ANNUAL REPORT

Project title:	Bedding plants: Evaluation of fungicides for the control of black root rot and downy mildew.
Reports:	Annual (1)
Project number:	HRI/SH/PP/98/E446 (PC 143)
Project leader:	Dr G M McPherson
Key workers:	Dr A Jackson, Dr D Teverson, Miss F Pomares, Mr N Glynn
Location:	Horticulture Research International Stockbridge House, Cawood, Selby, North Yorkshire, YO8 3TZ Tel: 01757 268275. Fax: 01757 268996.
Project co-ordinator:	Mr S Coutts
Date project commenced:	April 1997
Completion date:	March 2000
Keywords:	Bedding plants, pansy, viola, fungicides, downy mildew, *Peronospora,* black root rot, *Thielaviopsis,* fungicide resistance
Copies to:	HDC (3) Mr S Coutts (1) Dr G M McPherson (1) HRI Archive (1)

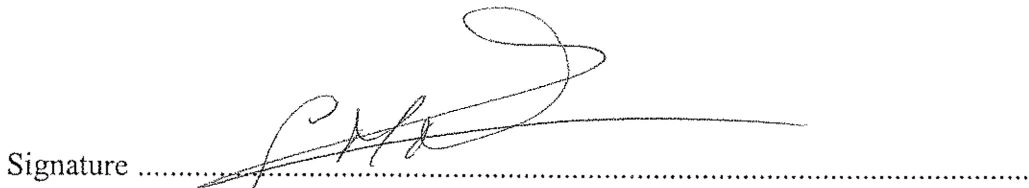
Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors or the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

©1998 Horticultural Development Council
No part of this publication may be reproduced in any form or by any means without prior permission from the HDC

The results and conclusions in this report are based on a number of laboratory based experiments. The conditions under which these experiments were carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

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 ....

Dr G M McPherson
Project Leader
Horticulture Research International
Stockbridge House

Date ...19/5/98........

Report authorised by ....

M R Bradley
Site Director
Horticulture Research International
Stockbridge House
Cawood
Selby
North Yorkshire
YO8 3TZ

Date ...27.5.98..........

©1998 Horticultural Development Council

CONTENTS

	Page No.
PRACTICAL SECTION FOR GROWERS	
Objectives and background	6-7
Summary of Results	8-9
Action points for growers	10
Practical and financial benefits from study	11
EXPERIMENTAL SECTION	
Introduction	12-13
Materials and Methods	14-22
Collection of fungal isolates	
Maintenance of isolates of *Thielaviopsis basicola*	
Maintenance of isolates of *Peronospora violae*	
Maintenance of plant material for bioassay tests	
Preparation of *Peronospora violae* inoculum	
Preparation of *Thielaviopsis basicola* inoculum	
Inoculation of plant material (*P. violae* only)	
Fungicide resistance testing	
Preliminary fungicide screening	
Assessments	
Statistical analysis	
Storage of data	
Official recognition and quality assurance	
Results	23-28
Discussion	29-31
Conclusions	32
References	33
Acknowledgements	34
Appendix I: Results from preliminary screens	

©1998 Horticultural Development Council

PRACTICAL SECTION FOR GROWERS

Objectives and background

Black root rot, caused by *Thielaviopsis basicola* and downy mildews (eg. *Peronospora violae* 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 on bedding plants, especially pansy, and cause a reduction in plant quality and severe plant losses. Results from research on the use of chemical disinfectants on contaminated plug trays, pots and standing areas (O'Neill, 1995 & 1996) 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.

Although there are reports of carbendazim (Bavistin) and prochloraz (Octave) giving good levels of control of black root rot (Scrace, 1993), more recently there have been reports of reduced efficacy of carbendazim, possibly due to the build up of resistant strains of *T. basicola* to this fungicide. In the last 3-4 years numerous fungicides with potential activity against both *T. basicola* and downy mildews have been approved on broad acre eg. cereal crops in the UK though little or no work has been done to identify those most effective and for use on bedding plants. Several fungicides but predominantly furalaxyl (Fongarid) have been used widely for downy mildew control in recent years. However, once again there is growing evidence that resistant strains are now present in pathogen populations though this remains unsubstantiated.

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

©1998 Horticultural Development Council

The objectives of this project were to:

a) determine whether isolates of both pathogens were resistant to the current approved fungicides and

b) evaluate a range of novel fungicides for the control of *Thielaviopsis basicola* (black root rot) and downy mildew in bedding plants alongside existing products to improve the level of disease control achieved and thus lower the financial losses incurred by bedding plant growers.

Pansy was selected as a typical bedding plant species prone to attack by both *T. basicola* and downy mildew (*Peronospora violae).* Information produced from this work relating to Pansy will have immediate application to similar disease problems in other bedding plant species so long as there are no differential phytotoxic reactions on different host species.

The aims of year 1 of the project were to:

a) collect a range of isolates of *T. basicola* and *P. violae* and to determine their relative sensitivity to carbendazim (eg. Bavistin) and furalaxyl (Fongarid) respectively.

b) screen a range of novel fungicides for activity against *T. basicola* and *P. violae.*

©1998 Horticultural Development Council

Summary of Results

A range of Pansy (*Viola* spp.) plant material, exhibiting characteristic symptoms of black root rot (*Thielaviopsis basicola*) and downy mildew (*Peronospora violae*), was collected from a diverse range of UK nurseries by Mr S Coutts.

From this material a selection of isolates of *T. basicola* and *P. violae* were secured for further study.

Some 15 isolates of *T. basicola* were tested for their sensitivity to the fungicide carbendazim (eg. Bavistin) in an *in vitro* agar plate test. Perhaps surprisingly, none of the isolates tested exhibited reduced sensitivity or resistance to this fungicide.

