EPIDEMIOLOGY OF BLACK ROOT ROT IN BEDDING PLANTS

Project PC 38a

Final Report July 1996

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Objectives and background

Black root rot, first known in the USA in early '90s on tobacco, affects a wide range of plant species, both woody and herbaceous. The disease has become a major problem in herbaceous plant species grown for bedding. Symptoms comprise loss of vigour, delayed flowering, reduced growth, leaf yellowing and poor root growth. Plants often die during the production stage, or after planting to their final positions. There is a trend in the UK to large-scale production and mechanisation of bedding plants particularly the use of modular trays and reduced volumes of growth media. The changes require high standards of management. Black root rot control has been mainly by the use of fungicides however results have been variable. Furthermore, the future marketing of some of the fungicides is in doubt for commerical reasons. There is a need for alternative control measures. In the past few years US scientists have given considerable emphasis to the role of plant stress in black root rot, and the use of environmental management techniques for control.

In the opinion of the author there was insufficient information on the biology of the disease as affecting bedding plants to understand the reasons for the variations in outbreaks observed in commercial glasshouses. The lack of standardisation in experiments also meant that it was impossible to make accurate interpretations of data. Also, very little experimental data could be found to substantiate the US reports about plant stress as a pre-disposing factor in black root rot. Hence it was not possible to interpret the US information in terms of the impact in UK conditions.
Project PC38 was set up following extensive discussion between the British Bedding and Pot Plant Association (BBPPA), HRI and the HDC. The Objectives were to (1) review the scientific literature particularly the US information on the relationship of environmental stress and black root rot; (2) determine the epidemiological characteristics of UK isolates of *Thielaviopsis*, the black root rot fungus; (3) investigate the effects of environment and plant stress on black root rot. A key part of the research was to devise standardised experimental methods that would provide the foundation for the detailed research in this project and also research by others.

Summary of Results

In a series of experiments we developed standard methods for the production of inoculum, infection of pansy seedlings, pH and EC measurement of growth compost, and the evaluation of root symptoms.

We investigated a variety of different forms of *Thielaviopsis* for inoculation and were able to induce black root rot by inoculation of pansy with the two spore types. Black root symptoms comprised leaf wilt, yellowing, reduced growth of aerial parts, and blackening of the roots and the stem base due to the presence of chlamydospores: the symptoms were typical of those found in commerce. Black root rot could be confused with symptoms caused by *Pythium* infection e.g. in samples sent for diagnosis, and informed microscopical and cultural studies are needed to distinguish the two; the control measures are radically different for the two diseases.

Low *Thielaviopsis* populations caused infection in pansy but without giving rise to black root rot symptoms. However, such healthy-looking plants with *Thielaviopsis*-infected roots died after transplanting. Clearly there is the danger that black root rot affected plants without symptoms could enter the trade and give problems later.

We found that temperatures of 15-25°C, and pH 5.5 favoured *Thielaviopsis* activity. Moist compost favoured black root rot development. Different *Thielaviopsis* isolates, however, varied in their response to environmental factors in controlled conditions. We were unable to find any published data to support the role of stress in black root rot. In experiments we found little evidence to suggest that environment stress e.g. temperature, pH, nutrient and low moisture affected black root rot. However, we did find that temperatures of 30°C and above stimulated endoconidium production and hence raised population levels of the fungus and the risk of spread.

We recorded temperatures of more than 30-35°C in compost in modular trays in a glasshouse in summer. These temperatures were higher than expected and would have had the dual effect of damaging Pansy and favouring *Thielaviopsis*. When combined with high compost wetness, the high summer glasshouse temperatures increased black root rot severity in pansy, probably due to the spread of the fungus. We were then able to demonstrate that irrigation water and the associated splash were factors in the spread *Thielaviopsis* to neighbouring plants. Raising modular trays off the bench matting markedly reduced the spread.

Unexpectedly, some experimental plants became affected by black root rot even though they had not been inoculated by the fungus. The source of infection was traced to outbreaks of insects. In controlled experiments, we were able to demonstrate that shore flies and fungus gnat larvae fed on *Thielaviopsis* and then spread the fungus in viable form to neighbouring plants. We think that this is the first report of the spread of *Thielaviopsis* by insects in bedding plants.

Finally we used information from the literature to prepare a protocol for black root rot control. However, we could find no published experimental evidence to support the protocol and feel that UK growers should observe caution. Some of the recommendations, e.g. the control of temperature and compost moisture appear impractical.

Action points for growers

- Pay careful attention to hygiene on the whole nursery e.g. use new or disinfected trays, cover unused stocks of growth compost, make sure that irrigation supplies are free from contamination by *Thielaviopsis*.

- Do not put trays or pots directly on soil in the glasshouse, nor allow roots to grow through plastic mesh covers into the soil beneath.

- Minimise potential sources of the black root rot fungus on the nursery e.g. hardy ornamental nursery stock, houseplants, bedding plants with subclinical symptoms.

- Avoid excessive watering and raise trays off the bench to minimise transfer of *Thielaviopsis* from infected to healthy plants.

- Control 'glasshouse insects' that spread black root rot.

Practical and financial benefits from study

The work has clearly identified the need to avoid excessive irrigation and high temperature and to control insects that spread *Thielaviopsis*. The work has also highlighted a lack of evidence that management of the glasshouse environment to reduce plant stress will control black root rot in UK conditions. At a wider scientific level the work has provided methods that will lead to more reproducible experimentation, and hence increase reliability in the evaluation of control methods.

Epidemiology of Black Root Rot in Bedding Plants

Introduction

Project PC38 was set up at the request of the British Bedding and Pot Plant Association (BBPPA) under the Chairmanship of Mr Brian Crosby, and involved extensive prior discussion between the BBPPA, HRI and the HDC. The two main reasons for the project were (1) black root rot was causing serious and unpredictable losses in bedding plants and (2) there were reports from the USA describing the role of environmental stress as a factor in black root rot, and crop and environment management methods as control measures.

The objectives were to (1) review the scientific literature particularly for information on the relationship of environmental stress and black root rot; (2) determine the epidemiological characteristics of UK isolates of *Thielaviopsis*, the black root rot fungus; (3) investigate the effects of environment and plant stress on black root rot.

Staff involved in PC38 were Sarah Jenkins, November 1992-January 1993; Jonathan E Biddulph July 1993-July 1996.

The work was accepted in 1997, for the degree of PhD at the University of Birmingham; graduation is expected in 1997 (Jonathan Biddulph).

Key findings

1 Black root rot was readily induced by inoculation of pansy with *Thielaviopsis*.

2 Black root symptoms comprised leaf wilt, yellowing, reduced growth of aerial parts, loss of turgor and root blackening and stem blackening.

3 Black root could be confused with symptoms caused by *Pythium* infection.

4 Standard methods were developed for the production of inoculum, for infection, pH and EC measurement, and the evaluation of root symptoms.

5 Low *Thielaviopsis* populations caused infection but not black root rot symptoms.

6 Healthy-looking plants with *Thielaviopsis*-infected roots died after transplanting.

7 *Thielaviopsis* activity was favoured by temperatures of 15-25°C, and pH 5.5.

8 *Thielaviopsis* isolates showed differences in their response to environmental factors in controlled conditions.

9 Moist compost favoured black root rot development.

10 No published data was found to support the role of stress in black root rot.

11 Little experimental evidence was obtained that suggested environmental stress affected black root rot e.g. temperature pH and nutrient stress.

12 Temperatures of 30°C and above stimulated endoconidium production.

13 Compost temperatures in the glasshouse exceeded 30-35°C in summer; these temperatures damage Pansy and favour *Thielaviopsis*.

14 High summer glasshouse temperatures and excessive watering increased black root rot severity in pansy.

15 Irrigation water and water splash spread *Thielaviopsis* to neighbouring plants.

16 *Thielaviopsis* survived ingestion by Shore Fly and Fungus Gnat larvae and spread in the glasshouse.

17 A protocol for black root rot control was prepared, based on information from the literature. No published experimental evidence was found to support the protocol.

Commonly used Terms and Abbreviations

(See also Glossary, page 56)

Compost: peat-based growth medium, either Fisons F1 (abbrev. FF1) or Fisons F2 (FF2) compost.

Thielaviopsis refers to *Thielaviopsis basicola* and includes any reference to *Chalara elegans* (see page 7).

PDA - potato dextrose agar solid growth medium; PDB - potato dextrose broth liquid medium.

TBCEN - *Thielaviopsis* selective isolation medium (see Specht & Griffin, 1985).

EC: Electrical conductivity in µS (microSiemens)

pH: measurement of alkalinity (pH>7) and acidity (pH<7)

P: probability value used in statistical analysis

SED: Standard Error of a Difference between two treatment means (statistical test).

The Project

1 Review

Literature review of (1.1) techniques available for the study of black root rot and (1.2) the characteristics of black root rot and the causal pathogen (Objective 1).

1.1 Techniques for study of black root rot

Information on techniques is described in Section 3 with the various experiments (see pages 12-21).

1.2 Characteristics of black root rot and the causal pathogen

Symptoms. Black root rot (BRR) reduces growth in bedding plants and causes leaf yellowing, damping-off, wilt, reduced and delayed flowering, and death. The responses are typical of a damaged root system and there is the possibility of confusion with other root diseases e.g. ***Pythium***. Black root rot-affected roots change from white to black due to the production of dark-coloured **chlamydospores** (Meyer *et al.,* 1989). The dark chlamydospores sometimes develop on the base of the bedding plant stems at soil level and this helps diagnosis (personal observations during project). The causal fungus (***Thielaviopsis***) also affects hardy ornamental nursery stock plants (**HONS**) and symptoms commonly develop in drought conditions after transplanting due to the effects of impaired water uptake in a mal-functioning root system. *Thielaviopsis* is implicated in **'decline'** symptoms and **replant disease** in woody plants in open ground e.g. cherry (Sewell & Wilson, 1975).

Causal organism. Black root rot is caused by ***Thielaviopsis basicola*** a fungus which is soil-borne (Tabachnik *et al.*, 1979). There are two spore forms: (1) the **chlamydospore**, the description of which forms the basis for the name *Thielaviopsis*, and (2) the **endoconidium** the features of which have, confusingly, given rise to a second name, ***Chalara elegans***; *Thielaviopsis* is now the accepted correct name (Shew & Meyer, 1992). The two spore forms have different functions in the life cycle: chlamydospores assist in **survival**; they comprise one-eight dark segments attached to groups of two or three thin-walled transparent basal cells the total unit being 38 x 12 µm; the segments may separate into single spore units (Patrick *et al.*, 1965). Endoconidia are thought to aid **dissemination** of the fungus within and between crops and do not survive for long; they are hyaline, cylindrical and 8-17 x 3-5 µm in size. In addition, there are three types of hyphae: (1) hyaline and thin-walled; (2) dark and thick-walled; (3) thick-walled, dark, dichotomously branched and 5-8µm diameter (Hawthorne & Tsao, 1970; Papavizas & Adams, 1969; Punja, 1993a; Shew & Meyer, 1992; Otani, 1962); apart from their involvement in growth and infection, there is no information that they have any specialised function.

Distribution and host range. *Thielaviopsis* is reported from 46 countries, and affects more than 137 species in 33 families, especially the Leguminosae and Malvaceae (Shew & Meyer, 1992; Otani, 1962). The first reports of *Thielaviopsis* were from tobacco in Europe and the USA where black root rot was very damaging (Tabachnik *et al.*, 1979). The disease also causes serious loss in landscape plantings of Japanese holly (*Ilex crenata*), *Prunus* and stored carrots (Punja, 1993b; Wicks, 1987; Sewell & Wilson, 1975). In the UK, black root rot occurs regularly in bedding plants including *Antirrhinum*, *Aster*, *Begonia*, *Cyclamen* and 'winter pansy' or *Viola* (Scrace, 1993). The role of *Thielaviopsis* in UK HONS is poorly documented and requires further study e.g. the prevalence of root infection and its effect on plant vigour, and the potential of infected HONS plants to be a source of inoculum for infection of bedding plants grown on the same nursery.

