BIOLOGY AND CONTROL OF PYRENOCHAETA LYCOPERSICI, *BROWN ROOT ROT OF TOMATO*

by

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ABSTRACT

A method for the isolation of grey sterile fungi from brown root rot infected tomato root systems was developed. Semi-selective media significantly reduced the growth of *Colletotrichum coccodes* and *Calyptella campanula* with little effect on the growth of grey sterile fungi. Pycnidia characteristic of *Pyrenochaeta lycopersici* were formed on V8-Juice agar (V8A) by twelve of the 19 grey sterile fungal isolates tested. A method for the routine production of pycnidia/conidia was developed: *P. lycopersici* cultures, inoculated onto V8A are incubated at 22°C with a 16h black light photoperiod. No vegetable constituent of V8-Juice, tested individually, could be shown to be solely responsible for sporulation on V8A.

Conidia require a temperature range of 20 to 26°C, pH range 5.0 to 8.0 and external nutrients to achieve germination levels greater than 90%. Conidial germination decreased with age. Incubation of *P. lycopersici* conidia in a dilute cirrus extract and under different light regimes did not affect germination. Conidia were shown to swell prior to germination and produce either single or dual germ tubes. Using mycelial inoculum, grey sterile fungal isolates caused lesions on tomato roots *in vitro* and *in vivo*. Conidia of *P. lycopersici* produced lesions on the roots of tomatoes grown in inoculated sterilised compost and unsterilised garden soil, within six weeks. Brown root rot and corky root symptoms were produced on tomatoes grown commercially in soils, infested with *P. lycopersici* conidia, for up to 21 weeks. Root lesions were produced on plants grown in conidia infested sterilised compost and unsterilised garden soil which had been stored at room temperature for up to 510 and 150 days respectively. Conidial germination was observed in soils, on cellophane and on polycarbonate membranes. On cellophane, conidia germinated in unsterilised and sterilised composts forming sclerotia on a "frond" type mycelium after three to seven days incubation. Conidial germination was lower in washed sand and greatly reduced in peat. Germination on polycarbonate membranes in compost was slower and sclerotial formation reduced compared to that observed on cellophane.

A soil assay has been developed to estimate soil infestation levels of brown root rot (BRR). Using the BRR assay, various species of bacteria and Streptomycetes were shown to reduce the number of lesions on tomato plants grown in soil infested with brown root rot. Potential fungal antagonists, applied as wheatbran cultures, did not significantly reduce disease levels. Bacteria and fungi increased the root dry weight of tomato plants grown in soil infested with brown root rot. Disease incidence, detected using the BRR assay, was directly related to subsequent levels of brown root rot on tomatoes eight weeks after transplanting into the glasshouse.

CONTENTS

Abbreviations used in this study

Ab.alc.	absolute alcohol
a.i.	active ingredient
ADAS	Agricultural Development and Advisory Service
A.T.C.C.	American Type Culture Collection
A	Ampicillin anhydrous
B	bacteria
BL	black light
BRR	brown root rot caused by *Pyrenochaeta lycopersici*
Cp.	*Calyptella campanula*
CRR	*Calyptella* root rot
°C	centigrade (degrees)
cm	centimetre(s)
C	Chloramphenicol
C.c.	*Colletotrichum coccodes*
c	crystal
day	day(s)
f.sp.	*forma specialis*
G.C.R.I.	Glasshouse Crops Research Institute
g	gram(s)
GSF	Grey sterile fungus (fungi)
h	hour(s)
Kg	kilogram(s)
lq	liquid
l	litre(s)
MBC	Methyl benzimidazol-2-yl carbamate
ml	millilitre(s)
mm	millimetre(s)
NT	not tested
O	other fungi
P	Penicillin
PST	Penicillin/Streptomycin/Tetracycline agar
PSTBay	Penicillin/Streptomycin/Tetracycline/Bayleton agar
Pm	Pimafucin
PDA	potato dextrose agar
RW	root dry weight
SW	shoot dry weight
SH	shoot height
S.D.	standard deviation
SED	standard error differences between the means
SDW	sterile distilled water
SME	sterile malt extract
S	Streptomycin sulphate
TW	tap water
TWA	tap water agar
T	Tetracycline anhydrous
U.V.	ultra violet
U.K.	United Kingdom
V	Vancomycin
V8A	V8-Juice agar
w.p.	wettable powder
WL	white light
>	greater than
<	less than
µm	micrometre(s)
%	percentage

CHAPTER ONE. INTRODUCTION

1.1 *Preface*

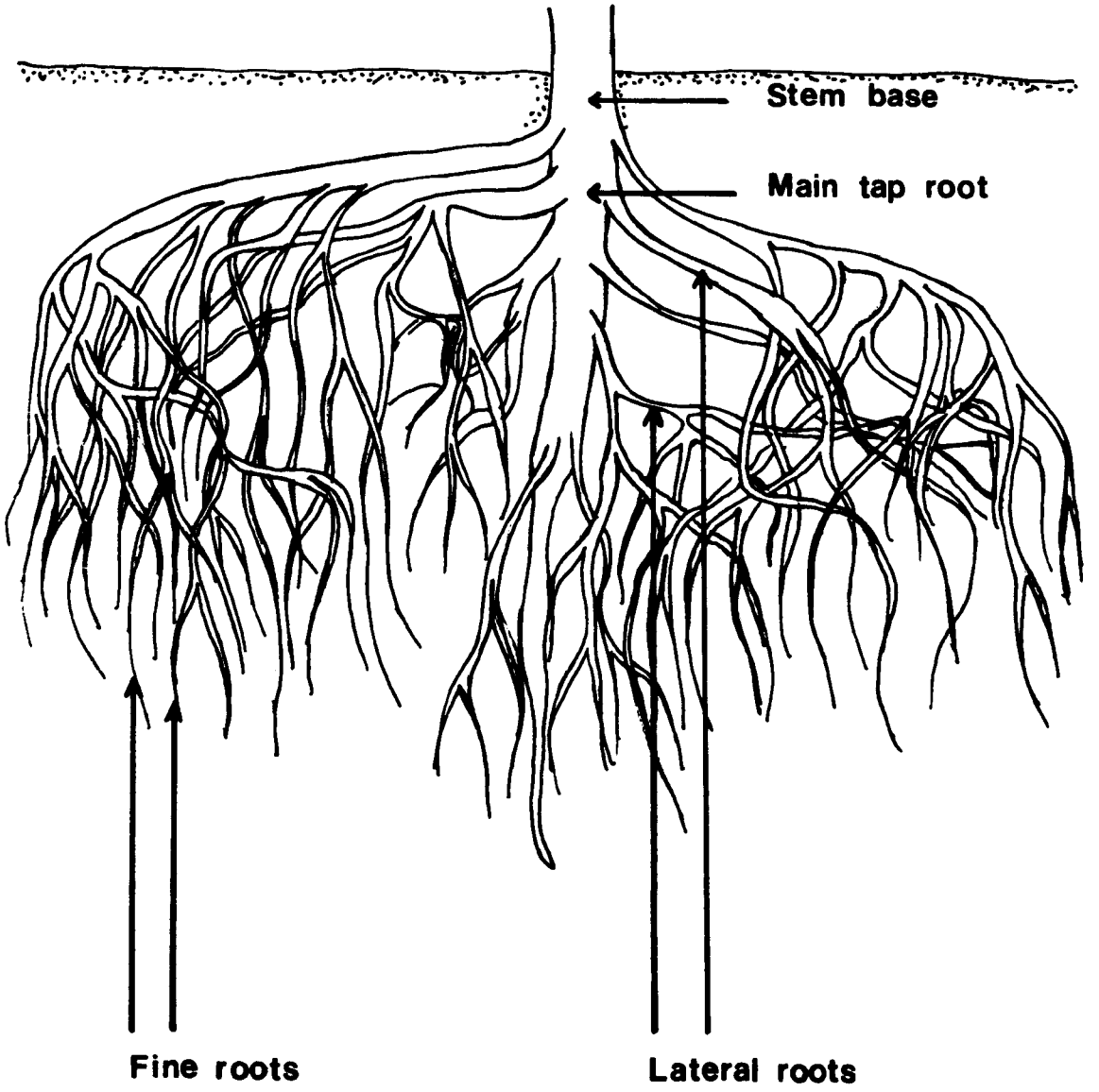
Brown root rot (BRR) caused by the fungus *Pyrenochaeta lycopersici* Schneider and Gerlach is a major disease of commercial glasshouse tomato (*Lycopersicon esculentum* Mill.) crops (Fletcher, 1973). The disease is characterised by the development of a brown furrowed bark causing "corkiness" on large roots (Figure 1). Dark lesions may develop on fine and medium sized lateral roots often girdling their entire length and causing cortical shredding. Stem base lesions may also occur (Ebben and Williams, 1956). The pathogen can attack tomatoes of all ages but is particularly damaging to young seedlings, the loss of fibrous roots at an early stage of growth leading to yield reductions of 45% (Last and Ebben, 1966).

The broad aims of this study were twofold. Firstly to examine certain aspects of the biology of *P. lycopersici* and secondly to investigate the potential of biological methods for the control of BRR.

Pyrenochaeta lycopersici is isolated from diseased roots and infested soil as a grey sterile fungus (GSF). However, many other fungi, including *Colletotrichum coccodes* (Wallr.) Hughes, *Calyptella campanula* (Nees ex Pers.) W.B. Cooke ss W.B. Cooke, *Fusarium* and *Phytophthora* species may also be associated with roots showing symptoms of BRR. The presence of these fungi, in particular *C.coccodes* and saprophytes such as *Trichoderma*,

Figure 1

Structure of a tomato root system.



Penicillium and *Mucor* species frequently make attempts to isolate the slow growing GSF unsuccessful. Most isolations for GSF have involved the use of chemical disinfectants to surface sterilise root sections infected with BRR (Last and Ebben, 1963; Manning and Vardaro, 1974). Termohlen (1962) tested calcium hypochlorite, mercuric chloride and alcohol, as surface sterilants, separately and in combinations to aid the isolation of GSF. Alcohol (75%) and calcium hypochlorite (7%) did not inhibit bacterial growth, which can hinder the transfer of GSF to pure culture. However, mercuric chloride retarded the growth of bacteria and other fungi, allowing colonies of GSF to develop.

In order to establish the causal agent of BRR many GSF isolates have been tested for their ability to reproduce BRR symptoms. The majority of *in vitro* pathogenicity tests have involved placing inoculum in direct contact with tomato seedling roots, maintained on a suitable substrate (generally nutrient agar or moist filter paper). Subsequent lesion formation on roots has been taken as an indication of pathogenicity. Inoculum for such tests has included mycelial agar discs (Last and Ebben, 1966; Manning and Vardaro, 1974), mycelial suspensions (Ebben and Williams, 1956) and micro-sclerotia (White and Scott, 1973). *In vivo* pathogenicity tests have involved the addition of inoculum to sterilised soils, potting composts or vermiculite. Brown root rot symptoms, which developed on tomato plants grown in these substrates, were taken as an indication of pathogenicity. Inoculum for *in vivo* pathogenicity tests has included the use of oat grain

(Richardson and Berkeley, 1944), wheat grain (Last and Ebben, 1966), vermiculite (Manning and Vardaro, 1974) and sand/maize meal (Preece, 1964) cultures of *P. lycopersici*.

Sporulation is necessary to identify GSF isolates as *P. lycopersici*. The fungus is identified by pycnidial and conidial measurements (Schneider and Gerlach, 1966) although Schneider (1976) also considers the presence of setae or hyphal hairs around the pycnidial ostiole, the mode of conidia formation and the characteristics of the conidiophores to be important taxonomic features of the genus *Pyrenochaeta*. However, pycnidia of *P. lycopersici* have only occasionally been observed in culture (Ebben and Williams, 1956; Gerlach and Schneider, 1964) or on BRR infected roots incubated under humid conditions (Giha, 1963). Clerjeau (1974) recorded pycnidia to form on roots of tomato and melon 12 days after inoculation with GSF isolates whilst Schneider and Gerlach (1966) reported that near ultra-violet (U.V.) light stimulated their production in agar culture. However, pycnidial production has been variable and unreliable (Manning and Vardaro, 1974) and, when formed, the quantity of pycnidia per culture has been low with the majority of isolates remaining sterile.

The importance of conidia in the life cycle of *P. lycopersici* is unknown. The pathogenicity of *P. lycopersici* conidia to tomato plants grown in vermiculite has been reported (McGrath and Campbell, 1983). However, the conditions favouring conidial germination and their ability to survive and act as inoculum for the disease in soil has not been investigated.

In this work studies were undertaken to develop reliable culturing and sporulation procedures to aid the isolation and identification of GSF isolates. A variety of techniques were used to study the germination and pathogenicity of conidia and to investigate their possible involvement in the survival and dispersal of the pathogen.

Brown root rot can be controlled by efficient soil sterilisation however this method is costly and, in the case of methyl bromide, application is limited by the danger of bromide residues in subsequent crops (Ebben *et al.*, 1978a). Alternative approaches to BRR control such as attempts to breed resistant varieties, grafting of resistant rootstocks (Termohlen, 1962) and the application of fungicides to the growing crop (Jeves and Smith, 1980) have not yet provided commercially acceptable procedures. Little work on the biological control of *P. lycopersici* has been reported. Davet (1976), studying interactions between fungi associated with corky root disease, observed certain antagonistic effects. *Fusarium oxysporum* (Wollenw.) and *Fusarium solani* (Mart.) Sacc. retarded the development of *P. lycopersici*, the intensity of the antagonistic effect varying with temperature. Termohlen (1962) reported that several bacteria and actinomycetes, isolated from roots infected with BRR, had a strong inhibitory effect against the growth of the corky root fungus on cherry agar. He suggested that these organisms competed with *P. lycopersici* in the soil and tomato roots possibly explaining the slow development of the fungus in naturally infected roots. Fargues *et al.* (1977) noted a complete lysis of the

fibrillar layer of *P. lycopersici* hyphal walls in soil, while the interhyphal mucus remained intact. He concluded that this was due to the action of enzymes originating from bacteria. Solberg (pers. comm.) isolated various micro-organisms from the rhizoplane of tomatoes grown in different soil types. A number of isolates exhibited various degrees of antagonism to *P. lycopersici in vitro*. However, he observed that application of these potential antagonists to tomatoes grown in rockwool gave erratic control of BRR. The possibility of decreasing the incidence of BRR using soil amendments was tested by Preece (1964). Incorporation of sawdust, grass clippings and compost with soil infested with BRR caused virtual complete suppression of disease symptoms. This effect may have been due to an increase in the volume of non-infested material around the plant roots. No attempts to screen micro-organisms for antagonistic effects against BRR in soil have been reported.

The identification of potential antagonists requires a suitable screening technique. *In vitro* agar culture studies involving dual inoculation tests etc. have been widely used to screen micro-organisms against plant pathogens. However, potential antagonists identified in such tests rarely show promise in field experiments (Linderman *et al.*, 1983). The disadvantages of *in vitro* agar plate tests are discussed by Baker and Cook (1974). In this work studies were undertaken to develop a method in which potential antagonists were tested for their ability to reduce disease levels of the pathogen in soil naturally infested with BRR. The relationship between disease levels detected in the laboratory and those subsequently

observed on tomato plants in the glasshouse was also investigated.

1.2 *Introduction*

The host

The tomato, *Lycopersicon esculentum* Mill., is a member of the nightshade family, *Solanaceae*, which contains many economically important plants including: potato, *Solanum tuberosum* L.; eggplant, *Solanum melongena* L.; tobacco, *Nicotiana tabacum* L. and several varieties of pepper belonging to the genus *Capsicum*. The tomato was first introduced into Europe around the beginning of the sixteenth century and appeared in the United Kingdom (U.K.) between 1570 and 1580 as a decorative plant. Its popularity as an edible fruit increased during the late part of the eighteenth century but it was not until the early 1870's that the plant was grown commercially. By 1946 it was estimated that approximately 1400ha were grown under glass and a further 1800ha were devoted to production of the crop in the open (Williams, 1973). This period represented the peak area of tomatoes, grown in England and Wales, with the cropped acreage gradually declining (Table 1) to approximately 700ha in 1983 (217ha heated early, 222ha heated late and 296ha cold). At present the tomato is considered to be the most important glasshouse crop in the U.K. (Tite, 1983). The value of the 1983 crop was approximately £56 million making it the second most important protected crop in the U.K.; mushrooms providing an output value of £69 million. Interest in outdoor tomatoes

Table 1

Value of the United Kingdom tomato crop in comparison to other important protected crops.

	1973	1976	1980	1982	1983
CROPPED AREA (hectares)					
Tomatoes	1,019	950	859	752	735
Cucumbers	138	235	238	239	231
Lettuce	1,083	1,194	1,384	1,435	1,621
Mushrooms	420	361	404	421	421
GROSS YIELD (tonnes per hectare)					
Tomatoes	117.8	137.3	149.7	157	162.9
Cucumbers	233.3	221.4	238.8	230.5	259.7
Lettuce	23.7	25.0	25.9	28.6	27.5
Mushrooms	132.9	141.5	160.6	158.4	165.3
GROSS PRODUCTION ('000 tonnes)					
Tomatoes	120.0	130.4	128.6	118.4	119.8
Cucumbers	32.2	52.0	56.8	55.1	59.9
Lettuce	25.7	29.8	35.9	41.0	44.5
Mushrooms	55.8	51.1	61.3	66.7	69.6
VALUE OF OUTPUT (£'000)					
Tomatoes	24,150	40,911	56,785	42,876	55,732
Cucumbers	5,200	13,450	19,105	16,457	21,056
Lettuce	8,392	17,392	18,910	22,778	30,091
Mushrooms	20,524	30,734	61,951	66,452	69,454

Basic horticultural statistics for the United Kingdom. Calendar and Crop Years 1974-1983 (Ministry of Agriculture, Fisheries and Food, London, 1984).

has recently been revived, particularly for the "pick-your-own" market; yields exceeding 50 tonnes per ha can be achieved in areas south of a line from Bristol to the Wash (Anon, 1982). The bulk of supplies of tomatoes exported to the U.K., excluding those from the Channel Islands, are from the Canaries, Spain and the Netherlands. Supplies from the Canaries and Spain arrive during October to March when home production is minimal whereas almost all supplies from the Netherlands, arrive during our normal season.

The disease

Brown root rot has a widespread distribution occurring on commercial tomato plants in a range of soil types (Last *et al.*, 1966). The disease is common in Europe (Table 2) but infrequently recorded in the United States or North America where, until recently, it seemed to be of little economic importance (Campbell *et al.*, 1982). Brown root rot has not been reported in the tropics (Holliday, 1980). The disease has been recorded on glasshouse heated (Last and Ebben, 1966), unheated (Last and Cole, 1969) and outdoor (Campbell *et al.*, 1982) tomato crops, but is most prominent in heated crops where optimum growth conditions favour BRR attack (Ebben *et al.*, 1978b). Wild tomato species, *Lycopersicon glandulosum* H.B. and K., *Lycopersicon hirsutum* Mill., and *Lycopersicon peruvianum* Mill. reported as resistant to BRR (Termohlen, 1962) can be attacked by the pathogen (Ebben, 1974). However, in these species the disease is restricted to the epidermis and first layer of the cortex parenchyma (Termohlen, 1962). The pathogen

Table 2

Geographical distribution and host range of tomato brown root rot (BRR)

Country	Host		Form of[A] pathogen	Reference
	Botanical Name	Common English Name		
Belgium	*Lycopersicon esculentum*	Tomato	GSF	Motmans (1955)
Bulgaria	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Bakhariev (1975)
Canada	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Jarvis (pers.comm.)
Canary Islands	*Lycopersicon esculentum*	Tomato	BRR	Milan (pers.comm.)
Denmark	*Lycopersicon esculentum*	Tomato	BRR	Gram (1944)
Egypt	*Lycopersicon esculentum*	Tomato	*Pyrenochaeta* sp.	El-Helaly *et al.* (1963)
France	*Cucumis melo* L.*	Melon	*Pyrenochaeta* sp.	Risser and Laugie (1968)
	Lycopersicon esculentum	Tomato	*P.lycopersici*	Clerjeau (1974)
Finland	*Lycopersicon esculentum*	Tomato	GSF	Termohlen (1962)
Germany	*Brassica oleracea* L. var *botyris*	Cauliflower	GSF	Termohlen (1962)
	*Capsicum annum longum L.**	Spanish pepper	GSF	Termohlen (1962)
	*Capsicum frutescens** L.	Paprika	GSF	Termohlen (1962)
	Cichorium endivia L.	Endive	GSF	Termohlen (1962)
	Cucumis sativus L.	Cucumber	GSF	Termohlen (1962)
	Lactuca sativus L.	Lettuce	GSF	Termohlen (1962)
	Lycopersicon esculentum	Tomato	*P.lycopersici*	Schneider and Gerlach (1966)
	Nicotiana glauca R.Grah.*		GSF	Termohlen (1962)
	Physochlaena orientalis C. Koch.*		GSF	Termohlen (1962)
	Solanum aviculare Forst.f.Prod.*		GSF	Termohlen (1962)
	Solanum cornutum Hort. Monsp.ex Dun.*		GSF	Termohlen (1962)
	Solanum dulcamara L.*	Woody nightshade	GSF	Termohlen (1962)
	Solanum mammosum L.		GSF	Termohlen (1962)
	*Solanum melongena**	Aubergine	GSF	Termohlen (1962)
	*Solanum ochrolsucum** Bast.		GSF	Termohlen (1962)
	Solanum villosum Mill.*		GSF	Termohlen (1962)
Greece	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Malathrakis *et al.* (1983)
Ireland	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Staunton and Cormican (1978)
Israel	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Katan (1980)
Italy	*Capsicum annum L.*	Pepper	*P.lycopersici*	D'Ercole and Nipoti (1978)
	Lycopersicon esculentum	Tomato	*P.lycopersici*	Holliday (1980)
	Solanum melongena	Eggplant	*P.lycopersici*	D'Ercole and Nipoti (1978)
Japan	*Lycopersicon esculentum*	Tomato	*P.lycopesici*	Morita *et al.* (1975)
Lebanon	*Capsicum annum*	Pepper	*P.lycopersici*	Davet (1969)
	Cucumis sativus	Cucumber	*P.lycopersici*	Davet (1969)
	Lycopersicon esculentum	Tomato	*P.lycopersici*	Davet (1969)
	Solanum melongena	Aubergine	*P.lycopersici*	Davet (1969)
Netherlands	*Lycopersicon esculentum*	Tomato	GSF	Termohlen (1962)
New Zealand	*Lactuca sativus*	Lettuce	*P.lycopersici*	Last (1969)
	Lycopersicon esculentum	Tomato	*P.lycopersici*	Newhook (pers.comm.)
	Nicotiana tabacum	Tobacco	*P.lycopersici*	Taylor *et al.* (1971)
Norway	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Solberg (pers.comm.)
Poland	*Lycopersicon esculentum*	Tomato	BRR	Pudelski *et al.* (1978)
Rumania	*Capsicum annum*	Pepper	*P.lycopersici*	Stan (1979)
	Cucumis sativus	Cucumber	*P.lycopersici*	Stan (1979)
	Lactuca sativus	Lettuce	*P.lycopersici*	Stan (1979)

Table 2 continued....