Nine isolates of *P. violae* were secured from the host plant tissues received. Unfortunately, difficulty was experienced in attaining consistent spore production and subsequent infection in *Viola* seedlings and this has delayed resistance testing against furalaxyl (Fongarid). Effort has concentrated on developing a robust methodology for achieving infection with this obligate pathogen (ie. it cannot be grown in artificial culture) and this aspect of the work has been re-scheduled.

During 1997, a range of approved (standard) and novel (experimental) fungicides were sourced from various agrochemical manufacturers and distributors.

©1998 Horticultural Development Council

Each fungicide was incorporated, at two concentrations, into an agar medium, and their relative performance in inhibiting mycelial growth of *T. basicola* determined. From these results several fungicides, including fenpropimorph (Aura), diethofencarb + carbendazim (Jonk), penconazole (Topas), difenconazole (Plover), cyproconazole (Alto 100), myclobutanil (Systhane) and prochloraz (Octave), were found to have potential against *T. basicola* in this *in vitro* study. Although Amistar (azoxystrobin) was not particularly effective in reducing the radial growth of *T. basicola* during the agar screening in this evaluation it is considered appropriate for further development in this crop primarily because of its activity on other important pathogens eg. *Ramularia* spp., *Alternaria* sp., powdery mildew and rust. It will be necessary in year 2 of the project to evaluate the potential phytotoxicity of these candidate products on a range of bedding plant species.

As with the resistance testing for *P. violae*, the initial efficacy screening studies have been inadvertently delayed because of the unexpected need to develop new techniques for handling this obligate pathogen. These problems have now been overcome and the isolates of *P. violae*, which have been stored at -20°C can be recovered and tested during the next few months.

©1998 Horticultural Development Council

Action points for growers

At this stage in the project there are few specific recommendations that can be made to growers until the fungicides selected during the preliminary screen on agar are screened for possible phytotoxic effects on bedding plant material is carried out as planned in year 2 of this project. The initial resistance testing of *T. basicola* with carbendazim would appear to indicate that resistant strains are not common in the population. Growers should perhaps therefore look closely at other factors which might explain poor performance of the fungicide eg. application methods.

©1998 Horticultural Development Council

Practical and financial benefits from the study

The identification of novel chemical fungicides with the ability to control either of these disease problems identified in this project should be of particular benefit to the bedding plant industry. Novel chemicals could play an important part in new integrated spray programmes to control these diseases, whilst at the same time minimising further resistance problems developing.

The value of the UK bedding industry is estimated to be in excess of £300 million. The 'farmgate' value of autumn and spring pansies alone is 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, but can be as high as 10% in some years. Losses due to downy mildew are again difficult to estimate accurately, but up to 10% of plants have been discarded in some seasons due to this disease. 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.

©1998 Horticultural Development Council

EXPERIMENTAL SECTION

Introduction

Black root rot caused by *Thielaviopsis basicola* is a common disease problem in ornamental and bedding plant crops in the UK. Symptoms of this disease include yellowing, stunting and even plant wilting and death. Examination of infected roots show dark-grey or black root lesions along the roots. For more information on the symptoms and biology of this disease refer to the recently published HDC Identification Card Series (McPherson, 1998b). 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 or 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 needs 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. *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

©1998 Horticultural Development Council

Identification Card Series (McPherson, 1997). 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 eg. lettuce is likely to be of some significance (McPherson, 1998a). Work in France (Boudier, 1987) assessed a range of chemical fungicides for control of downy mildew on pansy although most of the chemicals evaluated are 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, have been used widely for downy mildew control, although again here there is growing evidence that resistant strains are now present in the pathogen population.

The scientific targets of this project in the first year were to:

- collect a range of isolates of *Thielaviopsis basicola* and *Peronospora violae* from a range of commercial nurseries.

- evaluate levels of resistance among the *T. basicola* isolates towards the MBC fungicide carbendazim (Bavistin).

- evaluate levels of resistance among the *P. violae* isolates towards furalaxyl (Fongarid).

- conduct a preliminary efficacy screen using a range of novel fungicides against *T. basicola* (on agar) and *P. violae* (on host plants).

©1998 Horticultural Development Council

Materials and Methods

Collection of Fungal Isolates

Infected plant material received from commercial nurseries in the UK, courtesy of Mr S Coutts, was sampled for both *Thielaviopsis basicola* and *Peronospora violae*. The isolates collected from Pansy plant material and subsequently used in this project together with the geographic origin of the samples and the experimental codes are listed in Tables 1 and 2.

Table 1: Details of the *Thielaviopsis basicola* isolates collected for use in year 1 of this study.

Isolate-Code	Host	Cultivar	Geographic Origin	Date Isolated
1B	Pansy	cv. unknown	Liverpool	11.9.97
1C	Pansy	cv. unknown	Liverpool	11.9.97
3A	Pansy	Ultima	Lincolnshire	11.9.97
3B	Pansy	Ultima	Lincolnshire	11.9.97
5aA	Pansy	cv. unknown	Evesham	11.9.97
5aB	Pansy	cv. unknown	Evesham	11.9.97
5aC	Pansy	cv. unknown	Evesham	11.9.97
5B	Pansy	cv. unknown	Evesham	11.9.97
5C	Pansy	cv. unknown	Evesham	11.9.97
7B	Pansy	Universal White	Unknown	15.9.97
8A	Pansy	Ardross Gem	Unknown	11.9.97
8B	Pansy	Ardross Gem	Unknown	11.9.97
TB2*	Pansy	F1 Delft	Lancashire	6-92
TB4*	Pansy	Blue Blotch	Gloucestershire	12-92
TB8*	Pansy	cv. Unknown	Unknown	9-91

* Reference isolates collected by Dr J Biddulph and retained at HRI Wellesbourne.