Survival and spread of *Thielaviopsis*. *Thielaviopsis* occurs in cultivated and non-cultivated soils i.e. it appears to be a normal component of the soil microflora. However, there was little information on the relative importance of naturally occurring and commercially introduced sources of the fungus. The fungus survives as **mycelium** in dead plant residues (Shew & Meyer, 1992) and as chlamydospores that are produced in large numbers on infected host tissue (Sewell & Wilson, 1975). Endoconidia, which can only be seen through a microscope, are produced on roots but survival is limited to a few days. *Thielaviopsis* is thought to be spread by contaminated growth medium ('compost'), dust from infected plant residues, and by spores in contaminated **irrigation** e.g. water splash or bench matting (Lindquist & Powell, 1992).

Other diseases of Pansy. Specimens sent to Dr Entwistle's laboratory were commonly affected by ***Pythium***, **downy mildew**, **powdery mildew** and an unidentified leaf spot, possibly **bacterial** in nature. It was apparent that symptoms arising from Pythium were being confused with those of Thielaviopsis; therefore specialist identification is essential in order to select appropriate control measures.

Stress and black root rot. Black root rot has been regularly reported from the USA as being favoured by '**stress**' i.e. environmental conditions that adversely affect growth. Furthermore, the management of stress has been proposed as a control measure (Powell, 1989). Common stress factors are water, temperature, nutrition, salinity, pH and EC. We found no experimental data to support the involvement of stress in black root rot.

Water stress develops in dry conditions when leaf stomata open, transpiration rates are high and water availability is low. Turgor is lost, stomata close, cell wall and protein synthesis decline, cell division stops and leaf abscisic acid production increases. Water logging causes stress due to anaerobiosis at the root surface; water shortage occurs in high temperatures when humidity is low: in peat compost, water is held at low tensions and transpiration rates remain high until the water is almost exhausted. Shallow containers - <2.5 cm deep - tend to waterlog (Ball, 1991) therefore compost aeration is very important (Fonteno, 1988a, b; Koranski, 1987). Hence, water management is claimed to be the key to successful plant production in modular trays (Koranski & Karlovich, 1989; Koranski & Laffe, 1988). Compost temperature, moisture, nutrients, oxygen, pH and 'salt' accumulation are increasingly likely to fluctuate as module size declines.

Temperature stress develops when evaporative cooling ceases due to water shortage, or stomatal closure: leaf temperatures rise, proteins denature and the properties of membrane lipids change. By analogy with other fungi e.g. *Sclerotium cepivorum*, stress may reduce *Thielaviopsis* activity making it less able to resist competition from organisms in the root environment.

pH stress develops indirectly when a high pH reduces cation availability e.g. iron, zinc, copper and manganese in alkaline soils. Otherwise, most plant species grow well in the range pH 4-8, provided minor elements are sufficiently available.

Nutrient stress is caused by nutrient excess (toxicity), deficiency or imbalance, especially nitrate, molybdenum, zinc, phosphorus, calcium (Jarvis, 1992) and iron. These minerals variously affect hormone production and plant growth, pectin insolubility, hence resistance to fungal infection, or photosynthesis.

Salt (saline) stress occurs at salt concentrations that lower the water potential by 0.05 to 0.1 MPa; this leads to increased osmotic potential of the rooting medium and stunted growth. Plants do not wilt because the cell water potential is lowered by the increase in solutes that enables parenchyma cells to compete for water from the xylem.

2 Protocol for control

One aim of this project was to:

> **Devise a protocol for controlling black root rot based on the manipulation of cultural factors, the protocol to take into account the pH of the growth medium compost, composition of nutrients, EC and water quality. Test the protocol against black root rot in comparison with treatments which favour black root rot and relate results to changes in *Thielaviopsis* and *Viola* (Objectives 5.2, 6.2)**

A protocol for the control of black root rot in module-produced pansy was prepared with information obtained from a variety of sources including tape recordings of two presentations by Professor D Koranski at the Four Oaks Show, September 1990, and the scientific literature (Karlovich & Koranski, 1989; Karlovich *et al.*, 1989; Karlovich & Koranski, 1988; Koranski 1988a-c; Koranski & Laffe, 1988; Koranski, 1987). The following is a summary of the protocol and a full description is found in Appendix 2 (see page 52).

Protocol for the Control of Black Root Rot in Module-produced Pansy

Production of pansy is in four stages:

Stage 0: Assessment before seed sowing

1 Water should be pH 5.0-6.5, alkalinity 60 ppm bicarbonate, sodium <35 ppm.

2 Seed sowing medium should have a high buffering and water holding capacity, wide particle size distribution and good aeration, and a high cation exchange capacity (CEC), pH 5.5-5.8 or 5.8-6.5 and electrical conductivity (EC) of 750- 1000 µS, low nutrient status ratio of 1:1:1:0.5:1 N:P:Ca:Mg:Fe.

3 Seed quality should be 'good'. Primed seed is preferable because it tolerates 'warm' temperatures better than standard seed.

4 Containers should be new; the effects of small versus large module sizes should be evaluated - the former use small volumes of compost but there are greater fluctuations in compost moisture content, nutrients, oxygen, pH and soluble salts. Larger modules use more compost but reduce salt build-up, have greater aeration, and increase the production rates of high quality plants.

Stage 1a Seed Germination (4-7 days)

(1) Sow seed on top of compost and in the centre of the module in order to optimise

moisture, oxygen and temperature; (2) maintain water at 95-100 % relative humidity (RH) until radicle emergence; (3) maintain temperature in range 16.7-20°C; (4) adjust compost EC to < 750 µS and pH 6.5-8, and incorporate a fungicide e.g. benomyl; (5) do not stack the modular trays as this adversely affects germination.

Stage 1b Radicle emergence
(1) Reduce RH to 75-80 % using fog or mist; (2) raise temperatures to 20-23.9°C, (3) adjust compost to pH 5.5-5.8 and nutrient EC to <1000 µS, calcium 50 ppm, and magnesium 25 ppm; (4) transfer modular trays from germination chamber to glasshouse when cotyledons emerge from seed coat.

Stage 2 Stem and cotyledon emergence (next 7 days)
Maintain temperature at 16.7-20°C and apply 25-50 ppm of 20:10:20 fertilizer once a week following cotyledon expansion.

Stage 3 Growth and development of true leaves (next 14 days)
(1) Reduce watering and humidity; (2) maintain growth medium at pH 5.5-5.8 to control *Thielaviopsis* and control boron availability; (3) maintain nutrients at EC <1000µS, sodium <40 ppm, chlorine 30-40 ppm and ammonia <15 ppm; apply compound fertiliser at 50-100 ppm as needed.

Stage 4 Conditioning the plants in preparation for transplanting
(1) Keep compost moist and irrigate 2-3 hours before transplanting; (2) maintain plants at 12.8-15.6°C; apply fertilizer at 50-100 ppm applied as needed.

Transplanting
(1) Do not leave plants in modular trays longer than needed; (2) avoid using fungicides and fertilisers until roots emerge from the root ball. The transplant compost should be EC 1000 µS.

The above protocol is prepared from information on US conditions where mechanisation, and regular checking and adjustment of compost and irrigation pH and EC is routine. The protocol may need modification for UK production to take into account the different facilities available. ***It is emphasised that we found no experimental data to support or otherwise the above protocol. Certain of the recommendations eg. about pH varied between different versions of information and there would be practical difficulties implementing others to the accuracy described e.g. pH and temperature.***

Following discussions, and with the agreements of the Co-ordinator and Dr E Moorhouse, HDC, the final part of this Objective, to test the validity of the Protocol (see Appendix 1 para. 6.2), was deferred. The decision was made because the protocol included specific recommendations on environmental factors the implementation of which would have raised considerable practical difficulties both at HRI and in UK commerce.

3 Experimental

3.1 Devise standard methods

Devise methods for (3.1.1) identification of *Thielaviopsis*, (3.1.2) isolation and enumeration of *Thielaviopsis* from plant material and growth media, (3.1.3) production of *Thielaviopsis* inoculum and (3.1.4) production and enumeration of black root rot symptoms

A lack of quantitative experimental methods has previously given rise to variable experimental results that have been difficult to interpret, and this has hindered progress on black root rot research. ***The standardisation of methods was a key part of the project, and forms a foundation for future research.***

Origin of *Thielaviopsis* isolates

Thielaviopsis was obtained as purified isolates from ADAS Wolverhampton or from infected pansies sent to the author for diagnosis (see Appendix 3, page 54); *Thielaviopsis* was isolated on TBCEN selective medium (Specht & Grifffin, 1985) and maintained on potato dextrose agar solid medium (PDA) at 4°C. The potential for change in *Thielaviopsis* was minimised by using stock cultures as the source of *Thielaviopsis* in experiments. Isolate TB8c was used in experiments unless stated otherwise.

3.1.1 Identification of *Thielaviopsis*

Thielaviopsis isolates from infected plants and culture were grown on PDA or potato dextrose liquid medium (PDB) and compared with standard descriptions of the fungus.

3.1.2 Isolation and enumeration of *Thielaviopsis*

Experiments investigated: (a) different methods of isolation from diseased roots, some of them apparently healthy; (b) the isolation of *Thielaviopsis* from apparently healthy pansies from commercial nurseries and HRIW; (c) the enumeration of *Thielaviopsis* in compost.

Isolation. Roots from 6 week-old pansies were classified as: (a) heavily infected roots from diseased root systems; (b) apparently healthy roots from diseased root systems i.e. symptomless roots (see Appendix 1 para 6.3.3; (c) healthy roots from healthy, non-inoculated root systems. Roots were surface sterilised in sodium hypochlorite solution, washed in sterile distilled water (Shew & Meyer, 1992), and transferred to PDA, TBCEN selective medium or moist sterile filter paper and incubated in darkness at 25°C for two to four weeks.

Enumeration. Endoconidium suspensions, prepared as above, were serially diluted, counted with a haemacytometer count chamber and the populations calculated; spore populations that were below the threshold of accuracy of the haemacytometer (<approx. 10^7 spores/ml) were calculated by accurate dilution from known high spore populations.

A similar method was evaluated for the enumeration of *Thielaviopsis* in peat-based growth media. Aqueous suspensions of 10 to 10^5 endoconidia ml^{-1} were transferred to TBCEN, incubated at 25°C for 3 days then 20°C for 3 days, and colony numbers counted. One ml from each of a range of spore populations was transferred to Fisons F1 (FF1) compost and mixed, the mixture diluted 10 -10^3 times in water, aliquots incubated on TBCEN and the colony numbers counted.

3.1.3 Production of inoculum

Six experiments investigated the preparation of spore inoculum for experiments: (a) the effects of flooding on the removal of spores from PDA cultures; (b) combinations of filtration, differential centrifugation and desiccation on purification of spore preparations.

3.1.4 Production and enumeration of black root rot symptoms

Seedling infection test on filter paper. Ten *Thielaviopsis* isolates were produced on PDA and inoculated onto two week-old pansy seedlings on filter paper. Root symptoms were assessed after two weeks.

Young plants produced in compost. Modular trays containing FF1 were treated with 10^3 chlamydospore units/module of isolates Tb8c, Tb11, Tb12, Tb14 and Tb15; control modules contained equivalent volumes of water but no spores. The trays were sown with pansy, transferred to a glasshouse and the symptoms evaluated six weeks later.

Seedling infection test in compost with different spore populations. Populations of 25-250 chlamydospores g^{-1} soil or 100-1000 endoconidia g^{-1} soil are reported to cause a degree of black root rot symptoms that permits treatment effects to be distinguished (Shew & Meyer, 1992). Therefore, populations of up to 10^5 endoconidia/module or 10^4 chlamydospores/module were tested for their effect on black root rot in pansy seedlings in a glasshouse.

3.2 Characteristics of *Thielaviopsis*

(3.2.1) Investigate the capacity of *Thielaviopsis* to grow in growth media (compost). Determine the sensitivity of *Thielaviopsis* in vitro and in vivo, using controlled environments, to (3.2.2) temperature, (3.2.3) moisture and (3.2.4) pH.

