-Label Approval, use of the product is at their own risk and manufacturers carry no liability for poor performance or crop safety.

Practical and financial benefits from the study

The value of the UK bedding industry is estimated to be in excess of £300 million with the 'farmgate' value of autumn and spring pansies alone in the region of £70 million. Losses due to basal rots, of which black root rot is the primary cause, vary from season to season and from nursery to nursery, but overall can be as high as 10% in some years. With downy mildew losses are again difficult to estimate accurately, but up to 10% of plants have been discarded in some seasons due to this disease. Infection levels in individual nurseries can be much higher. In the ornamentals industry even a low level of infection by either pathogen can render plants unthrifty and therefore of lower marketable quality. If left untreated, disease severity increases and the plants become unsaleable. Therefore, the results from this project provide immediate practical benefit to bedding plant growers as the control of these diseases should be improved on individual nurseries which should increase financial returns.

SCIENCE SECTION

Introduction

Black root rot caused by *Thielaviopsis basicola* is a common disease problem in ornamental, bedding plant and several other crops in the UK. Symptoms of this disease on bedding plants include yellowing, stunting and even plant wilting and death. Examination of infected roots show dark-grey or black root lesions along their length. For more information on the symptoms and biology of this disease refer to the recently published HDC Identification Card Series (McPherson & Jackson, 1998a). The use of chemical disinfectants on contaminated plug trays, pots and standing areas (O'Neill, 1995 & 1996) against *T. basicola* have indicated the scale of the problems associated with this particular pathogen as well as demonstrated the efficiency of chemical disinfectants for reducing inoculum levels in these areas. However, the pathogen is a soil-borne fungus and is capable of living as a saprophyte in debris for many years as thick walled spores (chlamydospores) (Powell, 1991). *T. basicola* also produces asexual spores (conidia) which can be distributed on debris, by water splash or via drainage water (Biddulph, 1996). Therefore, effective control measures for established infections need to be sought.

Downy mildew diseases are generally host specific and the fungus attacking pansy *(Peronospora violae)* is no exception. Indeed, it is one of the most common diseases on pansy though does not cross-infect to other bedding plant species. *P. violae* is recognised by the production of large numbers of spores on the undersides of the leaves exhibited as a purple-brown 'felt' on the undersides of the leaves with an accompanying chlorosis on the upper leaf surface. Sometimes the stems are also infected and the whole plant may become distorted. The spores (sporangia) are readily wind-borne or spread by water splash and can germinate in water or at a high humidity to produce swimming spores (zoospores) which infect through the stomata on the lower leaf surface. This fungus grows and spreads through plant tissues. For more information on the symptoms and biology of this disease, refer to the recently published HDC Identification Card Series (McPherson & Jackson, 1998b). Little work to study resistance or fungicide efficacy has been conducted on downy mildews in bedding plants, although work on downy mildew on other hosts e.g. lettuce is likely to be of some significance (McPherson & Jackson, 1998c). Work in France (Boudier, 1987) assessed a range of chemical fungicides for control of downy mildew on pansy although most of the chemicals evaluated in the study reported were either not available in the UK or not approved for use on ornamentals under protection.

Whilst there are numerous fungicides with potential activity against *T. basicola* and *P. violae,* little work has been done to identify those most effective against these diseases in bedding plant species. Previous work has shown carbendazim and prochloraz to provide good levels of control of *T. basicola* (Scrace, 1993), although there have also been anecdotal reports that carbendazim is not giving the desired level of control, possibly due to the development of resistant strains of this fungus. Several fungicides, but particularly Fongarid (furalaxyl), have been used widely for downy mildew control, although again there is growing evidence that resistant strains are now present in the pathogen population.

The scientific targets of the final year of the project were to:

- Conduct a replicated experiment to evaluate the efficacy of fungicides selected during preliminary screening experiments towards *T. basicola*.

- Conduct a replicated experiment to evaluate the efficacy of fungicides using a range of novel fungicides towards *P. violae*.

Materials and Methods

Isolate Collection, Maintenance and Inoculum Preparation

Collection and maintenance of isolates of *Thielaviopsis basicola*

A virulent isolate of *T. basicola* isolated from Pansy plant root samples received in the Plant Clinic, HRI Stockbridge House in Autumn 1999 was used for all experiments involving *T. basicola.*

Isolates of *T. basicola* collected from infected plant material were maintained on Potato Dextrose Agar (PDA) and incubated at 20^{o}C and sub-cultured at 21 day intervals. In order to maintain pure cultures of each isolate of the fungus, a 3 mm diameter agar core was taken from the colony edge aseptically and placed centrally on a PDA plate. Agar plates were maintained in an incubator at 20^{o}C. Where long-term storage was required individual isolates were placed on agar slopes in sterile Universal Containers and placed in a refrigerator at 2-3^{o}C (+/- 1^{o}C).

