AN INVESTIGATION INTO THE EPIDEMIOLOGY AND CONTROL OF *RHIZOCTONIA* SPP. ON *CALLUNA VULGARIS* AND *ERICA* SPP.

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by

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This thesis is dedicated to
my husband Mark,
to The Ferranti Mountaineering Club,
especially Brian, Dave, Al, Campbell, Ian,
Rosemary, Neil and Clare
and to my parents.

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ABBREVIATIONS USED IN THE TEXT

AFP - air-filled-porosity

a.i. - active ingredient

CMI - Commonwealth Mycological Institute

cv - cultivar

IMI - International Mycological Institute

EHS - Experimental Horticultural Station

Expt.- Experiment

HDC - Horticultural Development Council

HONS - hardy ornamental nursery stock

Ltd. - limited

m.l. - magnesian limestone

N.S. - not significant

PCNB - pentachloronitrobenzene

PDA - potato dextrose agar

PDES - potato dextrose, erythromycin, streptomycin (agar)

plc - public limited company

r.h. - relative humidity

Rhiz.- Rhizoctonia

SEM - standard error of the difference between two means

sp. - species

spp. - species (plural)

SAC - The Scottish Agricultural College

UK - United Kingdom

WP - wettable powder

SUMMARY

Rhizoctonia spp. were isolated from 22% of cuttings and 10% of rooted plants of *C. vulgaris* and *Erica* spp., collected from UK nurseries, which showed browning of the foliage. *Rhizoctonia* spp. were most often isolated from cutting bases at compost level and from stem-base and foliage at compost level on rooted plants. Thirty two per cent of *Rhizoctonia* spp. isolates obtained from nursery stock species possessed multinucleate hyphal cells and were designated *R. solani* Kuhn. The remaining 68% which had two nuclei in each cell were termed binucleate *Rhizoctonia* spp.

Rhizoctonia spp. were isolated from between 3% and 13% of samples of used nursery materials including cutting trays, capillary matting, polythene and composts collected from UK nurseries. They were not isolated from any samples of new, unused materials or composts. Spread of *Rhizoctonia* spp. and subsequent disease development was initiated from contaminated plastic trays, polythene, capillary matting, gravel, sand, compost and infected cuttings.

Root development and the level of foliar browning due to disease caused by *Rhizoctonia* spp. on cuttings of *C. vulgaris* and *Erica* spp., varied significantly depending on isolate. There was evidence of a relationship between number of nuclei and the level of both foliar browning and root development.

The susceptibility of cuttings of *E. cinerea* and *C. vulgaris*, to disease caused by binucleate *Rhizoctonia* spp. differed significantly depending on both cultivar and environment. The susceptibility of *C. vulgaris* cvs Cuprea and Silver Queen cuttings to disease caused by binucleate *Rhizoctonia* sp. isolate D1, also varied depending on the environment in which stock plants were kept, and on whether cuttings were taken from shoot-tips or shoot-bases.

The level of disease caused by binucleate *Rhizoctonia* sp. isolate 48 on *C. vulgaris* and *E. vagans* cuttings was related to the quantity of inoculum present in the compost. Disease development was most severe when inoculum of binucleate *Rhizoctonia* sp. isolate D1 was spread on the surface, and least severe when it was spread on the base of cutting trays 5 cm below the compost surface.

The optimum pH for growth of isolates of *R. solani* and binucleate *Rhizoctonia* spp. *in vitro* lay between 5.4 and 7.4, and differed significantly depending on isolate. Disease development on *C. vulgaris* cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1, was significantly lower in unlimed propagation compost (pH 3.8) than in composts limed to pH values ranging from 4.9 to 6.0.

The optimum temperature for growth of *Rhizoctonia* spp. *in vitro* was either 20^{o} or 25^{o}C depending on isolate. Growth either stopped or was greatly reduced at 5^{o}C.

Disease development on *C. vulgaris* cuttings grown in compost amended with binucleate *Rhizoctonia* spp. isolates differed significantly depending on both temperature and isolate.

The level of controlled release fertiliser in propagation compost had a significant effect on foliar browning on *C. vulgaris* cuttings in infested composts, but had no significant effect on root development.

Growth of binucleate *Rhizoctonia* spp. isolates D1 and 64 was significantly faster in atmospheres of 90% to 100% r.h., than in atmospheres of 70% to 85% r.h.

Disease development was most severe on *C. vulgaris* cuttings in boxes placed on moist gravel (0 cm of water) and least severe on cuttings in boxes placed in 6 cm water.

There were significant differences between foliage scores recorded on *C. vulgaris* and *E. vagans* cuttings grown in different composts amended with binucleate *Rhizoctonia* sp. isolate D1. Root indices were greatest (in both infested and uninfested composts) in 1:1 (v/v) peat and bark compost.

Three isolates of *Penicillium* spp., three of *Mucor* spp. and twelve of *Trichoderma* spp. were obtained from propagation compost. Four of the isolates of *Trichoderma* spp. stopped the growth of one or both *Rhizoctonia* spp. isolates on PDA. None of these *Trichoderma* spp. isolates had any effect on the development of disease caused by *Rhizoctonia* spp. on *C. vulgaris* cuttings grown in compost

in which isolates of both *Trichoderma* and *Rhizoctonia* spp. had been incorporated.

No foliar browning was recorded on *C. vulgaris* plants grown in compost which contained *R. solani* isolate A (which was previously shown to infect *C. vulgaris* plants causing no symptoms), before they were planted in soil. However, 9 months after planting in soil, browning was significantly greater on plants grown as cuttings in composts amended with *R. solani* isolate A.

Only one type of composted pine bark, (produced by Melcourt Industries), significantly reduced the level of foliar browning on *C. vulgaris* cuttings, in comparison with those grown in standard propagation compost.

The growth of six *Rhizoctonia* spp. isolates on PDA was significantly reduced by the addition of 10 µg captan/ml PDA. Only three out of the six isolates were completely controlled by 1000 µg captan/ml PDA. The growth of eight *Rhizoctonia* spp. isolates was significantly reduced on PDA containing 5 µg of either iprodione or tolclofos-methyl/ml PDA. The presence of 500 µg tolclofos-methyl controlled all isolates tested and 500 µg iprodione controlled five out of the eight isolates.

The use of iprodione or tolclofos-methyl (compost incorporation followed by six, monthly drenches in the absence of pathogens) had no effect on the foliage or root development of *C. vulgaris* cv Gold Haze plants.

Of six fungicides tested, only tolclofos-methyl

completely controlled disease on *C. vulgaris* cuttings caused by binucleate *Rhizoctonia* spp. However, significant reductions in foliar browning were recorded on cuttings grown in *Rhizoctonia* spp.-amended composts which contained either captan or iprodione. A reduction in application rates (from those recommended for ornamentals by the manufacturers) of either tolclofos-methyl or captan, resulted in reduced disease control.

Both iprodione and tolclofos-methyl completely controlled disease caused by binucleate *Rhizoctonia* sp. isolate 64 on *C. vulgaris* cuttings in peat and grit, peat and perlite and peat and bark composts.

Disease caused by binucleate *Rhizoctonia* sp. isolate 48 was controlled when tolclofos-methyl was incorporated in the compost 0, 1, 2 or 3 weeks before cuttings were struck. However, disease control in composts which contained captan was only achieved when the fungicide was incorporated at striking.

The level of disease recorded on cuttings grown in compost amended with tolclofos-methyl and binucleate *Rhizoctonia* sp. isolate 64 depended on the quantity of water applied. Disease control was poorest on cuttings in compost irrigated with 60 l water at striking.

It was concluded that control of *R. solani* and binucleate *Rhizoctonia* spp. could be achieved through the use of a combination of hygienic production methods, manipulation of cultural and environmental conditions and fungicide programmes.

CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

The literature relating to Rhizoctonia diseases of *Erica* spp. and *Calluna vulgaris* (L.) Hull (commonly referred to as heathers), will be reviewed. To gain a full understanding of the results to be presented, work relating to other crop species and other pathogens will be discussed. A study of such work is necessary due to the lack of information currently available concerning Rhizoctonia diseases on heathers.

Although *Rhizoctonia* spp. have been known pathogens for over 100 years and have been shown to cause diseases on many species of crop plants, only in the past decade have they caused problems on nurseries which produce *C. vulgaris* and *Erica* spp. Changes in production methods have led to the creation of conditions whereby development of disease caused by *Rhizoctonia* spp. is often favoured. Heavy financial losses have been incurred as a result and the development of a control programme for the disease is necessary.

1.1 THE UK NURSERY STOCK INDUSTRY

The growth of gardening as a hobby has led to large increases in demand for plants of many types, in particular hardy ornamental nursery stock (HONS) species. HONS species include trees, large and small shrubs and herbaceous material, which are grown for ornament and are hardy in the British Isles.

Traditionally produced as one of a number of plant

families on general nurseries, Ericaceous species including the genera *Calluna, Erica* and *Daboecia* are now being produced increasingly on specialist nurseries growing only a limited number of species with similar requirements. Production techniques are then streamlined to suit the species concerned.

It is estimated that there are over 100 growers producing heathers in Great Britain, although the majority of heathers sold in this country are produced on 27 independently owned holdings, the proprietors of which belong to the British Heather Growers Association (BHGA). Specialist nurseries vary greatly in size from small concerns employing one or two people and producing 1000 to 2000 plants per year, to large nurseries with ten to twenty staff producing between 750 000 and 1.5 million plants annually.

Although no confirmed production figures exist, members of the BHGA estimate that around 12 million heathers are produced annually in this country, including rooted cuttings, liners, (i.e. plants in 7 and 8 cm pots), 1 and to a lesser extent 2 and 3 l pots. The retail sales value of the crop has increased greatly from around £5 M in 1979 to around £9.5 M in 1989. Heathers are produced in several continental countries, particularly Holland and West Germany, where large numbers of growers are served by research and development stations.

1.1.1 Species of heather

The term 'heaths and heathers' covers three genera, namely *Erica, Calluna* and *Daboecia*. These are contained in the family *Ericaceae*. *Erica* and *Daboecia* spp. are frequently referred to as heaths and *C. vulgaris* as heathers, however the terms are sometimes interchanged.

The genus *Calluna* contains only one species (*C. vulgaris* (L.) Hull) and is therefore monotypic. *Erica* contains over 600 species most of which are South African and are not hardy in the UK. Only fifteen species of hardy Ericas are produced in large quantities in Great Britain. The genus *Daboecia* has two species. There is a great deal of variation within a single species and many of these variations are treated as distinct varieties known as cultivars. Fifty per cent of heathers produced in the UK are cultivars of *C. vulgaris*. Cultivars of *E. carnea* L. and *Erica x darleyensis* Bean account for a further 30% of production. The remaining 20% of production is composed of cultivars of *E. cinerea* L., *E. vagans* L., *E. tetralix* L., *E. erigena* R. Ross and to a lesser extent other *Erica* and *Daboecia* spp. and their hybrids. There are several attractive and popular cultivars within these 'lesser' species. Table 1 lists the species of hardy heaths and heathers in cultivation, along with the number of cultivars commonly grown in Great Britain. Work in this project has concerned mainly *C. vulgaris* cultivars along with cultivars from *E. carnea*, *E. cinerea* and *E. vagans*, which are described along with *Erica x darleyensis* in more

detail in the following pages.

Table 1 Species and hybrids of hardy heathers and heaths grown in Great Britain and Northern Europe.

Species/hybrid	Common name	No. of cvs in commercial cultivation in Europe. (approx)
Calluna vulgaris (L.) Hull	Ling	320
Erica spp.		
Erica carnea L.	Winter heath	56
Erica cinerea L.	Bell heather	120
Erica ciliaris L.	Dorset heath	12
Erica erigena R. Ross	Mediterranean heath	17
Erica mackaiana Bab.	Irish heath	5
Erica tetralix L.	Cross leaved heath	30
Erica vagans L.	Cornish heath	30
Erica scoparia L.	Besom heath	rarely grown
Erica umbellata L.	-	" "
Erica hybrids		
Erica x praegeri Ostenf. *(E. mackaiana x E. tetralix)*	-	5
Erica x watsonii (Benth.)Bean *(E. ciliaris x E. tetralix)*	-	7
Erica x darleyensis Bean *(E. carnea x E. erigena)*	-	18
Erica x williamsii Druce *(E. vagans x E. tetralix)*	-	2
Dabeocia spp. and hybrid		
Dabeocia cantabrica (L.) K. Koch	St. Dabeoc's heath	22
Dabeocia azorica Tut & E.F. Warb.	-	1
Dabeocia x praegeri Tut & E.F. Warb.	-	7
Tree heaths		
Erica arborea L.	-	2
Erica australis L.	-	2
Erica lusitanica Rud.	-	1
Erica terminalis Salisb.	-	1
Erica x veitchii Bean *(E. arborea x E. lusitanica)*	-	3

1.1.1.1 *CALLUNA VULGARIS* - COMMON HEATHER

This is the common heather or ling found growing on the mountains and moorland in Great Britain and many other countries throughout the world. All *C. vulgaris* cultivars grow best in light, organic acid soil and an open, sunny situation, although many will grow well in a variety of conditions providing the soil is sufficiently acidic. A very wide range of variation can be observed amongst the cultivars of *C. vulgaris*. Some have grey, hairy foliage, (e.g. cv Silver Queen), some yellow foliage, (e.g. cv Beoley Gold), and others green, (e.g. cv Loch Turret). Many undergo a colour change depending on the season, e.g. cv Flamingo, which displays pink tipped foliage in spring and cv Spring Torch, the shoots of which turn creamy yellow in March.

The flowers of *C. vulgaris* are usually purple or purple-pink, although numerous white forms exist. Most are single flowered but some double forms such as cvs H.E. Beale and Kinlochruel are popular. The time of flowering differs depending on cultivar, although it always occurs between mid June and November. Growth habit also varies. Some such as cvs Long White and Tricolorifolia grow to around 70 cm tall, whereas others such as cvs Mousehole and Foxii nana grow to only 10 to 15 cm.

1.1.1.2 *E. CARNEA* - WINTER HEATH

Most cultivars of this species, which is native to several parts of mainland Europe, are very hardy, generally lime

tolerant, grow up to 20 cm tall and have a low spreading habit. Flowers are produced from late December to April depending on cultivar. Flower colour varies from white, (e.g. cv Springwood White), through pale pink (e.g. cv King George) to dark ruby red, (e.g. cv Myretoun Ruby).

1.1.1.3 *E. CINEREA* - BELL HEATHER

This species is widely distributed in Western Europe including the UK. It forms a stiffly branching shrub up to 60 cm tall and flowers from June to October. Foliage colour and type and flower colour vary depending on cultivar. Some examples include cv Golden Hue, (pale yellow foliage, with orange and red tints in winter, pale amethyst flowers, upright habit, up to 35 cm tall), cv Lilacina, (bright green foliage, pale mauve flowers, up to 30 cm tall), and cv Velvet Night, (dark green foliage, deep beetroot flowers, up to 20 cm tall). Plate 1. which follows page 14, shows a range of *C. vulgaris* and *Erica* spp. displayed in a private garden in Dorset.

1.1.1.4 *E. VAGANS* - CORNISH HEATH

This species is native to South West Cornwall and limited areas in Western Europe where soils are neutral or slightly alkaline. It forms an erect to spreading shrub, 20 - 80 cm tall and flowers from July to October. Flower colour varies from white (e.g cv Lyonesse), to deep pink-red (e.g. cv Mrs D.F. Maxwell)

Plate 1 A range of cultivars of *C. vulgaris* and *Erica* spp. grown in a private garden in Dorset.

1.1.1.5 *ERICA x DARLEYENSIS*

This is a natural hybrid between *E. carnea* and *E. erigena* which forms a vigorous shrub up to 40 cm tall. It flowers from December to May. Flower colour varies, e.g. cv Silberchmelze which has silver-white flowers, and cv Ghost Hills which has light purple-pink flowers.

1.1.2 Stock plants

C. vulgaris and *Erica* spp. can be propagated by layering, division and from seed, however these methods are rarely used by commercial nurserymen. Cuttings taken from stock plants provide the only reliable means of producing large numbers of uniform, quality plants from a small nucleus of mother plants. Stock plant management regimes differ depending on the situation of the nursery, in addition to the growers' personal preferences.

Stock plants were formerly maintained in open ground. However, such a system is only used where climate, site and soil are considered ideal, for example in Holland, Germany and parts of Southern England.

Several growers in England are now using an improved version of the open ground system developed at Efford EHS by Scott (1979). This involves planting the heathers in soil on raised beds covered with woven polypropylene, thus aiding drainage, ensuring good weed control and reducing the transmission of diseases from soil to plant. The beds in both the above systems are usually sterilised and re-planted every 7 - 15 years.

An increasing number of growers now keep their entire stock in containers, re-potting the plants each year until they reach the age of 6 - 10 years. They may be kept in the open, or under netting or polythene for all or part of the year. There are several advantages with this method. Firstly, it is easier to keep the plants in good condition as the grower retains complete control over nutrition (and watering if kept under protection), and can easily remove any unhealthy specimens before they affect neighbouring plants. Secondly, the older stock plants can be sold at a premium price once their useful life is over; and thirdly, when the plants are kept under protection, shoot growth commences earlier in the year, thereby allowing for larger and earlier cutting harvests. Plate 2 which follows page 16 shows stock plants maintained in a net-sided polythene tunnel on capillary beds on a nursery in Argyll.

All stock plants are trimmed annually to encourage shoot proliferation. Crop protection chemicals are used to varying degrees to control weeds, pests and diseases. (See section 1.1.9).

1.1.3 Propagation

Propagation techniques vary greatly depending on personal preferences. In general, simple, semi-ripe cuttings of 2.5 - 4 cm are taken from stock plant shoots, (either above or below the flowers), then inserted in compost where they root under protection in 3 to 6 weeks.

Most growers take cuttings from June to August,

Plate 2 Stock plants in a net-sided polythene tunnel on capillary beds on a nursery in Argyll.

Plate 3 *C. vulgaris* plants, 3 weeks after being potted, growing in a net-sided polythene tunnel on a nursery in Argyll.

although some are now extending the propagating season to cover 12 months of the year. Heated beds are often used to aid rooting during winter and early spring. Hormone rooting powder is not usually necessary. In some cases cuttings are struck in standard seed trays or polystyrene boxes with 100 to 200 cuttings per tray. However, many growers are now using modular or plug trays with 54 to over 300 cells per tray. It is thought that the use of such trays allows for faster establishment and growth of plants once potted. Trays of cuttings are placed on moist sand, gravel, peat or capillary matting in tunnels, frames or glasshouses, and are either covered in polythene or placed under mist until rooting takes place. Although cuttings taken at most nurseries are overwintered in trays, an increasing number of UK growers are potting in the summer or autumn, thereby producing a crop of heathers in one season. Cuttings are taken, rooted, potted, grown-on and sold in 6 - 9 months, i.e. less than half the time formerly taken on many nurseries, thereby giving increased turnover and profits.

1.1.4 Container production

Although the practice of planting rooted cuttings out to grow in open ground still continues in parts of Holland and Germany, all *C. vulgaris* and *Erica* spp. produced and sold in Great Britain are container grown. Container production systems are less labour intensive and less

dependent on soil type. In addition the product is easily transported, can be planted or re-potted all year round, and has a longer shelf-life in garden centres than do bare-root plants.

Rooted cuttings are potted into 7 - 9 cm pots by hand or machine. They are then set on gravel or sand outdoors or under net or polythene tunnels. They are sold as liners or re-potted following 4 - 9 months growth. Most container grown heathers are re-potted only once prior to sale, although an increased number are being re-potted and grown on for sale in 1, 2 and 3 l pots. Plate 3, which follows page 16, shows *C. vulgaris* plants, 3 weeks after potting, grown in a net-sided polythene tunnel on a nursery in Argyll.

1.1.5 Composts used in container production

The structure and composition of composts used in container production are acknowledged as being of great importance if quality plants are to be produced. (MacDonald, 1986). Ericaceous plants including heathers are in general calcifuge and salt sensitive (Thomas, 1983; Streit & Creed, 1986) and for this reason require composts with low pH and low levels of fertilisation. Compost structure must allow for good aeration and drainage whilst providing a stable environment for root growth and development.

Propagation media vary. Fine peat alone or fine peat amended with perlite or composted pine bark are often

used. Proprietary composts such as Bulrush Propagation Compost are also used. Few nurserymen add lime or fertiliser during propagation, liquid feeding usually being applied following rooting, prior to potting if necessary.

The nature of composts used for potting varies greatly depending on personal preference. Coarse sphagnum peats usually form the base of the compost (70 - 80% of the total volume) with one or more additives such as composted pine bark, perlite, grit or more rarely loam, sand, vermiculite, clay or rockwool being added, (5 - 30% of the volume). The list on page 20 shows several commonly used compost constituents, their characteristics and effects on compost properties.

1.1.6 Major compost constituents

PEAT - Usually the main constituent.

Young, coarse sphagnum types, pH 4.0 - 4.5 usually used.

Some growers use pure peat as compost. Wetters are necessary otherwise watering proves very difficult if compost is allowed to dry out.

BARK - Various types available, granulated composted pine bark is considered best of those available in the UK.

Used to increase compost AFP.

Also acts as a buffer by absorbing potentially damaging salt excesses.

Can result in excessive lock-up of fertiliser nitrogen which may necessitate addition of extra nitrogen.

Usually included as 20 - 30% of compost volume.

GRIT - Particle size 2 - 3 mm.

Must contain no lime.

Increases compost weight.

SAND - Rarely used now as seriously reduces compost AFP and reduces drainage if more than 10% by volume is used.

Helps to prevent compost drying out.

Must be lime free.

LOAM - Rarely used now as thought unnecessary and sources of good loam difficult to obtain.

Provides good buffering capacity.

PERLITE, POLYSTYRENE, VERMICULITE, ROCKWOOL etc. - Increase compost AFP (especially perlite) and improve drainage.

1.1.7 Compost fertilisation

Nutrition of container-grown heathers is a complex problem (Scott, 1981; Thomas, 1983). Deficiency symptoms become obvious if the level of fertilisation is too low, but an excess of salts will also produce a deterioration in plant quality. It has been shown that several crop species become increasingly susceptible to disease when suffering from nutrient deficiencies or excesses (Baker & Martinson, 1970). This may also occur with *C. vulgaris* and *Erica* spp., although there is no evidence in the literature to show that this is the case.

There are several approaches used in the nutrition of *C. vulgaris* and *Erica* spp. Some growers prefer to use only liquid fertiliser to supply both major nutrients and trace elements. They feed according to season, current weather and growth stage of the crop. However, this method demands considerable effort and expertise if success is to be achieved. Most UK nurserymen now use resin coated controlled-release fertilisers plus fritted trace elements. The fertilisers are added to the compost along with magnesian limestone (to give a pH of 4.5 - 5.5) prior to potting, and supply adequate nutrients until re-potting or sale.

Additives such as wetting agents (to increase wettability of peat) and crop protection chemicals, may also be added to compost during mixing.

1.1.8 Marketing, sales and use of heathers

In the UK, around 70% of heathers are sold directly to garden retail outlets. Only 30% are sold through mail order, to distributors, other nurseries or local authorities. The quality of heathers for sale in garden centres and shops is variable and often poor. After being removed from the nursery, plants are often subjected in the garden centre to unsuitable temperatures and lighting, and a lack or an excess of water or nutrients. When placed under such stresses, containerised heathers quickly lose condition and may be rendered unattractive or unsaleable.

Several workers have shown that stressed plants are more susceptible to disease (Baker & Martinson, 1970). For this reason heathers that harbour pathogens may rapidly develop disease symptoms in the event of unsuitable environmental conditions, following sale to retail outlets.

Following sale, almost all nursery-grown heathers are destined for use in large or small private or public gardens. They are frequently planted in drifts alongside conifers and other ericaceous species or often form part of alpine or rock gardens. If trimmed and kept free of weeds for 2 to 3 years following planting, they will provide a low maintenance, relatively trouble free display for 15 to 20 years.

1.1.9 Crop protection on heather nurseries

Only in the past decade have growers required to use crop

protection chemicals on *C. vulgaris* and *Erica* spp. The use of modern growing materials and techniques such as fertilisers, polythene tunnels, controlled irrigation and large scale monoculture, have led to the creation of conditions whereby disease development is often favoured. The presence of pests and weeds may also require remedial attention.

Heathers rarely suffer from pest attack, although chemicals are sometimes necessary to control leatherjackets, sciarid flies, vine weevils or red spider mite. Most growers control weeds on standing beds prior to setting plants out, with a herbicide such as diphenamid (Enide, ICI). Subsequent weeding may be done by hand or with herbicides such as simazine (Gesatop, Ciba Geigy).

Reports of shoot browning, root rot and plant death have increased over the past 10 years. Although little work has been published with reference to diseases on *C. vulgaris* and *Erica* spp., frequent reports of both root and foliar pathogens occur in annual reports of research stations involved in advisory plant pathology e.g. Boskoop Research Station, Boskoop, Holland; The Scottish Agricultural College (SAC), Auchincruive, Ayr; Bad Zwischenahn Research Station, Weser-Ems, West Germany.

The pathogen *Botrytis cinerea* Pers.:Fr. can cause severe, extensive foliar browning on heathers of all ages under conditions of high humidity (Smith & Evans, 1990), and can cause death of large numbers of cuttings during

propagation. Fungicides such as benomyl (Benlate, Du Pont), and iprodione (Rovral, Hortichem), are used to control the disease. *Pestalotiopsis guepinii* (Desm.) Stey. although less common, can also cause browning of shoots and flowers.

Several pathogens including *Rhizoctonia* spp., *Pythium* spp., *Cylindrocarpon destructans* (Zinssm.) Scholten and *Fusarium* spp. have been reported in papers, (Hutchinson, 1983; Humphreys-Jones, 1976), and in newsletters (Litterick & Holmes, 1988a, b & c; 1989 and 1990a). The incidence of diseases on heathers is also recorded in advisory records and annual reports of research stations including SAC, Auchincruive, Boskoop Research Station, Holland and Bad Zwischenahn Research Station, West Germany.

Pythium spp. are weak pathogens which are often isolated from roots of heathers showing foliar browning and root death (SAC, Auchincruive advisory case records, unpublished). *Phytophthora* spp. cause severe root rot and death of large numbers of plants on continental nurseries. Outbreaks of disease caused by *Phytophthora* spp. are uncommon on heathers in Great Britain. Growers can treat heather crops routinely with fungicides to control diseases caused by the phycomycete fungi *Phytophthora* and *Pythium* spp.

Cylindrocarpon destructans and *Fusarium* spp. have also been isolated from the roots of heathers showing foliar browning and root rot. Several broad spectrum fungicides

such as prochloraz (Octave, Fisons) benomyl and iprodione are thought to control these diseases. However, no results of work to confirm this have been published.

Rhizoctonia spp. are acknowledged as being the most important and damaging pathogens of commercially produced *C. vulgaris* and *Erica* spp. (Streit & Creed, 1986; Mygind, 1978). Although little work has previously been published regarding this pathogen on these genera, many growers both in the UK and in Europe have reported problems due to *Rhizoctonia* spp. through the advisory services.

The level of pesticide use varies greatly both between countries and between nurseries within countries. Discussions with several growers both in the UK and in Europe have revealed that pesticide use is in general, much greater in Holland and West Germany than in the UK. Most nurserymen in this country use herbicides and insecticides if required. However, the level of fungicide use varies, with some nurseries using no fungicides or a limited programme to control one or more pathogens. Others employ a full prophylactic fungicide programme at all growth stages to prevent infection by all pathogens which they consider to be potential problems.

Although the crop protection chemicals available for use on hardy nursery stock may be effective in controlling the pests, diseases and weeds for which they are intended, their use on heather crops can create problems (Litterick & Holmes, 1990b). Both *C. vulgaris* and *Erica* spp. are

extremely sensitive to many chemicals and the use of pesticides can result in severe phytotoxicity symptoms such as foliar browning and reduced root development. Table 2 shows the effects of three fungicides applied singly and in combination on the root development of *C. vulgaris* cuttings, and Plate 4, which follows page 27, shows *C. vulgaris* cv Golden Turret cuttings 12 weeks after striking in standard propagation compost which contained furalaxyl (Fongarid, Ciba Geigy) alone and furalaxyl plus tolclofos-methyl. No data regarding the effects of pesticides on the development of rooted liners or stock plants has been published.

The number of fungicides available to control diseases on *C. vulgaris* and *Erica* spp. in the UK is limited, due to recently introduced pesticide regulations (Food and Environment Protection Act, 1986), which state that no product may be used on any crop unless it has been proven to be both safe and effective. Although heathers and other HONS species are high value crops, the areas requiring fungicide treatment are small in comparison to those of arable crops. The revenue gained from the sale of pesticides to the horticulture industry is low compared with agricultural sales, therefore few chemical companies are prepared to pay for trials to be carried out to gain approval for the use of their products on ornamentals.

There is only one fungicide approved for use on heathers to control *Rhizoctonia* spp.; i.e. iprodione; several including benomyl and iprodione to control

Table 2 Foliage scores[a] and root weights of *C. vulgaris* cvs Mousehole and Loch Turret cuttings grown in standard propagation compost amended with tolclofos-methyl, furalaxyl and iprodione, assessed 7 weeks after cuttings were struck.

		Mousehole		Loch Turret	
Treatment	Rate (g a.i./l compost)	Mean root weight (g)	Mean foliage score	Mean root weight (g)	Mean foliage score
Control	-	1.42	0.00	1.10	0.00
Furalaxyl	0.100	0.79	0.00	0.31	0.00
"	0.200	0.33	0.83	0.24	1.33
Tolclofos-methyl	0.040	0.78	0.00	0.67	0.00
Iprodione	0.005	0.76	0.00	0.58	0.00
Furalaxyl + Tolclofos-methyl	0.100+ 0.040	0.70	0.00	0.61	0.00
Furalaxyl + Iprodione	0.100+ 0.005	0.63	0.00	0.40	0.50
Furalaxyl + Tolclofos-methyl	0.200+ 0.040	0.18	1.50	0.16	1.33
Furalaxyl + Iprodione	0.200+ 0.005	0.36	0.66	0.39	1.50

SED (root weight means, 8 d.f.) = 0.074; $p < 0.001$

[a] 0 = no foliar browning, 4 = totally brown foliage

NB. Data taken from Justice (1987, BSc Thesis)

Plate 4 *C. vulgaris* cv Golden Turret cuttings 12 weeks after striking in standard propagation compost: left, unamended; middle, 0.1 g furalaxyl incorporated/l compost right, 0.1 g furalaxyl + 0.04 g tolclofos-methyl incorporated/l compost.

Botrytis and four to control phycomycete fungi, including propamocarb hydrochloride (Filex, Fisons) and furalaxyl. There is a wider range of Rhizoctonia fungicides available on the continent, including tolclofos-methyl (Basilex, Fisons), pencycuron (Monceren, Bayer), PCNB (Quintozene, RP Environmental) and furmecyclox (Campogran, BASF).

Further chemicals could be made available to grower's through the 'off label approvals scheme', whereby the approval for use on edible protected crops can be extended for use on non-edible protected crops at the growers own risk. A greater range of chemicals than those currently available is necessary if good disease control is to be effected without the risk of fungicide resistance building up amongst pathogen populations.

1.2 THE GENUS *RHIZOCTONIA*

In the genus *Rhizoctonia* DC. ex Merat, over 100 often unrelated types of sterile mycelia have been described (Talbot, 1965). The best known of these, *Rhizoctonia solani* was discovered and named around 120 years ago by Julius Kuhn who found it growing on diseased potato (*Solanum tuberosum* L.) tubers (Menzies, 1970). Since then, this fungus along with many other *Rhizoctonia* spp. later recorded, have gained the reputation of being damaging, highly adaptable pathogens with widespread distribution (Parmeter & Whitney, 1970). *Rhizoctonia* spp. occur in all parts of the globe and are sometimes indigenous to

uncultivated areas (Ogoshi, 1985).

1.2.1 Taxonomy, nomenclature and morphology of *Rhizoctonia* spp. and *Thanatephorus* spp.

Rhizoctonia is considered a genus of basidiomycetous imperfect fungi without clamp connection. The genus is characterised as follows (Ogoshi, 1975):

1. Branching near the distal septum of cells in young vegetative hyphae.
2. Formation of a septum in the branch near the point of origin.
3. Constriction of the branch.
4. Dolipore septum.
5. No clamp connection.
6. No conidium (except monilioid cell).
7. Sclerotium not differentiated into rind and medulla.
8. No rhizomorph.

The genus *Rhizoctonia* is divided into two groups, namely multinucleate and binucleate species. Multinucleate types have many nuclei (usually more than three) in young vegetative cells. They have a larger hyphal width (about 6 - 10 um) and a teleomorph called *Thanatephorus* (Talbot, 1970). Binucleate *Rhizoctonia* spp. have only two nuclei per cell (rarely one or three) have a smaller hyphal width (4 - 7 um) and a teleomorph called *Ceratobasidium* Rogers 1935 (Burpee *et al.*, 1980b). The identification of

most *Rhizoctonia* spp. is very difficult due to the simplicity and similarity of vegetative structures belonging to the different species/groups (Ogoshi, 1975).

There has been a great deal of confusion in the past concerning the nomenclature of *Rhizoctonia* spp. and their teleomorphs. The genus *Ceratobasidium* now contains several species very similar to *T. cucumeris* (Frank) Donk, (the teleomorph of *R. solani*), but which have binucleate hyphal cells and generally no sclerotia (Parmeter *et al.*, 1967; Tu *et al.*, 1969; Burpee *et al.*, 1980a). Examples include *C. cornigerum* Rogers and *C. anceps* Jackson.

The genus *Thanatephorus* has several synonyms including *Corticium* Fr., *Hypochnus* Fr. and *Pellicularia* Cooke which are no longer in use (Domsch *et al.*, 1980). It contains about six species including *T. cucumeris*. *T. cucumeris* has several synonyms (no longer in use), including *Hypochnus cucumeris* Frank, *Corticium solani* (Prill. & Delacr.) Bourd & Galzin, *Hypochnus solani* Prill. & Delacr., *Pellicularia filamentosa* (Pat.) Rogers, *Hypochnus filamentosus* Pat. and *Ceratobasidium filamentosum* (Pat.) Olive.

In common with the mycelial state, *T. cucumeris* is regarded as a vaguely delimited species aggregate (Talbot, 1965). The combination of fast growth, monilioid hyphae, brown pigmentation, conspicuous dolipores, branching close to hyphal septa in young hyphae and multinucleate cells are considered as sufficient for the reliable

identification of plant pathogenic isolates or soil isolates as *T. cucumeris*.

R. solani is the only commonly found multinucleate species. *Thanatephorus sterigmaticus* Warcup & Talbot and *T. orchidicola* (Bourd.) Talbot are also multinucleate but are rarely isolated and have a limited distribution. *R. solani* is highly varied in its pathogenicity, morphology of sclerotia, cultural appearance *in vitro*, growth rate etc. (Ogoshi, 1985). Many attempts have been made to divide the species into logical groups. Two main methods have been used. One based on the differences in pathogenicity, morphology and ecology of the fungus, and the other by means of hyphal anastomosis on agar media.

1.2.1.1 MORPHOLOGICAL AND PATHOLOGICAL GROUPS

Takahashi & Matsuura (1954) divided *R. solani* into six groups by morphology on culture media and pathogenicity. They recognised six "formae speciales", namely *solani, microsclerotia, sasakii, timsii, betae* and *compacta*. Later, in 1966, Watanabe & Matsuda divided *R. solani* into seven groups based on ecological character, cultural type and character of pathogenicity. Although their nomenclature was different, six of their groups corresponded with those proposed by Takahashi & Matsuura in 1954. However, they created a seventh group which they named 'rush type' isolates.

1.2.1.2 HYPHAL ANASTOMOSIS GROUPS

When two different isolates of *R. solani* are placed 2 - 3 cm apart on agar media, their mycelia grow and overlap each other. If the isolates belong to the same anastomosis group (AG), then hyphal fusion occurs which is visible under the light microscope. Usually attraction of hyphae and death of fused cells occurs. If the isolates belong to different AGs, neither hyphal fusion nor cell death occur. Hyphal fusion occurs only among isolates of the same AG, therefore there is no movement of genetic factors between groups.

Schultz (1936) reported five AGs, namely Gruppe I (*hortensis*), II (*brassicae*), III (*typica*), IV (*cichorii endiviae*) and V (*fuchsiae*), all as varieties of *R. solani* (*Hypochnus solani*). His Gruppe V is now considered to be a binucleate *Rhizoctonia* spp. rather than a variety of *R. solani* (Parmeter & Whitney, 1970). Richter & Schneider, (1953) reported six AGs of *R. solani*; Fusiongruppen A, B, C, D (Cruciferen), E and F (Kartoffel). Gruppe E is not *R. solani*, but a binucleate *Rhizoctonia* spp. (Parmeter & Whitney, 1970). Parmeter *et al.* (1969) concluded that *R. solani* should be divided into AGs 1, 2, 3 and 4 based on hyphal fusion. They also discussed the possibility of the existance of additional anastomosis groups and the possibility of bridging isolates, i.e. those capable of anastomosis with members of two or more groups. This phenomenon has since been verified (Homma *et al.*, 1983; Neate & Warcup, 1985; Rovira *et al.*, 1988).

Ogoshi (1975) attempted to divide the Japanese isolates of *R. solani* into groups based on hyphal fusion. He concluded that each of the 255 isolates tested could be assigned to one of five AGs (AG - 1 to AG - 5). The remaining isolates were not assigned to any AG at that time.

AG - 2 was then further divided into type 1 and type 2 (AG - 2-1 and AG - 2-2), based on the relative frequency of hyphal fusion, with fusion between two isolates of the same type in AG - 2 being frequent, but that between two isolates of different types in AG - 2 being rare (Ogoshi, 1975).

Kuninaga *et al.* (1978) added AG - 6 and AG - BI (bridging isolates) to Ogoshi's six groups, following which Homma *et al.* (1983) reported an eighth group (AG - 7), and Neate & Warcup (1985) added a ninth group (AG - 8). Recently, Carling *et al.* (1987) reported a tenth group (AG - 9).

Thus there are currently ten AGs, one of which is further divided into two subgroups; (AG - 1, AG - 2-1, AG - 2-2, AG - 3, AG - 4, AG - 5, AG - 6, AG - 7, AG - 8, AG - 9 and AG - BI).

Not all AGs have been found throughout the world. AGs 1 to 4 have been reported in many countries in Asia, the Middle and Near East, Europe, (including the UK) and North America. However, AG - 5 occurs only in some countries, (not the UK), AGs 6, 7 and BI have been reported only in

Japan, AG - 8 only in Australia and AG - 9 only in Alaska. The work on distribution of AGs of *R. solani* is not yet complete, however it is clear that the arrangement of *R. solani* isolates in anastomosis groups is the most useful classification system yet developed for the fungus (Ogoshi, 1985; Anderson, 1982).

It has been shown that in a small area, the AGs that occur are limited. It is thought that the crop grown exerts an influence on the prevalence of a given AG in certain areas (Ogoshi, 1985). For example, AG - 1 has been isolated mainly from the *Leguminosae* and the *Graminae*. AG - 2 has been isolated from the *Cruciferae, Chenopodaceae* or *Graminae*, AG - 3 from the *Solanaceae* and AG - 4 from the *Chenopodaceae, Leguminosae* and *Solanaceae*.

The existence of pathogenic, binucleate *Rhizoctonia* spp. has been confirmed in the past two decades. The classification of such species has caused problems in the past (Parmeter & Whitney, 1970; Burpee *et al.* 1980a), and it is thought that some earlier references to diseases caused by *R. solani* in fact were caused by binucleate *Rhizoctonia* spp. (Parmeter *et al.*, 1967). These species possess mycelial characteristics identical to those of *R. solani* except that hyphal cells are predominantly binucleate and mycelium ranges from hyaline to brown. More than twenty form species of *R. solani*-like fungi have been described (Burpee *et al.*, 1980a), many of which cause serious plant diseases. Many workers have now begun to classify such species by means of AGs in a similar way to

that used for *R. solani* isolates.

Anastomosis groups of binucleate *Rhizoctonia* spp., (Ceratobasidium anastomosis groups or CAGs, as they have been termed by Burpee *et al.* 1980a), are distinct from the AGs of *R. solani* as proposed by Parmeter *et al.* (1969). Burpee *et al.* (1980a) have classified binucleate *Rhizoctonia* spp. into seven CAGs (CAGs 1 to 7). With the exception of CAG - 1, (which has been isolated mainly from *Graminae*), none of the groups can be defined by host range or geographic origin. Ogoshi (1985) has classified binucleate *Rhizoctonia* spp. into fifteen anastomosis groups (AG - A to O). There is evidence that many of the groups erected by Burpee *et al.* (1980a) correspond closely with those of Ogoshi (Ogoshi, 1985). However, no common nomenclature system has yet been agreed amongst workers.

1.2.2 Growth of *Rhizoctonia* spp. in soil

Previous work on Rhizoctonia diseases concerned the occurrence, morphology, epidemiology and control of strains of *R. solani*. There has been little work carried out on the growth and behaviour of binucleate *Rhizoctonia* spp. as saprophytes or pathogens in soils and composts. For this reason, much of the following discussion concerning *Rhizoctonia* spp. refers to *R. solani*. However, as previously stated, there was formerly much confusion regarding the nomenclature of *Rhizoctonia* spp. Binucleate species may have been mistakenly classified as *R. solani*.

Several workers demonstrated that *R. solani* could be isolated from natural soils by screened immersion plates (Thornton, 1956), by immersion tubes, (Chesters, 1948), by colonisation of organic substrate segments (Papavizas & Davey, 1959) or from seeds (Kendrick & Jackson, 1958). It was concluded that the parasite exists as active mycelium in soils. Boosalis & Scharen (1959) demonstrated that it can also exist in soil and organic materials as sclerotia.

Blair (1943) was the first person to study the growth of *R. solani* in soils. He demonstrated that the fungus could grow in unsterilised soil for relatively long distances without any energy sources other than those present in natural soils, and independently of the original inoculum.

R. solani and many other *Rhizoctonia* spp. are in general very efficient saprophytes (Papavizas, 1970), their saprophytism depending on several factors including growth rate, enzymatic ability, antibiotic and/or toxin production, tolerance of antibiotics and toxins produced by other micro-organisms, and the number and variety of antagonists present on the substrate being colonised. Both the speed and extent of growth and the ability of the fungus to cause disease are dependent on the quality and quantity of the substrates being colonised (Garret, 1956). For example, Boyle (1956) found that crop refuse served as a food base enabling a *Rhizoctonia* sp. to attack peanut (*Arachis hypogea* L.) plants. Agronomic practices designed to keep organic material out of the upper soil regions,

gave excellent control of peanut root rot and pod rot caused by the *Rhizoctonia* sp. and *Sclerotium rolfsii* Sacc.

It is difficult to classify *R. solani* as cellulose decomposers or sugar fungi, as the ability of isolates to decompose cellulose varies from very poor to highly efficient. *R. solani* behaves as a primitive sugar fungus in all other respects, which in theory means it cannot also be classed as a cellulose decomposer. It possesses the ability to decompose easily available sugars, it is amongst the first colonisers of injured or dead plant tissues in soil, it forms dormant or resting structures under some conditions, it infects living roots only occasionally depending on existing opportunities, and it has a high intrinsic mycelial growth rate. Garret (1956) concluded that *R. solani* should be classed as a sugar fungus, although somewhat unusual with regard to its cellulose decomposing abilities.

1.2.3 Survival of *Rhizoctonia* spp. in soil

Some *Rhizoctonia* spp. isolates may depend on parasitism for survival. For example *R. solani* did not survive in the absence of a susceptible host in Eastern Kansas when temperatures were too high for the survival of soil-borne mycelium or for inoculum production (Elmer, 1942). In addition, Pitt (1964b) found that *R. solani* declined rapidly after artificial introduction into non-sterile soil. It is unlikely that a fungus so variable in its

ability to colonise soil substrates, would depend on parasitic nutrition alone for its survival in soils.

There is evidence that *R. solani* isolates persist in soil primarily as saprophytes in tissue infected during parasitism, or by saprophytically colonising dead plant material in which it can remain alive for long periods (Boosalis & Scharen, 1959).

Growth and saprophytism may be influenced by non-biotic factors such as temperature, moisture and aeration, soil reaction and by biotic factors such as antibiosis, competition, lysis and available soil nutrients (Papavizas, 1970).

Survival and saprophytism of *R. solani* isolates was found to decrease with time in unsterile soil (Papavizas & Ayers, 1965). It was thought that decline of parasitism and saprophytism was perhaps brought about due to a depletion of certain necessary nutrients in the soil ecosystem. Parasitic activity was found to decline more rapidly than saprophytic activity. Garret (1955) proposed that this was because more energy is necessary for infection than for colonisation.

Conditions affecting the saprophytic existence of an organism such as *Rhizoctonia* spp., which during most of its life exists as a saprophyte in close association with antagonistic microflora, will be of considerable significance in the life cycle of the pathogen, and will greatly influence its subsequent disease potential.

1.2.4 Types of Rhizoctonia diseases and their occurrence

Rhizoctonia spp. cause several different types of disease on a wide variety of plants world-wide, under very diverse environmental conditions. For example, some species cause root rot of wheat (*Triticum* spp.) under semi-arid or temperate conditions, some cause damping off of seeds and seedlings under protection and outdoors, some destroy large areas of submerged aquatic plants and others produce web blight of aerial plant parts in the humid tropics and sub-tropics. *R. solani* and other *Rhizoctonia* spp. can produce varied effects including disfiguring superficial black sclerotia on potato tubers, brown patch on turf grasses, storage rots of vegetables and fruit, and root rot of seedlings, herbaceous plants and woody ornamentals.