Country	Host: Botanical Name	Host: Common English Name	Form of pathogen[A]	Reference
Rumania	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Stan (1979)
(continued)	*Solanum melongena*	Eggplant	*P.lycopersici*	Stan (1979)
Scandinavia	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Holliday (1980)
Sweden	*Lycopersicon esculentum*	Tomato	GSF	Termohlen (1962)
Tasmania	*Lycopersicon esculentum*	Tomato	*P.lycopersici*	Holliday (pers.comm.)
United Kingdom	*Allium cepa* L.	Onion	GSF	Chesters and Hornby (1965)
	Brassica oleracea L.	Cabbage	GSF	Menzies (1973)
	Capsella bursa-pastoris (L.) Medic	Shepherd's purse	GSF	Chesters and Hornby (1965)
	*Capsicum annum**	Capsicum	GSF	Chesters and Hornby (1965)
	*Capsicum frutescens**	Capsicum	GSF	Chesters and Hornby (1965)
	Chamaenerion angustifolium L.	Rosebay willowherb	GSF	Chesters and Hornby (1965)
	Chrysanthemum sp.	Chrysanthemum	GSF	Last and Ebben (1966)
	*Cucumis sativus**	Cucumber	GSF	Chesters and Hornby (1965)
	Cucurbita pepo L.	Marrow	GSF	Chesters and Hornby (1965)
	Lactuca sativus	Lettuce	GSF	Menzies and Colhoun (1973)
	Lepidium sativum L.	Garden cress	GSF	Chesters and Hornby (1965)
	Lycopersicon esculentum	Tomato	*P.lycopersici*	Last *et al.* (1966)
	Nicotiana tabacum	Tobacco	GSF	Chesters and Hornby (1965)
	Phaseolus vulgaris L.	Kidney bean	GSF	Chesters and Hornby (1965)
	Picea sitchensis Trantv. and May.	Sitka spruce	GSF	Last and Ebben (1966)
	Solanum capicastrum Link. ex. Schau.		GSF	Last and Ebben (1966)
	Solanum dulcamara	Woody nightshade	GSF	Chesters and Hornby (1965)
	Solanum nigrum L.	Black nightshade	GSF	Chesters and Hornby (1965)
	Sonchus oleraceus L.	Common sowthistle	GSF	Chesters and Hornby (1965)
	Trifolium hybridum L.	Alsike clover	GSF	Last and Ebben (1966)
	Triticum aestivum L.	Wheat	GSF	Last and Ebben (1966)
	Urtica dioica L.	Stinging nettle	GSF	Chesters and Hornby (1965)
United States	*Amaranthus retroflexus* L.	Pigweed	GSF	Wilhelm *et al.* (1969)
of America	*Chenopodium album* L.	Fat hen	GSF	Wilhelm *et al.* (1969)
	Cucumis melo *	Melon	*P.lycopersici*	Campbell and Moon (1979)
	Capsella bursapastoris	Shepherd's purse	GSF	Wilhelm *et al.* (1969)
	Fragaria grandiflora Crantz.	Strawberry	GSF	Wilhelm *et al.* (1969)
	Lycopersicon esculentum	Tomato	*P.lycopersici*	Campbell *et al.* (1982)
	Pinus pinea L.	Stone pine	GSF	Wilhelm *et al.* (1969)
	Solanum nigrum	Black nightshade	*P.lycopersici*	Campbell *et al.* (1982)
	Solanum nodiflorum Desv. ex. Dunn.		*P.lycopersici*	Campbell and Moon (1979)
	Solanum sarachoides		GSF	Wilhelm *et al.* (1969)
	Solanum tuberosum	Potato	GSF	Wilhelm *et al.* (1969)

[A] BRR = Brown root rot symptoms observed on roots of host plant
GSF = Grey sterile fungus isolated from roots of host plant
P.lycopersici = GSF isolated from host plant and identified as *Pyrenochaeta lycopersici*.

* Host pathogenicity test *in vitro*.

is capable of parasitising other species within the *Solanaceae* and can exist on the roots of a number of non-solanaceous plants (Table 2). Termohlen (1962) isolated the "corky root fungus" in varying amounts from 22 of 37 plant species inoculated with *P. lycopersici*. Davet (1969) isolated *P. lycopersici* from roots of aubergine (*S. melongena*), pepper (*Capsicum annum*, L.), cucumber (*Cucumis sativus*, L.) and courgette (*Cucurbita pepo*, L.) whilst Chesters and Hornby (1965) and Wilhelm *et al.* (1969) isolated a GSF similar to that causing BRR from the roots of common weeds.

The first above ground symptoms of BRR are a marginal leaf chlorosis accompanied by stunted growth and inhibition of leaf formation (Richardson and Berkeley, 1944; Malathrakis *et al.*, 1983). Leaf necrosis may develop causing a premature defoliation of infected plants. Infection can lead to wilting, particularly in warm weather, and is a reflection of the abnormal shoot to root ratio of BRR infected plants (Last and Ebben, 1963). Fruit set may be reduced and, in severe infections, flower shed can cause yield reductions (Termohlen, 1962). Plants with their fifth and sixth trusses in flower are particularly vulnerable to wilting due to the water demand of ripening fruit (de Mos, 1954). Root symptoms of BRR can generally be divided into three categories (Ebben, 1950; Ebben and Williams, 1956). Firstly a cortical rot of the fine roots and medium sized laterals (Plate 1). Secondly a characteristic dark brown furrowed bark (Plate 1) causing "cankers" on large roots (Richardson and Berkeley, 1944). Thirdly a brown cortical rot of the stem base at soil level (Plate 1). Rotting

Plate 1

Plate 1

Tomato root system showing symptoms of brown root rot (BRR) disease.

of the fine roots and laterals occurs within a couple of days of transplanting (Last and Ebben, 1963). Bakhariev (1980b) observed 22% of tomato roots to be infected within 15 days of sowing in soil infested with BRR. Lesions often girdle roots causing cortical shredding and the decay and loss of small fine roots (Last and Ebben, 1966). After approximately three months infection, cork may be visible on mature roots (Last and Ebben, 1966). These corky swollen areas are generally several centimetres in length and frequently girdle the root. The characteristic corky root is thought to be due to a considerable increase in the number of irregular suberized cells in the outer cortex (Ebben and Williams, 1956). These cause the cortex to split and furrow. Fungal hyphae have occasionally been seen in cortical cells but mycelial development is not extensive and usually confined to the outer cortex tissue. The development of cork may be a form of tolerance reaction when large roots are invaded by *P. lycopersici* (Last *et al.*, 1966). However, Mangenot and Diem (1979) consider the formation of cork by *P. lycopersici* to be a means of protecting the pathogen from saprophytes. Few micro-organisms are able to degrade the lignified cells and the corky tissue forms an effective protective envelope. Infection of the stem base is a frequent symptom (Richardson and Berkeley, 1944) which may develop concurrently with corkiness (Last *et al.*, 1968). Stem base lesions, first appearing as dry brown patches at the soil level, develop into a brown-black cortical rot girdling the stem and extending downwards to the upper part of the tap root system (Ebben, 1950). Diseased tissue is

usually confined to the cortex although a discolouration of the stem vascular system is noticeable. This rarely extends more than 20cm above soil level (Jones, 1983).

As disease severity of BRR increases, fruit yields decrease (Last *et al*., 1968; Ebben, 1971a). Yield reductions in excess of 60% have been recorded (Last and Cole, 1969; Bakhariev, 1980a) although losses in the region of 25 to 50% are more frequent (Last *et al*., 1966; Last *et al*., 1968; Ebben, 1974). Last *et al*. (1969) found yield reductions from BRR to increase progressively over five successive tomato crops. Ebben (1971b) showed yields from plants grown in unsterilised soils to be significantly reduced below those from sterilised plots by the fourth year of cropping. Yield reductions due to BRR were 14% in the fourth and 25% in the eighth crop.

Soil-borne inoculum of *P. lycopersici* can build up in sites cropped successively with tomatoes. Last *et al*. (1969), studying BRR levels over five seasons, showed the incidence of BRR to increase progressively to a maximum in the fifth season. Infected root tissue was considered to be an important source of the soil inoculum. Ebben (1971b) measured disease incidence of BRR on successive tomato crops grown on unsterilised soil for nine years. Disease levels reached a maximum in the sixth year; the decrease in root growth in soils severely infested with BRR, resulting in the production of less inoculum, was considered to be the cause of this levelling off in disease incidence.

The pathogen

The etiology of BRR was slow to be resolved due to the complex of micro-organisms associated with diseased tomato roots (Ebben and Williams, 1952, 1956). Reproduction of BRR symptoms whether by variation in cultural conditions or inoculation with known pathogens had little success in establishing the identity of the primary parasite (Williams, 1929; Ebben, 1950). Viruses, bacteria, fungi and soil fauna were suggested as the cause of BRR.

Thung (1953) suggested that corky root rot may be caused by a virus of the tobacco necrosis type, possibly after root wounding by nematodes. Termohlen (1962) frequently isolated tomato mosaic virus and occasionally tobacco necrosis virus from BRR infected tomato roots. Corky root symptoms could not be induced by inoculation of tomato roots with either virus. Application of the nematicide DD had no effect on corky root.

Experiments with bacteria were also negative (Klinkenberg, 1940). Williams (1929) isolated various bacteria from tomato roots showing both cortical root and corky scab. Stem inoculations with the bacteria caused slight lesions and a pith rot. Bacterial root drenches caused browning of the small laterals but the larger roots remained healthy.

It was assumed that several fungi were involved in the BRR disease (Ebben, 1950). Ebben and Williams (1952, 1956) isolated various fungi including *Colletotrichum atramentarium*, (Berk & Br.) Taubenh, *Volutella ciliata* Alb and Schwein. ex.

Fr., *Cephalosporium* spp., *Chaetomium* spp., *Fusarium* spp., *Pythium* spp., *Trichoderma* spp. and *Phytophthora* spp. from tomato roots showing BRR symptoms. Williams (1929) isolated several micro-organisms including a GSF from BRR infected tomato roots. Richardson and Berkeley (1944) and Termohlen (1962) demonstrated the pathogenicity of GSF isolates causing both BRR and corkiness on inoculated root systems. Morphologically similar GSF have been consistently reported as primary pathogens of BRR (Ebben and Williams, 1956; Last and Cole, 1969; Davet, 1973) and this fungus is now considered to be the most important fungal pathogen in the brown root rot complex (Termohlen, 1962; Last *et al.*, 1968).

Colletotrichum coccodes syn. *C.atramentarium*, frequently isolated from corky roots, was originally considered to be a primary pathogen in the brown root rot complex (Last and Ebben, 1963). The fungus causes black dot of tomato, so named because of the presence of micro-sclerotia at the stem base and on old roots (Plate 2). The disease rarely appears before 12 weeks growth (Last *et al.*, 1966), cannot be associated with any yield reductions (Last *et al.*, 1968) and is now considered to be a secondary pathogen (Last and Ebben, 1966; Hornby, 1968). Locally *Phytophthora* spp., *Fusarium* spp., *Cylindrocarpon radicicola* Wollenw. and *Thielaviopsis basicola* (Berk. and Br.) Ferraris can be important components of the brown root rot complex and may in certain soil types aggrevate the damage produced by GSF. *Calyptella campanula*, a recently recognised pathogen of tomatoes, has been shown to produce symptoms similar to GSF which may be confused with those of BRR (Clark

Plate 2

Plate 2

Part of a tomato root system showing the micro-sclerotia of Colletotrichum coccodes

et al., 1983).

Gerlach and Schneider (1964) established the taxonomic position of GSF to be *Pyrenochaeta* sp.. From pycnidial and biological characteristics they considered the corky root fungus to be morphologically distinct from *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker and Larson, pink root of onions (Gerlach and Schneider, 1966; Schneider and Gerlach, 1966). Since the fungus did not correspond to any other *Pyrenochaeta* species they described the tomato pathogen as a new species, *Pyrenochaeta lycopersici*. The genus *Pyrenochaeta* is a member of the suborder *Phialopycnidiineae* within the Blastodeuteromycete classification of the Deuteromycotina (Sutton, 1980) (Figure 2).

Disease control

Brown root rot can be controlled by efficient soil sterilisation using steam, gaseous or granular sterilants and/or solar radiation (solarisation) (Table 3). Commercially, soil partial sterilisation using steam was frequently used to reduce levels of BRR infestation (Termohlen, 1962; Ebben, 1974). Steam sterilisation reduced the incidence of BRR and gave higher yields than applications of methyl bromide (Last *et al.*, 1966), formalin or chloropicrin (Last and Ebben, 1963). However, steam sterilisation is more expensive than methyl bromide sterilisation: steam £5000/ha, methyl bromide £2500-3500/ha (Criddle, 1984, pers.comm.) and alternative cheaper soil sterilants are now used. Chloropicrin and methyl bromide

Figure 2

Suprageneric classification of the coelomycetes

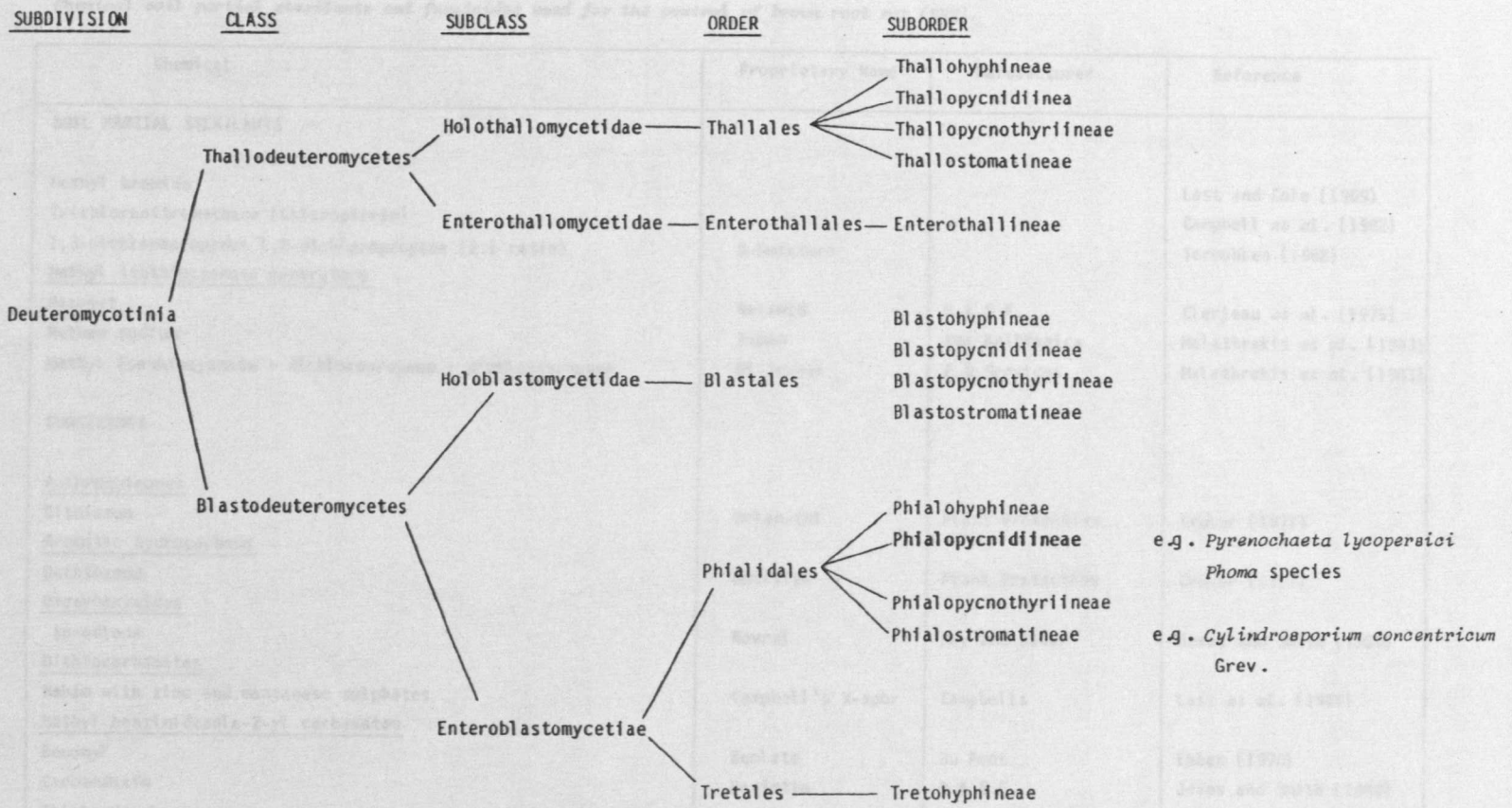


Table 3

Chemical soil partial sterilants and fungicides used for the control of brown root rot (BRR).

Chemical	Proprietary Name	Manufacturer	Reference
SOIL PARTIAL STERILANTS			
Methyl bromide			Last and Cole (1969)
Trichloronitromethane (Chloropicrin)			Campbell *et al.* (1982)
1,3-dichloropropene+ 1,2-dichloropropane (2:1 ratio)	D-Dmixture		Termohlen (1962)
Methyl isothiocyanate generators			
Dazomet	Basamid	B.A.S.F.	Clerjeau *et al.* (1975)
Metham sodium	Vapam	Pan Brittanica	Malathrakis *et al.* (1983)
Methyl isothiocyanate + dichloropropene + dichloropropane	Di-Trapex	P W Services	Malathrakis *et al.* (1983)
FUNGICIDES			
Anthraquinomes			
Dithianon	Delan-Col	Plant Protection	Crüger (1971)
Aromatic hydrocarbons			
Quintozene	Botrilex	Plant Protection	Crüger (1971)
Dicarboximides			
Iprodione	Rovral	May and Baker	Jeves and Smith (1980)
Dithiocarbamates			
Nabam with zinc and manganese sulphates	Campbell's X-spor	Campbells	Last *et al.* (1966)
Methyl benzimidazole-2-yl carbamates			
Benomyl	Benlate	Du Pont	Ebben (1970)
Carbendazim	Bavistin	B.A.S.F.	Jeves and Smith (1980)
Thiabendazole	Tecto 60	Mark, Sharpe & Dohme	Jeves and Smith (1980)
Thiabendazole/iodophor	Byatran	Wheatley Chemicals	Jeves and Smith (1980)
Thiophanate-methyl	Mildothane liquid	Mark, Sharpe & Dohme	Jeves and Smith (1980)
Phthalimides			
Captan	Orthocide concentrate	Murphy	Termohlen (1962)
Sulphamides			
Dichlofluanid	Elvaron	Bayer	Crüger (1971)

are commercially important true soil fumigants (Goring, 1962) which have been frequently used to reduce BRR levels. Last and Cole (1969) found little difference in efficiency between chloropicrin and methyl bromide for control of BRR. A combination of chloropicrin and methyl bromide gave greater disease control and higher yields than individual applications of the two fumigants (Campbell *et al.*, 1982). However, the use of chloropicrin has declined and methyl bromide application is limited by the danger of bromide residues in subsequent crops (Ebben *et al.*, 1978a). Dazomet and metham sodium (methyl isothiocyanate generators) are chemical partial soil sterilants currently approved for the control of BRR (Anon., 1983). However, their use is often commercially unacceptable because of the long treatment times necessary. Malathrakis *et al.* (1983) found dazomet and metham sodium to be equally effective against BRR giving comparable disease control to methyl bromide applications. However, Clerjeau *et al.* (1975) found dazomet to be less effective than methyl bromide in controlling BRR. Solar radiation has been used in warmer climates to partially sterilise soils infested with BRR (Katan, 1980). In Crete, soil solarisation was found to be equally effective as methyl bromide, metham sodium, dazomet and methyl isothiocyanate mixtures for the control of BRR (Malathrakis *et al.*, 1983). Under such conditions, soil solarisation during July to September could be an effective control against BRR. In Greece, soil solarisation was unsuccessful in controlling the disease, possibly due to low soil temperatures (Tjamos and Faridis, 1980).