©1998 Horticultural Development Council

Table 2: **Details of the *Peronospora violae* isolates collected for use in year 1 of this study.**

Isolate - Code	Host Plant and Cultivar	Geographic Origin	Date Isolated
15	Pansy cv. unknown	Cheshire F	2.10.97
16	Pansy cv. unknown	North Yorkshire	2.10.97
17A	Pansy cv. unknown	Cumbria A young plants	2.10.97
17B	Pansy cv. unknown	Cumbria B old plants	2.10.97
18	Pansy cv. unknown	Cumbria B	2.10.97
19	Pansy cv. unknown	Cheshire	2.10.97
20	Pansy Turbo Porcelain Blue	Cheshire	2.10.97
21	Pansy Universal Blue	Coventry	26.10.97
22	Pansy cv. unknown	York	1.12.97

Maintenance of isolates of *Thielaviopsis basicola*

Isolates of *T. basicola* collected from infected plant material were maintained on Potato Dextrose Agar (PDA) and incubated at 20°C and sub-cultured at every 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°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°C (+/- 1°C).

©1998 Horticultural Development Council

Maintenance of isolates of *Peronospora violae*

Samples of plant material infected with *P. violae* were sealed in individual polythene bags and stored at –20°C until needed. When spores of a specific *P. violae* isolate were required, infected leaf material was removed from the freezer and spores washed off the lower leaf surface using sterile distilled water. It was important to wash this material before it had completely defrosted, as the leaf material became difficult to handle. Also, spores of the fungus became lodged in the macerated leaf material and were not washed readily into solution.

Maintenance of Plant Material for Bioassay Tests

Two methods of raising and maintaining Viola plants were used.

1. Pansy seed cv. Forerunner were sown in a 5 mm deep layer of sterile vermiculite in glass crystallising dishes. The vermiculite was moistened with nutrient solution and incubated in an illuminated incubator set at 16°C +/- 2°C with a 16 hour day length. Seedlings were used for subsequent tests after ca. 14 days when the cotyledons had fully expanded.

2. Pansy seeds cv. Forerunner were sown in Levington F1 compost in new plastic plug trays and germinated in the propagation facility at Stockbridge House. After germination, trays of seedlings were transferred to a frost-free greenhouse. Fungicides were not incorporated into the compost or subsequently applied to the plants.

©1998 Horticultural Development Council

Preparation of *Peronospora violae* inoculum

Leaf material infected with *P. violae* develops a 'grey down' of *P. violae* spores on the undersides of the leaves. Spores were collected from either fresh or frozen infected leaf material by detaching the leaves, and washing sterile distilled water over the leaves while rubbing gently with a glass rod. About 30-35ml sterile distilled water was required for 8-10 heavily infected leaves. The washed solution was then collected in a 100ml beaker. This washing process was repeated re-using the water from the beaker until all the spores had been washed from the leaves.

Spore germination inhibitors occur naturally and can adversely affect the performance of inoculum. In order to remove these naturally occurring chemicals, the spore suspension was centrifuged at 2500 rpm for 15 minutes. The supernatant was then removed using a Pasteur pipette. This process was repeated before re-suspending the spore 'pellet' in sterile distilled water. The concentration of the spores in the solution was subsequently calculated using a haemocytometer and the concentration adjusted to 1×10^5 spores/ml.

Preparation of *Thielaviopsis basicola* inoculum

As *T. basicola* is a facultative pathogen, ie. it can be grown on artificial culture (agar) media, inoculum can be prepared easily. A 3 mm plug was taken from each isolate to be tested and placed in a fresh agar (PDA) plate. After 7 days growth 3 mm plugs from the outer colony edge were used in the resistance and fungicide efficacy studies.

©1998 Horticultural Development Council

Inoculation of plant material (*P. violae* only)

Two different methods for the production of pansy plant material for *in vivo* inoculation of *P. violae* were compared. In addition, because initial studies had failed to establish *P. violae* consistently, a number of different inoculation methods were evaluated in an attempt to devise a robust methodology for further studies with *P. violae*.

1. About 25 pansy seeds cv. Forerunner were sown on vermiculite (5mm deep) in glass covered crystallising dishes, moistened with nutrient solution and placed in an illuminated incubator at 16°C $\pm$ 2°C with a 16 hours day length. After ca. 14 days, once the seedlings had germinated and developed cotyledons, they were inoculated with a spore suspension of *P. violae* (1×10^5 spores/ml) using the methods described below and returned to the incubator.

2. Pansy seed cv. Forerunner were sown in Levington F1 compost in new plastic plug trays and germinated in the propagation facility at HRI Stockbridge House. After germination the trays were transferred to a frost-free greenhouse and used in subsequent inoculation studies (see below) when the cotyledons were fully expanded. After inoculation the seedlings (in modules) were enclosed in perspex inoculation trays to maintain conditions conducive to infection, and maintained in the greenhouse.

   Various inoculation techniques were evaluated on the two production systems in an attempt to secure a robust methodology that could reliably be used in all subsequent tests. These were:

   a. A 0.1 ml droplet of the spore suspension containing 1×10^5 spores/ml was placed on the underside of the cotyledons.

©1998 Horticultural Development Council

b. A 0.1 ml droplet of spore suspension containing 1 x 10^5 spores/ml was placed on the underside of the cotyledons and subsequently spread over the cotyledon lower surface with a sterile glass rod.

c. Infected cotyledons taken from a separate batch of plants were physically but gently abraded (rubbed) against the uninfected cotyledon tissue.