The apparent adaptability is chiefly dependent on the large numbers of diverse strains which constitute the *Rhizoctonia* spp. The adaptability does not extend to individual strains encountered in a single situation. However, the coincidence of a suitable environment, a susceptible host in a suitable stage of development and an adapted, aggressive pathogen does occur sufficiently often to make Rhizoctonia diseases some of the most important and damaging known to man.

Although there has been a great deal of work carried out on Rhizoctonia seed and seedling diseases, cereal root rots and tropical web blights, there is very little information available which is relevant to Rhizoctonia diseases of woody ornamentals. *R. solani* has been shown to

attack the roots of conifers (Hartley, 1921) and coffee trees (*Coffea* spp., Crandall & Arillaga, 1955) although the importance of this disease under field conditions has not been demonstrated. Burr *et al.* (1978) showed that *R. solani* was capable of causing severe root rot and death of young apple trees (*Malus domestica* Borkh. (*Malus sylvestris*)) in the USA. They found wilting and necrosis of 1-year-old plants. Numerous, distinct, reddish brown lesions developed on the white, newly formed roots. These lesions sometimes coalesced to produce large necrotic areas that finally encompassed the entire root resulting in collapse and death of trees. Infection was then seen to extend from the roots to the woody stem tissues. These became discoloured and the cortex sloughed off. Frisina & Benson (1987) obtained several isolates of both *R. solani* and binucleate *Rhizoctonia* spp. from the foliage of blighted azaleas (*Rhododendron* spp.) and other woody ornamentals in the USA. In this case the disease consisted of leaf blight and leaf lesions on foliage within the internal portions of the plant canopy. Diseased leaves abscised but remained attached to the plant by means of mycelial webs. Severely defoliated plants were rendered unsaleable.

Cooley (1942) showed that *R. solani* spread to the top of holly (*Ilex* spp.) cuttings from leaves in contact with the soil and caused browning and cutting death. Lambe & Wills (1980) found that *Rhizoctonia* spp. caused severe

aerial (foliar) web blight and root rot during propagation of ornamentals in the USA. The disease could be identified by the reddish-brown mycelium resembling fine threads or webs that appeared on diseased tissue. In addition, the mycelium could be noted on the soil surface growing among the soil particles. Mycelial threads fastened infected leaves to the particles of the propagation medium, thus making it difficult to lift the leaves.

Rhizoctonia diseases of HONS have been reported through advisory services in the UK and on the continent. They are mentioned in the trade press and in annual reports of research stations in the UK, Holland and Germany. No European papers have been published with reference to the species, strain characteristics, host range, symptoms or economic importance of diseases caused by the pathogen. Smith (1982) reported that Rhizoctonia is a serious disease of many woody species in the UK, but did not give detailed information.

R. solani has a very wide host range (Baker, 1970). No claims have apparently been made that any plant species is immune to the fungus, though many plants have been found not to be attacked by given strains of it.

The strains within *Rhizoctonia* spp. may differ in, aggressiveness, the hosts that they are able to attack, the temperature at which attack can occur, the ability to develop in the lower soil levels, the surface layers or the aerial habitat, the ability to tolerate CO_2, the ability to form sclerotia, the growth rate and the

survival ability in a given soil (Baker, 1970).

1.2.5 Infection and disease

Penetration into plants by *Rhizoctonia* spp. may be through intact cuticle and epidermis, either from complex organised infection structures or without defined morphological structures. The pathogen may also enter through natural openings such as lenticels and stomata and through wounds. Although fungal strains often penetrate in only one way, it is not uncommon for some isolates to penetrate the same host in a number of ways (Dodman & Flentje, 1970).

At present, little is known about the factors which lead to initiation of infection. Several workers have found that plant exudates stimulate growth and subsequent infection by *R. solani* (Flentje, 1957; Wyllie, 1962; Martinson, 1965). However, the nature of the host epidermis may also be important in determining whether infection occurs or not (De Silva & Wood, 1964).

The success of a given isolate in causing infection and disease is dependent on a number of host factors including host species and variety or cultivar, host age, (particularly in the case of seedling diseases), health, and the location of the tissue which is being attacked. There are several reports that some isolates of *R. solani* attack only certain parts of plants. Flentje (1957) demonstrated that different isolates may attack stems and

cotyledons but not roots and *vice versa*. He also showed that one isolate will infect the petioles of cabbage (*Brassica oleracea* L.) but not the stems.

Rhizoctonia spp. attack plant tissues with a combination of direct mechanical pressure, enzymes and toxins. Diseased tissues typically show an increased respiratory rate and in some cases a mobilisation of organic and inorganic metabolites to the infection site. Plant cells and cell walls become yellow-brown, tissue collapse occurs and the pathogen may proliferate profusely. Sclerotia may be produced within the decaying tissue.

1.2.6 Resistance to infection

The genetics of pathogenicity and host resistance range from simple 'gene to gene' relationships as seen in the relationship between flax (*Linum usitatissimum* L.) and rust (*Melampsora lini* (Pers.) Lev.; Flor, 1955), to complex situations where host resistance is polygenic as is the case with resistance to most Rhizoctonia diseases (Bateman, 1970).

Attempts to develop resistance to Rhizoctonia diseases in commercially important crop species have met with little success. However, many hosts are known to exhibit resistance to Rhizoctonia diseases with increasing age (Roth & Riker, 1943b; Bateman, 1970). Resistance invariably depends either on the possession of cell wall calcium complexes which the fungus cannot break down, or

the production of antifungal toxins which may kill or limit the speed of growth of the pathogen. The prospects for the breeding of crop varieties resistant to diseases caused by *Rhizoctonia* spp. has improved recently due to the concept of intra-specific groups and AGs (see section 1.2.8.2).

1.2.7 Epidemiology of Rhizoctonia diseases

Epidemiology concerns factors affecting incidence and severity of disease. Several workers have suggested that disease severity be expressed as 'the resultant of inoculum potential and disease potential' (Baker & Martinson, 1970). Inoculum potential concerns the factors influencing the energy available for colonisation of a host, and disease potential concerns the host and its susceptibility to disease.

1.2.7.1 INOCULUM POTENTIAL

Inoculum potential is basically a function of inoculum density which is modified as a result of environmental factors (Garret, 1960). Garret (1956) defined it as being "The energy of growth of a fungal parasite available for infection of the host at the surface of the host organ to be affected". Populations of *Rhizoctonia* spp. in soil are frequently significant although difficult to detect (Baker & Martinson, 1970). In many soils they are present in higher densities than other pathogens, even to depths of 10 to 15 cm (Takahashi & Kawase, 1964). The fungus can be

present as hyphae, sclerotia and basidiospores, (basidiospores are rare in the UK). The inherent pathogenicity of inoculum is dependent on genetic capacity and stored energy. Although parasitism is ultimately governed by genetic factors, nutrition, the presence of host plants and environmental factors are also known to affect pathogenicity (Sims, 1960).

In general, it is found that as inoculum density increases, disease severity increases (Boosalis & Scharen, 1959; Ui & Tochinai, 1955), although the reverse has also been demonstrated in a few cases (Das & Western, 1959).

Rhizoctonia spp. are unlike the classical foliar pathogens in that they are incapable of rapid secondary inoculum production and dissemination. The inoculum potential of *Rhizoctonia* spp. must be maintained in the soil by survival mechanisms.

Several investigators have noted survival of *R. solani* as sclerotia associated with plant debris or as thick-walled hyphae within debris particles (Pitt, 1964b; Chowdhury, 1944). Survival time in soil is dependent on soil moisture and temperatures. The survival of the fungus on the seed tubers of potato and in seeds of weeds and crop plants, is also well documented (Baker, 1947; Neergaard, 1958). In addition to the above mechanisms, *R. solani* has been shown to actively parasitise a wide range of weed species and alternative crops. For example Jager *et al.* (1982) found that 12 out of 52 weed species taken

from potato fields in the Netherlands were infected with *R. solani* and Pitt (1964b) found that the parasitic survival of sharp eyespot fungus (*R. solani*) was significant on other susceptible crops. He discovered that the fungus formed sclerotia on potato tubers and that high levels of sharp eyespot occurred on wheat following potato crops.

1.2.7.2 THE EFFECT OF ENVIRONMENTAL FACTORS ON INOCULUM POTENTIAL AND DISEASE POTENTIAL.

A set quantity of inoculum has a definite capacity for infection of a particular host only under a set of specified conditions. Under a different set of environmental conditions this capacity will usually differ (Dimond & Horsfall, 1960). Similarly disease potential is influenced by environmental factors. The effect of several such environmental factors on both pathogen and host are discussed below.

1.2.7.2.1 Nutrition

Numerous experiments have been performed to examine the effects of externally supplied nutrients on Rhizoctonia disease development. Results vary, but in general a deficiency or an excess of major nutrients pre-disposes plants to infection. For example, fertilisation experiments on agricultural soils revealed that potassium, calcium or nitrogen deficiencies increased disease potential. Disease potential may also increase with excessive nitrogen fertilisation (Baker & Martinson,

1970). The effects of organic manures on the development of Rhizoctonia diseases have also been studied by several workers (Davey & Papavizas, 1960; Snyder *et al.*, 1959). Fresh manures tended to increase disease and well decomposed manures tended to suppress disease, although again results were variable.

1.2.7.2.2 Temperature

The influence of temperature on the development of Rhizoctonia disease has been studied by many. However, an evaluation of the results is difficult because investigations have often been included with those concerning other environmental parameters. It can be concluded that disease severity is not a mere function of fungal growth rate. For example, Richards (1921) discovered that the optimum soil temperature for disease development on peas (*Pisum sativum* L.), potatoes and beans (*Vicia* spp.) was around 18°C and little disease developed above 21 to 24°C. The optimum temperature for fungal growth was around 26°C. In general the effect of temperature on disease development is mediated by both the response of the host and the pathogen.

Isolates vary in their response to temperature. For example Hunter *et al.*, (1960) isolated three strains of *R. solani* from cotton (*Gossypium* spp.), and found that the temperature optima for disease development varied between 24 and 32°C.

1.2.7.2.3 Soil moisture and aeration

The above two factors have been included together as it is very difficult to separate them in the literature. In damping off experiments, disease is usually found to be most severe at moisture concentrations of 20 to 80%. Disease is often less consistent at saturation. This is thought to be due to lack of aeration in the root zone. (Roth & Riker, 1943a; Wright, 1957). A lack of aeration can lead to the accumulation of CO_2 to which many strains of *Rhizoctonia* spp. are extremely sensitive (Blair, 1943). In field experiments, disease development is usually most severe in heavy poorly drained soils. For example, Rolfs (1904) found that such soils favoured the development of *R. solani* on potatoes. In such cases, plant growth may be more severely affected at high soil moisture levels than pathogen development.

R. solani grows optimally at 100% relative humidity (r.h.) and growth is definitely retarded at 99.5% r.h. (Baker & Martinson, 1970). Disease development on aerial plant parts is dependent on free moisture or near 100% r.h. For example sclerotia of *R. solani* causing brown patch of turf germinated at 98% r.h. but required free water or almost 100% r.h. for growth or infection.

1.2.7.2.4 pH

Most strains of *R. solani* grow well over a broad pH range which covers the range of most agricultural soils. Plants in general grow poorly at the extremes of pH which many *R.*

solani strains will tolerate. Most workers have found that pH has no effect on the severity of plant diseases (Sanford, 1947; Pitt, 1964a). However, Jackson (1940) found that damping off of Ponderosa pine (*Pinus ponderosa* L.) and Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco. decreased, as pH decreased from pH 6.5 to values as low as 3.5, and Weindling & Fawcett (1936) successfully controlled damping off of citrus seedlings (*Citrus* spp.) by *R. solani* through lowering the soil pH to 4.0.

1.2.7.3 DISEASE POTENTIAL

Disease incidence and severity is highly dependent on host susceptibility. The ability of a host to contract a disease depends on its genetic resistance to the disease, its growth stage and on environmental influences, as discussed previously.

Most of the hosts of *Rhizoctonia* spp. are particularly susceptible to penetration and tissue invasion during the seedling and juvenile stages of growth, and the plants develop resistance as they age. This is true with for example, crucifers and bedding plants, but it is not the case with HONS species, most of which are propagated by vegetative means and remain susceptible to attack by *Rhizoctonia* spp. to some extent throughout their lives. There has been no work published in English regarding the relative susceptibility of HONS species at different growth stages.

1.2.7.4 THE EFFECT OF MICROBIAL INTERACTIONS ON DISEASE SEVERITY.

Microbial interactions can affect both inoculum potential and disease potential. In some cases strains of *R. solani* can act synergistically with supposedly non-pathogenic organisms in soil (Wright, 1945) creating increased disease and damage. In other cases soil micro-organisms can suppress growth of *R. solani* and disease development through antagonism (Rich & Miller, 1962), thereby reducing plant damage.

1.2.8 Control of *Rhizoctonia* spp.

Rhizoctonia control is effected in cropping situations using one or more of the following methods: modification of cultural practices, host resistance, pathogen eradication or suppression, chemical agents.

1.2.8.1 MODIFICATION OF CULTURAL PRACTICES

By seedbed preparation (through minimising length of time which plant hypocotyls remain exposed to infection by *Rhizoctonia* spp.) judicious timing of sowing, correct cropping sequence and careful husbandry to allow for optimum host development and minimal pathogen development, the effects of Rhizoctonia diseases can be minimised in field crops.

1.2.8.2 RESISTANCE TO INFECTION BY *RHIZOCTONIA* SPP.

It was previously thought that the breeding of varieties resistant to diseases caused by *Rhizoctonia* spp. was

unlikely to be successful for a wide range of crops (Leach & Garber, 1970). *Rhizoctonia* spp. were thought to have a wide host range and the differences between isolates were not obvious. Potatoes (Hofferbert & Orth, 1951) and lettuce (*Lactuca sativa* L.) (Poole, 1952) are two of the few crops for which varieties with some degree of resistance to Rhizoctonia diseases have been developed.

With the concept of intra-specific groups and AGs however, the potential for breeding resistant varieties has improved; i.e. the group causing a distinct disease of a plant has been defined. In most cases, even if several AGs are isolated from a plant, the primary pathogen belongs to a specific AG or intra-specific group, and the target for breeding becomes clearer. For example Yamaguchi *et al.* (1977) have screened for varieties resistant to root rot of sugar-beet (*Beta vulgaris* L.). In this case only *R. solani* AG - 2-2 causes the disease. More recently, McCoy & Kraft, 1984a & b) compared pea varieties for resistance to stem-rot caused by *R. solani* using *R. solani* AG - 4, the primary pathogen.

1.2.8.3 ERADICATION OR SUPPRESSION

Although *Rhizoctonia* spp. are known to exist in most agricultural soils, strain differences make it worthwhile trying to reduce spread of the fungus around farms and nurseries. Avoiding transport of infested soil, transplants, nursery equipment or contaminated or infected seeds helps reduce spread of the disease.

The fungus can be eliminated from glasshouse soils and composts, plant beds, seed trays and nursery materials through the use of heat or chemicals such as soil fumigants and fungicides.

1.2.8.4 CHEMICAL CONTROL AGENTS

Chemical control of Rhizoctonia diseases is practical with many crops and is in many cases the principal method of control. Fungicidal control ranges from general and repeated applications to localised application of volatile or non-volatile chemicals. The nature of the host/ pathogen relationship and cultural methods used for the host, determines the way in which fungicide is best applied.

Selective fungicides such as tolclofos-methyl (e.g. Basilex, Fisons) may control the fungus but not other fungi. Broad spectrum materials such as dazomet (Basamid, BASF), will eradicate the entire soil flora within their range. All chemicals must be used at rates that will effectively control the pathogen without causing host phytotoxicity. Fungicidal control is only practical if the chemicals used are compatible with others (including fertilisers) necessary for crop production, and if the value of the crop merits their use.

A wide range of fungicides are used throughout the world for control of *Rhizoctonia* spp. The results of investigations into fungicide efficacy differ a great deal. The differences are probably due to variations in

experimental techniques and environmental/cultural conditions in the field as well as differences in sensitivity to fungitoxicants between the different *Rhizoctonia* spp. and strains (Kataria & Grover, 1975a).

The protectant organophosphorus fungicide tolclofos-methyl has been shown by several workers to be highly effective in controlling diseases caused by *Rhizoctonia* spp. For example, Inskeep & Filonow (1986) found that tolclofos-methyl applied at 5 - 6 kg a.i./hectare controlled pre-emergence damping-off in peanuts grown in Rhizoctonia-infested soil.

The protectant dicarboximide fungicide iprodione (e.g. Rovral WP, Embetec) is widely acknowledged as being effective in controlling many strains of *Rhizoctonia* spp. For example, Martin *et al.* (1984b) found that sprays of iprodione (100 and 1000 mg a.i./l) prevented infection of tall fescue (*Festuca arundinacea* Schreb.) by all *Rhizoctonia* spp. tested; and Frisina & Benson (1988) found that sprays or drenches of iprodione (900 µg a.i./ml) effectively limited the aerial mycelial growth of both *R. solani* and binucleate *Rhizoctonia* spp. on azaleas under greenhouse conditions.

There have been several reports of resistance of *Rhizoctonia* spp. to both tolclofos-methyl and iprodione (Gualco *et al.*, 1983; Ariena *et al.*, 1984; Gullino *et al.*, 1984). It is thought that this will not become a field problem, since fungicide resistant strains have reduced fitness and because fungicides with different mechanisms

of action can normally be alternated by growers in the field.

The protectant fungicide, quintozene (or pentachloronitrobenzene, e.g. Quintozene WP, Rhone Poulenc Environmental Products) has been found by many workers to be effective in controlling a range of diseases caused by *Rhizoctonia* spp. For example, Kataria & Grover (1975b) showed that quintozene (wettable powder) at 0.3% (0.225% a.i.) gave effective control of *R. solani* on sugar beet. There have been numerous instances where it has proved ineffective in controlling disease. For example, Martin *et al.* (1984b) reported that quintozene did not control disease caused by *R. zeae* Voorhees and an isolate of *R. solani* on tall fescue when sprayed on the plants as an aqueous solution (100 and 1000 mg a.i./l). Shatla & Sinclair (1963) reported a range of tolerance within *R. solani* isolates to quintozene when they were grown *in vitro*. Some isolates were highly tolerant (growth at 10 000 ppm), whereas others were sensitive (growth at 600 ppm).

Other fungicides which have been shown to be effective in controlling growth of *Rhizoctonia* spp. *in vitro* and diseases caused by *Rhizoctonia* spp. include: the carboximide derivatives carboxin, oxycarboxin, furmetamid, furmecyclox etc. (Huppatz *et al.*, 1982; Kataria & Sunder, 1986; Martin & Torres 1986); the benzimidazole fungicides carbendazim, thiophanate methyl and benomyl (Jones &

Belmar, 1986; Taneja & Grover, 1982); the urea fungicide pencycuron (Sumner, 1987; Yamada, 1986); the triazole fungicides, hexaconazole, propiconazole and triadimefon (Jones & Belmar, 1986; Carling *et al.*, 1990; Martin *et al.*, 1984a & b); the phthalimide fungicides captan captafol and chlorothalonil (Bains & Jhooty, 1983; Kataria & Sunder, 1986; Frisina & Benson, 1988)

Fungicide efficacy is dependent on a range of host and environmental factors (Hans *et al.*, 1981; Kataria & Grover, 1975b). The development of a successful fungicide programme for any crop must therefore take into account the production methods and likely environmental/cultural conditions necessary for crop growth.

1.3 OBJECTIVES OF THE STUDY

This investigation into the epidemiology and control of *Rhizoctonia* spp. on *Calluna vulgaris* and *Erica* spp. has several objectives.

a. To examine the sources of *Rhizoctonia* spp. on nurseries and to investigate the means by which they spread.

b. To examine the effects of a number of strains of *Rhizoctonia* spp. on a range of cultivars of *C. vulgaris* and *Erica* spp.

c. To investigate the effects of environmental and cultural parameters on diseases of *C. vulgaris* and *Erica* spp. caused by *Rhizoctonia* spp.

d. To examine the effects of fungicides on, *C. vulgaris* and *Erica* spp., *Rhizoctonia* spp. *in vitro* and on cuttings grown in Rhizoctonia-infested compost.

e. To investigate the possibility of biological control measures for *Rhizoctonia* spp. on *C. vulgaris* and *Erica* spp.

f. To develop an integrated control programme for Rhizoctonia diseases on *C. vulgaris* and *Erica* spp. based on information gained from experiments.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 CULTIVARS USED IN EXPERIMENTS

Cuttings were taken from stock plants of cultivars which are in current commercial cultivation. Cuttings for each experiment were always taken from a single source and where possible from a single plant.

Cuttings and stock plants were taken from a commercial wholesale nursery. Difficulty was often experienced in obtaining cuttings of the desired cultivar/cultivars due to demands of the nursery for cuttings of particular cultivars in large quantities. Although it was not possible to carry out all experiments using the same cultivars, an attempt was made to use only commercially important cultivars and to obtain cultivars used in previous experiments where possible. The following cultivars were used in experiments.

C. vulgaris

Silver Queen	Flamingo	Sister Anne
C.W. Nix	Loch Turret	Dart's Gold
Alba Praecox	Cuprea	Mousehole
Silver Knight	Salmon Leap	Robert Chapman
Beoley Silver	Alba Elata	Golden Feather
Foxhollow Wanderer	My Dream	Braemar
Hammondii	Tricolorifolia	K94
Beoley Gold	Bognie	Rosalind
Firefly	Darkness	J.H. Hamilton
Kinlochruel	Sir John Charrington	

Erica cinerea

Lilacina	Joseph Murphy	Rockpool
Purple Beauty	Golden Hue	Janet
Alba Major		

Erica carnea	*Erica vagans*
March Seedling	Lyonesse
Ruby Glow	Valerie Proudley

2.2 PROPAGATION

Cuttings were taken from the top 5 cm of shoots following flowering, or trimming where possible. Alternatively, cuttings were taken from shoot-bases immediately above branches when shoot-tips were unsuitable due to inadequate tip growth or development.

Cuttings were struck in standard propagation compost (see section 2.4) in black plastic trays (either 23 x 17.5 x 5.5 cm deep, Optipot half size seed trays, or 34 x 24 x 5.5 cm deep, Optipot full size seed trays, Congleton Plastics Ltd.) or 10 x 10 x 8 cm deep polythene boxes with five drainage holes punched in the base (Stewart Plastics Ltd.), and were set on moist gravel or capillary matting in a polythene tunnel or glasshouse. They were covered in white polythene (720 gauge, 180 micron, LBS Polythene) until rooting took place.

2.3 MAINTENANCE OF LINERS AND STOCK PLANTS

Cuttings were potted when rooted in standard potting

compost (see section 2.4) in 8 cm square pots, (Optipot 8K, Congleton Plastics Co. Ltd., then known as liners) and were set in Optipot trays (twenty pots per tray, Congleton Plastics Co. Ltd.) to facilitate easy handling. Stock plants were trimmed following flowering and were potted annually in the autumn in standard potting compost (see section 2.4), in pots 2 l larger than the previous ones. Stock plant pots were spaced 5 - 20 cm apart to allow air circulation and to prevent plant to plant contact. Liners and stock plants were kept in a net-sided polythene tunnel on free-draining gravel, with overhead irrigation.

2.4 COMPOSTS USED IN EXPERIMENTS

The composts used for propagation were as follows;

2.4.1 Standard propagation compost

Formulated as follows:- 1:1 (v/v) Vapo Sphagnum Peat (Finnfibre Horticulture Ltd.) + Fine Grade Cambark (Camland Products Ltd.) Used in Experiments 3 - 9 inclusive, 13, 15 - 17 inclusive and 21.

2.4.2 Bulrush Propagation Compost (Bulrush Peat Co. Ltd.)

Proprietary compost containing 4:1 (v/v) peat + perlite Used in Experiments 19, 24, 26, 27 and 28.

2.4.3 Standard potting compost

The standard compost used for potting of rooted cuttings, liners and stock plants was formulated as follows:-

- 3 parts (v/v) Bulrush Sphagnum Moss Peat (screened 22 mm, Bulrush Peat Company Ltd.)
- 1 part (v/v) Fine Grade Cambark
- 3 kg Ficote 140 14:14:14 (9 month controlled release fertiliser, Fisons plc)/m^3 compost.
- 1.8 kg Dolodust (magnesian limestone, $CaCO_3.MgCO_3$, Clydeside Trading Company Ltd.)/m^3 compost.
- 0.3 kg fritted trace elements (Ferro Chemical Division)/m^3 compost.

Standard propagation compost and standard potting compost were mixed dry in a 0.11 m^3 capacity (4 cu. foot) electric cement mixer for 10 minutes. Water was then added during mixing until the compost became moist.

2.5 DETERMINATION OF *RHIZOCTONIA* SPP. ISOLATED FROM NURSERY STOCK PLANTS

Rhizoctonia spp. were obtained from roots, lower foliage and stem-bases of several nursery stock species during the years 1985 to 1990 inclusive. Some isolates were obtained from plants grown in the UK. Others were isolated in Holland at the Boskoop Research Station, Boskoop, from Dutch-grown nursery stock. They were sent to the Crop Health Centre at Auchincruive (by courtesy of Dr N. Dolmans) in the form of cultures on potato dextrose agar (PDA) slopes. The number of nuclei present in mycelium of the *Rhizoctonia* spp. isolates was determined according to the method used by Bandoni (1979). Single hyphal tip cultures of isolates were grown on water agar (15 g/l

Difco agar) at 23°C in 9 cm diameter Petri dishes, until the fungal colony was 1 to 5 mm from the edge of the plate.

The following solutions were prepared:

2.5.1 Safranin stain solution

Distilled H_2O ---------- 79 ml

0.5% (w/v) Safranin O in distilled H_2O ------ 6 ml

3.0% (w/v) KOH in dist. H_2O ---------- 10 ml

Glycerine ---------- 5 ml

NB. Safranin O dye produced by Eastman Kodak Company

2.5.2 Potassium hydroxide solution

3.0% (w/v) KOH in dist H_2O

A drop of each solution was placed on a microscope slide and the two were mixed with a dissecting needle. Mycelium from the edge of a fungal colony was added and a coverslip placed over the material immediately to prevent overstaining. Microscopic examination (x 400) revealed areas where stain uptake was optimum and nuclei could be counted within cells.

Isolates with mycelial characteristics typical of *R. solani* (e.g. production of monilioid cells, pale yellow to brown cellular pigments and production of sclerotia, see section 1.2.1) and multinucleate hyphal cells were designated *R. solani* (Parmeter & Whitney, 1970), whereas those with mycelial characteristics identical to those of *R. solani* and hyphal cells containing two nuclei, were

classified as isolates of binucleate *Rhizoctonia* spp. (Frisina & Benson, 1987).

Thirty two percent of the *Rhizoctonia* spp. isolates obtained from nursery stock spp., including *C. vulgaris*, were multinucleate (Table 3). These isolates possessed the hyphal characteristics of *R. solani* (see section 1.2.1) and were therefore classified as isolates of this species. Three out of seven isolates taken from *C. vulgaris* were multinucleate. The remaining 68% of *Rhizoctonia* spp. isolates had two nuclei in each mycelial cell. They were therefore designated as binucleate *Rhizoctonia* spp. It had originally been intended to use a further one or two methods for nuclear staining to support the data gained through the use of the safranin stain. For example, Herr (1979) used an HCL-Giemsa staining procedure and two rapid direct staining methods. However, a lack of materials prevented these tests being carried out on the *Rhizoctonia* spp. isolates employed in our experiments.

2.6 MAINTENANCE OF CULTURES

Following isolation, the Rhizoctonia isolates were obtained by removing hyphal tips from mycelia actively growing on PDA. The hyphal tips were transferred to fresh PDA in 9 cm diameter Petri dishes which were incubated at 23^{o}C. Sterile 4 mm cork borers were then used to transfer agar plugs from fully colonised agar plates to test tubes containing approximately 2 g of 2 mm loam which had been

Table 3 Number of nuclei in, and sources of *Rhizoctonia* spp. isolates obtained from nursery stock, potatoes and soil (taken from a field where potatoes were grown two years previous to soil sampling date).

Rhiz. spp. isolate	No of nuclei	Geographic origin	Host/substrate	Source
A	many	UK	potato field soil	A. Litterick,
E	many	UK	potatoes	Crop Health Centre,
B1	many	UK	*C. vulgaris*	Auchincruive.
K1	many	UK	*C. vulgaris*	
52	many	Holland	*C. vulgaris*	N. Dolmans,
10	many	Holland	*Juniperis* spp.	Boskoop Research
55	many	Holland	*Cotoneaster* spp.	Station, Boskoop,
24A	many	Holland	*Chamaecyparis* spp.	Holland.
B	2	UK	potato field soil	A. Litterick, as
F	2	UK	potatoes	above
D1	2	UK	*C. vulgaris*	
64	2	UK	*C. vulgaris*	
M1	2	UK	*C. vulgaris*	
57	2	Holland	*C. vulgaris*	N. Dolmans, as
48	2	Holland	*Juniperis* spp.	above
58	2	Holland	*Juniperis* spp.	
Pr	2	Holland	*Microbiota* spp.	
Pd	2	Holland	*Pyracantha* spp.	
56	2	Holland	*Stephanandra* spp.	
72	2	Holland	*Astilbe* spp.	
24B	2	Holland	*Azalea* spp.	
87-18	2	Holland	*Cytisus* spp.	

sterilised by autoclaving at 1.05 kg/cm^2 and 120^{o}C for 40 minutes, three times at 24 hour intervals. The tubes were then incubated at 23^{o}C for 3 days prior to storage at 4 - 6^{o}C. This technique was adapted from that used by Jones (1974) to store *Helminthosporium victorae* Meehan & Murphy.

2.7 PREPARATION OF STANDARD INOCULUM

Milk bottles which contained 12 g of 5 - 10 mm long pieces of chopped straw + 35 ml distilled water were sterilised by autoclaving for 40 minutes at 120^{o}C and 1.05 kg/cm^2, three times at 24 hour intervals. *Rhizoctonia* spp. isolates were grown in 9 cm diameter Petri dishes on PDA for 7 to 10 days. The plate contents were then chopped into approximately 5 mm^2 sections and 1/4 of the plate contents were scraped into each milk bottle. Bottles were sealed and incubated at 23^{o}C for 7 - 14 days (depending on growth rate of individual isolates), until the mycelium had visibly colonised all the pieces of straw. Plate 5, which follows page 65, shows bottles containing standard Rhizoctonia inoculum and uninoculated straw ready for use. In order to keep the number of isolates of *Rhizoctonia* spp. used throughout the course of the investigation to a minimum, binucleate *Rhizoctonia* spp. isolates D1, 48 and 64 were used in experiments where possible. Occasional contamination of inoculum, (usually by *Penicillium* spp.), meant that other isolates were used, e.g. Expts. 4 and 17.

Plate 5 Bottles containing: left, standard inoculum of binucleate *Rhizoctonia* sp. isolate D1; right, uninoculated straw.

2.8 ASSESSMENT OF FOLIAGE

Foliage condition of both cuttings and liners was assessed using the following scale.

2.8.1 Foliage browning scale

0 - No foliar browning

1 - Tips or bases of a few branches brown

2 - As above, plus one or two branches totally brown

3 - Foliage almost totally brown, but some remaining green areas

4 - Foliage totally brown

In addition, when sufficient growth of shoots occurred, foliage dry weights were determined. The shoot was cut from the plant at soil level, was oven dried at 70^{o}C for 24 hours, then weighed.

2.9 ASSESSMENT OF ROOT SYSTEMS

Root systems of cuttings up to 12 weeks after striking were assessed using the following scale and index system:

2.9.1 Root index system for cuttings

1. The length of the longest root was measured (mm).
2. An assessment was made of the approximate number of roots and scored as follows:

0 = 0 - 10 roots	15 = 21 - 30 roots
5 = 11 - 15 roots	20 = 31 - 40 roots
10 = 16 - 20 roots	25 = > 40 roots

A root index was then calculated for each cutting.

Root index = length of longest root + root score

Root systems of cuttings assessed more than 12 weeks after striking were washed, removed from stem at soil level, oven dried at 70°C for 24 hours then weighed.

Root systems of liners were assessed using the following objective scale:

2.9.2 Scale for assessment of liner roots

Score Description

0 - No roots visible on compost surface after removing pot

1 - Fewer than twenty roots visible as above

2 - Between twenty and 80 roots visible as above

3 - More than 80 roots visible as above

2.10 ISOLATION OF *RHIZOCTONIA* SPP. FROM PLANT PARTS

The presence or absence of *Rhizoctonia* spp. in plant tissue was determined using three methods. These methods were developed by experimentation. For example, in order to devise an effective method for isolating *Rhizoctonia* spp. from plant tissue, a range of agar media, surface sterilants, surface sterilant concentrations and sterilisation times were examined.

The methods chosen for use in experiments (see sections 2.10.1, 2.10.2 and 2.10.3), gave the highest number of isolations of *Rhizoctonia* spp. both from

artificially inoculated and naturally infected plant tissue.

2.10.1 Agar plate isolation

Pieces of tissue (2 - 5 mm long, e.g. root, cutting or stem-base) were surface sterilised in an aqueous solution of Chloros [1:4 (v/v) sodium hypochlorite solution (8% available chlorine) : distilled water] for 20 seconds and were then placed on PDA containing 100 µg/ml erythromycin and 100 µg/ml streptomycin (PDES agar). Mycelium of *Rhizoctonia* spp., if present could be seen growing from the tissue sections within 48 hours (x 100 magnification).

2.10.2 Distilled water floats

Fine roots were removed from the cutting/plant and were washed once in a beaker containing 1 litre of sterile distilled water. They were then placed in 9 cm diameter Petri dishes containing 15 ml sterile distilled water. Mycelium of *Rhizoctonia* spp., if present could be seen growing from the roots within 24 - 48 hours (x 100 magnification).

2.10.3 Damp chambers

The upper 2 - 3 cm of cuttings or 2 - 10 cm branches from rooted plants were wrapped in moistened tissue paper and incubated at 15 - 20^{o}C in sealed polythene boxes (10 x 10 x 14 cm deep, Stewart Plastics Ltd.), for 24 - 72 hours. Mycelium of *Rhizoctonia* spp. was tentatively identified

confirmed on a microscope slide (x 100).

2.11 STATISTICS AND EXPERIMENTAL DESIGN

The experiments were of classical factorial design (Mead & Curnow, 1983, Chapter 6). The layout of treatments for each experiment was randomised within two or more replicate blocks.

Several of the experiments (i.e. those with cultivar as a factor) were of a "split-plot" design. The non-cultivar treatments were allocated to boxes/trays at random. Within each box/tray the cultivars were systematically placed. Despite the inappropriate randomisation, the experiments were analysed as randomised block designs. Evidence from the analysis of variance suggested that this procedure was acceptable for the foliage scores.

Experiments 2, 12 and 23 were assessed once, when treatment effects became apparent. Experiments 3 to 11, 13 to 22 and 24 to 28 were assessed on more than one occasion. The results from only one of these assessments were presented for each experiment. In each case the results which show the clearest treatment effects were included in the thesis. In most cases, such results were obtained on the final assessments.

The data obtained from assessments were of three types, namely binomial data, ordered categorical data and continuous data. The binomial data consisted of presence

and absence data. The ordered categorical data consisted of observations belonging to one of five discrete categories. The continuous data consisted of observations for which all non-negative values were possible.

All experiments were assessed by a single person, and boxes/trays and plants were removed in a random order from the experiment for assessment. All cuttings from each plot were assessed.

2.11.1 Statistical analysis of binomial data

Where isolations were made from cuttings or plants to determine the presence of Rhizoctonia, sampling (standard) errors were calculated for the percentage of infected cuttings in each treatment assuming binomial variation.

2.11.2 Statistical analysis of ordered categorical data

The means of scores of all cuttings from each plot were used for the analysis of variance. Data from foliage assessments, which were in the form of ordered categorical data, were analysed on the original scale, assuming that the differences between the scores were equal. Standard errors of means (SEMs) were used throughout the thesis in the final presentation of data, since this allowed the means to be compared readily with one another.

In cases where all (or almost all) foliage scores for a particular treatment were equal to either 4 or 0, the entire set of results from the treatment were omitted from the analysis of variance. The standard errors from the

means of such treatments were taken to be zero. Although this was not necessarily true, it was generally more accurate than the alternative assumption that the standard errors of the means of all treatments in an experiment were the same.

2.11.3 Statistical analysis of continuous data

2.11.3.1 ROOT INDICES

Standard analysis of variance were carried out on the data from root index assessments. The data were approximately of normal distributions.

2.11.3.2 ROOT WEIGHT MEASUREMENTS

Values comprising the data from root weight measurements were small and were not normally distributed. With data of this type, the smallest non-zero observation was located and added to each value. Analysis of variance was completed on the $\log_{10}$ of these values.

2.11.3.3 DATA FROM MYCELIAL GROWTH MEASUREMENTS IN MM

This was in general of approximately normal distribution. Standard analysis of variance was used.

2.11.4 Partition of treatment effects in analysis of variance

The opportunity to split cultivars and isolates into separate groups when doing analysis of variance was taken wherever possible. This allowed the partition of the variation between groups of treatments and helped to show where within each experiment the main differences

occurred. For example, Rhizoctonia isolates were divided into bi and multinucleate types and heather cultivars were classified according to both cultivar and species.

2.11.5 Diagnostic tests

Two diagnostic tests were carried out on all datasets following analysis of variance. The aim of these tests was to verify that the assumptions of the F and T tests were fulfilled.

2.11.5.1 PLOT OF RESIDUALS V FITTED VALUES

This gave an indication of whether the assumption of equality of variance was satisfied. If the distribution of the residuals obtained remained similar regardless of the fitted values, then this assumption was regarded as being satisfied. If the value of the residuals increased as the fitted values increased, (indicated by a cone-shaped distribution) then the F and T-tests may not have been reliable. Data were often transformed to remedy this defect.

2.11.5.2 QUANTILE V QUANTILE PLOT

This gave a measure of the normality of the distribution of the residuals. A straight-line relationship indicated that the residuals were distributed normally and therefore tests of significance could safely be applied. Any relationship other than a straight line indicated that the residuals were not normally distributed. In such cases the tests of significance were likely to be less reliable and were interpreted with caution.

CHAPTER 3

INVESTIGATION INTO THE SOURCES OF *RHIZOCTONIA* SPP. ON NURSERIES AND THE SYMPTOMS CAUSED BY THE FUNGUS ON *C. VULGARIS* AND *ERICA* SPP.

3.1 INTRODUCTION

It has been shown on several occasions that a knowledge of host pathogen relationships and disease epidemiology is necessary, before successful integrated control programmes for a disease can be formulated (Chase & Conover, 1987; Baker & Martinson, 1970). Since little research had been carried out on the epidemiology of *Rhizoctonia* spp. on hardy nursery stock, it was necessary to examine several aspects concerning the sources of the fungus, the means by which it spreads and the factors affecting its spread. The nature and extent of symptoms caused by a range of both *R. solani* and *Rhizoctonia* spp. isolates on a range of cultivars of *C. vulgaris* and *Erica* spp. were also examined.

The results and conclusions drawn from the experiments described in this chapter, provided basic information essential to the design and implementation of experiments in the following three chapters.

3.2 MATERIALS AND METHODS

3.2.1 Disease Survey - Incidence of *Rhizoctonia* spp. on plants of *C. vulgaris* and *Erica* spp. which showed foliar browning, collected from UK nurseries.

Between August 1987 and December 1989, 37 samples of diseased cuttings of *C. vulgaris* and *Erica* spp. and 42 samples of older plants from several nurseries in various parts of the UK, were examined. Symptoms were noted and the presence or absence of Rhizoctonia infection on the foliage, stem-base, woody roots and fine roots, was

determined as described in section 2.10.

3.2.2 Experiment 1 Investigation into the sources of *Rhizoctonia* spp. on nurseries producing *C. vulgaris* and *Erica* spp.

3.2.2.1 EXPERIMENT 1a PRODUCTION MATERIALS.

From three Scottish nurseries, a total of 30 samples of each of new and used capillary matting, several sizes of plastic propagation trays and white polythene (used for shading and as an anti-desiccant during propagation) were collected. The samples were cut into 2 cm^2 pieces and were examined under a dissecting microscope (x 40) for mycelium of *Rhizoctonia* spp. Pieces of material bearing mycelium tentatively identified as *Rhizoctonia* spp. were cut out, into 3 - 5 mm^2 pieces, washed and placed on PDES agar. The presence of *Rhizoctonia* spp. mycelium was then confirmed after 24 - 48 hours using a microscope (x 100).

3.2.2.2 EXPERIMENT 1b COMPOST CONSTITUENTS.

Fifty samples (250 ml) of each of the following growing media were placed in 8 cm square pots (Optipot 8K, Congleton Plastics Ltd.): Unused Vapo Peat (Finnfibre Horticulture Ltd.), unused Bulrush Peat (Bulrush Peat Company Ltd.), unused Cambark (Camland Products Ltd.), unused Scotbark (Scotbark, Inverness), soil from a field which had contained a potato crop for 2 years previous to sampling, soil from ground surrounding polythene tunnels on a nursery which produces *C. vulgaris* and *Erica* spp. and compost which had been used in propagation trays for *C.*

vulgaris cuttings. One glass microscope slide (2 cm x 5 cm) was pushed lengthways down the side of each pot until the 2 cm side was parallel to, and touching the base of the pot, and two 2 cm^2 nylon mesh bags (1 mm mesh, sealed on all four sides) containing three radish seeds (Carters, variety French Breakfast) were buried in each pot. In addition, a single 1 ml capillary blood collection vial (CB 1000, Sarstedt) containing Richard's agar (Johnson & Curl, 1972), and pierced at five points down the length of the vial using a red-hot dissecting needle to allow fungal access, was pushed downwards into the sample from the surface, until the top of the vial was level with the compost surface. Plate 6, which follows page 76 shows the slides, nylon mesh bags containing radish seeds and blood collection vials used in the experiment. The samples were kept moist in a glasshouse at 12^{o} (night) - $24^{o}C$ (day). After 5 days, the slides, radish seeds and vials were removed and examined using a microscope (x 100) for *Rhizoctonia* spp.

3.2.3 Experiment 2 Location and nature of damage caused by binucleate *Rhizoctonia* spp. on *C. vulgaris* and *E. cinerea* plants.

Thirty rooted cuttings of each of *C. vulgaris* cvs My Dream and Cuprea and of *E. cinerea* cv Golden Hue were potted, 12 weeks after striking, in 8 cm pots containing standard potting compost in which had been incorporated standard Rhizoctonia inoculum (one of five binucleate *Rhizoctonia*

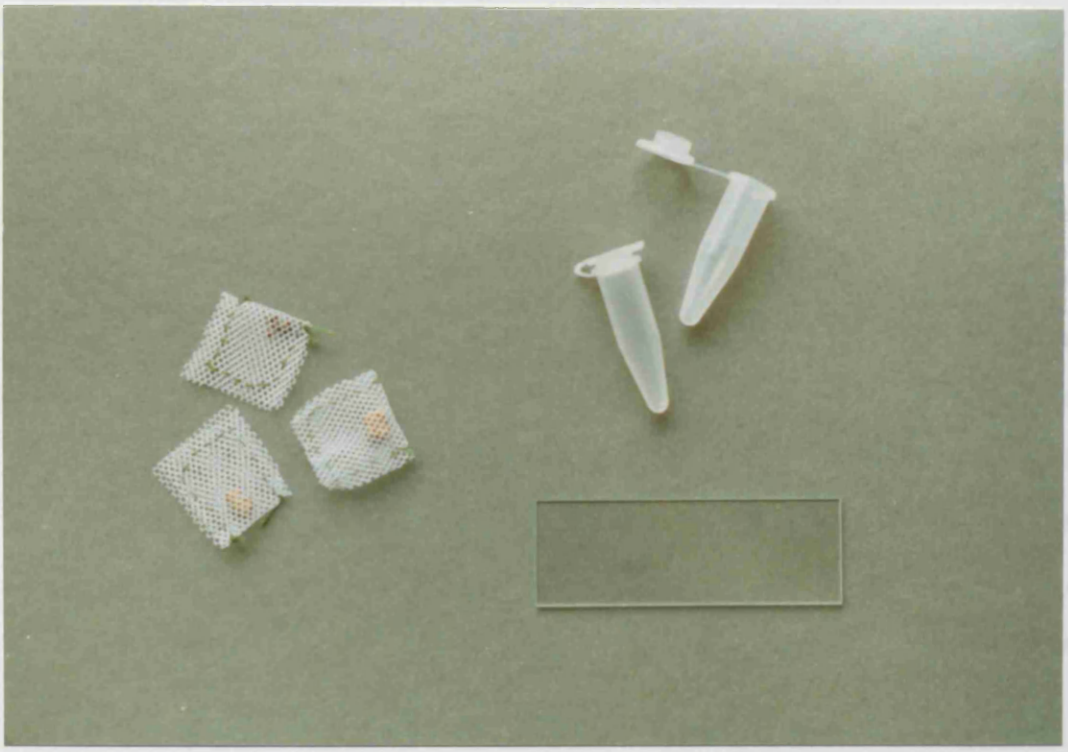

Plate 6 Nylon mesh bags (1 mm mesh, 2 x 2 cm), 1 ml capillary blood collection vials and microscope slides (2 x 5 cm) used to isolate *Rhizoctonia* spp. from soils and composts.

spp. isolates 72, D1, 64, 48 or 56) or uninoculated straw at 2 g straw/l compost. Within each of five replicate blocks, there were eighteen plots, each consisting of a single rooted cutting in a pot. The pots were randomly allocated to their positions in the blocks. Replicate blocks were arranged on gravel in a polythene tunnel. All blocks were surrounded individually by a single guard row of rooted cuttings in 8 cm pots.