Fungicide applications (Table 3) have generally been less effective than soil partial sterilisation for the control of BRR. Zineb is currently approved for the control of BRR. Termohlen (1962) found captan and zineb to be the most effective of six fungicides tested to reduce BRR levels throughout the season. Last *et al.*, (1966) recorded yield increases of 15% when nabam, a zineb/maneb tank mix, was applied to tomatoes grown in BRR infested soil. However, yields were significantly less than those obtained using steam or chloropicrin. Jeves and Smith (1980) found MBC generating fungicides to be active against *P. lycopersici in vitro* but less effective *in vivo*. Crüger (1971) found dithianon and dichlofluanid to have a high activity against BRR. However, probably more than one fungicide application per season would be required to control BRR and the use of fungicide drenches should not be considered as a substitute for soil partial sterilisation (Ebben, 1970).

Resistance to BRR has been found in various wild *Lycopersicon* species (Ebben, 1974). Termohlen (1962) found a high degree of tolerance to corky root in *L. peruvianum*, *L. glandulosum* and *L. hirsutum*. *Lycopersicon piminellifolium* Mill. was as susceptible as the cultivated tomato. Resistant wild species have been crossed with *L. esculentum* to produce F_1 hybrids tolerant of BRR infection.

The cultivars Piranto and Vicores are now grown on soils infested with BRR. These varieties are considered commercially resistant, but are physiologically tolerant and may give lower

yields than susceptible cultivars when grown in sterilised soil or in soils where the incidence of BRR is low (Ebben *et al.*, 1978b). However, when soil inoculum levels of BRR are very high disease tolerant cultivars may be infected and yields reduced; lesions are restricted and root and yield losses will be less than those of non tolerant plants grown under similar conditions.

Yield losses associated with BRR susceptible commercial tomato varieties can be minimized by grafting onto BRR resistant rootstocks derived from *L. hirsutum* (Bravenboer and Pet, 1962). Last *et al.* (1966) showed ungrafted plants to yield less than grafted plants when grown in BRR infested soil. However, increased yields from grafted plants are often offset by later cropping and the smaller proportion of high quality fruit (Last *et al.*, 1968). Resistant rootstocks, KVF, KNVF, KNVF-TMV, Identistock KVF and Hires - (Signaal) are resistant to *P. lycopersici* and are recommended for growth on BRR infested soils (Anon., 1980).

Last and Ebben (1966) tested the effects of cultural treatments on the incidence of BRR. Levels of BRR near the stem bases were significantly decreased by increasing the size of the propagating pot and the amount of compost around tomato seedlings. The added protection was sufficient to increase early yields and reduce BRR infection. Last *et al.* (1968) confirmed results demonstrating that increasing the volume of sterilised potting compost resulted in increased healthy roots. Termohlen (1962) used various cultural practices to

encourage the formation of new roots and limit the effect of BRR attack. Frequent applications of small quantities of water, earthing up at the stem base and covering the soil with a layer of organic material enabled plants to form new roots and minimised yield losses.

CHAPTER TWO. METHODS

SECTION 1. *GENERAL METHODS*

1.1 Preparation of media

(a) Agar media

Potato dextrose agar (PDA, Difco) and oatmeal agar (Difco) were prepared as per manufacturers instructions.

Tap water agar (TWA), V8-Juice agar (V8A, Campbells V8-Juice) and potato carrot agar were prepared, using Bacto-agar (Difco) according to Johnston and Booth (1983).

Kings B medium was prepared containing 20g proteose peptone, 15g Bacto-agar, 10g glycerol, 1.5g anhydrous potassium hydrogen phosphate and 1.5g magnesium sulphate per litre tap water (TW) (Robinson, pers. comm.).

Tomato juice agar (Campbells Tomato Juice) was prepared as per V8A substituting V8-Juice with tomato juice.

Beetroot, carrot, celery, lettuce, parsley, spinach, tomato and watercress agars were prepared at two concentrations: 100g of each vegetable were blended with 400ml TW, simmered for 30 minutes and strained through two layers of muslin. Ten grammes of Bacto-agar were added to 100ml (for the dilute medium) and 350ml (for the concentrated medium) of each strained vegetable extract and made up to 500ml with TW.

Home-made V8A was prepared at two concentrations: 10g Bacto-

agar added to a mixed vegetable extract prepared from 12.5ml (dilute) or 50ml (concentrated) of each strained vegetable extract and made up to 500ml with TW.

Home-made V8A, without parsley, was prepared from the remaining seven vegetables at the concentrated rate.

V8-Juice agar, amended with calcium carbonate, was prepared according to McGrath and Campbell (1983).

Stock suspensions of fungicides (excluding PCNB) and antibiotics, for incorporation into agar, were prepared in sterile distilled water (SDW). Suspensions of PCNB were prepared in absolute alcohol. Stock suspensions were added to sterilised PDA, cooled to 50°C.

Antibiotic medium was prepared containing 100µg ml^{-1} penicillin, 100µg ml^{-1} streptomycin and 100µg ml^{-1} tetracycline (PST), (Table 8).

The semi-selective medium, PSTBay, was prepared containing PST and 100µg ml^{-1} Bayleton (Table 7).

Agars, sterilised by autoclaving for 15 minutes at 1.055 Kg cm^{-2} (15lb in^{-2}), were cooled to 50°C and poured into sterile plastic Petri dishes (nine centimetre diameter, 16 ± 1ml per dish).

(b) Solid media

Perlite/maize meal: 15g perlite (Silvaperl, horticultural grade) mixed with 8g maize meal and 40ml TW (200ml

perlite/maize meal per 250ml flask).

Wheatbran: 1500ml (by volume) coarse wheatbran mixed with 400ml TW(1000ml wheatbran per 2000ml flask).

Solid media, sterilised by autoclaving for 15 minutes at 1.055 Kg cm^{-2}, were cooled and shaken before inoculation: 5 x 0.7cm mycelial plugs per flask of perlite/maize meal, 25 x 0.7cm mycelial plugs per flask of wheatbran.

(c) Liquid media

Malt extract broth (Oxoid) prepared at a concentration of 2% as per manufacturers instructions and TW were sterilised by autoclaving for 15 minutes at 1.055 Kg cm^{-2}. Sterilised malt extract (SME) and SDW were stored at room temperature.

(d) Soil media

(i) Sterilised compost: John Innes compost No. 2 (Gem, Joseph Metcalfe Ltd., Accrington) was sterilised by autoclaving at 1.055 Kg cm^{-2} for one hour. The sterilising procedure was repeated twice, on consecutive days. When cool, the sterilised John Innes compost No. 2 (sterilised compost) was placed in trays exposed to the laboratory atmosphere to allow possible volatile products to disperse and left overnight before use.

(ii) Soil naturally infested with brown root rot: Soil, naturally infested with BRR, used throughout

this study was collected from a commercial glasshouse site (Mr. Pickovant, Homefield Nurseries, Moss Lane, Burscough, Lancashire). A tomato/lettuce cropping system was used in the unheated glasshouse and the soil sterilised with methyl bromide, biannually. Soil was collected when necessary and was stored for short periods at room temperature in the dark.

(iii) Garden soil: The garden soil used throughout this study had previously been shown to have low levels of BRR. The soil was collected from the garden of Dr. J.M. Thomas, 49 The Park, Huyton, Merseyside. There was no history of tomato cropping in this soil.

(iv) Glasshouse Crops Research Institute (G.C.R.I.) propogation compost: Peat/grit (3:1 ratio peat to washed grit) with a base dressing of 85g magnesium limestone, 56g super phosphate, 42g ground lime chalk, 28g potassium nitrate, 15g trace element frit WM255 and 15g ammonium nitrate per 36l compost. pH = 6.0 to 6.2.

(v) Glasshouse Crops Research Institute plot compost: Loam/peat/grit/sand (1.75:5.25:1.5:1.5 ratio of loam to peat to grit to sand) with a base dressing of 2551g ground lime chalk, 680g base compound fertiliser (John Innes), 595g super phosphate, 595g magnesium limestone, 170g

potassium nitrate, 85g trace element frit WM255 and 85g ammonium nitrate per 324l compost.

(vi) Glasshouse soil (G.C.R.I. experiment 1982 treatment 4): Soil taken from G.C.R.I. glasshouse RA2 North 1. Base dressing of 453g potassium sulphate, 170g Epsom salts, 170g ground lime chalk, 128g triple super phosphate and 15g Nitroform applied per $0.84m^2$.

(vii) Field soil (G.C.R.I. experiment 1982, treatment 4): Soil taken from G.C.R.I. gardens. There was no history of tomato cropping in this soil.

The pH of soils was measured using a Chem-Mate™ pH meter (Beckman Instruments Ltd., California). The soil was mixed with SDW to form a thick slurry and the pH recorded after five minutes incubation.

1.2 *Maintenance and culture of isolates*

The origin of GSF and *P. lycopersici* isolates, used in this study, and their characteristics are given in Table 4. The origin of *C. coccodes* and *C. campanula* isolates are presented in Table 5 and those of potential antagonists in Table 13.

Stock cultures of fungi used in this study were maintained on potato carrot agar, bacteria on Kings B and *Streptomyces* species on oatmeal agar slopes at 10°C and sub-cultured once a year. When required for experiments, micro-organisms were sub-cultured onto PDA (fungi), Kings B (bacteria) or oatmeal agar

Table 4

Characteristics table for isolates of grey sterile fungi used in this study.

Isolate Number	Origin	Date isolated	Type species	Growth* rate (mm per day)	Pycnidial* size (µm)	Conidial* size (µm)	Pathogenicity		Colour on PDA at 26°C [A]
							In vitro Mean lesion length (cm)	*In vivo* Mean No. lesions/ Root	
AH1	Corky roots, Hesketh Bank	July 1984	-	2.66	-	-	-	-	Pale Olivaceous Grey
AH2	Tomato roots with BRR, Hesketh Bank	August 1984	-	1.68	-	-	-	-	Smoke Grey
AH3	Corky roots, Hesketh Bank	July 1984	-	1.72	-	-	-	-	Olivaceous Grey
AH4	Corky roots, Sandridge	June 1984	-	2.98	-	-	-	-	Olivaceous Grey
AH5	Corky roots, Cotton End	June 1984	-	3.57	-	-	-	-	Pale Olivaceous Grey
AH15	Tomato seedlings with brown lesions, Lancashire	17.7.81	-	3.42	-	-	0.61	22.2	Leaden Grey
AH24	Tomato seedlings, Peaty soil, Lancashire	3.10.81	-	2.53	333.8	5.16 x 1.72	0.91	-	Pale Olivaceous Grey
AH30	Tomato seedlings with brown lesions, Lancashire	3.10.81	-	2.67	455.0	5.16 x 1.72	1.02	14.4	Pale Olivaceous Grey
AH32	Corky roots in peaty soil, Lancashire	3.10.81	-	2.61	277.7	5.16 x 1.72	1.89	64.8	Olivaceous Grey
AH42	BRR_2 (G.C.R.I.)	4.10.81	-	2.62	473.67	6.31 x 1.72	0.65	18.8	Grey Olivaceous
AH56	R. Polley, Bedfordshire	18.6.81	-	2.92	326.7	4.78 x 1.72	2.20	64.0	Smoke Grey
AH57	R. Polley, Hertfordshire	18.6.81	-	2.67	-	-	-	-	Pale Mouse Grey
AH61	Tomato roots in peat block, Cheshire	3.11.81	-	3.04	-	-	-	-	Pale Olivaceous Grey
AH62	Tomato roots in peat block, Cheshire	3.11.81	-	2.52	329.0	5.54 x 1.72	-	-	Olivaceous Grey
AH64	Berlin	?	Schneider & Gerlach 62931	3.52	-	-	-	-	Pale Olivaceous Grey
AH65	Berlin	?	Schneider & Gerlach 62930	3.02	-	-	1.98	67.4	(Dark) Smoke Grey
AH89	Tomato roots with brown root rot, Yorkshire	4.1.82	-	2.89	315.0	5.16 x 1.72	-	-	Greenish White
AH97	Tomato seedlings in peaty soil, Lancashire	4.1.82	-	2.67	336.0	5.16 x 1.72	1.73	48.8	Olivaceous Grey
AH99	Tomato roots in peaty soil, Lancashire	7.1.82	-	2.70	347.7	5.16 x 1.72	1.85	36.0	Greenish White
AH100	Tomato seedlings in peaty soil, Lancashire	4.1.82	-	2.72	340.7	4.97 x 1.72	1.33	46.0	Olivaceous Grey
AH125	Jeves & Smith (1980)	March 1980	-	3.68	-	-	0.64	-	Smoke Grey
AH126	Jeves & Smith (1980)	March 1980	-	3.63	-	-	0.61	29.6	Smoke Grey
AH127	Jeves & Smith (1980)	March 1980	-	3.02	303.3	5.16 x 1.72	1.79	46.2	(Dark) Smoke Grey
AH128	Jeves & Smith (1980)	March 1980	-	2.90	331.3	5.16 x 1.72	1.65	59.0	Grey Olivaceous

*Measured on V8A at 22°C

[A] Rayner (1970)

Table 5

Origin of **Colletotrichum coccodes** *and* **Calyptella campanula** *isolates used in this study.*

Isolate	Origin	Date isolated
C. coccodes		
C.c.1	Tomato root lesion, Ball (Hesketh Bank)	July 1984
C.c.2	Tomato root lesion, Ball (Hesketh Bank)	August 1984
C.c.3	Corky root lesion, Howarth (Hesketh Bank)	July 1984
C.c.4	Corky root lesion, Sandridge	June 1984
C.c.5	Corky root lesion, Cotton End	June 1984
C.c.6	Tomato root lesion, Sandridge	June 1984
C. campanula		
Cp.1	Tomato stem base lesion, D. Rose (Hesketh Bank)	5.7.83
Cp.2	Tomato root , Stockbridge House Experimental Horticultural Station	1982
Cp.3	Tomato root lesion , D. Rose (Hesketh Bank)	5.7.83
Cp.4	Tomato root lesion , D. Rose (Hesketh Bank)	5.7.83
Cp.5	Tomato root lesion , D. Rose (Hesketh Bank)	5.7.83
Cp.6	Tomato root , Stockbridge House Experimental Horticultural Station	1982
Cp.7	Tomato root lesion , D. Rose (Hesketh Bank)	5.7.83

(Streptomycetes) plates and incubated at 22°C with a standard 16h photoperiod (Section 1.3). Agar plate inoculations were made using 0.7cm mycelial plugs taken from the leading edge of cultures actively growing on agar plates.

1.3 *Irradiation*

A standard 16h photoperiod was produced from cool white fluorescent lamps (Philips TL8W/35), a 16h black light photoperiod from near U.V. lamps (Philips TL8ON) and a 16h black/daylight photoperiod from a combination of lamps (2 x Philips TL8ON + 1 x Philips TL8W/35). All lamps were installed in cooled incubators, six lamps per incubator. Black light conditions at room temperature were provided from two near U.V. lamps (Philips TL20W/08) positioned 27.0cm from the subject.

Irradiation for light frames was produced from a combination of fluorescent lamps; warm white (Osram liteguard), daylight (Thorn) and grow lux (Thorn). The irradiation wavelength range for all light regimes (Table 6) was measured using a spectroradiometer (MSE Fisons, Model SR).

1.4 *Preparation of conidial suspensions of* **Pyrenochaeta lycopersici** *and suspensions of potential antagonists*

Conidia were harvested from pycnidia found in zone 3 (Figure 3) of *P. lycopersici* cultures growing on V8A, 21 to 40 days after inoculation. The age of conidia was expressed in terms of culture age, e.g. (AH24: 21 day) indicates conidia taken from isolate AH24 inoculated onto V8A 21 days earlier. Pycnidia

Table 6

Spectral intensity emitted from various light sources

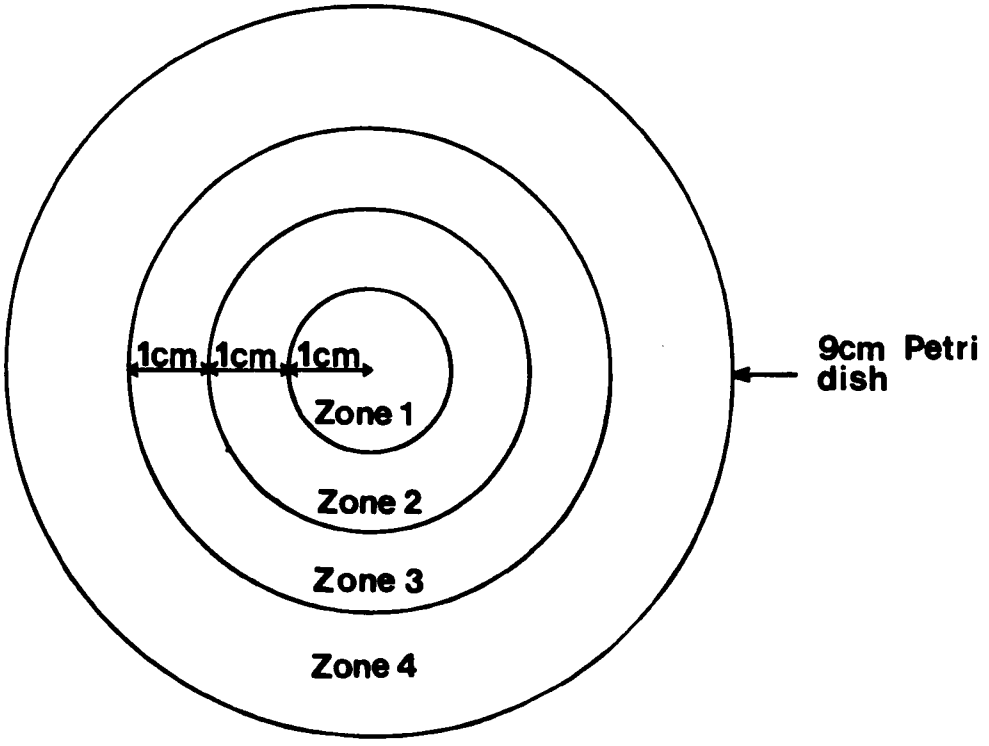
Wavelength (nm)	Spectral Intensity ($\mu w\ cm^{-2}\ nm^{-1}$)			
	Cool white* light	Black* light	Black* light/cool white light	Warm white/daylight[A] /grow lux
380	0.000	0.560	0.500	2.000
400	0.380	0.270	0.200	3.090
425	0.587	0.062	0.124	4.672
450	1.343	0.000	0.107	4.435
475	1.152	0.000	0.115	3.696
500	1.160	0.000	0.106	3.659
525	1.351	0.000	0.113	12.464
550	3.952	0.000	0.365	11.772
575	4.251	0.000	0.392	10.350
600	3.542	0.000	0.391	10.235
625	2.225	0.000	0.245	13.485
650	1.262	0.000	0.087	7.260
675	0.528	0.000	0.044	1.997
700	0.317	0.000	0.049	0.955
725	0.181	0.000	0.052	0.679
750	0.198	0.000	0.057	0.498

* present in illuminated cooled incubator (Gallenkamp), six lamps per incubator.

A combination from light frames

Figure 3

Colony regions used for the assessment of pycnidial and sclerotial production in* Pyrenochaeta lycopersici *isolates.



were picked off individually using a sterile needle and crushed in a sterile glass tube homogeniser containing SDW or SME. Conidial suspensions were filtered though one layer of muslin. When required, conidial suspensions were washed by centrifuging at mark 3 (MSE bench centrifuge) for five minutes and the supernatant poured off and replaced with fresh SDW or SME.

Preparations of potential antagonists were made by scraping inoculated plates, flooded with SDW (100ml SDW per plate) and filtering the resultant suspension through two layers of muslin. Three drops of Tween 80 were added, with the SDW, to fungal and actinomycete cultures before agitation.

1.5 *Preparation of the optical brightener, Calcofluor White M2R*

The diamino stilbene brightener, Calcofluor White M2R (70% active ingredient, American Cyanamid Company) was used to study the fate of conidia in soil. It is a disodium salt of 4, 4'-bis(4-anilino-6-diethylanino-s-triazin-2-ylamino)-2,2'-stilbene-disulphonic acid. An aqueous stock solution of Calcofluor White, 10000µg active ingredient per ml, prepared in 20% aqueous glycerol, was sterilised by Millipore membrane filtration (0.45µm pore size) and stored at 5°C until required.

1.6 *Germination of tomato seed*

Seeds from the tomato variety Moneymaker (Harrison's reselected), (Asmer Seeds Ltd.) were used throughout this

study. Tomato seeds, surface sterilised for three minutes in 7% sodium hypochlorite solution (10-14% w/v available chlorine, BDH Chemicals Ltd.) were washed in three changes of SDW and placed on moist filter paper in sterile plastic Petri dishes (approximately 100 seed per dish). Dishes, incubated at 22°C with a standard 16h photoperiod were checked regularly and any contaminated seed removed. Seeds usually germinated within three to five days.

1.7 *Assessment of the inoculum potential of soils naturally infested with brown root rot (BRR): The BRR assay*

Germinated tomato seeds were sown in a layer of perlite, overlying approximately 140ml (by volume) BRR infested soil, contained in a disposable plastic cup (7cm diameter). Cups were placed at ambient room temperature (15-25°C), under a light frame with a 16h photoperiod. Plants were harvested after three to four weeks growth, the root systems thoroughly washed in TW and examined under a dissecting microscope. Root lesions, visible as light brown flecks up to 3mm in length, were counted and expressed as number of lesions per tomato root. Seed quantity, replication and the period of tomato growth may vary.

