

**ASPECTS OF THE BIOLOGY AND CONTROL OF
PESTALOTIOPSIS ON
HARDY ORNAMENTAL NURSERY STOCK**

by

KATHERINE E. HOPKINS

**A thesis submitted for the degree of
Master of Science
in the Faculty of Science at the University of Glasgow**

**Department of Plant Science,
The Scottish Agricultural College,
Auchincruive, Ayr.**

June, 1996

ProQuest Number: 11007839

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 11007839

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Thesis
10581
Copy 1

ACKNOWLEDGEMENTS

This work was undertaken at SAC Auchincruive during the tenure of a studentship from The Horticultural Development Council. I would like to thank Dr George Marshall for providing research facilities in the Department of Plant Science at SAC Auchincruive.

Many thanks to my supervisor, Dr Mark McQuilken for his guidance, encouragement and support during the course of this project and in the preparation of this thesis.

I would also like to thank the staff of the Crop Health Centre for their friendship, help and encouragement. Thanks also to Dr Neil McRoberts for advice on all statistical matters.

Many, many thanks to Pilot Officer S. Kinloch for unrivalled (and continuing!) support, help and advice in many matters over the last 10 years - on connait l'ami au besoin.

Et à ma famille et mes amis - à chaque saint sa chandelle!

<u>CONTENTS</u>	<u>Page</u>
LIST OF TABLES	i
LIST OF PLATES	iii
ABBREVIATIONS USED IN THE THESIS	iv
ABSTRACT	v
1. GENERAL INTRODUCTION	1
1.1 The UK hardy ornamental nursery stock industry	1
1.1.1 Common species of HONS grown in the UK and their uses	1
1.1.2 Stock plants	3
1.1.3 Propagation	3
1.1.4 Container production	6
1.1.5 Diseases of container-grown HONS and their control	7
1.1.6 Control of diseases of container-grown HONS	12
1.2 The genus <i>Pestalotiopsis</i>	14
1.2.1 Taxonomy, nomenclature and morphology of <i>Pestalotiopsis</i> spp.	14
1.2.2 Growth and survival of <i>Pestalotiopsis</i> spp.	15
1.2.3 Diseases caused by <i>Pestalotiopsis</i> spp. and their occurrence	17
1.2.4 Infection processes and epidemiology of <i>Pestalotiopsis</i> spp.	19
1.2.5 Control of <i>Pestalotiopsis</i> spp.	21
1.3 Aims of the study	26
2. MATERIALS AND METHODS	27
2.1 Collection, identification and pathogenicity of <i>Pestalotiopsis</i> spp. on HONS	27
2.1.1 Isolation and maintenance	27

2.1.2 Preparation of spore suspensions of <i>Pestalotiopsis</i>	28
2.1.3 Colony morphology, spore measurements and radial growth rates	28
2.1.4 Pathogenicity assays	29
2.2 Effect of agar media on mycelial growth and acervuli production of <i>P. sydowiana</i>	30
2.3 Effects of environmental factors affecting growth of <i>P. sydowiana</i>	31
2.3.1 Effect of temperature on mycelial growth and conidial germination of <i>P. sydowiana</i>	31
2.3.2 Effect of pH on mycelial growth and conidial germination of <i>P. sydowiana</i>	32
2.3.3 Effect of water potential on mycelial growth and conidial germination of <i>P. sydowiana</i>	33
2.4 Preparation and growth of cuttings for glasshouse experiments	36
2.5 Inoculation of cuttings	36
2.6 Glasshouse experimental design and husbandry	37
2.7 Determination of host range of <i>P. sydowiana</i> on selected HONS	37
2.8 Determination of cultivar susceptibility of selected HONS to <i>P. sydowiana</i>	38
2.8.1 <i>Calluna vulgaris</i>	38
2.8.2 <i>Erica</i> spp.	38
2.8.3 <i>Rhododendron</i> spp.	39
2.9 Effect of water splash on transmission of spores of <i>P. sydowiana</i>	39

2.10 Effect of selected fungicides on the mycelial growth and conidial germination of <i>P. sydowiana</i> <i>in vitro</i>	40
2.11 Sensitivity of <i>P. sydowiana</i> to selected fungicides on HONS in the glasshouse	42
2.11.1 <i>Rhododendron</i> sp.	42
2.11.2 <i>Chamaecyparis lawsoniana</i>	43
2.11.3 <i>Calluna vulgaris</i>	43
2.12 Disease assessments	43
2.13 Statistical analysis	44
3. RESULTS	45
3.1 Collection, identification and pathogenicity of <i>Pestalotiopsis</i> spp.	45
3.1.1 Collection and identification of <i>Pestalotiopsis</i> spp.	45
3.1.2 Colony morphology, spore measurements and radial growth rates	45
3.1.3 Pathogenicity assays	48
3.2 Effect of agar media on mycelial growth and acervuli production of <i>P. sydowiana</i>	48
3.3 Effects of environmental factors affecting growth of <i>P. sydowiana</i>	51
3.3.1 Effect of temperature on mycelial growth and conidial germination of <i>P. sydowiana</i>	51
3.3.2 Effect of pH on mycelial growth and conidial germination of <i>P. sydowiana</i>	53
3.3.3 Effect of water potential on mycelial growth and conidial germination of <i>P. sydowiana</i>	53
3.4 Determination of host range of <i>P. sydowiana</i> on selected HONS	56

3.5 Determination of cultivar susceptibility of selected HONS to <i>P. sydowniana</i>	56
3.5.1 <i>Calluna vulgaris</i>	56
3.5.2 <i>Erica</i> spp.	59
3.5.3 <i>Rhododendron</i> spp.	59
3.6 Effect of water splash on the transmission of spores of <i>P. sydowniana</i>	61
3.7 Effect of selected fungicides on the mycelial growth and conidial germination of <i>P. sydowniana in vitro</i>	63
3.8 Sensitivity of <i>P. sydowniana</i> to selected fungicides on HONS in the glasshouse	63
3.8.1 <i>Rhododendron</i> sp.	63
3.8.2 <i>Chamaecyparis lawsoniana</i>	65
3.8.3 <i>Calluna vulgaris</i>	65
4. DISCUSSION	68
5. CONCLUSIONS	81
BIBLIOGRAPHY	83
APPENDICES	91
Appendix 1. Osmotic potential	91
Appendix 2. Matric potential	92
Appendix 3. Standard potting compost	93

LIST OF TABLES

Table 1. Origin of isolates of *Pestalotiopsis sydowiana*

Table 2. Colony morphology of isolates of *Pestalotiopsis sydowiana* grown on PDA for 3 weeks at 22°C

Table 3. Spore measurements and radial growth rates of isolates of *Pestalotiopsis sydowiana* on PDA

Table 4. Effect of different media on hyphal radial growth rate of four isolates of *Pestalotiopsis sydowiana* at 20°C

Table 5. Effect of different media on acervuli production by four isolates of *Pestalotiopsis sydowiana* at 20°C

Table 6. Effect of temperature on hyphal radial growth rate and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA

Table 7. Effect of pH on hyphal radial growth rate and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA at 20°C

Table 8. Hyphal radial growth rates and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA adjusted to various osmotic potentials with NaCl at 20°C

Table 9. Hyphal radial growth rates and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA adjusted to various osmotic potentials with glycerol at 20°C

Table 10. Hyphal radial growth rates of three isolates of *Pestalotiopsis sydowiana* on PDA adjusted to various matric potentials with polyethylene glycol 6000 at 20°C

Table 11. Susceptibility of rooted and unrooted cuttings of cultivars of *Calluna vulgaris* to infection by *Pestalotiopsis sydowiana*

Table 12. Susceptibility of rooted cuttings of cultivars of *Erica* spp. to two isolates of *Pestalotiopsis sydowiana*

Table 13. Susceptibility of rooted cuttings of cultivars of *Rhododendron* spp. to two isolates of *Pestalotiopsis sydowiana*

Table 14. Transmission of spores of *Pestalotiopsis sydowiana* by water splash from spore suspension and infected *Rhododendron* foliage

Table 15. IG₅₀ (concentration of active ingredient giving 50% linear growth inhibition) values for mycelial growth of two isolates of *Pestalotiopsis sydowiana* on fungicide amended PDA at 20°C

Table 16. IG₅₀ (concentration of active ingredient giving 50% inhibition of conidial germination) values for conidial germination of two isolates of *Pestalotiopsis sydowiana* on fungicide amended PDA at 20°C

Table 17. Effect of selected fungicides on levels of foliar browning caused by *Pestalotiopsis sydowiana* (isolate P16) on unrooted *Rhododendron* cuttings. Fungicides were applied pre- and post-inoculation.

Table 18. Effect of selected fungicides on levels of foliar browning caused by *Pestalotiopsis sydowiana* (isolate P16) on unrooted cuttings of *Chamaecyparis lawsoniana*. Fungicides were applied pre- and post-inoculation.

Table 19. Effect of selected fungicides on levels of foliar browning caused by *Pestalotiopsis sydowiana* (isolate P11) on unrooted cuttings of *Calluna vulgaris*. Fungicides were applied pre- and post-inoculation.

LIST OF PLATES

- Plate 1:** Cuttings of *Rhododendron* sp. infected with *Pestalotiopsis sydowiana*
- Plate 2:** Acervuli of *Pestalotiopsis sydowiana* on infected *Rhododendron* leaf
- Plate 3:** Acervuli of *Pestalotiopsis sydowiana* on infected *Chamaecyparis lawsoniana* cutting
- Plate 4:** Growth of isolates of *Pestalotiopsis sydowiana* on PDA
- Plate 5:** Growth of isolates of *Pestalotiopsis sydowiana* on selected agar media

ABBREVIATIONS USED IN THE THESIS

HONS	: Hardy ornamental nursery stock
PDA	: Potato dextrose agar
MEA	: Malt extract agar
cv(s)	: Cultivar(s)
PDAES	: Potato dextrose agar plus erythromycin and streptomycin
TWA	: Tap water agar
CMA	: Cornmeal agar
CDA	: Czapek Dox agar
SDA	: Sabouraud dextrose agar
V8	: V8 juice agar
PEG6000	: polyethylene glycol 6000

ABSTRACT

Aspects of the biology and control of *Pestalotiopsis* on hardy ornamental nursery stock (HONS) were studied. The species of *Pestalotiopsis* most commonly found on samples of diseased HONS collected from nurseries throughout the UK was identified as *Pestalotiopsis sydowiana* (Bresad.) B. Sutton. The colony morphologies, spore measurements and hyphal extension rates of all isolates of *P. sydowiana* collected were similar. Different isolates of *P. sydowiana* were able to grow well on commonly used agar culture media, including SDA, V8, MEA, CDA and PDA. Optimum temperatures for growth of several isolates were in the range of 15 to 25°C, with little or no growth occurring below 5°C or above 30°C. Conidial germination occurred over the range of 20 to 30°C, with an optimum of 30°C. Both growth and conidial germination occurred over the range of pH 2.6 to 7.6. Growth was optimum at pH 5.5. Although there were slight differences between osmotica and isolates of *P. sydowiana*, growth generally decreased with decreasing water potential. Growth was markedly reduced below -4.0 to -6.5 MPa, but it was still maintained at low osmotic potentials of -9.9 to -10.3 MPa. Similar results were obtained for conidial germination, but germination was completely inhibited at -9.9 MPa. Growth and conidial germination were affected more by low matrix than by low osmotic potentials.

A wide range of nursery stock species were infected by *P. sydowiana* including *Calluna vulgaris*, *Erica* spp., *Rhododendron*, *Chamaecyparis*, *Cupressocyparis*, *Pieris* sp. and *Euonymus*. Isolates of *P. sydowiana* were not

host specific and infected a number of nursery stock species other than those from which they were originally isolated. Typical symptoms included foliar browning, wilting, death of shoots, and root and stem-base rots. Black acervuli of *P. sydowniana* were typical on diseased leaves, shoots and stems. Unrooted cuttings of plants were generally more susceptible to infection than rooted. There were some slight differences in cultivar susceptibility of *Erica* spp. and *Rhododendron*, but the ranking of cultivars according to susceptibility was difficult because of differences in the pathogenicity of isolates. Transmission of conidia of *P. sydowniana* was shown to occur by water splash. Splashed conidia from infected foliage and spore suspension were capable of travelling up to distances of 0.7 m when water was dropped from above.

Fungicide trials indicated that foliar sprays of prochloraz and chlorothalonil were effective in reducing the severity of *Pestalotiopsis* on *Rhododendron*. Prochloraz, chlorothalonil, mancozeb and iprodione were also effective against *Pestalotiopsis* on *C. vulgaris*.

The importance of the findings to the integrated control of *Pestalotiopsis* on ornamental plant species is discussed.

1. GENERAL INTRODUCTION

1.1 The UK hardy ornamental nursery stock (HONS) industry

1.1.1 Common species of HONS grown in the UK and their uses

Many species of HONS are grown in the UK. Some of the most popular and easiest to grow include *Berberis*, *Buddleia*, *Calluna vulgaris* (L.) Hull, *Camellia japonica* (L.) and related spp., *Chaenomeles*, *Clematis*, *Cotoneaster*, *Cytisus*, *Erica* spp., *Euonymus*, *Forsythia*, *Hebe*, *Hedera*, *Ilex*, *Mahonia*, *Rhododendron* spp. (including Azaleas), *Ulex* and all the conifer species. Many of these can be seen growing in gardens throughout the UK and are easily obtained from nurseries.

Only *C. vulgaris*, *Erica* spp., *Rhododendron* spp. and the various types of conifer are considered here as they are of most importance to this project. *C. vulgaris* and *Erica* spp. are ericaceous plants that can be found growing in the country side as well as in cultivated gardens. Both shrubs can grow anywhere from 10 cm to 5 m in height depending on the species, and prefer to grow on acidic soils. They are normally described as hardy since they can survive in harsh conditions. The genus *Calluna* has only one recognised species whilst the genus *Erica* has over 600. It is also possible to obtain hybrids of these species. Many cultivars of *C. vulgaris* and *Erica* spp. are available. It is practically possible to have a garden of ericaceous plants that is flowering, or a source of colour, throughout the year since different cultivars will flower or change their foliage at different times. There is immense variation in flower colour and foliage between cultivars. Flowers can be dark purple, dark pink,

pale pink, white, cream, yellow or orange and different shades of these colours. Foliage may be silvery grey, dark green or lime green, and in autumn it is common to see foliage in shades of red or orange (Proudley, 1985).

The genus *Rhododendron* contains close to one thousand species and there are many more hybrids. These range from dwarf creeping alpines sometimes only 3 cm in height to trees of 24 m. There is also variation in leaf size and flower colour. Azaleas are included in this genus. *Rhododendron* sp. can be seen growing naturally over large areas of rain soaked mountains in the Northern hemisphere particularly in the temperate zone. They can be found growing in most situations including cliff tops, on and amongst boulders, by waterfalls, on moorlands and pasture, as well as in bogs and many types of forest. They tend to dislike soils that are rich in lime or those with a high clay content.

Conifers belong to the order *Coniferales*. They include the common species of *Chamaecyparis*, *Cupressus*, *Juniperus*, *Pinus* and *Cedrus* which can be seen in gardens throughout the UK (Proudley, 1984). They are evergreen plants which are desirable in gardens because they provide a source of colour all the year round. Their colours range from a variety of greens through to yellows, golds and bronze. Some species also have grey or blue foliage to add further variation. They grow in a wide range of soils apart from those with a high chalk or lime content.

An ornamental plant is one which is cultivated for its beauty rather than its use (Baker and Linderman, 1979). However, planting ornamentals can reduce erosion and provide shade. Garden environments can be improved by

controlling temperature, wind and traffic noise. Headlight glare can be reduced on roads. Such plantings can also help to purify the atmosphere from pollutants. For these reasons, it is important that ornamental plants remain free from disease.

1.1.2 Stock plants

Stock plants are an extremely important part of any nursery since they are a means by which new plant material can be produced. They are typically healthy and vigorously growing plants which must be of typical form and colour of their species to ensure uniformity in cuttings. Their main function is to provide new cutting material. As with any plant, stock plants must be maintained properly. It is important to keep them fertilised and pruned. They must also be kept free from pests, pathogens and weeds. When properly cared for they will maintain the juvenile state of recently propagated plants (Lamb, Kelly and Bowbrick, 1985).

1.1.3 Propagation

Young plants can be generated either from seed or by vegetative propagation. Vegetative propagation is probably the most common way of producing new plants of ornamental nursery stock, and it includes many different methods. However, the preparation of cuttings is probably one of the simplest and most common. *Erica* spp., *C. vulgaris*, *Rhododendron* spp. and conifers can all be prepared as cuttings. The process of propagation is not solely concerned with the preparation of cuttings. Other factors which must be considered are the preparation of suitable rooting media, correct aftercare of cuttings, and the use of capillary matting or the mist system to maintain

adequate irrigation. Pathogen control is also an important part of propagation. Ensuring that all these factors are correct for the propagation of young plants will allow propagators to achieve a high percentage of healthy young plants.

The type of cutting used will depend on the species of plant. There are three general types including softwood, semi-hardwood and hardwood. Softwood cuttings are those which are taken early in the season before lignification has occurred. These will wilt very rapidly and must be covered if rooting is to be successful. Semi-hardwood cuttings consist of young shoots whose stems are beginning to strengthen. Hardwood cuttings are taken late in the growing season, possibly in November, and these consist of growth from the previous season.

There are also different ways in which cuttings can be taken from the stock plant. Basal cuttings are taken by cutting through the base of a young shoot where it joins the stem. A heeled cutting is taken by pulling the young shoot in a downward motion, and ensuring that a piece of the stock plant is taken. Nodal cuttings are taken by cutting at right angles to the stem immediately below a leaf node, whereas an internodal cutting is sectioned between nodes of leaf stems. Stem cuttings are those which are removed from stock plants irrespective of the position of nodes. (Lamb *et al.*, 1985).

Cuttings should be struck in rooting media as soon as possible after removal from stock plants. They can be stored temporarily in polythene bags but should be kept away from sunlight to avoid extreme fluctuations in temperature (Lamb *et al.*, 1985). The lower foliage is usually removed to prevent rotting of the cutting in the rooting media. Care should be taken when

doing this so as to avoid tearing the stem. In some species, wounding the stem base is recommended as an aid to rooting. This may involve scraping the stem or removing a section of bark (Lamb *et al.*, 1985). It may be necessary to dip stems into hormone rooting substances before the cuttings are struck. Cuttings are usually kept under polythene on warmed benches or on capillary matting. Mist propagation is also a popular means of aiding rooting. Whichever method is used, it is important to ensure that irrigation is adequate at all times.

Other methods of propagation are available for use with ornamental nursery stock. Layering involves bending shoots over into the ground and pegging them down into the soil. The shoot is then brought up again. Covering the bend with soil encourages roots to grow at this point. Grafting involves joining a scion or bud of one plant with a seedling rootstock of another. The species must be closely related if the stock and scion are to develop into a single plant. Budding involves placing a bud beneath the bark of a rootstock where it is in contact with the cambium (Lamb *et al.*, 1985).