Preparation of *Thielaviopsis basicola* inoculum

For the glasshouse efficacy trial a spore suspension of *T. basicola* chlamydospores was prepared from 14-21 day old cultures of *T. basicola* grown on amended V8 agar (Scrace, 1993). The plates were flooded with l0 ml sterile distilled water and the surface of the colony scraped using a plastic spreader. The fungal suspension was transferred to a food blender and blended at the maximum speed for 20 mins (Biddulph, 1996) in order to create a single spore suspension of approximately 3,500 whole chlamydospores/ml.

Collection and maintenance of isolates of *Peronospora violae*

Infected plant material received from commercial nurseries in the UK, courtesy of Mr S Coutts and through the Plant Clinic, HRI Stockbridge House, was sampled for *Peronospora violae.* A number of isolates were used to inoculate live Pansy plant material maintained in an isolated glasshouse unit at Stockbridge House. The plants were maintained under day/night temperatures of 16^{o}C /10^{o}C and were covered in a polythene tent at night to increase humidity and encourage leaf infection. Plants were also watered from above to maintain leaf surface moisture to encourage spore dispersal and infection to surrounding plants.

Inoculation of plant material with *Peronospora violae*

The trials to assess the efficacy of experimental fungicides against *P. violae* were conducted in a polythene tunnel at HRI Stockbridge House. Pansy plant material infected with *P. violae* was placed in the experimental trial area to allow natural infection of downy mildew into the treated plants. The tunnel was closed overnight to increase the humidity and polythene sheeting was laid over the plant material to increase the chance of infection. In addition, to encourage infection and to allow natural dissemination of downy mildew spores the plants were watered from above to maintain leaf surface moisture.

Evaluation of fungicides for the control of *Thielaviopsis basicola*

The most promising fungicides selected during the first two years of this project were assessed for efficacy towards *T. basicola* on pansy plant material in a replicated glasshouse trial. This included two novel chemicals (Genie (flusilazole) and Dorado (pyrifenox)) that were screened *in vitro* in Year 2. Eleven fungicide treatments and a control treatment were laid out in a randomised block design with four replicates per treatment. Within each replicate plot, there were six individual pansy plants. Fungicide treatments were applied either as a drench at the time of inoculation or 14 days after inoculation at the rates detailed in Table 1.

Plant material and trial location

Pansy cv. Ultima plug plants were obtained from a commercial nursery. The trial was located in Glasshouse Unit 1, Headley Hall, Leeds University Field Station, Tadcaster.

Treatments

The candidate fungicides selected based on the results of the *in vitro* fungicide screen, the phytotoxicity trial and efficacy trial in Year 2 are listed below in Table 1.

Inoculation of the compost with *T. basicola* chlamydospores

A chlamydospore suspension containing 3500 spores/ml was prepared as described earlier. The suspension was mixed thoroughly with Levington M3 potting compost at a rate of 500 ml to 10 litres of compost prior to planting and application of the fungicidal treatments.

Table 1: Experimental fungicides screened on Pansy plants cv. Ultima for the control of *T. basicola.*

Treatment	Application Rate[+]	Time of Application	
		At inoculation	Post-inoculation
Control (water)	-	4	4
Bavistin (carbendazim)	1.0g product/litre water	4	4
Plover (difenoconazole)	1.0ml product/litre water	4	4
Folicur (tebuconazole)	1.875ml product/litre water*	4	4
Unix (cyprodinil)	0.67g product/litre water	4	4
Scala (pyrimethanil)	5ml product/litre water	4	4
Amistar (azoxystrobin)	0.8ml product/litre water	4	4
Stroby (kresoxim-methyl)	0.5g product/litre water	4	4
F279 (trifloxystrobin)	2ml product/litre water	4	4
Genie (flusilazole)	1.0g product/litre water	4	4
Dorado (pyrifenox)	0.25g product/litre water	4	4
Octave (prochloraz)	1.0g product/litre water	4	4

[+] All treatments were applied as a compost drench

* The treatment rate used in this experiment was reduced from the previous efficacy studies in year 2 in an attempt to maintain efficacy but to reduce the risk of phytotoxicity.

<u>Fungicide application</u>

Fungicides were applied using a hand held sprayer (Hozelock 31 Premier).

<u>Crop diary</u>

Inoculation:	2 December 1999
Planting:	2 December 1999
Fungicide application (at inoculation):	2 December 1999
Fungicide application (post-inoculation):	14 December 1999
Vigour, stunting and scorching assessment (1):	12 January 2000
Vigour, stunting and scorching assessment (2):	14 February 2000
Trial termination:	16 February 2000
Disease assessment of roots:	16 February 2000
Root dry weight:	18 February 2000
Foliage dry weight:	18 February 2000

Assessments

During the trial, individual plants were assessed for overall plant quality (0-3), the degree of foliage scorch (0-3) and degree of stunting (0-3) ranging from 0 - low quality/no symptoms to 3 - high quality/severely affected.