1.8 *Statistical analysis*

Results were analysed using standard methods. Where applicable results expressed as percentages were transformed (arc-sine) and the standard error of the differences between the means

(SED) calculated for the transformed data. Graphs of conidial germination tests (expressed as percentages) are presented as untransformed data with SED values for the transformed data displayed.

SECTION 2. *BIOLOGY OF* PYRENOCHAETA LYCOPERSICI

2.1 *Isolation of* Pyrenochaeta lycopersici

(a) Development of a semi-selective medium

Potato dextrose agar, amended with fungicides (Table 7) and/or antibiotics (Table 8) at various concentrations (Section 1.1) was inoculated with a 0.5cm mycelial plug of *P. lycopersici* or *C. coccodes* (three replicate plates per fungus per treatment). A control unamended PDA treatment was included for both fungi. Plates were incubated at 26°C with a 16h standard photoperiod and colony diameter recorded three, seven and 14 days after inoculation. The percentage inhibition of both fungi, compared to the controls (controls expressed as 0% inhibition), at each chemical concentration was calculated from the mean mycelial spread (mm per day, 14 days after inoculation). For each chemical concentration the *P. lycopersici* "selectivity value" was calculated:

Percentage inhibition value of *C. coccodes* − Percentage inhibition value of *P. lycopersici* = *P. lycopersici* selectivity value

Chemicals with high positive selectivity values (*P. lycopersici*

Table 7

Fungicides tested for effects on the linear spread of Pyrenochaeta lycopersici *and* Colletotrichum coccodes

Proprietary name	Active ingredient	Chemical group	Concentration and formulation of active ingredient	Manufacturer
Aaterra	Etridiazole	Thiadiazole	35% w.p.	Duphor-Midox Ltd.
Afugan	Pyrazophos	Organophosphate	30% lq.	Hoechst
Aliette	Fosetyl aluminium	-	80% w.p.	May & Baker Ltd.
Bavistin	Carbendazim	MBC	50% w.p.	BASF
Bayleton	Triadimefon	Ergosterol biosynthesis inhibitor (triazole)	25% w.p.	Bayer
Benlate	Benomyl	MBC	50% w.p.	Du Pont
Daconil 2787-W -75	Chlorothalonil	Phthalonitrile	75% w.p.	Duphor-Midox Ltd.
Dithane Wettable	Zineb	Dithiocarbamate	70% w.p.	Pan Brittanica
Nabam	Sodium salt of nabam	Dithiocarbamate	32% lq.	-
Orthocide Concentrate	Captan	Phthalimide	75% w.p.	Murphy
	Pentachloronitrobenzene (PCNB)	Aromatic Hydrocarbon	100% c.	Analar
Ridomil	Metalaxyl	Acylalanine	25% w.p.	May & Baker Ltd.
Ronilan	Vinclozolin	Dicarboximide	50% w.p.	BASF
Rovral	Iprodione	Dicarboximide	50% w.p.	May & Baker Ltd.
Sistan	Metham-sodium	Dithiocarbamate	38% lq.	Universal Crop Protection
Storite	Thiabendazole	MBC	60% lq.	Mark Sharp & Dohme Ltd.
Tecto 60	Thiabendazole	MBC	60% w.p.	Mark Sharp & Dohme Ltd.

MBC = Methyl benzimidazol-2-yl carbamate

w.p. = wettable powder

lq. = liquid

c. = crystal

Table 8

***Antibiotics tested for effects on the linear spread of* Pyrenochaeta lycopersici *and* Colletotrichum coccodes**

Name	Manufacturer
Ampicillin anhydrous	Sigma Chemical Company
Chloramphenicol	BDH Chemicals Ltd.
Nystatin	BDH Chemicals Ltd.
Penicillin (Benzyl-penicillin)	BDH Chemicals Ltd.
Pimafucin (25mg Natamycin per ml)	Brocades Great Britain Ltd.
Streptomycin sulphate	BDH Chemicals Ltd.
Tetracycline anhydrous	Sigma Chemical Company
Vancomycin (vacocin HCl)	Lilly

growth advantage) were selected for further examination at the effective concentrations.

(b) Evaluation of the semi-selective medium

Potato dextrose agar, PST and PSTBay media (Section 1.1) were inoculated with 0.5cm mycelial plugs of *P. lycopersici*, *C. coccodes* and *C. campanula* (five replicates per isolate per medium). Plates were incubated at 26°C with a 16h standard photoperiod and colony diameter recorded three, seven and 14 days after inoculation. The mean mycelial spread (mm per day) was calculated for each isolate on each medium.

(c) Isolation of grey sterile fungi from root systems showing brown root rot (BRR) symptoms

Isolations were made from brown and/or corky root lesions on tomatoes grown in soils, infested with BRR, for four weeks (in the laboratory) and eight weeks (after transplanting into the glasshouse).

Root sections possessing lesions were dissected from root systems (30 sections per lesion type) and surface sterilised for five minutes in a 7% solution of sodium hypochlorite (BDH Chemicals Ltd., 10-14% w/v available chlorine). Root pieces were washed in three changes of SDW, blotted dry on sterile filter paper and placed on PDA, PST and/or PSTBay agar plates (ten root sections per lesion type per medium). Plates were incubated at 26°C, with a 16h standard photoperiod and the species of fungi and their frequency of isolation recorded.

2.2 *Mycelial pathogenicity of grey sterile fungi*

(a) *In vitro*

Germinated tomato seeds (Section 1.6), grown until the radicles were two to three centimetres in length, were transferred to sterile plastic Petri dishes (nine cm diameter, five seeds per dish) containing two moist sterile filter papers. Each seedling was inoculated with a 0.5cm mycelial plug of *P. lycopersici*, uncolonised agar plugs were used as controls. Plugs were placed one centimetre behind the radicle tip (ten seedlings per isolate). Dishes were incubated at 22°C with a standard 16h photoperiod and lesion length recorded after 14 days. Care was taken to ensure filter papers were kept moist. Isolations were made from lesions (Section 2.1) to check the identity of the causal pathogen.

(b) *In vivo*

Cultures of *P. lycopersici*, grown on perlite/maize meal medium (Section 1.1) were incubated at 26°C with a standard photoperiod for six weeks. Inoculum of each isolate (200ml) or uninoculated perlite/maize meal (control) was mixed with 1800ml sterilised compost (Section 1.1). The inoculum potential of infested soils was determined using the BRR assay (Section 1.7): three seed sown, subsequently reduced to one per pot, ten pots per isolate. After four weeks growth the number of lesions per tomato root (five replicates) was recorded. Isolations were made from lesions on the remaining replicates of each isolate (Section 2.1) to check the identity of the

causal pathogen.

2.3 *Sporulation and identification of grey sterile fungal isolates*

(a) Induction of pycnidial production

Nineteen GSF isolates were inoculated onto V8A (Section 1.1) and incubated at various temperatures with a standard 16h photoperiod. An additional treatment of ambient temperature with a 16h black light photoperiod was tested. Each isolate had three replicates per treatment. Indexes were developed for the assessment of pycnidial and sclerotial numbers on agar plates (Table 9). Pycnidial and sclerotial counts were made, by eye, from the top of *P. lycopersici* colonies, within each of four zones per plate (Figure 3). A pycnidial and sclerotial rating for each isolate, per agar plate, was calculated by accumulating the production indexes in each zone. Colony diameter, pycnidial and sclerotial production (assessed using the arbitrary indexes) were recorded weekly.

(b) Effect of light on pycnidial production

Two *P. lycopersici* isolates (AH24 and 65) were inoculated onto V8A and incubated at 22°C and/or ambient room temperature under various light conditions. Each isolate had five replicate plates per treatment and plates were incubated in sealed polyethylene bags. Cultures for dark treatments were wrapped in aluminium foil immediately after inoculation. Colony diameter and pycnidial production, assessed using the arbitrary index (Table 9), were recorded weekly.

Table 9

Arbitrary indexes for pycnidial and sclerotial production in **Pyrenochaeta lycopersici** *isolates grown on agar*

Index	Number of pycnidia per zone	Number of sclerotia per zone
0	None	None
1	1-50	Sparse
2	51-100	Moderate
3	101-200	Many
4	201-300	Abundant
5	>300	-

(c) Effect of the vegetable constituents of V8-Juice on pycnidial production

Three *P. lycopersici* isolates (AH 24, 65 and 128) were inoculated onto V8A, PDA, TWA, home-made V8A, home-made V8A without parsley, tomato juice agar, V8A prepared according to McGrath and Campbell (1983) and media prepared from the vegetable constituents of V8-Juice (Section 1.1). Each isolate had five replicates per treatment. Plates were incubated in sealed polyethylene bags at 22°C with a 16h black light photoperiod. Colony diameter and pycnidial production, using an arbitrary index (Table 9), were recorded weekly.

2.4 *Germination of* Pyrenochaeta lycopersici *conidia* in vitro

In conidial germination tests, drops of conidial suspensions (10^7 conidia per ml) were placed on sterile cavity slides, supported on glass U-bends and incubated in sterile glass Petri dishes containing moist, sterile filter paper (one slide per dish). Conidia were defined as germinated when the germ tube length was equal or greater than half the minor conidium diameter.

(a) Effect of temperature and incubation period

Conidial suspensions of three *P. lycopersici* isolates (AH 24, 65 and 128; 41 day), prepared in SDW and SME (Section 1.4) were incubated at four temperatures. Conidial germination was recorded at four hourly intervals (500 conidia per isolate per temperature).

(b) Effect of temperature, nutrients and isolate

Conidial suspensions of three *P. lycopersici* isolates (AH24, 65 and 128; 22 day), prepared in SDW and SME were incubated at 12 temperatures. Conidial width and length and germ tube length (20 conidia per isolate per temperature) and conidial germination were recorded after 24h incubation.

(c) Effect of pH and isolate

For each buffer solution (Table 10) a range of pH values in SDW and SME was prepared. The pH of the solutions was recorded using a Philips (Model PW9 418/31) pH meter. The stability of the pH solutions was tested after 24h storage at 5°C in the dark. Conidial suspensions (one ml) of three *P. lycopersici* isolates (AH24, 65 and 128; 30 day), prepared in SDW, were added to each pH solution (nine ml) and the pH of the resulting suspension tested. Conidial germination and pH of the conidial suspension were recorded after 24h incubation at 22°C in the dark.

(d) Effect of light and isolate

Conidial suspensions of three *P. lycopersici* isolates (AH24, 65 and 128; 30 day), prepared in SDW and SME were incubated under various light conditions at 22°C. Suspensions for dark treatments were wrapped in aluminium foil immediately after preparation. Conidial germination was recorded after 24h incubation.

Table 10

Buffer solutions used to test the effect of pH on the germination of **Pyrenochaeta lycopersici** *conidia*

Buffer Solution	pH range tested
Universal: Citric acid, potassium dihydrogen orthophosphate and boric acid/ sodium hydroxide	2.6-12.0
Citric acid/di-sodium hydrogen orthophosphate*	2.6-8.0
Potassium dihydrogen orthophosphate/sodium hydroxide	4.7-9.2
Di-sodium hydrogen orthophosphate/sodium dihydrogen orthophosphate	5.7-8.0
Boric acid/sodium hydroxide	7.8-10.2
Sodium carbamate/sodium hydrogen carbamate	9.2-10.9

After Perin and Dempsey (1974)

*Coley-Smith (pers.comm.)

(e) Effect of age, conidial washing, cirrus extract and isolate

Three *P. lycopersici* isolates (AH24, 65 and 128) were inoculated onto V8A (20ml V8A per plate to reduce dessication, ten replicates per isolate) and incubated at 22°C, with a 16h black light photoperiod, in sealed polyethylene bags. Conidial suspensions (5ml) of each isolate were prepared in SDW and SME from pycnidia taken from the inoculated plates. Suspensions were washed three times (Section 1.4) and resuspended in fresh SDW, SME and/or cirrus extract obtained from the first conidial wash. Suspensions of unwashed, washed and cirrus extract conidial preparations were incubated at 22°C in the dark and conidial germination recorded after 24h. The experiment was repeated as the cultures aged, taking conidia from the same batch of V8A cultures.

(f) Effect of the optical brightener Calcofluor White M2R

Conidial suspensions of three *P. lycopersici* isolates (AH24, 65 and 128; 26 day), prepared in SDW, were centrifuged and resuspended in varying concentrations of Calcofluor White M2R (0 to 1000 µg active ingredient per ml) (Section 1.5). Suspensions were stored at 5°C for 24h, washed three times and resuspended in SDW and/or SME. Conidial germination was recorded immediately after 24h incubation at 22°C in the dark. The quality of fluorescence, stimulated by U.V. and blue-green illumination from a Vickers M41 Photoplan fluorescence microscope, was recorded after the germination counts and after a further one weeks incubation at 5°C.

(g) Observations on conidial germination

Conidial suspensions of one *P. lycopersici* isolate (AH128; 25 day), prepared in SDW and SME, were incubated at 22°C in the dark. Conidial width and length (20 conidia) was recorded at two hourly intervals over 24h.

General observations on conidial characteristics and behaviour during germination tests were recorded.

2.5 *Pathogenicity of* Pyrenochaeta lycopersici *conidia*

(a) Conidial pathogenicity in sterilised compost

A mixed *P. lycopersici* conidial suspension (AH24, 65 and 128; 32 day) was prepared (Section 1.4) and serially diluted (0 to 10^7 conidia per ml). Ninety millilitres of each conidial concentration and a SDW control were individually mixed with 1260 ml quantities of sterilied compost. The inoculum potential of infested soil was determined using the BRR assay (Section 1.7); seven seeds were sown, subsequently reduced to five per pot, nine pots per dilution. After four and five weeks tomato growth the number of lesions per tomato root (three pots per dilution) was recorded. Isolations were made from lesions (Section 2.1) and the causal agent identified (Section 2.3). The experiment was repeated with a further range of conidial concentrations.

(b) Conidial pathogenicity in unsterilised garden soil

The pathogenicity of conidia in unsterilised garden soil was

assessed as above. The number of lesions per tomato root was recorded after four, five and six weeks tomato growth.

(c) Conidial pathogenicity in a tomato crop grown under commercial conditions

A study was undertaken, at the Glasshouse Crops Research Institute, Littlehampton, Sussex, to assess the ability of *P. lycopersici* conidia to cause BRR symptoms in an unheated glasshouse tomato crop grown under commercial conditions. During 1983, the incidence of BRR on tomatoes grown in compost, field and glasshouse soils infested with conidia was assessed. The effects of BRR infection on the yield of tomato plants was also investigated. In a repeat experiment in 1984, conidial pathogenicity in propagation and plot composts was further investigated. In addition, the incidence of BRR on tomatoes grown in compost, originally infested with conidia in 1983, was assessed.

In both experiments a mixed *P. lycopersici* conidial inoculum (10^5 conidia per ml) was prepared from three isolates (AH24, 65 and 128; 25 day) and thoroughly mixed with compost, field and/or glasshouse soils at a standard rate; 90ml conidial inoculum per 1260ml soil.

Two week old tomato seedlings, sown in John Innes compost No. 2 (Table 11) were transplanted into the appropriate propagation compost (Figure 4); one seedling per 110mm pot, 38 pots per treatment. Plants were propagated in an unheated glasshouse and watered regularly.

Table 11

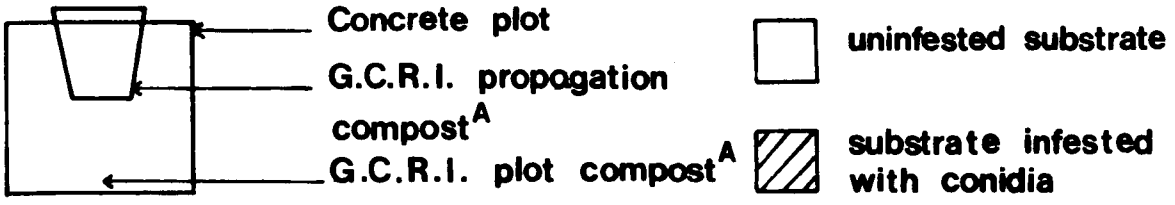
Pathogenicity of **Pyrenochaeta lycopersici** *conidia in a tomato crop grown under commercial conditions: summary of the experimental procedure.*

Date		Procedure
1983	1984	
8th April	2nd April	Tomato seeds (160-175) sown in John Innes compost No. 2
20th April	17th April	Tomato seedlings plotted into non-infested and conidial infested G.C.R.I. propogation compost
24th May	15th May	Plots filled with appropriate glasshouse treatments
26th May	23rd May	Plants, grown in appropriate propogation treatments, transplanted into the glass-house treatments
15th July	11th July	Plots sampled and recorded
7th Sept.	14th August	Plots sampled and recorded
18th Oct.	24th Sept.	Plots sampled and recorded

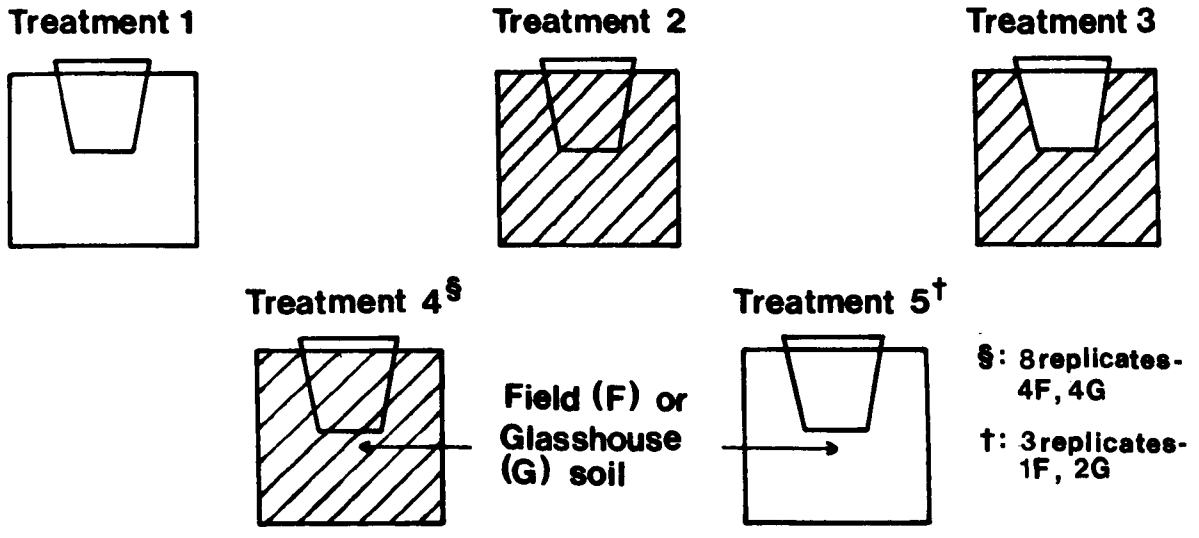
Figure 4

Pathogenicity of* Pyrenochaeta lycopersici *conidia in a tomato crop grown under commercial conditions: infestation of propogation and plot composts with conidia.

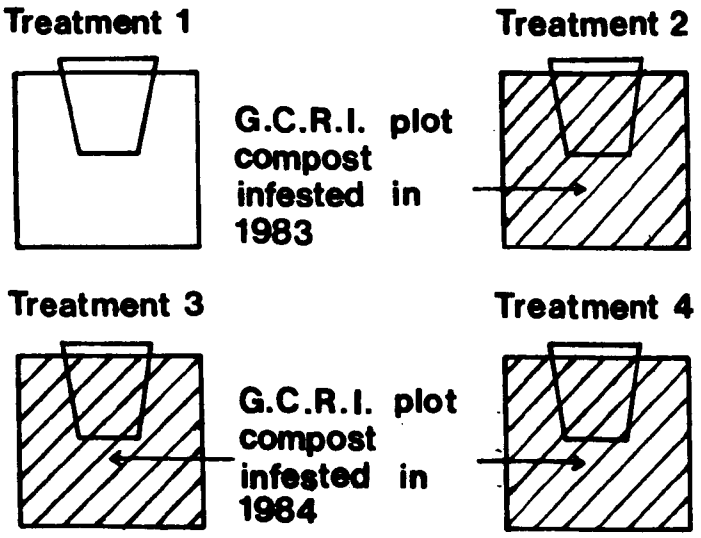
KEY



1983 EXPERIMENT



1984 EXPERIMENT



Note : No field or glasshouse soils were tested in 1984

A : Section 1.1

Washed concrete plots (Figure 5), in an unheated glasshouse, were filled with a layer of non-infested compost and overlaid with the appropriate plot treatment (Figure 4). Tomato plants, grown in the appropriate propagation treatment, were transplanted into the plots. Eleven replicate plots of each treatment (three plants per plot) were arranged in a randomised design. In 1984, plots containing soil infested with conidia in 1983 were selected from treatments 2 and 3 (Figure 4, 1983 Experiment). Soil from the selected plots was occasionally watered during the winter, rotovated and John Innes base compound fertiliser (28g) applied before planting in 1984.

Roots of surplus plants (five per treatment) from each propagation treatment were examined at the time of planting out and isolations made from root lesions (Section 2.1) to check the identity of the causal pathogen. Plots were watered regularly. In 1983 fruit yields were recorded from six replicate plots of treatments 1,2 and 3. In both 1983 (5 replicates) and 1984 (11 replicates) plants were harvested (one per plot) at three dates in each season. Roots were excised, washed and assessed for percentage fine roots, BRR, corkiness, stem base lesions and root size using arbitrary indexes (Table 12). In 1984, the number of trusses and the number of fruit set per truss was recorded at the first sampling date. Isolations were made from root lesions to check the identity of the causal pathogen. After harvest root systems, in sealed polyethylene bags, were placed under black light, daylight or kept in the dark (all at room temperature) and examined regularly for the presence of pycnidia.