Micropropagation is another method of producing young plants. This involves using methods which allow growers to clonally propagate a variety of plant species. Rapid clonal multiplication is normally used in commercial situations. This involves placing an explant which may be a stem apex, root, leaf or some other part of the plant. The explant is then placed on a synthetic media containing a range of nutrients and plant hormones such as auxin and cytokinin. In the first instance, callus will be formed followed by adventitious shoots. These can eventually be rooted to produce new plants (Murashige, 1974).

Whichever type of propagation is used, it is essential to use propagation medium which will allow the highest possible percentage of successfully rooted cuttings. A wide range of media are available for propagating ornamental nursery stock. Media with a reliable composition are preferable. Propagators have used peat moss, sand, perlite or vermiculite and combinations of these for many years. Prefabricated blocks, trays and slabs are also available for use in propagation (Matkin, 1971).

1.1.4 Container production

Container production of ornamental nursery stock involves planting cuttings or young seedlings in some type of container. This method of growing has replaced the method of planting cuttings or seedlings directly into the field. In 1984, 76.2 million hardy ornamental nursery stock plants were grown in the UK in containers (Bunt, 1988).

Types of container for the growth of young plants are continually being developed. The actual process of growing plants in containers can save growers time and money. Placing cuttings directly into containers rather than propagation trays saves production steps and delays from necessary transplanting. It also reduces the number of plants that may be affected by transplant shock. This can be caused by root disturbance in young plants. Containers may be made of metal, wood, plastic or clay. Plastic pots are probably the most advantageous since they are light, reusable and non-porous. They can also be stacked on top of one another thus reducing storage space. The major disadvantage is that they have a tendency to break easily. Fibre pots can also be used as can expanded foam blocks, which consist of container and

media in one unit.

If container-grown plants are to be successful, they must be properly maintained. Containers are normally placed on sand beds or damp mats so that water enters the pots via capillary action. Overhead sprinklers are also commonly used, but there is a problem with excess run-off. Trickle or drip irrigation is another widely used method. All plants must be protected from low temperatures and container grown plants are no exception to this. Plants that are well established before the onset of cold temperatures have an advantage, and placing pots closer together can also reduce damage from temperature fluctuations. It is essential that plants are not kept in containers for too long prior to planting out since this can result in constricted root systems (Hartman, Kester and Davies, 1990).

1.1.5 Diseases of container-grown HONS and their control

Soil-borne fungal plant pathogens cause the most problems to container grown woody plants. Research over the past 10 to 15 years, in the USA, has shown that brief exposure of plants to drought, flooding, extremes of temperature, nutrient deficiencies, salinity or air pollution can predispose plants to infection. These stresses particularly affect the roots of container-grown plants because the small soil volumes provide very little buffering. Containers are also usually fully exposed to climatic factors (Niedbalski-Cline, 1988). Species which have often been isolated from diseased ornamental nursery stock include *Pythium* spp., *Phytophthora* spp. and *Rhizoctonia solani* (Kühn). Other pathogens which have also been isolated include species of *Fusarium*, *Sclerotinia*, *Cylindrocladium*, *Thielaviopsis* and *Botrytis cinerea*

(Pers.) (Hartman *et al.*, 1990).

Pythium

A number of *Pythium* species have wide distributions and host ranges. They tend to infect the roots and stems of young plants. However, they can also infect the stems and foliage of older plants. Symptoms tend to develop suddenly and can result in the death of a large number of plants. Infection by *Pythium* spp. results in a soft rot of roots and stems.

Infection is influenced by the moisture, temperature, pH and cation composition of the growing media as well as the inoculum density. Light intensity and the presence and density of other plant pathogens can also influence infection. Of these, the temperature and moisture of the growing media are the most important with individual species responding more to one factor than to the others. Generally, a high soil moisture appears to be necessary for disease development; in soils which are frequently waterlogged, death of plants is often attributed to *Pythium* spp.. (Hendrix and Campbell, 1973). The pathogen is capable of surviving in the growing media without the presence of a host (MacDonald, 1986).

Phytophthora

Many species of *Phytophthora* infect the roots of a wide range of plants causing root rots. For example, *Phytophthora cinnamomi* (Rands) has been found to be pathogenic to, or has been recorded on, over 900 plant species (Hardy and Sivasithamparam, 1988). In the USA, this pathogen has been observed on *Rhododendron* spp., *C. vulgaris*, *Erica* spp. and other important ornamental plants. Symptoms on infected plants include dieback and wilting as

well as root rot and stem discoloration (Linderman and Zeitoun, 1977). Roots of susceptible hosts are often infected at injury points, producing a soft wet rot (McCully and Thomas, 1977). Alternatively, dry, very dark lesions can appear after infection.

Phytophthora spp. grow more readily at moderate to high levels of growing media moisture. Temperature requirements for growth and survival vary. Sporangia and oospores of *Phytophthora cactorum* (Leb and Cohn.) Schroet. can survive freezing temperatures, whilst *P. cinnamomi* has a minimum temperature range for growth of 5-16°C. Species of *Phytophthora* are capable of surviving in host debris in soil, and subsequently infecting susceptible hosts.

Rhizoctonia solani

Rhizoctonia solani (Kühn) has a large host range and pathogenic potential. It is one of the most widely distributed soil-borne fungi (Kataria, Hugelshofer and Gisi, 1991). It is pathogenic under many types of environmental conditions (Lambe and Wills, 1980). A range of disease symptoms are caused by this pathogen. Infection is indicated by the presence of reddish-brown mycelium. This can be seen on diseased tissue or on the surface of infected growing media. Mycelial threads can stick infected leaves to propagation media, and to other leaves, producing a web-like effect. The pathogen can cause root rots on ornamental plants including *Rhododendron* spp., *C. vulgaris* and *Erica* spp. and azaleas (Coyier and Roane, 1987; Litterick *et al.*, 1995).

Fusarium

Fusarium spp. are capable of causing economically important root rots. Infection of Douglas Fir seedlings with *Fusarium oxysporum* (Schlect.) has been associated with a needle infection, top blight and root rot which destroys the phloem and cortex of the roots (Bloomberg, 1971). Root and stem rots increase in severity when plants are stressed by high temperatures, intermittent drought or excess water and high nitrogen levels. The pathogen can also grow saprophytically on plant debris in soil. (McCully and Thomas, 1977).

Cylindrocladium

Cylindrocladium also causes root rots. *Cylindrocladium scoparium* (Morgan) was first described on azaleas in 1955, but remained of little interest until the 1960s (Baker and Linderman, 1979). At this time, azaleas suddenly began to wilt during shipping and subsequent forcing. Root rot symptoms also appeared. In the USA, the ornamental industry was soon faced with a widespread disease. Infection of plants had occurred during propagation, with between 30 and 60% of shipped liners being infected. Plants were symptomless prior to shipping. Control measures were started and, by the early 1970s, the disease had practically disappeared. The pathogen also causes important root rot diseases on spruce and pine seedlings. In soil inoculation experiments, 94% of spruce seedlings were killed within 8 weeks of transplanting to infected soil. Diseased seedlings were chlorotic, wilted and eventually died. Root rot was first noticed at the tips of young lateral roots which were eventually killed. Mycelium of *C. scoparium* could be seen growing on spruce needles following inoculation with the pathogen (Bugbee and Anderson, 1963).

Sclerotinia

Sclerotinia can infect roots, stems, leaves, petioles and flowers of host plants, producing a prominent mass of white cottony mycelium. Sclerotia persist in soil; these can germinate to produce mycelia or apothecia which in turn produce spores (Lambe and Wills, 1980). Stems infected by *Sclerotinia* develop pale or dark brown lesions (Agrios, 1988). The pathogen grows through the stem resulting in stem rots. Foliage above the lesion wilts and the plant dies fairly quickly. *Sclerotinia camelliae* (Hara) causes flower blight on azalea (Baker and Linderman, 1979).

Thielaviopsis

Thielaviopsis typically causes a black root rot of infected plants (Agrios, 1988). It is capable of attacking the roots and hypocotyls of many ornamentals (Lambe and Wills, 1980). *Thielaviopsis basicola* has been shown to cause severe damage to cultivars of Japanese holly (*Ilex crenata*; Thunb.). This pathogen produces chlamydospores which are capable of surviving for long periods in soil.

Botrytis cinerea

Botrytis cinerea produces a characteristic covering of grey mould on infected tissue. This pathogen is often found as a secondary infection after a damping-off disease. It can also be seen on senescing foliage or dropped flowers. Poor ventilation and warm humid conditions increase infection and spread as does overcrowding (MacDonald, 1986).

1.1.6 Control of diseases of container-grown HONS

To protect plants from becoming infected from potential plant pathogens, it is necessary to determine the sources of infection and the environmental factors affecting disease development. The following factors can all result in infection of planting stock: exposure to infected equipment, containers and tools; storage, planting or growth in infected locations or soils; use of contaminated fertilisers, water or soil amendments; exposure to flooding; exposure to windborne or rainborne inoculum or to vectors; use of unsuitable growing sites where inoculum sources may be present, and budding or grafting with infected plant material (Stout, 1962).

Maintenance of healthy stock plants is essential if cuttings are to be successful. Care of stock plants can be ensured by removing dead flowers and foliage, keeping plants under the correct environmental conditions and employing a fungicide programme. These may be broad spectrum fungicides or they could be aimed at a particular species of plant pathogen. Problems can arise if pathogens develop resistance to fungicides, though this may be alleviated by the alternate use of fungicides with a different mode of action.

Plant pathogens can easily become established during plant propagation because cuttings are under stress and, therefore, more likely to become infected. Infection of cuttings can be avoided by taking material only from healthy stock plants. Cuttings should be collected in clean polythene bags using disinfected knives or secateurs. It is essential that utensils do not come into contact with soil or diseased plant material since any pathogens could then be transmitted throughout the whole batch of cuttings. When possible, shoots

covered with soil particles should be avoided for use as cutting material as they may have spores of potential plant pathogens on their foliage. The working surface on which cuttings are to be prepared should be free from plant debris and kept clean. Surfaces can also be disinfected once a set of cuttings has been finished.

Cuttings are probably at most risk of infection during the preparation and striking stage. This is because they are being handled physically and cut tissue also provides a site for pathogens to infect. At this stage, it is important to discard cuttings which already appear to be unhealthy. Cuttings should be handled as gently as possible. Blades with sharp cutting edges should be used so that all cuts made are clean (Hartman, Kester and Davies, 1990). In some cases, it may be necessary to check the rooting media and sterilise it prior to use.

Chemical control can be started when cuttings are struck. Many types of cuttings are dipped in hormone rooting powder or liquid prior to rooting, and these substances often contain fungicides. Similarly, entire cuttings can be dipped in fungicides prior to striking or fungicides can be applied as drenches to the rooting media. After striking, a fungicide programme can be used to prevent infection. At this stage, cuttings should be inspected regularly and any dead tissue should be removed. Other cultural measures can be applied to ensure cuttings remain healthy. For example, a clean water supply is essential; *Pythium* and *Phytophthora* spp. are both capable of surviving in water tanks. Increased ventilation in glasshouses will reduce conditions of high humidity which may increase chances of infection (MacDonald, 1986).

1.2 The genus *Pestalotiopsis*

1.2.1 Taxonomy, nomenclature and morphology of *Pestalotiopsis* spp.

Pestalotiopsis spp. belong to the family Melanconiaceae of the order Melanconiales (Blanc, 1992). This order is found in the class Coelomycetes of the division Deuteromycotina. The characteristic of this order is that the conidia are borne on acervuli (Blanc, 1992). The genus is often referred to as *Pestalotia* and occasionally as *Pestallozia*. It was created in 1839 and is based on the species *Pestalotia pezizoides*. *Pestalotia guepini* was discovered in 1840 and since that time there have been many additions to the genus.

The genus *Pestalotia* has been divided into groups of species based on the number of cells constituting the conidium. These are referred to as quadriloculatae (4 cells), quinqueloculatae (5 cells) and sexloculatae (6 cells) (Guba, 1927). Problems have arisen concerning the taxonomy of *Pestalotia* and *Pestalotiopsis*, and whether or not the name *Pestalotia* should be restricted to a single species. Sutton (1969) has presented evidence favouring the approach of Steyaert (1949) which restricts *Pestalotia* to a single species and reassigns the many species formerly known as *Pestalotia* to other genera. Several of these were placed in two genera, *Pestalotiopsis* or *Truncatella*, but the majority of the more than 600 species described in the genus remained unexamined and therefore stayed in *Pestalotia* (Sutton, 1980). This has led to problems with nomenclature since a number of taxa which should now be referred to as *Pestalotiopsis* are not. Therefore, it is still possible to find many references to *Pestalotia* in the literature (Sutton, 1980).

Conidial morphology is important in the identification of different

species of *Pestalotiopsis*. For example, conidia of *P. sydowiana* are five celled, smooth walled and straight (rarely curved) (Sutton, 1961). They are 23-29 μm in length and 8-11 μm wide. The end cells are hyaline. The superior median cells are dark brown whilst the inferior cell is lighter in colour. The septa separating the superior median cells are almost black. Two to four appendages can be seen on the apical end cell with three being found most often. These are usually between 18-34 μm in length. The basal appendage is often straight, or slightly curved, and 3-6 μm in length. Identifying different species may only be possible by measuring the dimensions of the conidia and observing the colour of the spores.

1.2.2 Growth and survival of *Pestalotiopsis* spp.

Media

Experiments with different types of nutrient media have shown that colony diameters of *P. leucothoës* and *P. conigena* were greatest on potato dextrose agar (PDA) compared with malt extract agar (MEA) and mango leaf extract agar (Ibrahim, Satour and Elakaad, 1976). Comparisons of growth of these two species on synthetically based media indicated that Waksman's media results in greater colony diameters than Czapek media.

Temperature

Ibrahim *et al.* (1976) found that the optimum temperature for growth of *Pestalotiopsis* spp. is between 25 and 30°C and that conidial germination is also optimum at these temperatures as is the length of germ-tubes produced.

pH and osmotic potential

The effect of pH and osmotic potential on growth of *Pestalotiopsis* spp. have been studied using *Pestalotiopsis palmarum*. Experiments with this species have indicated that sporulation can be induced at high pH levels (pH 7-10) when 0.34 M of sodium chloride is present. Addition of sodium chloride to Czapek media, to simulate the effect of osmotic shock by altering the osmotic potential, inhibited growth of the pathogen. Concentrations of sodium chloride ranging from 0.08 to 2.7M inhibited growth (expressed as colony area) from 15 cm² to 1.5 cm², whilst sporulation was obtained only at concentrations between 0.34 - 1.02 M sodium chloride (Mani and Swamy, 1983).

Light

Effect of different lighting regimes on growth and sporulation of *P. herbarum* and *Pestalotia* sp. have been assessed on PDA. Results showed that violet light (410 nm) stimulated mycelial growth of the pathogen. However, diurnal light or continual fluorescent light provided the best conditions for maximum sporulation (Umalkur and Begum, 1976). This corresponds with the findings of other workers who have shown that continual light promotes growth whilst continual dark inhibits it (Ibrahim *et al.*, 1976).

Studies with *Pestalotiopsis theae* have shown that it is only the youngest part of a growing colony at the peripheral region that produces spores (Grover and Karvé, 1963). This occurred in colonies incubated in darkness for 60 h and then exposed to 1 h of light. These workers also showed that the breadth of the spore producing zone increased with an increase in the energy of light provided, suggesting that it is the youngest mycelium that is the most highly

photoreceptive.

Survival

Survival of *Pestalotiopsis guepini* has been examined at -8°C (Baxter and Fagan, 1986). The pathogen was grown on carrot juice agar and conidia were collected, incubated for 3 d on filter paper, dried and frozen for various lengths of time. At intervals of up to one year, 98.7% of the attempts to grow cultures from frozen samples were successful in forming recognisable colonies of *P. guepini*. After a period of three years, 100% of the attempts to grow recognisable colonies were successful indicating that this pathogen is capable of surviving for at least this length of time in frozen culture if not longer (Baxter and Fagan, 1986). However, germination of spores has been noted to decrease to total loss of viability after 12 months storage in host tissue. In contrast, mycelium from the same tissue was still viable after 18 months storage at -8°C (Ibrahim, Satour and Elakaad, 1976).

Problems with continual sub-culturing of *Pestalotiopsis* spp. have been recorded. For example, studies with *Pestalotiopsis annulata* have indicated that the pathogen seems to be capable of undergoing a differentiation, controlled by the genome, which once established becomes persistent (Chevaugnon, 1974).

1.2.3 Diseases caused by *Pestalotiopsis* spp. and their occurrence

Many species of *Pestalotiopsis* have been isolated from ornamental nursery stock in Great Britain. Examples of these include *P. guepini*, *P. truncata*, *P. fibricola*, *P. funerea*, *P. tumefaciens*, *P. caudata*, *P. neglecta*, *P. annulata* and *P. montellica* (Grove, 1937). Different species of *Pestalotiopsis*

have also been found to be capable of causing disease on many types of plants throughout the world. Not only are they capable of infecting ornamental nursery stock including *Rhododendron*, *Erica* spp., *Calluna vulgaris* and conifers, but they can also infect important tropical crops including tea, grapevines, bananas, coconuts and oil palms (Mordue, 1985). In general, these pathogens can be detected throughout the year, although there may be an increased likelihood of finding particular species at particular times. For example, *P. guepini* is more likely to be detected in October and November (Grove, 1937).

Pestalotiopsis spp. are primarily foliar pathogens. They are responsible for causing leaf spots and foliar browning. Disease is characterised by the presence of pin prick sized black or brown spots on leaves. These spots are due to the presence of acervuli. In severe cases, leaf fall may occur as a result of infection. However, certain species have been isolated from other parts of infected plants. *Pestalotiopsis theae* has been implicated in stalk rot of tea cuttings, whilst *Pestalotiopsis steyaertii* has been isolated from roots of *Eucalyptus viminalis* (Labill.) and *Solanum tuberosum* (L.). Fruits and flowers of plants have also been shown to be susceptible to infection (Mordue, 1985).

Disease symptoms on different species of ornamental nursery stock infected by *Pestalotiopsis* spp. are relatively similar. On *Rhododendron*, the early signs of infection are spots which are grey or white in the middle with a dark brown margin. Lesions increase in size until they become blotches (Plates 1 and 2). The surface of lesions is covered with pin prick sized small bodies, which are the acervuli produced by the pathogen (Pirone, 1978). The disease is

Plate 1. Cuttings of *Rhododendron* sp. infected with *Pestalotiopsis sydowiana*

Plate 2. Acervuli of *Pestalotiopsis sydowiana* on infected *Rhododendron* leaf



sometimes characterised by the appearance of the acervuli in concentric circles in the lesions. Needle blight is usually the result of an infection by *Pestalotiopsis* spp. on conifers. The foliage of infected plants turns brown resulting in twig die back (MacDonald, 1986). Again, it is possible to observe small black sporulating bodies on the foliage (Plate 3). The disease on *C. vulgaris* and *Erica* spp. is commonly identified by the presence of brown areas, often found at the tips of new growth. Browning progresses down the stem to the foliage at the centre of the plant (Edge, 1984). As with rhododendrons and conifers, it is possible to see black spots on infected foliage.

1.2.4 Infection processes and epidemiology of *Pestalotiopsis* spp.

Pestalotiopsis spp. are primarily thought to be transmitted from host to host by the action of water splash due to rainfall, or to overhead watering systems. Water landing on infected foliage will carry spores from the surface and then splash these on to healthy plants, thus effectively transporting the pathogen. There are reports of *Pestalotiopsis* spp. being seedborne and soilborne, and infection can also occur during physical contact between infected and healthy material (Mordue, 1985).