At trial termination, a destructive assessment was carried out to confirm whether *T. basicola* had infected the root system and was the cause of any root discoloration and plant decline. Dry weights of root and foliage plant material were calculated per plot.

The plant vigour and foliage scorch index, expressed in the Results section, was calculated from 0-3 assessments as follows:

$$\frac{1(\text{No in category 1}) + 2(\text{No in 2}) + 3(\text{No in 3})}{\text{No of plants assessed}} \times \frac{100}{3}$$

The range of this index was, therefore, zero (poor vigour or quality) to 100 (high vigour or quality).

Evaluation of fungicides for the control of *Peronospora violae*

Considerable difficulties were experienced in obtaining consistent infection of pansy plant material with downy mildew in early experiments in this project. Therefore, for this study an efficacy screen of candidate fungicides was carried out using 'live' infector plants as an inoculum under environmental conditions highly conducive to infection. Eleven fungicide treatments and a control treatment were laid out in a randomised block design with four replicates per treatment. Within each replicate plot, there were six individual pansy plants. Fungicide treatments were applied either prior to the introduction of infector plants or 14 days after their introduction to the trial area. All fungicidal treatments were applied at rates detailed in Table 2.

Plant material and trial location

Pansy plug plants cv. Ultima were obtained from a commercial nursery. The trials were located in a polythene tunnel at HRI Stockbridge House.

Treatments

The candidate fungicides selected for this screen against *P. violae* are listed in Table 2 below.

Fungicide application

Fungicides were applied using a hand held sprayer (Hozelock 31 Premier).

Table 2: Experimental fungicides screened on Pansy plants cv. Ultima for the control of *P. violae.*

Treatment	Application Rate+	Time of Application	
		Pre-inoculation	Post-inoculation
Control (water)	-	*4*	*4*
Fongarid (furalaxyl)	1.0g product/litre water	*4*	*4*
Favour (metalaxyl + thiram)	2ml product/litre water	*4*	*4*
Filex (propamocarb hydrochloride)	1ml product/litre water	*4*	*4*
Aliette (fosetyl-aluminium)	1.5g product/litre water	*4*	*4*
SL567A (enantiomeric metalaxyl)	1.3ml product/litre water	*4*	*4*
Trustan (cymoxanil + mancozeb + oxadixyl)	6.5g product/litre water	*4*	*4*
Curzate M (cymoxanil + mancozeb)	5.2g product/litre water	*4*	*4*
Invader (dimethomorph + mancozeb)	2.0g product/litre water	*4*	*4*
Shirlan (fluazinam)	1.5ml product/litre water	*4*	*4*
Amistar (azoxystrobin)	0.8ml product/litre water	*4*	*4*
Stroby (kresoxim-methyl)	0.5g product/litre water	*4*	*4*

+ All treatments were applied to the foliage to the point of run-off

Crop diary

Experiment 1

Planting:	27 March 2000
Fungicide application (pre- inoculation):	27 March 2000
Infector plants placed in trial area:	27 March 2000
Disease assessment (1):	11 April 2000
Fungicide application (post-inoculation):	11 April 2000
Plant vigour assessment:	18 April 2000
Disease assessment (2):	25 April 2000
Trial termination:	2 May 2000

Experiment 2

Planting:	14 June 2000
Fungicide application (pre-inoculation):	26 June 2000
Infector plants placed in trial area:	26 June 2000
Disease assessment:	20 July 2000
Trial termination:	20 July 2000

Assessments

During the trial individual plants were assessed for symptoms of phytotoxicity (foliage scorch and stunting). The level of infection of downy mildew (*P. violae*) and leaf spot caused by *Ramularia* spp. (where it occurred) were scored (0-3) depending on the degree of infection with 0 – no infection to 3 – severe infection on the foliage (Falloon *et al.*, 1995).

The disease index was calculated from 0-3 assessments as follows:

$$\frac{1(\text{No in category 1}) + 2(\text{No in 2}) + 3(\text{No in 3})}{\text{No of plants assessed}} \times \frac{100}{3}$$

The range of this index was, therefore, zero (no infection) to 100 (high infection).

Statistical analysis and Storage of Data

The data from these experiments was analysed by Biometrics Department, HRI Wellesbourne.

The raw data from these experiments will be archived for a period of not less than 5 years. Access to the data can only be made through a designated Archivist.

Official Recognition and Quality Assurance

The experiments reported were conducted in accordance with the guidelines for 'Official Recognition of Efficacy Testing Organisations in the United Kingdom' (Certificate Number ORETO 020) as outlined by the Pesticide Safety Directorate ("RD Ref. 2400/2996) and HRI's Standard Operating Procedures (SOPs).

A specific quality assurance audit was not undertaken during this work.