Figure 5

Pathogenicity of* Pyrenochaeta lycopersici *conidia in a tomato crop grown under commercial conditions: distribution and quantity of compost within each plot.

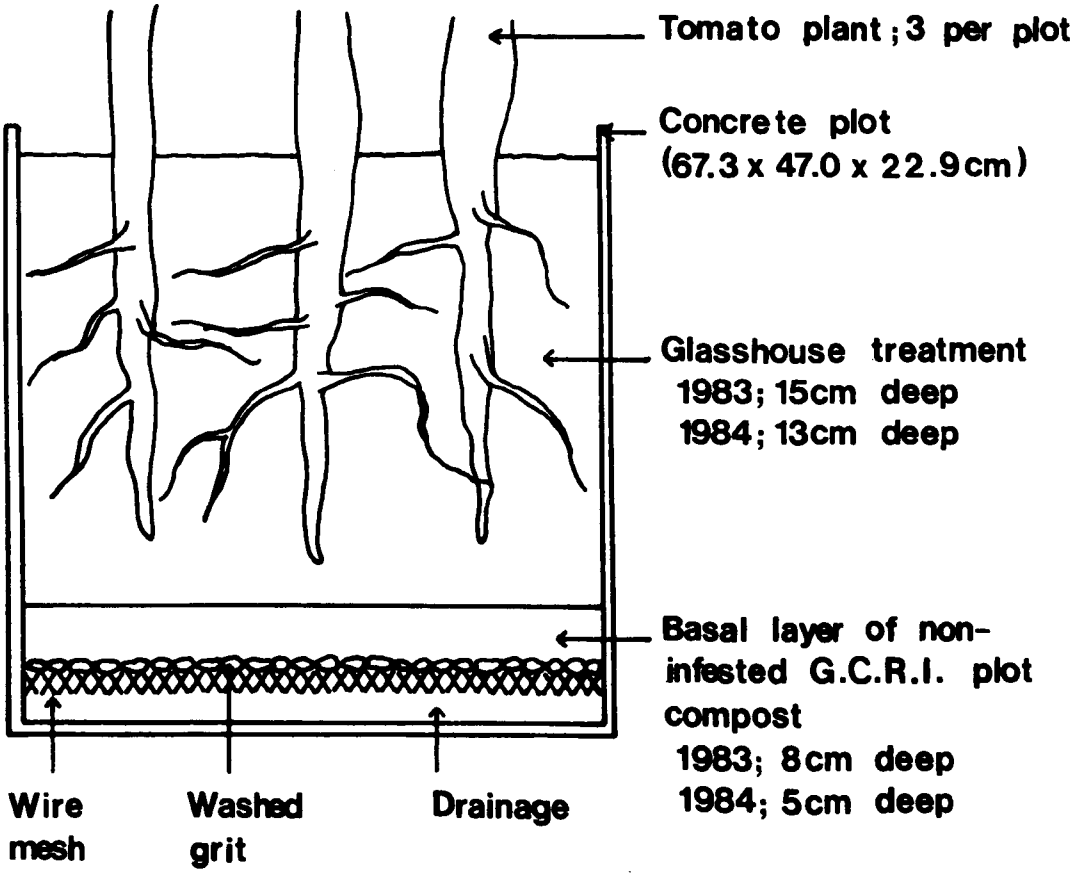


Table 12

Pathogenicity of **Pyrenochaeta lycopersici** *conidia in a tomato crop grown under commercial conditions: arbitrary indexes used to assess disease symptoms on tomato root systems infected with* **Pyrenochaeta lycopersici**

Index	Percentage BRR per root system	Percentage Corkiness per root system	Percentage fine roots per root system	Root size
0	None	None	None	-
1	40	1-25	1-40	Small
2	40-60	26-50	41-75	Medium
3	60	51-75	76-99	Large
4	-	>75	100	-

2.6 *Behaviour of* Pyrenochaeta lycopersici *conidia in soil*

(a) Survival of conidial inoculum in sterilised compost and unsterilised garden soil

Sterilised compost and unsterilised garden soil were inoculated with a mixed conidial suspension (AH24, 65 and 128; 26 day) (10^5 conidia per ml) at the rate of 90ml conidial suspension per 1260ml compost and/or soil. A control was prepared, using SDW, for each treatment; 90ml SDW per 1260ml compost. Soils were stored in the dark, at room temperature, in sealed polyethylene bags. The inoculum potential of each soil was tested periodically using the BRR assay (Section 1.7); five seed per pot, three pots per soil. After four, five (sterilised compost) and six (unsterilised garden soil) weeks isolations were made from lesions (Section 2.1) to check the identity of the causal pathogen.

(b) Germination and subsequent growth of *Pyrenochaeta lycopersici* conidia in various substrates

Washed cellophane film (British Sidac Ltd., quality 450PU) and polycarbonate membranes (Nuclepore Corporation, California, 1.0 µm) were cut into squares ($1cm^2$ and $5cm^2$) and autoclaved for 15 minutes at 1.055kg cm^{-2}. Sterile cellophane squares were immersed in an untreated conidial suspension and sterile polycarbonate membrane squares immersed in an optical brightener treated (Section 2.4) conidial suspension (500 µg per ml Calcofluor White M2R)(AH128; 26 day, 10^7 conidia per ml). Inoculated squares of cellophane and polycarbonate

membrane ($1cm^2$) were folded within squares ($5cm^2$) of the same material to form envelopes. Envelopes were buried at 2.5cm depths in various substrates (60% water holding capacity), contained in 127mm pots. Pots were enclosed in polyethylene bags and kept at room temperature. Envelopes were also incubated between layers of moist sterile filter paper at 22°C (control treatment). Drops of untreated and optical brightener treated conidial suspensions were incubated at 22°C in SME and conidial germination recorded after 24h. Treatments were watered regularly and the pH of the substrate measured after three days. Periodically squares were removed from each treatment and the conidia examined. Conidial germination (250 conidia per treatment) and fungal growth on the inner cellophane square were recorded. Conidia treated with the optical brightener were examined, under U.V. light, using a Vickers M41 Photoplan fluorescence microscope.

SECTION THREE. *BIOLOGICAL CONTROL OF* PYRENOCHAETA LYCOPERSICI

3.1 *Development of the screening technique*

Soil naturally infested with BRR, was mixed with sterilised compost (Section 1.1) to give a range of dilutions. The inoculum potential of infested soils was determined using the BRR assay (Section 1.7). The quantity of tomato seed, the number of replicates per dilution and the incubation period were varied. The number of lesions per root, root dry weight, and in one experiment shoot height and shoot dry weight per

plant were recorded.

3.2 *Screening potential antagonists against brown root rot (BRR)*

Potential antagonists (Table 13) were added to tomato seedlings and/or soil naturally infested with BRR:

Fungi: isolates, grown as wheatbran cultures (Section 1.1), were thoroughly mixed with soil infested with BRR (three parts soil: one part wheatbran culture). Control soils, amended with uninoculated, autoclaved wheatbran and/or sterilised compost were prepared.

Bacteria and Streptomycetes: isolates, prepared as suspensions (Section 1.4), were applied as root dip and soil drenches; isolate LW0547 was applied as a mycelial/spore homogenised suspension. Germinated tomato seeds (radicle length 0.5 to 2.0cm) were immersed in the suspensions immediately before planting in soil infested with BRR (three parts soil: one part sterilised compost). Seven and 15 days after sowing, tomato seedlings were drenched with spore suspensions of potential antagonists (ten ml spore suspension per tomato plant). Control seedlings were dipped and drenched with TW.

The product Biovegetal (AFM Horticultural Supplies, Kings Lynn) was tested for its ability to reduce BRR infection. Biovegetal was applied at three concentrations, 2g, 10g and 20g product per litre of soil infested with BRR (three parts soil: one part sterilised compost). Control soils, amended with sterilised

Table 13

Origin of potential antagonists screened for their ability to reduce brown root rot disease levels

Potential antagonist	Isolate number	Origin of potential antagonist
Trichoderma harzianum Rifai	AH119	Isolated from tomato roots with reduced levels of BRR, Liverpool Polytechnic
Trichoderma harzianum Rifai	AH101	Isolated from the soil of a Dutch glasshouse in which tomatoes had reduced levels of BRR, Liverpool Polytechnic
Trichoderma harzianum Rifai	AH113	As for AH101
Trichoderma sp.	TRC101	Known antagonistic ability, Tate and Lyle Group Research and Development, Reading
Trichoderma sp.	TRC26	As for TRC10
Trichoderma sp.	TRC28	As for TRC10
Gliocladium roseum Bainier	M588	Mycoparasite of *F. oxysporum*: carnation wilt, M. Ebben, G.C.R.I.
Cladosporium sp.	616	Frequent inhabitant of soil, containing crop leaf debris, in which disease levels of BRR were reduced, M. Ebben, G.C.R.I.
Fluorescent *Pseudomonas* sp.	B10	Increase in growth and yield of potato, radish, sugar beet, lettuce, bean and melon, Robinson (pers.comm.) ADAS, Leeds Regional Microbiology Office
Fluorescent *Pseudomonas* sp.	BK1	From potato rhizosphere, Robinson (pers.comm.)
Fluorescent *Pseudomonas* sp.	E6	As forB10
Fluorescent *Pseudomonas* sp.	RV3	Increase in growth and yield of sugar beet, potato and celery, Robinson (pers.comm.)
Bacillus cereus var *mycoides* (Flugge) Smith, Gordon and Clark	52B	Isolated from the rhizosphere of tomatoes grown in soil. Potential antagonist of *P. lycopersici* in pure culture, Solberg (pers. comm.), Norway
Bacterium sp.	40B	As for 52B
Bacterium sp.	53B	As for 52B
Bacterium sp.	23C	As for 52B
Bacterium sp.	50B	As for 52B
Streptomyces sp.	2C	As for 52B
Streptomyces sp.	34C	As for 52B
Streptomyces sp.	39C	As for 52B
Streptomyces sp.	61	Reduced disease levels caused by *Alternaria brassicicola*, *R. solani*, *Fusarium culmorum* and *Pythium debaryanum*; Tahvonen (1982b)
Streptomyces lavendulae (Waks. and Curt) Waksman and Henrici:	LW069	A.T.C.C.* 8664
Streptomyces subrutilis Arai, Kuroda, Yamagishi and Katoh	LW445	A.T.C.C.* 27467
Streptoverticillium septatum Prokop	LW0547	A.T.C.C.* 27467
Biovegetal^{R}	B10	Organic fertiliser derived from treated sewerage sludge. AFM Horticultural Supplies, Kings Lynn

*American Type Culture Collection

composts, at the same rates as Biovegetal, were prepared.

The ability of potential antagonists and Biovegetal to reduce the number of root lesions on tomato seedlings was assessed using the BRR assay (Section 1.7); one seed per pot, 25 pots per isolate. Plants were harvested after four weeks and the number of lesions per root and root dry weight recorded for 20 replicates per isolate. Isolations were made from lesions on remaining replicates to check the identity of the causal pathogen.

3.3 *Measurement of brown root rot (BRR) disease levels in soils naturally infested with* **Pyrenochaeta lycopersici**

The relationship between the number of lesions per root on seedlings grown in soil samples from commercial glasshouses and the subsequent disease levels of BRR on tomato plants eight weeks after transplanting into the soils was investigated.

Glasshouse sites in which the soil had not been sterilised for at least one year were selected (Table 14). Soils were sampled over two seasons, 1983 and 1984, with some soils sampled twice. At each glasshouse site approximately 200ml of soil were collected, at a depth of 15cm, at 20 regularly spaced intervals, along a W-sampling pattern and thoroughly mixed to provide a uniform sample. The inoculum potential of the soil was assessed using the BRR assay (Section 1.7); one seed per pot, 25 pots per dilution. Plants were harvested after four weeks and the number of lesions per root and root dry weight recorded for 20 replicates. Isolations were made from lesions

Table 14

Site details of soils assessed for BRR infestation

Soil	Soil type	Cropping history	Latest soil treatments	Cultivar	Planting date
Hesketh Bank (Control)	Ground-water gley soil (Hesketh complex)	1980 to 1983: Lettuce/Tomato	1983: Methyl bromide	1983: Vicores	Early June 1983
Cotton End	Heavy clay (Rowsham series)	1980 to 1984: Lettuce/Tomato	1981 and 1983: Methyl bromide	1983: Angela 1984: Sonatine	25.5.83 14.5.84
Sandridge	Organic silty loam (Charity series)	1980 to 1984: Tomato/Cabbage	1981 to 1983: Basamid	1983 and 1984: Piranto	18.4.83 1.5.84
Gustard Wood	Clay with flints (Batcombe series)	1980 to 1984: Tomato	1980: Steam	1983: Shirley 1984: Sonatine	12.5.83 21.4.84
Cheshunt	Chalky boulder clay (Oak series)	1981: Pepper 1982 and 1983: Tomato	1970: Steam	1983: Sonatine	15.3.83
Sharpenhoe	Calcarious gault clay (Bresham series)	1963 to 1983: Lettuce/Tomato	Nil (Grafting used as routine)	1983: Shirley	15.6.83
Wright	Surface-water gley soil (Salop series)	1980 to 1984: Lettuce/Tomato	1979: Methyl bromide	1984: Marathon	4.4.84
Howarth	Ground-water gley soil (Hesketh complex)	1980 to 1984: Tomato/Beans	Nil	1984: Moneymaker	28.5.84
Ball	Humic gley soil (Oaklands series)	1980 to 1984: Tomato/Radish	1983: Basamid	1984: Sonatine	1.5.84
Pickovant	Grey podzol (Sollom complex)	1975 to 1984: Lettuce/Tomato	1983: Methyl bromide	1983: Wilset	6.6.84
Hoddesdon	Chalky boulder clay (Oak series)	Before 1977: Cucumber 1977: Tomato 1978 to 1983: Cucumber 1984: Tomato	1984: Steam	1984: Moneymaker	3.5.84
Kimpton	Clay with flints (Batcombe series)	1976 to 1984: Tomato	1983: Basamid	1984: Sonatine	27.5.84

on remaining replicates to check the identity of the causal pathogen.

From each glasshouse site, 20 tomato plants were sampled (eight weeks after transplanting), five from each arm of the original W-transect. Roots were assessed for percentage brown root rot using the ADAS assessment key No. 9.2.1 (Ministry of Agriculture, Fisheries and Food Publications). A recently sterilised soil was included for comparison. Isolations were made from lesions to check the identity of the causal pathogen.

CHAPTER THREE. RESULTS

SECTION 1. *BIOLOGY OF* PYRENOCHAETA LYCOPERSICI

1.1 *Isolation of* Pyrenochaeta lycopersici

(a) Development of a semi-selective medium

Eighteen fungicides and seven antibiotics were tested individually and in combinations for effects on the growth (calculated as mycelial spread, mm per day) of *P.lycopersici* and *C.coccodes* (Tables 15 and 16). Storite and Tecto 60 (at concentrations <1.0µg a.i.ml^{-1}) and Bayleton (at concentrations >100µg a.i.ml^{-1}) restricted the growth of *C.coccodes* with little effect on that of *P.lycopersici* (Table 17). Certain combinations of PCNB and Nystatin also proved inhibitory to *C.coccodes* (Table 15). Bavistin and Benlate inhibited the growth of *P.lycopersici* at all concentrations. Further studies (Table 18) demonstrated that Bayleton (100µg a.i.ml^{-1}) significantly reduced the growth of *C.coccodes* (86% reduction in growth compared to the control) with little effect on *P.lycopersici* (8% reduction in growth compared to the control). At each PCNB concentration tested, increasing the concentration of Nystatin reduced the growth of *C.coccodes*.

The majority of antibiotics tested had little effect on the growth of either fungus. However, Penicillin/Streptomycin mixtures had some inhibitory effect on the growth of *C.coccodes* (Table 16).

Table 15

Effect of fungicides on the mycelial spread of Pyrenochaeta lycopersici *and* Colletotrichum coccodes

Fungicide concentration (µg a.i.ml^{-1})	Mean mycelial spread (S.D.) (mm per day)		Percentage inhibition *		*P.lycopersici*+ selectivity value
	P.lycopersici	*C.coccodes*	*P.lycopersici*	*C.coccodes*	
Aaterra 1	1.13 (0.15)	2.59 (0.31)	55.51	43.45	-12.06
Aaterra 10	1.45 (0.07)	2.27 (0.18)	42.91	50.44	+ 7.53
Aaterra 100	1.45 (0.01)	1.59 (0.17)	42.91	65.28	+22.37
Afugan 1	1.42 (0.08)	3.12 (0.36)	44.09	31.88	-12.21
Afugan 10	1.56 (0.05)	3.18 (0.20)	38.58	30.57	- 8.01
Afugan 100	1.59 (0.17)	2.65 (0.09)	37.40	42.14	- 4.74
Aliette 1	1.87 (0.10)	3.23 (0.34)	26.38	29.48	+ 3.10
Aliette 10	1.50 (0.11)	2.95 (0.31)	40.95	35.59	- 5.36
Aliette 100	1.56 (0.05)	3.05 (0.26)	38.58	33.41	- 5.17
Bavistin 1	0.00 (0.00)	1.24 (0.06)	100.00	78.28	-21.72
Bavistin 10	0.00 (0.00)	1.31 (0.06)	100.00	77.06	-22.94
Bavistin 100	0.00 (0.00)	1.39 (0.04)	100.00	75.66	-24.34
Bayleton 1	2.01 (0.08)	2.98 (0.22)	20.87	34.93	+14.06
Bayleton 10	2.01 (0.10)	2.81 (0.22)	20.87	38.65	+17.78
Bayleton 100	2.18 (0.04)	0.81 (0.04)	14.17	82.31	+68.14
Benlate 1	0.00 (0.00)	1.42 (0.03)	100.00	75.87	-24.13
Benlate 10	0.00 (0.00)	1.25 (0.05)	100.00	78.11	-21.89
Benlate 100	0.00 (0.00)	1.52 (0.04)	100.00	73.38	-26.62
Daconil 1	1.33 (0.04)	2.40 (0.21)	47.64	47.59	- 0.05
Daconil 10	1.06 (0.21)	2.12 (0.06)	58.27	53.71	- 4.56
Daconil 100	0.61 (0.12)	1.32 (0.15)	76.10	71.18	- 4.92
Dithane Wettable 1	1.02 (0.09)	2.58 (0.24)	59.84	43.67	-16.17
Dithane Wettable 10	1.14 (0.02)	2.60 (0.21)	55.12	43.23	-11.45
Dithane Wettable 100	0.97 (0.21)	2.09 (0.13)	61.81	54.37	- 7.44
Nabam 1	2.92 (0.26)	5.66 (0.07)	21.72	+0.18	+21.70
Nabam 10	2.74 (0.04)	3.73 (0.22)	26.54	33.98	+ 7.44
Nabam 100	0.00 (0.00)	0.00 (0.00)	100.00	100.00	0.00
Nystatin 1	1.17 (0.33)	0.87 (0.32)	53.94	81.00	+27.06
Nystatin 10	0.88 (0.15)	0.19 (0.07)	65.35	95.85	+30.50
Nystatin 100	0.36 (0.12)	0.00 (0.00)	85.83	100.00	+14.17
Orthocide Concentrate 1	1.19 (0.27)	2.92 (0.56)	53.15	36.25	-16.90
Orthocide Concentrate 10	0.91 (0.09)	2.28 (0.24)	64.17	50.22	-13.95
Orthocide Concentrate 100	0.51 (0.41)	1.64 (0.56)	79.92	64.19	-15.73
PCNB 1	1.45 (0.07)	2.84 (0.08)	42.91	37.99	- 4.92
PCNB 10	1.36 (0.13)	2.15 (0.09)	46.46	53.06	+ 6.60
PCNB 100	1.17 (0.15)	1.47 (0.13)	53.94	67.90	+13.96
Ridomil 1	1.61 (0.59)	2.45 (0.09)	36.61	46.51	+ 9.90
Ridomil 10	1.48 (0.42)	2.49 (0.07)	41.73	45.63	+ 3.90
Ridomil 100	1.56 (0.28)	2.09 (0.34)	38.58	54.37	+15.79
Ronilan 1	1.21 (0.12)	2.99 (0.07)	52.36	34.72	-17.64
Ronilan 10	0.85 (0.13)	2.31 (0.21)	66.54	49.56	-16.98
Ronilan 100	0.69 (0.24)	2.44 (0.08)	72.84	46.72	-26.12
Rovral 1	1.16 (0.14)	2.64 (0.36)	54.33	42.36	-11.99
Rovral 10	0.72 (0.29)	2.48 (0.09)	71.65	45.85	-25.80
Rovral 100	0.69 (0.04)	2.50 (0.17)	72.84	45.42	-27.42
Sistan 1	1.25 (0.20)	3.11 (0.45)	50.79	32.10	-18.69
Sistan 10	1.28 (0.16)	3.26 (0.22)	49.61	28.82	-20.79
Sistan 100	0.74 (0.19)	2.31 (0.12)	70.87	49.56	-21.31

Table 15 (continued)

Fungicide concentration (μg a.i.ml^{-1})	Mean mycelial spread (S.D.) (mm per day)		Percentage inhibition *		*P.lycopersici*+ selectivity value
	P.lycopersici	*C.coccodes*	*P.lycopersici*	*C.coccodes*	
Storite 1	1.69 (0.30)	0.92 (1.58)	0.34	79.91	+79.57
Storite 10	0.74 (1.04)	0.92 (0.23)	70.87	79.91	+ 9.04
Storite 100	0.12 (0.17)	0.92 (0.04)	95.23	79.91	-15.32
Tecto 60 1	1.63 (0.26)	0.98 (0.09)	35.83	78.60	+42.77
Tecto 60 10	0.00 (0.00)	1.11 (0.14)	100.00	75.76	-24.24
Tecto 60 100	0.00 (0.00)	1.05 (0.13)	100.00	77.07	-22.93
PCNB 100/Nystatin 25	0.76 (0.09)	0.00 (0.00)	70.08	100.00	+29.92
PCNB 10/Nystatin 25	1.95 (0.09)	0.24 (0.08)	23.23	94.76	+71.53

* Percentage inhibition compared to controls (controls expressed as 0% inhibition).