Infection of *Hymenaea coubaril* (L.) by *Pestalotiopsis subcuticularis* has been examined using electron microscopy (Fail and Langenheim, 1990). It was shown that penetration and ramification occurred rapidly in leaf disks, whole detached leaves and attached wounded leaves. Attached unwounded leaves showed no obvious symptoms other than some slight degradation of the cuticle. Spores were found to germinate on leaf surfaces between 6 and 12 h following inoculation, with the plant epidermis being penetrated by the conidial germ-

Plate 3. Acervuli of *Pestalotiopsis sydowiana* on infected *Chamaecyparis lawsoniana* cutting



tube some 12 to 24 h after germination. Hyphae were shown to enter via wounds, or in the absence of wounds, occasionally, by direct penetration of the epidermis. Penetration was not seen to occur via stomata. Hyphae grew in, and beneath the cuticle, causing degradation in these areas. When infections became active, leaf cells were killed three to ten cells in advance of the fungus, with hyphae then growing into disrupted cells. However, the fungus did not appear to enter living cells. The active infection gave rise to lesions which increased in size until almost the entire surface of the leaf was covered. Acervuli formed at lesion edges and, on reaching maturity, these erupted through the epidermis and cuticle to release spores (Fail and Langenheim, 1990).

This study of the infection process indicates that wounding is important if infection of plants is to take place, since no evidence was found to suggest that *P. subcuticularis* could enter via stomata. Other experimental work has also shown that the presence of wounds is an essential pre-requisite for infection. For example, symptoms of leaf spot on *Rhododendron* cuttings were only found following both synthetic and insect injuries (White, 1930). In order to induce symptoms of disease, it was necessary to injure the plants by burning or steam treating the leaves prior to inoculation. In experiments with *Pestalotiopsis rhododendri*, the pathogen was then capable of growing and sporulating in the dead areas produced as a result of the wounding treatment.

Pestalotiopsis spp. are also capable of infecting plants when they are subjected to various types of environmental stress. Lack of light, insufficient aeration, excess humidity, insufficient nutrient levels, sunscald, frost injuries

and too high levels of pH have all been suggested to weaken plants and increase their sensitivity to infection. These cultural conditions are generally unfavourable towards plant growth and render plants and cuttings less vigorous. They also provide the pathogen a means of penetrating the plant. For example, frost causes microlesions on plant surfaces. Excess humidity is thought to cause poor oxygenation of the roots which may then have the tendency to rot (Blanc, 1992).

As well as acting as pathogens in their own right, *Pestalotiopsis* spp. may also be able to act as secondary pathogens or in combination with other pathogenic organisms. For example, *P. sydowiana* has been found to cause a secondary infection on *Rhododendron* following initial infection by rust (Coyier and Roane, 1987).

The way in which cuttings are prepared in the process of propagation can also provide an entry for this pathogen. The removal of the distal portion of leaves of *Rhododendron* cuttings prior to insertion into propagation trays provides a ready entry site (Coyier and Roane, 1987). Similarly, the technique of removing the lower third of the foliage of rhododendron cuttings prior to striking can also provide easy entry for the pathogen.

1.2.5 Control of *Pestalotiopsis* spp.

There are three possible methods of controlling *Pestalotiopsis*. Firstly, it is essential to ensure that cultural conditions are ideal for the growth of the plant so as to reduce stress from unfavourable conditions. Secondly, it may be possible to select cultivars or species which have previously been shown to be less susceptible to the pathogen. Thirdly, fungicides can be applied.

Cultural control and use of cultivars

Information is available on the use of cultural control measures for the control of *Pestalotiopsis* spp.. However, this information does not directly relate to ornamental nursery stock grown in the United Kingdom. It is important to avoid high temperatures and high humidities under glass or polythene (Edge, 1984). Similarly, it is important to avoid low temperatures and prevent plants from becoming frosted. These conditions will weaken the plant and allow the pathogen a greater chance of becoming established. Plants should be watered only when necessary and a harsh water application should be avoided since this may increase the possibility of splashing spores from old material, or from contaminated soil, on to new growth. For similar reasons, dense spacing of cuttings should be avoided since this will increase the amount of foliage which is within the range of spore splash (Coyier and Roane, 1987). The removal of infected fallen leaves can also help to reduce inoculum potential.

Obtaining the correct levels of nutrients and a suitable pH in the growing media are essential if plants are to grow to their full potential. Disease caused by *Pestalotiopsis* spp. has been found to occur when concentrations of potassium and phosphonic acid (applied as fertilisers) are too low or when the pH is too high (Blanc, 1992).

During propagation, it is important that cuttings are handled as gently as possible to avoid wounding. Any wounds produced will provide the pathogen with an entry point. Previous work has indicated how much more likely wounded plants are to succumb to infection by this opportunistic pathogen (Fail and Langenheim, 1990).

Some cultivars and species of ornamental nursery stock have shown that they have a natural resistance to infection. Such is the case with those which have so far shown some resistance to *Pestalotiopsis* spp.. Of the many cultivars of *Erica* spp and *C. vulgaris* available to growers, very little information is available to indicate which are susceptible and which are not. However, *C. vulgaris* cvs of Golden Feather and Serlei Aurea have been shown to be more sensitive to *Pestalotiopsis* than *C. vulgaris* cv. Bonfire Brilliance, *Erica carnea* cv. Lesley Sparkes and *Erica x darleyensis* cv. Jack H. Brummage (Edge, 1984). Where Camellia sensitivity has been assessed, *Camellia x williamsii* cvs. Debbie and Donation have been shown to be particularly sensitive to *Pestalotiopsis* spp. (MacDonald, 1986).

A larger number of *Rhododendron* cultivars have been assessed for their susceptibility to *Pestalotiopsis* spp.. Those which appear to be the most sensitive include *Rhododendron x forestii repens* cvs. Baden Baden, Elisabeth and Golden Wonder. Those which have shown slightly less susceptibility include *Rhododendron catawbiense* cvs. Boursault, Grandiflora and Chikor (Blanc, 1992). Many more susceptible varieties are listed.

Chemical control

The ability of fungicides to inhibit growth of *Pestalotiopsis* spp. has been evaluated *in vitro*. *Pestalotiopsis palmarum* causes coconut grey blight and growth of this pathogen has been completely inhibited by carbendazim (Bavistin), tetramethyl thiuram disulphide (Hexathir) and thiabendazole (Tecto 60). Concentrations of the fungicides ranging from 100 to 500 ppm in Richards media all inhibited growth of the fungus (Das and Mahanta, 1985). Similar

experiments with *P. westerdijkii* have also indicated susceptibility to fungicides. Organomercury (Aretan 6), and a copper oxychloride and zineb mixture (Miltox) have been shown to be highly toxic to this species (Jain and Agrawal, 1974). Growth of *P. psidii* has been completely inhibited by Bordeaux mixture, benomyl (Benlate), carbendazim (Bavistin), zineb (Dithane-Z78) and a mixture of zineb and maneb (Dithane-M45) (Padule and Hande, 1980).

Limited trials undertaken in the United Kingdom and France have shown that fungicides are available which will control disease caused by *Pestalotiopsis* spp. on hardy ornamental nursery stock.

Trials on Camellias have indicated that 35% copper oxychloride (Colliodex), and a carbendazim and maneb WP mixture (Delsene M) are promising fungicides for the control of *Pestalotiopsis*. Application of these fungicides resulted in marked improvement of growth and quality of treated plants (Scott, 1978). Camellia plants heavily infected with *Pestalotiopsis* have been treated with benomyl (Benlate), but no control of the disease was observed. This fungicide appears to be more useful as a protectant against the pathogen rather than controlling the disease once it is established (Scott, 1978). In contrast, prochloraz has been shown to have potential both in the protection of plants against disease and eradication of disease (Scott, 1983).

Immersing rhododendron cuttings in fungicide prior to striking in the rooting medium has been shown to give good control of *Pestalotiopsis* spp., especially when the rooting medium has been previously drenched in fungicide. Benomyl (benlate: 0.01%), carbendazim (Bavistin: 0.06%), carbendazim and maneb (BAS 3501F: 0.08%) and captan (0.1%) all reduced the mean number

of cuttings which rotted due to *Pestalotiopsis* spp. Of these, the carbendazim and maneb mixture was the most successful (Smith, 1977). In established rhododendrons, however, applications of 50% prochloraz (Octave), 50% kocide (Kocide 101), 50% benomyl (Benlate) and 83% captan (Phytocap) have provided no control of the pathogen. New leaves on inoculated plants appeared healthy initially, but after one or two weeks, they were infected in the same way as the untreated control plants (Blanc, 1992).

Biological control

Very little information is available in the literature on the biological control of *Pestalotiopsis* spp.. However, antagonistic interactions have been demonstrated between *Pestalotiopsis psidii* and phylloplane fungi on guava (Pandey, Arora and Dubey, 1993). Dominant phylloplane fungi isolated from guava were screened for their antagonistic effect against *Pestalotiopsis psidii* *in vitro* and *in vivo*. Culture filtrates of *Cephalosporium roseo-griseum* and *Fusarium oxysporum* were effective in reducing growth of *P. psidii*. Volatile products produced in cultures of these two fungi together with *Trichoderma harzianum* have also been shown to reduce growth of *P. psidii*. In dual cultures, *Aspergillus terreus*, *C. roseo-griseum* and *Penicillium oxalicum* have all significantly reduced growth of *P. psidii*. Lesion development on guava leaves was inhibited when spore suspensions of *Aspergillus niger*, *A. terreus*, *C. roseo-griseum* and *T. harzianum* were applied. Inhibition of lesion development was also shown to increase as concentration of the antagonist was increased (Pandey, Arora and Dubey, 1993).

1.3 Aims of the study

The main aim of this study was to collect and identify species of *Pestalotiopsis* from HONS. Further aims were to investigate various aspects of the biology and control of *Pestalotiopsis sydowiana* on HONS. A series of *in vitro* experiments were conducted to determine the effects of media, temperature, pH and water potential on mycelial growth and spore germination of the pathogen. *In vitro* fungicide experiments were undertaken to examine the effect of various fungicides on the mycelial growth and spore germination on *P. sydowiana*.

Glasshouse trials were undertaken to investigate whether there were any differences in cultivar susceptibility of certain ornamental species to *P. sydowiana*. Trials also examined the efficacy of a range of fungicides against *P. sydowiana* on *C. lawsoniana* and *Rhododendron* sp.. Experiments to investigate the water splash dispersal of the pathogen were also conducted.

2 MATERIALS AND METHODS

2.1 Collection, identification and pathogenicity of *Pestalotiopsis* spp. on HONS

2.1.1 Isolation and maintenance

Between 1992 and 1994, hardy ornamental plants with symptoms of *Pestalotiopsis* (black spots on infected plant material often brown or silvery grey in colour) were collected from nurseries in the UK, or were submitted to the Crop Health Centre at the Scottish Agricultural College, Auchincruive. Sections of diseased plant tissue (3-5 mm²) were dissected from plants, surface sterilised in a 10% solution of sodium hypochlorite (10-14% available chlorine) for 5-10 s and rinsed with sterile distilled water. Surface sterilised sections of tissue were blot-dried on sterile filter paper and plated on Oxoid potato dextrose agar (PDA; Unipath, Basingstoke, Hampshire), modified to contain 100 µg ml⁻¹ of erythromycin and streptomycin (PDAES). Following growth at 20°C for 3-5 d, isolates were transferred onto PDA for preparation of single spore isolates.

Spore suspensions of each isolate were prepared by flooding Petri dish cultures with sterile distilled water and gently scraping the surface with a sterile inoculating loop. Spores were then inoculated onto 1 cm² segments of tap water agar (TWA; 2g Oxoid technical agar No. 3 in 100 ml tap water; Unipath) placed on sterile glass microscope slides. Agar segments were then examined under a light microscope (x10 magnification). Sections of agar containing single spores were excised using a sterile scalpel and placed on plates of PDAES.

Cultures of all isolates were maintained at 20°C in darkness.

Tentative identification of isolates of *Pestalotiopsis* (Table 1) was made based on morphological and cultural characteristics (Sutton, 1980). This consisted of measuring the dimensions of conidia and appendages, and examining the colour of both conidia and Petri dish cultures. Definitive identification was provided by experts at the International Mycological Institute (Egham, UK).

2.1.2 Preparation of spore suspensions of *Pestalotiopsis*

Isolates of *Pestalotiopsis* were grown on PDA in 9 cm diam. Petri dishes at 20°C in the dark for 21 d. Spore suspensions of each isolate were prepared by flooding Petri dishes with sterile distilled water and gently scraping the surface with a sterile inoculating loop. Spore concentrations were counted in a haemocytometer and dilutions made to give a concentration of $1-3 \times 10^6$ spores ml⁻¹.

2.1.3 Colony morphology, spore measurements and radial growth rates

The gross colony morphology of all isolates was examined after 21 d inoculation on PDA at 20°C in the dark. Colony colours of individual isolates were defined according to Raynor (1970) and the distribution of acervuli was recorded. Conidia harvested from 21-d-old colonies grown on PDA at 20°C were observed under a light microscope (x40 magnification). The following were measured for each of the 30-50 conidia using an eye-piece graticule: (i) length and width; (ii) length of median cells and, (iii) length of apical and basal appendages. Number of cells, appendages, and the colour of apical, basal and the two superior median cells were also recorded for each conidium.

The radial growth rate of each isolate was determined on PDA. Four replicate plates of PDA in 9 cm diam. Petri dishes were inoculated centrally with a 3 mm diam. mycelial disc cut from the periphery of a 3-5-d-old culture, and incubated in the dark at 20-22°C. At 24 h intervals for 6 d, two perpendicular colony diameters were measured on each plate excluding the diameter of the inoculum disc. Radial growth rates were then calculated.

2.1.4 Pathogenicity assays

All isolates identified as *Pestalotiopsis sydowiana* were assessed for pathogenicity to confirm that they were capable of infecting the host from which they were originally isolated. Mycelial discs and spore suspensions of each isolate were used to determine the pathogenicity of each isolate.

Leaves of the original host species of each isolate were cut from stock plants. These were surface-sterilised with a 10% solution of sodium hypochlorite, rinsed with sterile distilled water, and rubbed with sandpaper to create a damaged area for *P. sydowiana* to infect. Preliminary experiments indicated that infection of detached leaves and cuttings was higher when they were wounded by rubbing with sandpaper prior to inoculation with a spore suspension of the pathogen. Leaves were placed in Petri dishes on moist filter paper. Mycelial discs (3 mm diam.), cut from the periphery of a 3-5-d-old culture of each isolate, were each placed on a single excised leaf of the original host species of the corresponding isolate. All Petri dishes containing leaves were incubated at 20°C. Controls consisted of excised leaves from the host species rubbed with sandpaper and placed on moist filter paper in Petri dishes.

The assay was repeated as described above except that individual leaves

were each inoculated with a spore suspension (0.5 ml, $1-3 \times 10^6$ spores ml⁻¹) prepared as described in section 2.1.2. For both types of inoculation, two leaves were placed in each Petri dish, with the exception of *Erica* spp. and *C. vulgaris*, where two 3-4 cm sections of stem and foliage were placed in each Petri dish. Three replicate dishes were prepared for each isolate.

Leaves from both types of inoculation were examined for signs of disease after 14 d. Small pieces of diseased tissue were plated on Petri dishes of PDAES at 20°C, and after 3-5 d incubation they were observed for typical colony morphology of *P. sydowniana*.

2.2 Effect of agar media on mycelial growth and acervuli production of *P. sydowniana*

Information on the growth of *P. sydowniana* on different laboratory solid media is sparse. In this experiment, a range of the most common nutrient agar media available for growth of fungi under laboratory conditions were selected, and growth and acervuli production by isolates of *P. sydowniana* were tested on each.

Radial growth rates of four isolates of *P. sydowniana* (P6, P11, P16 and P21; see Table 1 for details) were compared on seven agar media: (i) Oxoid potato dextrose agar (PDA); (ii) Oxoid malt extract agar (MEA); (iii) Oxoid cornmeal agar (CMA); (iv) Oxoid Czapek Dox agar (CDA); (v) Oxoid Sabouraud dextrose agar (SDA); (vi) V8 juice agar (V8; 200 ml Cambells V8 vegetable juice, 20 g Oxoid Technical agar No. 3, 800 ml distilled water) and, (vii) tap water agar (TWA; 20 g Oxoid Technical agar No. 3 in 1 l tap water).

Sterile media were dispensed (15 ml) into 9 cm diam. Petri dishes. Petri dishes (five replicates per isolate) were inoculated centrally with a 3 mm diam. mycelial disc cut from the periphery of a 3-5-d-old culture, and incubated in the dark at 20-22°C. Colony diameters were measured and the radial growth rates calculated as described in section 2.1.3.

Production of acervuli on each medium was also assessed. In each case, the area of the plate covered by acervuli was estimated by a visual assessment.

2.3 Effects of environmental factors affecting growth of *P. sydowiana*

Fluctuations in temperature, pH and water potential play a major role in affecting the activity of fungal plant pathogens and the subsequent development of disease (Agrios, 1988; Griffin, 1994). There is no information currently available on the effects of these environmental factors on growth or conidial germination of *P. sydowiana*. Such information would allow a more rational approach to be taken in the control of the pathogen, especially in relation to manipulating environmental and cultural conditions. The effects of temperature, pH and water potential on the growth of *P. sydowiana* were therefore studied.

2.3.1 Effect of temperature on mycelial growth and conidial germination of *P. sydowiana*

The effect of temperature on the mycelial growth and conidial germination of three isolates of *P. sydowiana* (P7, P11 and P16; see Table 1 for details) was determined at five temperatures ranging from 5-30°C.

To study the effect of temperature on mycelial growth, plates of Oxoid

PDA were inoculated centrally with a 3 mm diam. mycelial disc cut from the periphery of a 3-5-d-old culture of each isolate. Plates were incubated in the dark at 5, 15, 20, 25 and 30°C, with five replicate plates for each isolate. Radial growth rates were determined as described in section 2.1.3.

To study the effect of temperature on germination of conidia, spore suspensions (0.5 ml, $1-3 \times 10^6$ spores ml⁻¹; prepared as described in section 2.1.2) of each isolate of *P. sydowiana* were spread evenly over the surface of agar media using a sterile glass spreader. Plates were placed in sealed plastic bags and incubated in the dark at 5, 15, 20, 25 and 30°C, with five replicates for each isolate. After 12 h, at least 100 conidia on each plate were examined microscopically (magnification x40) for germination. Only conidia with germ-tubes of length greater than spore diameter were considered to have germinated.

2.3.2 Effect of pH on mycelial growth and conidial germination of *P. sydowiana*

Changes in pH can influence respiration and growth of fungi by affecting enzyme systems at the cell surface, and by causing changes in membrane permeability (Griffin, 1972). The aim of these experiments was to determine both the pH range and optimum pH for growth and conidial germination of *P. sydowiana* on solid media.