Fungicide Resistance Testing

T. basicola

The sensitivity of a collected range of isolates of *T. basicola* to carbendazim (Bavistin) was evaluated in agar (PDA) culture. Each isolate was grown in agar culture at concentrations of 0, 2, 20 and 100 ppm carbendazim by placing a 3 mm agar plug containing the fungus on the amended agar. The growth of the fungus was subsequently measured at 2-3 day intervals over a period of 2 weeks. Growth was measured as the distance from the edge of the agar inoculation plug and the mycelial colony edge. Two measurements were made per replicate and the mean calculated. Two replicate plates were used for each concentration tested with each isolate. Results have been expressed as a percentage growth of the control.

P. violae

Fungicide resistance testing with the obligate pathogen, *P. violae*, was unavoidably delayed until a suitable robust methodology could be sought. A suitable method has now been developed and further tests to determine the relative sensitivity of the collected isolates of *P. violae* will be conducted during year 2 of the project.

©1998 Horticultural Development Council

Preliminary Fungicide Screening

T. basicola

The candidate fungicides listed in Table 3 were incorporated into agar (PDA) in Petri dishes and their inhibition on the growth of 12 isolates of *Thielaviopsis basicola* assessed compared to growth in the control amended with carbendazim. Concentrations of 2 and 20 ppm were used relative to a control unamended with any fungicide. The growth of the pathogen was measured at 2-3 day intervals over a period of 2 weeks. Growth was measured as the distance from the edge of the agar inoculation plug and the mycelial colony edge. Two measurements were made per replicate and the mean calculated. Two replicate plates were used for each concentration tested with each fungicide. Results have been expressed as a percentage growth of the control.

Table 3: Candidate fungicides used in the preliminary fungicide screen for the control of *Thielaviopsis basicola*.

Product	Active Ingredient	Concentrations Screened	
		0 ppm	20 ppm
Fongarid	furalaxyl	✓	✓
Bavistin	carbendazim	✓	✓
Bion	acibenzolar	✓	✓
Rovral	iprodione	✓	✓
Scala	pyrimethanil	✓	✓
Unix	cyprodinil	✓	✓
Aura	fenpropimorph	✓	✓
Jonk	diethofencarb + carbendazim	✓	✓
Folicur	tebuconazole	✓	✓
Topas	penconazole	✓	✓
Plover	difenconazole	✓	✓
Alto 100	cyproconazole	✓	✓
Opus	expoxiconazole	✓	✓
Systhane	myclobutanil	✓	✓
Octave	prochloraz	✓	✓
Amistar	azoxystrobin	✓	✓

©1998 Horticultural Development Council

Assessments

T. basicola

Assessments in the resistance tests towards carbendazim and preliminary fungicide screen on amended agar against *T. basicola* were based on the radial growth of the colony of each plate. Measurements were taken from the edge of the inoculation core to the leading mycelial edge. To allow easier comparison of each fungicide the mean radial growth measurements from all 12 *T. basicola* isolates screened were tabulated, thus giving a more accurate assessment of each fungicides performance. Results for individual isolates are presented in Appendix I. No other disease assessments were possible during the *in vitro* tests.

P. violae

Levels of infection by *P. violae* on the undersides of pansy leaves were scored as a percentage leaf area based on a key for downy mildew on pea (Falloon *et al.*, 1995). This assessment methodology will be used in subsequent tests with *P. violae*.

Statistical Analysis

The data from these experiments was not subjected to statistical analysis.

Storage of Data

The raw data from these experiments will be stored for a period of not less than 5 years in the HRI Archive at Stockbridge House. Access to the data can only be made by the designated Archivist.

©1998 Horticultural Development Council

Official Recognition and Quality Assurance

The experiments reported were conducted in accordance with the draft guidelines for Official Recognition of Efficacy Testing Organisations as outlined by the UK pesticide authority, PSD (PRD Ref. 2400/2996).

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

©1998 Horticultural Development Council

Results

Where various isolates of *T. basicola* were collected and tested for their resistance to carbendazim at various concentrations none was found. Measurements of radial growth taken after 7 (Table 4) and 14 days (Table 5) showed that of the 15 isolates tested failed to grow at all at concentrations of 2, 10, 20 and 100 ppm. One isolate of *T. basicola* (Code 5aA) exhibited slight growth (39% of the control) at the lowest concentration (2 ppm) of carbendazim tested. Interestingly, there was a marked variation in the growth rate between individual isolates from 13-25.5 mm (Table 4) after 5 days and 31.5-61.5 (Table 5) after 14 days.

In the preliminary efficacy screen a total of 16 different fungicides were evaluated against 12 individual isolates of *T. basicola*. The mean results for all isolates have been summarised to allow easier comparison of the relative fungicide performance (Tables 6 and 7). The complete results for individual isolates tested are presented in full in Appendix I.

Measurements of radial growth of *T. basicola* were taken after 7 and 14 days. The mean growth rate of the pathogen on unamended agar was 14.0 mm (7 days) and 44.8 mm (14 days). This data provided the baseline from which to measure the efficacy of the various fungicides incorporated into the agar medium.

Furalaxyl (Fongarid), a widely used fungicide for oomycete fungi (eg. *Pythium, Phytophthora, Peronospora*), as expected provided <u>no</u> inhibition of *T. basicola* (<u>not</u> an oomycete fungus - but a dematiacious hyphomycete). Bavistin (carbendazim), the fungicide used widely in bedding plants over many years for the control of black root rot and various leaf-spot fungi (eg. *Phoma, Phomopsis, Alternaria*) provided complete inhibition at 2 and 20 ppm and this inhibition was maintained at both assessment dates. This supports earlier work by Scrace (1993) who did not detect resistance in populations of *T. basicola*.