+ Percentage inhibition value of *C.coccodes* - Percentage inhibition value of *P.lycopersici* = *P.lycopersici* selectivity value

Table 16

Effect of antibiotics on the mycelial spread of Pyrenochaeta lycopersici *and* Colletotrichum coccodes.

Antibiotic concentration (μg a.i.ml^{-1})	Mean mycelial spread (S.D.) (mm per day)		Percentage inhibition *		*P.lycopersici*+ selectivity value
	P.lycopersici	*C.coccodes*	*P.lycopersici*	*C.coccodes*	
C50	2.48 (0.14)	4.45 (0.05)	10.10	9.55	- 0.55
C100	2.76 (0.08)	4.52 (0.17)	0.00	8.13	+ 8.13
C200	2.76 (0.10)	5.07 (0.23)	0.00	+ 3.05	- 3.05
P/S 50/100	2.71 (0.11)	4.91 (0.10)	1.81	0.20	- 1.61
P/S 100/100	2.74 (0.12)	4.19 (0.15)	0.73	14.84	+14.11
P/S/A 50/100/50	2.38 (0.33)	4.50 (0.18)	13.77	8.54	- 5.23
P/S/C 50/100/100	2.88 (0.14)	4.23 (0.16)	+ 4.35	14.02	+18.37
P/S/C 50/100/200	2.52 (0.19)	4.48 (0.10)	8.70	8.94	+ 0.24
T50	3.37 (0.05)	5.71 (0.00)	3.44	0.00	- 3.44
T100	3.00 (0.00)	5.71 (0.00)	14.04	0.00	-14.04
P/S/T 100/100/100	3.04 (0.07)	5.47 (0.12)	12.89	4.20	- 8.69
V50	2.25 (0.31)	4.29 (0.07)	18.48	12.81	- 5.64
C/V 50/50	2.57 (0.11)	4.37 (0.09)	6.89	11.18	+ 4.29
Pm 125	0.14 (0.11)	0.00 (0.00)	95.99	100.00	+ 4.01
Pm 250	0.00 (0.00)	0.00 (0.00)	100.00	100.00	0.00
P/S/A/V/C 50/100/50/50/50	2.52 (0.07)	4.45 (0.13)	8.70	9.55	+ 0.85
Ab.Alc. 100	2.88 (0.04)	5.36 (0.08)	17.48	6.13	-11.35

* Percentage inhibition compared to controls (controls expressed as 0% inhibition)

P Penicillin

S Steptomycin sulphate

A Ampicillin anhydrous

C Chloramphenicol

V Vancomycin

T Tetracyclin anhydrous

Pm Pimafucin

Ab.Alc. Absolute Alcohol

+ Percentage inhibition value of *C.coccodes* - Percentage inhibition value of *P.lycopersici* = *P.lycopersici* selectivity value

Table 17

Toxicity of fungicides tested against **Pyrenochaeta lycopersici** *and* **Colletotrichum coccodes** *in vitro.*

Proprietary name of fungicide		ED50 ($\mu g\ ml^{-1}$)*
P.lycopersici	*C.coccodes*	
Aaterra Afugan Bavistin Benlate Dithane Wettable Nystatin Orthocide Concentrate Ronilan Rovral Sistan	Bavistin Benlate Nystatin Storite Tecto 60	1.0
Daconil 2787-W-75 Storite Tecto 60	Aaterra Daconil 2787-W-75 Orthocide Concentrate PCNB	1.0 - 9.9
Nabam PCNB	Bayleton Dithane Wettable Nabam Ridomil	10.0 - 99.9
Aliette Bayleton Ridomil	Afugan Aliette Ronilan Rovral Sistan	100.0

* Concentration of fungicide inhibiting mycelial spread by 50%.

Table 18

Further studies on the effect of fungicides on the mycelial spread of Pyrenochaeta lycopersici *and* Colletotrichum coccodes.

Fungicide concentration (µg a.i.ml^{-1})	Mean mycelial spread (S.D.) (mm per day)		Percentage inhibition *		*P.lycopersici*+ selectivity value
	P.lycopersici	*C.coccodes*	*P.lycopersici*	*C.coccodes*	
Bayleton 100	2.86 (0.12)	0.78 (0.29)	8.33	86.02	+77.69
Bayleton 200	2.84 (0.04)	0.74 (0.21)	8.97	86.74	+77.77
Bayleton 500	2.52 (0.04)	1.52 (1.05)	18.23	72.76	+53.53
Storite 0.5	2.76 (0.13)	2.36 (0.10)	11.54	57.71	+46.17
Storite 1.0	2.96 (0.45)	2.00 (0.04)	5.13	64.16	+59.03
Storite 5.0	1.36 (0.12)	1.54 (0.07)	56.41	72.40	+15.99
Tecto 60 0.5	3.37 (0.17)	2.75 (0.13)	+ 8.01	50.70	+58.71
Tecto 60 1.0	2.88 (0.16)	2.41 (0.41)	7.67	56.81	+49.12
Tecto 60 5	0.48 (0.32)	1.59 (0.29)	84.62	71.51	-13.11
Nystatin 0.1	3.42 (0.05)	5.40 (0.04)	+ 9.62	3.22	+12.84
Nystatin 0.25	3.20 (0.14)	5.28 (0.20)	+ 2.56	5.38	+ 7.94
Nystatin 0.5	3.26 (0.19)	5.51 (0.10)	+ 4.49	1.25	+ 5.74
Nystatin 1.0	3.50 (0.09)	4.59 (0.13)	+12.18	17.74	+29.92
Nystatin 2.5	2.97 (0.04)	2.09 (0.07)	4.81	62.55	+57.74
Nystatin 10	2.86 (0.09)	2.55 (0.16)	8.33	54.30	+45.97
PCNB 0.5/Nystatin 0.125	2.99 (0.16)	5.33 (0.04)	4.17	4.48	+ 0.31
PCNB 1/Nystatin 0.25	3.28 (0.13)	5.42 (0.10)	+ 5.13	2.87	+ 8.00
PCNB 1/Nystatin 1.25	3.35 (0.17)	4.16 (0.13)	+ 7.37	25.45	+32.82
PCNB 1/Nystatin 25	3.30 (0.06)	3.25 (0.12)	5.77	41.21	+35.44
PCNB 5/Nystatin 0.25	3.03 (0.13)	5.13 (0.08)	2.88	8.07	+ 5.19
PCNB 5/Nystatin 1.25	2.68 (0.10)	4.14 (0.45)	14.1	25.81	+11.71
PCNB 5/Nystatin 2.5	2.51 (0.03)	0.81 (0.38)	19.55	85.48	+65.93
PCNB 7.5/Nystatin 2.5	2.66 (0.04)	2.32 (0.02)	23.78	59.37	+35.59
PCNB 7.5/Nystatin 5.0	2.67 (0.04)	1.95 (0.05)	23.49	65.85	+42.36
PCNB 10/Nystatin 0.25	2.42 (0.27)	4.59 (0.09)	22.44	17.74	- 4.70
PCNB 10/Nystatin 1.25	1.83 (0.06)	3.57 (0.12)	41.35	36.02	- 5.33
PCNB 10/Nystatin 2.5	1.81 (0.06)	0.19 (0.15)	41.99	96.59	+54.60
PCNB 10/Nystatin 25	1.81 (0.06)	0.19 (0.15)	41.99	96.59	+54.60
PCNB 100/Nystatin 2.5	2.64 (0.06)	2.88 (0.28)	24.36	49.56	+25.20
PCNB 100/Nystatin 25	1.36 (0.00)	0.38 (0.48)	56.41	93.19	+36.78
PCNB 2.5/Bayleton 100	2.56 (0.03)	0.48 (0.03)	26.65	91.59	+64.94
PCNB 5/Bayleton 100	2.44 (0.07)	0.50 (0.00)	30.09	91.24	+61.15
Bayleton 50/Benlate 0.5	0.00 (0.00)	0.69 (0.03)	100.00	87.79	-12.22
Bayleton 50/Benlate 1	0.00 (0.00)	0.63 (0.03)	100.00	88.85	-11.15
Nystatin 5/Ab.Alc. 100	2.55 (0.06)	1.29 (0.18)	26.93	77.41	+58.48

* Percentage inhibition compared to controls (controls expressed as 0% inhibition).

+ Percentage inhibition value of *C.coccodes* - Percentage inhibition value of *P.lycopersici* = *P.lycopersici* selectivity value

(b) Evaluation of the semi-selective medium

Growth of *P.lycopersici* on PST medium was similar to that on PDA (Table 19). Growth of *C.coccodes* and *C.campanula* isolates on PST medium tended to be lower than that on PDA and was significantly reduced by 88% and 100% respectively when inoculated onto PSTBay medium. Inoculation of *P.lycopersici* isolates onto PSTBay reduced the growth of the fungus by approximately 40%. An inherent variability in growth between isolates was observed on each medium.

(c) Isolation of grey sterile fungi from root systems showing brown root rot (BRR) symptoms

Tomato seedlings, after four weeks growth in soils naturally infested with BRR, possessed brown root lesions up to 5mm in length. The majority of isolations made from these lesions, onto PDA, PST and PSTBay media, yielded colonies of GSF (Table 20). Grey sterile fungal isolates were seen on the isolation plates approximately one week after inoculation. The fungus appeared as a dense, greyish white, sterile mycelium which rapidly darkened to an olivaceous grey (Rayner, 1970). Relatively few colonies of *C.coccodes* or *C.campanula* were observed. Bacterial contamination was reduced on PST and PSTBay media.

Discrete brown flecked lesions were observed on the root systems of many tomato plants, obtained from commercial crops, eight weeks after transplanting into the glasshouse soil. Lesions of corky root, 0.5 to 2.0 cm in length, could also be detected on larger roots. Isolations from brown and corky

Table 19

Effect of PDA, PST and PSTBay media on the mycelial spread of **Pyrenochaeta lycopersici, Colletotrichum coccodes *and* Calyptella campanula**

Fungal isolate	Mean mycelial spread (mm per day)		
	PDA	PST	PSTBay
P.lycopersici			
AH1	2.66	2.68	2.03
AH2	1.68	1.64	1.30
AH3	1.72	1.58	1.56
AH4	2.98	3.20	1.21
AH5	3.57	3.46	1.44
Mean (S.D.)	2.52 (0.82)	2.51 (0.87)	1.51 (0.32)
C.coccodes			
C.c.1	6.21	5.75	0.91
C.c.2	6.13	5.50	0.71
C.c.3	5.88	5.63	0.83
C.c.4	6.08	5.79	0.67
C.c.5	6.25	6.17	0.75
C.c.6	5.92	5.79	0.38
Mean (S.D.)	6.08 (0.15)	5.77 (0.23)	0.71 (0.18)
C.campanula			
Cp.1	7.26	7.14	0.00
Cp.2	5.76	4.74	0.00
Cp.3	7.13	7.00	0.00
Cp.4	3.24	5.29	0.00
Cp.5	7.03	5.02	0.00
Cp.6	4.03	4.91	0.00
Cp.7	3.49	2.96	0.00
Mean (S.D.)	5.42 (1.79)	5.29 (1.43)	0.00 (0.00)

Table 20

Isolation of grey sterile fungi from the roots of tomato seedlings grown for four weeks in soils naturally infested with brown root rot (BRR).

Soil	Frequency of isolation (Percentage out of ten root lesions per medium)														
	PDA					PST					PSTBay				
	GSF	C.c.	Cp.	B	0	GSF	C.c.	Cp.	B	0	GSF	C.c.	Cp.	B	0
Wright	40	-	-	10	20	60	-	-	-	20	50	-	-	-	-
Howarth	60	-	-	20	-	30	-	-	-	10	70	-	-	-	-
Ball	50	20	-	-	-	80	10	-	-	-	80	-	-	-	-
Sandridge	30	10	-	-	-	50	10	10	-	-	50	-	-	-	-
Hoddesdon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pickovant	70	-	-	30	-	70	-	10	-	-	30	-	-	-	-
Cotton End	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
Gustard Wood	30	-	-	20	-	60	-	-	-	-	40	-	-	-	-
Kimpton	50	-	10	-	-	70	-	-	-	-	70	-	-	-	-

Key

GSF Grey sterile fungi

C.c. *C.coccodes*

Cp. *C.campanula*

B Bacteria

0 Other fungi

lesions, onto PDA, yielded colonies of GSF (Table 21). Strains of *C.coccodes* and *C.campanula* were also isolated but at a lower frequency. On PDA, high levels of bacterial contamination, in particular from corky lesions, restricted the transfer of fungal colonies into pure culture. Incorporation of antibiotics into PDA significantly reduced bacterial growth. For the majority of soils, isolation of lesions onto PST and PSTBay increased the frequency of the appearance of GSF strains; the use of PST and PSTBay media never reduced the frequency of isolation of GSF below that observed on PDA. The growth of *C.coccodes* and *C.campanula* was inhibited on PSTBay. Colonies of *Fusarium*, *Penicillium* and *Trichoderma* species were occasionally observed on all isolation media.

1.2 *Mycelial pathogenicity of grey sterile fungi*

(a) *In vitro*

Root lesions were observed on tomato seedlings inoculated with GSF isolates (Table 22). A wide variation within and between isolates was observed. Colonies of GSF were isolated from root lesions. No lesions were observed in control treatments.

(b) *In vivo*

Root lesions were observed on tomato seedlings grown in composts infested with GSF isolates (Table 22). Differences in pathogenicity between isolates was observed. Colonies of GSF were isolated from root lesions. No fungi were isolated from the occasional lesions occuring in control treatments.

Table 21

Isolation of grey sterile fungi from the roots of tomatoes, eight weeks after transplanting into soils naturally infested with brown root rot (BRR).

Soil	Lesion type	Frequency of isolation (Percentage out of ten root lesions per medium)														
		PDA					PST					PSTBay				
		GSF	C.c.	Cp.	B	O	GSF	C.c.	Cp.	B	O	GSF	C.c.	Cp.	B	O
Wright	Brown	30	-	10	40	20	70	-	20	-	-	80	-	-	10	-
	Corky	40	-	-	30	20	40	-	-	-	60	60	-	-	-	10
Howarth	Brown	10	20	-	70	-	80	-	-	-	20	80	-	-	-	10
	Corky	60	-	-	10	30	60	20	-	-	10	60	-	-	-	40
Ball	Brown	70	-	-	10	-	80	-	-	-	-	100	-	-	-	-
	Corky	20	30	-	40	10	60	40	-	-	-	80	-	-	-	10
Sandridge	Brown	20	30	-	50	-	40	40	10	-	10	80	-	-	-	10
	Corky*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hoddesdon	Brown*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Corky*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pickovant	Brown	20	10	-	30	20	30	-	-	-	50	20	-	-	-	-
	Corky	30	-	-	60	10	30	-	-	10	50	70	-	-	-	30
Cotton	Brown	30	-	10	50	10	30	-	10	-	-	50	-	-	-	-
End	Corky*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gustard	Brown	50	-	-	10	30	80	-	10	-	-	100	-	-	-	-
Wood	Corky	-	-	-	80	20	90	-	10	-	-	100	-	-	-	-
Kimpton	Brown	80	10	-	-	-	80	20	-	-	-	100	-	-	-	-
	Corky	90	-	-	-	10	100	-	-	-	-	100	-	-	-	-

Key

GSF Grey sterile fungi
C.c. *C.coccodes*
Cp. *C.campanula*
B Bacteria
O Other fungi

* No lesions observed

Table 22

***Mycelial pathogenicity of grey sterile fungal isolates* in vivo *and* in vitro**

Isolate	*In vitro*	*In vivo*	
	Mean lesion length cm (S.D.)	Mean lesion number per root (S.D.)	Total number of lesions
15	0.61 (0.18)	22.2 (16.4)	111
24	0.91 (0.27)	-	-
30	1.02 (0.37)	14.4 (6.9)	72
32	1.89 (0.35)	64.8 (29.7)	324
42	0.65 (0.16)	18.8 (3.1)	94
56	2.20 (0.46)	64.0 (13.6)	320
65	1.98 (0.41)	67.4 (23.6)	337
97	1.73 (0.24)	48.8 (23.2)	244
99	1.85 (0.28)	36.0 (16.7)	180
100	1.33 (0.27)	46.0 (24.2)	230
125	0.64 (0.18)	-	-
126	0.61 (0.17)	29.6 (8.7)	148
127	1.79 (0.31)	46.2 (27.6)	231
128	1.65 (0.57)	59.0 (27.4)	295
Control	0.00 (0.00)	3.4 (3.1)	17

1.3 *Sporulation and identification of grey sterile fungal isolates*

(a) Induction of pycnidial production

The optimum temperature for the growth of 13 GSF isolates was 26°C and for the remaining six, 22°C (Table 23). Eleven isolates produced pycnidia and sclerotia, and one pycnidia alone, in one or more treatments (Table 24, Plate 3). A large variation in pycnidial and sclerotial production was observed between isolates although accumulated sclerotial and pycnidial indexes at 22 and 26°C showed no statistically significant differences. Six isolates remained sterile under all conditions tested. More pycnidia and sclerotia were produced under black light conditions, at room temperature, than at any other treatment. The optimum colony region (Figure 3) for pycnidial and sclerotial production depended on the incubation temperature (Table 25). More pycnidia and sclerotia were produced in the outer zones of the colony as the incubation temperature increased. Pycnidial production tended to be accompanied by sclerotial production, however there was no significant correlation between the two.

The characteristics of the pycnidia, conidiophores and conidia (Plates 4, 5 and 6) and pycnidial and conidial measurements (Table 26) confirmed the isolates as *P.lycopersici* (Table 27).

(b) Effect of light on pycnidial production

Pycnidial production under black light and black/daylight was significantly higher than under daylight treatments (Table 28). No pycnidia were produced in the dark treatments. Cultures

Table 23

Mycelial spread of Pyrenochaeta lycopersici *isolates incubated on V8 Juice agar at a range of temperatures*

Isolate	Mycelial spread* mm per day (S.D.)						
	Temperature (°C)						
	5°C	10°C	15°C	22°C	26°C	32°C[A]	Black light[B]
15	0.51 (0.07)	1.05 (0.06)	2.40 (0.06)	3.42 (0.05)	3.55 (0.04)	no growth	3.33 (0.07)
24	0.39 (0.03)	0.70 (0.14)	1.67 (0.05)	2.53 (0.13)	2.90 (0.26)	no growth	2.84 (0.03)
30	0.47 (0.04)	0.85 (0.04)	1.62 (0.08)	2.67 (0.09)	2.90 (0.09)	no growth	2.44 (0.11)
32	0.36 (0.03)	0.87 (0.08)	1.73 (0.09)	2.61 (0.18)	0.33 (0.07)	no growth	2.99 (0.09)
42	0.29 (0.05)	0.61 (0.06)	1.49 (0.21)	2.62 (0.08)	2.87 (0.10)	0.32 (0.09)	3.15 (1.50)
56	0.37 (0.11)	0.90 (0.10)	1.84 (0.04)	2.92 (0.14)	3.76 (0.07)	0.27 (0.14)	3.45 (0.04)
57	0.46 (0.07)	0.92 (0.09)	1.93 (0.17)	2.67 (0.24)	3.03 (0.13)	no growth	3.07 (0.17)
61	0.42 (0.04)	0.86 (0.02)	2.06 (0.39)	3.04 (0.06)	3.36 (0.25)	no growth	3.34 (0.17)
62	0.48 (0.07)	0.96 (0.06)	1.58 (0.19)	2.52 (0.12)	3.03 (0.25)	no growth	3.11 (0.16)
64	0.56 (0.05)	1.15 (0.06)	2.71 (0.10)	3.52 (0.07)	3.60 (0.25)	0.41 (0.02)	3.51 (0.06)
65	0.42 (0.04)	0.81 (0.07)	1.89 (0.05)	3.02 (0.19)	3.18 (0.18)	no growth	2.97 (0.07)
89	0.25 (0.03)	0.67 (0.08)	1.81 (0.04)	2.89 (0.08)	3.13 (0.11)	no growth	2.75 (0.09)
97	0.25 (0.03)	0.67 (0.08)	1.65 (0.09)	2.67 (0.14)	3.32 (0.06)	no growth	2.90 (0.23)
99	0.36 (0.05)	0.77 (0.03)	1.68 (0.07)	2.70 (0.07)	not recorded	0.17 (0.12)	3.06 (0.07)
100	0.47 (0.08)	0.80 (0.19)	1.78 (0.06)	2.72 (0.19)	3.36 (0.12)	no growth	2.96 (0.12)
125	0.56 (0.03)	1.30 (0.13)	3.03 (0.05)	3.68 (0.04)	not recorded	no growth	3.73 (0.09)
126	0.65 (0.07)	1.41 (0.06)	3.11 (0.01)	3.63 (0.04)	3.08 (0.10)	no growth	3.64 (0.12)
127	0.46 (0.07)	0.87 (0.09)	1.82 (0.10)	3.02 (0.03)	3.65 (0.03)	no growth	3.10 (0.09)
128	0.47 (0.07)	0.89 (0.04)	1.77 (0.11)	2.90 (0.06)	3.38 (0.24)	no growth	1.98 (0.11)

* Calculated after 21 days growth (includes initial inoculum plug).