The effect of medium pH on mycelial growth and germination of three isolates of *P. sydowiana* (P11, P16 and P21; see Table 1 for details) was determined on Oxoid PDA. Batches of sterile double-strength PDA were adjusted with N-NaOH or HCl to the required pH and an equal volume of

buffer was added to give the correct concentration of medium. The pH was maintained over the range of pH 2-7 and at pH 7.6 with citrate phosphate (0.05 M citric acid, 0.1 M Na₂HPO₄·7H₂O) and Tris (hydroxymethyl) aminomethane (0.1 M Tris, 0.1 M HCl) buffers, respectively (Gomori, 1955). Batches of amended medium were dispensed (20 ml) into four replicate Petri dishes (9 cm diam.). Controls consisted of unbuffered PDA (pH 5.5).

To study the effect of pH on mycelial growth, plates were inoculated centrally with a 3 mm diam. mycelial disc cut from the periphery of a 3-5-d-old colony on PDA. Radial growth rates were then determined at 20°C as described in section 2.1.3.

To study the effect of pH on germination of conidia, spore suspensions (0.5 ml, 1-3 x 10⁶ spores ml⁻¹) (prepared as described in section 2.1.2) were spread evenly over the surface of agar media with a sterile glass spreader. Plates were placed in sealed plastic bags and incubated in the dark at 20°C. After 12 h, plates were flooded with lactofuchsin and at least 100 conidia from each plate were examined microscopically for germination. Conidia with germ-tubes of a length greater than the spore diameter were considered to have germinated.

2.3.3 Effect of water potential on mycelial growth and conidial germination of *P. sydowiana*

The importance of substratum water potential on fungal growth is clearly recognised (Griffin, 1981). Water potential has both osmotic and matric components (Papendick and Campbell, 1981). Reductions in either of these components can result in reduced growth of fungi, although if reductions are

only slight fungal growth can be stimulated (Parr, Gardner and Elliot, 1981). Osmotic and matric potential affect the growth of fungi in a similar manner although growth appears to be more affected by lower matric potentials than by low osmotic potentials. In this series of experiments, the effect of osmotic and matric potential on the radial growth rates and conidial germination (osmotic potential only) of isolates of *P. sydowiana* was determined on media with modified water potential.

Osmotic potential

The effect of osmotic potential on mycelial growth and conidial germination of three isolates of *P. sydowiana* (P11, P16 and P21; see Table 1 for details) was determined on Oxoid PDA. PDA was adjusted osmotically over the range -0.3 to -9.9 MPa with NaCl (Lang, 1967) and -0.3 to -10.33 MPa with glycerol (Dallyn and Fox, 1980). The total water potential was the sum of the water potential of the PDA (-0.3 MPa) and the osmotic potential of the added osmotica (Appendix 1).

To study the effect of osmotic potential on mycelial growth, sterile medium (15 ml) adjusted with NaCl or glycerol was dispensed into 9 cm diam. Petri dishes. Five replicate plates were used for each treatment and these were inoculated centrally with a 3 mm diam. mycelial disc cut from the periphery of a 3-5-d-old colony on PDA. Plates were sealed in plastic bags and radial growth rates were determined at 20°C as described in section 2.1.3.

To study the effect of osmotic potential on germination of conidia, a spore suspension of each isolate, (1 ml; final concentration $1-3 \times 10^6$ spores ml⁻¹) was added to 19 ml sterile distilled water previously modified to the

required osmotic potential with either NaCl or glycerol. Samples of these spore suspensions (0.5 ml) were spread evenly over the surface of PDA (15 ml) of the same osmotic potential in five replicate 9 cm diam. Petri dishes. Plates were incubated at 20°C in the dark for 12 h, and were then flooded with lactofuchsin. At least 100 conidia from each plate were examined microscopically for germination. Conidia with germ-tubes of a length greater than the conidium diameter were considered to have germinated.

Matric potential

The matric potential of PDA was adjusted with polyethylene glycol (PEG 6000) (Appendix 2; Steuter, Mozafar and Goodin, 1981). PEG 6000 (g kg⁻¹ liquid) of different concentrations (equivalent to -0.8, -1.3, -2.1, -4.8, -6.5, -7.8, -9.1 MPa) for incubation temperatures of 20°C were determined from a formula derived by Michel and Kaufman (1973) (see Appendix 2 for details). The total water potential was the sum of the water potential of PDA (-0.3 MPa) and the matric potential of added PEG 6000. Concentrations of PEG 6000 were autoclaved separately, cooled to 50°C and added to PDA to give the required matric potential.

To study the effect of matric potential on mycelial growth, sterile medium (15 ml) of PDA/ PEG 6000 at each matric potential was dispensed into five replicate 9 cm diam. Petri dishes. Boiled autoclaved 9 cm diam. cellophane discs (PT 600; British Cellophane Co.) were placed on the matrically adjusted media to provide a suitable platform for growth because PDA/ PEG 6000 did not solidify completely below -1.5 MPa. Plates were inoculated centrally with a 3 mm diam. mycelial disc cut from the periphery of

a 3-5-d-old colony on PDA. Radial growth rates were determined at 20°C as described in section 2.1.3.

2.4 Preparation and growth of cuttings for glasshouse experiments

C. vulgaris and *Erica* spp..

Cuttings were taken from the top 3-5 cm of healthy shoots of stock plants. The lower third of the foliage on each was then removed. Treatments were applied to unrooted cuttings prior to striking into rectangular plastic trays (11 x 10 cm; Cookson Plantpak Ltd., Maldon, Essex) containing Bulrush Propagation Compost (Bulrush Propagation Company Ltd., Magherafelt, County Londonderry, N. Ireland). For experiments with rooted cuttings, treatments were applied to cuttings after rooting under polythene. Rooted cuttings were then planted in a standard potting compost (see Appendix 3 for details).

Rhododendron and *Chamaecyparis lawsoniana*.

Cuttings of *Rhododendron* were taken from the top 5 cm of healthy branches of stock plants. The lower leaves on each were then stripped. Cuttings of *C. lawsoniana* were taken from 10 cm sections of healthy new growth of stock plants, and the lower third of the foliage was removed.

2.5 Inoculation of cuttings

For most experiments, cuttings were rubbed with sandpaper for 5-10 s and dipped in a spore suspension (final concentration $1-3 \times 10^6$ spores ml⁻¹; prepared as described in section 2.1.2) of *P. sydowiana* for 10 s prior to

potting. For pre-inoculation fungicide experiments, cuttings were brushed with a spore suspension following treatment with fungicides.

Spore suspensions were applied to unrooted cuttings prior to dipping into Strike Rooting Powder (May and Baker, Ongar, Essex) and striking into pots (8 or 12 cm diam.) containing Bulrush Propagation Compost. Spore suspensions were applied to rooted cuttings after rooting. Cuttings were then re-potted into pots containing standard potting compost (see Appendix 3 for details).

2.6 Glasshouse experimental design and husbandry

For all glasshouse experiments, four cuttings of each ornamental plant were struck (or planted) in each replicate pot. There were at least three replicate pots for each treatment. Treatment pots were arranged in a randomised complete block design on moist gravel- or sand-covered benches in a glasshouse maintained at approximately 15-24°C, and watered when necessary. Pots of unrooted cuttings were covered in polythene for the duration of experiments.

2.7 Determination of host range of *P. sydowniana* on selected HONS

It is important to determine the range of HONS species that are susceptible to infection by *P. sydowniana*, and to determine whether individual isolates of the pathogen are capable of infecting more than one species of HONS.

The host range of four isolates of *P. sydowniana* (P7, P11, P16 and P22;

see Table 1 for details) on six species of HONS (*Erica cinerea*, *Calluna vulgaris*, *Chamaecyparis lawsoniana*, *Cupressocyparis leylandii* and two *Rhododendron* spp.) was determined in the glasshouse. Cuttings of each species were prepared as described in section 2.4 and inoculated as described in section 2.5. Disease assessments were made four weeks after striking as described in section 2.12.

2.8 Determination of cultivar susceptibility of selected HONS to *P. sydowniana*

The use of cultivars which show some degree of disease resistance can reduce the loss of plants to disease. In this series of experiments, the relative susceptibility of cultivars of selected HONS to isolates of *P. sydowniana* was examined.

2.8.1 *Calluna vulgaris*

Five cultivars of *C. vulgaris* (Iris van Leyen, John F. Letts, Kinlochruel, Silver Knight and White Star) were selected and their susceptibility to an isolate of *P. sydowniana* (P11; see Table 1 for details) was examined. The susceptibility of rooted and unrooted cuttings of each cultivar was also compared.

Rooted cuttings were prepared as described in section 2.4 and inoculated as described in section 2.5. Unrooted cuttings were prepared as described in section 2.4 and treatments applied as for rooted cuttings.

2.8.2 *Erica* spp.

Four cultivars of *Erica cinerea* (Purple Beauty, Violetta, Atrorubens

and Alba Major), one of *Erica vagans* (Lyonesse) and one of *Erica carnea* (Aurea) were examined for susceptibility to two isolates of *P. sydowniana*. Rooted cuttings were prepared as described in section 2.4 and inoculated as described in section 2.5.

2.8.3 *Rhododendron* spp.

Ten cultivars of *Rhododendron* spp. were examined for susceptibility to *P. sydowniana*: (i) Brilliant (cv. Elizabeth x *Ledum glandulosum*); (ii) Curlew (*R. ludlowii* x *R. fletcherianum*); (iii) Impeditum indigo; (iv) Lavendula ([*R. russatum* x *R. saluense*] x *R. rubiginosum*); (v) Campyloginum myrtleoides; (vi) Sarled (*R. sargentianum* x *R. trichostomum*); (vii) Shamrock (*R. keiskei* dwarf form x *R. hanceanum* cv. Nanum); (viii) Charmaine (cvs. Charm x Mayday); (ix) Bluett (*R. augustinii* ssp. *augustinii* cv. Lackamas Blue x *R. impeditum*) and, (x) Ginny Gee (*R. keiskei* cv. Yaku Fairy x *R. racemosum* Forrest 19404). Two isolates of the pathogen (P11, P16) were used to inoculate the rooted cuttings. Rooted cuttings of *Rhododendron* spp. were prepared as described in section 2.4 and inoculated as described in section 2.5.

For all cultivar susceptibility experiments, disease assessments were made four weeks after striking (or planting) as described in section 2.12.

2.9 Effect of water splash on transmission of spores of *P. sydowniana*

Spores of several plant pathogenic fungi can be efficiently dispersed in splashes of water droplets (Gregory, Guthrie and Bunce, 1959). Little is known about the dispersal abilities of conidia of *P. sydowniana* and the distances to which they may be dispersed in water droplets. Such information is likely to be

useful in designing strategies for the cultural control of the pathogen.

A spore suspension of *P. sydowniana* (20 ml; final concentration $1-3 \times 10^6$ spores ml^{-1} ; isolate P11) was prepared as described in section 2.1.2 and placed in a 9 cm diam. Petri dish. The Petri dish was surrounded by five further dishes, containing PDAES, arranged in concentric circles at intervals of 10, 25 and 50 cm from the spore suspension. Lids of Petri dishes were removed prior to dropping sterile distilled water, from a sterile 1 ml glass pipette from heights of 50 and 100 cm, onto the spore suspension. Ten drops of water were dropped from the pipette, and the method was repeated with three sets of Petri dishes. Petri dishes were incubated at 22°C for 3 d. After this period, numbers of colonies of *P. sydowniana* on each plate were counted.

The method was repeated using open Petri dishes containing *Rhododendron* leaves infected with *P. sydowniana* instead of spore suspension.

2.10 Effect of selected fungicides on the mycelial growth and conidial germination of *P. sydowniana in vitro*

Seven of the most commonly used fungicides were tested for their ability to inhibit mycelial growth and conidial germination of two isolates of *P. sydowniana* (P11, P16; see Table 1 for details). The fungicides tested were: (i) Fisons Basilex (50% w/w toclofos-methyl; Levington Horticulture, Ipswich, Suffolk); (ii) Bavistin DF (50% w/w carbendazim; BASF, Cheadle Hulme, Cheshire); (iii) Bravo 500 (40.4% w/w chlorothalonil; BASF); (iv) Dithane 945 (80% w/w mancozeb; PBI, Waltham Cross, Herts.); (v) Nimrod T (62.5 g l^{-1} bupirimate and 62.5 g l^{-1} triforine; Zeneca, Fernhurst, Haslemere, Suffolk);

(vi) Fisons Octave (50% w/w prochloraz manganese; Levington Horticulture) and, (vii) Rovral Flo (25% w/v iprodione; Rhône Poulenc Agriculture Ltd., Ongar, Essex).

Stock suspensions of fungicides were prepared and appropriate dilutions made to give a range of fungicide concentrations. Appropriate amounts were added to sterile molten PDA, cooled to 50°C. Amended PDA was then poured into 9 cm diam. Petri dishes. Three replicate plates for each fungicide concentration and each isolate were prepared. Plates were inoculated centrally with a 3 mm diam. mycelial disc cut from the periphery of a 3-5-d-old colony on PDA. Plates were sealed in plastic bags and radial growth rates were determined at 20°C as described in section 2.1.3, in the dark, and IG₅₀ values were calculated. To calculate the IG₅₀ value (concentration giving 50% linear growth inhibition) for each fungicide-isolate combination, linear regression equations were fitted to logarithmic probability data of fungicide concentration and percentage growth inhibition.

To examine the effect of the fungicides on germination of conidia, a second batch of amended PDA was prepared as before. Five replicate plates were prepared for each fungicide concentration and isolate. A spore suspension ($1-3 \times 10^6$ spores ml⁻¹) was prepared as described in section 2.1.2, and 0.5 ml was spread evenly over the surface of the amended PDA. Plates were placed in sealed plastic bags and incubated at 20°C in the dark. After 12 h, plates were flooded with lactofuchsin and at least 100 conidia on each plate were assessed for germination as described in section 2.3.1. Conidia with germ-tubes of a length greater than the breadth of the conidia were considered

to have germinated.

2.11 Sensitivity of *P. sydowniana* to selected fungicides on HONS in the glasshouse

Very few fungicides have been evaluated for the control of *P. sydowniana* on HONS. Therefore, a range of fungicides were applied as foliar sprays to selected cuttings of HONS pre- and post- inoculation to determine whether they had preventative or curative activity.

2.11.1 *Rhododendron* sp. (azalea)

Two trials were conducted to examine the ability of seven fungicides to control *P. sydowniana* (isolate P16) on unrooted *Rhododendron* sp. cuttings. The fungicides treatments were: (i) Basilex (50% w/w toclofos-methyl, 10g l⁻¹; Fisons); (ii) Bavistin DF (50% w/w carbendazim, 5.5g l⁻¹; BASF); (iii) Bravo 500 (40.4% w/w chlorothalonil, 10g l⁻¹; BASF); (iv) Dithane 945 (80% w/w mancozeb, 10g l⁻¹; PBI); (v) Nimrod T (62.5 g l⁻¹ bupirimate and 62.5 g l⁻¹ triforine, 100g l⁻¹; Zeneca); (vi) Octave (50% w/w prochloraz manganese, 50 g l⁻¹; Levington Horticulture) and, (vii) Rovral Flo (25% w/v iprodione, 10 g l⁻¹; Rhône Poulenc). Fungicide treatments were applied pre-inoculation in one trial and post-inoculation in a second. Fungicide sprays were applied at 200 lha⁻¹ using a Mardrive Precision Laboratory Sprayer (Marine Engineering Company, Stockport) at 3.0 bar with a medium spray. Cuttings were sprayed from over head to achieve good leaf cover.

Cuttings of *Rhododendron* were prepared and inoculated as described

in section 2.4. For the pre-inoculation trial, fungicides were applied 3h before inoculation. For the post-inoculation trial, fungicides were applied 3d after inoculation. Controls for both trials consisted of inoculated and uninoculated cuttings without fungicide treatments. There were five replicates of each treatment for each of the two trials and pots of cuttings were placed on wet capillary matting on plastic trays. Disease assessments were made four weeks after striking as described in section 2.12.

2.11.2 *Chamaecyparis lawsoniana*

Two trials were also conducted as described in section 2.11.1 above to examine the ability of the pre- and post-inoculation fungicide treatments to control *P. sydowiana* (isolate P16) on unrooted *C. lawsoniana* cuttings.

2.11.3 *Calluna vulgaris*

Two trials were also conducted as described in section 2.11.1 above to examine the ability of the pre- and post-inoculation fungicide treatments to control *P. sydowiana* (isolate P16) on unrooted *C. vulgaris* cuttings.

2.12 Disease assessments

Four weeks after striking (or planting), counts were made of numbers of cuttings infected by *P. sydowiana*. To confirm infection by *P. sydowiana*, small pieces of diseased tissue were cultured on plates of PDAES at 20-22°C for 3-5 d. Plates were then observed for typical colony morphology of *P. sydowiana*.

Plants were also assessed for foliar browning, using a scale of 0 - 6, where 0 = no foliar browning, 1 = trace -5% of leaf with foliar browning, 2 =

6-10%, 3 = 11-25%, 4 = 26-50%, 5 = 51 - 75%, and 6 = >76%. A foliar browning severity index was calculated for each replicate:

$$\text{Foliar browning severity index} = \frac{\text{Sum of individual ratings}}{\text{Number of plants assessed}} \times \frac{100}{6}$$

2.13 Statistical analysis

For most experiments, data were assessed using an analysis of variance (ANOVA). When necessary, percentage data were transformed prior to analysis by an angular transformation. Significant differences between means are indicated by 2 x SED (standard error of the difference between treatment means). Data from some experiments are presented as means \pm standard errors (SE) of counts or percentages.

3. RESULTS

3.1 Collection, identification and pathogenicity of *Pestalotiopsis* spp.

3.1.1 Collection and identification of *Pestalotiopsis* spp.

Nineteen isolates of *Pestalotiopsis* were collected between 1991 and 1994. All were collected from a range of diseased hardy ornamentals either in nurseries or in private gardens in England, Scotland and N. Ireland (Table 1). Isolates were obtained from the roots, stem or foliage of infected plants. All isolates were identified as *P. sydowniana*. Representative cultures of some of these have been lodged in the IMI culture collection for future reference.