Assessment was carried out after 4 weeks. The roots, stem-base and foliage were visually examined using a hand lens to determine the presence of browning. Five, 2 - 5 mm length pieces of surface sterilised root and stem-base from each plant were placed on PDES agar. Ten, 10 - 15 mm length pieces of fine root were taken from each plant and placed in sterile distilled water in 9 cm diameter Petri dishes. Foliage pieces (2 - 10 cm length branches) were incubated in sealed polythene boxes (see section 2.10). The presence of *Rhizoctonia* spp. on plant tissues was recorded after 24 - 72 hours.

3.2.4 Experiment 3 Pathogenicity of *R. solani* and binucleate *Rhizoctonia* spp. on *C. vulgaris* cuttings.

Four cuttings of each of *C. vulgaris* cvs Darkness, Rosalind and Alba Praecox were struck in standard propagation compost contained in plastic boxes (10 x 10 x 8 cm deep), in which standard inoculum of one of three isolates of *R. solani* (52, 55 or A) or one of seven isolates of binucleate *Rhizoctonia* spp. (F, 24B, D1, Pd,

48, 64 or 56) or untreated straw (control) had been introduced at 2 g straw/l compost.

Within each of three replicate blocks, there were eleven boxes (plots) comprising a four-cutting row of each cv (sub-plots). The boxes were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged along a glasshouse bench. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliage condition of cuttings was assessed after 2, 4 and 6 weeks (see section 2.8) and two, 2 - 5 mm length surface sterilised stem pieces from each cutting were placed on PDES agar following the final assessment, to determine the presence of Rhizoctonia.

3.2.5 Experiment 4 The susceptibility of *C. vulgaris* and *E. cinerea* cuttings to infection by binucleate *Rhizoctonia* spp.

Eight cuttings of each of *C. vulgaris* cvs My Dream, Cuprea, Rosalind, Beoley Silver, Serlei Aurea, Salmon Leap and Golden Feather and *E. cinerea* cvs Rockpool and Joseph Murphy were struck in standard propagation compost in seed trays (37 x 24 x 5.5 cm deep), in which had been incorporated standard inoculum of binucleate *Rhizoctonia* spp. isolates D1 or 56 or uninoculated straw (control) at 2 g straw/l compost.

Within each of four replicate blocks, there were three trays (plots) comprising an eight-cutting row of each cv (sub-plots). The trays were randomly allocated to their

positions within the blocks. Replicate blocks were separately covered in polythene and arranged along a glasshouse bench. As far as possible, each plot within each block received the same environmental/cultural conditions.

Foliage condition of cuttings was assessed after 2, 4 and 6 weeks (see section 2.8). Sixteen cuttings were taken at random from each cultivar/isolate combination following the final assessment, and two, 2 - 5 mm length surface sterilised stem pieces from each cutting were placed on PDES agar to determine the presence of Rhizoctonia.

3.2.6 Experiment 5 Effect of environment on the susceptibility of cuttings of *C. vulgaris* and *Erica* spp. to infection by binucleate *Rhizoctonia* spp.

Eight cuttings each of *C. vulgaris* cvs Firefly, K94, Hammondii, Foxhollow Wanderer, Silver Queen, Flamingo and Bognie and of *E. carnea* cv Ruby Glow, *E. cinerea* cv Rockpool and *E. vagans* cv Valerie Proudley were struck in standard propagation compost in seed trays (37 x 24 x 5.5 cm deep) in which had been incorporated standard inoculum of binucleate *Rhizoctonia* spp. isolate D1 or 48 or uninoculated straw (control) at 2 g straw/l compost. Three samples of each treatment were placed in the following three environments:

A. A growth room, (illuminated (150 $\mu mol.m^{-2}.s^{-1}$) for 16 hours in each 24 hour cycle. Mean temp. 20°C, temp. range 18° - 22°C.).

B. A glasshouse without supplementary lighting, (mean midday light level 210 $\mu mol.m^{-2}.s^{-1}$, mean temp. $12^{o}C$, temp. range 4^{o} - $25^{o}C$).

C. A glasshouse with supplementary lighting from 0600 to 2000 hours each day (mean midday light level 240 $\mu mol.m^{-2}.s^{-1}$, mean temp. $18^{o}C$, temp. range 12 - $24^{o}C$.)

Within each location, the trays were divided into three blocks. Within each block in each location, there were three trays (plots) comprising an eight-cutting row of each cv (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene. As far as possible, each plot within each block received the same environmental/cultural conditions.

Foliage condition of cuttings was assessed 2, 4 and 6 weeks after striking (see section 2.8). Two, 2 - 5 mm length stem pieces were taken from six cuttings removed at random from each isolate/cultivar/environment combination. Surface sterilised stem pieces were placed on PDES agar following the final assessment, to determine the presence of Rhizoctonia.

3.2.7 Experiment 6 Effect of stock plant environment and cutting type on susceptibility of *C. vulgaris* cuttings to infection by binucleate *Rhizoctonia* sp. isolate D1.

Stock plants of *C. vulgaris* cvs Silver Queen and Cuprea were grown in each of three locations for 6 months

previous to removal of cuttings, namely outdoors in sandy loam soil, or in pots in a glasshouse (supplementary lighting from 0600 - 2000 hours daily, mean midday light level 240 $\mu mol.m^{-2}.s^{-1}$, mean temperature 18°C, temperature range 12 - 24°C) or in a polythene tunnel. All three plants of each cultivar were grown from cuttings originating from one plant, (i.e. they belonged to a single clone.) Sixty shoot-tip cuttings (25-40 mm long, taken from soft tissue at the top of growing shoots), and 60 shoot-base cuttings (25-40 mm long, taken from semi-hardwood material 40-100 mm below the shoot-tip) were taken from each of a single *C. vulgaris* cv Cuprea or Silver Queen plant in each location. They were struck in standard propagation compost in seed trays (23 x 17.5 x 5.5 cm deep), in which had been incorporated standard inoculum of binucleate *Rhizoctonia* sp. isolate D1 or uninoculated straw at 2 g straw/l compost.

Within each of three replicate blocks, there were six trays (plots) comprising a five-cutting row of each cutting type of each cultivar from one of the three locations (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged on a tunnel floor. As far as possible, each plot within each block received the same environmental/cultural conditions. Foliage development of cuttings in the treatments was assessed 2, 4 and 6 weeks after striking (section 2.8).

3.2.8 Experiment 7 The effect of quantity of inoculum of binucleate *Rhizoctonia* sp. isolate 48 on the infection of *C. vulgaris* and *E. vagans* cuttings.

Five cuttings of *C. vulgaris* cvs Cuprea and Silver Knight and of *E. vagans* cv Lyonesse were struck in standard propagation compost (1 l compost/tray) in trays (23 x 17.5 x 5.5 cm deep), containing either 0, 1, 5, 20 or 50 pieces (i.e. 5 - 10 mm lengths of straw) of standard inoculum of binucleate *Rhizoctonia* sp. isolate 48. The inoculum plus compost for each tray were shaken for 2 minutes in a polythene bag to ensure random distribution of the straw pieces before the composts were placed in the trays. Within each of three replicate blocks, there were five trays (plots) comprising a five-cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and were arranged along a glasshouse bench. As far as possible, each plot within each block received the same environmental/cultural conditions.

The foliage condition of cuttings was assessed 1, 2, 4 and 6 weeks after striking (see section 2.8). Four cuttings were removed at random from each tray following the final assessment and two, 2 - 5 mm length surface sterilised stem pieces from each cutting, were placed on PDES agar to determine the presence of Rhizoctonia.

It was originally planned to use binucleate *Rhizoctonia* sp. isolate D1 in Experiments 6 to 9. However, problems with contamination of the inoculum (due to

Penicillium spp.) meant that there was insufficient inoculum of isolate D1 to carry out the experiments. Binucleate *Rhizoctonia* sp. isolate 48 inoculum was used in its place as it had been used in previous experiments

3.2.9 Experiment 8 Investigation into the potential for the infection of *E. vagans* cuttings from nursery materials contaminated with binucleate *Rhizoctonia* sp. isolate 48.

Fifteen cuttings of *E. vagans* cv Lyonesse were struck in standard propagation compost in seed trays (23 x 17.5 x 5.5 cm deep), two of which were placed in each of 28 plastic trays (32 x 42 x 8 cm deep, Petcraft Cat litter trays). The seed trays were placed in contact with a range of nursery materials contaminated with binucleate *Rhizoctonia* sp. isolate 48 within the cat trays as shown in the following treatments.

Treatment No

1. Seed trays set on contaminated capillary matting
2. Trays on contaminated sand
3. Trays on contaminated gravel
4. Contaminated compost fragments placed in seed trays
5. Infected cuttings (one/tray) placed in seed trays
6. Seed trays themselves contaminated with Rhizoctonia
7. " " covered with polythene contaminated with Rhizoctonia

Half of the cat trays contained uninfested materials, thereby acting as controls. Within each of two replicate blocks, there were fourteen cat trays (plots) containing

two seed trays (sub-plots) which each contained three rows of five cuttings. The cat trays were covered individually in white polythene to prevent spread of Rhizoctonia between trays. They were then randomly allocated to positions on the floor of a polythene tunnel. Foliage was examined visually twice weekly for 7 weeks and when foliar browning was observed, cuttings were removed and tissue pieces were examined under the microscope (x 100) to confirm the presence of mycelium of *Rhizoctonia* spp. Surface sterilised stem pieces from four cuttings in each treatment were placed on PDES agar to determine whether infection had taken place.

Nursery materials were contaminated with binucleate *Rhizoctonia* sp. isolate 48 as shown below.
Firstly, binucleate *Rhizoctonia* sp. isolate 48 was grown on PDA in 9 cm diameter Petri dishes until the plates were fully colonised. The plate contents were cut into 5 mm^2 blocks.

A. Two pieces of unused capillary matting, cut to fit the base of cat trays, were thoroughly soaked in tap water and were then squeezed out. They were placed in polythene bags with twenty 5 mm^2 blocks of agar colonised with binucleate *Rhizoctonia* sp. isolate 48. The bags were sealed, shaken and incubated at 23^{o}C for 3 days prior to the start of the experiment. Cutting trays were then placed on the matting within the cat trays.

B. Two, 1 l lots of each of sand, gravel and standard propagation compost were shaken in polythene bags with 40 ml of distilled water plus twenty, 5 mm^2 blocks of agar colonised with binucleate *Rhizoctonia* sp. isolate 48. The bags were sealed and incubated as in A. for 3 days prior to the start of the experiment. The sand or gravel in treatments 2 and 3 (1 l/tray) was then spread in the base of the cat trays and twenty compost fragments (1 - 5 mm long) were distributed evenly over the surface of the compost in each tray in treatment 4.

C. Four cuttings of *E. vagans* cv Lyonesse were placed in a polythene bag with twenty, 5 mm^2 blocks of colonised agar as above. The bags were sealed and incubated as above. One infected cutting was struck in the middle of each tray (treatment 5) at the start of the experiment.

D. Four seed trays (23 x 17.5 x 5.5 cm deep) were placed in separate polythene bags with 40 ml distilled water plus ten, 5 mm^2 blocks of colonised agar as above. The bags were sealed and incubated as above. The trays were then filled with compost, and cuttings were struck as for other treatments.

E. Two, 1 m^2 pieces of white polythene (120 gauge, 30 micron) were each placed in a polythene bag with 40 ml of distilled water and ten, 5 mm^2 blocks of colonised agar as above. The bags were sealed and incubated as above. The polythene was used to cover trays in treatment 7.

3.2.10 Experiment 9 The effect of depth of inoculum of binucleate *Rhizoctonia* sp. isolate D1 in the compost, on the infection of *C. vulgaris* and *E. vagans* cuttings.

Five cuttings of *C. vulgaris* cvs Mousehole and Loch Turret and of *E. vagans* cv Lyonesse were struck in seed trays (23 x 17.5 x 5.5 cm deep) containing 25 pieces (i.e. 5 - 10 mm lengths of straw) of standard inoculum of binucleate *Rhizoctonia* sp. isolate D1 or uninoculated straw spread evenly in a layer either below the compost (standard propagation compost), between the base of the tray and compost surface or on the compost surface.

Within each of three replicate blocks there were six trays (plots) comprising a five-cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and were arranged on the floor of a polythene tunnel. As far as possible, each plot within each block received the same environmental/cultural conditions.

The foliage condition of cuttings was assessed after 2, 4 and 6 weeks (see section 2.8). Two cuttings per cultivar were taken at random from each tray following the final assessment and two, 2 - 5 mm length stem pieces from each cutting were surface sterilised and placed on PDES agar, to determine the presence of Rhizoctonia.

3.3 RESULTS CHAPTER 3

3.3.1 Disease survey - Incidence of *Rhizoctonia* spp. on plants of *C. vulgaris* and *Erica* spp. which showed foliar browning, collected from UK nurseries.

Rhizoctonia spp. were isolated from 22% of cuttings exhibiting browning of the foliage. Cuttings infected with *Rhizoctonia* spp. in general showed browning of foliage at the base of the cutting which was often covered in fine pale yellow to brown web-like mycelium. Roots had either failed to form or had rotted once formed.

Rhizoctonia spp. were isolated from 10% of rooted plants of *C. vulgaris* and *Erica* spp. which showed foliar browning. Symptoms included browning of the foliage from the base of the plant upwards, and the presence of mycelium, (light microscope, x 100) on dead and dying foliage and on woody tissue near soil level. Browning of stem-base tissue and woody roots around soil-level was found on 20% of plants which showed foliar browning. However, extensive rotting of fine roots below the soil surface was found in only 3% of plants (which showed foliar browning) as a result of Rhizoctonia infection alone. Plate 7, which follows page 87 shows mycelium of binucleate *Rhizoctonia* sp. isolate 48 on foliage of *E. carnea* cv Springwood White (x 40), and plate 8, which also follows page 87 shows mycelium of *R. solani* isolate K1 on foliage of *C. vulgaris* cv Mousehole (x 40).

Several fungal genera including *Fusarium, Penicillium, Trichoderma* and *Cylindrocarpon* were also isolated from the

Plate 7 Mycelium of binucleate *Rhizoctonia* sp. isolate 48 on foliage of *E. carnea* cv Springwood White (x 40).

Plate 8 Mycelium of *R. solani* isolate K1 on dying foliage of *C. vulgaris* cv Mousehole (x 40).

roots and stem-bases of cuttings and plants showing foliar browning and/or root rot. These genera were isolated either singly, in combination or together with *Rhizoctonia* spp.

When present on rooted *C. vulgaris* and *Erica* spp., *Rhizoctonia* spp. were most frequently isolated from the stem-base and lower foliage up to 4 cm above soil level. The fine roots below soil level and foliage more than 4 cm above soil level were less frequently infected. Table 4 shows the regions from which *Rhizoctonia* spp. were isolated on a *C. vulgaris* cv My Dream plant, and Plate 9, which follows page 89 shows the same plant infected with binucleate *Rhizoctonia* sp. isolate D1.

3.3.2 Experiment 1 Investigation into the sources of *Rhizoctonia* spp. on nurseries producing *C. vulgaris* and *Erica* spp.

3.3.2.1 EXPERIMENT 1a PRODUCTION MATERIALS

Rhizoctonia spp. were isolated from 10% of used cutting trays, 13% of samples of used capillary matting and 3% of samples of used polythene taken from nurseries (Table 5). They were not isolated from any samples of new, unused materials.

3.3.2.2 EXPERIMENT 1b COMPOST CONSTITUENTS

Rhizoctonia spp. were not isolated from any unused compost component, (Table 6). They were isolated most frequently

Table 4 Isolation of a binucleate *Rhizoctonia* sp. from seven different areas on a two-year old *C. vulgaris* cv My Dream plant. (Disease survey)

Area on plant from which *Rhizoctonia* sp. isolated	Proportion of infected pieces (15 pieces from each area on the plant were tested)	Standard error of mean
Lower root zone (> 3 cm below soil level)	0.00	0.000
Upper " " (soil-level to 3 cm below)	0.07	0.064
Stem-base[a]	0.45	0.150
Lowest foliage 0 - 1 cm above soil-level	0.33	0.122
Lower " 1 - 2 cm " " "	1.00	0.000
Mid " 2 - 4 cm " " "	0.87	0.088
Upper " > 4 cm " " "	0.00	0.000

[a]Only 11 pieces from the stem-base were tested for infection

Plate 9 *C. vulgaris* cv My Dream plant infected with binucleate *Rhizoctonia* sp. isolate D1 showing browning of foliage from compost level upwards.

Table 5 Isolation of *Rhizoctonia* spp. from samples of materials collected from UK nurseries. (Experiment 1a)

Material	Proportion of samples on which *Rhizoctonia* spp. were detected (30 samples of each material were tested)			
	New	Standard error	Used	Standard error
Capillary matting	0	0.00	0.13	0.062
Cutting trays	0	0.00	0.10	0.055
Polythene	0	0.00	0.03	0.033

Table 6 Detection of *Rhizoctonia* spp. in samples of nursery composts and soils. (Experiment 1b)

Substrate	Proportion of samples in which *Rhizoctonia* spp. were detected (50 samples of each material were tested)	Standard error
Fresh Vapo Peat	0.00	0.000
Fresh Bulrush Peat	0.00	0.000
Fresh Cambark	0.00	0.000
Fresh Scotbark	0.00	0.000
Potato field soil[a]	0.50	0.071
Nursery soil	0.04	0.028
Used nursery compost	0.12	0.046

[a]Soil had been cropped with potatoes for 2 years previous to sampling.

from field soil which had been planted with potatoes for the 2 years previous to sampling. *Rhizoctonia* spp. were also isolated from 4% of samples of nursery soil tested, and from 12% of samples of used nursery compost.

3.3.3 Experiment 2 Location and nature of damage caused by binucleate *Rhizoctonia* spp. on *C. vulgaris* and *E. cinerea* plants.

Rhizoctonia spp. were most frequently isolated from the lower foliage, stem-base and woody roots of *C. vulgaris* plants growing in infested compost (Table 7). They were isolated only occasionally from the fine roots and upper foliage. Binucleate *Rhizoctonia* spp. isolates 72, D1, 64 and 48 caused varying levels of infection and browning of foliage depending on cultivar. *E. cinerea* cv Golden Hue was less severely infected and showed less foliar browning than the two *C. vulgaris* cultivars. Binucleate *Rhizoctonia* sp. isolate 56 was the least pathogenic of the isolates tested. It did not infect *E. cinerea* cv Golden Hue plants and although it infected the roots and stem-base of the *C. vulgaris* cultivars, it caused no foliar browning.

Plate 10 which follows page 92 shows foliar browning and stunted growth on *C. vulgaris* cv Cuprea plants and uninfected controls.

No foliar browning or root rot was observed on, and no *Rhizoctonia* spp. were isolated from, control plants grown in uninfested compost.

Table 7 Isolation of *Rhizoctonia* spp. from[a] and appearance[b] of *E. cinerea* and *C. vulgaris* plants assessed 4 weeks after potting. (Experiment 2).

Rhiz. spp. isolate	Fine roots	Woody roots	Stem base	Lower foliage	Upper foliage
None	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
72	0.1 (0.08)	0.4 (0.13)	0.6 (0.13)	0.8 (0.10)	0.0 (0.00)
D1	0.0 (0.00)	0.2 (0.10)	0.4 (0.13)	0.6 (0.13)	0.1 (0.08)
64	0.0 (0.00)	0.2 (0.10)	0.6 (0.13)	0.7 (0.12)	0.7 (0.12)
48	0.1 (0.08)	0.0 (0.00)	0.3 (0.12)	0.7 (0.12)	0.7 (0.12)
56	0.0 (0.00)	0.2 (0.10)	0.3 (0.12)	0.0 (0.00)	0.0 (0.00)
Isolation of *Rhiz.* spp.[a]. Mean (plants grown in *Rhiz.*-amended compost)	0.2 (0.05)	0.2 (0.05)	0.5 (0.06)	0.6 (0.06)	0.1 (0.03)
Proportion of plants showing areas of browning and necrotic tissue[b]	0.0 (0.00)	0.1 (0.03)	0.3 (0.05)	0.5 (0.06)	0.0 (0.00)

[a]A total of fifteen plants grown in compost infested with each isolate were tested, i.e. five of each of *C. vulgaris* cvs My Dream and Cuprea and *E. cinerea* cv Golden Hue. The proportion of plants from which *Rhizoctonia* spp. were isolated is recorded. Standard errors are in parenthesis.

[b]A presence or absence score of 1 for browning and 0 for no browning was given for each zone on each plant. Means were calculated from the 75 plants grown in Rhizoctonia-amended compost for each plant zone. Standard errors are in parenthesis.

Plate 10 *C. vulgaris* cv Cuprea plants: left, infected with binucleate *Rhizoctonia* sp. isolate 72 and showing stunted growth and foliage browning from compost level upwards; right, uninfected. (Experiment 2)

3.3.4 Experiment 3 Pathogenicity of *R. solani* and binucleate *Rhizoctonia* spp. on *C. vulgaris* cuttings.

When the statistical analysis was carried out on the foliage scores (final assessment), all treatments which had a mean score of zero were omitted. All plots were included in the analysis of the root indices (final assessment).

There were significant differences ($F_{7,52}$ = 174.74; P < 0.001) between the levels of foliar browning on cuttings grown in composts amended with different *Rhizoctonia* spp. isolates, (Table 8). Some isolates caused severe foliar browning, (e.g. *R. solani* isolate 55, mean foliage score on *C. vulgaris* cuttings grown in amended compost = 3.2; and binucleate *Rhizoctonia* sp. isolate D1, mean foliage score on cuttings = 3.6), whereas others caused little or no obvious damage (e.g. binucleate *Rhizoctonia* sp. isolate 64, mean foliage score on cuttings = 0.0). Plate 11, which follows page 94 shows *C. vulgaris* cvs Darkness, Alba Praecox and Rosalind, 4 weeks after striking in standard propagation compost amended with binucleate *Rhizoctonia* spp. isolates F, D1, 24B, Pd or 48.

There was evidence of a relationship between the number of nuclei per hyphal cell and the level of both foliar browning ($F_{1,52}$ = 7.09; P < 0.05) and root development ($F_{1,64}$ = 51.03; P < 0.001). In general, cuttings grown in compost containing binucleate isolates had higher foliar scores and lower root indices. A range of pathogenicity was observed within both binucleate and

Table 8 The effect of *Rhizoctonia* spp. isolates grown on sterile chopped straw (5 - 10 mm) and incorporated into compost on the foliage condition[a] of *C. vulgaris* cuttings assessed 6 weeks after striking. (Experiment 3)

	Foliage score[a]												
		Multinucleate isolates				Binucleate isolates							
Cultivar	Control[b]	52	55	A	mean	F	24B	D1	Pd	48	64[b]	56	mean
Darkness	0.0	1.0	3.3	2.9	2.4	3.0	1.9	3.6	3.0	2.3	0.0	0.7	2.4
Rosalind	0.0	0.7	2.8	2.5	2.0	2.2	1.3	2.9	2.8	1.3	0.0	0.3	1.8
Alba P.	0.0	0.7	3.3	2.8	2.3	3.7	3.4	4.0	3.3	3.1	0.0	0.4	3.0
		SEM[c] = 0.15			SEM = 0.09			SEM = 0.15					SEM = 0.06
Isolate mean	0.0	0.8	3.2	2.8	2.2	3.0	1.9	3.6	3.0	2.3	0.0	0.4	2.4
		SEM = 0.09						SEM = 0.09					

[a] 0 = no foliar browning, 4 = totally brown foliage

[b] Scores of cuttings grown in composts with no Rhizoctonia (controls), or isolate 64 were equal to zero. They were omitted from the statistical analysis and the SEM's were taken to be zero.

[c] SEM's with 52 degrees of freedom

Plate 11 *C. vulgaris* cvs, (left to right) Darkness, Alba Praecox and Rosalind 4 weeks after striking in standard propagation compost amended with isolates of binucleate *Rhizoctonia* spp.: Top, left to right, F, D1, unamended control; bottom, 24B, Pd, 48. (Experiment 3)

multinucleate isolates.

The level of foliar browning differed significantly ($F_{2,52}$ = 78.43; P < 0.001) between cultivars. In general *C. vulgaris* cv Rosalind was less susceptible to foliar browning than the other two cultivars.

Root development and the extent of root rot differed depending on isolate (Fig. 1). Root indices of cuttings grown in infested compost were significantly lower ($F_{1,64}$ = 185.43; P < 0.001) than those of the control cuttings grown in uninfested compost.

Some isolates such as binucleate *Rhizoctonia* spp. isolates D1 and Pd severely retarded or stopped root development whereas others such as *R. solani* isolate 52 and binucleate *Rhizoctonia* sp. isolate 64 had little effect. For example, cuttings grown in compost amended with isolates D1 and Pd produced 12% and 36% respectively of the root produced on control cuttings grown in unamended compost. However, cuttings grown in compost amended with isolates 52 and 64 produced 90% and 94% respectively of the root produced by control cuttings.

Rhizoctonia spp. were isolated from cuttings in all treatments except those grown in uninfested compost which showed no foliar browning and rooted in 3 weeks. This experiment was repeated at a later date and similar results were obtained.

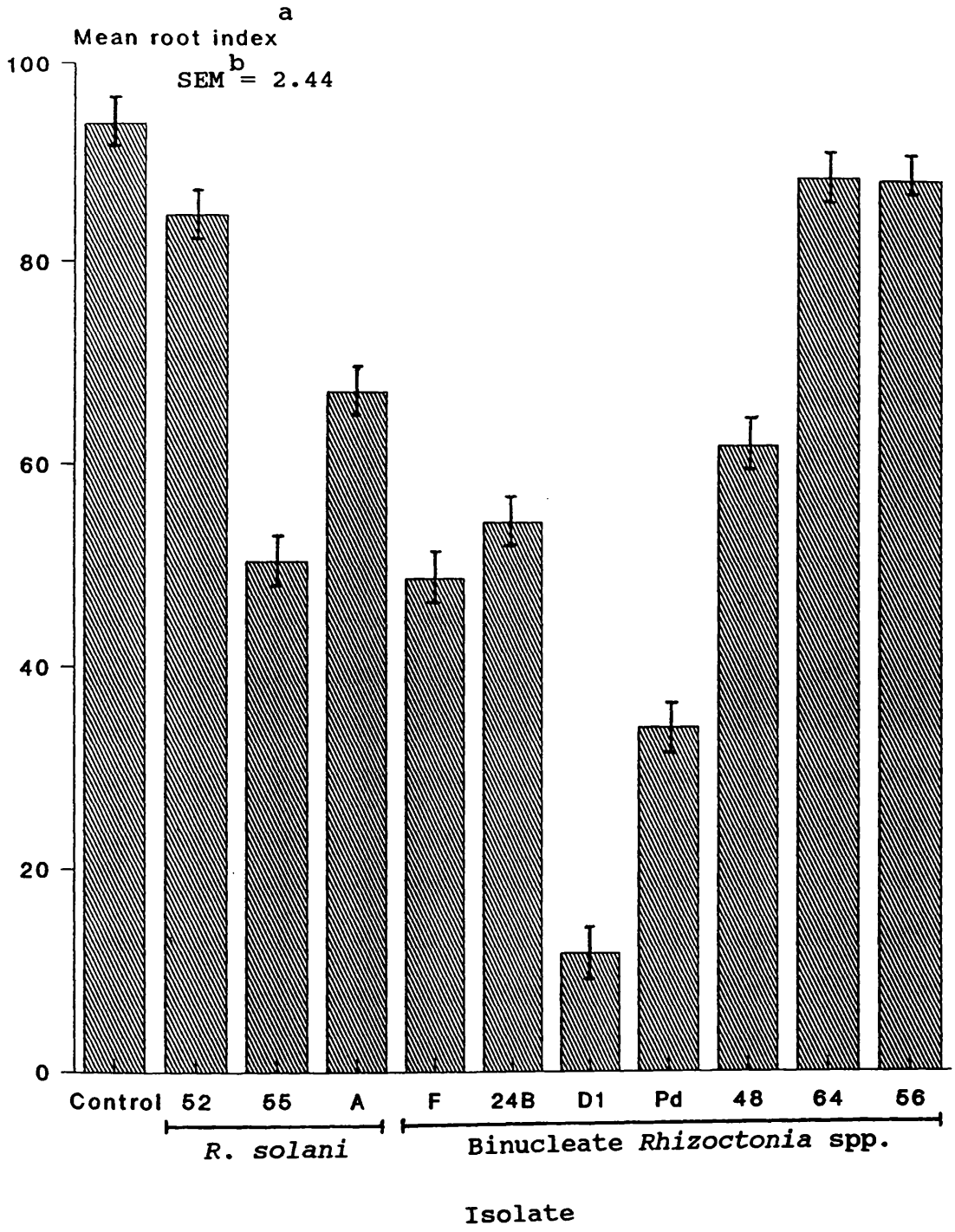

Fig. 1 The effect of several isolates of *Rhizoctonia* spp. on the mean root indices[a] of cuttings of *C. vulgaris* cvs Darkness, Rosalind and Alba Praecox (mean root indices of three cultivars) struck in standard propagation compost and assessed 6 weeks after striking. (Experiment 3)

[a]Root indices calculated as shown in section 2.9

[b]All SEM's with 64 degrees of freedom

3.3.5 Experiment 4 The susceptibility of *C. vulgaris* and *E. cinerea* cuttings to infection by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), all treatments which had a mean score of zero were omitted.

The level of foliar browning differed significantly ($F_{6,45}$ = 89.02; P < 0.001) between cultivars grown in compost amended with binucleate *Rhizoctonia* spp. (Table 9). The *E. cinerea* cultivars, (i.e. Joseph Murphy and Rockpool, mean foliage score of cuttings in treatments involving binucleate *Rhizoctonia* spp. isolates = 0.1) showed less foliar browning than the *C. vulgaris* cultivars (mean foliage score of cuttings in composts amended with binucleate *Rhizoctonia* spp. = 1.1). Of the seven *C. vulgaris* cultivars tested, My Dream showed most foliar browning with both isolates tested. A mean foliage score of 3.5 was recorded on cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1, and a mean foliage score of 0.6 was recorded on cuttings in compost containing binucleate *Rhizoctonia* sp. isolate 56. Plate 12, which follows page 98, shows foliar browning symptoms on *C. vulgaris* and *E. cinerea* cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1. The level of foliar browning observed differed significantly ($F_{1,45}$ = 1537.14; P < 0.001) between *Rhizoctonia* spp. isolates. Binucleate *Rhizoctonia* sp. isolate D1 (mean foliage score recorded on *C. vulgaris* and *Erica* spp. cuttings = 1.6) caused higher levels of

Table 9 The effect of binucleate *Rhizoctonia* spp. isolates D1 and 56 incorporated into compost on the foliage condition[a] of *C. vulgaris* and *E. cinerea* cuttings assessed 6 weeks after striking. (Experiment 4)

	Foliage condition score[a]		
	Rhiz. spp. isolate		Mean (infested treatments)
Cultivar	D1	56	
C. vulgaris			
My Dream	3.5	0.6	2.1
Cuprea	2.6	0.0	1.3
Rosalind	2.4	0.1	1.3
Beoley Silver	2.2	0.1	1.1
Serlei Aurea	0.5	0.3	0.4
Salmon Leap	1.3	0.0	0.6
Golden Feather	1.6	0.6	1.1
	SEM[c] = 0.08		SEM = 0.06
Mean (*C. vulgaris* cvs)	2.0	0.2	
	SEM = 0.02		
E. cinerea			
Joseph Murphy[b]	0.0	0.0	0.0
		SEM = 0.00	
Rockpool	0.5	0.0	0.3
	SEM = 0.08		SEM = 0.06
Overall mean	1.6	0.2	
	SEM = 0.02		

[a]Foliage score: 0 = no foliar browning; 4 = totally brown foliage.

[b]The scores of Joseph Murphy cuttings and cuttings of all cultivars grown in compost containing no Rhizoctonia were equal to zero. They were omitted from the statistical analysis and the SEM's were taken to be zero.

[c]SEM's with 45 degrees of freedom.

Plate 12 Cuttings in standard propagation compost amended with binucleate *Rhizoctonia* sp. isolate D1: left to right, vertical rows showing seven cuttings of each of *E. cinerea* cv Rockpool, *C. vulgaris* cv Golden Feather, *E. cinerea* cv Joseph Murphy, *C. vulgaris* cvs Salmon Leap, Serlei Aurea, Beoley Silver, Rosalind, Cuprea. (Experiment 4)

browning than binucleate *Rhizoctonia* sp. isolate 56 (mean foliage score recorded as above = 0.2). Control cuttings grown in unamended compost all received foliage scores of zero. There was a significant interaction ($F_{6,45}$ = 69.13; P < 0.001) between cultivar and *Rhizoctonia* spp. isolate.

The number of cuttings out of the total of sixteen taken from each cultivar/isolate combination which were infected, was in general related to the amount of foliar browning observed (Table 10). For example *Rhizoctonia* spp. were isolated from 94% of *C. vulgaris* cv My Dream cuttings struck into compost amended with binucleate *Rhizoctonia* sp. isolate D1. These cuttings showed severe foliar browning. No *Rhizoctonia* spp. were isolated from *E. cinerea* cv Joseph Murphy cuttings which showed no foliar browning.

By contrast, *Rhizoctonia* spp. were isolated from 38% of *C. vulgaris* cv Beoley Silver cuttings taken from compost amended with binucleate *Rhizoctonia* sp. isolate 56, although the cuttings showed no foliar browning. No *Rhizoctonia* was isolated from cuttings grown in unamended compost.

All plots were included in the analysis of the root indices (final assessment). There were significant differences ($F_{7,78}$ = 79.94; P < 0.001) between $\log_{10}$ root dry weights of cuttings of different cultivars (Fig. 2). *C. vulgaris* cv Rosalind produced the greatest weight of roots and the *E. cinerea* cvs Rockpool and Joseph Murphy the least.

Table 10 Isolation of *Rhizoctonia* spp. from *C. vulgaris* and *E. cinerea* cuttings, 6 weeks after striking into compost amended with binucleate *Rhizoctonia* spp. isolates D1 and 56. (Experiment 4)

	Proportion of cuttings from which *Rhizoctonia* spp. isolated (16 cuttings from each treatment were tested)			
Cultivar	*Rhiz.* sp. Isolate D1	Standard error	*Rhiz.* sp. Isolate 56	Standard error
C. vulgaris				
My Dream	0.94	0.059	0.25	0.108
Cuprea	1.00	0.000	0.19	0.098
Rosalind	0.63	0.121	0.06	0.061
Beoley Silver	0.50	0.125	0.38	0.121
Serlei Aurea	0.25	0.108	0.13	0.083
Salmon Leap	0.44	0.124	0.00	0.000
Golden Feather	0.50	0.125	0.13	0.083
E. cinerea				
Joseph Murphy	0.00	0.000	0.00	0.000
Rockpool	0.06	0.061	0.00	0.000

There was a significant interaction ($F_{14,78} = 8.60$; $P < 0.001$) between isolate and cultivar. In some cases the presence of a binucleate *Rhizoctonia* spp. isolate reduced the weight of roots produced in comparison with controls (e.g. *C. vulgaris* cvs Cuprea and Serlei Aurea with binucleate *Rhizoctonia* sp. isolate D1). However, in other cases cuttings produced a greater weight of roots when grown in the presence of binucleate *Rhizoctonia* spp., e.g.

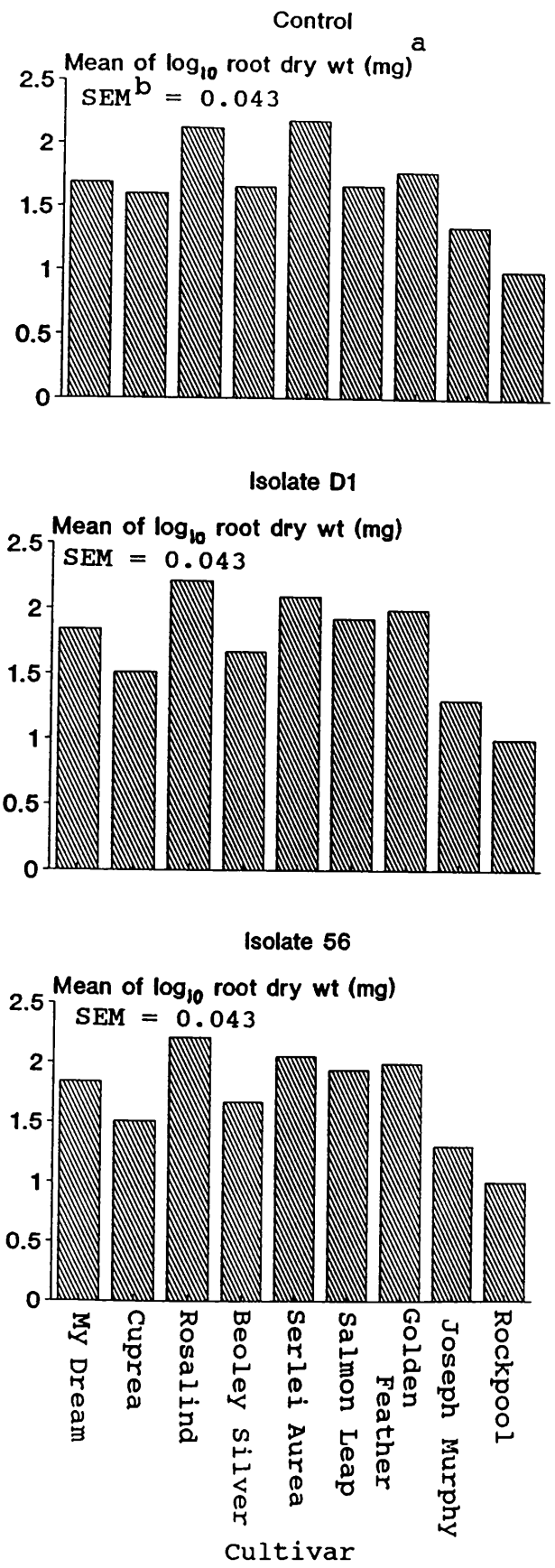

Fig. 2. The effect of binucleate *Rhizoctonia* spp. isolates D1 and 56 incorporated in standard propagation compost on the $\log_{10}$[a] of the root dry weight (mg) of *C. vulgaris* and *E. cinerea* cuttings assessed 6 weeks after striking. (Experiment 4)

[a]The value of the smallest non zero observation was added to all observations to ensure that all values were greater than zero prior to analysis.

[b]All SEM's with 78 degrees of freedom

C. vulgaris cvs My Dream and Salmon Leap with binucleate *Rhizoctonia* sp. isolate D1.

3.3.6 Experiment 5 Effect of environment on the susceptibility of cuttings of *C. vulgaris* and *Erica* spp. to infection by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), all treatments which had a mean score of zero were omitted. The treatments in environment B. (cool glasshouse) were also omitted since the mean foliage scores recorded from this environment were much lower than those recorded from the other two environments.

C. vulgaris and *Erica* spp. cuttings were maintained in each of three environments. Environment A. was a growth room (illuminated at 150 $\mu mol.m^{-2}.s^{-1}$ for 16 hours in each 24 hour cycle. Mean temp. $20^{o}C$, temp. range 18^{o} - $22^{o}C$). Environment B. was a glasshouse (no supplementary lighting, mean midday light level 210 $\mu mol.m^{-2}.s^{-1}$, mean temp. $12^{o}C$, temp. range 4^{o} - $25^{o}C$), and Environment C. was a glasshouse (with supplementary lighting from 0600 to 2000 hrs each day mean midday light level of 240 $\mu mol.m^{-2}\cdot-1$, mean temp. $18^{o}C$, range 12^{o} - $24^{o}C$).

There were no significant differences between foliage scores of cuttings grown in environments A. and C. (Figs. 3 and 4). Scores of cuttings grown in environment B. (cool glasshouse) were in general lower than those recorded on cuttings grown in the other two environments.

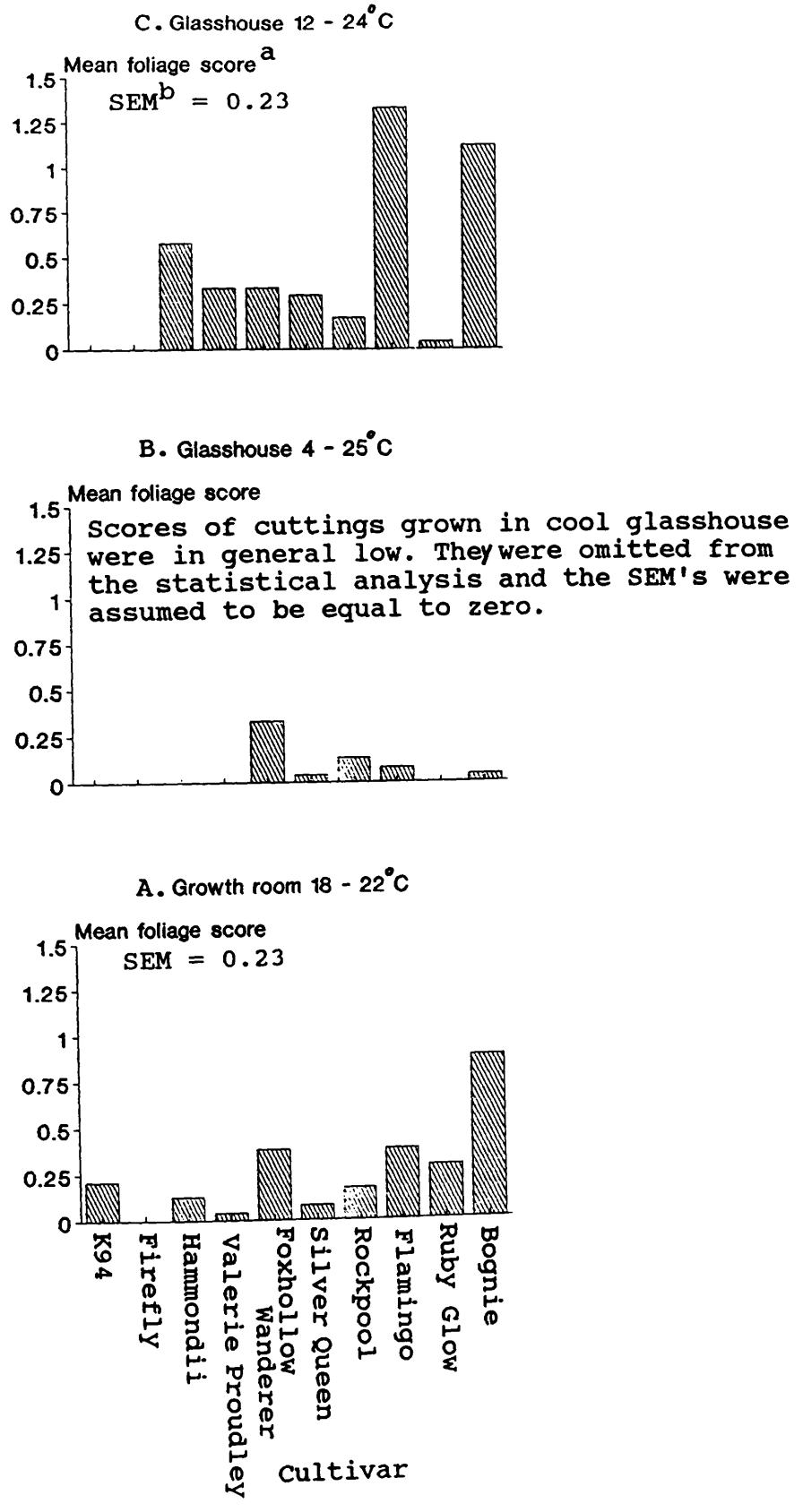

Fig. 3 The effect of binucleate *Rhizoctonia* sp. isolate D1 incorporated in standard propagation compost on the foliage condition[a] of *E. cinerea*, *E. vagans* and *C. vulgaris* cuttings grown in three different environments assessed 6 weeks after striking. (Experiment 5)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]SEM's with 78 degrees of freedom.