A Calculated after 82 days growth.

B Ambient room temperature.

Table 24

Sclerotia and pycnidia production for 19 **Pyrenochaeta lycopersici** *isolates at various temperatures.*

Isolate	Accumulated pycnidial/sclerotial Index*							
	Temperature (°C)							
	15		22		26		Black Light	
	s	p	s	p	s	p	s	p
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.33	0.33	0.00	3.67	5.33	3.00	6.00	9.67
30	0.00	0.00	0.00	0.00	0.00	0.00	1.33	2.00
32	0.67	0.33	2.67	2.67	3.67	2.33	3.67	9.33
42	0.00	2.00	0.00	2.00	0.00	0.00	0.00	1.67
56	1.33	2.00	4.33	4.67	0.00	1.33	8.67	0.00
57	1.33	1.67	0.67	0.00	0.00	0.00	0.00	0.00
61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
62	1.00	0.67	1.33	3.00	3.00	1.00	7.67	12.33
64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
89	0.67	1.67	0.67	2.67	2.33	0.67	0.67	0.00
97	0.33	0.67	7.67	2.67	6.33	4.33	8.00	13.00
99	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.67
100	1.67	1.67	5.33	3.00	1.67	4.33	3.00	13.33
125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
126	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
127	0.33	1.33	9.33	2.67	3.00	1.33	6.00	10.67
128	0.67	1.33	6.67	2.33	3.00	1.67	7.00	12.67
Mean	0.46	0.76	2.04	1.55	1.49	1.05	2.74	4.49
(SD)	(0.54)	(0.77)	(3.06)	(1.59)	(2.04)	(1.47)	(3.34)	(5.67)

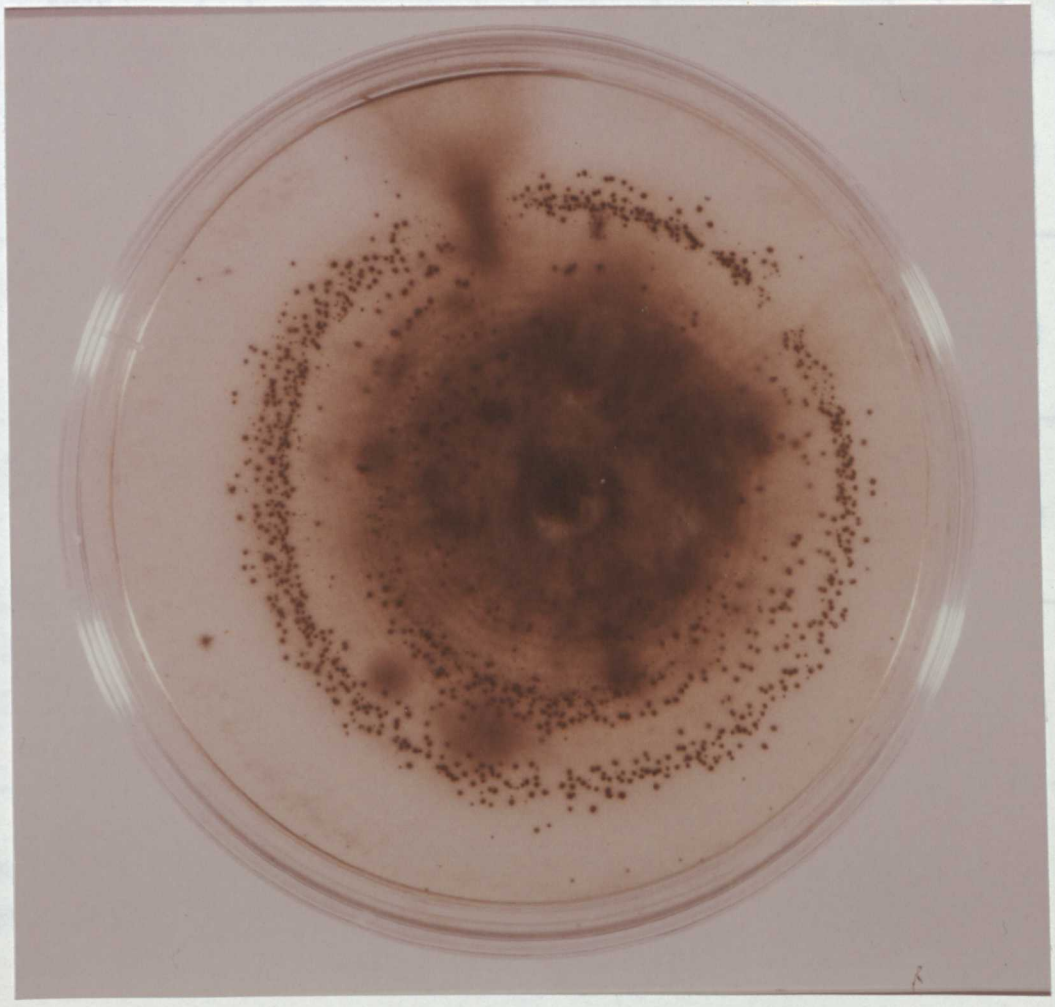
* Mean of three replicates calculated 35 days after inoculation.

s Sclerotia

p Pycnidia

Plate 3

Plate 3 **Pyrenochaeta lycopersici** *pycnidia forming on V8-Juice agar*



1cm

Table 25

Position of pycnidia and sclerotia produced by* Pyrenochaeta lycopersici *on V8-Juice agar at various temperatures.

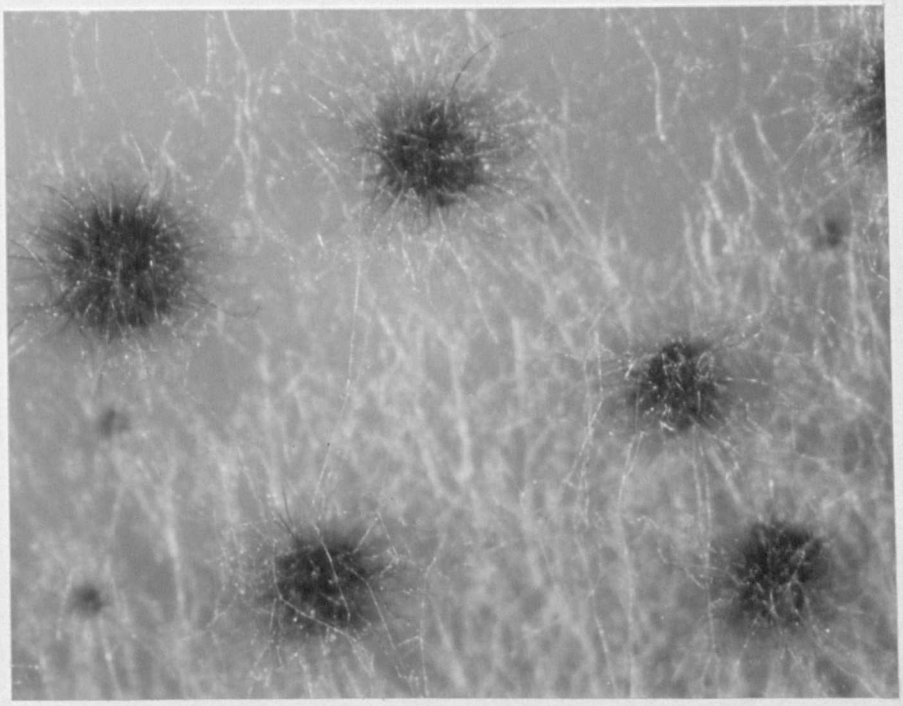
Temperature (°C) / Agar zone	Mean pycnidial Index*(S.D.)				Mean sclerotial Index*(S.D.)			
	1	2	3	4	1	2	3	4
15°C	0.51 (0.51)	0.56 (0.50)	0.03 (0.16)	0.00 (0.00)	0.23 (0.43)	0.41 (0.49)	0.00 (0.00)	0.00 (0.00)
20°C	0.33 (0.47)	0.79 (0.73)	0.69 (0.52)	0.44 (0.50)	0.31 (0.47)	0.83 (1.46)	1.33 (1.44)	1.13 (2.23)
26°C	0.05 (0.22)	0.26 (0.44)	0.41 (0.59)	0.72 (0.89)	0.33 (0.53)	0.74 (1.02)	0.90 (2.42)	0.87 (1.03)
Black Light	1.39 (2.48)	2.03 (1.95)	2.59 (2.39)	0.92 (1.01)	0.89 (0.75)	2.15 (2.79)	1.59 (0.18)	0.67 (1.55)

* Mean of 12 *P.lycopersici* isolates producing pycnidia and/or sclerotia.

Plate 4

Plate 4

Pyrenochaeta lycopersici *pycnidia forming on V8-Juice agar: solitary, globose to subglobose with papillate ostioles beset with three to twelve setae*



450µm

Plate 5

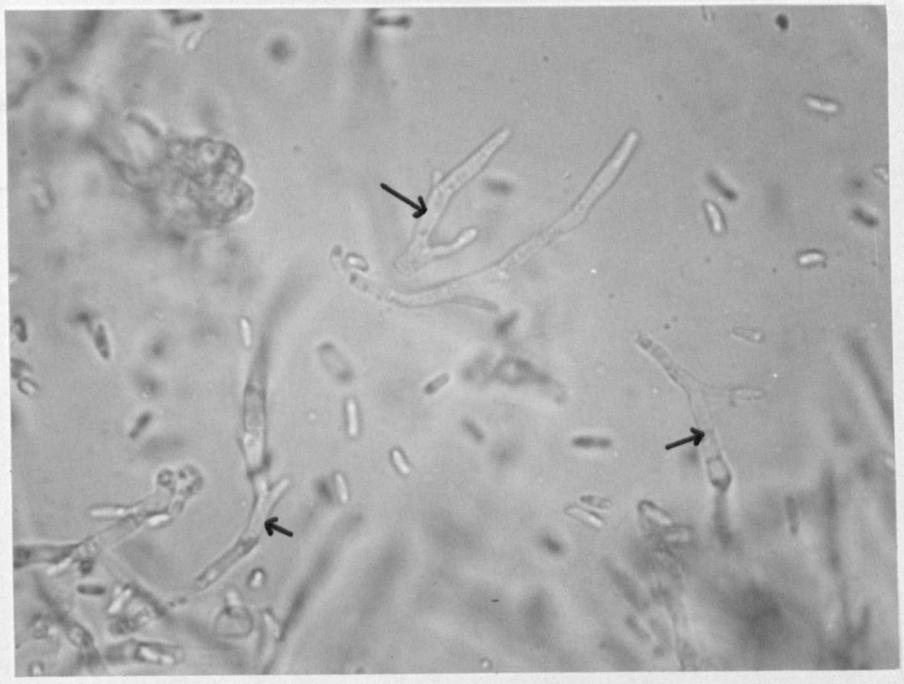
Plate 6

Plate 5

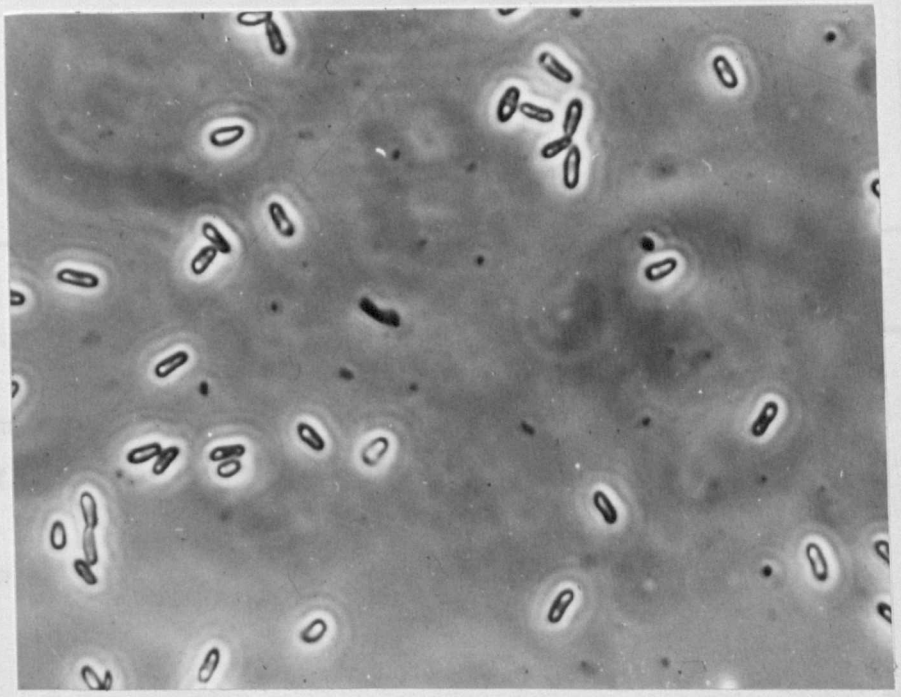
Conidiophores of Pyrenochaeta lycopersici: *simple, mostly branched, septate, arising from the hyaline walls lining the inside of the pycnidial cavity*

Plate 6

Conidia of Pyrenochaeta lycopersici: *hyaline, unicellular, acropleurogenous formed from the apex and short lateral branches immediately below the septa, cylindrical to allantoid.*



25µm



25µm

Phase positive

Table 26

Pycnidial and conidial measurements for GSF isolates[A]

Isolate	Mean pycnidial size (S.D.)* (µm)	Mean conidial size (S.D.)* (µm)
15	-	-
24	333.67 (77.16)	5.16 (0.00) x 1.72 (0.00)
30	455.00 (87.71)	5.16 (0.00) x 1.72 (0.00)
32	277.67 (46.61)	5.16 (0.00) x 1.72 (0.00)
42	473.67 (14.57)	6.31 (0.86) x 1.72 (0.00)
56	326.67 (24.58)	4.78 (0.26) x 1.72 (0.00)
57	-	-
62	329.00 (7.00)	5.54 (0.26) x 1.72 (0.00)
64	-	-
65	-	-
89	315.00 (35.00)	5.35 (1.35) x 1.72 (0.00)
97	336.00 (12.12)	5.16 (0.00) x 1.72 (0.00)
99	347.67 (81.13)	5.15 (0.00) x 1.72 (0.00)
100	340.67 (16.17)	4.97 (0.57) x 1.72 (0.00)
125	-	-
126	-	-
127	303.33 (4.04)	5.16 (0.00) x 1.72 (0.00)
128	331.33 (4.04)	5.16 (0.00) x 1.72 (0.00)
Mean	347.47 (57.81)	5.26 (0.38) x 1.72 (0.00)

A isolates incubated under black light, room temperature and measured 32 days after inoculation.

* Mean of ten pycnidia/conidia.

Table 27

Pycnidial and conidial measurements for **Pyrenochaeta lycopersici** *reported in the literature.*

Pycnidial size (μm)	Conidial size (μm)	Number of isolates measured	Reference
100-400	3.8-8.0 x 2.0	20	Schneider and Gerlach (1966)
150-300	4.5-8.0 x 1.5-2.0	1	Punithalingam and Holliday (1973)
90-260	3.2-5.2 x 1.2-1.6	25	Clerjeau (1974)
124.3-189.1	4.1-4.9- x 1.9-2.3	?	Morita *et al.* (1975)
277.7-455.0	4.8-6.3 x 1.72	12	Isolates examined in this study

Table 28

Effect of light on pycnidial production in **Pyrenochaeta lycopersici** *isolates at 22°C and room temperature (RT)*

Treatment	Mean accumulated pycnidial index (S.D.)*		Mean mycelial spread[A] (mm per day)	
	AH24	AH65	AH24	AH65
Blacklight, 22°C	3.0 (1.2)	8.4 (5.5)	3.16	3.26
Daylight, 22°C	0.4 (0.9)	1.0 (2.2)	3.06	3.24
Black/daylight, 22°C	5.2 (2.2)	10.3 (4.9)	3.12	3.04
Dark, 22°C	0.2 (0.5)	0.2 (0.5)	3.17	1.90
Blacklight, RT	16.8 (0.5)	16.6 (0.6)	3.04	3.25
Daylight, RT	0.0 (0.0)	0.4 (0.6)	2.98	2.88
Dark, RT	0.0 (0.0)	0.0 (0.0)	2.99	3.11

* Mean of five replicates calculated 35 days after inoculation.

A Calculated after 23 days growth. SED between isolate and treatment = 0.10

grown on V8A, under black light, tended to have higher growth rates than those under daylight or dark.

(c) Effect of the vegetable constituents of V8-Juice on pycnidial production

Differences in pycnidial production between isolates were observed within and between the media tested (Table 29). Proprietary V8A, home-made V8A, parsley (dilute) and celery (concentrated) media induced relatively high levels of pycnidial production. Increase in the concentration of parsley and carrot agars tended to increase sporulation. Sporulation on watercress, PDA and TWA agars was low. Pycnidial production on V8A and home-made V8A was significantly higher than that on V8A prepared according to McGrath and Campbell (1983). An inherent variability in pycnidial production was observed on V8A. Pycnidial production was greatest in zone 3 (dilute) and zone 4 (concentrated) of the vegetable agars. Increasing the concentration of vegetable agars tended to reduce the growth of *P.lycopersici* isolates (Table 30).

1.4 *Germination of* Pyrenochaeta lycopersici *conidia* in vitro

(a) Effect of temperature and incubation period

Significant variations in conidial germination were apparent after 12h following incubation at different temperatures (Figure 6). After 24h, germination at 22 and 26°C was significantly higher than that at 15 and 32°C.

(b) Effect of temperature, nutrients and isolate

Table 29

Effect of different media and medium concentration on pycnidial production in three isolates of **Pyrenochaeta lycopersici**

Medium / Isolate	Accumulated pycnidial production[A]					
	Dilute medium			Concentrated medium		
	24	65	128	24	65	128
Celery agar	1.0	6.8	1.2	10.2	9.0	4.6
Spinach agar	1.6	2.4	1.2	4.3	0.2	5.6
Parsley agar	9.0	8.8	5.8	10.0	1.6	3.8
Carrot agar	3.4	6.8	3.4	2.6	3.8	3.2
Watercress agar	0.0	0.0	0.8	0.6	0.0	2.2
Lettuce agar	5.2	7.0	4.0	5.2	5.2	4.8
Tomato agar	1.0	1.6	1.0	3.2	2.4	4.6
Beetroot agar	NT	NT	NT	3.2	9.2	5.8
Home-made V8 Juice agar	4.4	5.8	6.0	12.6	10.4	11.4
Home-made V8 Juice agar minus parsley	NT	NT	NT	15.4	14.6	10.0
McGrath and Campbell (1983)	NT	NT	NT	9.6	8.2	13.4
V8-Juice agar*	3.2	6.6	1.2	10.2	12.8	17.2
Potato dextrose agar*	1.0	0.8	1.0	1.0	1.0	0.8
Tap water agar*	0.8	0.0	1.0	0.6	0.0	1.0
Tomato juice agar	6.8	6.8	3.5	NT	NT	NT
SED	1.34			0.87		

NT Not tested.

* Concentration of these media remained unaltered.

A Measured 37 days after inoculation.