3.1.2 Colony morphology, spore measurements and radial growth rates

All isolates of *P. sydowniana* collected had the same basic morphological characteristics on PDA. It was possible to separate the isolates into three groups based on their morphological characteristics (Table 2). The majority of isolates (Group 1) had pale buff mycelia on the top of the colony and either pale buff or pale luteous mycelia from the reverse. Distribution, size and numbers of acervuli varied between isolates. Generally, acervuli were black and scattered over the surface of the colony, although in some cases they were concentrated in the centre and at the periphery (Plate 4). The second group of isolates had mycelia which was primarily pale luteous from the top, and luteous or pale luteous from the reverse. Distribution and numbers of acervuli varied between isolates but generally acervuli were sparse on these cultures. The third group of isolates consisted of miscellaneous cultures which could not be assigned to either of the first two groups. These isolates had significantly

Plate 4. Growth of isolates of *Pestalotiopsis sydowiana* on PDA

Plate 5. Growth of isolates of *Pestalotiopsis sydowiana* on selected agar media

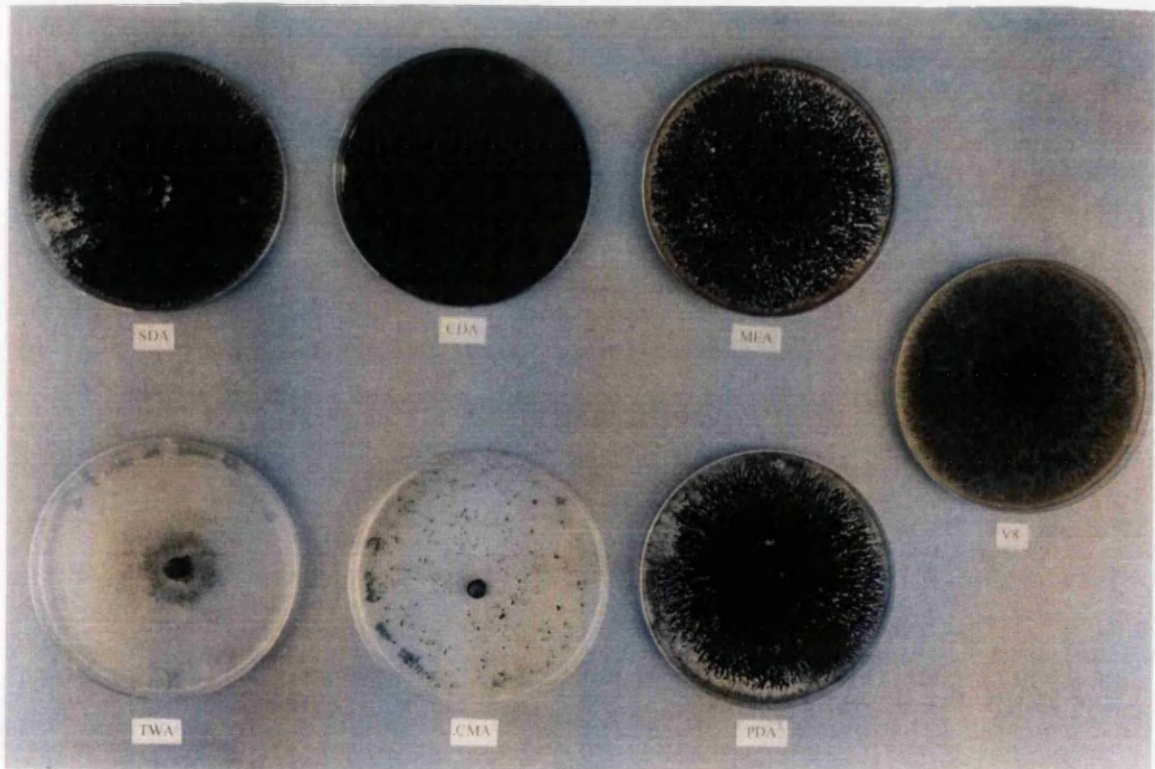
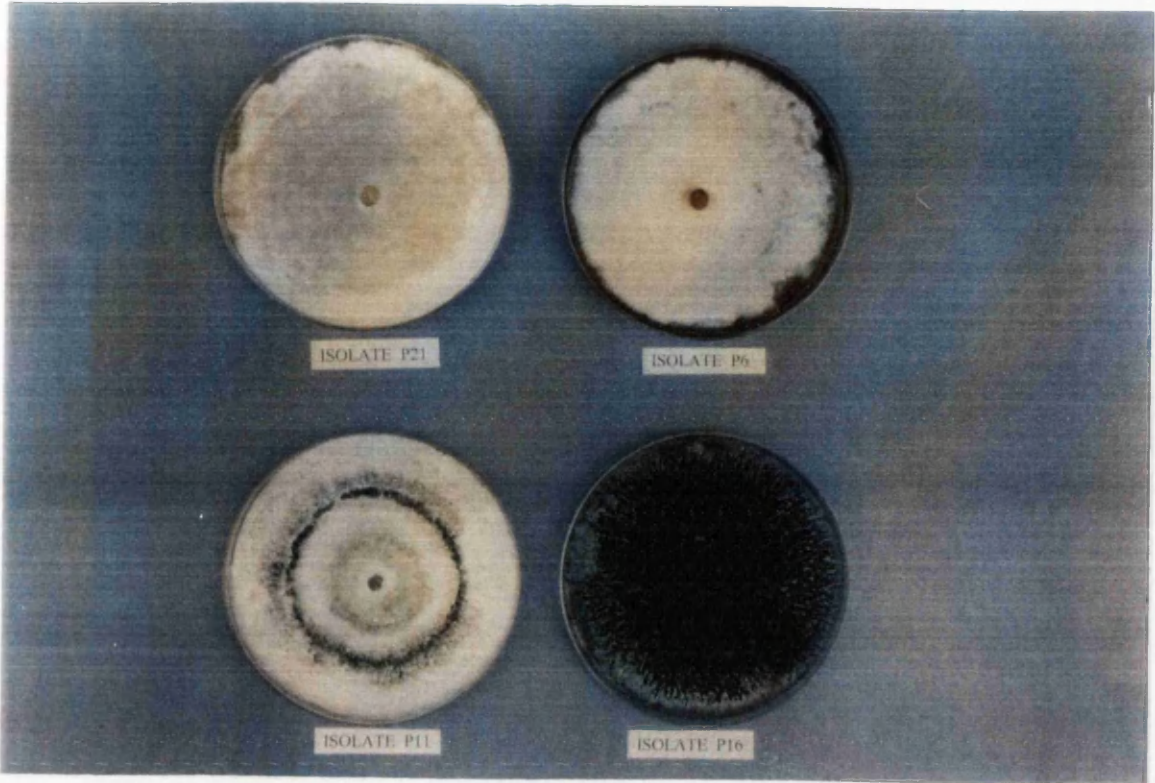


Table 1. Origin of isolates of *Pestalotiopsis sydowniana*

Isolate	Date	Collector	Host	Origin	IMI Number
P6	-	AML ¹	<i>Calluna vulgaris</i> cv. Rosalind	-	358103
P7	1991	- ⁴	Conifer	Lanark, Strathclyde, Scotland	356304
P9	1991	-	<i>Rhododendron</i>	Braevallich, Argyll, Scotland	356305
P10	1992	AML	<i>Rhododendron</i> cv. Princess Anne	Braevallich, Argyll, Scotland	-
P11	1992	AML	<i>Erica</i> sp. cv. Ruby Gem	Maghera, Co. Derry, N. Ireland	356306
P14	1991	-	<i>Rhododendron</i>	Perth, Tayside, Scotland	356308
P16	1992	AML	<i>Rhododendron</i>	Taynuilt, Argyll, Scotland	356309
P17	1992	AML	<i>Erica carnea</i> cv. Kramers Red	Lochgilphead, Argyll, Scotland	358104
P21	-	LCD ²	<i>Rhododendron</i> cv. Coral Velvet (stem)	-	356311
P22	1992	KEH ³	<i>Rhododendron</i> cv. Golden Torch	Dalmally, Argyll, Scotland	356312
P23	1993	KEH	<i>Rhododendron</i> cv. Hachmanns Rosita (roots)	Droitwich, Worcestershire, England	358105
P25	1993	KEH	<i>Rhododendron</i> cv. Cowslip (roots)	Braevallich, Argyll, Scotland	358106
P27	1993	KEH	<i>Dimorphantha</i> (foliage)	Edinburgh, Lothian, Scotland	-
P29	1993	KEH	<i>Juniperus</i> sp. (foliage)	Edinburgh, Lothian, Scotland	-
P32	1993	KEH	<i>Euonymus fortunei</i> cv. Emerald Gaiety (foliage)	Droitwich, Worcestershire, England	-
P33	1993	KEH	<i>Pieris</i> sp. (stem)	Barguilean, Argyll, Scotland	-
P34	1994	KEH	Azalea cv. Hinodegiri (roots)	Chobham, Surrey, England	-
P36a	1994	KEH	<i>Erica arborea</i> (roots)	Lochgilphead, Argyll, Scotland	-
P36b	1994	KEH	<i>Erica arborea</i> (stem)	Lochgilphead, Argyll, Scotland	-

¹ A M Litterick

² L C Dempster

³ K E Hopkins

⁴ Not known

Table 2. Colony morphology of isolates of *Pestalotiopsis sydowiana* grown on PDA for 3 weeks at 22°C

Isolate	Colony morphology
Group 1	
P6	Mycelia very pale buff from top, pale luteous from reverse. Sparse, scattered, black acervuli visible from top and reverse.
P7ai	Mycelia pale buff from top, pale luteous from reverse. Large, but sparse, black acervuli visible from top and reverse.
P10	Mycelia very pale buff from top and reverse. Many greenish-black acervuli visible from top and reverse.
P11	Mycelia pale buff from top, pale luteous from reverse. Many black acervuli visible from reverse.
P14	Mycelia very pale buff from top and reverse. Many black acervuli visible from top and reverse.
P17	Mycelia pale buff from top and reverse. Many black acervuli visible from top and reverse concentrated on periphery and centre.
P23	Mycelia pale buff from top, luteous from reverse. Many black mainly visible from reverse.
P25c	Mycelia pale buff from top, pale luteous from reverse. Many acervuli, black, visible from top and reverse.
P33	Mycelia pale buff from top, pale luteous from reverse. Many black acervuli visible from top and reverse.
P36a	Mycelia very pale buff from top, pale luteous from reverse. Acervuli large, black, scattered visible from top and reverse.
P36b	Mycelia pale luteous from top, luteous from reverse. Very sparse black acervuli visible from top and reverse.
Group 2	
P9	Mycelia pale luteous from top, luteous from reverse. Very sparse black acervuli visible from top and reverse.
P16	Mycelia a mixture of pale luteous and buff patches from top, from reverse pale luteous. Many greenish-black acervuli visible from reverse.
P21	Mycelia pale luteous from top, luteous from reverse. Acervuli black and very sparse, visible from top and reverse.
P27a	Mycelia pale buff with pale luteous patches from top, pale luteous with luteous patches from reverse. Acervuli rare, black, visible from top and reverse.
Group 3	
P22	Mycelia sparse, very pale buff from top and reverse. Many black acervuli, visible from top and reverse.
P29	Mycelia pale salmon from top and reverse with few pale buff patches. Many black acervuli in concentric circles visible from top and reverse.
P32a	Mycelia pale buff with salmon patches from top and reverse. Acervuli rare, scattered, black, visible from top and reverse.

different mycelia: isolate P22 had very sparse buff mycelia and consisted largely of acervuli; isolates P29 and P32a had pale salmon coloured mycelia or patches of pale salmon coloured mycelia. P29 also differed because it produced acervuli in concentric circles.

Measurements of conidia from cultures revealed few differences between the isolates (Table 3). The range of conidial length and width was 15.2-30.4 μm (mean 22.8 μm) and 5.7-9.5 μm (mean 6.7 μm), respectively. All isolates had 5-celled conidia. Basal appendage length was 2.5-7.0 μm (mean 4.4 μm). Numbers of apical appendages ranged from one to five, with three being the most common. Apical appendage length was 15.5-39.4 μm (mean 22.8 μm). Radial growth rates of isolates on PDA at 20-22°C varied between 1.1 and 7.4 mm per day, with an overall mean of 5.4 mm per day.

3.1.3 Pathogenicity assays

Leaves of the original host species of each isolate of *P. sydowniana* inoculated with mycelium or spore suspension showed symptoms of disease 14 d after inoculation. Disease symptoms included browning of leaf tissue and the presence of black acervuli. Pieces (0.5 mm²) of the diseased tissue placed on Petri dishes containing PDAES produced colonies with morphological characteristics typical of *P. sydowniana*.

3.2 Effect of agar media on mycelial growth and acervuli production of *P. sydowniana*

All four isolates of *P. sydowniana* grew on the seven different agars (Table 4). Radial growth rates of all four isolates ranged between 4.0-8.0 mm

Table 3. Spore measurements and radial growth rates of isolates of *Pestalotiopsis sydowiana* on PDA

Isolate	% plate covered with acervoli	Conidia size						Length of median cells (µm)			Appendage length (µm)			No. of Apical appendages	Radial growth rate ¹ (mm per day)
		Length (µm)		Width (µm)		Range		Mean	Max.	Min.	Apical	Basal	Range		
		Mean	Min.	Max.	Min.	Max.	Min.								
		Range	Max.	Min.	Range	Max.	Min.								
P6	1-2	24.8	28.5	20.9	6.7	7.6	5.7	17.9	20.9	15.2	22.6	3.8	2-3	(2)	5.6±0.80 ²
P7	5-10	23.8	26.6	20.9	6.5	7.6	5.7	17.2	19.0	15.2	39.4	7.0	1-3	(2)	1.1±1.22
P9	5-10	23.4	26.6	19.0	7.0	9.5	5.7	16.8	20.9	15.2	26.7	4.9	2-3	(3)	3
P10	80-90	23.4	26.6	19.0	6.5	7.6	5.7	16.9	19.0	15.2	24.5	4.1	2-4	(3)	5.6±0.77
P11	75-85	23.0	26.6	19.0	6.7	9.5	5.7	17.0	20.9	13.3	22.8	4.0	2-4	(3)	6.3±1.22
P14	80-90	22.2	24.7	19.0	6.8	7.6	5.7	15.7	17.1	13.3	23.6	2.8	2-4	(3)	6.1±1.33
P16	40-55	22.5	26.6	19.0	6.4	7.6	5.7	16.2	20.9	15.2	20.5	4.1	2-4	(3)	6.0±0.93
P17	65-75	23.5	30.4	19.0	6.3	7.6	5.7	16.7	22.8	15.2	23.1	4.4	3-4	(3)	-
P21	1-10	22.6	26.6	19.0	6.5	7.6	5.7	16.4	19.0	13.3	24.2	5.1	2-3	(2)	7.0±0.21
P22	80-90	23.0	28.5	19.0	7.0	7.6	5.7	16.0	19.0	11.4	19.3	4.1	2-4	(3)	7.4±0.57
P23	40-60	21.9	24.7	19.0	6.4	7.6	5.7	16.2	19.0	15.2	18.1	5.3	2-4	(3)	5.1±0.68
P25	55-70	22.6	24.7	20.9	6.1	7.6	5.7	16.6	17.1	15.2	18.9	4.0	2-4	(3)	7.0±0.42
P27	20-30	22.2	26.6	19.0	6.4	7.6	5.7	16.3	19.0	13.3	19.0	2.5	2-4	(3)	2.5±2.54
P29	75-85	22.4	26.6	19.0	6.5	7.6	5.7	14.3	19.0	13.3	15.5	4.3	2-4	(3)	4.5±0.46
P32	1-5	24.0	28.5	20.9	7.0	7.6	5.7	17.0	20.9	15.2	24.3	4.4	2-4	(3)	5.5±3.24
P33	40-50	22.4	24.7	20.9	7.1	7.6	5.7	16.6	19.0	15.2	22.4	3.2	2-3	(3)	4.3±1.66
P36a	7-10	23.6	26.6	20.9	7.3	9.5	5.7	16.7	19.0	15.2	24.4	5.0	2-4	(3)	-
P36b	10-15	23.8	26.6	20.9	7.0	7.6	5.7	16.8	19.0	15.2	23.6	5.8	2-4	(3)	-

¹ Growth rates were determined on potato dextrose (PDA) at 20-22°C

² Values are means ± sample standard error, based on the measurement of five replicate cultures

³ Not determined

Table 4. Effect of different media on hyphal radial growth rate of four isolates of *Pestalotiopsis sydowiana* at 20-22°C

Media	Radial growth rate (mm/day)			
	Isolate			
	P6	P11	P16	P21
PDA	5.6 ¹	5.6	7.6	7.4
MEA	5.2	7.6	6.8	6.4
CMA	4.8	4.0	5.4	6.4
CDA	5.4	5.8	5.2	7.4
SDA	6.4	8.0	6.6	7.6
V8	6.6	7.2	7.0	7.2
TWA	4.2	5.4	4.4	5.8
SED ² (10 d.f.)	0.16			

¹ Values are means of hyphal radial growth rate measured between 3-6 d

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived by analysis of variance)

Table 5. Effect of different media on acervuli production by four isolates of *Pestalotiopsis sydowiana* at 20-22°C

Media	Percentage area of agar surface covered by acervuli			
	Isolate			
	P6	P11	P16	P21
PDA	1-2 ¹	75-85	40-55	1-10
MEA	0	65-75	90	0
CMA	0	0	30-35	0
CDA	0-5	95	95	0-2
SDA	0	50-60	95	0-15
V8	0-2	10-25	70-75	0
TWA	0	0	10	0

¹ Values are based on the visual assessment of 10 d-old colonies growing on agar in 9 cm diam. Petri dishes.

per day, with relatively small but significant differences between isolates and media. Radial growth rates ranged between 5.2-8.0 mm per day on SDA, V8, CDA and PDA. Radial growth rates tended to be lower on CMA (4.0-6.4 mm per day) and TWA (4.2-5.8 mm per day) compared with the other five media.

There were differences in acervuli production between isolates and media (Table 5; Plate 5). Of the four isolates tested, only P16 produced acervuli on all seven media. Acervuli production was greatest on SDA, CDA and MEA (> 90% of the agar surface was covered); production was lower on V8, with further decreases in the order PDA, CMA and TWA. Isolate P11 produced high numbers of acervuli on CDA, PDA, MEA and SDA (> 50% of the agar surface was covered); production was lower on V8 and absent on TWA. Acervuli production on all media by P6 and P21 was either very low or absent.

3.3 Effects of environmental factors affecting growth of *P. sydowiana*

3.3.1 Effect of temperature on mycelial growth and conidial germination of *P. sydowiana*

Temperature significantly affected the hyphal radial growth rates of all three isolates of *P. sydowiana* (Table 6). P16 grew over the range 5-30°C with a maximum radial growth rate of 5.4 mm per day at 25°C. Both P7 and P21 grew between 15 and 30°C, although the optimum temperature for P7 was 20°C (4.5 mm per day) compared with 20-25°C (5.1 mm per day) for P21. Growth of both P7 and P21 was inhibited at 5°C.

Temperature significantly affected the conidial germination of all three

Table 6. Effect of temperature on hyphal radial growth rate and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA

Temperature (°C)	Radial growth rate (mm/day)			Conidial germination (% at 12 h)		
	Isolate			Isolate		
	P7	P16	P21	P7	P16	P21
5	0	0.5 ¹	0	0	0	0
15	3.2	3.3	3.1	0	0	0
20	4.5	4.6	5.1	86.2 (68.4) ²	96.4 (79.3)	91.4 (73.1)
25	4.0	5.4	5.1	86.0 (68.2)	96.0 (79.4)	96.4 (79.6)
30	0.6	0.3	2.8	98.4 (83.6)	98.4 (83.8)	98.8 (85.2)
SED ³ (56 d.f.)	0.20			(1.93)		

¹ Values are means of hyphal radial growth rate, measured between 3-6 d on five replicates

² Values in parentheses are angular transformations of percentage data based on five replicates

³ Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

Table 7. Effect of pH on hyphal radial growth rate and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA

pH	Radial growth rate (mm/day)			Conidial germination (% at 12 h)		
	Isolate			Isolate		
	P11	P16	P21	P11	P16	P21
2.6	4.1 ¹	3.7	4.6	97.2 (80.7) ²	98.2 (83.4)	94.0 (75.9)
3.8	5.4	3.8	1.4	96.7 (81.2)	98.2 (83.6)	97.2 (81.8)
5	4.9	4.5	4.7	98.7 (84.5)	99.0 (85.1)	97.2 (80.7)
5.5 (control)	6.5	5.6	6.2	99.2 (86.5)	99.2 (85.7)	98.7 (84.5)
6.3	3.6	2.6	2.2	97.0 (80.3)	98.2 (83.9)	98.2 (82.6)
7.6	3.5	3.0	2.9	98.7 (85.5)	98.5 (84.0)	98.2 (83.6)
SED ³ (60 d.f.)	0.30			(2.82)		

¹ Values are means of hyphal radial growth rate, measured between 3-6 d on five replicates

² Values in parentheses are angular transformations of percentage data based on five replicates

³ Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

isolates of *P. sydowiana* after 12h incubation (Table 6). Conidial germination of all three isolates was inhibited at 5 and 15°C. Conidia of all isolates germinated over 20-25°C (86-99% after 12h incubation), with a maximum germination of 98-99% at 30°C.