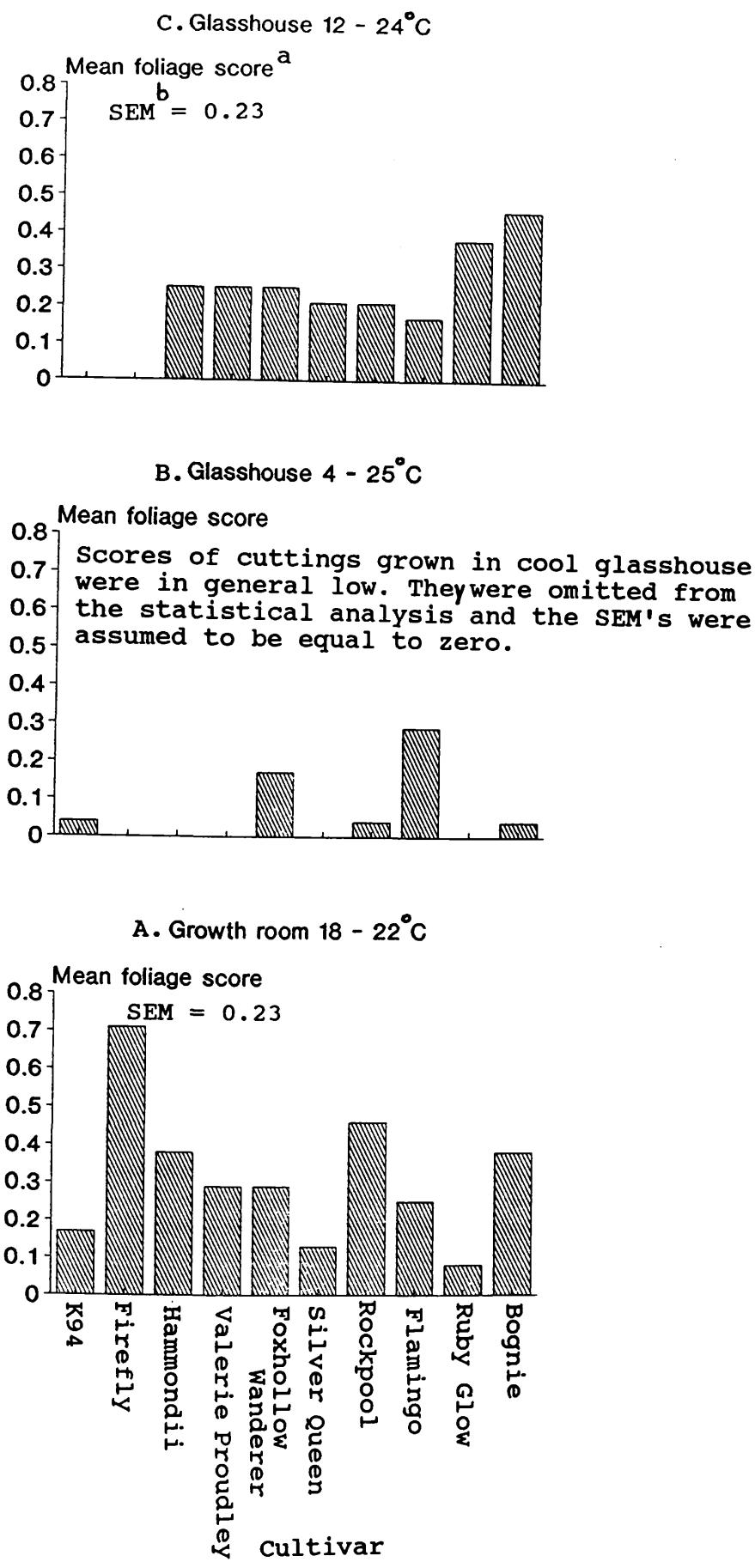

Fig. 4 The effect of binucleate *Rhizoctonia* sp. isolate 48 incorporated in standard propagation compost on the foliage condition[a] of *E. cinerea*, *E. vagans* and *C. vulgaris* cuttings grown in three different environments assessed 6 weeks after striking. (Experiment 5)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]SEM's with 78 degrees of freedom.

For example *C. vulgaris* cv Bognie, grown in binucleate *Rhizoctonia* spp.-amended compost showed very little foliar browning in the cool glasshouse, but showed severe foliar browning in the other two environments. Mean foliage scores of 0.88 and 0.04 were recorded on *C. vulgaris* cv Bognie cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1 placed in environment A and environment B respectively. The cultivars Hammondii and Flamingo also showed differences in foliar browning between treatments.

Isolations from cuttings revealed that all foliar browning was due to infection by *Rhizoctonia* spp. No foliar browning was observed on, and no *Rhizoctonia* spp. were isolated from control cuttings which rooted in 2 - 4 weeks depending on environment.

There were fewer foliage scores of 1, 2, 3 and 4 than expected, i.e. there were many scores of 0 recorded on cuttings grown in composts amended with binucleate *Rhizoctonia* spp. isolates D1 and 48.

3.3.7 Experiment 6 Effect of stock plant environment and cutting type on susceptibility of *C. vulgaris* cuttings to infection by binucleate *Rhizoctonia* sp. isolate D1.

When the statistical analysis was carried out on the foliage scores (final assessment), all treatments involving cuttings grown in uninfested compost were analysed together. Treatments involving infested compost were analysed separately. This was done because of the large difference in magnitude of foliage scores recorded

between cuttings grown in the two types of compost.

There were significant differences ($F_{1,22}$ = 12.71; P < 0.001) between foliage scores of cuttings taken from shoot-tips and shoot-bases and struck in compost amended with binucleate *Rhizoctonia* sp. isolate D1 (Table 11). There were no significant differences between foliage scores of cuttings taken from shoot-tips and shoot-bases which were struck into uninfested compost.

There were also significant differences between the foliage scores of cuttings taken from stock plants kept in different locations and struck into uninfested compost ($F_{2,22}$ = 61.58; P < 0.001) or compost containing binucleate *Rhizoctonia* sp. isolate D1 ($F_{2,22}$ = 3.44; P < 0.01). Foliage scores of cuttings taken from stock plants maintained in a polythene tunnel, were mainly lower than those of cuttings taken from stock kept outdoors or in a glasshouse. This was most often observed on cuttings struck in binucleate *Rhizoctonia* sp. isolate D1-amended compost. For example, mean foliage scores of 3.6 and 3.4 were recorded on cuttings taken from outdoor and tunnel-kept stock plants respectively, which were struck in compost amended with binucleate *Rhizoctonia* sp. isolate D1. Mean foliage scores of 0.9 and 0.1 were recorded on cuttings taken from outdoor and tunnel-kept stock plants respectively, which were struck in unamended composts.

Rhizoctonia spp. were isolated from 75% of tip cuttings and 95% of basal cuttings taken at random from

Table 11 The effect of stock plant environment and cutting type on the foliage condition score[a] of cuttings struck into compost amended with binucleate *Rhizoctonia* sp. isolate D1, assessed 6 weeks after striking. (Experiment 6)

	Foliage score[a]				
	Tip cuttings[b]		Basal cuttings[c]		
Stock environment	Silver Knight	Cuprea	Silver Knight	Cuprea	Mean
Uninoculated					
Outdoors	1.5	0.1	1.9	0.0	0.9
Tunnel	0.1	0.3	0.0	0.0	0.1
Glasshouse	0.0	0.0	0.1	0.0	0.0
		SEM[d] = 0.12			SEM = 0.06
Inoculated with *Rhiz.* sp. isolate D1					
Outdoors	3.9	3.5	3.9	2.9	3.6
Tunnel	3.9	3.2	3.5	2.8	3.4
Glasshouse	3.9	3.4	4.0	3.0	3.6
		SEM = 0.14			SEM = 0.07
Mean (inoculated treatments)	3.9	3.4	3.8	2.9	
		SEM = 0.08			

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]Tip cuttings - 25-40 mm taken from soft tissue at the top of grown shoots.

[c]Basal cuttings - 25-40 mm taken from semi-hardwood material 40-100 mm below the shoot tip.

[d]Data from treatments involving *Rhizoctonia* and no *Rhizoctonia* were analysed separately. All SEM's with 22 degrees of freedom.

cuttings struck in compost amended with binucleate *Rhizoctonia* sp. isolate D1 following the final assessment.

Foliar browning occurred on control tip cuttings of *C. vulgaris* cv Silver knight grown in unamended compost. This browning was not due to *Rhizoctonia* spp., as no *Rhizoctonia* spp. were isolated from any of the control cuttings struck in unamended compost. *Pythium, Cylindrocarpon* and *Fusarium* spp. were isolated from several of these cuttings.

3.3.8 Experiment 7 The effect of quantity of inoculum of binucleate *Rhizoctonia* sp. isolate 48 on the infection of *C. vulgaris* and *E. vagans* cuttings.

All plots were included in the statistical analysis of the foliage scores (final assessment). The differences between scores of cuttings in composts containing the five inoculum levels were significant ($F_{4,28} = 47.46$; $P < 0.001$). The extent of foliar browning on *C. vulgaris* and *E. vagans* cuttings was related to the level of binucleate *Rhizoctonia* sp. isolate 48 inoculum incorporated into the compost immediately before striking the cuttings (Fig. 5). The mean foliage score of cuttings grown in compost amended with the highest rate of Rhizoctonia inoculum, (i.e. 50 pieces of straw per seed tray 23 x 17.5 x 5.5 cm deep) was approximately eight times greater than that recorded for cuttings grown in compost containing only one piece of Rhizoctonia-infested straw per tray.

There were no significant differences between levels of browning on the three cultivars, and no significant interaction between cultivar and inoculum level.

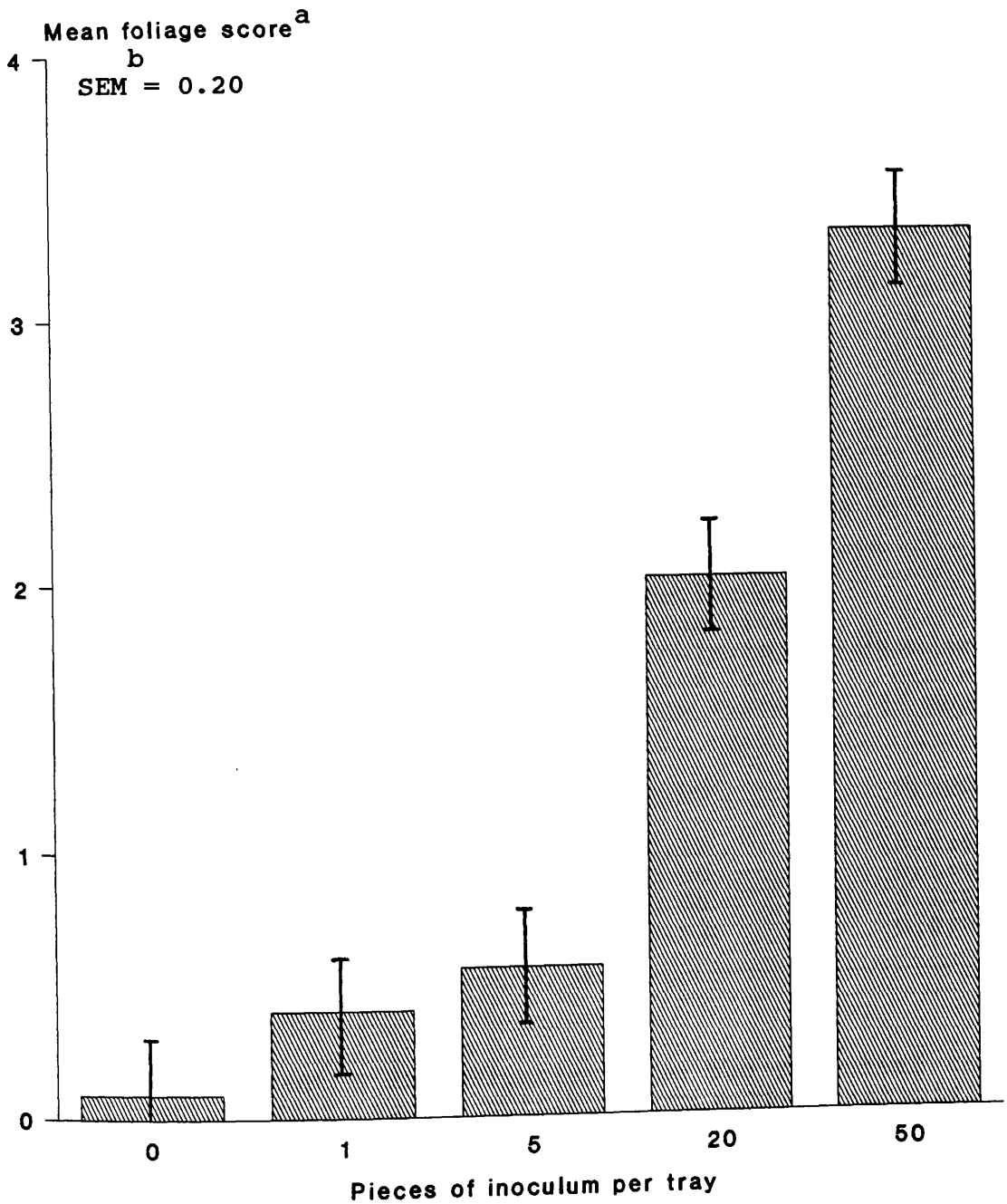

Fig. 5 The effect of quantity of inoculum of binucleate *Rhizoctonia* sp. isolate 48 in standard propagation compost on the foliage condition[a] of cuttings of *C. vulgaris* cvs Silver Knight and Cuprea and *E. vagans* cv Lyonesse (mean foliage scores of three cultivars) assessed 6 weeks after striking. (Experiment 7)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]All SEM's with 28 degrees of freedom

Isolations from cuttings showed that the level of foliar browning in each tray was related to the number of cuttings which were infected in each tray (Table 12). A high level of browning was associated with high numbers of infected cuttings. For example, *Rhizoctonia* spp. were isolated from 33% of cuttings grown in compost containing one piece of inoculum per tray (mean foliage score 0.40), whereas they were isolated from 92% of cuttings grown in compost containing 50 pieces of inoculum per tray (mean foliage score 3.33).

Table 12 Isolation of *Rhizoctonia* spp. from *C. vulgaris* cuttings (random sample of 12) 6 weeks after striking into compost amended with different rates of inoculum of binucleate *Rhizoctonia* sp. isolate 48. (Experiment 7)

Treatment	Inoculum level (pieces of straw/tray	Proportion of cuttings from which *Rhiz.* sp. isolated (12 cuttings tested)	Standard error of mean
Uninoculated	0	0.00	0.000
Rhizoctonia sp. isolate 48	1	0.33	0.136
	5	0.50	0.144
	20	0.58	0.142
	50	0.92	0.078

3.3.9 Experiment 8 Investigation into the potential for the infection of *E. vagans* cuttings from nursery materials contaminated with binucleate *Rhizoctonia* sp. isolate 48.

No statistical analysis was carried out on the results of Experiment 8, since the data obtained was limited. Binucleate *Rhizoctonia* sp. isolate 48 was grown on seven different substrates, (including plastic trays, capillary

matting, gravel, sand, 1 - 5 mm compost fragments, cuttings and polythene) which were then placed in contact with trays of cuttings as detailed in section 3.2.9.

Rhizoctonia disease was initiated from all seven inoculum sources (Table 13). Infection in the newly struck cuttings took place most quickly from infested compost fragments and infected cuttings (6 days) and most slowly from contaminated capillary matting at the base of the trays (46 days).

Table 13 Foliar browning due to Rhizoctonia infection of cuttings grown in trays in contact with nursery materials and substrates contaminated with binucleate *Rhizoctonia* sp. isolate 48. (Experiment 8)

Material or substrate[a]	Number of days (following striking) after which foliar browning due to *Rhizoctonia* sp. was observed on cuttings
Capillary matting	46
Sand	32
Gravel	32
Compost fragments	6
Cuttings	6
Trays	9
Polythene	11

[a]See section 3.2.10 for details of treatments.

Isolations from cuttings revealed that all foliar browning which developed in this experiment was due to infection by *Rhizoctonia* spp. There was no foliar browning on, and no *Rhizoctonia* spp. isolated from control cuttings grown in contact with uncontaminated nursery materials.

3.3.10 Experiment 9 The effect of depth of inoculum of binucleate *Rhizoctonia* sp. isolate D1 in compost, on the infection of *C. vulgaris* and *E. vagans* cuttings.

When the statistical analysis was carried out on the foliage scores (final assessment), all treatments which had a mean score of zero (including all controls) were omitted. Cuttings grown in compost with infested straw spread on the surface, showed severe foliar browning due to *Rhizoctonia* spp., whereas those grown in compost with inoculum spread on the base of the trays showed no foliar damage after 6 weeks (Fig. 6). Differences between scores of cuttings in the treatments involving infested straw spread on the top and through the middle of the compost were significant ($F_{1,10} = 13.68$; $P < 0.001$).

There were no significant differences between the scores recorded on different species or different cultivars of heather.

Isolations from cuttings showed that the trays containing surface inoculum yielded the greatest number of infected cuttings (83%, Table 14). Twenty-eight percent of cuttings grown in compost containing Rhizoctonia inoculum spread on the base of the tray were infected. None of the cuttings in control treatments were infected with *Rhizoctonia* spp.

3.4 DISCUSSION

Foliar browning and death of *C. vulgaris* and *Erica* spp. may result from a variety of causes. Adverse environmental conditions such as high compost salt concentrations, lack

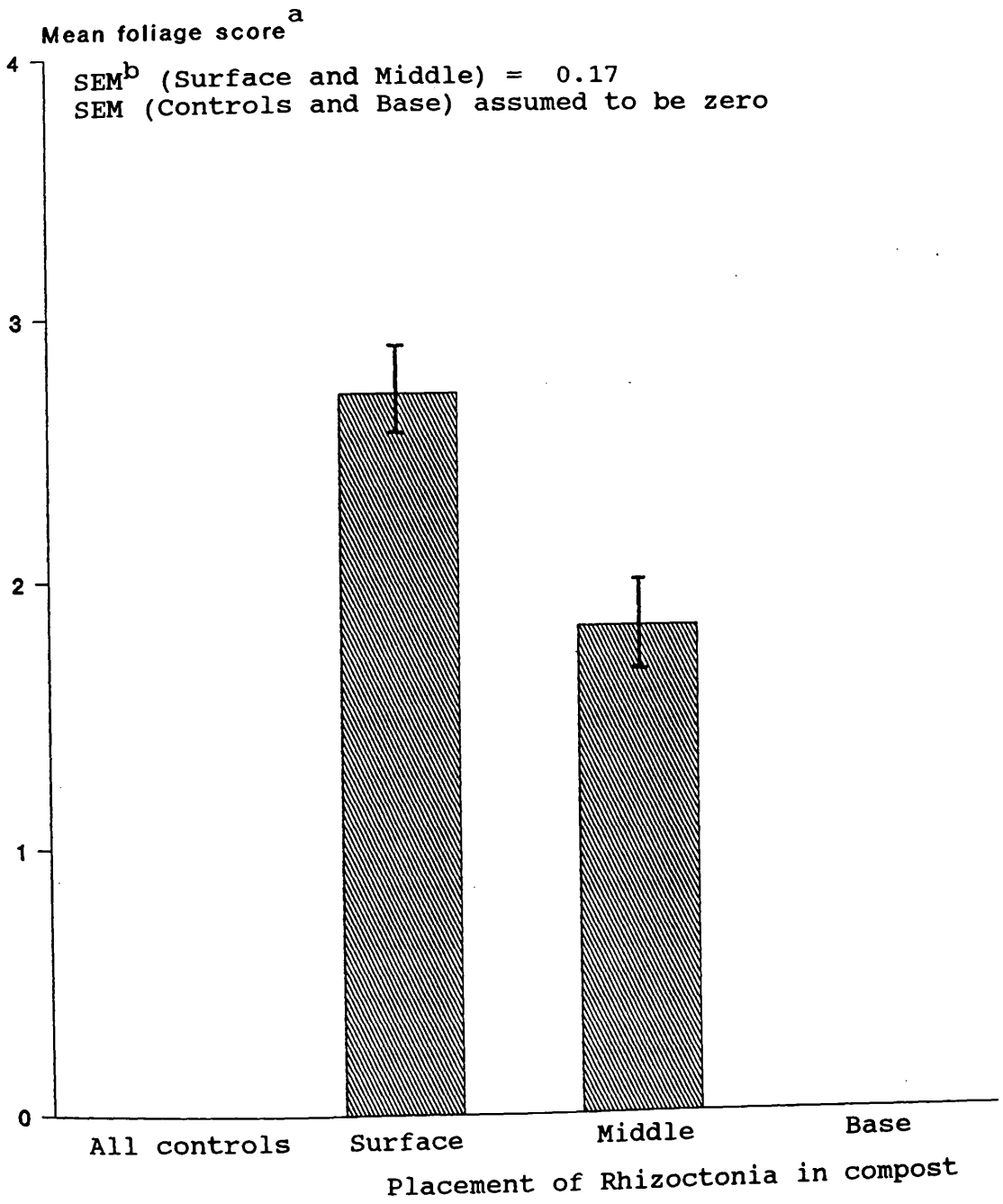

Fig. 6 The effect of depth of inoculum of binucleate *Rhizoctonia* sp. isolate D1 in the compost, on the foliage condition[a] of *C. vulgaris* cv Loch Turret cuttings struck in standard propagation compost and assessed 6 weeks after striking. (Experiment 9)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]Scores of cuttings grown in uninfested composts and composts containing binucleate *Rhizoctonia* sp. isolate D1 inoculum spread on the base of the tray were equal to zero. They were omitted from the statistical analysis and the SEM's were assumed to zero. All other SEM's with 10 degrees of freedom.

Table 14 Isolation of *Rhizoctonia* spp. from *E. vagans* and *C. vulgaris* cuttings 6 weeks after striking into compost amended with binucleate *Rhizoctonia* sp. isolate D1 inoculum at different depths within the cutting trays. (Experiment 9)

Treatment	Position of inoculum (depth in cm)	Proportion of cuttings from which *Rhizoctonia* spp. isolated (18 cuttings tested)	Standard error of mean
Uninoculated	0.0 (surface)	0.00	0.000
	2.5 (middle)	0.00	0.000
	5.0 (bottom)	0.00	0.000
Rhizoctonia sp. isolate D1	0.0 (surface)	0.83	0.089
	2.5 (middle)	0.67	0.111
	5.0 (bottom)	0.28	0.106

of compost aeration, and diseases caused by pathogens other than *Rhizoctonia* spp. give rise to symptoms which may be confused with those caused by *Rhizoctonia* spp. Visual diagnosis of Rhizoctonia disease of *C. vulgaris* and *Erica* plants is difficult because the disease manifests itself in several ways, due in part to the diverse array of growth habits and foliage types exhibited by the various host cultivars.

Infection of *C. vulgaris* and *Erica* spp. however, was often associated with several characteristic symptoms. Cuttings turned brown from the base upwards, formed poor roots (or no roots) and sometimes became covered in pale yellow to brown web-like mycelium. Rooted plants showed foliar browning from soil level upwards and rotting of

roots and stem-base at soil level. Mycelium was often visible on stem-base and lower foliage under X 40 magnification. Such symptoms have also been observed on other ornamental and nursery stock species, (Burr *et al.*, 1978; Lambe & Wills, 1980; Frisina & Benson, 1988).

Several workers have shown that inoculum potential, (defined by Johnson & Booth, 1983 of CMI) as 'the energy for growth of a fungus available for colonisation of a substratum at the surface of the substratum to be colonised'), is highly dependent on a range of environmental factors. For example, Kerr (1956) and Shurtleff (1953) showed that almost 100% relative humidity was necessary for growth of mycelium and for infection to take place. Blair (1943) found that a carbon dioxide concentration of 2.5% in the atmosphere retarded the linear growth rate of *R. solani* on PDA and that concentrations of 20 - 25% carbon dioxide greatly inhibited saprophytic growth.

The fact that *Rhizoctonia* spp. infection of *Erica* spp. and *C. vulgaris* cultivars occurred most frequently at or around soil level, suggests that conditions for infection and therefore inoculum potential, are frequently suitable in this region. Carbon dioxide levels may be too low for frequent high levels of infection in fine roots, most of which lie more than 2 cm below the compost surface, and air relative humidity may be too low for infection to take place on foliage higher than 4 cm above compost level. Since it has been shown that *Rhizoctonia* spp. infection

takes place most frequently in the foliage and roots surrounding the compost surface, the harvesting of propagating material from outwith this region, (i.e. more than 4 cm above compost surface), may reduce the possibility of disease transmission from the stock plant to cuttings.

Strains of *Rhizoctonia* spp. may differ in their tolerance of adverse environmental conditions and therefore in their inoculum potential under different situations. For example, Durbin (1959) found that strains of *R. solani* showed different levels of tolerance to high carbon dioxide concentrations depending on their ecological niche. Strains which mainly caused root rots, grew better than isolates which attack foliage or stem in the presence of high carbon dioxide concentrations.

The strains of *Rhizoctonia* spp. examined in Experiment 2 all caused infection which gave rise to browning which was concentrated on the lower foliage, stem-base and woody roots. However, strains may exist which primarily attack different areas of *C. vulgaris* and *Erica* spp. plants, for example fine roots below compost level or foliage several centimetres above soil level. This is possible considering the high numbers of *Rhizoctonia* spp. strains which exist, differing in many characteristics (Baker, 1970; Ogoshi, 1985). Infection may also spread within the plant canopy or down through the compost, if environmental conditions change to favour pathogen development or if host

susceptibility increases (Baker & Martinson, 1970).

Rhizoctonia spp. were isolated only from nursery soil and used nursery materials and compost components (Experiment 1). They were not found on new materials. *R. solani* is capable of considerable spread through soil in the absence of a host (Papavizas, 1970) and can also grow and survive on inert media such as plastics, sand and perlite. It is clear that spread of *Rhizoctonia* spp. is only partially dependent on the presence of suitable host plants. These findings are in accordance with those of Stephens *et al.* (1983), who found that *Rhizoctonia* spp. could be isolated from glasshouse soil, walkways and from seedling flats, but not from fresh, unused peat, perlite or vermiculite. Jager *et al.* (1982) found that *R. solani* could be isolated from the roots of 52 weed species in the Netherlands. Lack of time prevented such a study being carried out on UK nurseries as part of this investigation.

Spread of *Rhizoctonia* spp. and subsequent infection can be initiated from diseased plants and cuttings and from infested compost fragments (Experiment 8). It can also be initiated from several inert sources including contaminated capillary matting, polythene, plastic, sand and gravel. Although several workers have found that *Rhizoctonia* spp. can spread rapidly from one plant to another in a favourable environment (Singh, 1955), and it has been shown that *Rhizoctonia* spp. infection can be initiated from and spread through contaminated soil/compost (Stephens *et al.*, 1983), there has been

little work carried out to examine the role of contaminated equipment/nursery materials in the spread of Rhizoctonia diseases.

The fact that *R. solani* mycelium can absorb, translocate and store nutrients, reduces continuous nutritional dependence on food bases for survival and growth. For example Papavizas (1970), reported that *R. solani* was capable of growing to a depth of 30 - 40 cm in soil from infected potato sprouts. Between exhaustion of a particular food base and appearance of another, *Rhizoctonia* spp. can survive as thick-walled mycelium or sclerotia or on the exhausted food base (Boosalis & Scharen, 1959). New nutritional bases may be made on the addition of fresh compost to the area surrounding the fungus, and in this way growth may re-commence after a resting period.

It can be concluded that the use of new or sterilised composts and materials is of great importance in preventing the spread of *Rhizoctonia* spp. throughout heather nurseries. Efforts made to reduce or eliminate the presence of weeds and to reduce contact of composts, nursery materials and plants with nursery soil, will also help to reduce the quantity of Rhizoctonia inoculum coming into contact with *C. vulgaris* and *Erica* spp., thereby reducing disease incidence.

Thirty six percent of *Rhizoctonia* spp. isolated from nursery stock (including *C. vulgaris*), soil and potato

tubers, were assigned to the species *R. solani* on the basis of hyphal characteristics and nuclear number (*R. solani* isolates possess multinucleate cells). The remaining 64% were binucleate and will subsequently be referred to as such. Species determination amongst binucleate *Rhizoctonia* spp. isolates is complex and time consuming, hence many workers have attempted to classify binucleate *Rhizoctonia* spp. into anastomosis groups, (see section 1.2.1). It was intended to determine the AGs and CAGs of both *R. solani* and binucleate *Rhizoctonia* spp. respectively. However, this proved impossible in the time available. IMI were unable to provide the full range of *R. solani* tester isolates necessary to determine AGs and no other UK source was found. There is no current UK source of tester isolates for determination of CAGs within binucleate *Rhizoctonia* spp. Requests were made to Dr A. Ogoshi, (Hokkaido University, Sapporo, Japan), Dr L.L. Burpee, (Pesticide Research Laboratory, Pennsylvania State University, USA) and Dr D.M. Benson, (North Carolina State University, USA) for tester isolates of both *R. solani* and binucleate *Rhizoctonia* spp. It is intended to carry out determination of AG and CAG groups as soon as possible.

Although it will be of scientific interest to determine the anastomosis groups to which the *Rhizoctonia* spp. isolates used in this work belong, it is doubtful whether the information will have immediate practical relevance. Burpee *et al.* (1980b) showed that none of the CAGs found in America were defined by host range or

geographic origin, therefore the knowledge of which CAGs the isolates belong to would be unlikely to aid the development of control measures.

Work in this project has shown that great differences exist between levels of disease and damage caused by different *Rhizoctonia* spp. isolates taken from HONS spp. both in the UK and in Holland, therefore control measures must be developed for these *Rhizoctonia* spp. regardless of the anastomosis groups to which they belong.

Three of seven strains taken from *C. vulgaris* were multinucleate. These results differ from those of Frisina & Benson (1987), who found that 16% of isolates taken from woody ornamental plants were of *R. solani* and 84% were of binucleate *Rhizoctonia* spp. However, the sample examined in our survey was small. A larger sample may have yielded different proportions of *R. solani* and binucleate *Rhizoctonia* spp.

There were significant differences between levels of foliar browning and between root development on cuttings grown in composts amended with different *Rhizoctonia* spp. isolates (Experiment 3). Several workers have reported that both *R. solani* and binucleate *Rhizoctonia* spp. are composed of a great many diverse strains, differing in several characteristics including pathogenicity (Bateman, 1970). Some strains may be specific only to one plant, whereas others attack a wide range of families. Different strains may cause different symptoms in the same host (Le

Clerg, 1939).

Frisina & Benson (1987) found that disease levels were significantly greater on plants infected with *R. solani* than on those infected with binucleate *Rhizoctonia* spp. isolates. However, this was not found with strains isolated from Dutch or UK nurseries, (Dolmans, 1988, personal communication). A range of pathogenicity was found within both *R. solani* and the binucleate *Rhizoctonia* spp. isolates.

Although the results obtained in Experiment 3 show that several of the *Rhizoctonia* spp. isolates tested caused little or no damage, some workers have suggested that populations of *Rhizoctonia* spp. are continuously varying in pathogenicity (Daniels, 1963). *Rhizoctonia* spp. are pathogens with immense capabilities for variation and flexibility under a wide range of host plants and environmental and cultural conditions. It is therefore possible that host/fungal relationships in which the fungus exists as a weak parasite, may alter as a result of changing environmental/cultural conditions to allow rapid fungal development, disease and plant damage.

The severity of *Rhizoctonia* spp. infection was found in Experiment 4 to differ significantly between cultivars. This was as expected due to the wide variety of growth habits, foliage and leaf types present in *C. vulgaris* and *Erica* spp. However, the differences in cultivar susceptibility were found to differ significantly depending on environment (Experiment 5). This can be

understood when considering the ways in which host/pathogen relationships change under differing environmental conditions. For example it has been shown on numerous occasions that changes in temperature, air relative humidity and soil moisture levels can affect both pathogen growth and development and the growth rate of the host, thereby affecting disease development, (Agrios, 1969; Dimond & Horsfall, 1960). Initially, the intention was to rank cultivars for their susceptibility to disease caused by *Rhizoctonia* spp. However, this has proved impossible both due to the large number of diverse *Rhizoctonia* spp. strains and the variety of environmental conditions in which host and fungus are brought into contact with one another.

Several workers have noted the extreme difficulty encountered in developing varieties or species which are resistant to diseases caused by *Rhizoctonia* spp. (Leach & Garber, 1970). This can be understood when the nature of parasitism of the fungus is considered along with its wide host range and its large number of diverse strains. Leach & Garber (1970) stated that, "On theoretical grounds, the odds would seem to be against finding in a single variety, resistance to a fungus such as *R. solani* which is not selective in its parasitism. In this particular area of plant protection, chemical control seems to offer greater promise of success than control by resistant varieties." Although a few workers have noted improved resistance in

sugar beet to root-rot pathogens, (Downie *et al.*, 1952; Afanasiev & Morris, 1954) and Creager (1945) described varietal resistance in gladiolus (*Gladiolus* spp.), there are few incidences of resistance within susceptible species.

Although the *E. cinerea* cultivars used in Experiments 4 and 5 were less susceptible than the *C. vulgaris* cultivars, further comparisons must be made before a definitive statement on the relative susceptibilities of *E. cinerea* and *C. vulgaris* to *Rhizoctonia* spp. infection can be put forward. It is unlikely that *C. vulgaris* cultivars will be found with a significant degree of resistance to strains of *R. solani* and binucleate *Rhizoctonia* spp. under the range of environmental conditions likely to be found on heather nurseries.

Significantly higher foliage scores were measured on *C. vulgaris* cuttings taken from shoot-tips in comparison with shoot-bases (Experiment 6). This may have been a function of the age of the plant material. Shoot-tip cuttings are softer and therefore may present less of a barrier to fungal attack than older, firmer shoot-base cuttings.

Foliage scores of cuttings grown in *Rhizoctonia*-infested compost which were taken from stock kept outdoors or in the glasshouse, were higher than those of similar cuttings taken from stock maintained in a tunnel. This may be as a result of the fact that the tunnel provided the best environment (of the three) for vigorous, healthy

shoot growth. Plant growth outdoors was slower than that in the tunnel. In addition, rain may have splashed soil micro-organisms into the foliage, some of which may have acted synergistically with binucleate *Rhizoctonia* sp. isolate D1.

Although no work has been carried out on the effects of synergism between *Rhizoctonia* spp. and saprophytes on cuttings of *C. vulgaris* or *Erica* spp. it has been postulated that species of several fungal genera such as *Trichoderma, Penicillium* and *Gliocladium* can act synergistically with known root pathogens on cuttings of *Rhododendron, Juniperis* and *Camellia* spp., to produce higher levels of disease than when single root pathogens are present, (Smith, 1982). Conversely, however, saprophytic fungi such as *Trichoderma* spp. have been shown to prevent infection and disease caused by *Rhizoctonia* spp. (see sections 5.1 and 5.2, also Lewis & Papavizas, 1985; and Henis *et al.*, 1978).

Shoot growth in the glasshouse was abundant but soft. This may have allowed easy penetration of fungal infection pegs in plant tissue. For example, damping-off of brassicas is most severe during seedling stages of growth when stem tissues are soft, (Agrios, 1969).

Results from Experiment 7 demonstrated that disease severity was related to inoculum density. The highest level of foliar browning was observed in compost which contained the greatest number of pieces of straw infested

with binucleate *Rhizoctonia* sp. isolate 48. These results are in accordance with those of studies carried out by several workers. Rich & Miller (1963) counted the number of mycelial fragments on 5 cm segments of strawberry (*Fragaria* spp.) roots and found that very significant linear regressions were obtained between number of fragments per unit area of roots, and the number of surviving plants at the end of the growing season. Boosalis & Scharen (1959) compared the occurrence of plant debris particles infested with *R. solani* in two areas of a field previously containing sugar beet. Approximately 8.5% of the plant residue particles (PRP) from soil with a high incidence of crown rot, yielded *R. solani* pathogenic to sugar beet, whereas only 2% of the PRP from part of the field with a low incidence of crown rot yielded *R. solani*.

Since inoculum density has been shown to affect the amount of damage caused by binucleate *Rhizoctonia* sp. isolate 48, measures taken to reduce the quantity of Rhizoctonia inoculum present on plants, compost and nursery equipment will help to reduce disease incidence and severity.

There was evidence of a relationship between disease severity and depth of Rhizoctonia inoculum in the compost (Experiment 9). Foliar browning was most marked where inoculum of binucleate *Rhizoctonia* sp. isolate D1 was spread on the surface and least severe where it was spread on the base of trays (approximately 5.0 cm beneath compost surface). Since temperature, levels of nutrient elements

and pH are unlikely to have differed greatly between the compost surface and compost at the tray bases, it is thought that carbon dioxide/oxygen levels may have affected saprophytic growth and/or inoculum potential of the fungus.

Oxygen depletion and carbon dioxide build-up result from respiration in the soil/compost, and are accentuated by high soil moisture and low compost air-filled-porosity (high bulk density). Both oxygen depletion and carbon dioxide build-up increase with compost depth.

Several workers have demonstrated the effects which carbon dioxide/oxygen levels can have, both on the saprophytic growth of *R. solani* and on disease incidence.

Blair (1943) found that a concentration of 2.5% carbon dioxide in the atmosphere retarded growth of *R. solani* on PDA and that concentrations of 20 - 25% greatly inhibited its growth. Papavizas & Davey (1962) found that oxygen supply does not become limiting to *R. solani* growth *in vitro* or in soil until the concentration in the atmosphere is 1%. Thus from the available literature on oxygen and carbon dioxide concentrations in soil, (Baker & Martinson, 1970), it seems that under restricted aeration, carbon dioxide toxicity is a more probable explanation for reduced growth of *R. solani* than oxygen deficiency.

Durbin (1959) showed that *R. solani* strains demonstrated a differential tolerance to high concentrations of carbon dioxide depending on their

ecological niche. Strains that mainly incited root rots grew much better than foliar or stem-base attacking isolates in the presence of high carbon dioxide levels. For this reason, the effect of inoculum depth must be studied using a range of *Rhizoctonia* spp. strains before definitive conclusions can be reached. Binucleate *Rhizoctonia* sp. isolate 48 which was used in Experiment 7 was isolated from the stem-base of Juniper cuttings, therefore its growth may be restricted by carbon dioxide toxicity to a greater extent than that of *Rhizoctonia* spp. strains such as K1 and E, which were isolated from roots and tubers respectively.

CHAPTER 4

THE EFFECT OF CULTURAL AND ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF DISEASE CAUSED BY *RHIZOCTONIA* SPP. ON NURSERY-GROWN *C. VULGARIS* AND *ERICA* SPP.

4.1 INTRODUCTION

Numerous successful disease control programmes have been developed, which are dependent on the changing responses of pathogens to differences in environmental or cultural conditions, for example, clubroot of brassicas (caused by *Plasmodiophora brassicae* Woronichin) can be controlled through manipulation of soil pH (Jones, 1987). Good soil drainage reduces the number and activity of several fungal pathogens including *Pythium* spp. (Agrios, 1969) and careful attention to humidity levels in glasshouses will minimise disease caused by *Botrytis cinerea* (Nelson, 1991)

The effect of several environmental parameters including compost pH, air relative humidity, temperature, compost moisture content, compost air-filled porosity and level of controlled-release fertiliser on the infection of cuttings of *C. vulgaris* and *Erica* spp. by *Rhizoctonia* spp. was examined. The aims were firstly to determine whether or not the severity of infection and foliar browning caused by *Rhizoctonia* spp. on cuttings was affected by changes in the above environmental factors, and secondly to propose control measures for *Rhizoctonia* spp. based on the differential responses of the pathogen to the changes in these factors.

4.2 MATERIALS AND METHODS

4.2.1 Experiment 10 The effect of pH on the growth of *Rhizoctonia* spp. *in vitro*.

Seven, 250 ml lots of autoclaved PDA were amended using

lactic acid or sodium hydroxide to give pH values of 4.0, 4.8, 5.4, 5.9, 6.2, 7.4 and 8.8. The agar was then poured into 9 cm diameter Petri dishes (nine plates of each pH value) and allowed to set.

One, 11 mm diameter agar disc from a 2 week old culture of *R. solani* isolate A or E or binucleate *Rhizoctonia* sp. isolate F on PDA, was placed in the centre of each dish. There were three plates (plots) of each *Rhizoctonia* spp./agar pH combination within each of three replicate blocks. Plates from each replicate block were sealed in polythene bags in a random order and were incubated at 23°C. As far as possible each plate within each block was maintained under the same conditions.

Radial growth of mycelium was measured 24, 48 and 72 hours after the start of incubation. Growth was taken as the mean of three measurements taken at 120° from one another on each plate (Fig. 7).

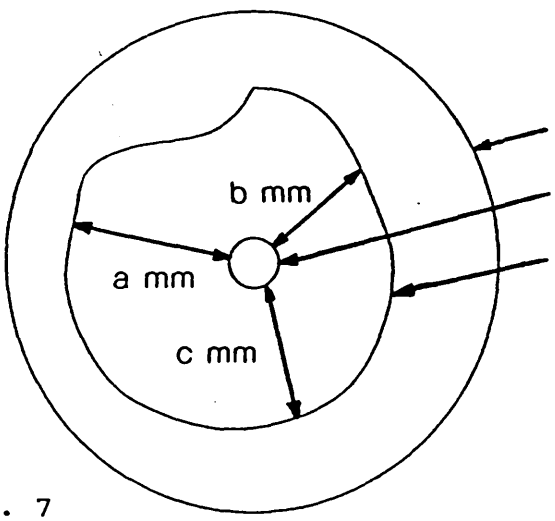

Fig. 7

4.2.2 Experiment 11 The effect of temperature on the growth of *Rhizoctonia* spp. *in vitro*

Potato dextrose agar discs (11 mm diameter) from 2 week old cultures of each of the two binucleate *Rhizoctonia* spp. isolates 72 and 48 and each of the two *R. solani* isolates E and A were placed in the centre of 9 cm diameter Petri dishes which contained PDA.

Within each of six incubators set at 5°, 10°, 15°, 20°, 25° and 30°C, the plates were divided into three replicate blocks, each comprising four plates (plots). The plates from each replicate were sealed in separate polythene bags. As far as possible each plot within each block received the same environmental/cultural conditions. Growth of mycelium was measured (as described for Experiment 10), 24, 48 and 72 hours after the start of incubation.

4.2.3 Experiment 12 The effect of relative humidity on the growth of binucleate *Rhizoctonia* spp. on *C. vulgaris* cv Cuprea plants.

Standard inoculum of either binucleate *Rhizoctonia* spp. isolate D1 or 64 or uninoculated straw was incorporated into standard potting compost (2 g straw/1 compost). Twelve, 8 cm square pots (Optipot 8K, Congleton Plastics Co. Ltd.), each of which contained a rooted cutting of *C. vulgaris* cv Firefly (potted 12 weeks after striking in one of the above composts), were placed in each of eighteen plastic trays (42 x 32 x 8 cm deep, Petcraft Cat litter trays). One half of the trays were placed on a

propagation bed in a polythene tunnel and sealed under a single sheet of white polythene (120 gauge, 30 micron, LBS Polythene), to give a measured relative humidity (r.h.) of 90-100% surrounding the plants. The others were placed under a further sheet of polythene (in the same tunnel), which had been cut with twenty regularly placed slits (20 mm in length) per tray to give a measured r.h. of 70-85%. Relative humidity was recorded daily in mid-afternoon, from gauges (Brannan Ltd.) taped to plant labels which were pushed into the optipots under the white polythene.

The trays in each humidity level were divided into three blocks. Within each block there were three trays (plots) comprising twelve pots of uninoculated controls and twelve pots inoculated with each of the two binucleate *Rhizoctonia* spp. isolates (sub-plots). The trays and pots within them were randomly allocated to their positions within the blocks. As far as possible each plot within each block received the same environmental/cultural conditions.

The growth of *Rhizoctonia* spp. mycelium vertically up the foliage of the *C. vulgaris* plants was measured as follows, 8 weeks after the start of the experiment. The foliage including main stem was cut at compost level and examined under a dissecting microscope (x 40) to confirm the presence of *Rhizoctonia* spp. mycelium. The height at which mycelium grew above compost level was measured on each plant using a ruler.

4.2.4 Experiment 13 The effect of controlled release fertiliser on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

Five cuttings of *C. vulgaris* cvs Beoley Gold, Kinlochruel and Cuprea were struck in standard propagation compost contained in seed trays (23 x 17.5 x 5.5 cm deep), in which standard inoculum of one of binucleate *Rhizoctonia* spp. isolates D1 or 64 (or uninoculated straw) had been incorporated at 2 g straw/l compost together with either 0, 1 or 3 g of Ficote 140 14:14:14 controlled release fertiliser/l compost.

Within each of three replicate blocks, there were nine trays (plots) comprising a five-cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged along a glasshouse bench. As far as possible, each plot received the same environmental/cultural conditions.

Foliage condition of cuttings was assessed after 2, 3, 6 and 7 weeks (see section 2.8). Twenty cuttings were taken at random from each treatment following the final assessment and two, 2 - 5 mm length pieces of the stem-base from each cutting were placed on PDES agar to determine the presence of Rhizoctonia.

4.2.5 Experiment 14 The effect of compost type on the infection of *C. vulgaris* and *E. vagans* cuttings by binucleate *Rhizoctonia* sp. isolate D1.

Four composts were made up as follows to give a range of air-filled porosity (AFP) values.

1:1 (v/v) Bulrush Sphagnum Moss Peat (screened 22 mm) + Standard Grade Horticultural Perlite (Silvaperl)	AFP 31
1:1 (v/v) peat (as above) + Cambark Fine Grade Bark	AFP 16
5:1 (v/v) peat (as above) + fine sand	" 9
1:1 (v/v) peat (as above) + fine sand	" 4

Five cuttings of *C. vulgaris* cvs Beoley Gold and Silver Queen and *E. vagans* cv Lyonesse were struck in seed trays (23 x 17.5 x 5.5 mm deep) which contained one of the four composts and standard inoculum of binucleate *Rhizoctonia* sp. isolate D1 or uninoculated straw incorporated at 2 g straw/l compost.

Within each of three replicate blocks, there were eight trays (plots) comprising a five-cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered with polythene and arranged on a tunnel floor. As far as possible, each plot within each block received the same environmental/cultural conditions.

Foliage condition was assessed 2, 3, 4 and 6 weeks after striking (see section 2.8). A final destructive assessment was made after 6 weeks. Root development was assessed (see section 2.9) and two, 2 - 5 mm stem-base pieces taken at random from twelve cuttings in each treatment, were placed on PDES agar to determine if Rhizoctonia infection had occurred.