3.2.1 Growth of *Thielaviopsis* in growth media

Two drops of a suspension of chlamydospores, endoconidia and mycelia, prepared from an 8 week-old culture, were transferred to nylon gauze pieces (3 x 3 cm). and placed on FF1 in a Petri dish; compost was watered with DW or Vitafeed (1:200 dilution) and incubated at 25°C. After 2 weeks, the gauzes were removed, and stained with cotton blue in lactophenol.

Suspensions of 5,000 chlamydospores were also transferred to Millipore (AP25) filters and transferred to FF1. Pansy seeds were placed on the compost, watered and the dish sealed and incubated at 25°C. The filter papers were removed after three weeks and the inner surfaces examined. A second dish was prepared without pansy seeds.

3.2.2 Effects of temperature on growth of *Thielaviopsis* and infection of pansy

Black root rot is favoured by high temperatures (Bateman & Dimock, 1959; Lucas, 1955; Johnson & Hartman, 1919) and by temperatures that are unfavourable to host growth.

The effects of temperature were investigated on: (a) *Thielaviopsis* growth in culture; (b) spore germination in culture; (c) black root rot severity in pansy.

a Colony growth in culture. Petri dishes containing PDA were inoculated with Tb8a, incubated at 10, 15, 20, 25, 30 and 35°C and the colony diameters recorded for 17 days. The experiment was repeated with 16 *Thielaviopsis* isolates.

Growth of Tb8a, Tb11, Tb12, Tb13, Tb14 and Tb15 in PDB was investigated at similar temperatures by measuring the oven dry weights of mycelium after 17 days.

b Spore germination in culture. **Endoconidia** were added to Petri dishes containing PDA, incubated at 10-35°C and germination assessed after 16 hours. Germination was also tested in PDB on glass microscope slides. **Chlamydospore** suspensions of approx. 10^2 ml^{-1} were transferred to TBCEN, incubated at 10 or 20°C and germination assessed 3-21 days later. Chlamydospore chains of Tb8c, Tb11, Tb12, Tb14 or Tb15 were transferred to TBCEN, incubated at 10-30°C and germination assessed after 3 and 7 days.

Endoconidiophore production (Additional objective). Differences in the rate of spore production, rather than spore activity, have the potential to affect black root rot development. The effects of constant and alternating temperatures on the production of endoconidiophores - the fungal structures that produce the spores - were studied: (a) endoconidia of Tb8c, Tb11, Tb12, Tb14 and Tb15 were incubated at 20, 25, 30 or 35°C; (b) endoconidia were incubated at 35°C for 6 h, then at 20°C for 14 h, or 40°C for 3 h, followed by 20°C; (c) endoconidia were incubated at 20°C for 8 h, then at 30 or 35°C for 6 h, or at 40°C for 3 h; the spores were returned to 20°C for the remainder of the 24 hour period. After treatment, the production of the new generation of endoconidiophores produced by germinating endoconidia was also evaluated.

3.2.3 Effects of water availability on *Thielaviopsis* growth and pansy infection

Water potential, the water energy status in plants and microorganisms, is the best measure of water availability. Water potential comprises **osmotic potential**, resulting from the nature and quantity of solutes, and **matric potential,** which results from the surface tension of water on particles. Osmotic potential is important in plants and matric potential is important in soil or compost. Other components of water potential, pressure (turgor) potential and gravitational potential, were not investigated as they are not relevant in the present context.

a Colony growth in culture. PDAs at water potentials of -0.25 to -3.5 MPa were prepared with mannitol, checked with a hygrometer/Dew point microvoltmeter, inoculated with *Thielaviopsis*, and the colony growth measured for 18 days. Growth in PDBs at similar water potentials was also evaluated by measuring mycelial dry weight.

b Spore germination. **Endoconidia** were transferred to PDA or PDB at similar water potentials as above, and colony development measured after 15-43 hours. **Chlamydospore** chains of five *Thielaviopsis* isolates were transferred to PDAs of similar water potentials and colony development measured after 7-11 days.

c Infection of pansy. Pansy seedlings were produced on filter paper of different moistures, inoculated with endoconidia or chlamydospores and black root rot symptoms assessed two weeks later. In a second experiment, FF1 in modular trays received 1, 2 or 3 ml water/module to give 'dry', 'moist' and 'wet' (i.e. saturated) treatments; all treatments comprised *Thielaviopsis* inoculated and non-inoculated FF1. Water was applied when compost receiving the 'moist' treatment appeared dry. Stomatal conductance and leaf water potential were measured at intervals for six weeks. After seven weeks, plants were examined for the presence of black root rot; plants that appeared healthy were transplanted to FP7 pots containing FF2. The effects of chlamydospore inoculum was tested in two experiments (27 February-21 April 1995; 5 June-21 July; endoconidial inoculum was tested in a third experiment (12 June-28 July).

3.2.4 Effects of pH on growth of *Thielaviopsis* and pansy infection

Measurement of compost pH

Various methods were compared for their accuracy and suitability for measuring the pH of small volumes of compost as found in modular trays.

'ADAS' method. Compost sieved <6 mm was moistened in the ratio 400 ml water + 1/15 mass of 1 litre compost, shaken for 1 hour and the pH recorded. The '**Fisons loose fill bulk density method**' differed in that (a) preparations were shaken for 30 minutes and (b) the bulk density was calculated on the basis of moist compost. A third method comprised compost:glass distilled water ratios of 1:2.5 or 1:1.5 by weight and the pHs of the slurries were recorded immediately (Metson, 1971).

The effects on pH measurement of compost preparation in slurries with 0.01M calcium chloride solution, and different volumes of compost, were also studied.

a Colony growth in culture. PDAs with target values pH 5.0-7.0 (0.5 pH increments), prepared by the addition of 50 mM citric acid or bipotassium hydrogen phosphate (K_2HPO4) solution, were inoculated with *Thielaviopsis*, incubated at 21°C and colony growth measured at intervals for 24 days; 16 *Thielaviopsis* isolates were tested.

Interaction between pH and temperature on colony growth. *Thielaviopsis* cultures produced on PDA at pH 5.0-7.0 were incubated at temperatures of 15-35°C and colony growth measured as before.

Mycelial growth in liquid medium. PDBs were prepared with similar pHs to the above, inoculated with Tb8c, Tb11, Tb12, Tb14, or Tb15, incubated at 21°C, and mycelial dry weights determined.

b Spore germination. PDAs at pH 5.0-7.0 were inoculated with standard populations of endoconidia, incubated at 25°C and germination assessed after 48 hours. PDBs of similar pHs were inoculated with endoconidia of Tb11, Tb12, Tb14 or Tb15 and germination assessed after 15 hours. Chlamydospores were tested by transferring spore chains of Tb8c, Tb11, Tb12, Tb14 or Tb15 to TBCEN at pH 5.0-7.0, incubating at 20°C and recording colony development after 10 days.

c Infection of pansy. Experiments to test the effects of stress on black root rot are described in the Section 3.3 (see pages 18-20).

3.2.5 Effects of fertilizer nutrients on growth and infection of pansy by *Thielaviopsis*

Measurement of Electrical Conductivity (EC) of peat-based compost

Suspensions of FF1 were prepared as for pH measurement (see Section 3.2 page 8) and the EC of filtered and unfiltered suspensions measured using a conductivity meter (Jenway, model 4070).

Effects of compost EC on seed germination

Pansy seed germination was tested in FF1 or FF2 in a growth room (18°C; warm white fluorescent light; 8 days) followed by transfer to a glasshouse. Trays were watered (pH=6.6; EC=1108 iS) every 2-4 days. Seed germination and development was recorded from 8 to 19 days after sowing. ECs and pHs of the compost were measured at the start and end of the experiment. For comparison, 50 pansy seeds were also tested for germination on a Copenhagen Tank at 15°C.

Effects of fertiliser on EC and pH of compost

Modular trays with FF1 were sown with pansies or left unsown. Seven days after sowing, trays received: (a) distilled water; (b) Vitafeed fertiliser at 1:200 dilution in DW (Appendix 4); (3) Peters Professional fertiliser at 1:200 dilution in DW; (4) no water, no fertiliser. Treatments 1-3 were applied to the trays containing seeds and treatment 4 to trays without seeds. Treatment was repeated every 2-4 days. The compost EC and pH were measured at intervals starting 17 days after sowing and using the Fisons method. The experiment was repeated.

Effects of nutrient concentration on growth and infection of pansy by *Thielaviopsis*

Modular trays were filled with FF1 and either inoculated with 10^3 chlamydospores/module or not inoculated. Plots of 36 modules received applications of 2 ml/module Vitafeed at: (a) 1:200 (EC=465µS, pH=6.24, 95 ppm N); (b) 1:100 (EC=901µS, pH=6.12, 190 ppm N); (c) 1:400 (EC=247µS, pH=6.20, 47.5 ppm N). Treatments were alternated with applications of 2 ml distilled water (EC=1µS, pH=5.64). Trays were sown with pansy (20°C; dark), transferred to a humidity chamber in the glasshouse after 9 days, then removed from the chamber at 14 days and the Vitafeed treatments applied thereafter. Compost and air temperatures were recorded in a second tray containing compost but no plants. Leaf water potential was measured (SKPM 1400 Portable Plant Moisture System) at 1400 h after 5 weeks. After 6 weeks some seedlings were transplanted to FP7 pots with FF2, watered as per normal commercial practice and the number of flowers recorded at weekly intervals. Fifteen weeks after sowing (9 October '95) plants were washed and roots examined for black root rot and the shoot dry weights determined. The experiment was repeated with endoconidia.

3.3 Environment and plant stress

(3.3.1) Identify types and duration of environment-plant stress in Viola-Black root rot, (3.3.2) devise methods of simulating environment-plant stress in Viola-black root rot, (3.3.3) investigate the interactions between the effects of stress on bedding plants and infection by *Thielaviopsis*.

3.3.1 Types and duration of environment-plant stress

Winter pansies are produced in modular trays with small cell volumes hence the compost has a limited capacity for buffering environmental changes: water, temperature, pH and EC are thought to be the factors most likely to fluctuate to the extent that they could give rise to stress.

Modular trays with FF1 were transferred to a glasshouse on 26 June 1994 when the weather was 'hot'. Temperatures were recorded at 30 minute intervals at a depth of 1 and 2 cm in the compost. The effects of module position (edge versus centre of tray) and compost moisture content (irrigated versus non-irrigated) were compared. Changes in water content were observed to be those encompassing the range from dry to saturated therefore detailed measurements were not made. Host stress was measured as stomatal conductance of the first-formed leaves using a Steady-State Porometer/EGM-1.

3.3.2 Methods for simulating environment-plant stress

Stress was simulated by: (a) alternating 'favourable' with 'unfavourable' temperatures; (b) varying the compost pH; (c) varying the compost moisture content. Details of the methods are described in the appropriate experimental sections that follow.

3.3.3 Effects of temperature stress

a Endoconidium germination and endoconidiophore production

Endoconidia were incubated in PDB at: (1) at constant temperatures of 20-40°C in 5°C increments (isolate Tb8c); (2) at various combinations of alternating temperatures: (a) 20°C x 7 h + 30°C x 6 h + 20°C x 7 h; (3) 20°C x 7 h + 35°C x 6 h + 20°C x 7 h + 35°C x 6 hours (h); (4) 20°C x 8.5 h, 40°C x 3 h, 20°C x 8.5 h (isolates Tb8c and Tb12). Germination and endoconidiophore production were assessed after 20 hours.

b Production of endoconidia on infected pansy roots

FF1 in modular trays was inoculated with 10^5 endoconidia or chlamydospores of Tb8c, TB12, TB14 or Tb15, and sown with pansy. Eight weeks later, the FF1 was removed and the pansy plants treated as follows: (1) plants transferred to glass tubes with the roots

dipping in 1:400 aqueous Vitafeed solution (pH 5.5; EC 234 µS), incubated at alternating temperatures of 20°C x 18 h + 35°C x 6 h daily and the solution examined for the presence of endoconidia after 4-9 days incubation; (2) as (1) but plants incubated at constant temperatures of 20, 25 or 30°C for 7 days or daily for a similar period in alternating temperatures of (a) 20 x 18 h, + 30 or 35°C x 6 h; (b) 20°C x 21h + 40°C x 3 hours.