3.3.2 Effect of pH on mycelial growth and conidial germination of *P. sydowiana*

Medium pH had significant effects on the hyphal radial growth rates of all three isolates of *P. sydowiana* (Table 7). All three isolates grew on PDA over the range pH 2.6-7.6, while the optimum pH for hyphal radial growth was pH 5.5 (unbuffered control).

There were no significant differences in the effect of pH on conidial germination of conidia of all three isolates of *P. sydowiana* (Table 7). Conidial germination after 12h incubation was greater than 94% over pH 2.6-7.6.

3.3.3 Effect of water potential on mycelial growth and conidial germination of *P. sydowiana*

Osmotic potential

Sodium chloride

The fastest radial growth rates of isolates of *P. sydowiana* on PDA osmotically adjusted with NaCl occurred at -1.2 MPa for isolates P16 and P21, and at -0.3-2.1 MPa for isolate P11 (Table 8). Below these optima osmotic potentials for growth of each isolate, radial growth rates declined markedly with decreasing osmotic potential. Complete inhibition of growth did not occur

Table 8. Hyphal radial growth rates and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA adjusted to various osmotic potentials with NaCl at 20°C

Osmotic potential -MPa	Radial growth rate (mm/day)			Conidial germination (% at 12 h)		
	Isolate			Isolate		
	P11	P16	P21	P11	P16	P21
0.3	5.7 ¹	4.9	5.3	97.8 (81.7) ²	96.4 (79.2)	88.8 (70.5)
0.8	5.9	5.4	5.3	97.2 (80.4)	97.0 (80.4)	77.0 (61.4)
1.2	4.9	6.2	6.9	91.8 (73.5)	96.9 (79.5)	31.4 (34.0)
2.1	5.6	5.5	5.9	85.8 (68.0)	97.8 (81.6)	31.2 (33.0)
4.9	2.1	5.5	4.6	42.0 (40.4)	35.8 (36.7)	32.6 (34.7)
6.5	1.6	3.4	4.1	15.0 (22.5)	17.0 (24.3)	19.6 (26.7)
8.3	0.6	2.0	1.8	0	3.8 (10.9)	0
9.9	0.7	1.4	1.1	0	0	0
SED ³ (92 d.f.)	0.21			(2.13)		

¹ Values are means of hyphal radial growth rate, measured between 3-5 d on five replicates

² Values in parentheses are angular transformations of percentage data based on five replicates

³ Significant differences between means are given by 2 x SED (standard error of the difference between means derived by analysis of variance)

Table 9. Hyphal radial growth rates and conidial germination of three isolates of *Pestalotiopsis sydowiana* on PDA adjusted to various osmotic potentials with glycerol at 20°C

Osmotic potential -MPa	Radial growth rate (mm/day)			Conidial germination (% at 12 h)		
	Isolate			Isolate		
	P11	P16	P21	P11	P16	P21
0.3	4.9 ¹	5.5	5.0	41.2 (39.9) ²	97.8 (82.5)	95.8 (78.6)
0.8	6.2	6.2	5.7	40.0 (39.2)	99.2 (86.1)	94.2 (76.1)
1.2	6.0	6.7	5.6	31.4 (34.0)	99.0 (85.7)	96.0 (78.6)
2.1	6.0	5.4	6.0	29.2 (32.6)	98.2 (83.4)	94.4 (76.4)
4.8	4.1	4.0	4.1	11.4 (19.6)	96.8 (79.8)	91.0 (72.6)
6.5	3.5	3.0	2.0	6.4 (14.6)	94.2 (76.4)	40.2 (39.3)
7.8	1.7	2.6	2.0	1.8 (6.5)	65.0 (53.7)	4.2 (11.6)
9.1	1.0	2.1	1.6	0	7.4 (15.6)	1.0 (4.3)
10.3	1.3	1.3	1.0	0	0	0
SED ³ (102 d.f.)	0.24			(1.83)		

¹ Values are means of hyphal radial growth rate, measured between 3-5 d on five replicates

² Values in parentheses are angular transformations of percentage data based on five replicates

³ Significant differences between means are given by 2 x SED (standard error of the difference between means derived by analysis of variance)

within the range of osmotic potentials (-0.3 to -9.9 MPa) tested.

The germination of conidia after 12h was maximum on PDA osmotically adjusted over the range -0.3 to -0.8 MPa for isolates P11 and P21, and -0.3 to -2.1 MPa for P16. Below these optima osmotic potentials, conidial germination of all isolates declined markedly with decreasing osmotic potential. Conidial germination of isolates P11 and P21 was completely inhibited at -8.3 MPa, whereas germination of P16 was inhibited at -9.9 MPa.

Glycerol

The fastest radial growth rates of all three isolates of *P. sydowiana* on PDA osmotically adjusted with glycerol occurred at -0.8 to -2.1 MPa (Table 9). As osmotic potential decreased over the range -2.1 to -10.3 MPa, radial growth rates became significantly slower. Complete inhibition of growth did not occur within the range of osmotic potentials tested. There was little difference in the response of the individual isolates of *P. sydowiana* to decreasing osmotic potential.

Conidial germination after 12 h was maximum at -0.3 to -0.8 MPa for isolate P11 and -0.3 to -4.8 MPa for isolates P16 and P21 (Table 9). Below these optima osmotic potentials, conidial germination of all isolates declined markedly with decreasing osmotic potential. Conidial germination of P11 was completely inhibited at -9.1 MPa, whereas germination of isolates P11 and P16 was inhibited at a lower osmotic potential of -10.3 MPa.

Matric potential

The fastest radial growth rates on matrically adjusted PDA occurred at -2.1 MPa for isolate P11, and on unamended PDA (-0.3 MPa) for isolates P11 and P21 (Table 10). With further decreases in matric potential, radial growth rates declined markedly.

3.4 Determination of host range of *P. sydowniana* on selected HONS

The four isolates of *P. sydowniana* (P7, P11, P16 and P21) were able to infect all of the species of HONS (*Erica cinerea*, *Calluna vulgaris*, *Chamaecyparis lawsoniana*, *Cupressocyparis leylandii* and two *Rhododendron* species) artificially inoculated. Symptoms of infection occurred within 28 d after inoculation. These included the browning of leaves and stems, and the presence of black acervuli. Death of cuttings commonly occurred following infection. Fungal colonies produced on PDAES from diseased segments of plant tissue were typical of *P. sydowniana*.

3.5 Determination of cultivar susceptibility of selected HONS to *P. sydowniana*

3.5.1 *Calluna vulgaris*

There were very few significant differences between the percentage of cuttings infected and the levels of foliar browning between rooted and unrooted cuttings of different cultivars of *C. vulgaris* (Table 11). Percentage of rooted cuttings infected was significantly lower for John F. Letts compared with rooted cuttings of Iris van Leyen, Kinlochruel and Silver Knight. However,

Table 10. Hyphal radial growth rates of three isolates of *Pestalotiopsis sydowiana* on PDA adjusted to various matric potentials with polyethylene glycol 6000 at 20°C

Matric potential	Radial growth rate (mm/day)		
	Isolate		
	P11	P16	P21
-MPa			
0.3	5.7 ¹	5.7	5.6
0.8	5.1	4.0	3.4
1.3	5.5	3.6	3.5
2.1	6.4	2.9	2.4
4.8	1.0	1.4	1.2
6.5	0	0	0
7.8	0	0	0
9.1	0	0	0
SED ² (87 d.f.)	0.30		

¹ Values are means of hyphal radial growth rate, measured between 3-6 on four replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived by analysis of variance)

Table 11. Susceptibility of rooted and unrooted cuttings of cultivars of *Calluna vulgaris* to infection by *Pestalotiopsis sydowniana*³

Cultivar	% Cuttings infected		Foliar browning severity index	
	Rooted	Unrooted	Rooted	Unrooted
Iris van Leyen	75.0 (64.4) ¹	75.0 (68.8)	25.0 (29.7)	18.7 (24.8)
John F. Letts	8.3 (11.2)	75.0 (68.8)	1.4 (5.1)	39.6 (38.6)
Kinlochruel	66.7 (59.4)	50.0 (45.0)	11.1 (19.2)	25.0 (24.4)
Silver Knight	66.7 (59.4)	66.7 (59.7)	19.4 (25.4)	25.0 (25.6)
White Star	25.0 (25.6)	100.0 (88.2)	4.2 (10.4)	79.2 (63.9)
SED ² (18 d.f.)	(19.83)		(8.43)	

¹ Each value is the mean of three replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived by analysis of variance)

³ Assessments were made 8 weeks after inoculation

Table 12. Susceptibility of rooted cuttings of cultivars of *Erica* spp. to two isolates of *Pestalotiopsis sydowniana*³

Cultivar	% Cuttings infected		Foliar browning severity index	
	P11	P16	P11	P16
Purple Beauty	25.0 (24.7) ¹	45.0 (39.4)	4.2 (8.3)	11.7 (17.3)
Violetta	45.0 (39.4)	30.0 (30.4)	11.7 (17.3)	19.2 (19.2)
Atrorubens	25.0 (24.7)	45.0 (39.4)	10.8 (13.6)	21.6 (24.3)
Alba Major	70.0 (59.7)	55.0 (50.7)	15.8 (22.7)	15.0 (19.9)
Lyonesse	85.0 (74.0)	40.0 (36.4)	16.7 (23.7)	6.7 (10.7)
Aurea	0 (1.8)	10.0 (13.1)	0 (1.8)	8.3 (11.9)
SED ² (44 d.f.)	(17.72)		(8.49)	

¹ Each value is the mean of five replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived by analysis of variance)

³ Assessments were made 3 weeks after inoculation

there was no significant difference in the level of foliar browning between the cultivars. Of the five cultivars tested, only rooted cuttings of John F. Letts and White Star were less susceptible to infection compared with the corresponding unrooted cuttings of the same cultivars.

3.5.2 *Erica* spp.

There were very few significant differences between the percentage of cuttings infected and the levels of foliar browning between rooted cuttings of different cultivars of *Erica* spp. (Table 12). Percentage of rooted cuttings infected by isolate P11 and levels of foliar browning were significantly lower for Aurea compared with Alba Major, Lyonesse and Violetta. When cuttings were inoculated with P16, however, percentage infection and levels of foliar browning were significantly lower for Aurea compared with only Alba Major. There were no significant differences between any of the other cultivars. In general, there was no significant difference in the pathogenicity of the two isolates on each cultivar tested.

3.5.3 *Rhododendron* spp.

There were some significant differences between the percentage of cuttings infected and the levels of foliar browning between rooted cuttings of different cultivars of *Rhododendron* spp. (Table 13). Cultivars Curlew and Campyloginum myrtleoides were the most susceptible to infection, whereas cvs. Impeditum indigo and Charmaine showed no signs of infection to either isolate of *P. sydowniana*. There were also some differences in the levels of foliar

Table 13. Susceptibility of rooted cuttings of cultivars *Rhododendron* spp. to two isolates of *Pestalotiopsis sydowiana*

Cultivar	% cuttings infected		Foliar browning severity index	
	P11	P16	P11	P16
Brilliant	31.2 ¹ (30.5) ²	12.5 (12.6)	8.3 (14.4)	3.1 (6.5)
Curlew	50.0 (45.0)	75.0 (66.6)	14.6 (17.3)	27.1 (28.1)
Impeditum indigo	0	0	0	0
Lavendula	37.5 (34.2)	31.2 (30.5)	6.2 (11.1)	5.2 (9.8)
Campyloginum myrtleoides	75.0 (66.6)	68.7 (59.6)	14.6 (20.0)	12.5 (18.5)
Sarled	6.2 (8.9)	6.2 (8.9)	1.0 (4.3)	3.1 (6.5)
Shamrock	18.7 (16.4)	12.5 (12.6)	3.1 (6.5)	2.1 (5.6)
Charmaine	0	0	0	0
Bluett	56.2 (52.1)	0	9.4 (15.4)	0
Ginny Gee	12.5 (15.9)	12.5 (12.3)	2.1 (6.8)	2.1 (5.6)
SED ³ (57 d.f.)	(17.60)		(5.80)	

¹ Each value is the mean of five replicate values

² Values in parentheses are angular transformations of percentage data based on five replicates

³ Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

Table 14. Transmission of spores of *Pestalotiopsis sydowiana* by water splash from spore suspension and infected *Rhododendron* foliage

Distance from source	Number of colonies of <i>Pestalotiopsis sydowiana</i>			
	Spore suspension		Infected <i>Rhododendron</i> foliage	
	Height of water droplets			
	50 cm	100 cm	50 cm	100 cm
10 cm	>200	>200	>200	>200
25 cm	>200	>200	5.8±0.91 ¹	>200
50 cm	0.46±0.50	2.8±1.44	2.0±3.46	1.86±0.90

¹ Values are means ± sample standard error, based on observation of 15 replicates 9 cm diam. Petri dishes

browning and percentage of cuttings infected between the two different isolates of *P. sydowniana*. For example, on the cv. Bluett, 56.2% of cuttings were infected by P11 whereas none were infected by P16.

3.6 Effect of water splash on the transmission of spores of *P. sydowniana*

Spores of *P. sydowniana* were capable of being transmitted over distances up to 50 cm from a source containing a spore suspension (Table 14). Numerous colonies of *P. sydowniana* were obtained from splash droplets at distances of 10 and 25 cm from the source irrespective of the height from which water was dropped. At a distance of 50 cm from the source, more colonies were produced from water dropped from a height of 100 cm than from 50 cm.

Similar results were obtained using infected *Rhododendron* foliage in place of a spore suspension. More colonies of *P. sydowniana* were present when water was dropped onto the infected foliage from a height of 100 cm than from 50 cm.

3.7 Effect of selected fungicides on the mycelial growth and conidial germination of *P. sydowniana in vitro*

The effect of selected fungicides on the mycelial growth of two isolates of *P. sydowniana* was determined by calculating IG₅₀ (concentration of active ingredient giving 50% linear growth inhibition) values (Table 15). The IG₅₀ values for the two isolates were different. In most cases, the IG₅₀ values were higher for isolate P16 than for isolate P11, the exception to this being

Table 15. IG_{50} (concentration of active ingredient giving 50% inhibition of mycelial growth) values for mycelial growth of two isolates of *Pestalotiopsis sydowiana* on fungicide amended PDA at 20°C

Fungicide	Fungicide concentration ($\mu\text{g a.i. ml}^{-1}$)	
	P11	P16
Tolclofos-methyl	2.03 ¹	7.94
Carbendazim	4.58	6.01
Chlorothalonil	2.99	6.48
Mancozeb	31.5	71.5
Bupirimate+triforine	3.07	9.41
Prochloraz	0.12	0.20
Iprodione	2.90	1.46
SED ² (97 d.f.)	3.47	

¹ Each value is the mean of five replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

Table 16. IG_{50} (concentration of active ingredient giving 50% inhibition of conidial germination) values for conidial germination of two isolates of *Pestalotiopsis sydowiana* on fungicide amended PDA at 20°C

Fungicide	Fungicide concentration ($\mu\text{g a.i. ml}^{-1}$)	
	P11	P16
Tolclofos-methyl	6.33x10 ⁷⁷	831.8 ¹
Carbendazim	1.1x10 ⁶⁹	891.2
Chlorothalonil	0.14	0.01
Mancozeb	1.17	0.52
Bupirimate+triforine	1318.2	257.0
Prochloraz	8.90	33.80
Iprodione	6.76	3.02
SED ² (97 d.f.)	3.78	

¹ Each value is the mean of five replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

iprodione, where P16 was more sensitive to this fungicide. Significant differences were obtained in the response of the isolates to the different fungicides. Mycelial growth was most inhibited by prochloraz followed by iprodione, and the least inhibited by mancozeb.

The effect of selected fungicides on the germination of conidia of *P. sydowniana* is summarised in Table 16. The IG_{50} values for the two isolates were different. Significant differences were obtained in the response of the isolates to the different fungicides. Tolclofos-methyl and carbendazim had no effect on conidial germination of either isolate of *P. sydowniana*, and this is reflected in high IG_{50} values. For both isolates, conidial germination was most inhibited by chlorothalonil followed by mancozeb.

3.8 Sensitivity of *P. sydowniana* to selected fungicides on HONS in the glasshouse

3.8.1 *Rhododendron* sp (Azalea)

There were very few significant differences in the levels of foliar browning between *Rhododendron* cuttings treated with fungicides pre- and post- inoculation with *P. sydowniana* (Table 17). Of the seven fungicides tested, only prochloraz significantly reduced the levels of foliar browning compared to the inoculated control when applied either pre- or post- inoculation. Chlorothalonil also significantly reduced foliar browning, but only when applied after inoculation. Uninoculated control cuttings which were rubbed with sandpaper recorded low levels of foliar browning.

Table 17. Effect of selected fungicides on levels of foliar browning caused by *Pestalotiopsis sydowniana* (isolate P16) on unrooted *Rhododendron* cuttings. Fungicides were applied pre- and post-inoculation.

Treatment	Foliar browning severity index	
	Pre-inoculation	Post-inoculation
Uninoculated		
Control	13.3 (21.3) ¹	12.5 (20.2)
Inoculated		
Control	35.0 (36.1)	34.2 (35.3)
Tolclofos-methyl	32.5 (34.6)	31.7 (34.1)
Carbendazim	33.3 (35.1)	35.0 (36.0)
Chlorothalonil	24.2 (29.0)	16.7 (23.8)
Mancozeb	30.0 (32.9)	30.0 (32.6)
Bupirimate + triforine	38.3 (38.10)	35.8 (36.1)
Prochloraz	20.8 (26.6)	15.8 (22.0)
Iprodione	36.7 (37.2)	30.0 (33.1)
SED ² (32 d.f.)	(3.85)	(4.36)

¹ Figures in parentheses are angular transformations of percentage data based on five replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

3.8.2 *Chamaecyparis lawsoniana*

None of the pre- and post- inoculation fungicide treatments reduced the levels of foliar browning on unrooted cuttings of *C. lawsoniana* compared with the inoculated control (Table 18). However, cuttings which were treated with fungicides after inoculation with *P. sydowiana* resulted in slightly lower levels of foliar browning than those treated before inoculation. Uninoculated control cuttings rubbed with sandpaper recorded low levels of foliar browning.