4.2.6 Experiment 15 The effect of compost moisture content on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

Plastic boxes, 10 x 10 x 8 cm deep with five drainage holes in the base, were filled with standard propagation compost which contained 2 g standard inoculum of either binucleate *Rhizoctonia* spp. isolate D1 or 64 or uninoculated straw/1 compost. The boxes were then placed on gravel (1 cm deep) in one of four trays (42 x 32 x 8 cm deep, Petcraft cat litter trays) which were filled to either 0, 2, 4 or 6 cm (above the depth of the gravel) with tap water. Water levels within compost in the boxes were allowed to reach these levels, following which four cuttings of *C. vulgaris* cvs Cuprea, Beoley Silver and Robert Chapman were struck in the boxes. Water levels were maintained in the trays throughout the course of the experiment.

Within each water level, the boxes (plots) were divided into three blocks. Within each block, there were three boxes (plots) containing one of the two isolates or control, comprising a four-cutting row of each cultivar (sub-plots). The boxes were randomly allocated to their positions within the blocks. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliar assessments were made after 2, 4 and 6 weeks (see section 2.8). Following the final assessment, six cuttings were taken at random from each treatment and two, 2 - 5 mm stem-base pieces from each cutting were placed on

PDES agar to determine if Rhizoctonia infection had taken place.

4.2.7. Experiment 16 The effect of pH on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* sp. isolate D1.

Five cuttings of *C. vulgaris* cvs Cuprea, Flamingo and Silver Queen, were struck in seed trays (23 x 17.5 x 5.5 cm deep) of standard propagation compost which contained standard inoculum of binucleate *Rhizoctonia* sp. isolate D1 or uninoculated straw incorporated at 2 g straw/l compost. Magnesian limestone was included prior to addition of inoculum/straw at either 0, 2, 4 or 6 g/l to give measured compost pH values of 3.8, 4.9, 5.7 and 6.0 respectively.

Within each of three replicate blocks, there were eight trays (plots) comprising a five-cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered with polythene and arranged on a tunnel floor. As far as possible, each plot within each block received the same environmental/cultural conditions.

Foliar assessments were made 2, 4 and 6 weeks after striking, (see section 2.8) and total roots in each treatment were washed after 6 weeks, detached from the stem and dried at 60^{o}C for 48 hours prior to weighing.

4.2.8 Experiment 17 The effect of temperature on the infection of *C. vulgaris* and *E. cinerea* cuttings by binucleate *Rhizoctonia* spp.

Five cuttings of *C. vulgaris* cvs Darkness and Cuprea and the *E. cinerea* cv Lilacina were struck in standard propagation compost in seed trays (23 x 17.5 x 5.5 mm deep) which contained standard inoculum of binucleate *Rhizoctonia* spp. isolates 48 or 72 or uninoculated straw incorporated at 2 g straw/l compost. Each tray was placed in one of three controlled environment cabinets maintained at 95% relative humidity and temperature/light regimes as detailed in Table 15.

Within each temperature, the trays were divided into three blocks. Within each block there were three trays (plots) comprising a five-cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered with polythene and arranged in the environmental cabinets. As far as possible, each plot within each block received the same environmental/cultural conditions.

Foliar assessments were carried out after 3 and 4 weeks. (see section 2.8)

4.3 RESULTS

4.3.1 Experiment 10 The effect of pH on the growth of *Rhizoctonia* spp. *in vitro*.

All plots were included in the statistical analysis of the radial growth measurements (final assessment). The growth

Table 15 Light levels ($\mu mol.m^{-2}.s^{-1}$) and temperatures (^{o}C) during 24 hour cycle in controlled environment cabinets. (Experiment 17)

	Hour																							
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Light level	2400 to 0600 hrs = 0, 0900 to 2200 hrs = 210													0600 to 0900 hrs = 140, 2200 to 2400 hrs = 140										
Cabinet 1 temps.	6	5	5	5	5	7	8	9	10	10	10	10	10	10	10	10	10	10	10	10	10	9	8	7
Cabinet 2 temps.	12	10	10	10	10	12	14	15	16	17	17	17	17	17	17	17	17	17	17	17	17	16	15	14
cabinet 3 temps.	18	15	15	15	15	18	21	22	23	25	25	25	25	25	25	25	25	25	25	25	25	23	22	21

rate of isolates of *Rhizoctonia* spp. on PDA taken as an average over all pH values, differed significantly ($F_{2,40}$ = 528.46; P < 0.001) from one another (Table 16). *R. solani* isolate A showed the highest growth rate at all pH values except 6.2.

Significant differences ($F_{6,40}$ = 120.83; P < 0.001) were found between the growth rates of *Rhizoctonia* spp. isolates on media of different pH values. There was also evidence of a significant interaction ($F_{12,40}$ = 35.29; P < 0.001) between *Rhizoctonia* spp. isolate and pH. Binucleate *Rhizoctonia* sp. isolate F grew fastest on agar of pH 6.2 (colony diameter 36.9 mm after 72 hours), *R. solani* isolate A on agar of pH 5.4 (colony diameter 40.1 mm after 72 hours) and *R. solani* isolate E on agar of pH 7.4 (colony diameter 34.9 mm after 72 hours).

4.3.2 Experiment 11 The effect of temperature on the growth of *Rhizoctonia* spp. *in vitro*.

When the statistical analysis was carried out on the radial growth measurements (final assessment), the treatments involving isolates kept at 5°C were omitted due to the large number of zero's in the data. Temperature had a significant effect ($F_{4,38}$ = 1436.75; P < 0.001) on the growth of isolates of *Rhizoctonia* spp. *in vitro* (Fig. 8). There were significant differences ($F_{3,38}$ = 32.09; P < 0.001) between the growth rates of the four *Rhizoctonia* spp. isolates.

There was also evidence of an interaction ($F_{12,38}$ =

Table 16 Growth of *Rhizoctonia* spp. on potato dextrose agar amended with lactic acid or sodium hydroxide to give a range of pH values. (Experiment 10)

*Rhiz.*spp. isolate	Number of nuclei	Mean colony radius[a] pH 4.0	pH 4.8	pH 5.4	pH 5.9	pH 6.2	pH 7.4	pH 8.8	mean
A	many	30.0	34.0	40.1	35.8	32.8	36.3	31.6	34.4
E	many	19.7	18.0	20.0	22.0	18.2	34.9	12.8	20.8
F	2	20.2	33.0	33.6	35.7	36.9	35.3	17.2	30.3
					SEM[b] = 0.80				SEM = 0.30
Mean		23.3	28.3	31.2	31.2	29.3	35.5	20.5	
					SEM = 0.46				

[a]Means of three measurements of colony radius from original 11 mm culture disc to edge of colony in mm taken 72 hr after inoculation and incubation at 23°C.

[b]All SEM's with 40 degrees of freedom

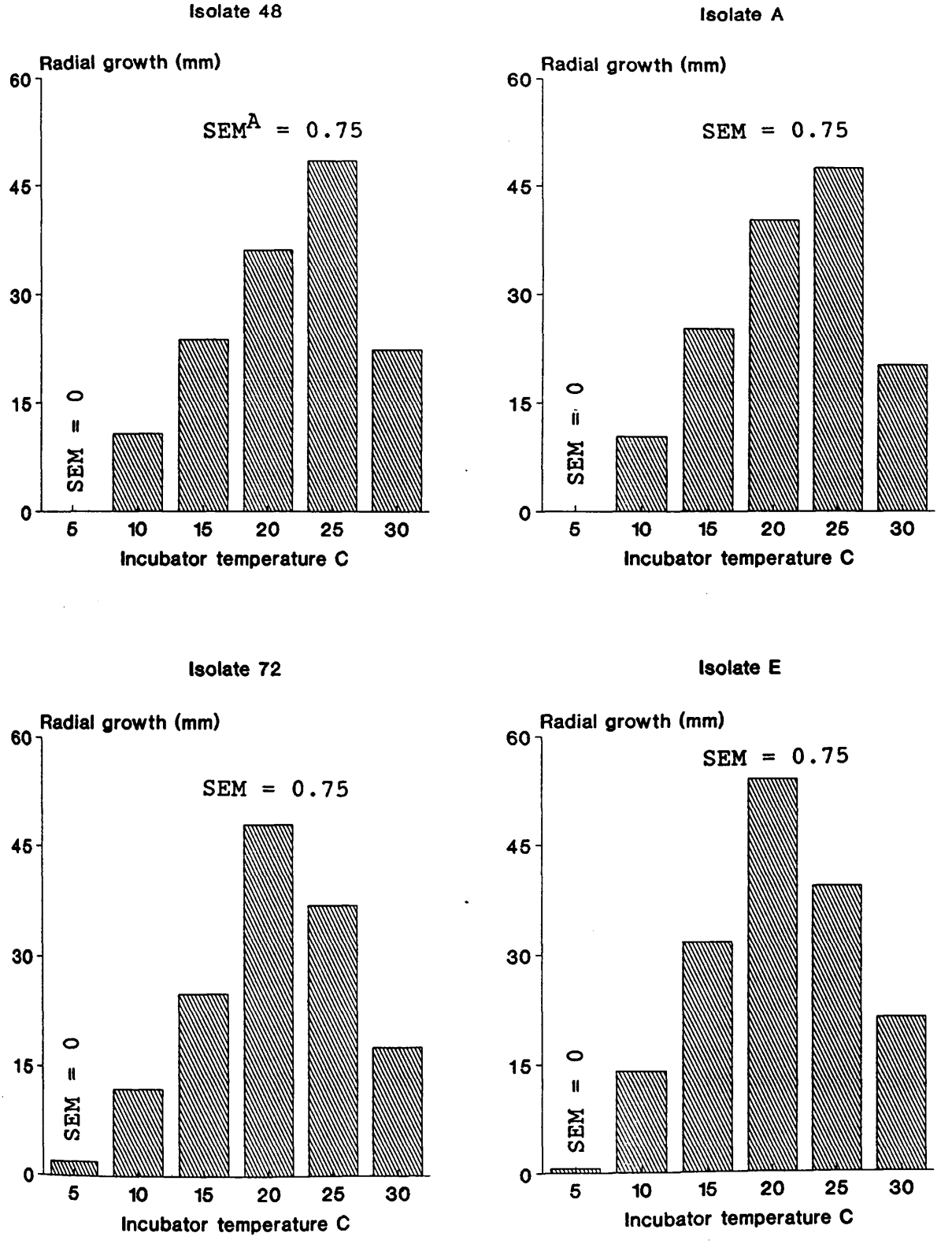

Fig. 8 Growth of *R. solani* isolates A and E and binucleate *Rhizoctonia* spp. isolates 72 and 48 at a range of temperatures on potato dextrose agar. (Means of three measurements of colony radius from original 11 mm culture disc to edge of colony in mm, taken 72 hours after inoculation). (Experiment 11)

[a]SEM of treatments at temperatures 10° to 30°C is 0.75. Data from treatments at 5°C were omitted from the statistical analysis. In these cases the SEM was taken to be zero. All other SEM's with 38 degrees of freedom.

43.22; P < 0.001) between isolate and temperature. That is the four isolates showed different growth responses at the six temperatures tested. *R. solani* isolate A and binucleate *Rhizoctonia* sp. isolate 48 did not grow at 5°C. However, *R. solani* isolate E and binucleate *Rhizoctonia* sp. isolate 72 grew slowly at 5° (approx. 0.7 mm and 1.0 mm respectively in 72 hours). *Rhizoctonia* spp. isolates 48 and A grew optimally at 25°C (approx. 48mm in 72 hours) and isolates E and 72 grew optimally at 20°C (approx. 54 and 48 mm respectively in 72 hours). This experiment was carried out again at a later date and similar results were obtained.

4.3.3 Experiment 12 The effect of relative humidity on the growth of binucleate *Rhizoctonia* spp. on *C. vulgaris* cv Cuprea plants.

When the statistical analysis was carried out on the pathogen growth measurements, the control treatments, (which contained no *Rhizoctonia* spp. and had a mean measurement of zero) were omitted. Growth of isolates of binucleate *Rhizoctonia* spp. was significantly faster ($F_{1,6}$ = 2568.39; P < 0.001) in an atmosphere of 90 - 100% r.h. than in that of 70 - 85% r.h. Binucleate *Rhizoctonia* sp. isolate D1 grew an average of 1.1 mm vertically from the compost surface in an atmosphere of 70 - 85% r.h. in 8 weeks, whereas it grew 10.9 mm in 90 - 100% r.h. Binucleate *Rhizoctonia* sp. isolate 64 grew an average of 3.1 mm vertically in an atmosphere of 70 - 85% r.h. in

8 weeks, whereas it grew 14.9 mm in 90 - 100% r.h.

Differences between growth rates of isolates D1 and 64 were significant ($F_{1,6}$ = 197.97; P < 0.001) and there was a significant interaction ($F_{1,6}$ = 20.79; P < 0.001) between humidity and isolate. No *Rhizoctonia* spp. were observed on, or isolated from control plants.

4.3.4 Experiment 13 The effect of controlled release fertiliser on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), the control treatments were omitted because they had mean scores equal to zero. There were significant differences ($F_{2,34}$ = 8.35; P < 0.001) between the extent of foliar browning on cuttings grown in composts which were infested with binucleate *Rhizoctonia* spp. and contained the equivalent of 0, 1 or 3 g of controlled release fertiliser/l compost (Table 17). There were significant differences both between mean foliage scores obtained from the three cultivars ($F_{2,34}$ = 9.44; P < 0.001) and between foliage scores obtained from the two binucleate *Rhizoctonia* spp. isolates ($F_{1,34}$ = 279.37; P < 0.001). No *Rhizoctonia* spp. were isolated from cuttings grown in composts which had not been amended with the fungus.

The control treatments were omitted from the analysis of the root indices since the values obtained were much higher than those from other treatments. There was no significant effect of fertiliser level on root indices of

Table 17 Mean foliage scores[a] of *C. vulgaris* cuttings grown in peat and bark compost which contained different levels (g/l compost) of controlled release fertiliser (Ficote 140) and was amended with binucleate *Rhizoctonia* spp. isolates D1 and 64, assessed 7 weeks after striking. (Experiment 13)

Rhiz. sp. isolate	Mean foliage scores[a]			
	0 g Ficote	1 g	3 g	Mean
Uninoculated control	0.0	0.0	0.0	0.0
		SEM = 0		
D1	1.2	1.3	1.5	1.4
64	3.0	2.6	3.4	3.0
		SEM = 0.12		SEM = 0.07
Mean (Rhizoctonia-infested treatments	2.1	2.0	2.4	
		SEM = 0.08		

[a]Foliage scores: 0 = no browning; 4 = totally brown foliage. Foliage scores were mean values taken from the three *C. vulgaris* cvs Beoley Gold, Kinlochruel and Cuprea.

[b]Data from control treatments was omitted from the analysis of variance and their SEM's were assumed to be equal to zero. All other SEM's with 34 degrees of freedom.

cuttings grown in compost amended with binucleate *Rhizoctonia* spp. isolates D1 or 64 (final assessment, Fig. 9). Root indices of cuttings in infested composts were lower than those of the controls in all three fertiliser levels. There were no significant differences between the mean root indices recorded on cuttings grown in treatments containing binucleate *Rhizoctonia* spp. isolates D1 and 64. The plot of residual versus fitted values which was completed following the analysis of variance indicated that the results of the

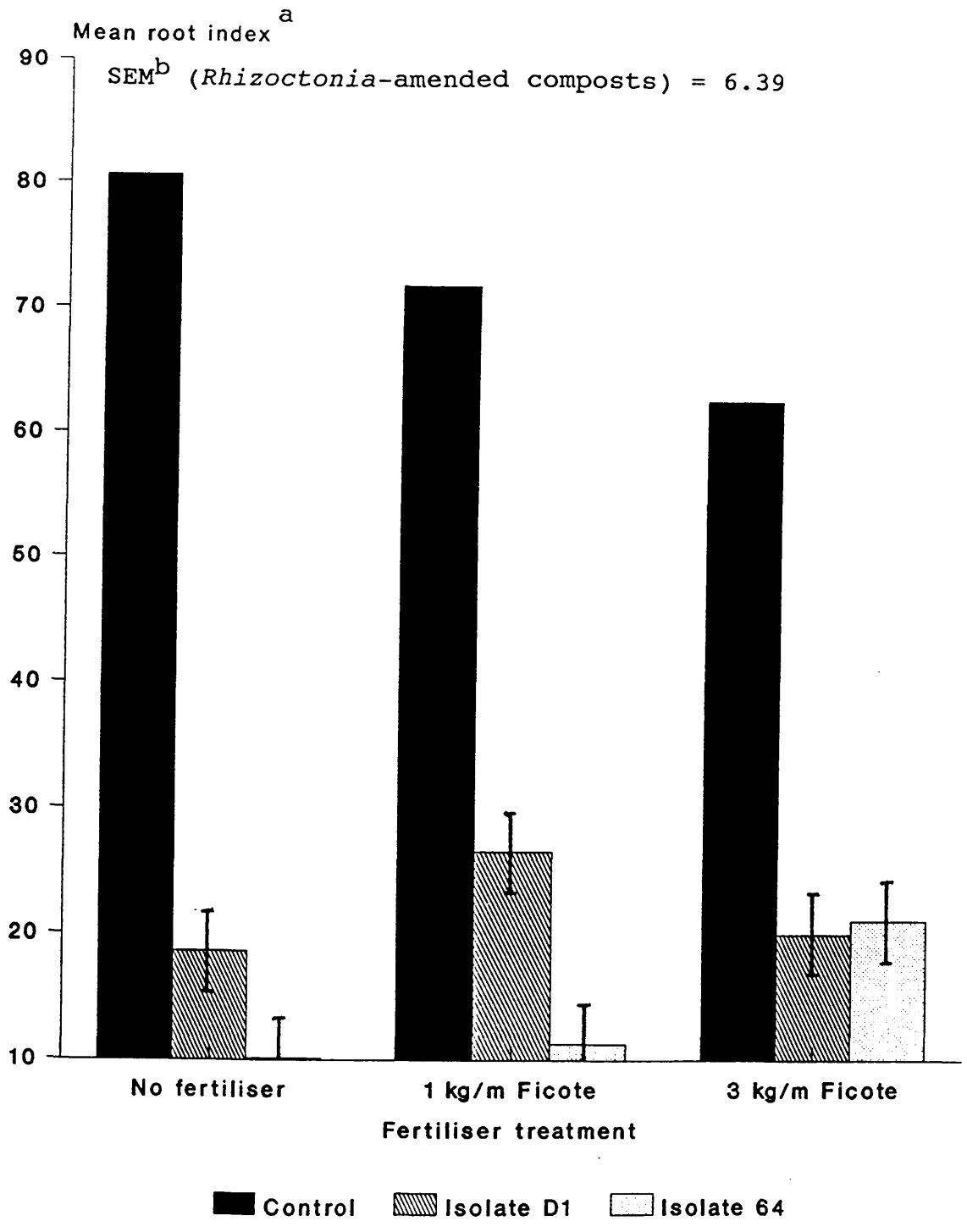

Fig. 9 The effect of controlled release fertiliser on the mean root indices of *C. vulgaris* cvs Cuprea, Kinlochruel and Beoley Gold (means of three cultivars) grown in standard propagation compost amended with binucleate *Rhizoctonia* spp. isolates D1 or 64 assessed 7 weeks after striking. (Experiment 13)

[a]Root indices calculated as shown in section 2.9

[b]Controls were omitted from the analysis of variance. All other SEM's (*Rhizoctonia*-amended composts) with 34 degrees of freedom

analysis should be treated with caution. *Rhizoctonia* spp. were isolated from 90% of cuttings grown in infested composts. There was no effect of fertiliser level on the percentage of infected cuttings from each *Rhizoctonia* spp. infested compost. This experiment was carried out again at a later date and similar results were obtained.

4.3.5 Experiment 14 The effect of compost type on the infection of *C. vulgaris* and *E. vagans* cuttings by binucleate *Rhizoctonia* sp. isolate D1.

When the statistical analysis was carried out on the foliage scores (final assessment), the control treatments were omitted since they had mean scores equal to zero. Significant differences ($F_{3,22} = 14.65$; $P < 0.001$) were recorded between foliage scores of cuttings grown in different composts amended with binucleate *Rhizoctonia* sp. isolate D1 (Fig. 10). The lowest foliage scores on cuttings grown in Rhizoctonia-amended compost were recorded on cuttings grown in 5:1 peat and sand, (mean score taken from all three cultivars tested was equal to 1.5). The highest foliage scores were recorded on cuttings grown in 1:1 peat and sand, (mean score taken from all three cultivars tested was 3.0). There were no significant differences between cultivars and there was no significant interaction between cultivar and compost type.

All treatments were included in the analysis of the root indices (final assessment). Root indices of cuttings grown in composts amended with binucleate *Rhizoctonia* sp.

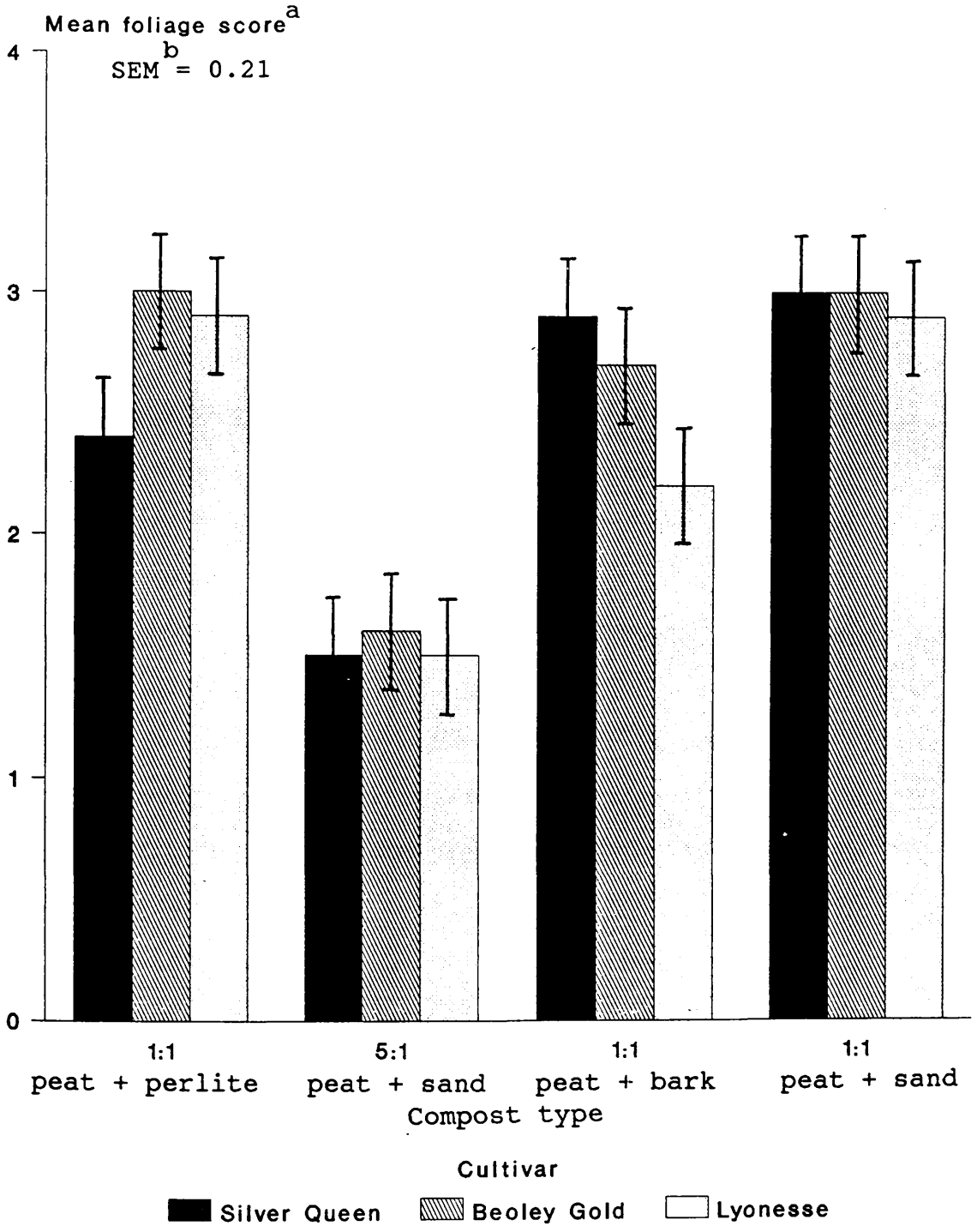

Fig. 10 The effect of compost type on the foliage condition[a] of *C. vulgaris* and *E. vagans* cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1, assessed 6 weeks after cuttings were struck. (Experiment 14)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]All SEM's with 22 degrees of freedom

isolate D1 (Table 18) were significantly lower ($F_{1,46}$ = 215.31; P < 0.001) than those of cuttings grown in unamended compost. Differences between root indices recorded on cuttings grown in the four types of compost were significant ($F_{3,46}$ = 19.00; P < 0.001). There was also a significant interaction ($F_{3,46}$ = 9.79; P < 0.001) between the isolate (control or D1) and the compost type. Of the composts used, peat and bark promoted the best root growth, both in the presence and absence of Rhizoctonia. Cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1, produced least root in 1:1 peat and fine sand (AFP 4, *C. vulgaris* cvs Silver Queen, root index = 4 and Beoley Gold, root index = 2) or 1:1 peat and perlite (AFP 31, *E. vagans* cv Lyonesse root index = 0), whilst control cuttings produced least root in the 5:1 peat and fine sand compost (AFP 9, mean root index = 18). There were significant differences ($F_{2,46}$ = 18.24; P < 0.001) between root indices recorded on the three cultivars.

Isolations were made from twelve cuttings in each treatment. *Rhizoctonia* spp. were isolated from cuttings grown in the following composts; 83% of cuttings (peat and perlite), 58% (peat and bark), 75% (5:1 peat and fine sand) and 75% (1:1 peat and fine sand). No *Rhizoctonia* spp. were isolated from control cuttings, none of which showed foliar browning. This experiment was carried out again at a later date and similar results were obtained.

Table 18 The effect of compost type and binucleate *Rhizoctonia* sp. isolate D1 incorporated into compost on the root indices[a] of *C. vulgaris* and *E. vagans* cuttings assessed 6 weeks after cuttings were struck. (Experiment 14)

Compost	Compost air-filled porosity	*Rhiz.* sp. isolate	Root index[a]			
			C. Vulgaris Silver Queen	*C. vulgaris* Beoley Gold	*E. Vagans* Lyonesse	Mean
1:1 (v/v) peat + perlite	31	none	46	46	16	36
1:1 (v/v) peat + bark	16		61	54	41	52
5:1 (v/v) peat + fine sand	9		29	26	1	18
1:1 (v/v) peat + fine sand	4		38	31	22	30
				SEM[b] = 4.7		SEM = 2.7
Mean (control)			44	39	20	
				SEM = 2.3		
1:1 (v/v) peat + perlite	31	D1	13	4	0	6
1:1 (v/v) peat + bark	16		11	9	13	11
5:1 (v/v) peat + fine sand	9		7	6	5	6
1:1 (v/v) peat + fine sand	4		4	2	1	3
				SEM = 4.7		SEM = 2.7
Mean (D1)			9	5	5	
				SEM = 2.3		
Overall mean			26	22	12	
				SEM = 1.7		

[a] Root indices calculated as shown in section 2.9
[b] All SEM's with 46 degrees of freedom

4.3.6 Experiment 15 The effect of compost moisture content on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), the control treatments were omitted because they had very low mean scores. There were significant differences ($F_{3,46}$ = 132.80; P < 0.001) between the amount of foliar browning on cuttings grown in trays of compost amended with binucleate *Rhizoctonia* spp. isolates with different moisture contents (Fig. 11). Most foliar browning occurred in composts amended with binucleate *Rhizoctonia* spp. isolates in boxes placed in cat trays which contained moist gravel (0 cm of water). Foliar browning was least severe when cuttings were grown in boxes placed in cat trays which contained 6 cm water. For example, a mean foliage score of 3.6 was recorded on *C. vulgaris* cv Robert Chapman cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1 in trays placed on moist gravel, whereas a mean foliage score of 0.8 was recorded on cuttings of the same cultivar grown in compost amended with the same isolate, grown in trays water-logged to 6 cm depth (i.e. to the compost surface).

Binucleate *Rhizoctonia* sp. isolate D1 caused significantly greater ($F_{1,46}$ = 282.64; P < 0.001) amounts of foliar browning than binucleate *Rhizoctonia* sp. isolate 64. For example, a mean foliage score of 3.6 was recorded on *C. vulgaris* cv Beoley Silver cuttings grown in compost water-logged to 2 cm and containing *Rhizoctonia* sp. isolate D1, whereas a score of 1.5 was recorded on

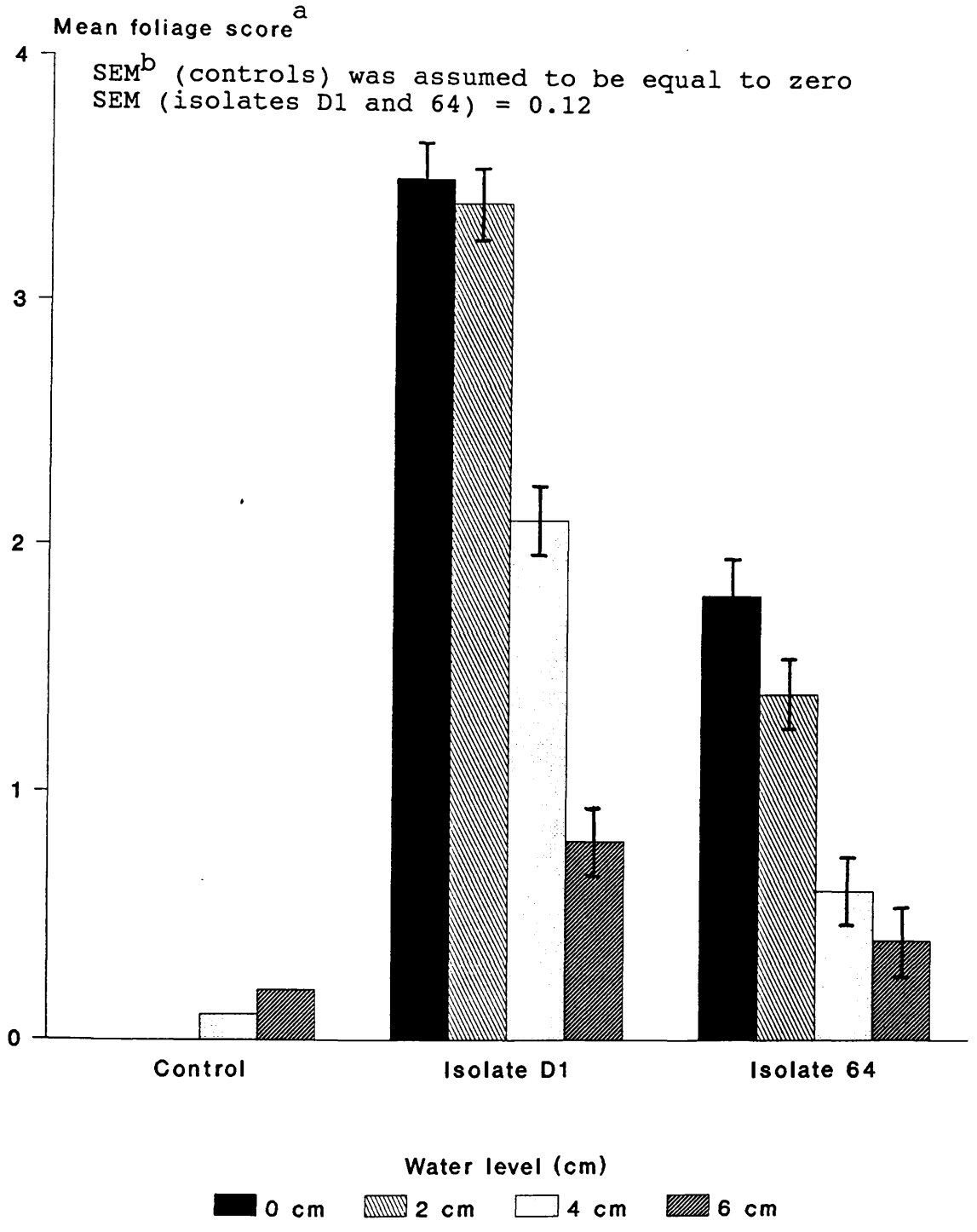

Fig. 11 The effect of water level (cm above gravel in base of tray) on the foliage condition[a] of *C. vulgaris* cvs Robert Chapman, Cuprea and Beoley Silver cuttings (mean scores taken from all three cultivars), assessed 6 weeks after striking in standard propagation compost amended with binucleate *Rhizoctonia* spp. isolates D1 or 64. (Experiment 15)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]Data from control treatments was omitted from analysis of variance. All other SEM's with 46 degrees of freedom

cuttings of the same cultivar grown in similar conditions in compost amended with binucleate *Rhizoctonia* sp. isolate 64.

All three cultivars of *C. vulgaris* (Robert Chapman, Beoley Silver and Cuprea) displayed similar patterns of foliar browning throughout the experiment, although significant differences ($F_{2,46}$ = 8.20; P < 0.001) were recorded between their foliage scores on the final assessment.

A small amount of browning was observed on control cuttings grown in composts in trays which contained 2, 4 and 6 cm water (mean score = 0.1). No *Rhizoctonia* spp. were isolated from control cuttings. *Rhizoctonia* spp. were isolated from between 66 and 100% of the six cuttings taken at random from each treatment involving Rhizoctonia-amended compost.

4.3.7 Experiment 16 The effect of pH on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* sp. isolate D1.

When the statistical analysis were carried out on the foliage scores (second assessment), the control treatments were omitted since they received mean scores equal to zero. Foliage scores recorded on cuttings of *C. vulgaris* cvs Cuprea, Flamingo and Silver Queen grown in composts of different pH values and amended with binucleate *Rhizoctonia* sp. isolate D1, were significantly different ($F_{3,22}$ = 44.61; P < 0.001) from one another (Fig. 12).

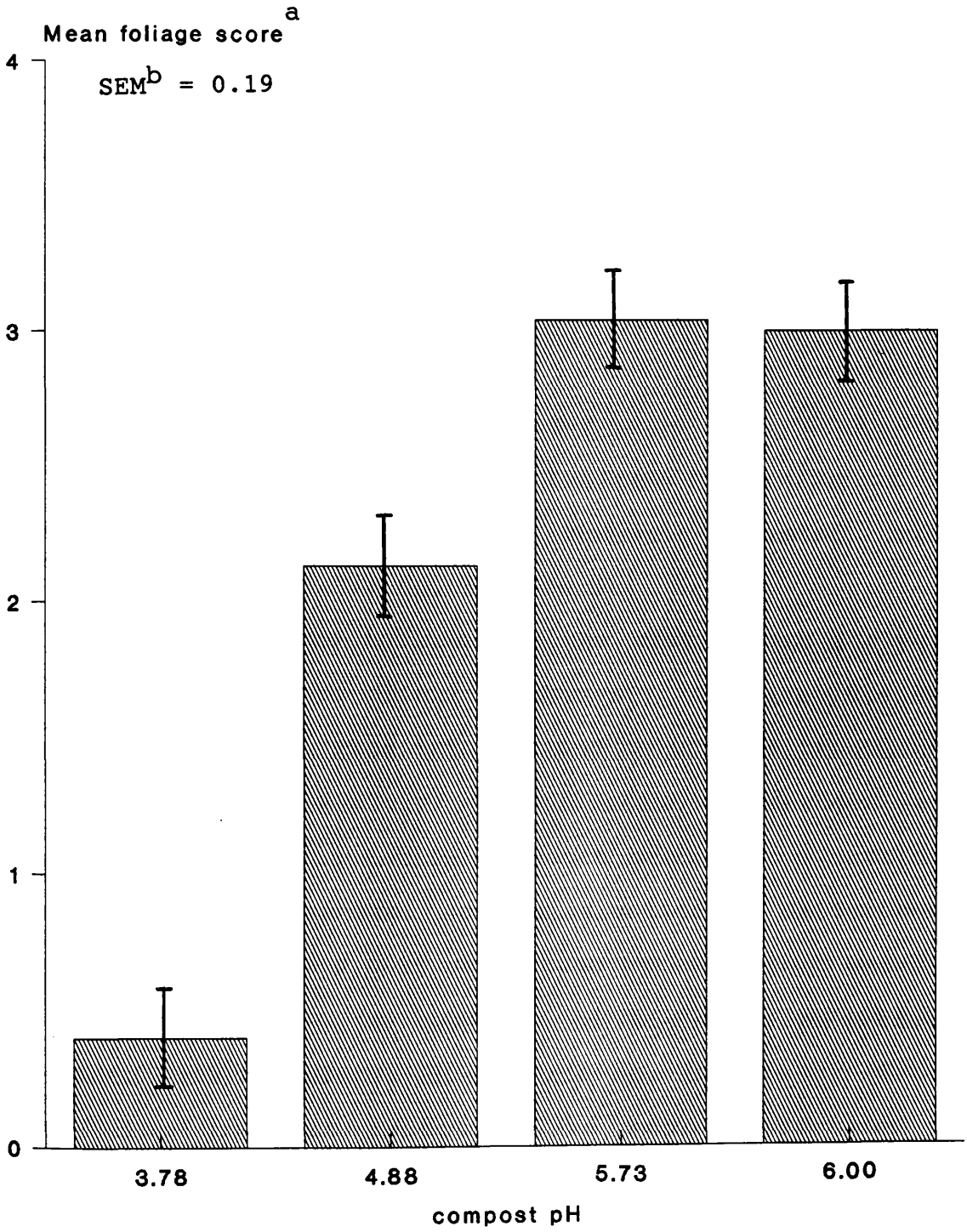

Fig. 12 The effect of compost pH on the foliage condition[a] of cuttings of *C. vulgaris* cvs Flamingo, Silver Queen and Cuprea (mean scores taken from all three cultivars), grown in standard propagation compost amended with binucleate *Rhizoctonia* sp. isolate D1 assessed 4 weeks after striking. (Experiment 16)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]All SEM's with 22 degrees of freedom

Foliar browning was least severe in unlimed compost and was most severe in composts limed to pH values of 5.73 and 6.00. For example, a mean foliage score of 0.3 was recorded on *C. vulgaris* cv Flamingo cuttings grown in unlimed compost amended with binucleate *Rhizoctonia* sp. isolate D1, whereas a foliage score of 3.4 was recorded on cuttings of the same cultivar grown in compost limed to pH 6.0 and amended with binucleate *Rhizoctonia* sp. isolate D1. No foliar browning occurred in cuttings in uninfested composts. Plate 13 which follows page 154, shows the effect of compost pH on the foliage condition of *C. vulgaris* cuttings grown in compost infested with binucleate *Rhizoctonia* sp. isolate D1.

All values were included in the statistical analysis of the root weights (final assessment). The analysis was split, with values from infested and uninfested treatments being analysed separately. No significant differences were found between the quantity of roots produced on cuttings in uninfested composts. However, significant differences ($F_{3,6} = 66.48$; $P < 0.001$) were found between root weights of cuttings grown in composts of the four pH values amended with binucleate *Rhizoctonia* sp. isolate D1 (Table 19). The greatest reductions in root production in Rhizoctonia-amended composts (compared with controls), were measured in composts of pH 5.7 and 6.0. The root weight of cuttings in the above composts was 51% of that recorded for cuttings grown in unamended composts of pH 5.7. or 6.0. The smallest reduction was recorded on

Plate 13 *C. vulgaris* cvs, left to right; Flamingo, Silver Queen and Cuprea in standard propagation compost and amended with binucleate *Rhizoctonia* sp. isolate D1 and magnesian limestone (m.l.). Top: left, unlimed, pH = 3.78; middle, 2 g m.l./l compost, pH = 4.88; right, 4 g m.l./l compost, pH 5.73. Bottom: left, 6 g m.l./l compost, pH = 6.00; right, control, no *Rhizoctonia* sp. 2 g m.l./l compost, pH = 4.88. (Experiment 16)

cuttings grown in unlimed compost. The root weight of cuttings in unlimed compost which had been amended with binucleate *Rhizoctonia* sp. isolate D1, was 68% of that recorded for cuttings grown in unamended unlimed compost.

This experiment was carried out again at a later date, using two *E. cinerea* cultivars and one *E. carnea* cultivar. Similar results were obtained.

Table 19 Mean root weight (g x 100) of *C. vulgaris* cuttings grown in composts of different pH values amended with binucleate *Rhizoctonia* sp. isolate D1, assessed 6 weeks after cuttings were struck. (Experiment 16)

		Mean root weight (g x 100)				
Cultivar	Isolate	pH 3.8	pH 4.9	pH 5.7	pH 6.0	Mean
Cuprea	Control	8.0	7.9	8.6	8.2	8.2
Flamingo	"	8.2	8.0	8.2	8.2	8.2
Silver Queen	"	5.3	7.4	5.3	5.2	5.8
			SEM[a] = 0.71			SEM = 0.36
Mean (controls)		7.2	7.8	7.4	7.2	
			SEM = 0.41			
Cuprea	D1	3.2	0.4	0.3	0.4	1.1
Flamingo	"	2.8	0.8	0.4	0.2	1.1
Silver Queen	"	2.0	0.0	0.0	0.0	0.5
			SEM = 0.25			SEM = 0.13
Mean (infested composts)		4.9	4.1	3.8	3.7	
			SEM = 0.15			

[a]All SEM's with 6 degrees of freedom

4.3.8 Experiment 17 The effect of temperature on the infection of *C. vulgaris* and *E. cinerea* cuttings by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), the control treatments were omitted since they received mean scores equal to zero. Foliage scores of cuttings grown in infested composts differed significantly both between temperatures, ($F_{2,51}$ = 38.63; P < 0.001) and between isolates ($F_{1,51}$ = 2.84; P < 0.01, Table 20). There was a significant interaction between isolate and temperature ($F_{2,51}$ = 71.08; P < 0.001). Binucleate *Rhizoctonia* sp. isolate 72 caused most foliar browning in the medium temperature growth cabinet (10° - 17°C), whereas binucleate *Rhizoctonia* sp. isolate 48 caused most foliar browning at the highest temperature (15° - 25°), and least at the lowest temperature (5° - 10°C).

Foliage scores of cuttings of different species were significantly different from one another ($F_{1,51}$ = 170.84; p < 0.001). Scores of *E. cinerea* cv Lilacina were lower than those of the two *C. vulgaris* cvs Darkness and Cuprea. In the medium temperature cabinet, a mean foliage score of 1.7 was recorded on *E. cinerea* cv Lilacina cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 72, whereas a score of 3.6 was recorded on both of the *C. vulgaris* cvs Darkness and Cuprea cuttings which were grown in similarly amended compost. There was no foliar browning on control cuttings grown in uninfested composts.

This experiment was repeated at a later date using

Table 20 Mean foliage scores[a] of *C. vulgaris* and *E. cinerea* cuttings grown in composts amended with binucleate *Rhizoctonia* spp. isolates 48 and 72 and kept in three different temperature regimes, assessed 4 weeks after cuttings were struck. (Experiment 17)

Temperature regime	Mean foliage score[a]				
	C. vulgaris		*E. cinerea*		
	72	48	72	48	Mean
5° - 10°C	2.6	1.1	0.5	0.9	1.4
10° - 17°C	3.6	2.9	1.7	1.2	2.6
15° - 25°C	1.2	3.4	0.2	1.7	1.8
	SEM[b] = 0.16		SEM = 0.23		SEM = 0.26
Mean	2.5	2.5	0.7	1.3	
	SEM = 0.09		SEM = 0.13		

[a]Foliage scores: 0 = no browning; 4 = totally brown foliage. Foliage scores of cuttings in uninfested controls were equal to zero. The results were omitted from the analysis of variance and SEM's were assumed to be equal to zero.

[b]SEM's with 51 degrees of freedom

binucleate *Rhizoctonia* spp. isolates D1, 48 and 64. The results obtained were similar.

4.4 DISCUSSION

Results in Experiment 10 showed that the optimum pH for growth of *Rhizoctonia* spp. isolates A, E and F on PDA lay between 5.4 and 8.8. In accordance with this, most workers have found that pH optima for growth of *R. solani* isolates, lies between 5 and 8, (Sherwood, 1970). A bimodal growth pattern was observed with *R. solani* isolate A which grew optimally at pH 5.4, and less at 6.2, than at either 7.4 or 5.4. Similar growth patterns were observed by Bateman (1962) when examining the growth rates of *R. solani* on buffered PDA.

Although several other workers have shown that significant differences may exist between pH optima for growth of different *R. solani* isolates (Sherwood, 1970), care must be taken in interpreting the results of this experiment. We used unbuffered PDA in our experiments due to a difficulty in obtaining appropriate buffers. It is known that *R. solani* isolates are capable of altering the pH of the media in which they are grown (Weber, 1939; Barker & Walker, 1962). Media pH can be altered by up to 2 pH units either up or down in 60 hours, depending on isolate (Sherwood, 1970). Changes in pH of the medium during culture are influenced by the isolate used, composition of the medium, initial pH value and length of

culture period.

Hence although growth of *Rhizoctonia* spp. isolates was recorded at pH values of 8.8 and 4.0, no measurement of media pH was taken either during or at the end of Experiment 10. Therefore it is possible that the fungal isolates may have altered the pH of the media to values more suitable for their growth.