3.3.4 Effects of pre-treatment temperature stress on chlamydospore germination

Chlamydospore suspensions were air-dried for 4 days and treated at 15-35°C in intervals of 5°C for 8 hours. The spore chains were transferred to TBCEN, incubated at 20°C and spore germination and colony development evaluated after 6-11 days.

3.3.5 Effects of temperature pre-treatment 'stress' of pansy seedlings on infection by *Thielaviopsis*

Pansy seedlings were produced on moist filter paper at 20°C and inoculated with 2 x 10^3 chlamydospores as follows: (a) plants inoculated and incubated at 20°C for 2 weeks; (b) plants treated at 30°C for 6 hours **before** inoculation then incubated at 20°C for 2 weeks; (c) plants inoculated and treated at 30°C for 6 h followed by incubation at 20°C for 2 weeks; (d) plants inoculated with chlamydospores that had been pre-treated at 30°C for 6 h, and the inoculated plants incubated at 20°C for 2 weeks; (e) plants inoculated and treated daily for 7 days at 20°C x 18 h + 30°C x 6 h then incubated at a constant 20°C. Black root rot severity was assessed as a root rot index. The experiment was repeated with endoconidia.

3.3.6 Effects of temperature stress on infection of pansy by *Thielaviopsis*

Modular trays with FF1 were transferred to seed trays lined with Vatex capillary matting, sown with pansy (6 July '94) and transferred to an illuminated growth room at 16-20°C for 7 days. Trays were transferred to a glasshouse for three weeks and inoculated with 10^3 endoconidia or chlamydospores per module, or with water, and transferred to an illuminated incubator at: (a) a constant 20°C; (b) alternating temperatures of 20°C x 16 h + 30°C x 8 hours. The plants were removed after 8 weeks and the black root rot index determined. Compost pH and EC were measured at regular intervals. The experiment was repeated two and four weeks later.

3.3.7 Effects of pH stress on pansy growth and infection by *Thielaviopsis*

Adjustment of the compost pH. FF1 in modular trays was treated with calcium hydroxide solution (pH 12.2), ferrous sulphate solution (pH 3.7) or distilled water until the compost pH was 6.5 or 5.0.

Effects of pH on pansy growth. Modular trays with FF1 at the recommended, higher or lower pHs than the recommendation were inoculated with 1000 chlamydospores/module,

sown with pansy and black root rot symptoms assessed at harvest. The experiment was repeated.

3.4 Spread of *Thielaviopsis*

(Additional objective)

3.4.1 Drainage water from infected pansies

Experiments were prepared as follows: 1) Hassy 308 trays containing healthy or infected pansies, were watered and the leachates from each tray collected. The leachates were left to settle for one day, the supernatant removed and the residual suspension centrifuged and re-suspended. The preparations were mixed with melted TBCEN and transferred to a total of 34 Petri dishes. The dishes were incubated at 21°C in darkness for 8 days and examined for the presence of *Thielaviopsis*; 2) three Hassy 308 trays were loosely filled with sieved FF1, one tray was inoculated with 1000 chlamydospores/module and DW was added to modules in the other two trays. The trays were sown with pansy (15 May '95), kept in darkness for 5 days at 20±1°C, transferred to a fogging chamber for 5 days in a glasshouse. After fogging, the inoculated tray was placed on Vatex capillary matting, non-inoculated trays placed alongside, one tray raised off the matting and one placed in contact with the matting. Trays were hand watered from above and fertilised twice a week. Plants were assessed for black root rot after 8 weeks; 3) five severely infected roots from the inoculated tray in the previous experiment were placed in separate beakers with 20 ml water for 5 days at room temperature and the suspensions examined. Centrifuge tubes were also placed under individual modules containing infected plants and the irrigation lea. Twenty ml of DW was passed through each module, the drainage liquid collected, centrifuged and the pellets examined for the presence of spores; 4) 500 ml DW was added to an FP7 pot containing an infected pansy plant and the leachate collected. The leachate was added to plants produced in spore-free compost and the effects compared with plants receiving *Thielaviopsis*-free water. The roots were assessed after 2 weeks; 5) pansy seeds were germinated on filter papers in Petri dishes and inoculated with aliquots of leachate from an infected plant and examined for the presence of infection after 7 days.

3.4.2 Spread by insects

A large infestation of shore flies and fungus gnats appeared in the glasshouse during August 1995 when transplanted pansies were growing. A series of experiments were prepared to test for the presence of *Thielaviopsis* on shore flies and fungus gnats.

1) Ten shore flies were collected from around the transplanted infected pansies growing in FP7 pots. Each fly was placed in separate universal bottle containing 1 ml of SDW and shaken on a wrist action shaker for 30 minutes. The flies were removed and the 1 ml suspension was spread over Petri dishes containing TBCEN. Dishes were incubated for 8 days at 21°C and examined.

2) Eleven shore flies were collected and transferred individually to Petri dishes containing TBCEN. Dishes were incubated for 8 days at 21°C and examined.

3) Two hundred shore flies were collected, 100 of which were directly transferred to 10 Petri dishes containing TBCEN. The other flies were squashed and then transferred to another 10 Petri dishes containing TBCEN. All dishes were incubated at 21°C for 8 days and examined.

4) Fifty shore flies were collected and transferred to 5 Petri dishes and left for a week in which all the flies died. Flies were removed and molten TBCEN poured into the dishes. This was repeated with another 50 flies except that the flies were removed after 2 days and TBCEN poured into the dishes. This was repeated with sciarid flies.

5) Two hundred fungus gnats were collected, 100 were squashed and distributed between 10 Petri dishes, and the others left intact and transferred to 10 Petri dishes. Molten TBCEN was poured over the flies and the dishes incubated as before. This was repeated with shore flies except that they were less numerous and only 100 were collected, squashed and distributed between to 10 Petri dishes.

Next, 100 sciarid larvae were collected from around dead plants and washed in SDW three times. Larvae were then transferred to 10 Petri dishes containing TBCEN. Another 100 larvae were washed and surface sterilised in sodium hypochlorite (0.5% v/v) for 30 seconds, then washed three times in SDW. Larvae were squashed and then transferred to 10 Petri dishes containing TBCEN. All dishes were incubated at 21°C for 8 days and then examined.

Another 200 sciarid larvae were collected from around dead plants, washed in SDW, surface sterilised for 30 seconds in sodium hypochlorite and washed three times in SDW. Half the larvae were squashed and transferred to 10 Petri dishes using aseptic technique and the others were transferred to another 10 dishes. Molten TBCEN was then poured over the larvae and the dishes incubated for 8 days.

Finally, 40 sciarid larvae were collected, separately squashed and examined under the phase contrast microscope for *Thielaviopis* propagules. Squashed larvae were then transferred to three Petri dishes containing TBCEN. Dishes were incubated for 8 days at 21°C and then examined.

3.4.3 Spread in dust and compost

First, 20 Petri dishes containing TBCEN were placed on the floor in a glasshouse where black root rot experiments had been carried out. The floor was vigorously brushed around the dishes. The dishes were then incubated for 9 days at 20°C and examined for *Thielaviopis* colonies. This was repeated in a passage outside the glasshouse and in another glasshouse where no black root rot experiments had been set up.

Next, 7 Petri dishes containing TBCEN were placed along the length of a 'clean' potting bench and the area around them lightly brushed (27 November '95). Dishes were then incubated at 25°C and examined after 7 days for *Thielaviopis* colonies. This was repeated (14 December '95) using ten dishes. Finally, 20 g of FF1 was added to 500 ml of molten TBCEN and the suspension divided into 40 Petri dishes. Dishes were incubated for 9 days and examined. This was repeated using Fisons F2 compost.

4 RESULTS

4.1 Standard methods

4.1.1 Identification of *Thielaviopsis*

Thielaviopsis isolates from artificial and natural substrates produced hyphae and spores whose shape, size and colour were in agreement with the descriptions in the literature. Colony growth on agar varied considerably between isolates, and the colonies often developed sectors of different growth as has been described elsewhere (Corbaz, 1985).

4.1.2 Isolation and enumeration of *Thielaviopsis*

Thielaviopsis was successfully isolated from roots using a combination of washing, surface sterilisation in sodium hypochlorite, and incubation on TBCEN. Symptoms were enumerated by a root rot index (see below).

4.1.3 Production of inoculum

Pure chlamydospore suspensions were successfully prepared by scraping the surface of 6 to 8 week-old colonies produced on PDA at 20°C followed by sieving and centrifugation of the mixture of mycelium, chlamydospores and endoconidia. Pure endoconidium suspensions were obtained by a combination of flooding young *Thielaviopsis* colonies, gentle agitation of the agar surface, removal of the supernatant and re-suspension of the spore pellet.

The various methods described in the literature were unsatisfactory because they gave low chlamydospore populations, the chlamydospores were produced in clumps that prevented accurate counting; desiccation and talcum powder for the purification of spore preparations was ineffective.

4.1.4 Production and enumeration of black root rot symptoms

Seedling infection test on filter paper. Black root rot symptoms were easy to produce and were similar to those described in the literature (see Section 1: Review, page 7) and included yellowing of the cotyledons and young leaves, collapse of the root and stems and blackening of roots due to the production of chlamydospores.

A **root rot index (RRI)** was effective for quantifying symptoms:

RRI = 100 x ((C1 x 1) + (C2 x 2) + (C3 x 3) / (total number of plants assessed x number of categories))

Where **C1** = number of roots with <25% infection; **C2** = roots with >25% <50% infection; **C3** = roots with >50% infection.

The root rot index was similar to that used for the evaluation of cereal take-all (Scrace, 1993).

Young plants produced in compost. Symptoms were readily produced and comprised leaf wilt, yellowing, reduced growth of aerial parts, loss of turgor and blackening of roots due to the production of chlamydospores. Dark chlamydospores were also produced on the stem region at the junction with compost and proved to be a positive distinguishing feature from symptoms caused by *Pythium* infection.

Seedling infection test in compost with different spore populations. By 6 weeks the percentage infection and RRI ranged from 2% and 1.1 (100 endoconida/ml) to 98% and 73 (10^5 endoconida/ml). With chlamydospores, infection and RRI were 21% and 7 at 100 spores/ml and 93% and 64 with 10^4 chlamydospores/ml. Thus, low populations of chlamydospores caused more root rot than endoconidia. The chlamydospores may have separated into constituent single spores and hence exceeded the target population, or endoconidia may have lysed because of their thin walls, hence reducing populations below target.

Populations of 10^3 chlamydospores per plant allowed detection of differences in virulence between isolates Tb8c, Tb11, Tb12, Tb14 and Tb15 as measured by RRI.

Endoconidiophores were produced on infected pansy roots and gave rise to endoconidia; the newly-formed endoconidia are a potential source of spread of *Thielaviopsis* during pansy production. Chlamydospores were also produced on infected roots. Newly-produced chlamydospores were likely to be an important source of *Thielaviopsis* in following production cycles of pansy and other bedding plants but to be of lesser importance to the in-season spread of black root rot.

Plants inoculated with 10^2 or 10^3 spores became infected but did not show symptoms i.e. low populations gave rise to symptomless (sub-clinical) infection.

4.2 Characteristics of *Thielaviopsis*

4.2.1 Growth of *Thielaviopsis* in peat-based growth medium

Irrespective of the method of testing, chlamydospores and endoconidia were present in compost but had not germinated; the presence of pansy (Millipore filter test) had no effect on chlamydospores (endoconidia were not tested). In the absence of fertiliser, the majority of hyphal fragments had lysed whereas with fertiliser the hyphae appeared healthy; there was no evidence of growth from the hyphae. Many of the chlamydospore chains had separated into individual spore segments.