Results

Evaluation of fungicides for the control of *Thielaviopsis basicola*

At the termination of the trial all of the root systems were examined under a low power microscope and scored on the level of *T. basicola* chlamydospores present on the roots. The results show that chlamydospores were found on root material examined from all of the fungicide treatments in the trial and that there was consistent spread of the inoculum in the compost at the time of inoculation. This is important to ensure there was an even inoculum pressure on all of the experimental treatments. The results presented in Table 3 demonstrate that a number of fungicides including Folicur, Octave, Genie, Bavistin, Unix, Plover, Stroby, F279 and Amistar all significantly reduced the symptoms on the plant roots. However, both Folicur and Genie caused severe phytotoxicity symptoms including scorching and stunting which significantly reduced the plant quality.

The dry weight of plant foliage and roots were also recorded at the end of the trial. The only fungicide treatment that significantly reduced the root dry weight was Genie (Table 4). Folicur and Scala also resulted in a decrease in root dry weight although this was not significant. There was no reduction in the root dry weight in the inoculated control treatment. This is surprising due to the significant increase in *T. basicola* spores and discoloration seen on the pansy roots and in the severity index recorded (Table 3). The fungicides Folicur, Scala and Genie that recorded the largest reduction in plant quality measured as degree of scorching and stunting led to significant reductions in foliage dry weight (Table 5).

Assessment of plant vigour following treatment with each of the fungicides evaluated was made on two occasions during the experiment. Folicur, and to a lesser degree Scala and Genie significantly reduced the vigour of the treated plants at the first assessment (Table 6) while at the second assessment Folicur and Scala (post-inoculation drench only) significantly reduced plant vigour (Table 7). Surprisingly, there was no significant reduction in vigour in the untreated inoculated control plants.

Levels of scorching of the plant foliage after treatment with the experimental fungicides indicated significant scorching symptoms on plants treated with Folicur, Scala, Genie and

Unix at both application times (Table 8). Following fungicide application, it was noted that plants treated with Folicur did not recover from the initial scorch damage while plants treated with Scala, Genie and Unix did, to a certain extent, recover from the original damage caused when the fungicides were applied. The assessments of scorch symptoms due to the fungicide treatments were made more complex due to a natural invasion of a leaf spotting pathogen (*Ramularia* spp.). Detailed assessments of this disease are detailed in a later section of this report.

Measurements of stunting during the trial (Table 9) indicated that Folicur, F279 and Genie all caused a significant degree of stunting of the plants. All of the plants treated with Folicur exhibited some degree of stunting. None of the plants treated with Bavistin or Plover were stunted while the very low levels of stunting recorded on plants treated with Stroby, Amistar, Unix and Octave were insignificant.

Table 3: Final assessment of the severity of *T. basicola* infection on pansy plant roots at the termination of the trial on 16 February 2000.

Treatment		Infection Severity Index (0-100)		
		Drench at Inoculation[a]	Post-Inoculation Drench[b]	Mean
T1	Inoculated control, water	59.1	56.4	57.8
T2	Bavistin	29.3	37.7	33.5
T3	Plover	38.7	44.3	41.5
T4	Folicur	26.9	19.9	23.4
T5	Unix	35.6	37.0	36.3
T6	Scala	52.3	52.3	52.3
T7	Amistar	54.3	36.3	45.3
T8	Stroby	39.8	43.9	41.8
T9	F279	39.4	45.0	42.2
T10	Genie	32.1	34.9	33.5
T11	Dorado	41.4	38.4	39.9
T12	Octave	33.2	31.8	32.5
	Compare 2 different fungicide treatments			
	Significance	**		*
	SED (21 df)	4.71		4.92
	Compare different spray times per treatment			
	Significance	NS		-
	SED (84.29 df)	6.74		-

[a] The fungicidal drench was carried out at the time of planting/inoculation.
[b] The post-inoculation fungicidal drench was applied 14 days after inoculation.

Infection Severity Index; 0 = no infection, and, 100 = severe infection level.

Table 4: Assessment of root dry weight of pansy plants at trial termination on 18 February 2000.

Treatment		Root Dry Weight (g)	
		Drench at Inoculation[a]	Post-Inoculation Drench[b]
T1	Inoculated control, water	2.5	2.5
T2	Bavistin	1.6	1.8
T3	Plover	3.1	1.6
T4	Folicur	1.4	0.9
T5	Unix	2.3	3.0
T6	Scala	1.2	1.6
T7	Amistar	1.8	1.2
T8	Stroby	2.7	1.4
T9	F279	2.1	2.8
T10	Genie	0.3	1.0
T11	Dorado	1.9	1.0
T12	Octave	1.7	1.8
	Compare 2 different fungicide treatments		
	Significance	*	
	SED (21 df)	0.90	
	Compare different spray times per treatment		
	Significance	NS	
	SED (37.98 df)	1.06	

[a] The fungicidal drench was carried out at the time of planting/inoculation.
[b] The post-inoculation fungicidal drench was applied 14 days after inoculation.

Table 5: Assessment of foliage dry weight at trial termination on 18 February 2000.