The effect of pH on the growth *in vitro* of a very large number of isolates would have to be examined before definitive conclusions could be reached, on the optimum pH range for growth *in vitro* of isolates of *Rhizoctonia* spp. which occur on or are pathogenic to, *C. vulgaris* or *Erica* spp. *R. solani* isolates have been shown to grow on buffered media at pH values of 3.5 to 8.5 (Jackson, 1940), with reports of growth on unbuffered media of pH values of 2.5 to 11 (Sherwood, 1970). Therefore, although the pH optima of the three *Rhizoctonia* spp. isolates taken from potatoes and field soil all lay between 5.4 and 8.8, it is probable that the pH optima of *Rhizoctonia* spp. isolates taken from *C. vulgaris* and *Erica* spp. would differ not only from those of *Rhizoctonia* spp. isolates A, E and F, but also from one another.

When grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1, cuttings of *C. vulgaris* cvs Cuprea, Flamingo and Silver Queen showed significantly less foliar browning in compost of pH 3.8 than in composts of pH 4.9 to 6.0 (Experiment 16). Root production on cuttings grown in binucleate *Rhizoctonia* sp. isolate

D1-infested compost of pH 3.8 was significantly greater than that in limed infested composts (pH values 4.9 to 6.0). Similar results were obtained in experiments with *R. solani* isolates 48 and 64 and in work concerning *E. carnea* and *E. cinerea* cuttings. These results are in accordance with those of Jackson (1940), who found that damping-off of Ponderosa pine and Douglas fir decreased when the pH of the root zone was reduced from pH 6.5 to 3.5. Weindling and Fawcett (1936), also found that by reducing the pH to 4.0, damping-off of citrus seedlings (*Citrus* spp.) by *R. solani* was successfully controlled.

The reduction in inoculum potential of *Rhizoctonia* spp. may be due to several reasons. Jackson (1940) obtained evidence that aluminium ions at low pH decreased the pathogenicity of *R. solani*, and Bateman (1970) concluded that since the processes of host penetration by *R. solani* are primarily enzymatic, then the pH of the soil rhizosphere solution may affect both enzyme production and enzymatic activity. In addition, the availability of mineral ions such as nitrate, ammonium, potassium, calcium and iron is influenced by soil reaction, and as such may affect both inoculum potential and host susceptibility or disease potential.

Many plant species grow poorly at the extremes of pH (both acidity and alkalinity) which *Rhizoctonia* spp. will tolerate. However, *C. vulgaris* and many *Erica* spp. including *E. cinerea* and *E. tetralix* are calcifuge and

thrive in soils and composts with pH values between 4.0 and 5.5.

Although the pH optima for growth of *Rhizoctonia* spp. are known to vary significantly between isolates, the fact that for most these lie between pH values of 5.0 and 8.0 suggests that the growth of most isolates and subsequent infection, will be reduced or controlled in composts of pH values of less than 4.0.

The optimum pH range for growth and development of rooted *C. vulgaris* and *Erica* spp. lies between 4.5 and 5.5. Growth is not satisfactory below pH 4.0. This range is within that tolerated by many strains of *Rhizoctonia* spp., hence pH manipulation as a disease control measure is unlikely to be practical beyond the propagation stage.

There were significant differences between the growth responses of *R. solani* isolates E and A, and binucleate *Rhizoctonia* spp. isolates 72 and 48 in cabinets of each of the six temperatures used in Experiment 11. These results are consistent with those of other workers who have found extreme variability in response to temperature within the species *R. solani* (e.g. Sherwood, 1970). The minimum temperature for growth has been shown to vary from 2 to 12^{o}C. Some isolates will not grow at less than 10^{o}C, although care must be taken when determining minimum growth temperatures, as it has been shown (Lauritzen, 1929), that the initiation of growth at low temperatures may be delayed for several days.

The optimum temperature for growth of *R. solani*

isolates *in vitro* has been found by several investigators to lie between 18 to 33°C, and most frequently between 23 to 28°C depending on isolate. Maximum temperature for growth lies between 30 and 40°C. The type of incubators available to individual workers, governed to a large extent the temperature intervals tested, therefore the experimentally gained cardinal temperatures were usually only approximate. No data was available on the response of binucleate *Rhizoctonia* spp. isolates to temperature *in vitro*.

Although the results of Experiment 11 which determined the growth responses of some isolates of *Rhizoctonia* spp. to temperature *in vitro*, revealed the broad temperature ranges over which growth occurred, and the fact that growth responses differed depending on isolate, the practical relevance of fungal growth rates *in vitro* is limited. Firstly it has been shown that the cardinal temperatures for growth of *R. solani in vitro* may depend on the medium used (Sherwood, 1970). Secondly several workers have shown that the optimum temperature for infection and disease differed from that required for growth, therefore it is possible that both the cardinal temperatures and the optimum temperature for growth of *Rhizoctonia* spp. *in vitro*, will differ from those required for growth of the fungus in peat composts and infection of *C. vulgaris* and *Erica* spp.

The amount of foliar browning caused by binucleate

Rhizoctonia spp. isolates 72 and 48 differed significantly depending on both isolate and temperature (Experiment 17). Similar results have been obtained by other workers. For example Hunter *et al.* (1960), isolated three strains of *R. solani* from cotton, and found that disease development varied with the strains depending on temperature. The mildly aggressive strain caused most disease at 24°C, the moderately aggressive strain caused most disease at 32°C, and the highly aggressive strain caused most disease at 24, 27.5 and 32°C.

Diseases caused by *Rhizoctonia* spp. can occur over very wide temperature ranges. It has been shown that certain strains of *R. solani* incite diseases at very low temperatures where the fungal growth rate is slow. Disease severity then decreases at higher temperatures. For example, lettuce damping-off strains are most active near 8°C (Shephard & Wood, 1963). Others cause disease at higher temperatures for example strains causing bottom-rot of lettuce show most activity above 24°C (Townsend, 1934). Strains such as those which cause Rhizoctonia root-rot of alfalfa (*Medicago sativa* L.) are most damaging at around 30°C (Baker & Martinson, 1970).

Several earlier investigators have successfully correlated growth rates from *in vitro* studies with disease development (Sherwood, 1970). However, most workers accept that disease severity is not a simple function of fungal growth rate. For example, Richards (1921) found that the optimum soil temperature for disease development (caused

by *R. solani*) on potatoes, peas and beans was near 18°C, with little disease development occurring above 20 - 24°C. However, he found the optimum temperature for fungal growth was 26°C.

Leach (1947) found that disease incidence did not conform closely with the growth rate of either the host or the pathogen at different temperatures. The strain of *R. solani* which he used, grew on agar media from 8 - 40°C with optimal growth occurring between 25 - 30°C. With cool temperature crops such as sugar beet, there was a low incidence of pre-emergence damping-off at the lower temperatures (4 - 12°C) where host growth was favoured over pathogen growth. At higher soil temperatures, the relative growth rates favoured the pathogen, particularly at 20 - 30°C where damping-off was severe. Leach concluded with all of the many host/pathogen combinations which he tested, that the host/pathogen growth ratio was a good basis from which to predict the incidence of disease caused by *Rhizoctonia* spp.

It would prove difficult if not impossible to manipulate temperatures within polythene tunnels and glasshouses in order to restrict growth of *Rhizoctonia* spp., because *C. vulgaris* and *Erica* spp. grow poorly at the extremes of temperature which *Rhizoctonia* spp. will tolerate. However, if temperatures are set to allow for balanced healthy growth of *C. vulgaris* and *Erica* spp., then host/pathogen growth ratio should remain high thereby

minimising disease development. The precise temperatures at which host/pathogen growth ratio is highest, may differ depending on other environmental parameters, *Rhizoctonia* spp. and strain, and on *C. vulgaris* or *Erica* spp. cultivar. Further work is required to determine such temperatures.

Vertical growth of binucleate *Rhizoctonia* spp. isolates D1 and 64 on *C. vulgaris* plants was slow (1.1 - 3.1 mm in 8 days) when relative humidity lay between 70 and 85% (Experiment 12). However, fungal growth was 10.9 - 14.9 mm in 8 days when relative humidity lay between 90 - 100%. This corresponds with results of research carried out by several investigators. Sherwood (1970) described *R. solani* as a hygrophile, i.e. a fungus with a straight line or concave growth curve with an optimum relative humidity of 100%; and Shurtleff (1953) found that the strains of *R. solani* which caused brown patch of turf, required free water or almost 100% humidity for growth and infection.

Current practices for production of *C. vulgaris* and *Erica* spp. involve growing large numbers of plants closely packed in polythene tunnels for several months (or years in the case of stock plants used for the production of propagation material). Such practices result in situations where the relative humidity often rises to 100%, particularly around plant bases near soil/compost level and lower foliage. When other environmental conditions, in particular temperature are favourable, rapid spread of *Rhizoctonia* spp. on above-compost plant parts, both on

individual plants and from one plant to another can occur. Attempts to reduce humidity where possible, will result in a reduction in spread of *Rhizoctonia* spp. This is particularly important since most strains of *Rhizoctonia* spp. isolated from *Erica* spp. and *C. vulgaris* have been taken from plant parts at and around soil/compost level.

High humidities are necessary during propagation to prevent foliage desiccation. However, timely reduction of humidity following rooting, will restrict the spread of any *Rhizoctonia* spp. which may be present. The provision of ventilation in polythene tunnels and adequate spacing of stock, to prevent plant to plant contact if possible , will allow air circulation and prevent high humidities developing around foliage thus restricting mycelial spread and infection.

The level of controlled release fertiliser incorporated in propagation compost, had a significant effect on foliar browning due to *Rhizoctonia* spp. in Experiment 13. Numerous experiments have been performed to determine the effects of supplementary nutrients on development of disease caused by *Rhizoctonia* spp. Results vary widely depending on *Rhizoctonia* spp. strains, crop and cropping situation.

Fertilisation experiments on agricultural soils with inorganic fertilisers, revealed that potassium, nitrogen or calcium deficiencies increased the disease potential (Baker & Martinson, 1970). Several workers have found that

fertilisation with potash greatly decreased disease potential (Baker & Martinson, 1970), and the addition of calcium to soil was found to prevent Rhizoctonia root rot of wheat (Hynes, 1937).

In contrast, an excess of nitrogen or high salt concentrations, has also been associated with an increase in disease. Hearn (1943) showed that an excess of nitrogen increased the damping-off of broad-leaved tree seedlings by *R. solani* and *Pythium* spp. He found that if the excess nitrates were tied up by micro-organisms which were decomposing organic amendments, then damping-off decreased considerably.

Based on experimental considerations, inorganic mineral nutrients seem to affect disease potential more than inoculum potential. High salt concentrations predispose plants to disease caused by *R. solani*, (Baker & Martinson, 1970). Beach (1949), found that damping-off of tomatoes (*Lycopersicon esculentum* Mill.) and cucumbers *(Cucumis sativus* L.) was very severe when the plants were cultured in 8 X or 16 X Knop's solutions (solutions contained inorganic salts of nitrogen, potassium, phosphorus, calcium, magnesium and iron). It was concluded that the effects were probably on disease potential rather than on inoculum potential, since damping-off occurred at 8 X and 16 X normal nutrient solution concentration whether the pathogen was present or not.

C. vulgaris and *Erica* spp. in general, are salt sensitive and require relatively small quantities of

essential nutrients for growth. Foliar browning has been shown to occur (in later experiments, in the absence of pathogens) as a result of excessive salt concentrations in the growing medium. Foliar browning occurred on cuttings in both uninfested compost and compost amended with binucleate *Rhizoctonia* spp. isolates D1 and 64 which contained 3 g of controlled release fertiliser/l compost.

No increase in foliar browning or decrease in root production, was recorded on cuttings (in Experiment 13) grown in fertilised *Rhizoctonia* spp.-infested composts in comparison with those grown in unfertilised infested composts. Although the use of fertilised propagation composts may allow for rapid plant development following initial rooting, the use of fertiliser in propagation composts is not practical due to the extra expense involved, and the possible risk of slight foliar browning and retarded root development.

Controlled-release fertilisers are necessary at potting following rooting, for the rapid production of quality *C. vulgaris* and *Erica* spp. plants. Although it is likely that a balanced sufficiency of mineral nutrients allowing for healthy, vigorous growth will minimise the likelihood of development of Rhizoctonia disease on rooted plants, the exact levels of nutrients, application methods, and crop husbandry, requires to be examined before the effects of nutrients on incidence and development of disease caused by *Rhizoctonia* spp. on

rooted *C. vulgaris* and *Erica* spp. can be fully evaluated.

C. vulgaris and *E. vagans* cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1 received lower foliage scores when grown in 1:1 peat and sand compost (air-filled porosity [AFP] = 4), than when grown in the other three peat-based composts (Experiment 14). Cuttings grown in both unamended compost and compost amended with binucleate *Rhizoctonia* sp. isolate D1 produced the greatest quantity of roots in a mix of 1:1 peat and bark (AFP = 16). Soil structure and texture have been shown by several investigators to affect development of diseases caused by *R. solani* (Baker & Martinson, 1970). Disease development and severity is dependent on the relative abilities of host and pathogen to tolerate conditions associated with low/high compost AFP. It has been shown that the optimum AFP value for composts used for production of *Erica* spp. and *C. vulgaris* lies between 15 and 20% (Bunt, 1988), thus allowing for free drainage and adequate aeration in the medium. *Erica* spp. and *C. vulgaris* are very susceptible to damage resulting from water-logging/poor aeration in the root zone, but in addition will not tolerate high soil-moisture tensions. Compost with an AFP of 15 - 20% and a balance of small, medium and large pores (30 - 300 µm in size), provides adequate aeration without a serious risk of it drying out rapidly in the absence of irrigation.

The use of propagation compost which contained binucleate *Rhizoctonia* spp. isolates with either a higher

(31) or lower (9 or 4) AFP value than the optimum (around 16), may have affected both the disease potential and the inoculum potential. Although root production by *C. vulgaris* and *E. vagans* cuttings was retarded in these composts, saprophytic growth of binucleate *Rhizoctonia* sp. isolate D1 may also have been retarded thus restricting infection and subsequent disease development. For example, several workers have shown that the growth of *R. solani* through soil, was restricted by poor aeration (Blair, 1943; Das & Western, 1959).

Restriction in saprophytic growth of binucleate *Rhizoctonia* sp. isolate D1 in composts of low AFP values was thought to be due to a build-up of carbon dioxide rather than a lack of oxygen. Papavizas & Davey (1962) found that oxygen supply did not become limiting to *R. solani* growth *in vitro* or in soil until the concentration in the atmosphere was 1%, whereas it has been shown (Blair, 1943) that carbon dioxide concentrations of 20 - 25% greatly inhibited growth of *R. solani* in soil.

The available literature on soil oxygen/carbon dioxide concentrations suggests that carbon dioxide toxicity, rather than oxygen deficiency, was the more probable cause of limitation of growth of binucleate *Rhizoctonia* sp. isolate D1. Durbin (1959) found that *R. solani* strains differed greatly in their tolerance to carbon dioxide. Strains which primarily attacked roots showed a much greater tolerance of high carbon dioxide concentrations

than those which mainly caused rot of stem-base or foliage. Since binucleate *Rhizoctonia* sp. isolate D1 was taken from foliage of *C. vulgaris* and has been shown to attack mainly stem-base and lower foliage of *C. vulgaris* and *Erica* spp., (Experiments 1 and 2), it may be less tolerant of high carbon dioxide concentrations than isolates taken from roots. For this reason the effect of compost AFP on development of disease caused by other isolates of *Rhizoctonia* spp., should be examined before conclusions regarding the behaviour of the pathogen in composts of different AFP values can be reached.

Difficulty was experienced in formulating a peat based compost with an inert additive to give an AFP value of 15 - 20%, hence bark was mixed with peat to provide a compost of this nature. Peat and bark (1:1 v/v) is the most commonly used commercial propagation compost. Bark, which (unlike perlite and sand) is not inert, may have contained micro-organisms which affected binucleate *Rhizoctonia* sp. isolate D1 or the cuttings in the compost. It may also have contained organic compounds, for example terpenes and phenolics, which affected either the fungal isolate or the cuttings. For this reason and to re-examine further the discrepancies in the data, (for example the widely varying root indices recorded on *E. vagans* cuttings), the experiment requires to be repeated using peat composts amended only with inert ingredients, before conclusions can be reached regarding the effect of compost AFP on the infection of cuttings of *Erica* spp. and *C. vulgaris* grown

in composts infested with *Rhizoctonia* spp.

Difficulty was experienced in maintaining peat and bark compost at specific moisture contents, since such composts have a high percentage of large (> 100 um) pores and have been developed to drain quickly. Experiment 15 was designed to provide a range of water levels in the compost within the propagation boxes, to give an idea of the effect of moisture on infection by *Rhizoctonia* spp. and subsequent disease development. The use of loam or fine sand in composts, may aid the production of composts which can be more easily maintained at specific moisture contents than the peat and bark mixture used.

Cuttings grown in trays of compost amended with binucleate *Rhizoctonia* spp. isolates, showed significantly different levels of foliar browning depending on the level of water in the cutting boxes. Most foliar browning occurred on cuttings in boxes placed in trays placed on moist gravel.

The effects of soil moisture on infection by *Rhizoctonia* spp. and disease development are closely associated with those of aeration, as one of the primary effects of high soil moisture is reduced aeration. Moisture stress is generally more important below container capacity, (defined by Bunt, 1988, as "the amount of water held in a container after the compost within has been irrigated and allowed to drain"), whereas aeration is possibly most important above container capacity. The

effects of moisture concentration on development of diseases caused by *R. solani* have been demonstrated by several workers. For example in damping-off experiments, Wright, (1957) showed that maximum damping-off occurred at moisture concentrations of 20 - 80% of saturation. Disease was less consistent at saturation. Flentje & Hagedoorn (1964) recorded a greater influence of tip-blight (caused by *R. solani*) on pea seedlings if the relative humidity at the soil surface was very high. Since the pathogen was active near the soil surface, aeration was not a limiting factor.

Binucleate *Rhizoctonia* spp. isolates D1 and 64 have been shown to attack *C. vulgaris* and *Erica* spp. plants primarily at or around soil/compost level and lower foliage. For this reason it was thought that compost aeration/moisture level would not be a limiting factor in infection and disease development. However, since the Rhizoctonia inoculum was mixed throughout the peat and bark medium, compost moisture level may have affected the saprophytic growth of binucleate *Rhizoctonia* spp. mycelium through the compost to the *C. vulgaris* cuttings.

If growth of binucleate *Rhizoctonia* spp. mycelium was restricted or stopped by high compost moisture levels, then infection and disease development would be largely dependent on the quantity of inoculum which was contained in the compost which was not water-logged, (i.e. 0, 2, 4 or 6 cm depth in each box). This explains why disease severity increased with decreasing compost water level.

Although high compost moisture contents may restrict saprophytic growth and spread of *R. solani* and binucleate *Rhizoctonia* spp. mycelium within composts, it is likely that growth and development of *C. vulgaris* and *Erica* spp. plants and cuttings would be retarded in compost which contained very high moisture levels, therefore the application of large volumes of water to propagation or potting composts is unlikely to prove practical.

CHAPTER 5

INVESTIGATIONS INTO THE POSSIBILITY OF BIOLOGICAL CONTROL OF *RHIZOCTONIA* SPP ON *C. VULGARIS* AND *ERICA* SPP.

5.1 INTRODUCTION

There have been several cases of successful biological control of *Rhizoctonia* spp. using fungal antagonists such as *Trichoderma* spp. (Strashnov *et al.*, 1985; Elad *et al.*, 1980), and several successful instances of suppression of disease caused by *Rhizoctonia* spp., when certain compost amendments such as composted sewage sludge (Lumsden *et al.*, 1983) and composted hardwood bark (Nelson & Hoitink, 1982 & 83; Nelson *et al.*, 1983 and Stephens *et al.*, 1981) have been used. In addition, Ichielevich-Auster *et al.* (1985) reported control of *Rhizoctonia* spp. on several hosts using a 'non-pathogenic' isolate of *R. solani*.

The experiments in Chapter 5 were set up to examine the possibility of developing biological control measures for *Rhizoctonia* spp. on *C. vulgaris* and *Erica* spp.

5.2 MATERIALS AND METHODS

5.2.1 Experiment 18 The effects of several isolates of saprophytic compost fungi on the growth of binucleate *Rhizoctonia* spp. *in vitro*.

Eighteen isolates of saprophytic fungi including *Trichoderma* spp. (isolates labelled 1 to 12), *Penicillium* spp. (isolates labelled A to C) and *Mucor* spp. (isolates labelled a to c), were obtained from compost and heather roots. Hyphal tip cultures were made from each of the isolates which were grown on PDES agar. A 5 mm diameter agar disc bearing an isolate of one of the saprophytic fungi was placed at the edge of a 9 cm diameter Petri dish

containing PDES. A 5 mm diameter agar disc bearing binucleate *Rhizoctonia* spp. isolate D1 or 64 was placed at the opposite edge. Growth of both isolates on each plate was monitored over a 2 week period. Plates were examined under a microscope (x 40 and x 100) for evidence of parasitism or antibiosis by either isolate.

5.2.2 Experiment 19 The effect of *Trichoderma* spp. incorporated in propagation compost on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

Five isolates of *Trichoderma* spp., (four of which were shown to inhibit the growth of binucleate *Rhizoctonia* spp. isolates D1 or 64 *in vitro*, see section 5.3.1) were grown on PDES agar in 9 cm diameter Petri plates until the surface of the agar was fully colonised. They were then grown on sterile, 5 - 10 mm length pieces of chopped straw as described for *Rhizoctonia* spp. (see section 2.7). Isolates of *Trichoderma* spp. (or uninoculated straw) were then mixed individually with moist Bulrush Propagation Compost at 2 g straw/l compost. Twelve polythene boxes (10 x 10 x 8 cm deep, Stewart Plastics Ltd.) were filled with each of the above compost mixtures and left for 4 days covered in white polythene (120 gauge, 30 micron, LBS Polythene), within a polythene tunnel. The composts were then removed and mixed with either uninoculated straw or standard inoculum of binucleate *Rhizoctonia* spp. isolate D1 or 64 at 2 g straw/l compost. The boxes were left for a further 2 days under polythene, following which three cuttings of each of *C. vulgaris* cvs Mousehole, Cuprea and

Silver Queen were struck in each box.

Within each of four replicate blocks, there were eighteen boxes (plots) comprising a three-cutting row of each cultivar (sub-plots). The boxes were randomly allocated to their positions within the blocks. Replicate blocks were separately covered with polythene and arranged on capillary matting on a glasshouse bench. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliar assessments were made weekly for 4 weeks (see section 2.8). After the final assessment, two, 2 - 5 mm length stem pieces were taken from each cutting and placed on PDES agar to determine the presence of Rhizoctonia, (see section 2.10).

5.2.3 Experiment 20 The effects of several compost additives on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* sp. isolate 48.

Four cuttings each of *C. vulgaris* cvs Beoley Gold, Mousehole and Cuprea were struck in seed trays (23 x 17.5 x 5.5 cm deep) which contained one of the following composts plus standard inoculum of binucleate *Rhizoctonia* sp. isolate 48 or uninoculated straw incorporated at 2 g straw/l compost.

1. Bulrush Sphagnum Moss Peat (screened 22 mm)
2. 1:1 (v/v) peat (as in 1.) + Fine Grade Cambark
3. 1:1 (v/v) peat (as in 1.) + Horticultural Bark (composted pine bark, Melcourt Industries)

4. 10:1 (v/v) peat (as in 1.) + liquid sewage sludge (New Cumnock Sewage Works, New Cumnock, Ayrshire.)
5. 3:1 (v/v) peat (as in 1.) + solid sewage sludge (Carbarns Sewage Works, Wishaw, Glasgow.)
6. peat (as in 1.) + 15 g calcium peroxide granules (Fertilox, Interox Chemicals Ltd.)/l

Within each of five replicate blocks, there were twelve trays (plots) comprising a four-cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered with polythene and arranged on a tunnel floor. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliage condition of cuttings was assessed after 2 and 4 weeks. Two, 2 - 5 mm length stem pieces were removed from twelve cuttings taken at random from each treatment to determine the presence of Rhizoctonia.

5.2.4 Experiment 21 The effect, over a 1 year period, of *R. solani* isolate A (which was previously shown to infect *C. vulgaris* cuttings, causing no visible symptoms), on *C. vulgaris* plants.

Three hundred cuttings (100 each of *C. vulgaris* cvs Mousehole, Robert Chapman and Silver Knight), were struck in seed trays (37 x 24 x 5.5 cm deep) of standard propagation compost. The trays contained standard inoculum of *R. solani* isolate A or uninoculated straw at 2 g straw/l compost. Within each of two replicate blocks,

there were two trays (plots) each comprising five, five-cutting rows of each cultivar (sub-plots). The boxes were randomly allocated to their positions within the blocks on the floor of a polythene tunnel. The blocks were separately covered with polythene. As far as possible each plot within each block received the same environmental/cultural conditions.

After 10 weeks, the foliage condition of cuttings was assessed (see section 2.8) and two, 2 - 5 mm long root and two, 2 - 5 mm stem pieces were taken from five cuttings of each cultivar/isolate combination (treatment), and were placed on PDES agar to determine if *R. solani* infection had taken place.

Forty of the remaining cuttings per treatment were taken at random and potted into 8 cm square pots (Optipot 8K, Congleton Plastics Co. Ltd.). They were then placed in a polythene tunnel. Within each of two replicate blocks there were 120 pots (twenty pots from each treatment). The pots were randomly allocated to their positions within the blocks. After 9 months, foliage condition of plants was noted. Six, 2 - 5 mm long pieces were taken from woody roots and stem-bases of twenty plants from each treatment and placed on PDES agar as above.

The remaining twenty heathers from each treatment were planted in clay loam soil 25 cm apart. Within each of twenty replicate blocks there were six plants (one plant from each treatment). The plants were randomly allocated to their positions within the blocks. At all times

throughout the course of the experiment, each plot within each block received (as far as possible), the same cultural/environmental conditions. After 9 months, the foliage condition was noted, and root and stem pieces were placed on PDES agar as above. The number of nuclei in all *Rhizoctonia* spp. isolates taken from roots of *C. vulgaris* cuttings and 9 month old plants were counted using the method detailed in section 2.5 to determine whether the isolates were *R. solani* or binucleate *Rhizoctonia* spp.

5.3 RESULTS

5.3.1 Experiment 18 The effects of several isolates of saprophytic compost fungi on the growth of binucleate *Rhizoctonia* spp. *in vitro*.

No isolate of *Penicillium* spp. or *Mucor* spp. demonstrated antagonism towards binucleate *Rhizoctonia* spp. isolates D1 or 64, i.e. growth of binucleate *Rhizoctonia* spp. isolates was not stopped (Table 21). In some cases binucleate *Rhizoctonia* spp. isolates D1 or 64 restricted the growth of *Mucor* or *Penicillium* spp.

A variety of interactions was observed between *Trichoderma* spp. and binucleate *Rhizoctonia* spp. isolates 64 and D1. *Trichoderma* spp. isolates 4, 7 and 9 caused the growth of both binucleate *Rhizoctonia* spp. isolates to stop when mycelia of the two species met *in vitro*. *Trichoderma* sp. isolate 10 also stopped growth of binucleate *Rhizoctonia* sp. isolate 64. Plate 14, which follows page 182, shows the interaction between binucleate

Table 21 Interactions[a] between binucleate *Rhizoctonia* spp. isolates D1 and 64 and *Trichoderma, Penicillium* and *Mucor* spp., inoculated as 5 mm diameter agar culture discs on extreme opposite sides of 9 cm diameter potato dextrose agar plates, assessed 1 week after inoculation. (Experiment 18)

Saprophytic isolate		Interaction[a] *Rhiz.* sp. isolate D1	Interaction[a] *Rhiz.* sp. isolate 64
Penicillium	A	none	none
	B	none	none
	C	none	c
Mucor	a	none	none
	b	c	c
	c	none	none
Trichoderma	1	c	none
	2	c	c
	3	none	none
	4	b	b
	5	none	none
	6	none	none
	7	b	b
	8	none	none
	9	b	b
	10	d	b
	11	d	d
	12	none	none

[a]Interactions

None - i.e. both isolates grew across whole plate, no obvious damage or alteration to normal growth of either isolate.

b - Growth of *Rhizoctonia* spp. isolate stopped when contact made with saprophytic isolate. Saprophytic isolate grew over *Rhizoctonia* spp. mycelium to colonise whole plate.

c - Growth of saprophytic isolate stopped when contact made with *Rhizoctonia* spp. *Rhizoctonia* spp. grew over whole plate.

d - Growth of both isolates stopped when two isolates were 0 - 2 mm apart.

Plate 14 Interaction between binucleate *Rhizoctonia* sp. isolate 64 (bottom right) and *Trichoderma* sp. isolate 4 (top left) on potato dextrose agar. Growth of the *Rhizoctonia* sp. isolate stopped when contact was made with *Trichoderma* sp. mycelium, which grew over the *Rhizoctonia* sp. mycelium to colonise the entire plate. (Experiment 18)

Rhizoctonia sp. isolate 64 and *Trichoderma* sp. isolate 4. Growth of binucleate *Rhizoctonia* sp. isolate 64 stopped when contact was made with *Trichoderma* sp. mycelium, which then grew over the binucleate *Rhizoctonia* sp. mycelium to cover the entire plate.

Microscopic examination (x 40 and x 100) revealed that hyphae of *Trichoderma* spp. isolates 4, 7, 9 and 10 coiled around and penetrated binucleate *Rhizoctonia* spp. mycelium when two isolates met *in vitro*.

5.3.2 Experiment 19 The effect of *Trichoderma* spp. incorporated in propagation compost on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), the controls involving no *Rhizoctonia* spp. were omitted since they had mean scores equal to zero. There were small but significant differences ($F_{5,33} = 2.63$; $P < 0.05$) between the levels of foliar browning on cuttings in *Rhizoctonia*-infested composts amended with *Trichoderma* spp. isolates (Table 22). There was no obvious pattern to the levels of browning on cuttings however. In some cases the addition of a *Trichoderma* spp. isolate to Rhizoctonia-amended compost reduced the level of foliar browning in comparison to that on cuttings grown in compost containing only a *Rhizoctonia* sp. isolate. For example cuttings grown in compost containing *Trichoderma* sp. isolate 10 and *Rhizoctonia* sp. isolate 64 had a mean score of 2.7. Controls grown in compost containing only *Rhizoctonia* sp.

Table 22 The effect of *Trichoderma* spp. incorporated into compost on the foliage condition[a] of *C. vulgaris*[b] cuttings grown in compost amended with binucleate *Rhizoctonia* spp. isolates, assessed 3 weeks after cuttings were struck. (Experiment 19)

	Foliage condition score[a]			
Trichoderma sp. isolate	*Rhiz.* sp. isolate None	*Rhiz.* sp. isolate 64	*Rhiz.* sp. isolate D1	(mean infested treatments)
None	0.0	3.0	3.5	3.3
4	0.0	3.1	3.6	3.3
7	0.0	3.1	3.6	3.4
9	0.0	3.2	3.8	3.5
10	0.0	2.7	3.4	3.1
11	0.0	2.9	3.3	3.1
	SEM[c] = 0	SEM = 0.14		SEM = 0.10
Mean	0.0	3.0	3.5	
	SEM = 0	SEM = 0.06		

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]Scores are the mean from three *C. vulgaris* cvs Mousehole, Cuprea and Beoley Gold

[c]Scores of treatments containing no Rhizoctonia were omitted from the analysis of variance. Their SEM's were assumed to be equal to zero. All other SEM's with 33 degrees of freedom.

isolate 64 had a mean score of 3.0. However, in other cases, the addition of a *Trichoderma* sp. isolate increased the level of browning on cuttings in *Rhizoctonia*-infested composts. For example a score of 3.2 was recorded on cuttings grown in compost containing *Rhizoctonia* sp. isolate 64 and *Trichoderma* sp. isolate 9.

Foliage browning of cuttings grown in composts amended with binucleate *Rhizoctonia* sp. isolate D1 was significantly worse ($F_{1,33}$ = 48.31; P < 0.001) than in those grown in compost amended with binucleate *Rhizoctonia* sp. isolate 64. *Rhizoctonia* spp. were isolated from all cuttings grown in *Rhizoctonia*-amended compost.

All cuttings grown in unamended compost and compost containing saprophytic fungi in the absence of binucleate *Rhizoctonia* spp., remained healthy, had foliage scores of 0 and rooted in 3 to 4 weeks.

5.3.3 Experiment 20 The effects of several compost additives on the infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* sp. isolate 48.

When the statistical analysis was carried out on the foliage scores (final assessment), the uninfested controls were omitted because they had mean scores equal to zero. There were significant differences ($F_{5,68}$ = 12.97; P < 0.001) between foliage scores of cuttings grown in different composts amended with binucleate *Rhizoctonia* sp. isolate 48, (Table 23). Cuttings grown in peat and bark (Melcourt Industries) compost amended with binucleate *Rhizoctonia* sp. isolate 48 had lower foliage scores, than those grown in pure peat or any other compost which had been amended with binucleate *Rhizoctonia* sp. isolate 48. For example, a mean foliage score of 2.5 was recorded on *C. vulgaris* cv Beoley Gold cuttings grown in peat + M.I. bark compost, whereas scores of 3.4 and 3.6 were recorded on cuttings of the same cultivar grown in peat + Cambark

Table 23 The effect of compost type on the foliage condition[a] of *C. vulgaris* cvs Mousehole, Cuprea and Beoley Gold cuttings struck into compost amended with binucleate *Rhizoctonia* sp. isolate 48, assessed 4 weeks after cuttings were struck. (Experiment 20)

	Foliage score[a]			
Compost	Mousehole	Cuprea	Beoley Gold	Mean
Bulrush Peat	3.4	3.2	3.4	3.3
1:1 (v/v) Peat + Cambark	3.1	3.4	3.4	3.3
1:1 (v/v) Peat + M.I.[b] bark	2.6	3.0	2.5	2.7
10:1 (v/v) Peat + liquid sludge	3.5	3.4	3.6	3.5
3:1 (v/v) Peat + solid sludge	3.3	3.4	3.5	3.4
Peat + Fertilox granules (15 g/l)	3.4	3.7	3.5	3.5
		SEM[c] = 0.14		SEM = 0.08
Mean	3.2	3.3	3.3	
		SEM = 0.06		

[a]Foliage score: 0 = no browning; 4 = foliage totally brown

[b]Bark produced by Melcourt Industries

[c]SEM's with 60 degrees of freedom

and peat + liquid sludge composts respectively.

Isolations from the stem-bases of cuttings revealed that all foliar browning was due to infection by *Rhizoctonia* spp. No *Rhizoctonia* spp. were isolated from control cuttings grown in unamended composts which rooted in 2 to 4 weeks and showed no foliar browning.

There was considerable growth of algae and moss on the surface of the compost containing solid sewage sludge. This experiment was carried out again on two subsequent occasions but the results obtained from Experiment 20 were not confirmed.

5.3.4 Experiment 21 The effect, over a 1 year period, of *R. solani* isolate A (which was previously shown to infect *C. vulgaris* cuttings, causing no visible symptoms), on *C. vulgaris* plants.

An analysis of variance was carried out on the foliage scores recorded on the final assessment only. All values were included. *R. solani* was isolated from 40% of the five *C. vulgaris* cv Mousehole cuttings and 60% of each of the five *C. vulgaris* cv Robert Chapman and Silver Knight cuttings which had been struck into *R. solani*-amended compost and assessed 10 weeks after striking. It was also isolated from 30% of Mousehole plants, 60% of Robert Chapman plants and 65% of Silver Knight plants (twenty plants /cultivar were tested) which had been struck into compost amended with *R. solani* isolate A and were assessed 9 months after potting. No foliar browning was observed on cuttings grown in *R. solani*- amended compost or on plants

9 months after potting. No *Rhizoctonia* spp. were isolated from control cuttings or plants and no binucleate *Rhizoctonia* spp. were isolated from cuttings or plants grown as cuttings in infested compost. No foliar browning was observed on control cuttings or plants.

The foliar browning observed on *C. vulgaris* plants grown as cuttings in compost amended with *R. solani* isolate A and then planted in clay loam soil, was significantly greater ($F_{1,95} = 14.65$; $P < 0.001$) than for those grown as cuttings in unamended composts (Table 24). Foliar browning was observed on 50% of plants grown in infested propagation compost. Isolations from the roots showed that 68% of the browning was due to *Rhizoctonia* spp. Foliar browning was observed on 20% of the control plants growing in soil. Isolations from the roots showed that 8% of this browning was due to *Rhizoctonia* spp.

Rhizoctonia spp. were isolated from 43% of plants which had been struck (as cuttings) into infested compost prior to planting in soil. The fungus was isolated from 2% of control plants growing in soil.

5.4 DISCUSSION

Four of the twelve isolates of *Trichoderma* spp. tested in Experiment 18 were antagonistic to one or both of binucleate *Rhizoctonia* spp. isolates D1 and 64 *in vitro*. Several other investigators have demonstrated that many isolates of *Trichoderma* spp. have shown antagonism towards

Table 24 Isolation of *Rhizoctonia* spp.[a] from, and foliage condition[b] of *C. vulgaris* plants[c] 9 months after planting in garden soil. (Experiment 21)

		C. vulgaris cultivar			
		Mousehole	Robert Chapman	Silver Knight	Mean
Control	Mean foliage score	0.4	0.5 SEM = 0.213	0.5	0.5 SEM = 0.123
	Proportion of cuttings from which *Rhiz.* sp. isolated[a]. (Standard errors in parenthsis)	0.00 (0.000)	0.05 (0.049)	0.00 (0.000)	0.02
Rhiz. sp. isolate A	Mean foliage score	0.7	1.0 SEM = 0.213	1.0	0.9 SEM = 0.123
	Proportion of cuttings from which *Rhiz.* sp. isolated. (Standard errors in parenthesis)	0.40 (0.110)	0.40 (0.110)	0.50 (0.111)	0.40

[a]Twenty plants from each treatment were tested for *Rhizoctonia*. spp.

[b]Foliage score: 0 = no browning; 4 = totally brown foliage

[c]Plants had been grown from cuttings which had been struck into either uninfested (control) compost or compost which had been amended with *R. solani* isolate A.

isolates of *R. solani in vitro*, (Hadar *et al.*, 1979; Elad *et al.*, 1982).

Fungal biological control agents have essentially three modes of action, namely; mycoparasitism, antibiosis and competition. None of the three processes is mutually exclusive and in nature all three may occur simultaneously. Microscopic observations of fungal interactions between *Trichoderma* spp. and *Rhizoctonia* spp., revealed that mycoparasitism occurred between *Trichoderma* spp. isolates 4, 7, 9 and 10 and one or both of binucleate *Rhizoctonia* spp. isolates D1 or 64. Mycoparasitism is the predation of one fungus upon another. It is usually visible under X 400 magnification.

Elad *et al.* (1987) stated that in interactions between *T. harzianum* and *R. solani*, "*T. harzianum* hyphae were shown to coil around their hosts hyphae or produce appressorium-like structures which adhere to the hosts walls. The mycoparasite then digests the wall of the attacked fungus and its hyphae penetrate." In our experiments, growth of *Rhizoctonia* spp. mycelium was stopped. However, due to a lack of time and an inability to obtain certain specific ingredients for an agar medium selective for growth of *Rhizoctonia* spp., no further work was carried out to see whether or not the original *Rhizoctonia* spp. mycelium was killed. When working on interactions between *Trichoderma* spp. and *R. solani in vitro*, Bell *et al.* (1982) removed the original *R. solani* inoculum discs from Petri dishes containing both *R. solani*

and *Trichoderma* spp. following fungal interactions. They placed the original *R. solani* inoculum disc on an agar medium which was selective for the growth of *Rhizoctonia* spp., and found that all twelve isolates of *R. solani* which had been parasitised by *Trichoderma* spp. had been killed.

Growth of *Trichoderma* spp. isolates 10 and 11 stopped when the fungus reached a point 0.5 to 2.0 mm from the advancing *Rhizoctonia* spp. mycelium. Antibiotic production by one or both fungal isolates may have been responsible for the cessation in growth. Several workers have reported that both *Trichoderma* spp. and *R. solani* are capable of producing antibiotics in *in-vitro* interactions (Henis, 1984; Sivan *et al.*, 1984). However, competition for nutrients may also have been involved in such interactions.

Successful biological control of *Rhizoctonia* spp. by *Trichoderma* spp. *in vitro*, does not necessarily mean that similar control will be effected in soil or compost. Several investigators have demonstrated that isolates of *Trichoderma* spp. which were shown to stop growth of *Rhizoctonia* spp. isolates *in vitro*, did not control the pathogen in soil (Lewis & Papavizas, 1985).

None of the *Trichoderma* spp. isolates which were effective in controlling binucleate *Rhizoctonia* spp. *in vitro* (Experiment 19) controlled the fungus when inoculated in the compost on sterile straw pieces. This

may have been due to one or more of several reasons. Firstly, the agar plate tests were carried out in the absence of other fungal species, whereas a wide range of micro-organisms normally present in peat and bark composts, would have been present in the propagation trays in addition to the *Rhizoctonia* spp. Such micro-organisms may have affected the metabolism of the *Trichoderma* spp. either through competition for nutrients, or antibiosis.

Secondly, the environment surrounding the *C. vulgaris* cuttings may have been unsuitable for the growth of *Trichoderma* spp. Temperature, relative humidity, moisture levels, pH and nutrient levels would have differed considerably from those in agar media. Growth of the binucleate *Rhizoctonia* spp. may have been favoured over that of the *Trichoderma* spp. isolates.

Thirdly, the form in which *Trichoderma* spp. were introduced into compost may have been unsuitable to support the growth of mycelium capable of parasitising mycelium of binucleate *Rhizoctonia* spp. Lewis & Papavizas (1985), demonstrated that the form in which *Trichoderma* spp. were applied to soil, determined whether or not control of damping-off caused by *R. solani* was achieved. They found that mycelial preparations (grown on wheat bran), but not conidia of most isolates of *Trichoderma* spp. tested, prevented damping-off of sugar beet, cotton and radish (*Raphanus sativus* L.) seedlings by *R. solani*.

Although no control of binucleate *Rhizoctonia* spp. isolates D1 or 64 was achieved on this occasion, the

screening of a large number of possible antagonistic fungal isolates, (in particular isolates of *Trichoderma* spp.), and the examination of a range of application methods for the fungal antagonists, may lead to the development of a practical biological control strategy for use against *Rhizoctonia* spp. on *C. vulgaris* and *Erica* spp. in peat composts. Potential antagonists were identified to genus level. Species determination was to be carried out only if successful antagonists were found.

It has been shown by several investigators that certain compost ingredients can suppress diseases caused by soil-borne pathogens such as *Rhizoctonia* and *Pythium* spp. Tahvonen (1982a) found that half of the Finnish sphagnum peat samples tested, significantly reduced or inhibited damping-off caused by *R. solani* on cauliflowers. Lumsden *et al.* (1983) showed that the addition of 10% composted sewage sludge to soil significantly reduced the level of Rhizoctonia root rot on cotton, bean and radish; and Nelson & Hoitink, (1983) demonstrated that the addition of composted hardwood bark to peat and perlite composts, significantly reduced the damping-off of Celosia seedlings caused by *R. solani*.

In Experiment 20, the addition of composted pine bark (Melcourt Industries) reduced the level of foliar browning on *C. vulgaris* cuttings grown in Rhizoctonia-infested compost. However, in a similar repeat of the experiment, this reduction in foliar browning was not recorded. No

reductions in foliar browning were recorded on cuttings grown in peat composts amended with other ingredients in comparison to pure sphagnum peat.

Workers investigating the mechanisms behind suppression of pathogens in container media, have concluded that suppression is frequently due to antagonistic micro-organisms. Tahvonen (1982b) showed that *Trichoderma viride* and *Streptomyces* spp. isolated from peat, were responsible for the suppression of *R. solani* in peat composts. Lumsden *et al.* (1983) suggested that populations of micro-organisms such as *Penicillium, Sepedonium* and *Trichoderma* spp., which were higher in soil amended with composted sewage sludge than in unamended soil, were responsible for the suppression of disease caused by *R. solani*. Nelson & Hoitink (1983) concluded that the suppression of damping-off caused by *Rhizoctonia* spp. on radish grown in compost amended with composted hardwood bark, was induced by microbial activity and to a lesser extent chemical inhibitors, the activity of which was dependent on the age of the bark.

The fact that no reduction in infection or disease development was observed on cuttings grown in peat composts amended with various additives, suggests firstly that there were no chemical inhibitors present, capable of restricting growth of *Rhizoctonia* spp. through composts, and secondly, that there were no micro-organisms (or sufficient populations of micro-organisms), which were

antagonistic to the binucleate *Rhizoctonia* spp. isolates used. Although the compost additives tested were not suppressive to the binucleate *Rhizoctonia* spp. isolates, the screening of a large number of peat types, sewage sludges and barks, may yield an additive/additives which would be useful in controlling disease caused by *Rhizoctonia* spp. on *C. vulgaris* and *Erica* spp.

Sewage sludge composition varies greatly depending on the policies of local authorities which process it, and on the nature of the industry and residential settlements in the catchment area. Although use of the sludge obtained for Experiment 20 led to an undesirable copious growth of moss and algae on the compost surface, such effects may not occur with all sludges. The quantity of sludge added to peat may also affect suppression of pathogens in the peat.

No UK source for composted hardwood bark (as used by Nelson & Hoitink, 1982; and Stephens *et al.*, 1981) was found, hence two proprietary brands of composted pine bark were used. Judging by the numerous reports of successful control of *Rhizoctonia* spp. in media amended with composted hardwood bark (Daft *et al.*, 1979; Stephens *et al.*, 1981; Hoitink & Kuter, 1986), it is possible that composts amended with this type of bark may aid in the control of diseases of *C. vulgaris* and *Erica* spp. caused by *Rhizoctonia* spp.