4.2.2 Effects of temperature on growth of *Thielaviopsis* and infection of pansy

a Colony growth in culture. When tested on **PDA** solid medium, all isolates grew best at 25°C and 20°C and showed least growth at 10°C (Table 1). Tb12 and Tb16 grew faster at 30°C than at 15°C in contrast to the remainder of the isolates where there was either little difference or 15°C was the more favourable temperature.

Table 1. Effects of temperature on *Thielaviopsis* growth on PDA (18 days).

Isolate	Colony diameter (mm) incubation temperature (°C)				
	10	15	20	25	30
Tb1	5	39	63	75	26
Tb2	4	43	71	80	50
Tb3	6	44	80	80	40
Tb4	3	23	54	67	20
Tb5	12	49	81	80	33
Tb6	5	34	65	76	21
Tb7	3	41	73	79	17
Tb8	7	37	59	80	28
Tb9	3	29	40	47	16
Tb10	8	47	78	75	30
Tb11	8	45	66	77	20
Tb12	7	31	62	77	61
Tb13	5	52	76	81	21
Tb14	6	42	80	82	21
Tb15	6	41	71	79	18
Tb16	9	48	78	80	64

SED = 1
($P<0.05$; d.f.=400)

When measured in **PDB** liquid medium, isolates varied considerably in their growth response as mycelial dry weight: Tb8b and Tb15 showed much less growth at 10°C in comparison with other isolates (Table 2). All isolates grew well at 30°C; However, the temperature response was variable: isolates TB11, 12 and 15 grew best in the range 10-15°C, Tb14 grew best at 10-15°C and isolate Tb8b growing best at 15-20°C (Table 2).

Table 2. Effects of temperature on growth of *Thielaviopsis* in PDB

Temp (°C)	Mycelium dry weight ($x10^{-3}g$)					
	Tb8b	Tb11	Tb13	Tb12	Tb14	Tb15
10	19	183	nd	141	257	106
15	214	231	nd	252	269	261
20	235	188	158	186	226	197
25	186	142	137	160	148	183
30	127	186	147	203	205	139

SED = 16
(P<0.05; df=112)

nd - no data.

b Spore germination. **Endoconidium** germination on PDA was a maximum at 25°C, followed by 20°C and a minimum at 10°C (Table 3). The subsequent development of hyphae was greatest at 20 and 25°C but few endoconidiophores were produced; in contrast, hyphal branching was limited at 30°C but endoconidiophore production was abundant. With PDB, endoconidia of all isolates showed maximum and minimum germination at 25°C and 10°C respectively (Table 4). However, isolates varied in their response at other temperatures: Tb12 showed the greatest overall capacity for germination at all temperatures, but especially at 10°C; at 30°C, Tb8c and Tb12 produced endoconidiophores with secondary endoconidia, and thus had the potential for in-season spread of the pathogen.

Table 3. Effects of temperature on Tb8c endoconidium germination on PDA

Temp (°C)	No. of endoconidia germinated	Appearance of endoconidia
10	32	germ tubes just emerging from a few spores
15	123	many endoconidia with germ tubes, some branching
20	142	extensive hyphal branching
25	170	extensive hyphal branching
30	109	hyphal branches bearing endoconidiophores

SED 11
(P < 0.05; d.f. = 16)

Table 4. Effects of temperature on endoconidium germination in PDB

Temp (°C)	% germination (15 hours) Tb8c	Tb11	Tb12	Tb14	Tb15
10	<1 (2)[a]	<1 (1)	20 (26)	<1 (1)	0 (0)
15	46 (43)	21 (28)	76 (61)	34 (36)	14 (22)
20	57 (50)	29 (33)	74 (60)	30 (33)	31 (33)
25	70 (57)	35 (36)	94 (77)	43 (41)	49 (45)
30	22 (27)	17 (24)	60 (51)	12 (20)	28 (31)

SED = (4)[a]
(P<0.05; df=125)

[a] values in parenthesis are angle transformed data.

The majority of Tb8c chlamydospores germinated at 15-30°C and most produced colonies; chlamydospores failed to germinate at 10°C (Table 5).

Table 5. The effects of temperature on chlamydospore germination and colony production on TBCEN (7 d)

Temp (°C)	No. chlamydospores germinated	Appearance of colonies
10	0	no growth
15	17	colonies pale
20	18	spreading colonies producing chlamydospores
25	18	spreading colonies producing chlamydospores
30	18	small and compact dark colonies

SED 1
(P<0.05; df=16)

There was little difference between isolates except that Tb8c germinated better at 15°C than other isolates (Table 6).

Table 6. Effects of temperature on germination of chlamydospores of different isolates on TBCEN (7 d)

Temp (°C)	% chlamydospore germination				
	Tb8c	Tb11	Tb12	Tb14	Tb15
10	0 (0)[a]	12 (19)	8 (14)	6 (13)	5 (11)
15	85 (72)	38 (37)	40 (39)	19 (25)	37 (37)
20	94 (78)	91 (73)	82 (65)	72 (59)	83 (66)
25	94 (81)	94 (79)	61 (52)	80 (64)	84 (67)
30	91 (79)	83 (66)	72 (59)	80 (64)	75 (60)

SED = 6
($P<0.05$; df=100)

[a] values in parenthesis are angle transformed data.

Endoconidiophore production (Additional objective). Endoconidiophore production in isolates Tb8c and Tb12 was high at 30°C but not in Tb11, Tb14 and Tb15 probably because the rate of spore gemination in the latter isolates was also low (Table 7). Similar results were obtained in a second experiment except that Tb14 endoconidiophore production was a maximum at 30°C.

Table 7. Effects of temperature on endoconidiophore production

Temp (°C)	% germinated endoconidia producing endoconidiophores				
	Tb8c	Tb11	Tb12	Tb14	Tb15
20	<1	<1	-	0	-
25	2	1	0	0	<1
30	37	<1	35	4	<1

no statistical analysis

4.2.3 Effects of water availability on *Thielaviopsis* growth and pansy infection

a Colony growth in culture. Maximum growth occurred at -0.8 to -1.0 MPa on PDA and declined thereafter.

b Spore germination. Tb8c endoconidium germination, as measured by colony production on PDA, was a maximum at -0.7 to -1.2 MPa (Table 8). In PDB, endoconidium germination was a maximum at -0.25 to -0.8 MPa and showed a progressive decline at lower potentials (5 isolates tested). Isolates varied considerably in their response to differing water potentials with Tb8c and Tb12 having approximately twice the germination of other isolates.

Table 8. Effects of water potential on colony production from germinated **endoconidia** (PDA)

Water potential (-MPa)	Mean colony number[a]
0.25	53
0.4	48
0.7	61
1.0	46
1.2	68
1.7	52
2.0	43

SED=6
($P<0.05$; df=35)

[a] approximately 100 spores tested.

Chlamydospore germination was high at water potentials of -0.25 to -1.5 MPa. The majority of germinated chlamydospores developed into colonies at -0.25 and -1.2 MPa; at other water potentials colony development was sparse.

c Infection of pansy. On filter paper, endoconidium-induced root symptoms were most severe in the dry/dry, moist/dry and wet/wet regimes. Symptom development from chlamydospores was less affected by differences in water status but was most severe in the dry/dry treatment.

In compost, the percentage plants with healthy roots decreased and the disease severity (RRI) increased as compost moisture content increased (Table 9). RRI more than doubled in the summer experiments and was associated with higher summer temperatures (Table 10). Root infection, even when severe, had no consistent effect on shoot and root dry weight (Table 9). In the summer experiments, a few plants produced in inoculum-free compost became infected and this was associated with an outbreak of shore flies; this aspect of dissemination is discussed in Section 4.4.2.

Table 9. Effects of watering regime on pansy infection by *Thielaviopsis* chlamydospores (C) or endoconidia (E) (1995)

Water regime	Shoot dry wt ($x10^{-3}$g/plant)		Root dry wt ($x10^{-3}$g/plant)		% plants with healthy roots		RRI	
Experiment 1: 27 Feb - 21 April								
	+C	-C	+C	-C	+C	-C	+C	-C
Dry	30	23	18	13	77 (62)[a]	100	12	0
Moist	41	40	32	21	44 (41)	100	29	0
Wet	48	41	32	28	31 (34)	100	43	0
SED (P<0.05; df=24)		3		4		(4)		4
Experiment 2: 5 June - 21 July								
	+C	-C	+C	-C	+C	-C	+C	-C
Dry	23	33	14	18	22 (28)	99 (88)	59	<1
Moist	33	46	18	23	7 (146)	99 (88)	76	<1
Wet	37	55	16	26	2 (7)	98 (83)	86	<1
SED (P<0.05; df=20)		4		2		(4)		3
Experiment 3: 12 June - 28 July								
	+E	-E	+E	-E	+E	-E	+E	-E
Dry	25	28	15	16	51 (45)	99 (88)	37	<1
Moist	38	45	24	19	33 (35)	100 (90)	51	0
Wet	44	53	18	23	12 (20)	98 (83)	71	1
SED (P<0.05; df=20)		7		4		(4)		4

[a] values in parenthesis are angle transformed data; +C: inoculated with chlamydospores; +E: inoculated with endoconidia; -C, -E: spore free.

Increasing the amount of water to the maximum, raised the pH to 5.5-6.3 and lowered the EC to 98-144 µS.

Table 10. Temperatures in compost (C) in modular trays or air, 1995

Temperature (°C)	Number of hours at each temperature range					
	Experiment 1 4/03-22/04		Experiment 2 10/06-21/07		Experiment 3 17/06-28/07	
	C	AIR	C	AIR	C	AIR
<20	929	1024	513	556	466	475
20<25	245	169	285	250	272	274
25<30	25	7	154	152	200	191
30<35	1	0	45	45	59	63
>35	0	0	11	5	11	5

There was evidence of plant stress in the dry treatment as shown by a 50% reduction in leaf water potential and a reduction in stomatal conductance at the 1500h assessment.

The effects of the pre-transplanting water regimes on infection persisted after transplanting, even though all plants now received similar amounts of water: in Experiment 3, plants that had previously received a 'wet' treatment showed higher death rates and fewer flowers than the 'dry' treatment (Table 11). A large infestation of shore flies and fungus gnats occurred in mid August and were thought to be implicated in the presence of black root rot in the non-inoculated control.

Table 11. Effects of module watering regimes on black root rot in pansy following transplanting, 1995

Previous treatment in modules	shoot dry wt (g)		% plants dead		Nos. flowers (15-16 wks)	
Experiment 3: June 12-October 2: endoconidia (E)						
	+E	-E	+E	-E	+E	-E
Dry	10	21	78 (49)[a]	14 (9)	10	38
Moist	10	19	100 (52)	26 (16)	5	42
Wet	11	18	98 (43)	60 (7)	2	35
SED (P<0.05; df=20)	3		(7)		6	

[a] values in parenthesis are angle transformed data; compost differed in moisture content at module stage; moisture regime constant after transplanting.

4.2.4 Effects of pH on growth of *Thielaviopsis* and pansy infection

Measurement of compost pH. pH values of 5.27, 5.28, 5.02, 5.07 and 5.27 were obtained for the ADAS, Fisons, CaCl2, and 1:2.5 and 1:5 compost:water ratios respectively i.e. the ADAS and Fisons methods gave slightly higher values than other methods; for comparison, the Manufacturer's specified range for FF1 is pH 5.3-6.0. When left overnight, there was a 0.23 pH rise irrespective of preparation method; changes in preparation volumes had no effect on pH.

The 'Fisons method' proved to be suitable for small compost volumes, was simple and the pH values were independent of compost moisture content.

a Colony growth in culture. Growth of isolates Tb1, Tb3, Tb5, Tb6, Tb8, Tb14, Tb15 and Tb16 was best on PDA at pH 4.8, and least at pH 6.4 (Table 12).