©1998 Horticultural Development Council

Several of the experimental fungicides including fenpropimorph (Aura), diethofencarb + carbendazim (Jonk), penconazole (Topas), cyproconazole (Alto 100), myclobutanil (Systhane) and prochloraz (Octave) all provided 100% inhibition of mycelial growth, measured after 7 days at 2 and 20 ppm.

Where the plates were incubated for a further 7 days a slightly different result emerged and growth was evident on agar plates amended with fenpropimorph, cyproconazole and myclobutanil but at 2 ppm only.

Many of the other fungicides tested provided some suppression of mycelial growth compared to the untreated. These included iprodione (Rovral), pyrimethanil (Scala), epoxiconazole (Opus), azoxystrobin (Amistar) and difenconazole (Plover).

The novel 'plant activator' (Bion) from Novartis was not effective in reducing mycelial development in this agar plate test, though this is not surprising given its unique mode of action within the host.

©1998 Horticultural Development Council

Table 4: **Sensitivity of a range of isolates of *T. basicola* to carbendazim (Bavistin) in agar culture. Results recorded 5 days from inoculation.**

T. basicola Isolate	Concentration of carbendazim - Colony diameter (mm)				
	0 ppm	2 ppm	10 ppm	20 ppm	100 ppm
1B	16.5	0	0	0	0
1C	13	0	0	0	0
3A	17.5	0	0	0	0
3B	19	0	0	0	0
5aC	19	0	0	0	0
5aA	20.5	0	0	0	0
5aB	20	0	0	0	0
5B	18.5	0	0	0	0
5C	25.5	0	0	0	0
7B	21.5	0	0	0	0
8A	20	0	0	0	0
8B	18.5	0	0	0	0
TB2*	16.0	0	0	0	0
TB4*	21.5	0	0	0	0
TB8*	19.0	0	0	0	0

* Reference isolates collected by Dr J Biddulph and stored at HRI Wellesbourne.

©1998 Horticultural Development Council

Table 5: Sensitivity of a range of isolates of *T. basicola* to carbendazim (Bavistin) in agar culture. Results recorded 14 days from inoculation.

T. basicola Isolate	Concentration of carbendazim - Colony diameter (mm)				
	0 ppm	2 ppm	10 ppm	20 ppm	100 ppm
1B	45.5	0	0	0	0
1C	31.5	0	0	0	0
3A	47.5	0	0	0	0
3B	52.5	0	0	0	0
5aC	55.0	0	0	0	0
5aA	60.0	8	0	0	0
5aB	57.5	0	0	0	0
5B	53.5	0	0	0	0
5C	61.5	0	0	0	0
7B	39.5	0	0	0	0
8A	55.0	0	0	0	0
8B	53.5	0	0	0	0
TB2*	44.5	0	0	0	0
TB4*	37.0	0	0	0	0
TB8*	51.5	0	0	0	0

* Reference isolates collected by Dr J Biddulph and stored at HRI Wellesbourne.

©1998 Horticultural Development Council

Table 6: Summary results for the inhibition of radial growth of *T. basicola* in agar culture by the candidate experimental fungicides. Results recorded 7 days after inoculation.

Fungicide	Colony diameter (mm)*		
	0 ppm (control)	2 ppm#	20 ppm#
Fongarid	21.5	22.2 (103)	21.6 (100)
Bavistin	19.1	0 (0)	0 (0)
Bion	11.0	10.1 (92)	9.5 (86)
Rovral	13.6	7.8 (57)	7.3 (54)
Scala	13.6	9.6 (71)	4.0 (29)
Unix	11.0	10.0 (90)	8.7 (79)
Aura	21.5	0 (0)	0 (0)
Jonk	21.5	0 (0)	0 (0)
Folicur	11.8	9.4 (80)	0 (0)
Topas	12.8	0 (0)	0 (0)
Plover	12.8	12.3 (96)	0 (0)
Alto 100	13.6	0 (0)	0 (0)
Opus	11.8	2.0 (17)	0 (0)
Systhane	11.8	0 (0)	0 (0)
Octave	12.8	0 (0)	0 (0)
Amistar+	17.0	13.9 (82)	9.4 (55)
Mean	14.0	- (-)	- (-)

Notes

\# Agar amended with each fungicide at concentrations of 2 and 20 ppm.

* Mean results for 12 *T. basicola* isolates screened.

\+ Mean of 3 isolates of *T. basicola* only.

Figures in parentheses represent growth of *T. basicola* on the fungicide amended agar expressed as a percentage of the unamended agar control.

NB. Raw data for individual isolates of *T. basicola* are presented in Appendix I.

©1998 Horticultural Development Council

Table 7: **Summary results for the inhibition of radial growth of *T. basicola* in agar culture by the candidate experimental fungicides. Results recorded 14 days after inoculation.**

Fungicide	Colony diameter (mm)*		
	0 ppm (control)	2 ppm#	20 ppm#
Fongarid	50.7	54.2 (107)	53.6 (106)
Bavistin	51.0	0 (0)	0 (0)
Bion	52.9	46.8 (88)	49.6 (94)
Rovral	45.4	26.7 (59)	26.0 (57)
Scala	45.4	15.1 (33)	6.9 (15)
Unix	52.9	48.8 (92)	45.0 (85)
Aura	50.7	5.0 (9.9)	0 (0)
Jonk	50.7	0 (0)	0 (0)
Folicur	37.1	26.9 (72)	16.1 (43)
Topas	40.7	0 (0)	0 (0)
Plover	40.7	11.0 (27)	0 (0)
Alto 100	45.4	5.5 (12)	0 (0)
Opus	31.1	22.5 (61)	12.9 (35)
Systhane	37.1	18.2 (49)	0 (0)
Octave	40.7	0 (0)	0 (0)
Amistar+	39.1	28 (72)	23 (59)
Mean	44.8	- (-)	- (-)

Notes

Agar amended with each fungicide at concentrations of 2 and 20 ppm.