Several investigators have reported that non-

pathogenic isolates of *Rhizoctonia* spp. or 'Rhizoctonia-like' fungi can suppress diseases caused by *R. solani*. For example Ichielevich-Auster *et al*. (1985) demonstrated that an isolate of *R. solani* which was shown to be non-pathogenic to cotton, radish and wheat, (*Triticum* spp.) suppressed damping-off caused by pathogenic strains of *R. solani*. In addition Cardoso & Echandi (1987) showed that certain isolates of 'binucleate Rhizoctonia-like' fungi protected bean seedlings from Rhizoctonia root rot in field experiments.

Experiments carried out to determine the effects of a range of *Rhizoctonia* spp. isolates on *C. vulgaris* and *Erica* spp. (Experiment 3) revealed that *R. solani* isolate A infected *C. vulgaris* cuttings, but caused no obvious damage symptoms. It was thought that if cuttings were infected with a non-pathogenic isolate of *R. solani*, protection against infection by pathogenic isolates may be achieved. Experiment 21 was set up to find out whether *R. solani* isolate A was non-pathogenic towards *C. vulgaris* under a range of conditions, and whether infection by other isolates of *Rhizoctonia* spp. occurred at any stage of plant growth.

R. solani was isolated from rooted cuttings and 10 week-old plants of *C. vulgaris*, but no symptoms of infection by *R. solani* were observed. However, 50% of *C. vulgaris* plants which were propagated in *R. solani*-infested compost showed browning 9 months after planting in garden soil. *Rhizoctonia* spp. were isolated from 43% of

the above plants. *R. solani* isolate A was shown earlier to have infected *C. vulgaris* cuttings and plants but caused no disease symptoms. This isolate may have become pathogenic towards the *C. vulgaris* plants due to changes in host susceptibility. Alternatively foliar browning may have been due to infection by isolates of *Rhizoctonia* spp. other than *R. solani* isolate A. In which case, isolate A failed to protect the plants against such infection.

Several factors require to be investigated if conclusions regarding the possible suppression of Rhizoctonia diseases on *C. vulgaris* and *Erica* spp. by non-pathogenic *Rhizoctonia* spp. isolates, are to be reached.

Firstly, isolates of *Rhizoctonia* spp. taken from plants originally infected with supposedly non-pathogenic *Rhizoctonia* spp. isolates showing foliar browning symptoms (as recorded in Experiment 21), require to be characterised as to both species and anastomosis group. In this way it will be established whether browning is due to the original (supposedly non-pathogenic) *Rhizoctonia* spp. isolate or to another isolate of *Rhizoctonia* spp. Although it was originally intended to do this, a lack of time prevented it from being done.

Secondly, a large number of isolates of *Rhizoctonia* spp. require to be screened under a wide range of conditions in order to select potentially non-pathogenic isolates.

Thirdly, if found, it would be necessary to show that

the non-pathogenic *Rhizoctonia* spp. isolate was capable of causing infection in all cultivars of *C. vulgaris* and *Erica* spp., and remaining active against a wide range of isolates of pathogenic *Rhizoctonia* spp. throughout the life of the plant, which may be up to 20 years in length.

The difficulties involved in carrying out such procedures mean that the development of practical control measures, using non-pathogenic *Rhizoctonia* spp. isolates for control of disease caused by *Rhizoctonia* spp. on *C. vulgaris* and *Erica* spp., is highly unlikely at least in the near future.

CHAPTER 6

THE DEVELOPMENT OF FUNGICIDE PROGRAMMES FOR THE CONTROL OF *RHIZOCTONIA* SPP. ON *C. VULGARIS* AND *ERICA* SPP.

6.1 INTRODUCTION

There has been little work carried out on the development of fungicide programmes for use on hardy ornamental nursery stock. Frisina & Benson (1988) found that benodanil, iprodione and benomyl effectively controlled disease caused by both *R. solani* and binucleate *Rhizoctonia* spp., and Lambe & Wills (1980) reported that PCNB and benomyl gave control of Rhizoctonia root-rot of ornamentals. However, the safety and efficacy of fungicides on any crop is dependent on many factors including cultivar, crop management regimes and fungicide application rates and timings.

The experiments in Chapter 6 were designed to examine several aspects relating to fungicide application on *C. vulgaris* and *Erica* spp., with a view to developing a safe and effective programme to control disease caused by *Rhizoctonia* spp.

6.2 MATERIALS AND METHODS

6.2.1 Experiment 22 The effect of captan, tolclofos-methyl and iprodione on the growth of *Rhizoctonia* spp. *in vitro*.

6.2.1.1 EXPERIMENT 22a CAPTAN. Sterile, molten PDA (50^{o}C contained in a 500 ml flask) was mixed with captan (in Captan 83, Dow Agriculture) on a laminar flow bench to give a concentration of 1000 µg a.i./ml agar. Media from this batch was then serially diluted using further sterile, molten PDA to give agar containing 100, 10 and 1 µg/ml of captan. The agar lots were then poured under

sterile conditions, on the flowbench giving approx. eighteen Petri dishes (9 cm diameter) containing approx. 16 ml of each concentration of captan.

Single 10 mm diameter discs, cut from 10 day old PDA cultures of each of the three binucleate *Rhizoctonia* spp. isolates D1, 72 and 48 and the three *R. solani* isolate K1, A and B1 were then placed in the centre of three replicate Petri dishes of each concentration of fungicide and control plates containing unamended agar. The plates from each replicate were placed in a random order in separate polythene bags which were sealed and incubated at 23°C. Measurement of growth was carried out after 72 hours as described in section 4.2.1. Analysis of variance was used on mean radial growth measurements to calculate SEM's in a similar way to previous experiments.

Linear regression analysis was also used with the data and the estimated effective concentration (dose) of captan to give 50% inhibition of radial growth (ED_{50}) for each isolate, was determined by interpolation from computer generated graphs of fungal radial growth versus fungicide concentration. This allowed comparisons to be made with results of other workers.

6.2.1.2 EXPERIMENT 22b TOLCLOFOS-METHYL AND IPRODIONE. Sterile, molten PDA was mixed with tolclofos-methyl (in Basilex 50% WP, Fisons plc.) and iprodione (in Rovral 50% WP, Rhone Poulenc Environmental Products) as in Expt. 22a, and serial dilutions were made to give agar containing

500, 50, 5 and 0.5 µg a.i. of either fungicide/ml agar. The agar lots were then poured and inoculated as in Expt. 22a with each of the four binucleate *Rhizoctonia* spp. isolates D1, 72, 56 and 48, and each of the four *R. solani* isolates A, B1, 24A and 55. The plates from each of three replicates were placed in a random order in separate polythene bags which were sealed and incubated at 23°C. Measurement of growth was carried out after 72 hours as described in section 4.2.1.

Analysis of variance was carried out only on the data from treatments containing 0.5 and 5 µg/ml of iprodione and tolclofos-methyl due to the large differences in growth measurements obtained throughout the data. No ED_{50} values were calculated because both iprodione and tolclofos-methyl when used at 50 and 500 µg/ml, strongly inhibited the growth of most of the *Rhizoctonia* spp. isolates tested, i.e. there were insufficient growth measurements which were greater than zero, (necessary for regression analysis).

6.2.2 Experiment 23 The effect of iprodione and tolclofos-methyl on the development of potted, rooted *C. vulgaris* plants.

One hundred and twenty five rooted cuttings (12 weeks old) of *C. vulgaris* cv Cuprea were potted in standard potting compost in 8 cm square pots (Optipot 8K, Congleton Plastics Ltd.). Each of five fungicide treatments were applied to twenty-five plants as follows.

1. Control (no fungicide).

2. Tolclofos-methyl incorporated in compost at 0.04 g a.i. in 0.3 l water/l compost immediately before cuttings were struck. Also drenched on compost at 1 g a.i./l water/m^2 monthly for 6 months.

3. Tolclofos-methyl as above at 0.08 g a.i. in 0.3 l water/l compost and drenches at 2 g a.i./l water/m^2.

4. Iprodione (in Rovral Dust, 1.25% w/w, Hortichem) incorporated in compost at 0.005 g a.i./l compost immediately before cuttings were struck. Also drenched on compost (Rovral 50% WP.) at 1 g a.i./l water/m^2.

5. Iprodione as above at 0.01 g a.i./l compost and drenched at 2 g a.i./l water/m^2.

Within each of five replicate blocks, there were five treatments (plots) comprising five plants in separate pots (sub-plots). The pots were randomly allocated to their positions within the blocks. Replicate blocks were surrounded with a single guard row of plants. The experiment was maintained in a polythene tunnel. Every effort was made to ensure that each plot within each block received the same environmental/cultural conditions

Foliage condition and root development were assessed 28 weeks after potting, (see sections 2.8 and 2.9). Foliage dry weights were measured and five root and five stem-base pieces from ten plants/treatment taken at random were placed on PDES agar to determine if infection by soil-borne pathogens had taken place.

6.2.3 Experiment 24 Efficacy of a range of fungicides in controlling infection of cuttings of *C. vulgaris* cv Alba Praecox by binucleate *Rhizoctonia* spp.

Twelve cuttings of *C. vulgaris* cv Alba Praecox were struck in 10 x 10 x 8 cm deep polythene boxes, which contained Bulrush Propagation Compost which had been treated with one of seven fungicides. Standard inoculum of one of the three binucleate *Rhizoctonia* spp. isolates D1, 64 or 48, or uninoculated straw at 2 g straw/l compost had also been incorporated in the composts prior to cutting insertion.

1. Control (0.3 l water/l compost)
2. Tolclofos-methyl (0.04 g in 0.3 l water/l compost, i.e. 40 µg a.i./ml)
3. Iprodione (in Rovral 50 WP, 50% w/w, 0.04 g as above, i.e. 40 µg a.i./ml)
4. PCNB (in Quintozene WP, 50% w/w, Rhone-Poulenc Environmental Products, 0.04 g as above, i.e. 40 µg a.i./ml)
5. Oxycarboxin (in Plantvax 75, 75% w/w, Fargro Ltd., 0.03 g as above, i.e. 40 µg a.i./ml)
6. Benomyl (in Benlate Fungicide, 50% WP w/w, [UK] Ltd. 0.04 g as above, i.e. 40 µg a.i./ml)
7. Captan (in Captan 83, 83% WP w/w, Dow 0.066 g as above i.e. 66 µg a.i./ml)

Within each of four replicate blocks, there were 28 boxes (plots) each comprising three, four cutting rows. The boxes were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged along a glasshouse

bench. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliar assessments were made 2 and 3 weeks after cuttings were struck (see section 2.8). Two, 2 - 5 mm long stem-base pieces from twelve cuttings taken at random from each treatment, were placed on PDES agar following the final assessment, to determine the presence of Rhizoctonia.

6.2.4 Experiment 25 The effect of compost composition on the efficacy of iprodione and tolclofos-methyl in controlling infection of *C. vulgaris* and *E. cinerea* cuttings by binucleate *Rhizoctonia* sp. isolate 64.

Five cuttings of *C. vulgaris* cvs Bognie and Alba Praecox and of *E. cinerea* cv Golden Hue were struck, in seed trays (23 x 17.5 x 5.5 cm deep) which contained one of the following three composts.

1. 1:1 (v/v) Bulrush Peat (screened 22 mm) + Silvaperl Standard Grade Horticultural Perlite. (Silvaperl Products Ltd.).
2. 7:3 (v/v) Bulrush Peat (as above) + 2 - 3 mm grit.
3. 1:1 (v/v) Bulrush Peat (as above) + Cambark Fine Grade Bark.

Trays containing each compost were then drenched with either tap water (1 l/m^2), iprodione (in Rovral 50 WP, at 1 g a.i./l water/m^2) or tolclofos-methyl (1 g a.i./l water/m^2) and were left covered with white polythene (120 gauge, 30 micron, LBS Polythene) in a glasshouse, (temperature 12^o - 24^oC) for 4 days. Sixteen pieces of

standard inoculum (i.e. 5 - 10 mm lengths of straw) of binucleate *Rhizoctonia* sp. isolate 64 or uninoculated straw were then spread evenly across the surface of the compost in each tray, and were pushed 5 - 10 mm down into the compost. The trays were re-covered with polythene.

Within each of three replicate blocks, there were eighteen trays (plots) comprising a five cutting row of each cultivar (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged on a tunnel floor. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliar assessments were made 4 and 6 weeks after cuttings were struck (see section 2.8). Two, 2 - 5 mm length stem-base pieces from each of twelve cuttings taken at random from each treatment, were placed on PDES agar following the final assessment, to determine the presence of Rhizoctonia.

6.2.5 Experiment 26 The effect of application rates on the efficacy of tolclofos-methyl and captan applied to control infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

Four cuttings of each of *C. vulgaris* cvs Bognie, Silver Queen and K94 were struck in 10 x 10 x 8 cm deep polythene boxes containing standard inoculum of binucleate *Rhizoctonia* spp. isolates D1 or 64 or uninoculated straw incorporated at 2 g inoculum/l Bulrush Propagation Compost

plus either tolclofos-methyl or captan as follows.

1. no fungicide (0.3 l water incorporated/l compost)
2. tolclofos-methyl 0.010 g a.i. in 0.3 l water/l compost
3. 0.020 g a.i. as above
4. 0.040 g a.i. as above
5. 0.080 g a.i. as above
6. captan 0.017 g a.i. in 0.03 l water/l compost
7. 0.033 g a.i. as above
8. 0.066 g a.i. as above
9. 0.132 g a.i. as above

Within each of three replicate blocks, there were 27 boxes (plots) each comprising a four cutting row of each cultivar. The boxes were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged along a glasshouse bench. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliar assessments were made 3 and 5 weeks after cuttings were struck (see section 2.8). Roots were washed and root indices calculated on the final assessment (see section 2.9). Two, 2 - 5 mm long stem-base pieces from each of twelve cuttings taken at random from each treatment, were placed on PDES following the final assessment, to determine the presence of Rhizoctonia.

6.2.6 Experiment 27 The effect of tolclofos-methyl and captan incorporated in compost 0, 1, 2 and 3 weeks before cuttings were struck, on the infection and foliar browning of *C. vulgaris* cv Cuprea cuttings caused by binucleate *Rhizoctonia* sp. isolate 48.

Seventy two seed trays (23 x 17.5 x 5.5 cm deep) were filled with Bulrush Propagation Compost in which had been incorporated tolclofos-methyl (0.04 g a.i. in 0.3 l water/l compost), captan (0.066 g a.i. in 0.3l water/l compost) or tap water (0.3l/l compost) on one of four dates, at weekly intervals. Standard inoculum of binucleate *Rhizoctonia* sp. isolate 48 or uninoculated straw was added to the compost and mixed into each tray (sixteen, 5 - 10 mm lengths of straw per tray), on the day which the final trays were filled (i.e. 3 weeks after the start of the experiment), and fifteen cuttings of *C. vulgaris* cv Cuprea were struck in each tray.

Within each of three replicate blocks, there were 24 trays (plots) comprising three rows of five cuttings. The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged on a tunnel floor. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliage assessments were made after 1 and 3 weeks (see section 2.8). Two, 2 - 5 mm stem-base pieces from each of twelve cuttings taken at random from each treatment, were placed on PDES agar following the final assessment, in order to determine the presence of Rhizoctonia.

6.2.7 Experiment 28 The effect of irrigation on the efficacy of tolclofos-methyl in controlling infection by binucleate *Rhizoctonia* sp. isolate 64 on *C. vulgaris* cv Cuprea cuttings.

Bulrush Propagation Compost mixed with 0.3 l water/l compost, or compost treated with tolclofos-methyl (0.04 g a.i./l in 0.3 l water/l compost) was used to fill 48 seed trays (23 x 17.5 x 5.5 cm deep). Water was then applied at either 1.25, 20, 40 or 60 l/m^2 (using 5 l watering cans with a fine rose) to each of twelve seed trays placed within a 1 m^2 quadrat frame (six from each fungicide treatment), and the trays were allowed to drain for 3 hours. Standard inoculum of binucleate *Rhizoctonia* sp. isolate 64 or uninoculated straw was spread over each tray (sixteen, 5 - 10 mm straw lengths per tray), and mixed through the compost. Fifteen cuttings of *C. vulgaris* cv Cuprea were then struck in each tray.

Within each of three replicate blocks, there were sixteen trays (plots) each comprising three, five cutting rows (sub-plots). The trays were randomly allocated to their positions within the blocks. Replicate blocks were separately covered in polythene and arranged along a tunnel floor. As far as possible each plot within each block received the same environmental/cultural conditions.

Foliage assessments were made 2 and 3 weeks after cuttings were struck (see section 2.8). Two, 2 - 5 mm length stem-base pieces from each of eight cuttings taken at random from each treatment, were placed on PDES agar following the final assessment, to determine the presence

6.3 RESULTS

6.3.1 Experiment 22 The effect of captan, tolclofos-methyl, and iprodione on the growth of *Rhizoctonia* spp. *in vitro*.

6.3.1.1 EXPERIMENT 22a CAPTAN. All data was included in the statistical analysis. There were significant differences ($F_{4,58}$ = 2398.83; P < 0.001) between the growth of *Rhizoctonia* spp. isolates on media containing different concentrations of captan (Table 25). For example, the addition of 10 µg captan/ml agar restricted growth of *Rhizoctonia* spp. isolates to 42 to 90% of that of isolates growing on unamended agar, and the addition of 100 µg captan/ml agar restricted fungal growth to 8 - 18% of that of controls on unamended agar. There were significant differences ($F_{5,58}$ = 113.26; P < 0.001) between the growth of *Rhizoctonia* spp. isolates. None of the isolates used were completely controlled by concentrations of 1, 10 or 100 µg captan/ml agar. Only *R. solani* isolates A and K1 and binucleate *Rhizoctonia* sp. isolate D1 were controlled in agar containing 1000 µg captan/ml. Diagnostic plots showed that the value of the residuals increased as the fitted values increased. For this reason the results of the analysis of variance were treated with caution.

Significant linear relationships ($F_{6,23}$ = 38.38; P < 0.001) were observed between the mean radial fungal growth (mm) and fungicide concentration (Fig. 13 and Table 26). The regression lines had a common slope but different intercepts.

Table 25 Radial growth (mm)[a] on potato dextrose agar (72 hours after inoculation), of binucleate *Rhizoctonia* spp. isolates D1, 48 and 72 and *R. solani* isolate K1 exposed to captan. (Experiment 22a)

Rhiz. spp. isolate	No of nuclei	Captan (µg a.i./ml) 0	1	10	100	1000	Mean
D1	2	40	30	17	4	0	19
48	2	40	38	35	7	5	25
72	2	39	33	22	4	1	19
B1	many	39	39	35	3	1	23
K1	many	33	23	14	3	0	14
A	many	36	28	16	6	0	17
				SEM[b] = 0.8			SEM = 0.4
Mean		38	32	23	4	1	
				SEM = 0.3			

[a]Means of three measurements of colony radius from original inoculum to edge of colony in mm.

[b]SEM's with 58 degrees of freedom

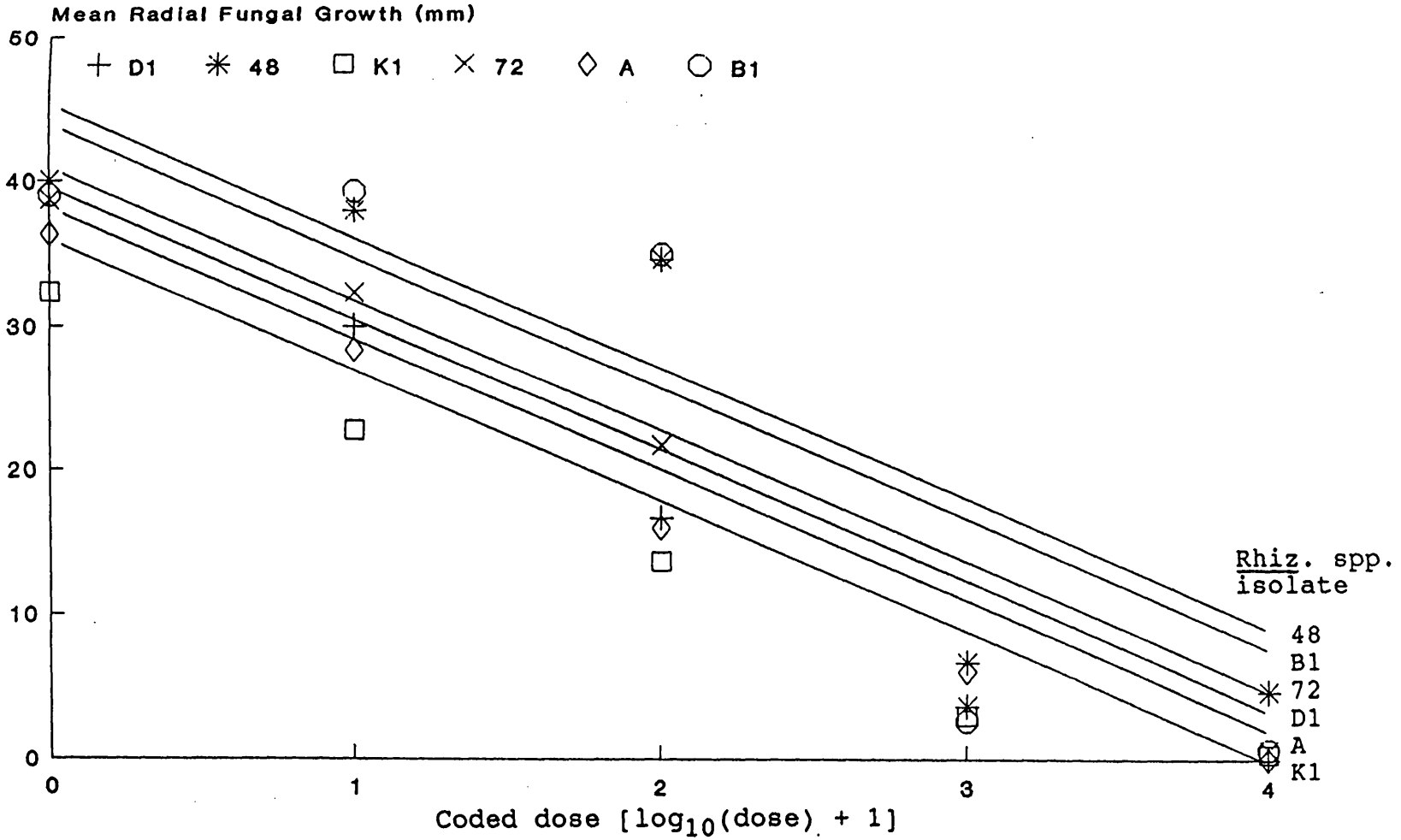

Fig. 13 Linear regression of fungal growth on $\log_{10}$ fungicide concentration. Each point is the mean growth of three replicates. Y = 45 - 10.1x for isolate 48; Y = 43.5 - 10.1x for isolate B1; Y = 40 - 10.1x for isolate 72; Y = 38.2 - 10.1x for isolate D1; Y = 37.5 - 10.1x for isolate A and Y = 34.6 - 10.1x for isolate K1, where Y = mean radial fungal growth (mm) and x = $\log_{10}$ of fungicide concentration. (Experiment 22a)

Table 26 ED_{50} values[a] (µg a.i./ml) of *Rhizoctonia* spp. isolates exposed to captan. (Experiment 22a)

Rhiz. spp. isolate	No. of nuclei	Regression equation	ED_{50}	Sensitivity
D1	2	Y = 38.2 - 10.1x	7.76	moderate
48	2	Y = 45.0 - 10.1x	16.82	slight
72	2	Y = 39.6 - 10.1x	9.09	moderate
B1	many	Y = 43.5 - 10.1x	14.24	slight
K1	many	Y = 34.6 - 10.1x	5.14	moderate
A	many	Y = 37.5 - 10.1x	7.19	moderate

[a] ED_{50} values were determined by interpolation from computer generated graphs of fungal radial growth (mm) versus fungicide concentration. For purposes of comparison, isolates were considered tolerant of the fungicide if the ED_{50} value was > 50 µg a.i./ml, slightly sensitive if it was 10 - 50 µg a.i./ml, moderately sensitive if it was 1 - 10 µg a.i./ml and very sensitive if the ED_{50} value was < 1 µg a.i./ml.

[b] Standard error on gradient (x) = $\pm$ 0.685; standard error on intercepts = $\pm$ 2.74

ED_{50} values from 5.14 to 16.82 were calculated from the regression analysis and graphs.

Plate 15, which follows page 214, shows binucleate *Rhizoctonia* spp. isolates D1, 72 and 48 and *R. solani* isolates K1, B1 and A on PDA amended with 100 µg captan/ml agar after 72 hours incubation. Plate 16 (same page), shows binucleate *Rhizoctonia* sp. isolate D1 on PDA amended with 0, 1, 10, 100 and 1000 µg captan/ml agar after 72 hours incubation.

6.3.1.2 EXPERIMENT 22b TOLCLOFOS-METHYL AND IPRODIONE.

When the statistical analysis was carried out on radial growth measurements, only data from treatments involving either 0.5 or 5 µg fungicide/ml agar were included. There were significant differences ($F_{1,62}$ = 9.20; $P < 0.001$) between the growth of *Rhizoctonia* spp. isolates on media containing different fungicides. There were also significant differences ($F_{7,62}$ = 15.08; $P < 0.001$) between *Rhizoctonia* spp. isolates and a significant three-way interaction ($F_{7,62}$ = 9.04; $P < 0.001$) between isolate, fungicide treatment and dose rate. None of the isolates of *Rhizoctonia* spp. used were controlled *in vitro* by concentrations of 0.5 µg/ml of either iprodione or tolclofos-methyl (Table 27). *R. solani* isolate 24A and binucleate *Rhizoctonia* spp. isolates 72 and D1 were controlled by a concentration of 5 µg/ml iprodione, and *Rhizoctonia* spp. isolates 24A, D1 and 48 were controlled by a concentration of 5 µg/ml tolclofos-methyl. All

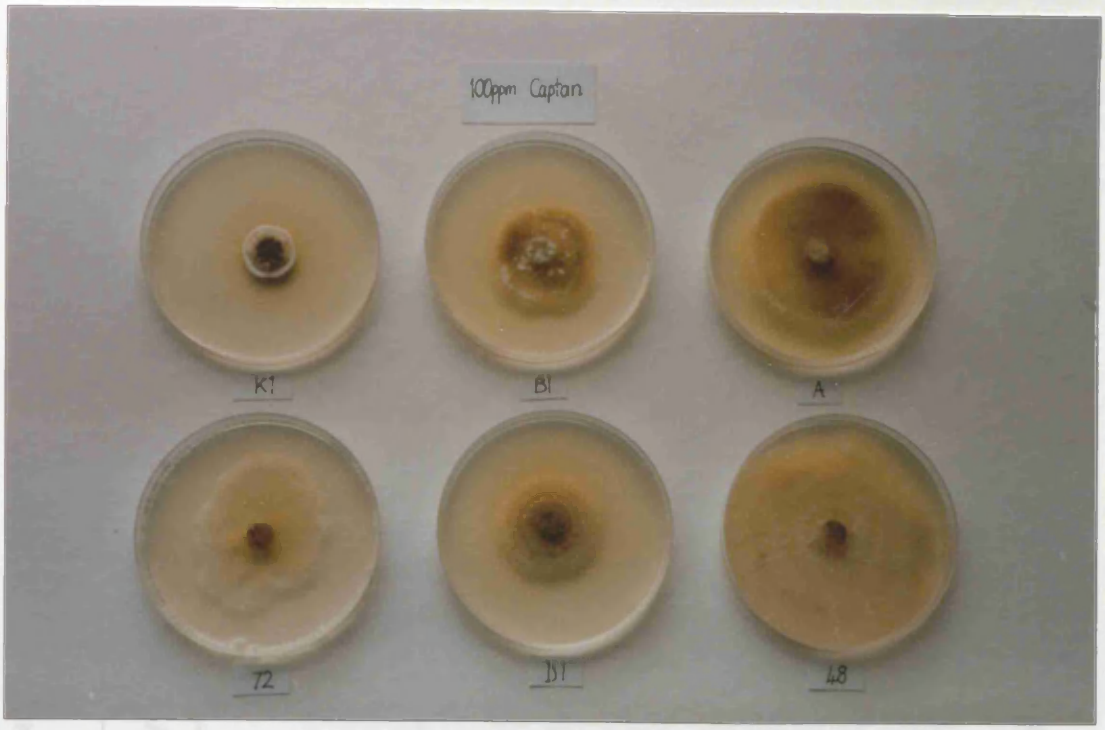

Plate 15 Binucleate *Rhizoctonia* spp. isolates D1, 72 and 48 and *R. solani* isolates K1, B1 and A on potato dextrose agar amended with 100 µg captan/ml agar after 72 hours incubation at 23°C. (Experiment 22a)

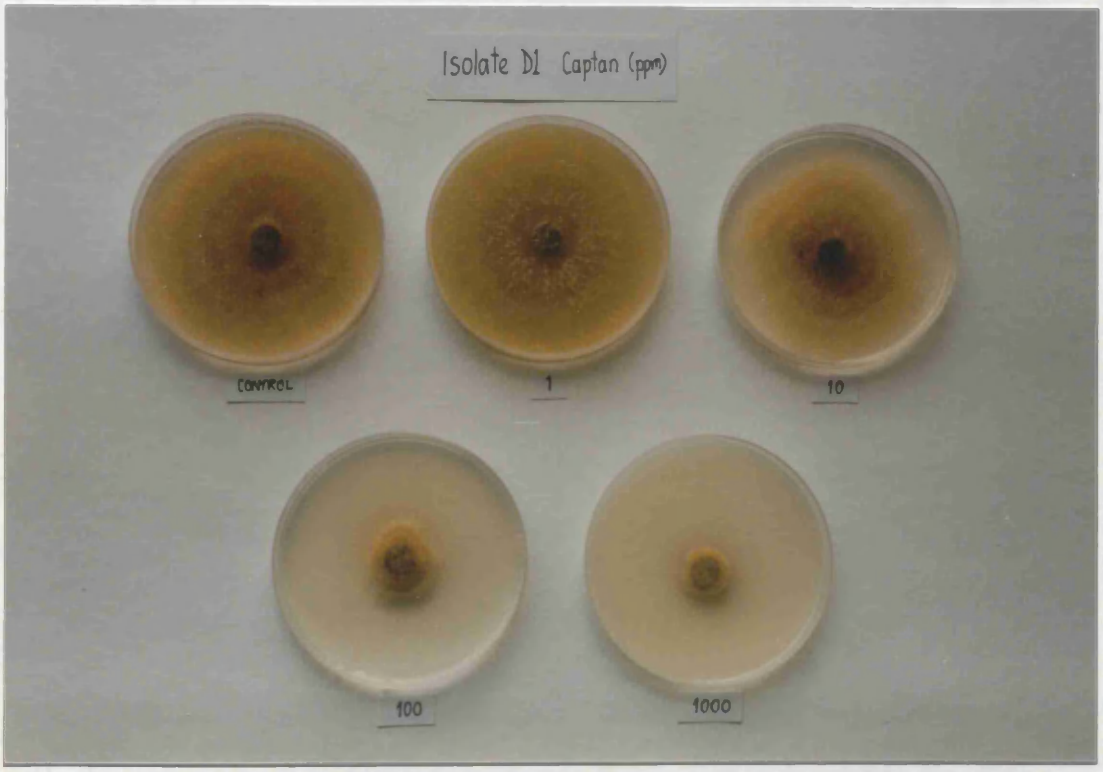

Plate 16 Binucleate *Rhizoctonia* sp. isolate D1 on potato dextrose agar amended with 0, 1, 10, 100 and 1000 µg captan/ml agar after 72 hours incubation at 23°C. (Experiment 22a)

Table 27 Growth of *Rhizoctonia* spp. on potato dextrose agar amended with iprodione or tolclofos-methyl. (Experiment 22b)

			Mean colony radius (mm)[a]							
			Iprodione (µg/ml)				Tolclofos-methyl (µg/ml)			
Rhiz. spp. isolate	No of nuclei	Unamended control	0.5	5	50	500	0.5	5	50	500
A	many	40	26	4	3	0	37	4	6	0
B1	many	40	26	6	3	1	38	6	6	0
24A	many	40	40	0	0	0	40	0	0	0
55	many	40	39	3	0	0	40	3	0	0
D1	2	40	33	0	0	0	40	0	0	0
72	2	40	40	0	3	1	40	2	0	0
56	2	40	40	2	2	2	40	4	4	0
48	2	40	40	16	0	0	40	0	0	0
			SEM[b] = 1.1				SEM = 1.1			
Mean			36	4	1	1	39	2	2	0
			SEM = 0.4				SEM = 0.4			

[a]Means of three measurements of colony radius from original 10 mm agar disc to edge of colony in mm taken 72 hours after inoculation and incubation at 23[C].

[b]Only data from treatments involving 0.5 or 5 µg/ml of either tolclofos-methyl or iprodione were included in the analysis of variance. The SEM's had 62 degrees of freedom.

isolates were controlled on PDA amended with 500 µg/ml tolclofos-methyl, and all isolates except binucleate *Rhizoctonia* spp. isolates 56 and 72 and *R. solani* isolate B1 were controlled by 500 µg/ml iprodione.

6.3.2 Experiment 23 The effect of iprodione and tolclofos-methyl on the development of potted, rooted *C. vulgaris* plants.

All values were included in the statistical analysis. There were no significant differences between the foliage dry weights of plants which were given different fungicide treatments, 28 weeks after potting (Table 28).

Table 28 The effect of iprodione and tolclofos-methyl on the dry weight (g) of foliage of *C. vulgaris* cv Cuprea plants assessed 28 weeks after potting. (Experiment 23)

	Application rates		
Fungicide treatments	Compost incorporation (g a.i./l)	Six x monthly drenches (g a.i./l water/m^2	Foliage dry weight (g)
Control	-	-	0.79
Iprodione[a]	0.005	1	0.68
"	0.010	2	0.81
Tolclofos-methyl[b]	0.040	1	0.73
"	0.080	2	0.64
Mean			0.73 SEM = 0.144

[a]incorporated in Rovral Dust (1.25% a.i.), drenched as Rovral 50 WP.

[b]incorporated and drenched as Basilex 50% WP.

No foliar browning was observed on plants in any treatment, and no differences were observed between root development of plants in different treatments. Root systems of all plants received a score of 3, i.e. there were more than eighty roots visible on the root ball surface when pots were removed. No fungal pathogens were isolated from the roots of plants taken at random for examination following the final assessment.

6.3.3 Experiment 24 Efficacy of a range of fungicides in controlling infection of cuttings of *C. vulgaris* cv Alba Praecox by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), all treatments with a mean score of zero (i.e. uninfested controls and treatments involving tolclofos-methyl) were omitted. The incorporation of any fungicide in infested compost resulted in reduced foliar browning of cuttings (compared with those grown in untreated infested composts, Table 29). This reduction was most marked in treatments involving tolclofos-methyl, where cuttings had a mean score of zero. Significant differences ($F_{5,51} = 56.82$; $P < 0.001$) were recorded between the levels of foliar browning on the other fungicide treatments. Cuttings in composts containing captan showed a large (significant) reduction in foliar browning (mean score recorded on cuttings was equal to 0.2), whereas those in composts containing benomyl, oxycarboxin, iprodione or PCNB (mean scores of 2.8, 3.0, 2.4 and 3.2 respectively) showed smaller (but

Table 29 The effect of fungicides on the development of foliar browning[a] on *C. vulgaris* cv Alba praecox cuttings grown in compost infested with binucleate *Rhizoctonia* spp. isolates assessed 3 weeks after cuttings were struck. (Experiment 24)

Fungicide treatment	Rate (µg a.i./ml compost)	Foliage scores [a]				
		No *Rhiz.* sp.	*Rhiz.* sp. isolate D1	*Rhiz.* sp. isolate 48	*Rhiz.* sp. isolate 64	Mean
Control	-	0.0	3.5	3.2	3.6	3.4
Benomyl	40	0.0	2.8	3.6	3.0	2.8
Iprodione	40	0.0	2.4	2.0	2.8	2.4
Captan	66	0.0	0.1	0.1	0.4	0.2
Oxycarboxin	40	0.0	2.7	3.1	3.1	3.0
PCNB	40	0.0	3.1	3.1	3.4	3.2
				SEM[b] = 0.27		SEM = 0.13
Tolclofos-methyl	40	0.0	0.0	0.0	0.0	0.0
				SEM = 0.00		
Mean		0.0	2.1	2.0	2.3	
				SEM = 0.10		

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]Data from uninfested controls and treatments containing tolclofos-methyl had mean scores equal to zero and were omitted from the statistical analysis. Their SEM's were assumed to be equal to zero. All other SEM's with 51 degrees of freedom.

significant) reductions.

There were no significant differences between the levels of disease caused by the three binucleate *Rhizoctonia* spp. isolates tested and there was no significant interaction between isolate and treatment.

No infection took place in cuttings grown in compost treated with tolclofos-methyl or captan (Table 30). Eighty-four to one hundred percent of cuttings tested from all other treatments where compost was inoculated with binucleate *Rhizoctonia* spp., were infected with the fungus. No foliar browning was observed on, and no *Rhizoctonia* spp. were isolated from cuttings struck in uninfested compost.

6.3.4 Experiment 25 The effect of compost composition on the efficacy of iprodione and tolclofos-methyl in controlling infection of *C. vulgaris* and *E. cinerea* cuttings by binucleate *Rhizoctonia* sp. isolate 64.

When the statistical analysis was carried out on the foliage scores (final assessment), data from all treatments with mean scores equal to zero (i.e. treatments containing no *Rhizoctonia* spp. and/or a fungicide were omitted. Fungicides which contained either iprodione or tolclofos-methyl gave complete control of disease caused by binucleate *Rhizoctonia* sp. isolate 64 in all three composts. No foliar browning was observed on cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 64 and drenched with either iprodione (1 g/l water/m^2) or tolclofos-methyl (1 g/l water/m^2, Table 31).

Table 30 Isolation of *Rhizoctonia* spp. from *C. vulgaris* cv Alba Praecox cuttings 3 weeks after cuttings were struck in binucleate *Rhizoctonia* spp.-amended compost treated with fungicides. (Experiment 24)

Treatment	Rate (µg a.i./ml compost)	Proportion of cuttings from which *Rhizoctonia* spp. isolated (twelve cuttings from each treatment were tested).		
		Isolate D1	Isolate 64	Isolate 48
Control	-	1.0 (0.00)[a]	1.0 (0.00)	1.0 (0.00)
Benomyl	40	1.0 (0.00)	0.8 (0.11)	0.9 (0.08)
Iprodione	40	0.8 (0.11)	0.9 (0.08)	0.8 (0.11)
Tolclofos-methyl	40	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
Captan	66	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
Oxycarboxin	40	1.0 (0.00)	1.0 (0.00)	0.9 (0.08)
PCNB	40	1.0 (0.00)	1.0 (0.00)	1.0 (0.00)

[a]Standard errors are in parenthesis

Table 31 The effect of compost type and the addition of iprodione or tolclofos-methyl on the foliage condition[a] of *C. vulgaris* and *E. cinerea* cuttings[b] grown in compost amended with binucleate *Rhizoctonia* sp. isolate 64, 6 weeks after cuttings were struck. (Experiment 25)

Rhiz. sp. isolate	Fungicide treatment	Drench rate g a.i./l water/m^2	Foliage score[a] Peat + grit	Peat + bark	Peat + perlite
	none	-	0.0	0.0	0.0
Control	tolclofos-methyl	1	0.0	0.0	0.0
	iprodione	1	0.0	0.0	0.0
	none	-	0.6	0.6	0.6
				SEM[c] = 0.08	
64	tolclofos-methyl	1	0.0	0.0	0.0
	iprodione	1	0.0	0.0	0.0

[a]Foliage scores: 0 = no browning; 4 = totally brown foliage

[b]Foliage scores were taken as the mean scores obtained from the three cultivars *C. vulgaris* cvs Bognie and Alba Praecox and *E. cinerea* cv Golden Hue.

[c]Data from all treatments with mean scores of zero were omitted from the analysis of variance. All other SEMs with 22 degrees of freedom.

No *Rhizoctonia* spp. were isolated from cuttings grown in infested compost which contained either iprodione or tolclofos-methyl.

There were no significant differences between the levels of foliar browning on cuttings grown in untreated infested composts of the three types used. Mean foliage scores of cuttings grown in composts amended with binucleate *Rhizoctonia* sp. isolate 64, but which received no fungicide treatments, were lower than expected, (scores of all cuttings were 0, 1 or 2 and mean foliage scores obtained from cuttings in each compost were all less than 1). No *Rhizoctonia* spp. were isolated from cuttings grown in uninfested composts. These cuttings rooted in 2 to 3 weeks.

6.3.5 Experiment 26 The effect of application rates on the efficacy of tolclofos-methyl and captan applied to control infection of *C. vulgaris* cuttings by binucleate *Rhizoctonia* spp.

When the statistical analysis was carried out on the foliage scores (final assessment), all treatments with no foliar browning (mean scores equal to zero) were omitted (i.e. uninfested treatments and treatments containing captan at 0.132 g/l compost or tolclofos-methyl at 0.04 g or 0.08 g/l compost). Significant differences ($F_{5,70} = 116.23$; $P < 0.001$) existed between the scores of cuttings in different fungicide treatments (Figs. 14 and 15).

Foliar browning was recorded on all cultivars in all

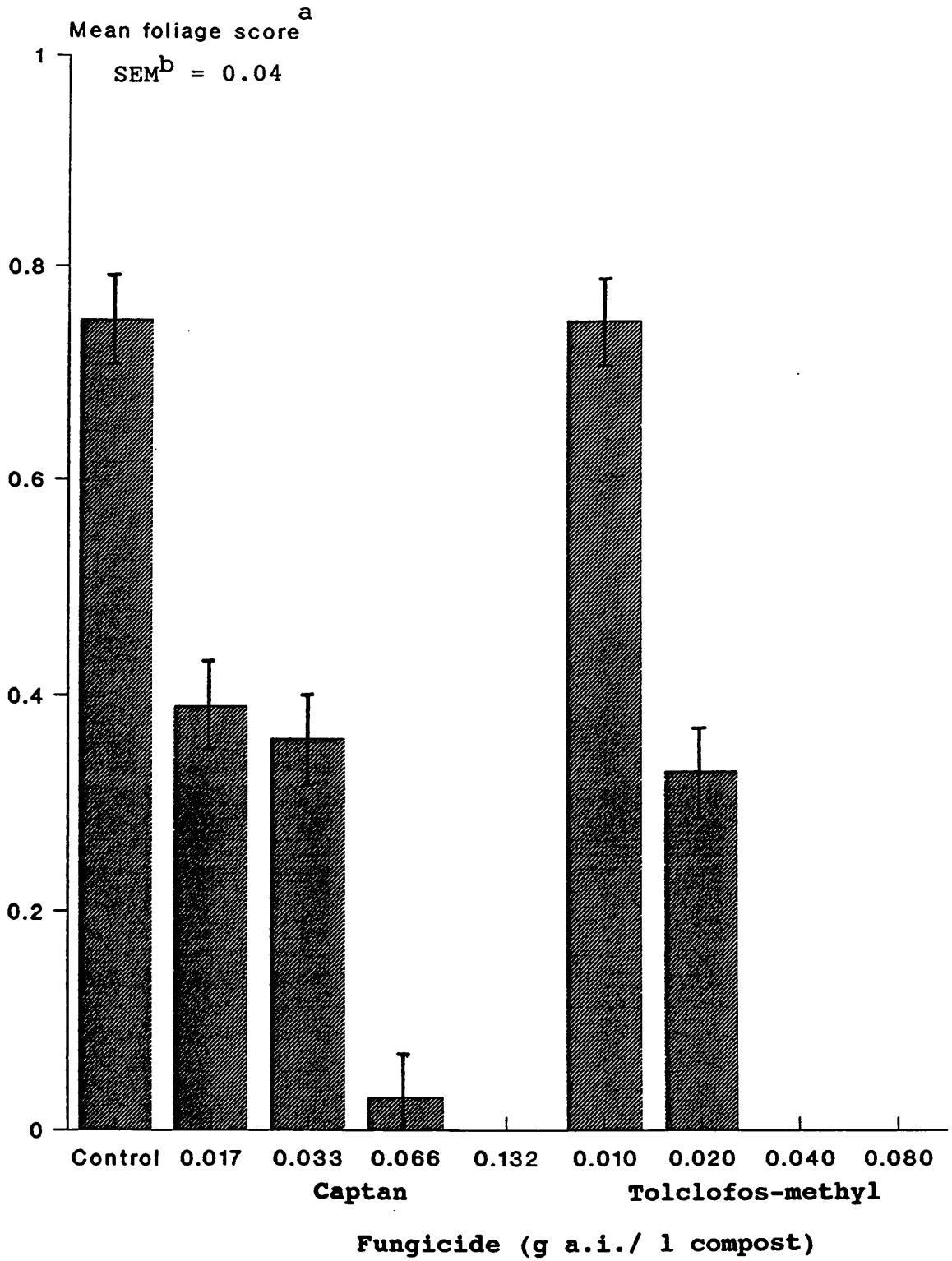

Fig. 14. The effect of different compost incorporation rates of tolclofos-methyl and captan on the mean foliage score[a] of *C. vulgaris* cvs Bognie, Silver Queen and K94 grown in Bulrush Propagation Compost amended with binucleate *Rhizoctonia* sp. isolate D1, assessed 5 weeks after striking. (Experiment 26)

[a]Foliage score (means taken from three cultivars) : 0 = no browning; 4 = totally brown foliage

[b]Treatments with mean scores equal to zero were omitted from the statistical analysis. Their SEM's were assumed to be zero. All other SEM's with 70 degrees of freedom.