Table 12. Effects of pH on colony growth on PDA (18 days)

Isolate	Colony diameter (mm)				
pH:	4.8	5.4	5.8	6.4	6.6
Tb1	58	32	27	26	25
Tb2	43	28	23	23	25
Tb3	59	32	24	26	26
Tb4	37	20	19	19	19
Tb5	60	41	30	27	25
Tb6	62	36	29	27	28
Tb7	53	32	27	27	27
Tb8	62	37	29	27	26
Tb9	30	20	17	15	14
Tb10	55	37	28	25	26
Tb11	40	26	21	21	18
Tb12	45	30	28	20	21
Tb14	69	38	28	28	29
Tb15	61	37	29	28	27
Tb16	62	43	26	24	24

SED = 1
(P<0.05; df=375)

Interaction between pH and temperature on colony growth. There was no evidence of an interaction between pH and temperature. Growth was optimum at pH 4.8 irrespective of temperature.

b Spore germination. High rates of endoconidium germination occurred in the range pH 5.1-6.1, with pH 5.6 being optimum for all isolates. Tb12 had 89% germination at pH 5.1 compared to 13-29% for other isolates. Germination at pH> 6.5 was negligible except for Tb12 which showed 40% germination at pH 6.5.

Chlamydospore germination, as measured by the proportion that produced colonies on PDA, was unaffected in the range pH 5.0-7.0. Following germination, colony growth was a maximum at pH 5.5 to 5.9 and production of new chlamydospores on the developing colonies was a maximum at pH 6.5 to 7.0.

c Infection of pansy

Adjustment of compost pH. Seven applications of ferrous sulphate or calcium hydroxide were needed to change the compost from pH 5.4 to the targets of pH 5.2 or pH 6.5 respectively. In a first experiment in July, pH had little effect on seed germination, shoot or root dry weights, percentage plants affected by black root rot and RRI. In a second experiment, RRI was a minimum and the percentage healthy plants and shoot root dry weights a maximum at pH >6.0. It was evident that black root rot severity was affected by differences in the rate of compost drying brought about by the proximity of the trays to the ventilation fans: trays that were furthest from the fans remained 'wet' and black root incidence was double that in the 'dry' trays irrespective of compost pH. A greater severity of black root rot in the first experiment was probably due to the higher temperatures.

4.2.5 Effects of fertilizer nutrients on growth and infection of pansy by *Thielaviopsis*

Measurement of Electrical Conductivity (EC) of peat-based compost

The mean EC values of FF1 obtained by the ADAS and Fisons methods differed by 1 µS which was less than the variation between individual samples.

Effects of compost EC on seed germination

The ECs of FF1 and FF2 were 301 and 307 µS respectively. Seed germination in FF1 was 21-28% after 8 days, compared with 5% in FF2. Eight days later, germination was 85-91% and 84-86% respectively. For comparison, germination on filter paper on a Copenhagen Tank was 76% by 7 days and 100% by 14 days.

Effects of fertiliser on EC and pH of compost

The final EC of FF1 treated with Peters Professional fertiliser, Vitafeed or distilled water were 311, 228 and 119 μS respectively (Table 13). When plants were omitted, the ECs rose progressively e.g. to 586μS with Peters Professional after 63 days.

Table 13. Effects of fertiliser on compost EC and pH

Treatments		EC (µS) and pH during sampling						
		0 days	17	31	39	49	56	63
Vitafeed	EC	363	331	334	261	260	283	228
	pH	5.3	5.6	5.6	5.7	5.6	5.5	5.5
Peters	EC	363	343	361	262	296	298	311
	pH	5.3	5.6	5.6	5.7	5.5	5.3	5.5
Water	EC	363	250	226	175	165	157	119
	pH	5.3	5.8	5.9	6.0	6.1	5.9	6.2

Treatments applied 16 times.

Effects of nutrient concentration on growth and infection of pansy by *Thielaviopsis*

With chlamydospores (Expt 1: C), the lowest rate of fertiliser treatment resulted in RRIs of 81; higher fertiliser rates had similar effects (Table 14). With endoconidia (Expt 2: E), the RRIs were reduced by about half and this was associated with higher temperatures (Table 15). Black root rot affected some plants produced in inoculum-free compost, indicating that there had been cross contamination.

Table 14. Effects of fertiliser on black root rot in pansy, 1995

Fertiliser rate[a]		Shoot dry wt ($x10^{-3}$g)	Root dry wt ($x10^{-3}$g)	% plants with healthy roots	RRI
Experiment 1 (with chlamydospores, C, 26 June-August 11)					
Low	+C	32	17	5 (10)[b]	81
	- C	51	21	98 (85)	1
Medium	+C	32	15	8 (15)	83
	- C	54	23	100 (90)	0
High	+C	32	13	9 (14)	82
	- C	57	20	98 (85)	<1
Experiment 2 (with endoconidia, E, 10 July-25 August)					
Low	+E	32	14	53 (47)	43
	- E	51	21	100 (90)	0
Medium	+E	40	15	47 (43)	47
	- E	51	20	99 (88)	<1
High	+E	42	14	50 (45)	45
	- E	56	19	99 (88)	<1
SED (P<0.05; df=20)		4	1	(4)	5

[a] fertiliser rates: low, EC=247μS, medium EC=465μS, high EC=901μS;
[b] values in parenthesis are angle transformed data; compost inoculated with chlamydospores (C) or endoconidia (E).

Table 15. Temperatures of compost (A) and air (B,) 1995

Temp1 (°C)	Number of hours at each temperature			
	Experiment 1 1 July-11 August		Experiment 2 15 July-25 August	
	A	B	A	B
<20	420	413	377	376
20<25	277	289	253	273
25<30	212	199	221	198
30<35	..78	83	119	126
>35	..21	24	38	35

Leaf water potential. Fertiliser rate had no effect on leaf water potential irrespective of inoculum (Table 16). Water potentials were lower in a second experiment but did not appear to be related to fertiliser rate, compost EC or pH (Table 17).

Table 16. Effects of fertiliser rate on plant water potential (records at 1400h)

Fertiliser rate	Leaf water potential (-MPa) Expt. 1		Expt. 2	
	+C	-C	+E	-E
Low	9.3	8.3	5.2	4.0
Mod	9.4	9.5	5.8	4.7
High	9.2	7.8	4.8	4.3
SED (P<0.05; df=20)	1.5		0.7	

[a] fertiliser rates: low, EC=247µS, medium EC=465µS, high EC = 901µS; [b] values in parenthesis are angle transformed data; compost with chlamydospores (C) or endoconidia (E).

In both experiments, pH was least and EC greatest for compost treated at the highest fertiliser rate (Table 17). EC was higher in *Thielaviopsis*-free compost with a high nutrient rate than in inoculated compost especially in Experiment 1 (Table 17).

Table 17. Effects of fertiliser rate on compost EC and pH

Fertiliser rate	Experiment 1 EC (µS)		pH		Experiment 2 EC (µS)		pH	
	+C	-C	+C	-C	+E	-E	+E	-E
Low	302	391	5.2	5.1	254	276	5.2	5.3
Mod	424	461	5.1	5.0	332	313	5.1	5.1
High	497	608	4.8	4.9	399	481	4.8	4.9
SED (P<0.05; df = 20)	46		<1		36		<1	

[a] fertiliser rates: low, EC=247µS, medium EC=465µS, high EC = 901µS; [b] values in parenthesis are angle transformed data; compost inoculated with chlamydospores (C) or endoconidia (E).

Following transplanting, plants produced in the presence of *Thielaviopsis* had lower shoot dry weights, lower flower production, and lower survival (Table 18) with chlamydospores having the greater effect. Plant survival and flower production were higher with endoconidia in comparison with chlamydospores. In the absence of *Thielaviopsis*, flower production in Experiment 2 was almost double that in Experiment 1. Differences in fertiliser rates at the module stage had no effect on survival, shoot weight and flower production after transplanting.

Table 18. Effects of fertiliser rate on black root rot in pansy, 1995

Fertiliser rate	Shoot dry wt (g)		% death (15 wks)		Nos. flowers produced (10 plants, 15 wks)	
Experiment 1: June 26-October 9, chlamydospores (C)						
	+C	-C	+C	-C	+C	-C
Low	1	15	90 (79)[a]	0 (0)	4	35
Moderate	<1	14	98 (86)	10 (14)	2	36
High	<1	14	100 (90)	10 (14)	2	30
SED (P<0.05; df=20)	2		(8)		<1	
Experiment 2: July 10-October 23, endoconidia (E)						
	+E	-E	+E	-E	+E	-E
Low	10	21	56 (49)[a]	6 (9)	27	63
Moderate	10	19	60 (52)	12 (16)	28	62
High	11	18	46 (43)	4 (7)	34	64
SED (P<0.05; df=20)	3		(7)		8	

[a] fertiliser rates: low, EC=247μS, medium EC=465μS, high EC = 901μS;
[b] values in parenthesis are angle transformed data; compost inoculated with chlamydospores (C) or endoconidia (E).

4.3 Environment and plant stress

4.3.1 Types and duration of environment-plant stress

Temperatures of peat-based compost in modular trays. Maximum and minimum compost temperatures of 46.2°C and 8.8°C were recorded in 1994. On a 'hot' sunny day (30 June 95), compost temperatures exceeded 30°C for 8 hours and 22-30°C for 4 hours. In contrast, on a 'cool' overcast day (26 June '94), temperatures were 22-30°C for 9 hours and 12-22°C for 15 hours. Temperature changes, in response to changes in cloud cover, were rapid especially on 'hot' days. Watering reduced maximum temperatures by up to 4°C on a 'hot' day but had little effect on a 'cool' day. Module position within the tray had little effect on temperature.

Differences in compost moisture content varied from saturation shortly after watering to dry in hot conditions near to forced ventilation.

The compost pH changed very slowly during the course of experiments.

4.3.2 Methods for simulating environment-plant stress

These comprised the use of temperature controlled environment cabinets, adjustment of compost to a range of different pHs, nutrient rates, and watering regimes. The methods are described under the appropriate experimental sections.

4.3.3 Effects of temperature stress

a Endoconidium germination and endoconidiophore production

Temperature stress, as tested by varying the temperatures from 20°C to 30°C, 35°C or 40°C had little effect on endoconidium germination which exceeded 70%; a constant 40°C stopped germination (Table 19). Hyphal growth from spore germ tubes was greatest at constant temperatures of 20, 25 and 30°C and temperature stress had little effect. Thirty eight percent of the germinated Tb8c endoconidia produced endoconidiophores at constant temperatures of 25°C, 30°C and alternating temperatures of 20-30°C). Endoconidiophore production for Tb8c was zero at 35, 40°C and alternating temperatures of 20-40°C. In a second experiment, endoconidiophore production by both *Thielaviopsis* isolates was <2% irrespective of treatment.

Table 19. Effects of temperature on endoconidium germination and hyphal growth (Isolate Tb8c)

Temp (°C)	Germination and hyphal growth (20 hours)
20	germination, hyphae branched, >20 hyphal segments
25	germination, hyphae branched, >20 hyphal segments
30	germination, hyphae branched, 4-16 hyphal segments
35	germination, hyphal growth distorted, 1-2 hyphal segments
40	no germination
20-30	germination, hyphae branched, >20 hyphal segments
20-35	germination, hyphae branched, >10 hyphal segments
20-40	germination, poor hyphal growth, 1-4 hyphal segments

Table 20. Effects of temperature stress on endoconidiophore formation

Temp (°C)	% germinated spores producing endoconidiophores			
	Experiment 1		Experiment 2	
	Tb8c	Tb12	Tb8c	Tb12
20	2	<1	3	0
25	15	2	4	0
30	38	4	1	0
35	0	0	0	0
40	0	0	0	0
20-30	12	0	<1	0
20-35	3	0	<1	0
20-40	0	0	0	0

b Production of endoconidia on infected pansy roots

Endoconidium production of Tb8c was 180,000/plant 4 days after inoculation and rose to 1.2 million after 9 days. In a second experiment, endoconidium production of Tb8c was maximum at constant temperatures of 25 and 30°C and of Tb14 at 30°C; temperature stress at alternating temperatures of 20/35°C and 20/40°C drastically reduced endoconidium production of both isolates; a similar effect was also seen at 20/30°C with Tb14 but the inhibitory effect on Tb8c was much less (Table 21).