3.8.3 *Calluna vulgaris*

There were significant differences in the levels of foliar browning between *C. vulgaris* cuttings treated with fungicides pre- and post- inoculation with *P. sydowiana* (Table 19). Of the seven fungicides tested, chlorothalonil, mancozeb, prochloraz and iprodione significantly reduced the level of foliar browning compared to the inoculated control when applied pre-inoculation. These four fungicides were equally effective in reducing the level of foliar browning when applied pre-inoculation. All fungicides significantly reduced the levels of foliar browning compared to the inoculated control when applied post-inoculation. However, there were very few significant differences between the efficacy of the fungicides. Prochloraz was equally effective as iprodione, bupirimate+ triforine, mancozeb, chlorothalonil and carbendazim, but more effective than tolclofos-methyl.

Table 18. Effect of selected fungicides on levels of foliar browning caused by *Pestalotiopsis sydowiana* (isolate P16) on unrooted cuttings of *Chaemacyparis lawsoniana*. Fungicides were applied pre- and post-inoculation.

Treatment	Foliar browning severity index	
	Pre-inoculation	Post-inoculation
Uninoculated		
Control	21.7 (27.1) ¹	1.7 (5.8)
Inoculated		
Control	56.7 (49.2)	19.2 (25.2)
Tolclofos-methyl	72.5 (59.5)	29.2 (32.5)
Carbendazim	65.8 (54.5)	19.2 (25.8)
Chlorothalonil	59.2 (51.2)	12.5 (20.4)
Mancozeb	56.7 (48.9)	25.8 (29.1)
Bupirimate + triforine	53.3 (46.0)	34.2 (35.6)
Prochloraz	68.3 (56.1)	14.5 (22.4)
Iprodione	65.8 (54.4)	19.2 (25.5)
SED ² (32 d.f.)	(7.75)	(4.59)

¹ Figures in parentheses are angular transformations of percentage data based on five replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

Table 19. Effect of selected fungicides on levels of foliar browning caused by *Pestalotiopsis sydowniana* (isolate P11) on unrooted cuttings of *Calluna vulgaris*. Fungicides were applied pre- and post-inoculation.

Treatment	Foliar browning severity index	
	Pre-inoculation	Post-inoculation
Uninoculated		
Control	2.5 (4.1) ¹	5.0 (8.2)
Inoculated		
Control	67.5 (55.5)	83.3 (67.0)
Tolclofos-methyl	51.7 (46.0)	48.3 (43.9)
Carbendazim	40.8 (39.6)	37.5 (36.9)
Chlorothalonil	31.6 (33.1)	36.7 (36.7)
Mancozeb	33.3 (34.4)	34.2 (35.2)
Bupirimate + triforine	17.5 (46.1)	31.7 (34.1)
Prochloraz	35.8 (21.3)	20.8 (26.2)
Iprodione	51.7 (32.8)	24.2 (29.2)
SED ² (32 d.f.)	(8.61)	(6.58)

¹ Figures in parentheses are angular transformations of percentage data based on five replicates

² Significant differences between means are given by 2 x SED (standard error of the difference between means derived from analysis of variance)

4. DISCUSSION

There is little information available on the biology and control of *Pestalotiopsis* spp. on HONS. No detailed disease control programmes are currently available. In view of this lack of information, the major aim of this project was to study aspects of the biology and control of *Pestalotiopsis* species on HONS. Findings from such a study are likely to be useful in the implementation of an integrated disease control programme in the future.

All 19 isolates of *Pestalotiopsis* collected in the UK from HONS were identified as *P. sydowiana*. These isolates have morphological and other characteristics which closely resemble those of isolates of the fungus from other parts of Europe (Sutton, 1961, 1980). More recently, a further 13 isolates have been obtained from HONS in England and Scotland and these have been shown to be *P. sydowiana* (Litterick, personal communication). Using molecular biology techniques such as DNA fingerprinting, it may be possible to differentiate between the isolates of *P. sydowiana* collected during this study.

The isolates of *P. sydowiana* were obtained from a wide range of HONS species collected from locations throughout the UK. The majority of these isolates were obtained from *Calluna vulgaris*, *Erica* spp., *Rhododendron* and conifers. These results suggest that *P. sydowiana* may be the main species associated with *C. vulgaris*, *Erica* spp., *Rhododendron* spp. and conifers, in the UK. However, *P. sydowiana* is not the only species of *Pestalotiopsis* which has been isolated from these HONS species. For example, in France, Vegh and LeBerre (1992) isolated *Pestalotiopsis versicolor* from *Erica* spp. and

Pestalotiopsis funerea from *Cupressus*. These and other species of *Pestalotiopsis* have also been isolated from a wide range of other plant species including *Camellia* (Pirone, 1978), *Pieris japonica* (Gerlach, Hoitink and Ellett, 1974), *Cissus rhombifolia* (Bissett, 1982). *Oenothera laciniata* (Evening primrose) (Venkatasubbaiah, Grand and Van Dyke, 1991) and strawberry (Howard and Albrechts, 1973). Species of *Pestalotiopsis* have been isolated from many countries in different continents where they cause disease on a wide range of crops including coconut, grapevines, guava and tea to name just a few. These findings and the results presented in this thesis indicate the wide range of ornamental (and other) plant species which are susceptible to infection by species of *Pestalotiopsis*.

Pestalotiopsis species are primarily thought to act as opportunistic pathogens, i.e. they will infect a plant only when it is growing under stress or it is infected by another pathogen (Coyier and Roane, 1987). Results from this study have shown that in order for isolates of *P. sydowiana* to infect, it was necessary to damage the foliage of both rooted and unrooted cuttings prior to inoculation with a spore suspension of the pathogen. Previous workers have also found this with other species of *Pestalotiopsis*. For example, White (1930) has shown that infection with *P. macrotricha* occurred only when *Rhododendron* leaves were subjected to abrasions, scalding or pinpricks. Similarly, Vegh and LeBerre (1992) reported that *Camellia*, *Rhododendron* and *Thuja* showed symptoms of infection by different species of *Pestalotiopsis* after the foliage had been injured by either pesticide damage or sun scald.

Isolates of *Pestalotiopsis sydowiana* were obtained from the foliage,

stems and roots of HONS. However, species of *Pestalotiopsis* can infect other parts of plants including flowers and petals (Johnson *et al.*, 1991; Mordue, 1985) as well as fruits (Howard and Albrechts, 1973). Results from experiments with rooted and unrooted cuttings indicated that unrooted cuttings were more susceptible to infection by *P. sydowniana*. *P. sydowniana* has previously been isolated from rotted *Rhododendron* cuttings (Smith, 1977). The reason for the increased susceptibility of unrooted cuttings may be due to *P. sydowniana* being an opportunistic pathogen.

Both the pathogenicity tests and host range studies have shown that isolates of *P. sydowniana* are not host specific and that they will infect a number of HONS species other than those from which they were originally isolated. This indicates that *P. sydowniana* has the potential to infect a wide range of nursery stock species. Isolates of *P. sydowniana* were collected from a variety of locations throughout the UK suggesting that the pathogen is very widespread.

All isolates of *P. sydowniana* were able to grow on all the seven media tested. However, SDA, V8, MEA, CDA and PDA were consistently the best for hyphal extension. This confirms the suitability of these media for routine culture of *P. sydowniana* and also suggests that these may be good media on which to study the fungus.

With regard to acervuli production and hyphal extension, some differences were observed with the different isolates. For example, isolate P21 did not produce acervuli at all on MEA, CMA, V8 and TWA. However, the isolate grew well on all of these media. This observation may simply reflect

differences in nutritional requirements for hyphal growth and acervuli production. Additional studies on the effect of nutritional factors on acervuli production would be useful. These could include studies on the vitamin, carbon and nitrogen requirements of isolates of *P. sydowniana*.

This investigation has identified the temperature, pH and water potential optima for growth of different isolates of *P. sydowniana*. Physical environmental factors such as pH, temperature and water potential are known to influence the activity of fungal plant pathogens both *in vitro* and on the surfaces of aerial plant parts. Although the cultural studies described here using temperature, pH and water potential systems do not in any way directly simulate the conditions of the natural environment, the results from such studies may give an insight to the likely behaviour of *P. sydowniana* in the environment.

The isolates were typical mesophiles with optimum hyphal radial growth between 15-25°C, with little or no growth occurring at or below 5°C, and at or above 30°C. Conidial germination occurred over the range of 20-30°C, whilst the optimum temperature was 30°C. Similar results have been obtained with a number of species of *Pestalotiopsis* isolated from HONS by Vegh and LeBerre (1992). Optimum temperatures for growth were between 23 and 28°C, and mycelial growth was inhibited at 35°C, although germination of conidia did occur at this temperature. It would appear from these results that *P. sydowniana* is well suited as a pathogen of HONS grown in the glasshouse or under polythene tunnels where temperatures are normally maintained in the region of 10 to 25°C. Disease development is likely to be inhibited at temperatures

below 10-15°C. However, further experiments are required to study the effect of temperature on the development of *P. sydowniana* on nursery stock grown under protection.

The effects of pH on hyphal growth and conidial germination of *P. sydowniana* have not been examined before. In this study, hyphal growth occurred over a wide pH range (pH 2.6-7.6), with an optimum at pH 5.5 for most isolates. Conidial germination was greater than 94% over pH 2.6-7.6. These results suggest that *P. sydowniana* is likely to be pathogenic in most horticultural soils and growing media. Trials are required to study the effect of growing media pH on the development and survival of *P. sydowniana*. Once environmental conditions favouring the growth, development and survival of *P. sydowniana* have been determined, it may be possible to inhibit the activity of the pathogen by manipulating growing medium pH as well as glasshouse temperatures. Unfortunately, adjusting environmental and growing conditions to render them unfavourable for the pathogen may be unfavourable to the plants that are being protected.

The water potential of the environment is recognised as an important factor in the ecology of plant-pathogenic fungi (Woods and Duniway, 1986). In soil, pathogens are exposed mainly to matric potential forces, but as they infect plants they become dependent on the water relations of the surrounding plant tissues. The investigation reported in this study has revealed for the first time the effects of osmotic and matric potentials on the growth and conidial germination of *P. sydowniana*. Although there were slight differences between osmotica and isolates of *P. sydowniana*, growth generally decreased with

decreasing osmotic potential. Radial growth rates were reduced markedly below -4.0 to -6.5 MPa, but growth of the three isolates of the pathogen still occurred at -9.9 to -10.3 MPa (0.6 to 1.4 mm per day). Similar results were obtained for conidial germination, but germination of all three isolates was completely inhibited at -9.9 MPa.

The growth and spore germination responses of *P. sydowiana* to different water potentials are similar to those previously observed for other fungi belonging to the Deuteromycotina. Lower limits for mycelial growth of a number of Deuteromycotina, including plant pathogens, have been shown to be in the range of -9 to -12 MPa (Cook and Duniway, 1981).

Growth of *P. sydowiana* was affected more by low matric than by low osmotic potentials, with the matric potential range for growth being markedly narrower than the osmotic range. Growth of other plant pathogens including *Alternaria*, *Phytophthora cinnamomi* and *Fusarium* have also been shown to occur over a wider osmotic than matric range (Adebayo and Harris, 1971; Duniway, 1979; Brownell and Schneider 1985; Magan, 1988; Jorge-Silva *et al.*, 1989).

Some speculation can be made as to why *P. sydowiana* is more sensitive to matric potential than to an equivalent low osmotic potential. At low matric potentials, liquid films absorbed to the substrate may be extremely thin which may reduce diffusion of nutrients. It is possible, at low osmotic potentials that hyphae of *P. sydowiana* may be able to take up solutes to reduce their internal osmotic potential. This mechanism may not exist when water potential is matric. PEG 6000 was used in experiments to adjust matric

potential. It has a larger molecular size than the solutes NaCl or glycerol and, unless broken down into smaller units, would not be able to enter the hyphae.

The range of water potentials enabling growth of *P. sydowiana* has important practical implications. The majority of horticultural growing media are maintained naturally or artificially at water potentials greater than the permanent wilting point of mesophytic plants, which is approximately -1.5 MPa (Slayter, 1967). As growth of *P. sydowiana* occurred at lower water potentials, it is likely that the pathogen can grow in dry growing media that restrict plant growth. The growth of *P. sydowiana* at low water potentials might help to explain the greater pathogenicity of the fungus which has often been observed when plants are grown in growing media of low water potential. It could also explain growers' observations which have revealed a higher incidence and severity of disease in plants subjected to irregular water regimes. The ability of *P. sydowiana* to grow at low water availability must be one of the factors involved in its success as a pathogen of plants grown under water stress.

Infective propagules are one way in which plant pathogens can be transmitted from one host to another (Garrett, 1966). Transmission of spores of a number of plant pathogens has been shown to occur via splash droplets from rainfall (or from overhead watering). For example, conidia of *Pseudocercospora herpotrichoides*, *Pyrenopeziza brassicae* and *Botrytis fabae* are typically dispersed by rainfall (Fatemi and Fitt, 1983; Fitt, Creighton and Bainbridge, 1985). These pathogens cause serious disease on cereals, oilseed rape and field beans, respectively. Similarly, the spread of fungal pathogens by overhead watering could lead to problems for nursery growers.

Spores of *Pestalotiopsis* spp. are known to be spread by water splash (Mordue, 1985). This study has shown that splash droplets of water from a spore suspension of *P. sydowiana* or infected *Rhododendron* foliage can carry conidia up to a distance of 50 cm from the source. Generally, the greater the height from which the water hit the infective sources resulted in droplets being carried further. Greater numbers of colonies of *P. sydowiana* were observed on Petri dishes closer to the source materials. Gregory, Guthrie and Bunce (1959) reported similar results with spores from a number of fungal plant pathogens. They determined that the number of droplets deposited per unit area on a horizontal plane decreased rapidly with increasing distance from the source. Fatemi and Fitt (1983) recorded similar results with *P. herpotrichoides* and *P. brassicae*. They estimated that in one splash more than 240 spore carrying droplets, and more than 10000 spores, travelled less than 15 cm, whereas less than 10 spore carrying droplets, and less than 100 spores, travelled beyond 85 cm.

For disease control, it is important to understand the significance of spore carrying water splash droplets in transmitting the pathogen. In many modern nurseries, stock in polythene tunnels or glasshouses is watered from above. Given that spores of *P. sydowiana* have been shown in this study to travel up to 70 cm in splash droplets, the number of plants which could become infected is considerably high. Possible control measures to counteract the effect of transmission of spores by water splash would be to increase the space between plants. Unfortunately, however, this is often impossible for growers with limited space. Nevertheless, further work to investigate plant

spacing patterns on disease development would be beneficial. Other options would be to avoid overhead watering, reduce the height from which watering takes place and to water only when necessary (Blanc, 1992). Further experimental work to investigate irrigation regimes in more detail would be useful.

Differences in the susceptibility of cultivars of HONS to *Pestalotiopsis* spp. have been reported by previous workers. The most extensive of these studies appears to have been undertaken by Blanc (1992). The study provided a list of cultivars of *Rhodendron* spp. which are resistant to *P. guepini* as well as some cultivars which have been shown to be susceptible. However, very little other major work appears to have been undertaken to determine the susceptibility of cultivars of other HONS to species of *Pestalotiopsis*.

The use of resistant cultivars of HONS can be an important first step in preventing disease. Cultivars of *C. vulgaris*, *Erica* spp. and *Rhodendron* spp. were examined in this study for their susceptibility to *P. sydowniana*. Cultivars of *C. vulgaris* did show differences in their susceptibility to *P. sydowniana*. However, the differences in foliar browning and percentages of cuttings infected were not significant. When cultivars of *Erica* species were examined, differences in susceptibility were shown, but only Aurea was significantly less susceptible to one of the two test isolates. Cultivars of *Rhododendron* species also exhibited differences in foliar browning and levels of cuttings infected. Cultivars Curlew and Campyloginum myrtleoides were the most susceptible to infection, whereas cvs. Impeditum indigo and Charmaine showed no signs of infection to either of the test isolates.

The results from these experiments, coupled with the information from Blanc (1992) suggest that cultivars of HONS do exist that exhibit some resistance to species of *Pestalotiopsis*. However, a greater number of cultivars for each HONS species would have to be tested repeatedly for continual resistance to the pathogen before a definitive list of resistant cultivars could be produced. Problems can arise when testing cultivars for resistance to plant pathogens. For example, it can be difficult to achieve consistency in results during a series of repeated experiments. Differences in pathogenicity of isolates can influence levels of disease and isolates may become less pathogenic due to repeated sub-culturing over the course of study. It may also be difficult to find a suitable, consistent method of inoculation or to produce a standard inoculum. If natural inoculation is to be relied upon in nursery trials, then a long wait may be required before any disease becomes apparent. There is the further problem that initial cultivar resistance may be overcome by the pathogen.

More research has been undertaken on the effect of fungicides against *Pestalotiopsis in vitro* rather than *in vivo*, especially as far as HONS are concerned. *In vitro* experiments can provide a useful indication on whether fungicides are active against plant pathogens.

In vitro agar plate bioassays with two isolates of *P. sydowiana* indicated that prochloraz and iprodione were the most effective fungicides at inhibiting the growth of the pathogen, whilst mancozeb was the least efficient. Chlorothalonil was the most efficient at inhibiting germination. Tolclofos-methyl and carbendazim had no effect on conidial germination.

In vitro studies on species of *Pestalotiopsis* by other workers have indicated varying responses to different fungicides. Complete inhibition of mycelial growth of *P. palmarum* was observed with carbendazim at concentrations ranging from 100-500 ppm. Inhibition of mycelial growth with zineb and maneb was less successful (Das and Mahanta, 1985). In contrast, studies on *P. funerea* (isolated from *Eucalyptus globulus*) indicated that zineb and maneb significantly reduced growth of the pathogen *in vitro*. Concentrations of the fungicide were present in the nutrient media ranging from 0.05-1.0%w/v (Upadhyay, 1984). Similarly, a 0.25% concentration of zineb provided complete inhibition of *P. psidii*. Benlate and carbendazim (0.05%w/v final concentration) also provided complete inhibition of mycelial growth (Padule and Hande, 1980). It can be concluded from these contrasting results that different species of *Pestalotiopsis* vary in their response to different fungicides.

There appears to be little information available on the control of *Pestalotiopsis* species on HONS using fungicide programmes. Trials carried out by ADAS in the 1970s suggest that copper oxychloride and a mixture of carbendazim plus maneb were promising fungicides for the control of *Pestalotiopsis* on *Camellia* (Scott, 1977; 1978). Further trials, utilising benlate, revealed that this fungicide did not achieve satisfactory control, although greater success was achieved when the fungicide was applied as a protectant during the propagation of cuttings.

Control of *P. sydowiana* causing rotting of *Rhododendron* cuttings was most successful using carbendazim and a carbendazim plus maneb mixture.

Applications of these fungicides to both cuttings and the rooting media reduced the mean number of rotted cuttings by 46 and 39% respectively, (Smith, 1977). In contrast, Blanc (1992) describes trials on *Rhododendron* in France involving the application of prochloraz, kocide, benomyl and captan. The different fungicide treatments had no effect on the disease, despite repeated applications over a three month period. These results were corroborated by Coyier and Roane (1987) who found that control of *Pestalotia* leaf spot, on rhododendron and azalea foliage, by the use of fungicides was generally unsuccessful.