* Mean results for 12 *T. basicola* isolates screened.

+ Mean of 3 isolates of *T. basicola* only.

Figures in parentheses represent growth of *T. basicola* on the fungicide amended agar expressed as a percentage of the unamended agar control.

NB. Raw data for individual isolates of *T. basicola* are presented in Appendix I.

©1998 Horticultural Development Council

Discussion

Among all of the isolates of *T. basicola* screened in this first year study no evidence was gained to suspect the development of resistance towards the benzimidazole fungicide, carbendazim. In addition, under these controlled conditions carbendazim successfully inhibited mycelial growth of *T. basicola* during the agar screen.

It is interesting that, although there is anecdotal evidence of poor control of *T. basicola* with carbendazim, no resistance (or reduced sensitivity) was detected on a range of isolates gathered from commercial nurseries. Whilst it will continue to be important to monitor this it would appear more likely that there are other factors affecting fungicide performance eg. timing, inoculum pressure, environmental conditions, host susceptibility. It will be important to look at other factors in more detail on individual nurseries, though it should be noted that this is not built-in to the current project.

The results from the preliminary screen identified a number of fungicides exhibiting good efficacy against *T. basicola*. These fungicides can be taken forward to the next stage of the project. It is important that the most promising fungicides from this work are assessed on whole plant material as the preliminary screen was carried out in agar plates under controlled conditions. The next stage will allow us to assess whether there is any potential risk from phytotoxicity with these fungicides on various bedding plant subjects.

It should be noted that the preliminary screen against *T. basicola* has measured inhibition of mycelial growth only. If the fungicides evaluated have alternative modes of action, eg. spore germination inhibitors, then their performance in this respect will not have been measured. It is somewhat imprecise therefore to select fungicides on the basis of the agar test only. Azibenzolar or 'Bion' for example is a novel 'plant activator' from Novartis which is claimed to operate by 'switching on' host defence responses. The poor results

©1998 Horticultural Development Council

using the agar plate method are therefore not surprising. Specific host tests would need to be devised and conducted to evaluate its performance. It should not therefore be eliminated from further studies on the basis of this initial screen.

Similarly, the relatively poor *in vitro* test result with the novel strobilurin fungicide azoxystrobin (Amistar) should not be eliminated on the basis of this initial result. Amistar has already been demonstrated to be effective in other studies against a broad range of pathogens, some of which are likely to occur occasionally eg. *Sclerotinia, Rhizoctonia*. If it is demonstrated to be safe to a broad range of bedding plants it could still be beneficial as a component of an anti-resistance strategy on this crop sector.

An important characteristic of *P. violae*, as an obligate pathogen, is that it can only survive in the presence of living plant material. With current available techniques it cannot be grown in the laboratory on agar medium. The use of crystallising jars to produce plant material for inoculation with *P. violae,* as used routinely for *Bremia lactucae*, another downy mildew on lettuce, was not very successful in this study. It was hoped that this method would provide a relatively straightforward and convenient technique for inoculation and infection that would have allowed larger numbers of isolates and fungicides to be screened easily in the laboratory. However, in initial tests the levels of infection on the cotyledons were poor and not sufficient to allow successful fungicide screening. Alteration of the growing environment by increasing/decreasing temperature and light levels during the inoculation and infection period did not improve the levels of infection by *P. violae* either. In all tests the seedlings had a tendency to be heavily colonised by saprophytic fungi eg. *Oedocephalum* spp.

©1998 Horticultural Development Council

In contrast, the use of compost raised older pansy plant material grown in module trays and incubated under high RH by conditions by enclosing in a Perspex box did allow significant levels of infection by *P. violae* to be achieved successfully. Unfortunately, this method is more time-consuming and labour intensive than any laboratory based method as spacial separation of individual isolates of the fungus is more difficult. The delay in obtaining a successful methodology for this inoculation procedure has delayed certain scientific targets of year 1 of this project. These include the resistance tests of *P. violae* isolates to furalaxyl (Fongarid) as well as the preliminary fungicide screen. These aspects of the project will be included in the schedule for year 2 of the project.

©1998 Horticultural Development Council

Conclusions

1. A range of isolates of *T. basicola* and *P. violae* were sourced from commercial Viola crops during 1997.

2. After screening a range of *T. basicola* isolates there was no evidence of any resistance towards the benzimidazole fungicide, carbendazim (Bavistin).

3. From the preliminary fungicide efficacy screen on agar, a range of novel fungicides were identified with activity against *T. basicola* and these have been selected for further studies.

4. Promising experimental fungicides identified in year 1 of this project will be evaluated for crop safety in a phytotoxicity screen during 1998.

5. Additional novel fungicides, unavailable in 1997, will be included in the project during 1998.

6. Initial difficulty was encountered in securing consistent infection with isolates of *P. violae*, as originally planned, and this has delayed progress of this component of the project.

7. A robust methodology for generating consistent infection with *P. violae* has been developed during 1997.

8. Fungicide resistance testing with furalaxyl (Fongarid) and a preliminary efficacy screen against *P. violae* will now be conducted during 1998.

©1998 Horticultural Development Council

References

Biddulph, J.E. (1996) Epidemiology of black root rot in winter pansy. *PhD Thesis, University of Birmingham*. 230pp.

Boudier, B. (1987) Le mildiou de la pensee. Methode de lutte et sensibilities varietales. *Horticulture-Francaise* 189:7-8.

Falloon, R.E., Viljanen-Rollinson, S.L.H., Coles, G.D. & Poff, J.D. (1995) Disease severity keys for powdery mildew and downy mildews of pea, and powdery scab of potato. *New Zealand Journal of Crop and Horticultural Science* 23:31-37.