Table 21. Effects of temperature on the production of endoconidia on infected pansy roots

Temp (°C)	Number of endoconidia ($x10^4$) Tb8c	Tb14
20	11	6
25	31	15
30	28	30
20-30	19	6
20-35	7	3
20-40	4	4

SED=6
(P<0.05; d.f.=106)

4.3.4 Effects of pre-treatment temperatures on chlamydospore germination

Pre-incubation of Tb8c chlamydospores at 10-30°C had little effect on their subsequent germination, which was 81% or higher, or on the subsequent colony development which was profuse.

4.3.5 Effects of temperature pre-treatment 'stress' of pansy seedlings on infection by *Thielaviopsis*

Pre-incubation of pansy at 30°C prior to inoculation with endoconidia had no effect on RRI which was 17-18. With chlamydospore inoculum, pre-incubation reduced the RRI from 25 to 5. When endoconidia were pre-treated at 30°C, RRIs were reduced to 7 but chlamydospores were unaffected. Treatment of plants or spores at alternating temperatures of 20/30°C for 7 days had no effects on RRI compared with constant temperatures of 30°C.

4.3.6 Effects of temperature stress on infection of pansy by *Thielaviopsis*

Shoot dry weights of non-inoculated plants were up to 18 times higher in the glasshouse compared with an illuminated incubator. In two glasshouse experiments (6 July-28 August; 20 July-11 September '94) RRIs were 62-64 and 56-78 respectively, and in a third experiment (9 August-27 September) the RRIs were 16-30 (Table 22). In the first

two experiments, black root rot severity was much higher in the glasshouse than the controlled environment chamber. The effects of incubator temperature were variable and alternating temperatures sometimes gave higher RRIs in comparison with a constant 20°C but at other times there was no effect. There was no apparent correlation between black root rot severity and plant size as measured by shoot dry weight.

Temperatures exceeded 30°C for a considerable period in Experiment 1 (July-August) but were lower in the other two experiments (Table 23).

Table 22. Effects of temperature, stress and inoculum type on plant size and black root rot severity

Experiment (1994)	Temperature (°C)	Shoot dry wt (10^{-3}g)			RRI		
		Inoculum			Inoculum		
		Endo	Chlam	None	Endo	Chlam	None
6 July-28 August	20[a]	7	6	7	18	11	0
	20/30[a]	5	5	6	24	26	0
	Glasshouse	38	41	57	62	64	0
		SED=3 (P<0.05; df=96)			SED=5 (P<0.05; df=96)		
20 July 11 Sept.	20[a]	5	5	5	19	27	0
	20/30°C[a]	3	3	4	12	31	0
	Glasshouse	58	56	68	56	78	0
		SED=3 (P<0.05; df=96)			SED=5 (P<0.05; df=96)		
9 August 27 Sept.	20	4	4	5	15	12	0
	20/30	3	3	3	37	27	0
	Glasshouse	23	23	26	30	16	0
		SED=3; (P<0.05; df=96)			SED=5 (P<0.05; df=96)		

Endo - endoconidia, Chlam - chlamydospores; RRI root rot index
[a] experiments in LEEC incubators.

Table 23. Glasshouse temperatures in modular trays, 1994

Temperature (°C)	Hours at each temperature		
	Experiment 1 (18 July-28 Aug)	Experiment 2 (1 Aug-11 Sept)	Experiment 3 (22 Aug-27 Sept)
<15	146	239	317
15-20	310	352	279
20.1-25	194	142	52
25.1-30	67	11	0
30.1-35	26	0	0
>35	1	0	0

4.4 Spread of *Thielaviopsis*

4.4.1 Spread from infected pansies via drainage leachings

A total of 200 *Thielaviopsis* colonies in 34 Petri dishes developed from leachings from a modular tray with infected pansies. It was not possible to tell whether the colonies had developed from endoconidia, chlamydospores or hyphae. Leachings from healthy pansies did not contain *Thielaviopsis*.

When pansies were grown in *Thielaviopsis*-free compost, 12% became infected when the trays were in contact with capillary matting next to trays containing *Thielaviopsis*-inoculated compost. Raising the trays off the matting stopped the cross infection (Table 24). Leachings from infected pansies contained hyphae, chlamydospores and endoconidia (Table 25). Pansies developed black root rot when watered with the leachings from infected plants.

Finally endoconidia produced from infected roots caused infection in 50% of pansy seedlings in Petri dishes.

Table 24. Spread of *Thielaviopsis* via capillary matting

Treatment	No. pansies assessed	Infection by *Thielaviopsis*			Total no. plants infected
		Slight	Moderate	Severe	
Inoculated plants, trays on matting	212	0	0	212	212
Un-inoculated plants, trays raised off matting	212	0	0	0	0
Un-inoculated plants, trays on matting	212	7	8	10	25

Root area infected: slight < 25%, moderate > 25 < 50%, severe > 50%.

Table 25. *Thielaviopsis* content of leachate from infected plants produced in modular trays

Test	Endoconidia	Chlamydospores	Hyphae
1	5-15	0	0
2	5-15	1	0
3	10-20	0	0
4	5-15	3 chains	0
5	5-10	2 chains	0
6	5-15	0	0

4.4.2 Spread by insects

Out of a total of 100 intact and 100 squashed shore flies tested on TBCEN, 2 intact and 12 squashed flies gave rise to *Thielaviopsis*. *Thielaviopsis* was found in Petri dishes that had contained shore flies but not in washings from the flies. *Thielaviopsis* developed from 19% fungus gnat larvae that had been washed and 55% larvae that were surface sterilised and squashed. *Thielaviopsis* developed from 6% sciarid larvae that been washed and surface sterilised and 35% larvae that had been surface sterilised and squashed and 31% larvae that were dissected. Endoconidia or structures

resembling endoconidia and hyphae were seen in 50% larvae examined. Chlamydospores were never seen.

4.4.3 Spread in dust and compost

A total of 29 *Thielaviopsis* colonies developed in 20 Petri dishes in a glasshouse containing black root rot experiments but not when dishes were placed in an adjoining passage or in a glasshouse where black root rot experiments had not been done. A total of 197 *Thielaviopsis* colonies developed in 7 Petri dishes on a potting bench. When the experiment was repeated, 99 colonies developed on 10 Petri dishes. *Thielaviopsis* was not isolated from FF1 or FF2 compost.

TBCEN was the most successful medium for isolating *Thielaviopsis* from roots. There was clear evidence that roots were infected by *Thielaviopsis* even though they did not show symptoms. Possibly the roots were at an early stage of infection and symptoms would have developed at a later date. The appearance of many of the colonies strongly suggested that the fungus originated from roots that had been colonised by hyphae.

General Discussion

The increased production of winter flowering pansies as bedding plants in modular trays was associated with an increase in black root rot severity and incidence. Professor D Koranski, USA said at Colegrave Seeds Ltd. (Banbury), 1995 and the Four Oaks Trade show, UK, September 1990 that black root rot was increased by high pH, high nutrient concentration and high temperature stress and that attention to these factors would give control. However, we have found no data to substantiate or otherwise his claims. In any case some of the recommendations would be difficult to put into practice in the UK, for example temperature control, pH and electrical conductivity.

The present project investigated: (a) whether stress affected black root rot; (b) whether control measures recommended in the USA were applicable to the UK.

The most likely stresses in module-grown plants are temperature, water availability, pH and nutrients. It is difficult to distinguish the effects of stress on the host, the pathogen and the root microflora. Furthermore, growers and scientists recognise stress symptoms differently: extreme stress is recognised by growers as a wilting or reduced growth; slight stresses are recognised by scientists as changes in growth processes involving plant hormones, root exudates, protein synthesis, stomatal conductance and leaf water potential. A major problem was the actual measurement of stress. Most instruments are for large woody plants and are expensive e.g. tensiometers, hygrometers and leaf area recorders. They were not immediately applicable to small pansies grown in a modular trays.

In our experiments, temperature had the largest effects on black root rot and this was in accordance with reports from the USA. Root disease was always greater in the 'warm' summer months in comparison with the 'cooler' spring months. An increased production of endoconidia hence the spread between adjacent plants is the most likely explanation for

the increased severity of black root rot in summer. There was no evidence that temperature stress was a factor. This has implications for the production of winter pansy because module-grown pansies produced in summer, and that were already infected by *Thielaviopsis*, died when they were transplanted irrespective of watering or nutrient regimes. Similarly, plants with roots that appeared healthy but which were actually in the early stages of infection also died after transplanting. Summer temperatures frequently exceeded 25°C, temperatures that would be favourable to hyphal growth, and endoconidium and chlamydospore germination. The highest endoconidium populations in leachates also occurred in summer and were capable of infecting pansies and causing stunting. Temperature effects may also occur indirectly via the host: black rot is reported to be more severe in low temperature plants -growth optima 13-17°C - that experience high temperatures and in high temperature plants - growth optima 26-31°C - that experience low temperatures. Pansy grows best at 18-23°C and hence is midway between the extremes.

Water potential was a second factor that had major effects on black root rot. Plant water potential vessels (pressure chambers) were successful at evaluating the water stress in plant tissue and demonstrated consistent differences in water potential between dry and wet treatments at 0600 h and, as expected, the effects increased at 1400 h when temperatures were higher. Attempts to measure plant stress by recording the water vapour diffusion rate from the leaf surface (stomatal conductance) with a porometer were not successful except in extreme conditions. Often the leaves of pansy seedlings were too small for the instrument to measure. Surprisingly, stress had little consistent effect on shoot and root dry weight. High summer temperatures in combination with abundant watering increased black root rot severity. We do not know whether stress was the main factor or whether stress had indirect effects on dispersal or infection. Thus, endoconidium germination and hyphal growth of *Thielaviopsis* were a maximum at high water potentials, whereas chlamydospore germination tolerated lower water potentials. The increased severity of black root rot in wet conditions may have resulted from a combination of anaerobic plant stress, release of root nutrients used as an nutrient source by *Thielaviopsis* and of conditions that favoured spore germination and hyphal growth. Whatever the reason, irrigation needs to be carefully controlled. Modules dry out quickly and hence large quantities of irrigation may be applied to prevent this. The present study demonstrated that sub-irrigation via capillary matting helped to disseminate *Thielaviopsis* endoconidia within and between modular trays. Once infected, the chlamydospores produced on infected roots will remain on the matting and be a source of inoculum source for future crops. Endoconidia and chlamydospores did not germinate in peat-based compost when plants were absent either because of the physical conditions of the compost or to a lack of nutrients. It seems likely that the plant provides nutrients which favour germination and growth of *Thielaviopsis*.

Moisture also affected the prevalence of insects in the compost. Green algae which require moist conditions for growth are the main food for shore fly larvae; however the larvae do not appear to damage the plants. Shore fly larvae probably increased black root incidence by ingesting *Thielaviopsis* which was isolated from 13% of adult flies and from faeces. In contrast, fungus gnat larvae damage plants by feeding on roots and tunnelling into the stems. *Thielaviopsis* was isolated from 45% of fungus gnat larvae probably from endoconidia in the gut. Thus wet conditions favour the spread of *Thielaviopsis* spores in irrigation water, capillary matting and during insect feeding. pH is a plant stress factor

because of the effects on nutrient availability and the soil microflora. However, we were unable to demonstrate any effects of pH on black root rot. Thus, compost in the range pH 5.0-5.5, 5.6-5.9 and 6.0-6.5 had no effect on black root rot severity in 'warm' summer conditions although there was some reduction at pH 6.0-6.5 in 'cooler' conditions. Our evidence conflicts with the scientific literature where pHs of 5.0, 5.5, 5.5-5.8 are said to restrict black root rot (Powell, 1989; Powell, 1995).

We found no evidence that nutrients affect black root rot severity (Koranski & Karlovich, 1989; Koranski & Laffe, 1988). Nor did we find any evidence that accumulating nutrients in compost would become damaging to the plant. The leaf yellowing resulting from low nutrient conditions could be a source of confusion with black root rot.