Treatment		Foliage Dry Weight (g)	
		Drench at Inoculation[a]	Post-Inoculation Drench[b]
T1	Inoculated control, water	5.6	5.6
T2	Bavistin	5.7	6.1
T3	Plover	5.2	5.2
T4	Folicur	1.9	1.4
T5	Unix	6.0	5.2
T6	Scala	5.0	3.2
T7	Amistar	5.1	5.2
T8	Stroby	5.3	4.8
T9	F279	5.2	4.8
T10	Genie	4.4	4.6
T11	Dorado	5.2	5.5
T12	Octave	5.3	5.6
	Compare 2 different fungicide treatments		
	Significance	***	
	SED (21 df)	0.35	
	Compare different spray times per treatment		
	Significance	NS	
	SED (49.69 df)	0.48	

[a] The fungicidal drench was carried out at the time of planting/inoculation.
[b] The post-inoculation fungicidal drench was applied 14 days after inoculation.

Table 6: Interim assessment of the mean plant vigour of pansy plants infected with *T. basicola* to evaluate the efficacy of fungicide treatments on 13 January 2000.

Treatment		Plant Vigour Index (0-100)	
		Drench at Inoculation[a]	Post-Inoculation Drench[b]
T1	Inoculated control, water	100.0	100.0
T2	Bavistin	100.0	100.0
T3	Plover	98.6	98.6
T4	Folicur	65.9	65.9
T5	Unix	99.9	95.7
T6	Scala	93.9	77.3
T7	Amistar	99.6	99.6
T8	Stroby	99.6	96.7
T9	F279	98.8	98.7
T10	Genie	93.4	92.1
T11	Dorado	100.0	97.3
T12	Octave	100.0	100.0
	Compare 2 different fungicide treatments		
	Significance	***	
	SED (21 df)	2.59	
	Compare different spray times per treatment		
	Significance	***	
	SED (34.23 df)	2.96	

[a] The fungicidal drench was carried out at the time of planting/inoculation.
[b] The post-inoculation fungicidal drench was applied 14 days after inoculation.

Plant Vigour Index; 0 = very poor quality/low vigour, and, 100 = high quality/good vigour.

Table 7: Interim assessment of the mean vigour of pansy plants infected with *T. basicola* to evaluate the efficacy of fungicide treatments on 14 February 2000.

Treatment		Plant Vigour Index (0-100)	
		Drench at Inoculation[a]	Post-Inoculation Drench[b]
T1	Inoculated control, water	100.0	100.0
T2	Bavistin	100.0	100.0
T3	Plover	98.2	99.6
T4	Folicur	48.6	43.1
T5	Unix	100.0	100.0
T6	Scala	100.0	94.8
T7	Amistar	99.3	97.5
T8	Stroby	99.3	96.5
T9	F279	98.8	98.8
T10	Genie	100.0	100.0
T11	Dorado	98.7	100.0
T12	Octave	99.1	99.1
	Compare 2 different fungicide treatments		
	Significance	***	
	SED (21 df)	2.29	
	Compare different spray times per treatment		
	Significance	**	
	SED (27.39 df)	2.45	

[a] The pre-inoculation fungicidal drench was applied at the time of planting/inoculation.
[b] The post-inoculation fungicidal drench was applied 14 days after planting/inoculation.

Plant Vigour Index; 0 = very poor quality/low vigour, and, 100 = high quality/good vigour.

Table 8: Interim assessment of the percentage plants scorched after application of the fungicide treatment on 13 January 2000.

Treatment		Percentage plants displaying scorch symptoms	
		Drench at Inoculation[a]	Post-Inoculation Drench[b]
T1	Inoculated control, water	0	0
T2	Bavistin	13.0	4.7
T3	Plover	1.5	14.0
T4	Folicur	81.8	90.2
T5	Unix	20.5	37.2
T6	Scala	85.2	93.5
T7	Amistar	11.2	11.2
T8	Stroby	0	16.3
T9	F279	8.7	4.5
T10	Genie	72.2	93.0
T11	Dorado	7.5	32.5
T12	Octave	0	15.8
	Compare 2 different fungicide treatments		
	Significance	***	
	SED (21 df)	8.85	
	Compare different spray times per treatment		
	Significance	NS	
	SED (53.25 df)	12.55	

[a] The fungicidal drench was carried out at the time of planting/inoculation.
[b] The post-inoculation fungicidal drench was applied 14 days after inoculation.

Table 9: Interim assessment of stunting of the plant material after application of the fungicide treatments on 13 January 2000.

Treatment		Plant Stunting Index (0-100)	
		Drench at Inoculation[a]	Post-Inoculation Drench[b]
T1	Inoculated control, water	0	0.4
T2	Bavistin	0.6	0.6
T3	Plover	2.6	2.6
T4	Folicur	58.5	61.3
T5	Unix	4.4	3.1
T6	Scala	8.4	12.5
T7	Amistar	4.2	6.9
T8	Stroby	0	0
T9	F279	22.5	4.2
T10	Genie	6.0	42.6
T11	Dorado	0.4	0
T12	Octave	4.8	6.1
	Compare 2 different fungicide treatments		
	Significance	***	
	SED (21 df)	9.59	
	Compare different spray times per treatment		
	Significance	*	
	SED (23.47 df)	9.87	

[a] The fungicidal drench was carried out at the time of planting/inoculation.
[b] The post-inoculation fungicidal drench was applied 14 days after inoculation.