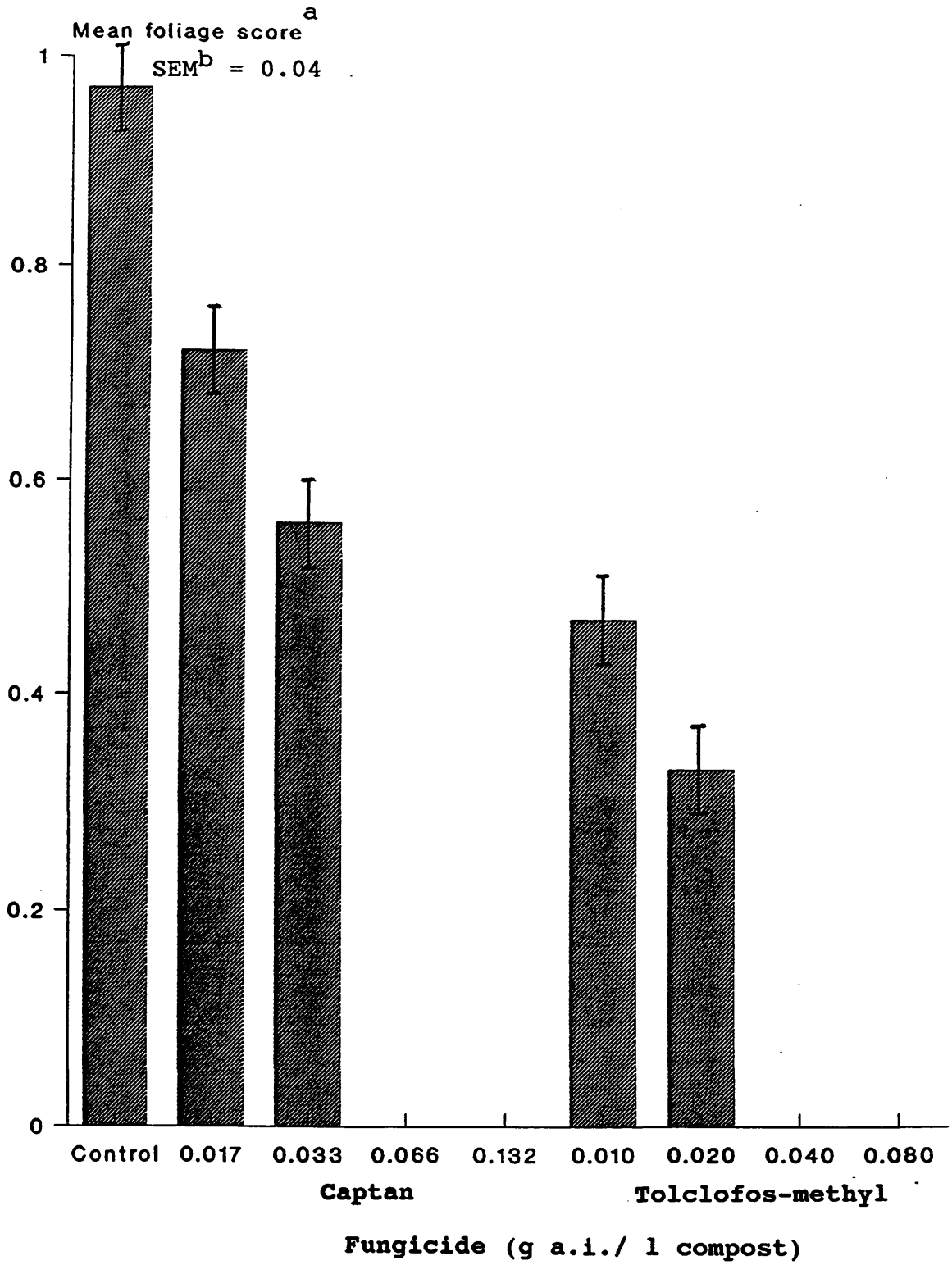

Fig. 15. The effect of different compost incorporation rates of tolclofos-methyl and captan on the mean foliage score[a] of *C. vulgaris* cvs Bognie, Silver Queen and K94 grown in Bulrush Propagation Compost amended with binucleate *Rhizoctonia* sp. isolate 64, assessed 5 weeks after striking. (Experiment 26)

[a]Foliage score (means taken from three cultivars) : 0 = no browning; 4 = totally brown foliage

[b]Treatments with mean scores equal to zero were omitted from the statistical analysis. Their SEM's were assumed to be zero. All other SEM's with 70 degrees of freedom.

treatments which involved either binucleate *Rhizoctonia* spp. isolate D1 or 64, and either captan at 0.017 g or 0.033 g/l compost, or tolclofos-methyl at 0.010 g or 0.020 g/l compost. For example, the mean foliage score of cuttings grown in compost which contained no fungicide or binucleate *Rhizoctonia* spp. isolate was 0.0, whereas the mean scores of cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1 and treated with 0.01g and 0.02g tolclofos-methyl/l compost, were 0.8 and 0.3 respectively. Although reductions in browning of cuttings were observed on these treatments in comparison to the browning seen on untreated infested treatments, heavy cutting losses were still incurred. There were no significant differences between the levels of browning caused by the two binucleate *Rhizoctonia* spp. isolates. However, significant differences were recorded between the amount of foliar browning on the three cultivars ($F_{2,70}$ = 15.52; $P < 0.001$). There were also significant interactions between cultivar and fungicide ($F_{10,70}$ = 3.87; $P < 0.01$) and fungicide and isolate ($F_{5,70}$ = 20.66; $P < 0.001$). The degree of foliar browning was lower than expected (as with Experiment 25).

All values were included in the analysis of the root indices. Root production was greatest in uninfested compost which was not treated with fungicide (Table 32, mean root score of cuttings was 27.3). Differences between root scores of cuttings in the nine fungicide treatments

Table 32 The effect of captan and tolclofos-methyl (incorporated in compost at different rates) on the root development[a] of *C. vulgaris* cuttings[b] grown in compost amended with binucleate *Rhizoctonia* spp. isolates D1 and 64 and assessed after 5 weeks. (Experiment 26)

		Root index[a]			
Fungicide	Rate (g a.i./l compost)	Rhiz. sp. none	*Rhiz.* sp. isolate D1	*Rhiz.* sp. isolate 64	Mean
None	-	27.3	17.2	10.4	18.3
Captan	0.017	13.1	21.6	16.1	16.9
	0.033	18.1	17.7	14.2	16.7
	0.066	13.5	13.1	10.5	12.4
	0.132	2.1	6.2	5.1	4.4
Tolclofos-methyl	0.010	18.0	15.1	16.9	16.7
	0.020	15.0	17.6	18.2	16.9
	0.040	17.9	16.5	19.3	17.9
	0.080	18.2	14.8	17.7	16.9
			SEM[c] = 2.07		SEM = 1.20
Mean		15.9	16.5	14.3	
			SEM = 0.69		

[a]Root indices were calculated as shown in section 2.9

[b]Root indices shown above were means taken from the three *C. vulgaris* cultivars, Bognie, Silver Queen and K94

[c]All SEM's with 160 degrees of freedom

were significant ($F_{8,160} = 13.46$; $P < 0.001$). The addition of any fungicide to uninfested compost was associated with a reduction in root production. Such reductions were most marked where captan was added to the compost at the highest rate (mean root score of cuttings was 2.1). The addition of either strain of binucleate *Rhizoctonia* spp. to compost untreated with fungicide, also resulted in a reduction in root production. For example, the root

indices of cuttings grown in compost amended with binucleate *Rhizoctonia* spp. isolate D1 or 64, were 63% and 38% respectively, of that recorded on cuttings grown in uninfested compost which had not been treated with fungicide. When fungicides were added to infested composts, root production was increased in comparison with untreated controls which contained no fungicides in some cases, but not all.

Both fungicides, (except captan when used at 0.132 g/l) increased root production of cuttings grown in compost infested with binucleate *Rhizoctonia* sp. isolate 64 when compared with inoculated control. For example, the mean root index of cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 64 which contained no fungicide, was 38% more than that of control cuttings grown in untreated compost with no binucleate *Rhizoctonia* spp. isolate. The mean scores of cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 64 which contained 0.01 g, 0.02 g or 0.04 g tolclofos-methyl/l compost, were 62%, 67% and 71% respectively more than that of inoculated controls. Only the addition of captan at 0.017 g/l compost resulted in significantly increased root production of cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate D1. There were no significant differences between the scores of cuttings recorded on the three cultivars.

No infection was found in control cuttings in uninfested composts or in cuttings grown in compost

treated with captan at the highest rate, (0.132 g/l compost) or with tolclofos-methyl at the two higher rates, (0.04 g and 0.08 g/l compost, Table 33). Captan used at 0.017 g, 0.033 g or 0.066 g/l compost and tolclofos-methyl used at 0.01 g or 0.02 g/l compost did not prevent infection of cuttings grown in infested compost, although percentage infection of cuttings grown in fungicide treated infested compost was lower than that of cuttings grown in untreated infested compost.

Table 33 Isolation of *Rhizoctonia* spp. from *C. vulgaris* cuttings, 5 weeks after striking in compost amended with binucleate *Rhizoctonia* spp. isolates D1 and 64 and treated with captan or tolclofos-methyl at several rates. (Experiment 26).

Fungicide	Rate g a.i./l compost)	Proportion of cuttings from which *Rhizoctonia* spp. isolated	
		D1	64
None	-	0.8 (0.13)[a]	0.7 (0.14)
Captan	0.017	0.5 (0.14)	0.6 (0.14)
	0.033	0.4 (0.14)	0.3 (0.14)
	0.066	0.1 (0.08)	0.0 (0.00)
	0.132	0.0 (0.00)	0.0 (0.00)
Tolclofos-methyl	0.010	0.1 (0.08)	0.2 (0.11)
	0.020	0.0 (0.00)	0.1 (0.08)
	0.040	0.0 (0.00)	0.0 (0.00)
	0.080	0.0 (0.00)	0.0 (0.00)

[a]Standard errors are in parenthesis

6.3.6 Experiment 27 The effect of tolclofos-methyl and captan incorporated in compost 0, 1, 2 and 3 weeks before cuttings were struck, on the infection and foliar browning of *C. vulgaris* cv Cuprea cuttings caused by binucleate *Rhizoctonia* sp. isolate 48.

When the statistical analysis was carried out on the foliage scores (final assessment), the uninfested controls and treatments involving tolclofos-methyl were omitted because the cuttings had no foliar browning. The scores on cuttings in these treatments were either equal to zero or were very low (Table 34).

Foliar browning occurred in infested composts which received no fungicide treatment and in compost treated with captan at 0.066 g/l compost 1, 2 and 3 weeks before striking. There were no significant differences between scores of cuttings grown in infested compost containing captan and those in infested compost containing no fungicide. There were significant differences ($F_{3,14}$ = 21.08; $P < 0.001$) between the scores of cuttings treated with captan (or water) on different weeks. For example, the mean foliage score recorded on cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 48 and treated with captan at striking of cuttings was zero, whereas a score of 3.1 was recorded on cuttings growing in similar compost which had been treated with captan 1 week prior to striking. Plate 17 which follows page 230, shows *C. vulgaris* cv Cuprea cuttings in compost amended with binucleate *Rhizoctonia* sp. isolate 48 and treated with captan at striking and 1, 2 and 3 weeks prior to striking.

No infection (by *Rhizoctonia* spp.) of cuttings

Table 34 The effect of captan and tolclofos-methyl incorporated in compost 0, 1, 2 and 3 weeks before cuttings were taken, on the foliage condition[a] of *C. vulgaris* cv Cuprea cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 48. Assessment made 3 weeks after striking. (Experiment 27)

Rhizoctonia sp. isolate	Fungicide	Rate (g a.i./compost)	Foliage score[a] Timing of fungicide incorporation (weeks before striking) 3	2	1	0	Mean
None	-	-	0.0	0.0	0.0	0.0	0.0
None	captan	0.066	0.1	0.0	0.0	0.0	0.0
None	tolclofos-methyl	0.040	0.0	0.0	0.0	0.0	0.0
				SEM[b] = 0.00			SEM = 0.00
48	-	-	1.6	1.5	1.4	1.8	1.6
48	captan	0.066	1.4	2.9	3.1	0.0	1.9
				SEM = 0.20			SEM = 0.10
48	tolclofos-methyl	0.040	0.0	0.0	0.0	0.0	0.0
				SEM = 0.00			
Mean (infested treatments)			1.0	1.5	1.5	0.6	
				SEM = 0.08			

[a]Foliage score: 0 = No browning; 4 = Totally brown foliage

[b]All uninfested controls and treatments containing tolclofos-methyl were omitted from the statistical analysis and the SEM's were assumed to be equal to zero. All other SEM's with 46 degrees of freedom.

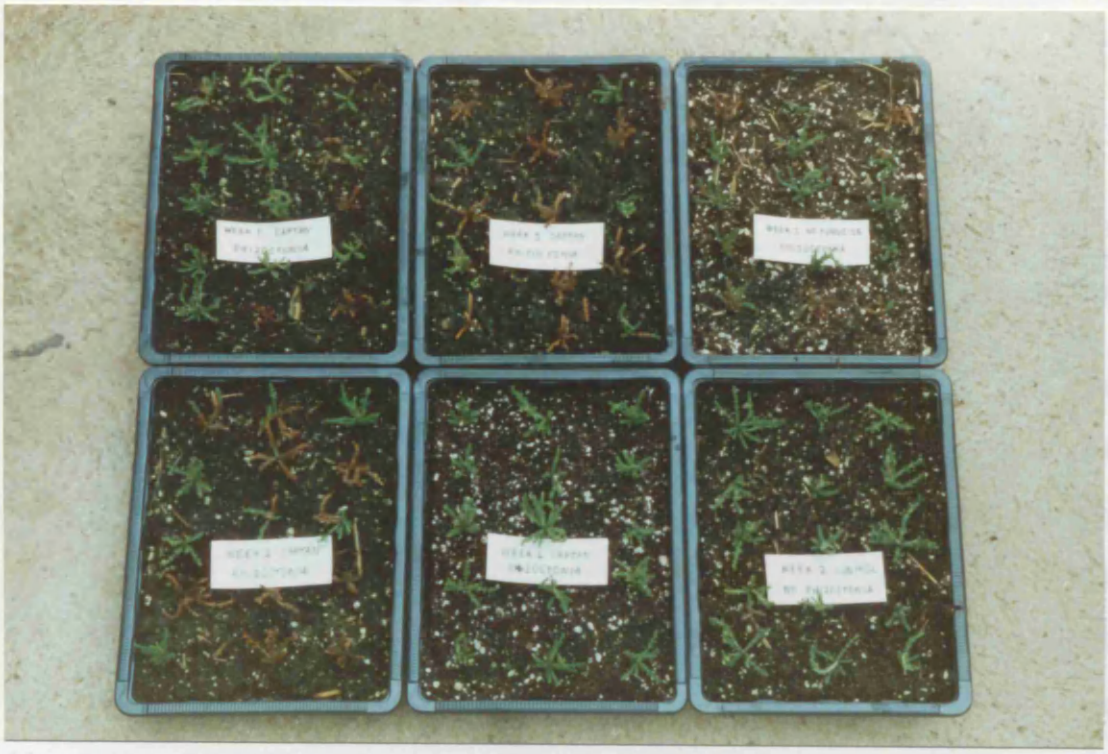

Plate 17 *C. vulgaris* cv Cuprea cuttings in Bulrush Propagation Compost amended with binucleate *Rhizoctonia* sp. isolate 48 and 0.066 g captan incorporated/l compost. Top: left, 3 weeks before striking; middle, 1 week before striking; right, no fungicide. Bottom: left, 2 weeks before striking; middle, at striking; right, no fungicide or *Rhizoctonia* sp. (Experiment 27)

occurred in uninfested composts or composts treated with either fungicide at striking, or with tolclofos-methyl 1 or 3 weeks prior to striking (Table 35). Infection was reduced compared with controls, in infested compost treated with tolclofos-methyl 2 weeks before striking. However, no such reductions were observed with captan.

6.3.7 Experiment 28 The effect of irrigation on the efficacy of tolclofos-methyl in controlling infection by binucleate *Rhizoctonia* sp. isolate 64 on *C. vulgaris* cv Cuprea cuttings.

When the statistical analysis was carried out on the foliage scores (final assessment), the uninoculated control treatments were omitted because they had mean scores of zero. The remaining data was split into treatments containing fungicide or no fungicide. The data was analysed separately due to large differences between the mean scores.

Tolclofos-methyl used at 0.04 g/l compost, gave complete control of binucleate *Rhizoctonia* sp. isolate 64 when cuttings were watered with 1.25 l of water/m^2, (Fig. 16). That is foliage scores of zero were recorded on all cuttings in this treatment. However, the level of control was significantly reduced ($F_{3,30} = 18.94$; $P < 0.001$) when 20, 40 or 60 l of water was applied. The greatest reduction was observed where 60 l of water/m^2 was applied following fungicide incorporation. For example, the mean foliage scores of cuttings grown in composts amended with binucleate *Rhizoctonia* sp. isolate 64, treated with

Table 35 Proportion of *C. vulgaris* cv Cuprea cuttings from which *Rhizoctonia* spp. were isolated (samples of twelve cuttings/treatment) 3 weeks after the cuttings were struck in compost amended with binucleate *Rhizoctonia* sp. isolate 48 and treated with fungicide 0, 1, 2 and 3 weeks before cuttings were struck. (Experiment 27)

Fungicide	Rate (g a.i./l compost)	Timing of fungicide incorporation (weeks before cuttings were struck)			
		3	2	1	0
None	-	0.6 (0.14)[a]	0.5 (0.14)	0.4 (0.14)	0.6 (0.14)
Captan	0.066	0.4 (0.14)	0.7 (0.14)	0.8 (0.13)	0.0 (0.00)
Tolclofos-methyl	0.040	0.0 (0.00)	0.1 (0.08)	0.0 (0.00)	0.0 (0.00)

[a]Standard errors are in parenthesis

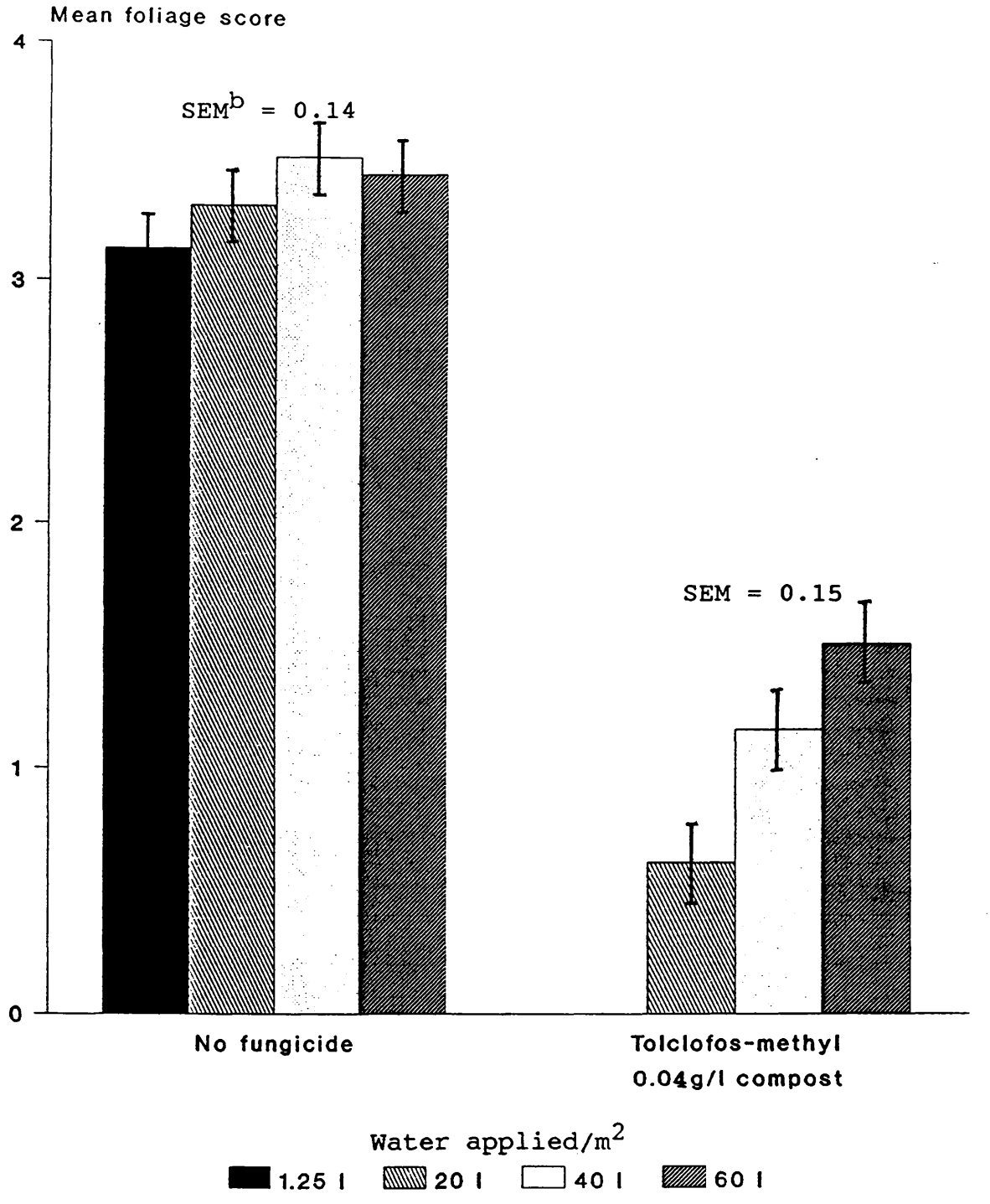

Fig. 16. The effect of irrigation and tolclofos-methyl incorporated in compost on the foliage condition of *C. vulgaris* cv Cuprea cuttings struck in Bulrush Propagation Compost amended with binucleate *Rhizoctonia* sp. isolate 64 and assessed 3 weeks after striking. (Experiment 28)

[a]Foliage score: 0 = no browning; 4 = totally brown foliage

[b]All SEM's with 62 degrees of freedom

tolclofos-methyl and drenched with 20, 40 and 60 l of water/m^2 were 0.6, 1.2 and 1.5 respectively. Plate 18, which follows page 234, shows *C. vulgaris* cv Cuprea cuttings in compost amended with binucleate *Rhizoctonia* sp. isolate 64, treated with tolclofos-methyl and irrigated with different quantities of water, 3 weeks after striking.

Rhizoctonia spp. were isolated from 25%, 92% and 83% of cuttings from infested composts which had been treated with fungicide and watered with 20, 40 and 60 l of water respectively (eight cuttings/treatment were tested). No *Rhizoctonia* spp. were isolated from cuttings in uninfested control treatments or cuttings grown in compost treated with tolclofos-methyl which received 1.25 l of water/m^2 before striking.

6.4 DISCUSSION

ED_{50} values of 5.14 to 16.12 μg a.i./ml were calculated for isolates of *R. solani* and binucleate *Rhizoctonia* spp. grown on PDA amended with captan. It was concluded that binucleate *Rhizoctonia* sp. isolate 48 and *R. solani* isolate B1 were slightly sensitive to the fungicide and that binucleate *Rhizoctonia* spp. isolates D1 and 72 and *R. solani* isolates A and K1 were moderately sensitive. The results are similar to those of Kataria & Grover (1978), who obtained an ED_{50} value of 11.7 for an isolate of *R. solani* grown on agar containing captan. They found captan to be

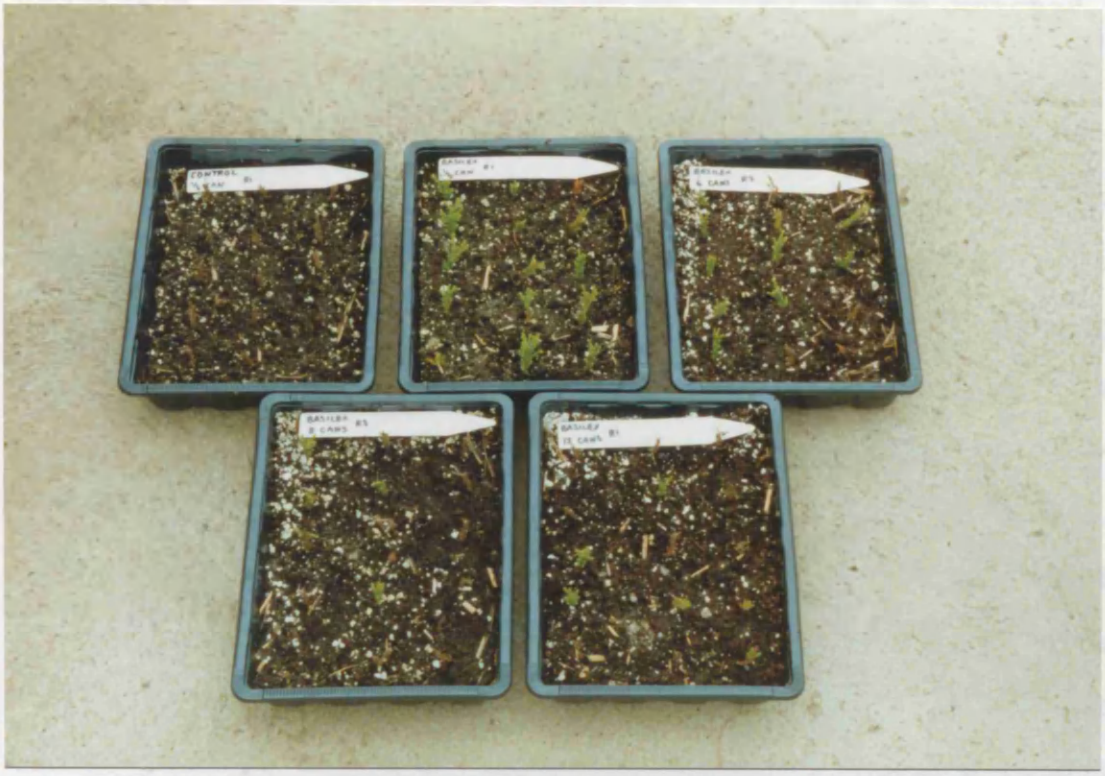

Plate 18 *C. vulgaris* cv Cuprea cuttings 3 weeks after striking in Bulrush Propagation Compost amended with binucleate *Rhizoctonia* sp. isolate 64 and 0.04 g tolclofos-methyl incorporated/l compost and irrigated with tap water using a fine rose at striking. Top: left, control (no fungicide), 1.25 l water/m^2; middle, 1.25 l water/m^2; right, 20 l water/m^2. Bottom: left, 40 l water/m^2; right, 60 l water/m^2. (Experiment 28)

one of the least effective fungicides (out of a total of 41 tested) in controlling this isolate *in vitro*. A larger number of isolates of both binucleate *Rhizoctonia* spp. and *R. solani* require to be examined before conclusions regarding the efficacy of captan *in vitro* can be reached.

According to results obtained in Experiment 22, captan, when incorporated at 1000 µg a.i./ml agar, controlled fungal growth of three out of six *Rhizoctonia* spp. isolates tested. However, it has been shown on several occasions that the inhibition of fungal growth caused by fungicides *in vitro*, is not always found *in vivo*. For example Kataria & Grover (1978) found that thiophanate-methyl, which caused only slight growth inhibition of a *R. solani* isolate *in vitro* (ED_{50} 22.4 µg/ml), gave good control of the pathogen on mung bean (*Phaseolus aureus* Roxb.). However, they also found that captafol and Conen (S-benzylbutyl-s-ethylphosphorodithionate), which strongly inhibited the growth of *R. solani in vitro* (ED_{50} 1 - 10 µg/ml) gave poor control of the disease *in vivo*. Their earlier study (1976) suggested that the activity of fungicides against a host/pathogen combination was likely to be governed by both host and soil factors. For the above reasons, it can be concluded that although studies of the effect of captan on growth rate of isolates of *R. solani* and binucleate *Rhizoctonia* spp. isolates *in vitro* provide a useful guide to whether the fungicide may be effective in controlling the disease in soil/compost, additional glasshouse tests are also necessary.

Both iprodione and tolclofos-methyl, when used at 50 and 500 µg a.i./ml PDA (Experiment 22b) strongly inhibited the growth of most of the *Rhizoctonia* spp. isolates tested. For this reason, there were insufficient values for percentage growth (which were greater than zero) for regression analyses, which were necessary to calculate ED_{50} values.

Martin *et al.* (1984a) calculated ED_{50} values of less than 1 for fourteen out of sixteen isolates of *Rhizoctonia* spp. (both *R. solani* and binucleate *Rhizoctonia* spp.) grown on PDA amended with iprodione, and Frisina & Benson (1988) obtained values of less than 1 for an isolate of *R. solani* and two binucleate *Rhizoctonia* spp. isolates, (isolated from *Ilex crenata* and *Rhododendron* spp. respectively) and grown on similar media. It was therefore surprising that growth of both binucleate and multinucleate *Rhizoctonia* spp. isolates was recorded on agar which contained 50 µg of either iprodione or tolclofos-methyl/ml. This suggests that such isolates may have ED_{50} values of greater than 50 and could be classed as tolerant of the fungicide. Although resistance to both iprodione and tolclofos-methyl has been recorded in the laboratory (Gullino *et al.*, 1984), it has not yet been reported in the field. Gullino *et al.* maintained that the fungicide resistant strains have reduced fitness in nature and would be unlikely to survive in the field. Before conclusions can be drawn regarding the

tolerance/sensitivity of the above isolates to iprodione and tolclofos-methyl, the experiment requires to be repeated, using lower concentrations of fungicide in order to calculate ED_{50} values.

Work on the effect of fungicides on *C. vulgaris* cuttings (Litterick & Holmes, 1990b) has shown that the addition of tolclofos-methyl, iprodione or furalaxyl to propagation compost at 0.04, 0.04 and 0.02 g a.i./l compost respectively, resulted in reduced root production and sometimes foliar browning. In addition, the incorporation of a combination of two fungicides resulted in much greater levels of foliar browning and reduced root development, than when fungicides were used singly.

Experiment 23 aimed to determine whether rooted *C. vulgaris* cuttings showed similar phytotoxicity symptoms when potted in compost treated with fungicide and given monthly fungicide drenches. No significant differences in foliage dry weight or root production were recorded between *C. vulgaris* plants receiving no fungicide, and those receiving iprodione or tolclofos-methyl (compost incorporation at potting plus six, monthly drenches).

Although it is generally acknowledged amongst nurserymen that *C. vulgaris* and *Erica* spp. are very sensitive to crop protection chemicals including fungicides, there is a lack of data available to support such views. This is largely because little work has been done on the subject. From the limited evidence which is available however, Litterick & Holmes, 1990b;

Experiment 23), it can be concluded that *C. vulgaris* and *Erica* spp. are particularly susceptible to phytotoxicity damage during the propagation stage. For this reason, disease control measures other than those involving fungicides may prove to be useful, particularly where more than one pathogen is to be controlled.

It is common practice to use fungicides to control both *Rhizoctonia* spp. and phycomycete fungi (*Pythium* and *Phytophthora* spp.) during production of *C. vulgaris* and *Erica* spp. Since the fungicides used to control these pathogens are highly specific in action, and control only the target organisms, two fungicides are necessary to control them.

If *Rhizoctonia* spp. were successfully controlled by the use of hygienic production methods (see Chapter 3) and cultural control measures (see Chapters 4 and 5) then the need for fungicide applications during propagation would be reduced, thereby reducing the extent of phytotoxicity damage. Fungicide use could be restricted to growth stages where phytotoxicity damage is least likely, i.e. on rooted plants.

The use of regular fungicide drenches on stock plants may reduce substantially (or eliminate) the presence of *Rhizoctonia* spp. on cuttings of *C. vulgaris* and *Erica* spp., thereby reducing the need for fungicide applications to control disease caused by the fungus during propagation. However, the safety of using regular fungicide

drenches on rooted plants requires further investigation before this approach can be recommended. The plants used in Experiment 23 were 12 weeks old and were growing vigorously. Stock plants of *C. vulgaris* and *Erica* spp. are commonly used for cutting production for up to 10 years. The susceptibility of such stock plants to fungicide phytotoxicity may change as the plants age. In addition a range of cultivars of both *C. vulgaris* and *Erica* spp. require to be tested for their susceptibility to damage from fungicides.

The incorporation of tolclofos-methyl (0.04 g a.i./l compost) in standard propagation compost, controlled foliar browning due to binucleate *Rhizoctonia* spp. isolates D1, 48 and 64 on *C. vulgaris* cv Alba Praecox cuttings (Experiment 24). The incorporation of captan (0.066 g a.i./l compost), also significantly reduced the level of foliar browning.

Several investigators have found tolclofos-methyl to be effective in controlling diseases caused by *Rhizoctonia* spp. on other crops. Dolmans & Looman (1984) found that tolclofos-methyl when drenched (2 g Basilex, 50% WP/l water) on propagation compost, gave complete control of disease on *C. vulgaris* cv H.E. Beale cuttings caused by a binucleate *Rhizoctonia* spp. isolate. Koster (1983) found that tolclofos-methyl, when mixed with soil at the manufacturers' recommended rate before planting bulbs, gave good control of disease caused by *R. solani* on forced flower bulbs, and D'Aquila *et al.* (1981) reported that

disease caused by *R. solani* on carnations was controlled when tolclofos-methyl was applied to soil (2 g a.i./m^2 soil) prior to transplanting of young plants.

Reports of the efficacy of captan as a fungicide for control of diseases caused by *Rhizoctonia* spp. vary considerably, although most workers report that disease control is poor or that no control is effected. For example Dolmans & Looman (1984) found captan to be totally ineffective in controlling disease caused by a binucleate *Rhizoctonia* spp. on *C. vulgaris* cv Long White and *Juniperis x media* cv Plumosa Aurea. In addition, Al-Beldawi *et al.* (1976) found that captan failed to control *R. solani* disease on okra (*Hibiscus esculentus* L.) seedlings when applied as a dust to seeds. However, El-Din *et al.* (1975) reported that disease caused by *R. solani* on strawberry transplants was reduced if the plants were dipped in a captan suspension prior to planting in a *R. solani* inoculated soil. Although foliar browning was recorded in Experiment 24 on *C. vulgaris* cv Alba Praecox cuttings grown in composts amended with binucleate *Rhizoctonia* spp. isolates and treated with captan, isolations from cutting bases revealed that the browning was not due to *Rhizoctonia* spp. or any other pathogen. The browning may have been due to fungicide phytotoxicity, although if this was the case, it is unclear why there was no browning in uninfested compost.

There are many reports in the literature of the

excellent control which the fungicide iprodione gives of Rhizoctonia diseases of horticultural crops, (Dolmans & Looman, 1984; Frisina & Benson, 1988; Martin *et al.*, 1984b). For this reason, the poor control of binucleate *Rhizoctonia* spp. isolates D1, 64 and 48 given by iprodione in Experiment 24 was unexpected. Although fungicide resistance to iprodione has been reported with *R. solani* strains, the investigators who discovered the phenomenon, (Gualco *et al.*, 1983) maintained that resistance to iprodione would probably not become a problem in the field because of the reduced fitness of resistant strains, and because several fungicides with different mechanisms of action are often alternated in the field. It may be the case that binucleate *Rhizoctonia* spp. isolates are less sensitive to iprodione than are *R. solani* isolates. However, further work will be necessary to ascertain this.

Although several investigators have reported good control of disease caused by *Rhizoctonia* spp. through the use of benomyl (Frisina & Benson, 1988; Kataria & Grover, 1975b), oxycarboxin (Reddy & Bharathudu, 1980; Oyekan, 1979) and PCNB (Kataria & Grover, 1978; Shatla & Sinclair, 1965), all three fungicides were ineffective in controlling such diseases on C. vulgaris cv Alba Praecox cuttings.

Of the six fungicides tested, tolclofos-methyl was most effective at controlling infection and the development of disease caused by binucleate *Rhizoctonia* spp. Both captan and iprodione also showed potential.

It has been shown that efficacy of fungicides applied to control *R. solani* can vary significantly depending on soil type, (Kataria & Sunder, 1987). For example, Alexander, (1957) reported that quintozene drenches controlled Poinsettia root rot in sandy soil but failed in soil containing organic matter, and Kataria & Grover (1975b), found that quintozene, chloroneb, benomyl and thiophanate-methyl gave better control of *R. solani* on mung bean in light-textured sandy soil, than in heavy textured soils.

Complete control of binucleate *Rhizoctonia* sp. isolate 64 was achieved in standard propagation compost (1:1 v/v Bulrush Peat and Cambark), 1:1 v/v peat and perlite and 1:1 v/v peat and grit with both iprodione and tolclofos-methyl, i.e. there was no effect of compost type on fungicide efficacy (Experiment 25).

However, the conclusions drawn from this experiment were limited for several reasons. The mean foliage scores recorded on cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 64 and receiving no fungicide treatments were very low (i.e. all below 1.0). This was unexpected, since in Experiment 24, mean foliage scores of 3.6 and 2.8 were recorded on *C. vulgaris* cv Alba Praecox cuttings grown in compost amended with the same isolate, and treated with no fungicide and iprodione respectively. It is thought that disease development may have been slow due to an accidental reduction in

glasshouse temperatures which occurred at this time. The entire experiment requires to be repeated in order to further examine the efficacy of iprodione and tolclofos-methyl in controlling infection of cuttings of *C. vulgaris* and *Erica* spp. by binucleate *Rhizoctonia* sp. isolate 64 in different composts.

Complete control of disease on *C. vulgaris* cuttings caused by binucleate *Rhizoctonia* spp. isolates D1 and 64 was achieved in Experiment 26 with 0.040 or 0.080 g tolclofos-methyl/l compost and 0.132 g captan/l compost. Good disease control was also acheived with 0.066 g captan/l compost. No foliar browning was recorded on cuttings growing in fungicide treated composts containing no binucleate *Rhizoctonia* spp. Captan applied at 0.017 or 0.033 g/l compost and tolclofos-methyl applied at 0.010 or 0.020 g/l compost did not control infection and subsequent disease development.

It has been shown that different fungicide application rates are necessary for different crops and cropping situations. For example, concentrations of 0.14, 0.18 and 0.21% a.i. (w/w) of thiophanate-methyl are required to give control of disease caused by *R. solani* on long-melon (*Cucumis melo* Roxb.), mung bean and egg plant (*Solanum melongene* L.) respectively (Kataria & Grover, 1975b). Reductions in manufacturers recommended application rates may give adequate disease control with increased economy and less risk of phytotoxicity . However, the results obtained in Experiment 26 suggest that it is unlikely that

the use of either tolclofos-methyl or captan at less than the manufacturers' recommended application rates, which give 40 µg and 66 µg a.i./g compost respectively, will give adequate disease control.

Tolclofos-methyl gave good control of disease caused by binucleate *Rhizoctonia* sp. isolate 48 on *C. vulgaris* cv Cuprea cuttings, when incorporated in propagation compost at 0.04 g/l compost either at striking or 1, 2 or 3 weeks prior to striking of cuttings (Experiment 27). This indicates that it is a relatively stable compound since it remained effective throughout the 6 week period during the experiment.

Captan gave good control of binucleate *Rhizoctonia* sp. isolate 48 when incorporated in propagation compost at 0.066 g/l compost at striking of cuttings, but failed to control disease if incorporated 1, 2 or 3 weeks prior to striking.

Several investigators have found that the persistence of captan in soils is low. For example Agnihotri (1971) found that it was fully degraded within a week in forest nursery soil when applied at or below 250 µg a.i./g soil. Similarly at 125 µg a.i./g soil it had a half-life of between 1 and 2 days. The lack of persistence may be due to several reasons. The fungicide may have been broken down through exposure to light (compost surface only). For example it was shown that 50% of the fungicide was transformed to inactive material after 3 days of sunshine,

(Mitchell, 1961). It may have been degraded by micro-organisms or through reactions with compounds in the propagation composts, or the captan molecules may have become adsorbed to the compost particles, rendering the fungicide inactive.

Although captan provides good control of disease caused by binucleate *Rhizoctonia* spp. when applied at striking of *C. vulgaris* cuttings, the fact that it is quickly decomposed to inactive compounds, suggests that it will not continue to provide control of *Rhizoctonia* spp. as the cuttings root and develop in propagation compost prior to potting. Hence its use as a fungicide during production of *C. vulgaris* and *Erica* spp. is limited.

The efficacy of tolclofos-methyl was significantly reduced when 20, 40 or 60 l of water/m^2 was applied at striking to groups of twelve trays of *C. vulgaris* cv Cuprea cuttings grown in compost amended with binucleate *Rhizoctonia* sp. isolate 64 (Experiment 28). No foliar browning was recorded on cuttings in infested fungicide treated composts watered with 1.25 l water/m^2. Maximum foliar browning was recorded on cuttings in infested, fungicide treated composts which received 60 l water/m^2.

The application of water to propagation compost, resulted in loss of efficacy of the fungicide, probably as a consequence of leaching. This would not normally cause a problem during the propagation of *C. vulgaris* and *Erica* spp., as the application of large volumes of water is not necessary at this stage, since the cutting trays are

normally covered in polythene whilst rooting. Hence appreciable water loss through transpiration and evaporation does not occur. However, once potted, crops of *C. vulgaris* and *Erica* spp. are often irrigated daily, using overhead irrigation systems for up to an hour, particularly during spring and summer. Loss of fungicide through leaching in such situations may present a serious problem. Careful attention to avoid overwatering, or the placement of plants on capillary sand-beds (where overhead irrigation is unnecessary as plants receive water from below the pots by capillary action) may help to conserve fungicide within the compost. The frequency of watering may also be taken into account when formulating fungicide programmes. Plants watered with overhead irrigation in summer may require more frequent fungicide applications than those grown on capillary beds in winter.

CHAPTER 7

CONCLUSIONS AND FUTURE WORK

Successful control of *Rhizoctonia* spp. on *C. vulgaris* and *Erica* spp. is dependent on a number of factors. The use of new or sterilised nursery materials including trays, polythene, capillary matting, etc., is of great importance in limiting the spread of Rhizoctonia. Similarly composts which consist only of fresh or sterilised components should be used, and contact of plant pots with nursery soil should be minimised. Used nursery materials which cannot be sterilised, should be either burnt or removed from the nursery along with old or diseased plants, to reduce the quantity of inoculum of *Rhizoctonia* spp. on the nursery.

The way in which stock plants are maintained is central to the health of the entire crop of *C. vulgaris* and *Erica* spp. Plants should be potted and trimmed annually to maintain vigorous healthy growth. Spacing of pots is also required in order to minimise plant to plant contact, and to reduce humidity levels around the base of the foliage.

Timely removal of polythene (or removal of cuttings from mist where used) following rooting of cuttings, will ensure that r.h. around cutting tops is reduced as soon as possible, thereby making conditions for spread of Rhizoctonia less favourable. Unlimed propagation composts with a pH value of around 4.0 should be used for propagation.

During the growth of *C. vulgaris* and *Erica* spp. prior

to sale, every effort should be made to maintain plant vigour through the use of good cultural practices. Adequate irrigation, air circulation around the plants and a balanced nutrition in a well-drained compost with an AFP value of 15% to 20%, combined with optimal levels of fertiliser, will combine to favour development of the host over that of the pathogen.

Fungicides provide a useful means of controlling *Rhizoctonia* spp., particularly during growth of rooted plants being produced for sale and stock plants. The use of fungicides to control *Rhizoctonia* spp. during propagation has been shown to cause damage and is not thought necessary, providing hygienic production methods are used along with cultural measures as described above.

Although tolclofos-methyl was the most effective of the fungicides tested in controlling *Rhizoctonia* spp. (captan and iprodione were less effective), it is recommended that the use of tolclofos-methyl is alternated with use of another fungicide, in order to prevent resistance developing amongst populations of *Rhizoctonia* spp.

Fungicide applications at growth stages following propagation, should be minimised to reduce the likelihood and extent of phytotoxicity damage. However, care must be taken when formulating programmes, to take into account the facts that fungicides may decompose within short periods, (particularly captan) and that they may be leached from compost in irrigation water. Both of the

above will result in loss of efficacy.

Further work is necessary to develop an integrated control programme for diseases caused by pathogens other than *Rhizoctonia* spp., which commonly affect *C. vulgaris* and *Erica* spp., i.e. *Botrytis cinerea, Pythium and Phytophthora* spp. and possibly *Pestalotiopsis* and *Cylindrocarpon* spp. Although the hygienic production methods recommended for control of *Rhizoctonia* spp. may aid in the control of these pathogens, cultural control measures may differ. Fungicide programmes must be formulated with care, since different fungicides are necessary to control different pathogens, and phytotoxicity symptoms are more severe when fungicides are used in combination.

A useful crop protection programme for *C. vulgaris* and *Erica* spp. would take into account all pathogens, pests and weeds which would be likely to cause economic loss. The use of pesticides and cultural/biological control measures could then be balanced to minimise crop loss through disease and phytotoxicity damage.

Future work may consider the need for a wider range of fungicides to control *Rhizoctonia* spp. There are several fungicides, for example pencycuron (Monceren DS, Bayer) which are currently used to control *Rhizoctonia* spp. on other crops but are not approved for use on ornamentals.

The high cost of fungicides and the sensitivity of *C. vulgaris* and *Erica* spp. to crop protection chemicals may

lead to further research on biological control measures. The success of other workers in formulating control programmes for *Rhizoctonia* spp. based on the use of composted sewage sludge and composted hardwood bark may lead to the development of similar measures for use on *C. vulgaris* and *Erica* spp.

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