Summarising, black root rot severity is increased by 'warm' (>20°C) temperatures and wet conditions. Close control of temperatures in glasshouses on 'hot' summer days is difficult, and may be impracticable. Water is more easily controlled and this would have affects on endoconidia as a source of spread. Black root rot problems are often blamed on environmental stress, however no one has actually reported exactly why these stresses supposedly make the plant more susceptible to attack. The term stress seems to have been used loosely for explaining the occurrence of plant diseases. Environmental stress is known to influence release of plant root exudates stimulating growth of the pathogen or change the microbial flora allowing growth of the pathogen. Thus, further research is required to assess if environmental stresses result in different quantitative and qualitative release of plant exudates from pansy roots and why this occurs.

Finally, hygiene in a glasshouse is very important since *Thielaviopis* can be introduced and survive in dust, insects such as shore flies and fungus gnats may spread and aid survival of the fungus between host crops. Restricting irrigation is likely to reduce endoconidia survival and insect activity.

This study has provided the basic ground work for further studies, allowing comparisons to be made due to the use of standardised methods throughout all experiments. Further studies could include: testing different pansy varieties on susceptibility to *Thielaviopis*, using different growth media with different degrees of peat decomposition, testing different *Thielaviopis* isolates against environmental stresses and infection, and adding possible biological control agents to peat-based growth media.

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Appendix 1. Project PC38b

1 Project Description

2 Title of Project: Epidemiology of Black Root Rot in Bedding Plants

3 Background and Commercial Objective
Black root rot (BRR) (*Thielaviopsis basicola*; TB) causes considerable problems in the production of bedding plants. The fungus is very common and capable of infecting a wide range of plants species. However, the production of black root rot symptoms is thought to occur mainly when the environmental conditions cause stress in the host plant. In bedding plants, stress is associated with high summer temperatures hence may also involve lack of soil moisture and low relative humidity. Bedding plants are increasingly being grown as 'plugs' hence the small volume of the module may be a factor pre-disposing the plant to black root rot. There appears to be no data on the role of stress on black root rot in the scientific literature.

4 Potential Financial Benefit to the Industry
Little information is available on the financial losses caused to the industry by black root rot. However, the information provided by UK bedding plant producers indicates that there has been an increase in the occurrence of black root rot in the past two years (before 1992) when summer temperatures have been unusually high. Should the recent high temperatures in summer continue the problems from black root rot are likely to increase. Black root rot has been reported to be a serious problem in bedding, pot and container grown plants in the USA.

5 Scientific and technical targets of the work
5.1 Review the scientific literature
5.2 Devise a protocol for controlling BRR based on information supplied by Professor Koranski etc
5.3 Determine the epidemiological characteristics of UK isolates of TB

5.4 Determine the effect of environment and plant stress on BRR

: identify types and duration of environment-plant stress in Viola-BRR; test methods of simulating an environment-plant stress in Viola-BRR

: investigate the effects of stress on the growth of bedding plants in standard conditions

: investigate the interactions between the effects of stress on bedding plants and infection by TB

6 Description of the work

6.1 Literature review of (a) techniques available for the study of BRR and (b) the characteristics of BRR and the causal pathogen

6.2 Devise protocol for controlling BRR based on the manipulation of cultural factors, the protocol to take into account pH of the growth medium compost, composition of nutrients, EC and water quality,

Test this protocol against BRR in comparison with treatments which possibly favour BRR and relate results to changes in TB and *Viola*

6.3 Investigate characteristics of the BRR pathogen

6.3.1. Devise standard methods for (a) identification of TB; (b) isolation and enumeration of TB from plant material and growth media, (c) production of inoculum and (d) production and enumeration of BRR symptoms

6.3.2 Investigate the capacity of TB to grow in growth media (compost) Determine the sensitivity of TB to temperature, moisture and pH *in vitro* and *in vivo* using controlled environments

6.3.3 Investigate the potential of plants as symptomless carriers of TB.

Fresh isolates of TB will be obtained from UK growers

6.4 Environment and plant stress

6.4.1 Identify types and duration of environment-plant stress un Viola-BRR e.g. air temperature, root and leaf surface temperature nutrient pH and moisture of growth media

6.4.2 Devise methods of simulating environment-plant stress in Viola-stress.

6.4.3 Investigate the interactions between the effected of stress on bedding plants and infection by TB.

Appendix 2. Protocol for controlling black root rot of pansies grown in modular trays.

The Protocol

Stage 0 An assessment stage before seed sowing

1. **Water quality**. This affects germination, pH of the growing medium, nutrient availability, root production. pH should be 5.0-6.5. Optimum alkalinity (bicarbonate concentration) is 60 ppm. Sodium concentration should be less than 35 ppm.
2. **Seed sowing medium.** The medium should have a high buffer capacity, a high water holding capacity, broad distribution of particle size to ensure full drainage, a high cation exchange capacity (CEC) and good air porosity which determines the amount of oxygen available for root development, gas exchange, nutrient and water absorption. pH should be 5.5-5.8 or 5.8-6.5 and electrical conductivity (EC) between 750 and 1000 µS. The medium should be low nutrient status in the ratio of 1:1:1:0.5 for nitrogen, potassium, calcium and magnesium (N, K, Ca & Mg). The manganese to iron ratio should be 1:2.
3. **Seed quality.** Good quality seed is recommended. Primed seed is more tolerant to 'warm' temperatures than standard seed.
4. **Containers** New modular trays, pots and flats should be used. The smaller the module size, the more vulnerable the compost is to fluctuations in moisture, nutrients, oxygen, pH and soluble salts in the medium. The deeper the module, the better drained is the medium, which allows better leaching of nutrients, reduced salt build-up and greater aeration. Larger modules produce a higher quality finished plant in a shorter period of time than smaller modules. Square modules are preferred to round modules as they hold more growing medium and water is distributed more evenly.

Stage 1 Germination of seed (4-7 days)

Stage 1A Imbibition of water

1. **Sowing:** Sow seed on top of the medium to allow optimum levels of moisture, oxygen and temperature. Most seeds germinate better when exposed to the air. Do not stack the trays as this prevents optimum oxygen requirement for good rooting. Seeds should be placed in the centre of the module.
2. **Water:** Water after sowing and maintain a 95-100% relative humidity until the radicle emerges, seeds should not be saturated.
3. **Temperature:** 16.7-20°C.
4. **Nutrients:** EC <750 µS.
5. **Fungicide:** Include a fungicide such as benomyl (Benlate).
6. **pH of growth medium:** 5.8-6.5 to allow availability of both major and minor elements.

Stage 1B Radicle emergence

1. **Water:** Essential to reduce moisture to 75-80% relative humidity. Use fog or mist to maintain high humidity. A droplet size of 15 µm is best. Apply sufficient water to cover the trays uniformly, and to leach out soluble salts. When saturated, the growth medium contains almost no oxygen (0-2%) therefore the medium should be allowed to dry out to allow increased oxygen for optimum germination and growth.

2. Light: Light is required for hypocotyl and cotyledon development.
3. Temperature: 20-23.9°C.
4. pH of growth medium: 5.5-5.8.
5. Nutrients: EC < 1000 µS, calcium at 50 ppm which promotes root growth, and magnesium at 25 ppm. Do not add ammonium fertilisers.
Remove modules from germination chamber when cotyledons emerge from seed coat.

Stage 2 Stem and cotyledon emergence (next 7 days)
1. Temperature: 16.7-20°C.
2. Nutrients: Apply a light 'feed', 25-50 ppm of 20-10-20, once per week when cotyledons are fully expanded. Pansies are light feeders and do not like high concentrations of soluble salts.

Stage 3 Growth and development of true leaves (next 14 days)
1. Water: Reduce the amount of moisture, but never let the modules dry out completely. When the medium drips water only when squeezed, this indicates the correct combination of moisture and oxygen for plant growth.
2. pH: Maintain at 5.5 or 5.8 if *Thielaviopsis* is present. Low pH is an effective control measure for this disease. Boron availability is controlled by maintaining a pH between 5.5-5.8.
3. Nutrients: EC < 1000 µS. Sodium < 40 ppm otherwise it harms the root hairs. Chlorine between 30-40 ppm. Fertilisers should be used with low or no ammonium as ammonium weakens the roots, concentrations of ammonium should be less than 15 ppm. Moderate levels of compound fertiliser can be applied as needed at 50-100 ppm.

Stage 4 Conditioning the plants and preparation for the next growth stage (14-21 days)
1 Water: Keep moist to maintain turgor pressure. Water modules 2 - 3 hours before transplanting to aid removal of modules.
2. Temperature : 12.8-15.6°C.
3. Nutrients: Moderate levels (50-100 ppm) of fertiliser can be applied as needed.

Transplanting
Do not keep plants in modular trays longer than is necessary or there will be a decrease in the time to flowering and in the number and size of flowers. Do not use fungicides and fertilisers after transplanting until after roots have emerged from the root ball. The transplant growth medium should have an EC of 1000 µS.

Appendix 3: Origin of *Thielaviopsis* isolates

Isolate	Host plant	Origin	Isolated	Stored
Tb1	Pansy Universal series	Warwickshire	10/91	2/93
Tb2	Pansy F1 'Delft'	Lancashire	6/92	2/93
Tb3	Fuchsia 'Pink Galore'	Worcestershire	2/92	2/93
Tb4	Pansy 'Blue Blotch'	Gloucestershire	12/92	10/92
Tb5	Poinsettia cv. unknown	Bristol	11/92	10/92
Tb6	Pansy cv. unknown	Somerset	11/92	10/92
Tb7	Pansy cv. unknown	Yorkshire	11/92	10/92
Tb8a	Pansy cv. unknown	Unknown	9/91	10/92
Tb8b	Pansy 'Universal Beaconsfield'	HRI	5/94	5/94
Tb8c	Pansy 'Universal Beaconsfield'	HRI	10/94	10/94
Tb9	Pansy cv. unknown	Unknown	10/91	2/93
Tb10	Tomato cv. unknown	Hampshire	11/91	10/92
Tb11	Viola 'Cottage Garden'	Unknown	9/94	10/94
Tb12	Pansy F1 'Red Blotch'	Unknown	9/94	10/94
Tb13	Pansy 'Universal Ultima'	Unknown	9/94	10/94
Tb14	Pansy 'Universal Ultima'	Cheshire	9/94	10/94
Tb15	Pansy 'Universal Orange Blotch'	Unknown	9/94	10/94
Tb16	Pansy cv. unknown	Cheshire	9/94	10/94

Appendix 4. Composition and dilutions of Vitafeed 111 (19-19-19) and Peters Professional (20-10-20) fertilisers

	% composition	
	Vitafeed	**Peters Professional**
Nitrogen	19	20
Phosphorus	19	10
Potassium	19	20
Magnesium	0.15	0.15
Iron	0.05	0.1
Manganese	0.025	0.056
Boron	0.013	0.02
Copper	0.025	0.01
Molybdenum	0.0003	0.01

Strength of feed (ppm)

Dilutions	1:100	1:200	1:400	1:100	1:200	1:400
Nitrogen	190	95	47.5	200	100	50
Phosphorus	190	95	47.5	100	50	25
Potassium	190	95	47.5	200	100	50

Appendix 5. Outcomes of project

Three Concept Notes about Black Root Rot were submitted to the HDC.

GLOSSARY

Electrical conductivity (EC): concentration of soluble salts, measured in Siemen (S) or MicroSiemen (μS), that are electrolytes in solution. The greater the concentration of fertilisers or salts, the greater the conductance and hence the EC. Thus, EC measures the concentration of nutrient salts in the soil or compost solution (see Bunt, 1988)

[Mho: the reciprocal of the unit of resistance was previously used to measure conductance but has been replaced by the Siemen as an SI unit. Both units have the same value and are large in relation to the salt concentration found in plant growth media. Therefore, EC is generally described as microSiemen ($1\mu S = S10^{-6}$)**]**

pH: lorgarithm to the base 10 of the reciprocal of the hyrdrogen ion concentration. Thus $pH = log1/[H^+]$. pH measures the degree of acidity or alkalinity of a solution.

Matric potential: the energy required to remove water from soil particles.

Osmotic concentration: the concentration of solutes (nutrient salts) that, in water, make a soil solution.

Osmotic potential: the energy required to remove water against a solute gradient.