Plant Stunting Index; 0 = no stunting, and, 100 = severe stunting.

Evaluation of fungicides for the control of *Peronospora violae*

In the first experiment, conducted during the period of March - May 2000, the levels of downy mildew recorded in the trial area were very low. About 2 weeks after the first application of the fungicide treatments (pre-inoculation) and introduction of the infector plants, a low level of sporulation of downy mildew was evident on a few trays of pansy plants. These mildew symptoms were only recorded on untreated plant material, which was due to be sprayed on the second fungicide application time (the post-inoculation treatment). However, following this second fungicide application no further symptoms of downy mildew infection were recorded. The fungicide treatments that eliminated the downy mildew infection were Amistar, Favour and Fongarid. This result gives an indication of the potential effectiveness of these fungicides to control mildew although the levels of mildew were low.

Interestingly, during this first experiment, there was a dramatic build up of another foliage pathogen *Ramularia* leaf spot (*Ramularia* spp.). This fungal pathogen quickly spread through the trial area affecting all treatments. The conditions of high humidity and overhead watering, which are conducive to the spread of downy mildew also favoured the rapid spread of this leaf spot pathogen. However, the foliage symptoms were significantly reduced on plants treated with Amistar prior to infection whilst the other fungicides failed to reduce the development of the pathogen. The development of *Ramularia* leaf spot led to defoliation of the plants and this in turn was likely to have reduced downy mildew infection.

A second experiment was established during June-July 2000 to further examine the efficacy of the same fungicide treatments against downy mildew. On this occasion the infector plants were introduced to the trial area prior to application of the fungicide treatments and this helped enormously in securing plant infection to conduct a stern test with the fungicides under evaluation. The results presented in Table 10 indicate that all of the fungicide treatments reduced the levels of downy mildew compared to the untreated control. Favour and Shirlan completely controlled the development of downy mildew. SL567A, Filex, Trustan, Curzate M, Amistar, Stroby, Fongarid and Aliette significantly reduced the levels of mildew compared to the control. Both fungicides with metalaxyl/metalaxyl-M as the active component (Favour and SL567A) controlled the development of downy mildew on pansy during these trials. Results from previous efficacy trials and observations have indicated that in other downy mildew pathogen/host combinations there has been a development of metalaxyl resistance within fungal populations (eg *Bremia* in lettuce and possibly *Peronospora* in onions). Although there was no indication of resistance during the one isolate used in this study the development of metalaxyl resistant strains cannot be ruled out. Whilst still present, the levels of *Ramularia* during this experiment were much lower than in Experiment 1.

Table 10: Assessment of downy mildew infection on pansy plants on 20 July 2000 (Experiment 2).

Treatment		Disease Index (0-100)
T1	Inoculated control, water	18.93
T2	Fongarid	5.03
T3	Favour	0
T4	Filex	2.97
T5	Aliette	4.33
T6	SL567A	0.17
T7	Trustan	2.96
T8	Curzate M	2.27
T9	Invader	8.0
T10	Shirlan	0
T11	Amistar	3.13
T12	DUK9700F	3.13
	Compare 2 different fungicide treatments	
	Significance	*
	SED (21 df)	4.90

Disease Index; 0 = no disease, and, 100 = severe disease.

Evaluation of fungicides for the control of *Ramularia* spp.

Infection with the leaf spot pathogen *Ramularia* spp. was common-place in the series of efficacy trials conducted during the third year of this project. When assessing the effectiveness of fungicides against *T. basicola* there was an invasive infection of *Ramularia*. The results presented in Table 11 demonstrated that Amistar and Octave both displayed a significant reduction in leaf spot symptoms on treated plants. Amistar was the most effective treatment with a six-fold reduction in symptom development compared with the untreated control. The currently approved fungicide Bavistin although reduced the levels of leaf spotting it was not significant from the untreated control. Some of the other treatments reduced *Ramularia* leaf spot but not significantly.

During the first experiment to screen fungicides against downy mildew, there was a dramatic build up of *Ramularia* leaf spot. The pathogen quickly spread through the trial area affecting all treatments to a greater or lesser extent. The conditions of high humidity and overhead watering, which are conducive to the spread of downy mildew also favoured the rapid spread of this leaf spot pathogen. However, the foliage symptoms were significantly reduced on plants treated with Amistar prior to infection. The other fungicides in this trial failed to reduce the development of the pathogen.

Table 11: Assessment of *Ramularia* leaf spot infection on pansy plants in February 2000.