McPherson, G.M. (1998a) Protected Lettuce: Evaluation of novel fungicides and fungicide programmes for the control of downy mildew (*Bremia lactucae*). Contract Report for the Horticultural Development Council (PC 20a), 44pp.

McPherson, G.M. (1998b) Pansy; black root rot. Diseases and Pests of Bedding Plants: Identification Cards (D14.0). Horticultural Development Council.

McPherson, G.M. (1997) Pansy: Downy mildew. Diseases and Pests of Bedding Plants: Identification Cards D12.0). Horticultural Development Council.

McPherson, G.M. (1997) Evaluation of novel fungicides for the control of pink rot (*Sclerotinia sclerotiorum*) in protected celery. Contract report for the Horticultural Development Council (PC 131), 31pp.

O'Neill, T. (1996) A clean start in pot and bedding plant production. *HDC Project News, June 1996*. p14-16.

O'Neill, T. (1995) Chemical disinfectants for treatment of plastic plugs trays contaminated with *Thielaviopsis basicola*. Contract Report for the Horticultural Development Council (PC 38c), 13pp.

Powell, C (1991) Understanding and controlling black root rot disease. *Grower Talks* 52 (11):34-38.

Scrace, J.M. (1993) The effect of pH, plug nutrition and fungicide timing on control of black root rot in Autumn pansy). Contract Report for the Horticultural Development Council (PC 38b), 32pp.

©1998 Horticultural Development Council

Acknowledgements

The Horticultural Development Council sponsored this work and their financial assistance is greatly acknowledged. Mr S Coutts kindly provided many of the plant samples infected with either *T. basicola* and *P. violae* and his assistance and technical support to the project was invaluable.

©1998 Horticultural Development Council

APPENDIX I: Results from the Preliminary Fungicide Efficacy Screen on Agar against *Thielaviopsis basicola*.

Table 8(i): Results of the effects of candidate fungicides (Octave, Plover and Topas) on radial growth of *T. basicola* in agar culture. Results recorded (a) 7 days and (b) 14 days from inoculation.

(a)

ISOLATE	Concentration of Chemical/Colony diameter (mm)						
	Control	Octave		Plover		Topas	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	11.25	0	0	10.5	0	0	0
1C	12.25	0	0	10.5	0	0	0
3A	12.25	0	0	13	0	0	0
3B	11.5	0	0	14	0	0	0
5aC	11	0	0	11.75	0	0	0
5aA	9.75	0	0	0	0	0	0
5aB	13.5	0	0	16.25	0	0	0
5B	18.5	0	0	14.25	0	0	0
5C	14.5	0	0	13.75	0	0	0
7B	12.25	0	0	14.5	0	0	0
8A	11.5	0	0	13.75	0	0	0
8B	16	0	0	14.75	0	0	0
MEAN	12.8	0	0	12.3	0	0	0

(b)

ISOLATE	Concentration of Chemical/colony diameter(mm)						
	Control	Octave		Plover		Topas	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	33.5	0	0	10.5	0	0	0
1C	36	0	0	10.5	0	0	0
3A	52.75	0	0	13	0	0	0
3B	11.5	0	0	0	0	0	0
5aC	40.5	0	0	11.75	0	0	0
5aA	31.75	0	0	0	0	0	0
5aB	50.75	0	0	16.25	0	0	0
5B	57.25	0	0	14.25	0	0	0
5C	53.25	0	0	13.75	0	0	0
7B	45.5	0	0	14	0	0	0
8A	37.5	0	0	13.75	0	0	0
8B	38	0	0	14.75	0	0	0
MEAN	40.7	0	0	11.0	0	0	0

Table 8(ii) Results of the effects of candidate fungicides (Aura, Jonk and Fongarid) on radial growth of *T. basicola* in agar culture. Results recorded (a) 7 days and (b) 14 days from inoculation.

(a)

ISOLATE	Concentration of Chemical/Colony diameter (mm)						
	Control	Aura		Jonk		Fongarid	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	12.75	0	0	0	0	14.5	13.5
1C	11.5	0	0	0	0	14.5	17.25
3A	22.75	0	0	0	0	24.5	24.5
3B	22.5	0	0	0	0	22	20.5
5aC	25.75	0	0	0	0	24.25	23.5
5aA	26.25	0	0	0	0	23.5	23.75
5aB	23	0	0	0	0	24	24.5
5B	22	0	0	0	0	24.5	22.5
5C	21.25	0	0	0	0	23.5	22.75
7B	24.5	0	0	0	0	24.25	22.75
8A	22	0	0	0	0	24	21.5
8B	23.5	0	0	0	0	23.5	22.5
MEAN	21.48	0	0	0	0	22.25	21.65

(b)

ISOLATE	Chemical concentration/Colony diameter (mm)						
	Control	Aura		Jonk		Fongarid	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	30.25	8.5	0	0	0	37.75	43.25
1C	36.5	8.25	0	0	0	41.25	43.25
3A	53.25	0	0	0	0	54.25	55.5
3B	51.25	8	0	0	0	54	52.75
5aC	62	4.5	0	0	0	57.75	57
5aA	45	8	0	0	0	56.25	57
5aB	58.25	0	0	0	0	57	55
5B	51	10	0	0	0	57.5	53.75
5C	52.25	9.25	0	0	0	57	55
7B	60.75	0	0	0	0	61	59.75
8A	53.75	4	0	0	0	59	54.75
8B	54.25	0	0	0	0	58.25	56.5
MEAN	50.7	5.0	0	0	0	54.25	53.6

Table 8(iii): Results of the effects of candidate fungicides (Opus, Systhane and folicur) on radial growth of *T. basicola* in agar culture. Results recorded (a) 7 days and (b) 14 days from inoculation.