Experiments on *Rhododendron* sp., *Chamaecyparis lawsoniana* and *Calluna vulgaris* during this project have provided slightly more encouraging results. On *Rhododendron* sp., foliar sprays of either chlorothalonil or prochloraz significantly reduced the levels of foliar browning when applied either pre- or post-inoculation. On *C. lawsoniana*, no significant differences were recorded in the levels of foliar browning following treatment with any of the test fungicides. On *C. vulgaris*, chlorothalonil, mancozeb, prochloraz and iprodione all reduced the levels of foliar browning significantly when applied both pre- and post-inoculation. The results indicate that prochloraz and chlorothalonil are both important fungicides to be included in a fungicide programme for the control of *Pestalotiopsis*, especially during the propagation of *Rhododendron* and *C. vulgaris*. Prochloraz would be especially useful because of its protectant and eradicant activity. Further trials are required to determine the optimum levels of fungicide which provide maximum disease control, and to test for phytotoxicity on both rooted and unrooted cuttings.

This study has provided some useful information on the biology and control of *P. sydowiana* on *C. vulgaris*, *Erica* spp., *Rhododendron* and conifers. However, further research on aspects of the biology, epidemiology and control of *Pestalotiopsis* sp. are still required before an integrated control programme can be implemented. A detailed study on the spread of the pathogen on nurseries would be useful. It would be beneficial to determine whether the pathogen can be transmitted by air currents as well as by water splash. A study on the effect of environmental conditions on disease development on nurseries would also be useful. Experiments to determine the survival of the pathogen in growing media, on old trays, pots and other nursery equipment are essential. It may also be possible to rank cultivars of selected HONS according to their susceptibility which have exhibited resistance to *Pestalotiopsis* spp. following repeated trials on nurseries where infection occurs naturally. Furthermore, a series of detailed fungicide trials are required before an integrated disease control programme can be implemented. These could involve repeated trials on nurseries with natural infection to compare fungicide products, methods of application (for example, a drench with a high volume spray application), and different fungicide programmes.

In conclusion, further research should help to determine the relative importance of irrigation timing, plant spacing, hygiene and fungicide treatment in the control of *Pestalotiopsis* on HONS, and therefore where management effort for disease control is best directed.

5. CONCLUSIONS

The main conclusions to be drawn from the work undertaken include:-

1. *Pestalotiopsis sydowiana* (Bresad.) B. Sutton is likely to be the main species of *Pestalotiopsis* associated with diseased hardy ornamentals in the UK.
2. Colony morphologies, spore measurements and hyphal extension rates of isolates of *P. sydowiana* collected from diseased hardy ornamentals are similar.
3. Isolates of *P. sydowiana* are able to grow well on commonly used agar culture media including SDA, V8, MEA, CDA and PDA.
4. A wide range of nursery stock species can be infected by *P. sydowiana* including *Calluna vulgaris*, *Erica* spp., *Rhododendron*, *Chamaecyparis*, *Cupressocyparis*, *Pieris* sp. and *Euonymus*.
5. Isolates of *P. sydowiana* can cause foliar browning, wilting, death of shoots, and root and stem-base rots on nursery stock species. Black acervuli of the pathogen are typical on diseased leaves, shoots and stems.
6. *P. sydowiana* is a typical mesophile, with optimum growth between 15 to 25°C. Little or no growth occurs above 30°C, or below 5°C.
7. *P. sydowiana* grows over a wide pH range (pH 2.6-7.6), with an optimum at pH 5.5.
8. Optimum growth of *P. sydowiana* occurs at -0.3 to -2.1 MPa. *P. sydowiana* can grow at low water potentials (-9.9 to -10.3 MPa), suggesting that the fungus can grow in dry growing media that restrict plant growth.

9. Temperature, growing media pH and water availability are likely to be important in the incidence and development of *Pestalotiopsis*.
10. Ranking cultivars of ornamental species according to their susceptibility to *P. sydowiana* is likely to be difficult as the susceptibility of individual cultivars can vary markedly under different environmental conditions and when exposed to different isolates of the fungus.
11. The incidence of *Pestalotiopsis* is likely to be more severe on plants which are suffering from stress. For example, those which are damaged or growing under extreme temperature and low water availability.
12. The incidence and spread of *Pestalotiopsis* may be reduced by watering from below rather than by overhead, and by increasing plant spacing.
13. *Pestalotiopsis* on cuttings of *Calluna vulgaris* and *Rhododendron* may be controlled through the use of foliar-applied fungicides such as prochloraz and chlorothalonil. Prochloraz is likely to be especially useful because of its protectant and eradicant activity.
14. Future research should help to determine the relative importance of irrigation timing, plant spacing, hygiene and fungicide treatment in the control of *Pestalotiopsis*, and therefore where management effort for disease control is best directed.

BIBLIOGRAPHY

- Adebayo, A.A. and Harris, R.F. (1971). Fungal growth responses to osmotic as compared to matric water potential. *Soil Science Society of America* 35, 465-469.
- Agrios, G. (1988). *Plant Pathology*. Academic Press, New York.
- Baker, K.F. and Linderman, R.G. (1979). Unique features of the pathology of ornamental plants. *Annual Review of Phytopathology* 17, 253-277.
- Baxter, L.W.Jr. and Fagan, S.G (1986). Method for maintaining three selected fungi. *Plant Disease* 70 (6), 499-500.
- Bissett, J. (1982). *Pestalotiopsis menezesiana* on greenhouse plantings of *Cissus rhombifolia* with notes on related fungi occurring on Vitaceae. *Canadian Journal of Botany* 60, 2570-2574.
- Blanc, F. (1992). L'Anthracnose du Rhododendron due au *Pestalozzia guepini* Desm.. *Revue Horticole* 323, 31-36.
- Bloomberg, W.J. (1971). Diseases of Douglas Fir seedlings caused by *Fusarium oxysporum*. *Phytopathology* 61, 467-470.
- Brownell, K.H. and Schneider, R.W. (1985). Roles of matric and osmotic components of water potential and their interaction with temperature in the growth of *Fusarium oxysporum* in synthetic medium and soil. *Phytopathology* 75, 53-57.
- Bugbee, W.M. and Anderson, N.A. (1963). Infection of Spruce seedlings by *Cylindrocladium scoparium*. *Phytopathology* 53, 1267-1270.

- Bunt, A.C. (1988). Media and mixes for container grown plants. Unwin Hyman, London.
- Chevaugon, J. (1974). Stability of the differentiated state in *Pestalozzia annulata*. *Transactions of the British Mycological Society* 63(2), 371-379.
- Cook, R.J. and Duniway, J.M. (1981). Water relations in the life cycles of soilborne plant pathogens. In: Parr, J.F., Gardner, W.R. and Elliott, L.F. (Eds.). *Water Potential Relations in Soil Microbiology*. Soil Science Society of America, Madison, Wisconsin.
- Coyier, A.L. and Roane, M.K. (Eds.). (1987). *Compendium of Rhododendron and Azalea Diseases*. APS Press, New York.
- Dallyn, H. and Fox, A. (1980). Spoilage of material of reduced water activity by xerophilic fungi. In: Gould, G.H. and Corry, C.E.L. (Eds.). *Society of Applied Bacteriology Technical Series no. 15* pp. 129-139. Academic Press, London.
- Das, C.M. and Mahanta, I.C. (1985). Evaluation of some fungicides against *Pestalotia palmarum* Cke., incitant of grey blight of coconut. *Pesticides* 19, 37-38.
- Duniway, J.M. (1979) Water relations of water molds. *Annual Review of Phytopathology* 17, 431-460.
- Edge, D (1984) Yearbook of the Heather Society pp 34-35
- Fail, G.L. and Langenheim, J.H. (1990). Infection processes of *Pestalotia subcuticularis* on leaves of *Hymenaea courbaril*. *Phytopathology* 80(11), 1259-1265.

- Fatemi, F. and Fitt, B.D.L (1983). Dispersal of *Pseudocercospora herpotrichoides* and *Pyrenopeziza brassicae* spores in splash droplets. *Plant Pathology* 32, 401-404.
- Fitt, B.D.L., Creighton, N.F. and Bainbridge, A. (1985). Role of wind and rain in dispersal of *Botrytis fabae* conidia. *Transactions of the British Mycological Society* 85 (2), 307-312.
- Garrett, S.D. (1966). Spores as propagules of disease. In: Madelin, M.F. (Ed.). *The Fungus Spore*. Butterworths, London.
- Gerlach, W.W.P., Hoitink, H.A.J. and Ellett, C.W. (1974). Shoot blight and stem dieback of *Pieris japonica* caused by *Phytophthora citricola*, *P. citrophthora* and *Botryosphaeria dothidea*. *Phytopathology* 64, 1368-1370.
- Gomori, G. (1955). Preparation of buffers for use in enzyme studies. In: Colowick, S.P. and Kaplan, N.O. (Eds.). *Methods in Enzymology*. p. 138 Academic Press, New York
- Gregory, P.H., Guthrie, E.J. and Bunce, M.E. (1959). Experiments on splash dispersal of fungal spores. *Journal of General Microbiology* 20, 328-354.
- Griffin, D.M. (1972). *Ecology of soil fungi*. Chapman and Hall, London.
- Griffin, D.M. (1981). Water and microbial stress. In: Alexander, M. (Ed.). *Advances in Microbial Ecology*. pp. 91-136. Plenum Publishing Co., New York.
- Griffin, D.M. (1994). *Fungal Physiology*. Wiley-Liss, New York.
- Grove, W.B. (1937). *British Stem and Leaf Fungi II*. C.U.P pp. 345-352.

- Grover, R.K. and Karve, A.D. (1963). Induction by light of sporulation in *Pestalotia theae* Sawada.. *Canadian Journal of Botany* 41, 569-571.
- Guba, E.F. (1927). Monograph of the genus *Pestalotia de notaris*. *Phytopathology* 19, 191-231.
- Hardy, G.E. and Sivasithamparam, K. (1988). *Phytophthora* spp. associated with container grown plants. *Plant Disease* 72, 435-437.
- Hartmann, H.T., Kester, D.E. and Davies, F.T. (1990). Plant Propagation: Principles and Practices. Prentiss Hall, New York.
- Hendrix, F.F. and Campbell, W.A. (1973). *Pythiums* as plant pathogens. *Annual Review of Phytopathology* 11, 77-98.
- Howard, C.M. and Albrechts, E.E. (1973). A strawberry fruit rot caused by *Pestalotia longisetula*. *Phytopathology* 63, 862-863.
- Ibrahim, A.N., Satour, M.M. and Elakkad, E.M. (1976). Variability of *Pestalotia* spp with special reference to the effect of media, temperature and light on their growth, sporulation and spore gemination. *Acta Mycologia* 12(1), 141-151.
- Jain, P.C. and Agrawal, S.C. (1974). Fungicides in relation to the growth of some anthracnose pathogens. *Proceedings National Academy of Science India* 44(B) IV, 223-226.
- Johnson, G.I., Mead, A.J., Cooke, A.W. and Dean, J.R. (1991). Mango stem end rot pathogens - infection levels between flowering and harvest. *Annals of Applied Biology* 119, 465-473.
- Jorge-Silva, M.L., Rodrigues, M.L., Ferraz, J.F.P and Ricardo, C.P.P. (1989) Effect of water availability on growth of *Fusarium oxysporum* f. sp.

melonis and on host-parasite relations. *Mycological Research* 92 (2), 157-161.

Kataria, H.R., Hugelshofer U. and Gisi, U. (1991). Sensitivity of *Rhizoctonia* species to different fungicides. *Plant Pathology* 40, 203-211.

Lamb, J.G.D., Kelly, J.C. and Bowbrick, P. (1985). Nursery Stock Manual. Grower Books, London.

Lambe, R.C. and Wills, W.H. (1980). Soilborne fungus diseases of ornamentals. Proceedings of the International Plant Propagation Society 30, 485-492.

Lang, A.R.G. (1967). Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40°C. *Australian Journal of Chemistry* 20, 2017-2023.

Linderman, R.G. and Zeitoun, F. (1977). *Phytophthora cinnamomi* causing root rot and wilt of nursery grown native Western azalea and Salal. *Plant Disease Reporter* 61, 1045-1048.

Litterick, A.M., McQuilken, M.P. and Holmes, S.J. (1995). *Rhizoctonia* spp. associated with container-grown Ericaceous plants in Scotland. *Journal of Phytopathology* 143, 509-510.

MacDonald, B. (1986). Practical Woody Plant Propagation for Nursery Growers. pp. 369-381. Batsford, London.

Magan, N. (1988). Effects of water potential and temperature on spore germination and germ-tube growth *in vitro* and on straw leaf sheaths. *Transactions of the British Mycological Society* 90, 97-107.

Mani, K. and Swami, R.N. (1983). Induction of sporulation in *Pestalotiopsis palmarum* by sodium chloride. *Transactions of the British Mycological*

Society 80(1), 151-156.

Matkin, O.A. (1971). Soil mixes today. *Proceedings of the International Plant Propagation Society* 21, 162-163.

McCully, A.J. and Thomas, M.B. (1977). Soilborne diseases and their role in propagation. *Proceedings of the International Plant Propagation Society* 27, 339-350.

Michel, B.E. and Kaufmann, M.R. (1973). The osmotic potential of polyethylene glycol 6000. *Plant Physiology* 51, 914-916.

Mordue, J.E.M. (1985). Descriptions of pathogenic fungi and bacteria. CMI, Cambian News, Aberystwyth.

Murashige, T. (1974). Plant cell and organ cultures as horticultural practices. *Acta Horticulturae* 78, 17-30.

Niedbalski-Cline, M. (1988). Current and future research directions of ornamental pathology. *Plant Disease* 72, 926-934.

Padule, D.N. and Hande, Y.K. (1980). Laboratory evaluation of some fungicides against *Pestalotia psidii* Pat., the cause of guava canker. *Pesticides* 14, 37-38.

Pandey, R.R., Arora, D.K. and Dubey, R.C. (1993). Antagonistic interactions between fungal pathogens and phylloplane fungi of guava. *Mycopathologia* 124, 31-39.

Papendick, R.I. and Campbell, G.S. (1981). Theory and measurement of water potential. In: Parr, J.F., Gardner, W.R. and Elliott, L.F. (Eds.). *Water Potential Relations in Soil Microbiology*. pp. 1-23 Soil Science Society of America, Madison, Wisconsin.

- Parr, J.F., Gardner, W.R. and Elliott, L.F. (Eds.). (1981). *Water Potential Relations in Soil Microbiology*. Soil Science Society of America, Madison, Wisconsin.
- Pirone, P.P. (1978). *Diseases and Pests of Ornamental Plants*. Wiley Interscience, New York.
- Proudley, B. and V. (1984) *Garden Conifers*. Blandford, London.
- Proudley, B. and V. (1985). *Heathers in Colour*. Blandford, London.
- Raynor, R.W. (1970). *A Mycological Colour Chart*. Commonwealth Mycological Institute, Kew, Surrey.
- Scott, M.A. (1977, 1978). ADAS Reference Book 235.
- Scott, M.A. (1983). *Camellia*. *Combined Proceedings of the International Plant Propagation Society* 33, 222-225.
- Slayter, R.D. (1967) *Plant-water relationships*. Academic Press. New York.
- Smith, P.M. (1977). *Glasshouse Crops Research Institute Annual Report* pp. 121-122.
- Steuter, A.A., Mozafar, A. and Goodin, J.R. (1981). Water potential of aqueous polyethylene glycol. *Plant Physiology* 67, 64-67.
- Steyaert, R.L. (1949). Contribution à l'étude monographique de *Pestalotia* de Not. et *Monochaetia* Sacc. *Bulletin du Jardin botanique de l'Etat Brux.* 19, 285-354.
- Stout, G.L. (1962). Maintenance of 'pathogen free' planting stock.

Phytopathology 52, 1255-1258.

Sutton, B.C. (1961). Coelomycetes I. Mycological Papers IMI 80:14-15.

Sutton, B.C. (1969). Forest Microfungi III. The heterogeneity of *Pestalotia de Not.* section *sexloculatae* Klehbahn sensu Guba. *Canadian Journal of Botany* 47, 2083-2094.

Sutton, B.C. (1980). The Coelomycetes. pp. 263-266 Commonwealth Mycological Institute Kew, Surrey.

Upadhyay, R.K. (1984). Evaluation of fungicides and antibiotics against *Pestalotiopsis funerea in vitro* causing leaf spot of *Eucalyptus globulus*. *Indian Forester* 110, 209-217.

Umalkur, G.V. and Begum, S. (1976). Effect of light spectra on growth, sporulation and pectic enzyme secretion in some pathogenic fungi. *Biovigyanam* 2, 21-25.

Vegh, I. and LeBerre, A. (1992). Étude de quelques champignons rencontrés sur ligneux d'ornement. *Phytoma - La defense des végétaux* 435, 66-68.

Venkatasubbaiah, P., Grand, L.F. and Van Dyke, C.G. (1991). A new species of *Pestalotiopsis* on *Oenothera*. *Mycologia* 83 (4), 511-513.

White, R.P. (1930). Pathogenicity of *Pestalotia* spp on rhododendron. *Phytopathology* 20, 85-91.

Woods, D.M. and Duniway, J.M. (1986). Some effects of water potential on growth, turgor and respiration of *Phytophthora cryptogea* and *Fusarium moniliforme*. *Phytopathology* 76, 1248-1253.

APPENDICES

Appendix 1.

Osmotic Potential

Amounts of solute (g) added l⁻¹ distilled water to give the final osmotic potentials (-MPa) of PDA at 20°C. Double strength PDA was added to the appropriate volume of osmotically maintained solution to give the required osmotic potential.

Osmotic potential (-MPa)	NaCl	Osmotic potential (-MPa)	Glycerol
0.3*	-	0.3	-
0.8	5.9	0.8	18.4
1.2	11.9	1.2	36.8
2.1	23.8	2.1	64.5
4.9	59.5	4.8	165.8
6.3	77.3	6.5	230.2
8.3	101.1	7.8	276.3
9.9	118.9	9.1	322.4
		10.3	368.4

* Unmodified medium

Appendix 2.

Matric potential

Formula derived by Michel and Kaufman (1973):-

$$\psi_m = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 \\ + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2 T$$

where ψ_m is the matric potential in bars, C is the concentration of PEG 6000 in $\text{g kg}^{-1} \text{H}_2\text{O}$ and T is the temperature in $^{\circ}\text{C}$. For specific values of ψ_m and T, the equation becomes a simple quadratic and C may be obtained as the positive value in a quadratic solution.

Amounts of PEG 6000 ($\text{g kg}^{-1} \text{H}_2\text{O}$) added to distilled water to give final matric potentials (-MPa) of PDA at 20°C . Double strength PDA was added to the appropriate volume of matrically maintained solution to give the required medium matric potential.

Matric potential (-MPa)	PEG 6000 ($\text{g kg}^{-1} \text{H}_2\text{O}$)
0.3*	-
0.8	222.5
1.3	316.5
2.1	420.5
4.8	668.0
6.5	781.9
7.8	861.1
9.1	930.4

* Unmodified medium

Appendix 3.

Standard potting compost

10 l fine grade bark (SHL, Lincoln)

30 l Irish moss peat (Bulrush Propagation Company Ltd.)

100 g Osmocote Plus (Grace Sierra, Haslemere, Surrey)

72 g Dolofil (Redland Minerals, Worksop)

pH = 5