Treatment		Disease Index (0-100)
T1	Inoculated control, water	29.23
T2	Bavistin	22.30
T3	Plover	18.83
T4	Folicur	17.16
T5	Unix	30.73
T6	Scala	31.30
T7	Amistar	5.03
T8	Stroby	25.16
T9	F279	26.63
T10	Genie	22.20
T11	Dorado	21.06
T12	Octave	10.66
	Compare 2 different fungicide treatments	
	Significance	*
	SED (21 df)	9.55

Disease Index; 0 = no disease, and, 100 = severe disease

Discussion

The results from the efficacy trials carried out in the third year of this project have identified a number of experimental products with efficacy towards black root rot caused by *Thielaviopsis basicola*, downy mildew (*Peronospora violae*) and *Ramularia* spp. on Pansy.

Octave, Bavistin, Plover, Stroby, F279, Unix and Amistar all reduced the level of root infection by *T. basicola* without causing a reduction in overall plant growth and quality. However, although significant differences were recorded when examining the level of chlamydospores on the root tissue it was not possible to ascertain the viability of these spores due to the slow growth of *T. basicola in vitro* and the occurrence of secondary contaminants. Folicur was included in the third year's work as it proved to be the most effective treatment during the second year of this project. However, even after further reductions in the application rate it still led to severe phytotoxicity symptoms of pansy foliage and so whilst it provided the greatest reductions in infection levels by *T. basicola* its use cannot be developed further.

During this final years work, the introduction of 'infector plants' to the trial area allowed us to identify a number of fungicides with good efficacy against downy mildew on bedding plants. Favour and Shirlan completely inhibited the development of downy mildew on the plant material; SL567A, Filex, Trustan, Curzate M, Amistar, Stroby, Fongarid and Aliette also performed well. Trials with downy mildew pathogens, including *P. violae*, are complex because as an obligate pathogen it can only survive in the presence of living pansy plant material and cannot be maintained in a laboratory. It is possible that with a higher level of infection it would have provided an even sterner test by which to evaluate the experimental fungicides under test.

During the trials a natural but severe infection by a fungal leaf spot pathogen (*Ramularia* sp.) quickly led to scorching symptoms on plant material in the trial area. Where the infection was not controlled by fungicide application severe defoliation of the plants occurred reducing the potential spread of downy mildew on the plant material. However, from these studies two products, Amistar and Octave, were identified that effectively controlled *Ramularia* leaf spot.

Bavistin, the currently approved fungicide to control this pathogen did not perform well. It is possible that insensitive strains of this pathogen have developed although further work would be required to investigate this.

In conclusion, a number of fungicides have been identified during this study which are efficacious towards the primary target pathogens studied (Table 12). The fungicides that can currently be applied to protected bedding plants are listed above. One fungicide, Amistar (azoxystrobin) which has a broad spectrum of activity performed well against all three pathogens in this study. Its use could allow reductions in overall fungicides use whilst maintaining disease control. Since November 2000 when new SOLA's were issued for Amistar for Tomato and Cucumber grown under protection growers can potentially now use Amistar on ornamentals via The Long-Term Arrangements at their own risk. There are however specific restrictions and further work is necessary to restore certain aspects of the approval as it related to ornamental crops. It is important to ensure that all fungicide use is considered as part of an overall anti resistance strategy, where there is alternation of active ingredients with different modes of action to try and avoid the development of resistance.

Table 12: Efficacy and approval status of the fungicides evaluated in this study[1]

Fungicide Product	Active Ingredients	Products evaluated in this study			Status on Protected Crops (2001)[2]
		Black Root Rot	**Downy Mildew**	***Ramularia* leaf spot**	
Aliette	fosetyl-aluminium	-	4	-	Approved
Amistar	azoxystrobin	4	4	4	Approved*
Bavistin	carbendazim	4	-	**X**	Approved*
Curzate M	cymoxanil + mancozeb	**X**	-	**X**	Not Approved
Dorado	pyrifenox	**X**	-	**X**	Not Approved
Favour	metalaxyl + thiram	-	4	-	Approved[3]
Filex	propamocarb hydrochloride	-	4	-	Approved
Folicur	tebuconazole	**X**	-	X	Not Approved
Fongarid	furalaxyl	-	4	-	Approved[4]
F279	trifloxystrobin	4	-	**X**	Not Approved
Genie	flusilazole	**X**	-	**X**	Not Approved
Invader	dimethomorph + mancozeb	-	4	-	Not Approved
Octave	prochloraz	4	-	4	Approved
Plover	difenoconazole	4	-	**X**	Approved*
Unix	cyprodinil	4	-	**X**	Not Approved
Scala	pyrimethanil	**X**	-	**X**	Approved*
Shirlan	fluazinam	-	4	-	Not Approved
SL567A	enantiomeric metalaxyl	-	4	-	Not Approved
Stroby	kresoxim-methyl	4	4	**X**	Not Approved
Trustan	cymoxanil + mancozeb + oxadixyl	-	4	-	Not Approved

Key: 4 = Efficacious and no phytotoxicity, X = Not efficacious, - = not tested.