(a)

ISOLATE	Concentration of Chemical/Colony diameter (mm)						
	Control	Opus		Systhane		Folicur	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	9.5	0	0	0	0	0	0
1C	8.5	0	0	0	0	9.5	0
3A	14	0	0	0	0	7.5	0
3B	11.25	0	0	0	0	11	0
5aC	11.25	0	0	0	0	10.75	0
5aA	10.75	0	0	0	0	0	0
5aB	15.5	11.75	0	0	0	16	0
5B	13.5	0	0	0	0	14.5	0
5C	14.5	3.25	0	0	0	17.75	0
7B	11.25	0	0	0	0	13.5	0
8A	12	9.5	0	0	0	12	0
8B	9.25	0	0	0	0	0	0
MEAN	11.8	2.0	0	0	0	9.4	0

(b)

ISOLATE	Concentration of Chemical/Colony diameter (mm)						
	Control	Opus		Systhane		Folicur	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	31.25	0	0	20.5	0	23.5	22.5
1C	31.5	17.5	16.5	26.5	0	13.5	18
3A	43.5	19	28	36	0	38.5	30.75
3B	37.75	29.75	26	0	0	28	0
5aC	32	7.5	0	21	0	19.25	23
5aA	35	29.5	0	16.25	0	0	23.25
5aB	44.5	28	18	22.5	0	29.5	26.5
5B	35.5	30	15.5	36	0	45.5	37.5
5C	42.5	31.75	28	27.5	0	43.5	0
7B	40	26.5	22.5	0	0	37.75	0
8A	39.25	29	0	12.75	0	43.5	12
8B	32	21	0	0	0	0	0
MEAN	37.1	22.5	12.9	18.25	0	26.9	16.1

Table 8(iv): Results of the effects of candidate fungicides (Scala, Alto 100 and Rovral) on radial growth of *T. basicola* in agar culture. Results recorded (a) 7 days and (b) 14 days from inoculation.

(a)

ISOLATE	Concentration of Chemical/Colony diameter (mm)						
	Control	Scala		Alto 100		Rovral	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	11.75	10.75	3	0	0	4	4.5
1C	7.5	7.75	0	0	0	0	0
3A	13.75	5	0	0	0	0	0
3B	15.75	3.75	0	0	0	8	11
5aC	14.75	4	0	0	0	8.75	0
5aA	12.25	13	11.25	0	0	10.5	11.25
5aB	16.25	10.5	0	0	0	11.25	11
5B	19	13.5	0	0	0	13	12.75
5C	15.25	12	10.25	0	0	10.5	11
7B	12.25	14	8.75	0	0	9.25	9.25
8A	11.5	10.7	7.5	0	0	9.75	5
8B	17.5	10.25	7	0	0	8	12
MEAN	13.6	9.6	3.98	0	0	7.75	7.3

(b)

ISOLATE	Concentration of Chemical/Colony diameter (mm)						
	Control	Scala		Alto 100		Rovral	
	0ppm	2ppm	20ppm	2ppm	20ppm	2ppm	20ppm
1B	38.5	9.25	9.5	0	0	18.75	19
1C	24.75	10.5	0	0	0	14.25	15.75
3A	39	10.5	0	0	0	32.25	25.5
3B	50.25	16.5	13	0	0	20	29
5aC	51	10.25	0	0	4	23.5	18.25
5aA	44.5	20	14.5	11	0	35.75	31.25
5aB	56.25	17	9.5	8.5	0	42.5	29.5
5B	58.25	21.5	9	12	0	30.75	39.25
5C	53.5	26.5	13	10	0	31.25	34.75
7B	50.5	16.75	6.5	10.5	0	22.25	25.25
8A	39	11	0	9.25	0	16.5	25.75
8B	39.5	11.75	7.5	5.25	0	32.75	18.5
MEAN	45.42	15.1	6.9	5.5	0	26.7	26.0

Table 8(v): Results of the effects of candidate fungicides (Bion and Unix) on radial growth of *T. basicola* in agar culture. Results recorded (a) 7 days and (b) 14 days from inoculation.

(a)

ISOLATE	Concentration of Chemical/Colony diameter (mm)				
	Control	Bion		Unix	
	0ppm	2ppm	20ppm	2ppm	20ppm
1B	7	8.25	8	0	5
1C	7.5	7	7.5	7.5	8.5
3A*					
3B	13.5	10	9.25	12	11.75
5aC	11.5	8.75	8.5	10.75	5.25
5aA	9.25	8.5	8	9.5	8.5
5aB	16.5	12.25	12	16.5	12.5
5B*					
5C	14.25	13	11.75	12.5	12.5
7B	7.25	10.75	9.75	8.5	4.5
8A	8.75	9.75	8.5	8.75	7.75
8B	14.25	12.5	12	13.5	10.75
MEAN	10.98	10.1	9.5	9.95	8.7

(b)

ISOLATE	Concentration of Chemical/Colony diameter (mm)				
	Control	Bion		Unix	
	0ppm	2ppm	20ppm	2ppm	20ppm
1B	40.25	28.75	31	27	27.5
1C	40.5	39.75	40.5	33.75	41
3A*					
3B	65.25	58.5	56.5	61.25	59.5
5aC	56.75	31.25	27.25	37	28
5aA	38	33.75	19.75	48.25	28.25
5aB	64.75	68.5	67.5	71	68.75
5B*					
5C	65	66.75	65	69.75	69.75
7B	58.75	63.75	64.25	50.25	46.5
8A	52.5	44.5	39.25	44.5	39.25
8B	47.25	32.25	85	45.25	42
MEAN	52.9	46.8	49.6	48.8	45.05

* No data available due to contamination of the agar plates.