[1] It is important for Growers to check the current approval status of any fungicide, before application to the crop, with the current edition of the Long-Term Arrangements for Extension of Use. Fungicides are applied at the grower's risk and it is recommended to apply to a small area of crop to check for potential phytotoxic symptoms.

[2] Where the approval is based on either the Long Term Arrangements or Specific Off-Label Approval they are signified by an asterisk (*).

[3] Metalaxyl based products to be replaced with metalaxyl-M, an entantiomeric form of the same molecule. Future for metalaxyl-M based products for use under protection to be determined by manufacturers

[4] Product unsupported at Round 1 of EU Review Programme.

Conclusions

<u>Black root rot (*Thielaviopsis basicola*)</u>

- Inoculation of the compost with a spore suspension of *T. basicola* chlamydospores prior to planting led to even inoculum throughout the trial area and therefore an even disease pressure for all of the experimental fungicides screened.
- Screening test carried out *in vitro* for resistance of *T. basicola* towards carbendazim indicates that resistant strains were not common in the fungal population sampled during this study.
- Seven fungicides, Octave, Bavistin, Plover, Stroby, F279, Unix and Amistar all reduced levels of black root rot on Pansy after inoculation with a suspension of *T. basicola* chlamydospores.
- Octave (prochloraz), Bavistin (carbendazim), Plover (difenconazole) and Amistar (azoxystrobin) are the only fungicides identified in this study that can currently be used on ornamentals under protection.
- Although application of Folicur led to the greatest reduction of black root rot infection, plant quality was severely reduced through scorching and stunting of the treated plants.

<u>Downy mildew (*Peronospora violae*)</u>

- Difficulty was encountered earlier in this project to consistently secure infection of downy mildew (*P. violae*) on pansy plants largely because, as an obligate pathogen, it cannot be grown in agar culture. However, during the final year significant progress was made using an *in vivo* study which screening work using infector plants within the trial area.
- Experiments to screen novel chemicals against downy mildew (*P. violae*) on Pansy identified a number of effective fungicides including Favour and Shirlan which completely inhibited downy mildew. Other chemicals including SL567A, Filex, Trustan, Curzate M, Amistar Stroby, Fongarid and Aliette also significantly reduced the levels of downy mildew compared to the untreated control.
- Favour (metalaxyl + thiram), Filex (propamocarb HCl), Amistar (azoxystrobin) and Fongarid (furalaxyl) are the only fungicides identified in this study that are currently recommended on protected crops.

- No evidence of metalaxyl resistant strains of *P. violae* was recorded during the screening trials on Pansy plants.
- A higher level of infection within the trial area could have provided a sterner test by which to evaluate the experimental fungicides against downy mildew (*P. violae*).

Leaf spot (*Ramularia* spp.)

- During the trials in the third year of this project, severe infection of a fungal leaf spot pathogen (*Ramularia* sp.) caused extensive leaf spot symptoms, which led to partial defoliation of the plants in the trials.
- Where infection levels of *Ramularia* leaf spot were assessed during these efficacy trials, Amistar was found to provide the best control, followed by Octave. Bavistin, which is currently registered to control *Ramularia*, did not significantly reduce the foliar symptoms caused by *Ramularia* on this occasion.
- Octave (prochloraz) and Bavistin (carbendazim) are currently recommended for control of foliage diseases, such as *Ramularia* leaf spot on protected ornamentals.
- Further work is needed to assess the relative sensitivity of *Ramularia* to carbendazim and other fungicides.

Overall conclusion

- From the work in this study a number of novel fungicides have been identified with activity towards the target pathogens. It is imperative that either via the manufacturer's support for On-Label recommendation or through the SOLA scheme that registration for new active products is actively sought to maintain effective control and assist in the development of a sustainable anti-resistance disease control strategy.

Technology Transfer

Articles

McPherson, G.M. (1997) The identification and control of root-rot and leaf spot diseases of bedding plants. HRIA Study Day. Understanding Pest and Diseases of Ornamentals. February 1997, HRI Wellesbourne.

McPherson, G.M. & Jackson A.J. (1998) Pansy: Downy mildew. Diseases and pests of bedding plants: Identification cards (D12.O). Horticultural Development Council.

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Presentations at grower meetings

1. Diseases of Bedding Plants, their identification and control. International Plug Workshop, January 1998. Colgrave Seeds Ltd (Martin McPherson).
2. Nursery Hygiene and Disease/Pest Control. Presentation and practical training session to nursery staff at Crystal Heart Plant Raisers Ltd, 25 February 1999 (Andrew Jackson).
3. Evaluation of fungicides for the control of downy mildew and black root rot in pansies. HDC Bedding Plant Conference at HRI Wellesbourne, 26 October 2000. (Andrew Jackson)

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