Table 30

Effect of different media and medium concentration on the growth of three **Pyrenochaeta lycopersici** *isolates*

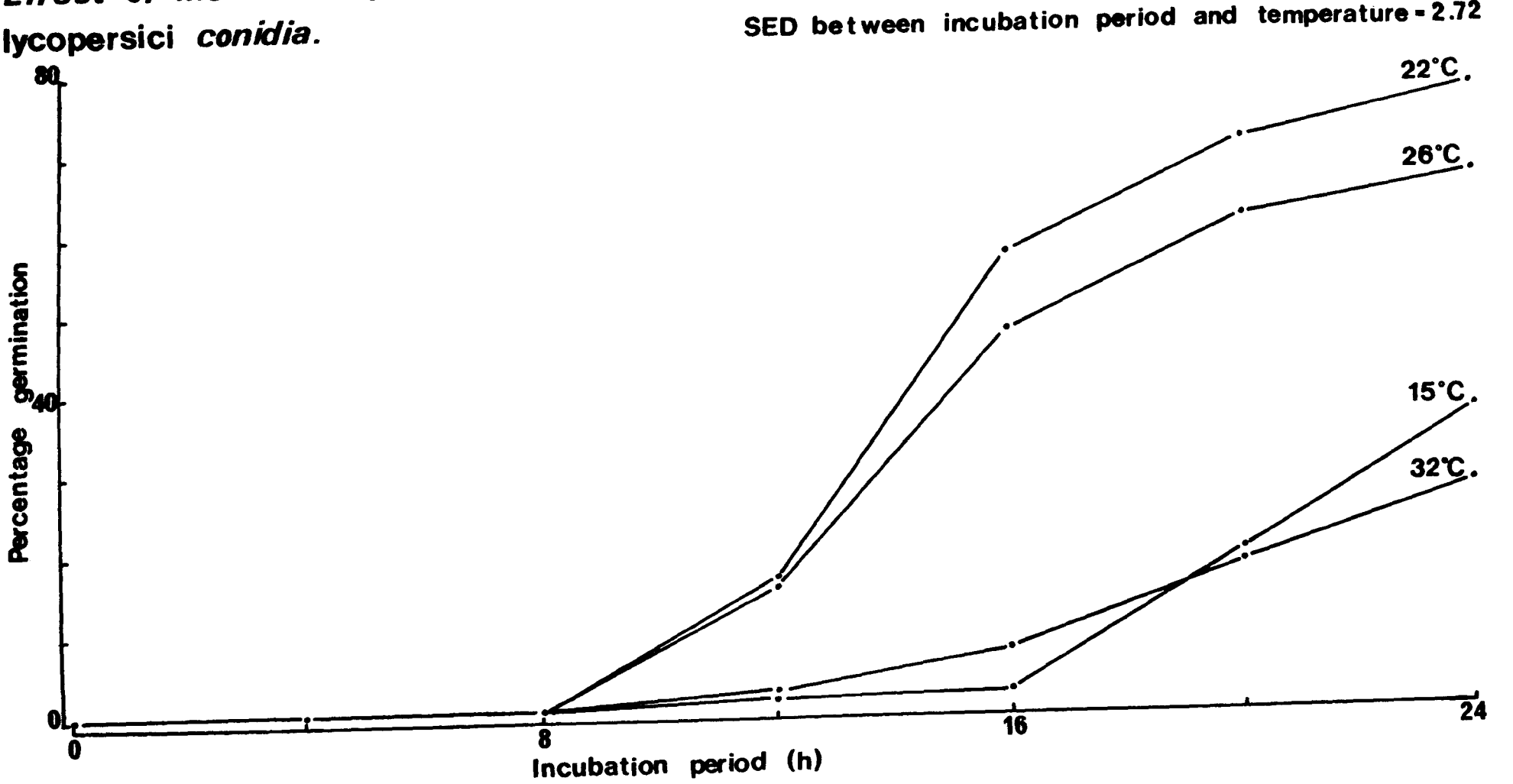
Medium / Isolate	Mycelial spread (mm per day)					
	Dilute medium			Concentrated medium		
	24	65	128	24	65	128
Celery agar	4.08	4.34	3.68	3.18	2.57	2.94
Spinach agar	3.81	4.11	3.51	3.76	3.92	2.95
Parsley agar	3.45	4.33	3.81	3.32	3.45	3.46
Carrot agar	3.82	3.19	4.12	4.03	4.04	3.44
Watercress agar	4.23	4.27	4.13	3.95	4.01	3.57
Lettuce agar	4.18	4.35	3.90	4.09	4.36	3.6
Tomato agar	3.18	1.93	2.54	2.33	2.38	2.02
Beetroot agar	NT	NT	NT	3.55	3.84	2.81
Home-made V8 agar	3.88	3.90	3.33	3.25	3.60	3.01
Home-made V8 agar minus parsley	NT	NT	NT	3.38	3.63	3.04
McGrath and Campbell (1983)	NT	NT	NT	3.39	3.62	3.05
V8-Juice agar*	3.00	3.38	3.00	3.17	3.24	2.74
Potato dextrose agar*	3.42	2.45	1.51	3.59	2.63	1.85
Tap water agar*	3.89	3.60	3.11	3.76	3.79	3.18
Tomato juice agar	3.05	3.41	2.79	NT	NT	NT
SED	0.14			0.12		

NT Not tested.

* Concentration of these media remained unaltered.

Figure 6

Effect of incubation period and temperature on the germination of **Pyrenochaeta lycopersici** ***conidia.***



Conidia germinated over the temperature range 5 to 28°C with an optimum between 20 and 26°C (Figure 7). Variability in germination between isolates was observed at all temperatures. Addition of external nutrients significantly increased germination between 15 and 28°C. The degree of conidial swelling and germ tube length was greatest over the optimum temperature range for germination (Table 31).

(c) Effect of pH and isolate

Conidial germination was affected by the presence of nutrients and the pH of the medium (Figures 8, 9, 10, 11, 12 and 13). The optimum pH range for conidial germination was 5.0 to 8.0. The level and pH range over which conidia germinated was increased by the addition of nutrients. A variability in germination was observed between isolates. Buffer solutions affected the degree of germination in SDW and SME (Figure 14).

(d) Effect of light and isolate

The level of germination varied when conidia were incubated, in SDW and SME, under various light conditions (Table 32). However no treatment reduced conidial germination below 75%. Germination in SDW was lower and more variable than that in SME. Variability in conidial germination between isolates was observed.

(e) Effect of age, conidial washing, cirrus extract and isolate

Conidial germination decreased with increasing culture age (Figure 15). Addition of external nutrients significantly

Figure 7

***Effect of temperature on the germination of* Pyrenochaeta lycopersici *conidia*.**

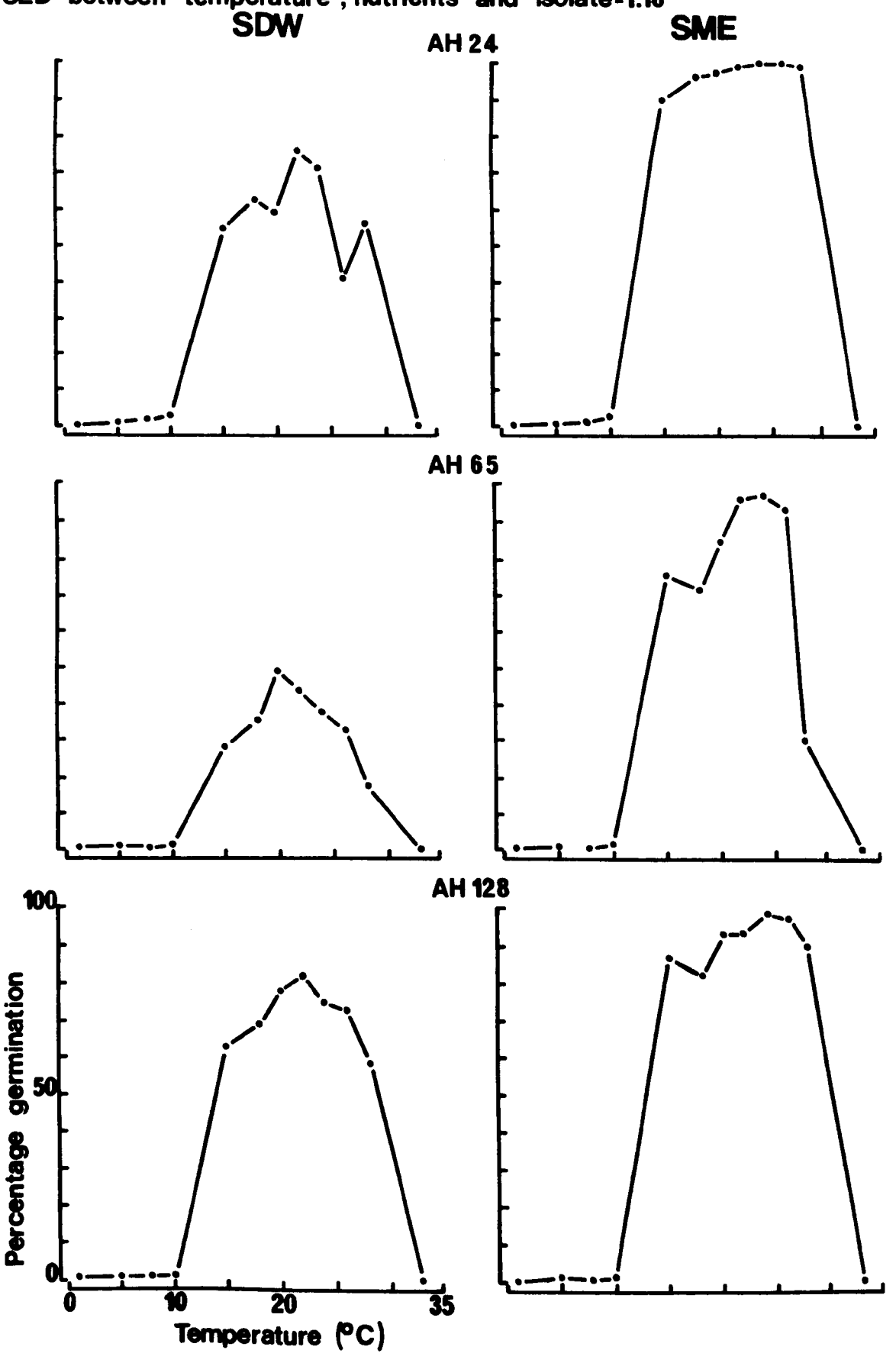


Table 31

Conidial width and length and germ tube length of three Pyrenochaeta lycopersici *isolates incubated at various temperatures*

Temperature (°C)	Isolate AH24						Isolate AH65						Isolate AH128					
	Conidial Length		Conidial Width		Germ tube Length		Conidial Length		Conidial Width		Germ tube Length		Conidial Length		Conidial Width		Germ tube Length	
	SDW	SME	SDW	SME	SDW	SME	SDW	SME	SDW	SME	SDW	SME	SDW	SME	SDW	SME	SDW	SME
1	5.14	5.14	2.57	2.57	-	-	5.14	5.14	2.57	2.57	-	-	5.14	5.14	2.57	2.57	-	-
	(0.00)	(0.00)	(0.00)	(0.00)			(0.00)	(0.00)	(0.00)	(0.00)			(0.00)	(0.00)	(0.00)	(0.00)		
5	5.14	5.14	2.57	3.43	-	-	5.14	5.14	2.57	2.57	-	-	5.14	5.14	2.57	2.57	-	-
	(0.00)	(0.00)	(0.00)	(0.00)			(0.00)	(0.00)	(0.00)	(0.00)			(0.00)	(0.00)	(0.00)	(0.00)		
8	5.14	6.34	3.43	3.43	-	-	5.14	5.14	2.57	2.57	-	-	5.14	6.81	2.57	3.43	-	-
	(0.00)	(0.70)	(0.00)	(0.00)			(0.00)	(0.00)	(0.00)	(0.00)			(0.00)	(0.44)	(0.00)	(0.00)		
10	5.14	5.74	2.57	3.43	-	-	5.27	5.14	2.57	3.43	-	-	5.27	6.94	2.57	4.16	-	-
	(0.00)	(0.40)	(0.00)	(0.00)			(0.75)	(0.00)	(0.00)	(0.00)			(0.32)	(0.27)	(0.00)	(0.32)		
15	5.23	7.54	2.57	4.11	6.34	19.15	5.23	5.14	2.57	2.57	-	-	5.14	6.98	2.57	3.86	4.46	21.21
	(0.27)	(1.02)	(0.00)	(0.35)	(2.69)	(5.92)	(0.27)	(0.00)	(0.00)	(0.00)			(0.00)	(2.03)	(0.00)	(0.71)	(1.95)	(12.44)
18	5.14	8.06	2.57	4.71	10.49	52.71	5.49	8.66	2.57	4.29	7.28	14.78	5.14	6.64	2.57	4.24	7.07	17.99
	(0.00)	(1.09)	(0.00)	(0.44)	(3.33)	(20.01)	(0.51)	(0.62)	(0.00)	(0.00)	(2.83)	(5.59)	(0.00)	(1.96)	(0.00)	(0.44)	(2.39)	(11.39)
20	5.14	8.96	2.57	5.36	10.10	>120	5.14	8.39	2.66	4.29	10.10	12.06	5.06	8.31	2.66	4.29	7.76	41.69
	(0.00)	(0.65)	(0.00)	(0.62)	(4.01)		(0.00)	(0.59)	(0.26)	(0.00)	(2.03)	(4.58)	(0.55)	(0.69)	(0.62)	(0.39)	(3.06)	(17.51)
22	5.27	8.57	2.57	5.36	13.69	>120	5.23	8.57	3.17	4.63	9.89	21.17	5.14	8.09	2.57	5.14	11.66	15.26
	(0.32)	(0.00)	(0.00)	(0.38)	(4.72)		(0.27)	(0.00)	(0.40)	(0.49)	(3.37)	(4.62)	(0.00)	(0.52)	(0.00)	(0.00)	(3.31)	(8.60)
24	5.99	8.69	3.21	5.61	15.68	>170	5.14	8.69	2.57	4.52	9.51	24.13	5.14	8.69	2.57	5.19	9.92	>85
	(0.79)	(0.32)	(0.38)	(0.44)	(5.23)		(0.00)	(0.32)	(0.00)	(0.49)	(3.62)	(4.51)	(0.00)	(0.32)	(0.00)	(0.59)	(1.93)	
26	5.69	8.74	2.91	5.44	9.98	>120	5.14	9.17	2.57	4.29	8.96	20.09	5.18	8.93	2.83	5.20	7.11	>80
	(0.75)	(0.53)	(0.64)	(0.85)	(2.55)		(0.00)	(0.84)	(0.00)	(0.00)	(2.83)	(4.29)	(0.59)	(0.32)	(0.40)	(0.62)	(2.98)	
28	5.14	9.29	2.57	5.74	5.91	>170	5.14	9.34	2.83	5.01	6.13	25.32	5.18	8.14	2.57	4.29	8.23	41.78
	(0.00)	(0.51)	(0.00)	(0.88)	(2.91)		(0.00)	(0.87)	(0.40)	(0.69)	(2.56)	(4.83)	(0.52)	(0.99)	(0.00)	(0.00)	(4.47)	(15.84)
32	4.88	5.53	3.73	4.29	-	-	5.14	5.14	2.66	2.57	-	-	5.14	5.14	2.57	2.57	-	-
	(0.40)	(0.44)	(0.42)	(0.00)			(0.00)	(0.00)	(0.40)	(0.00)			(0.00)	(0.00)	(0.00)	(0.00)		

Measurements in µm.
Standard deviation, mean of 20 spores, in parenthesis.

Figure 8

Effect of pH on the germination of **Pyrenochaeta lycopersici** ***conidia: Universal buffer.***

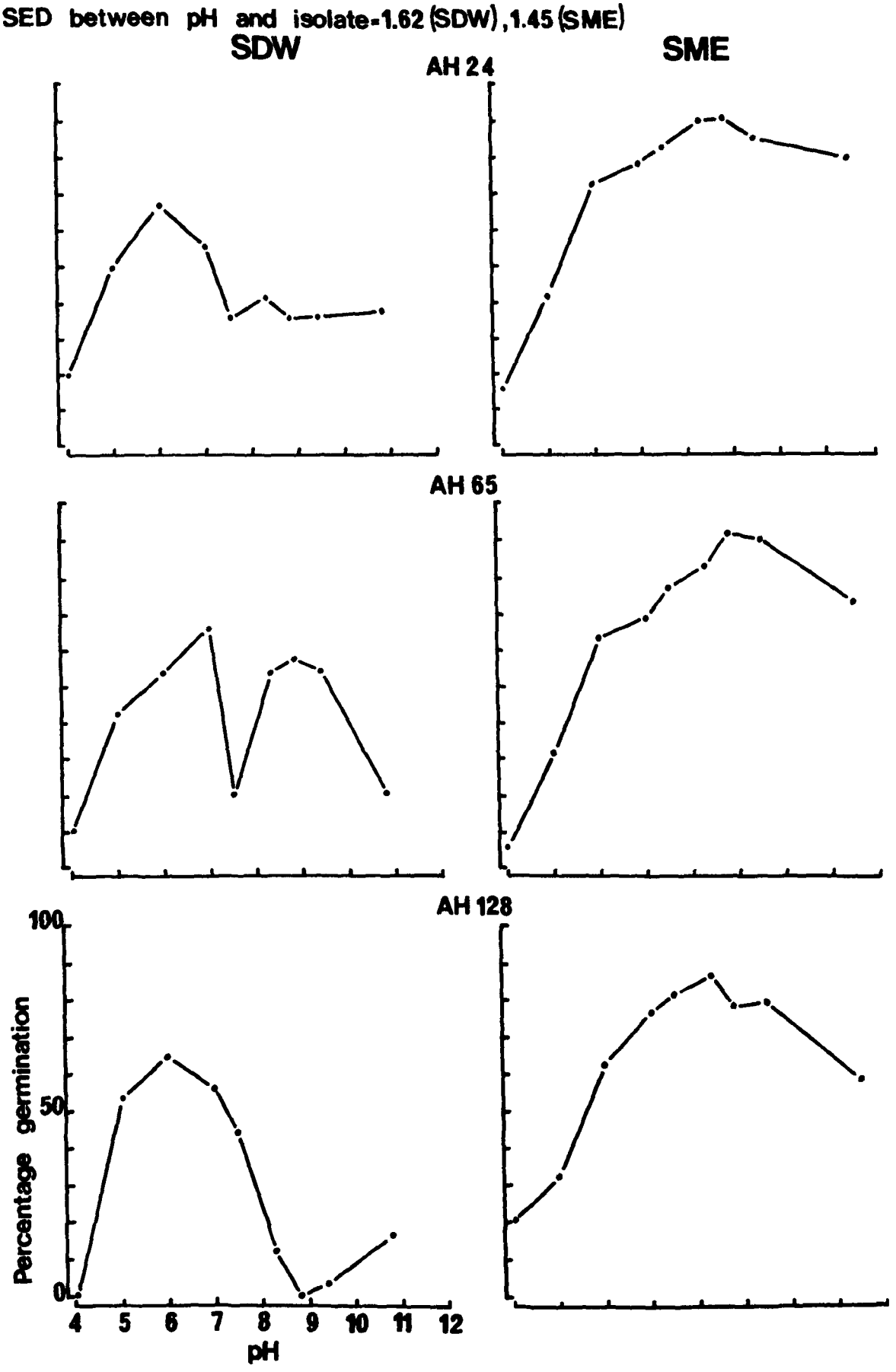


Figure 9

Effect of pH on the germination of* Pyrenochaeta lycopersici *conidia: Citric acid/ di-sodium hydrogen orthophosphate buffer.

SED between pH and isolate=1.30 (SDW) , 0.33 (SME)

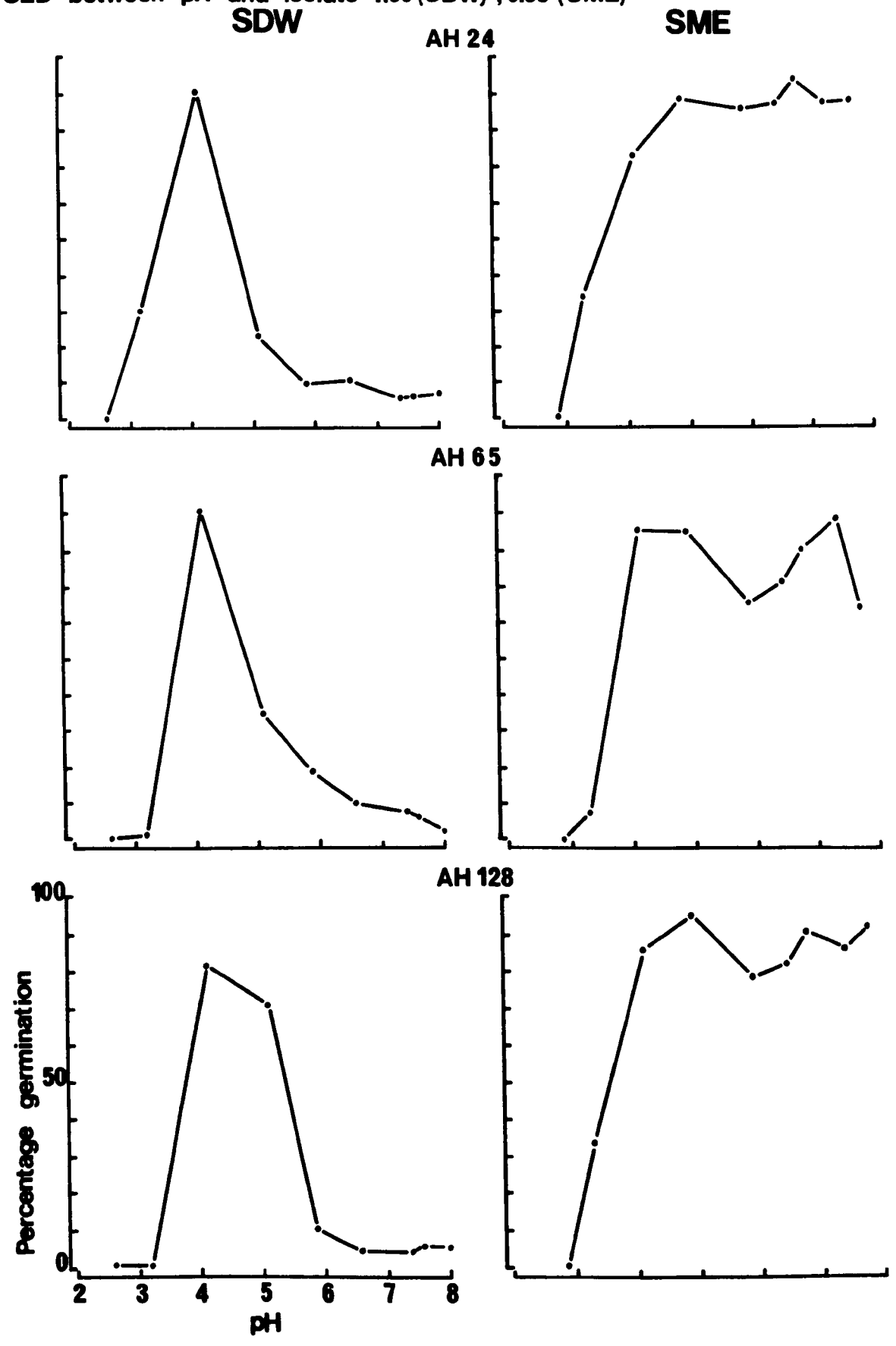


Figure 10

Effect of pH on the germination of **Pyrenochaeta lycopersici** ***conidia: Potassium dihydrogen orthophosphate / sodium hydroxide buffer.***

SED between pH and isolate=2.42 (SDW), 2.46 (SME)

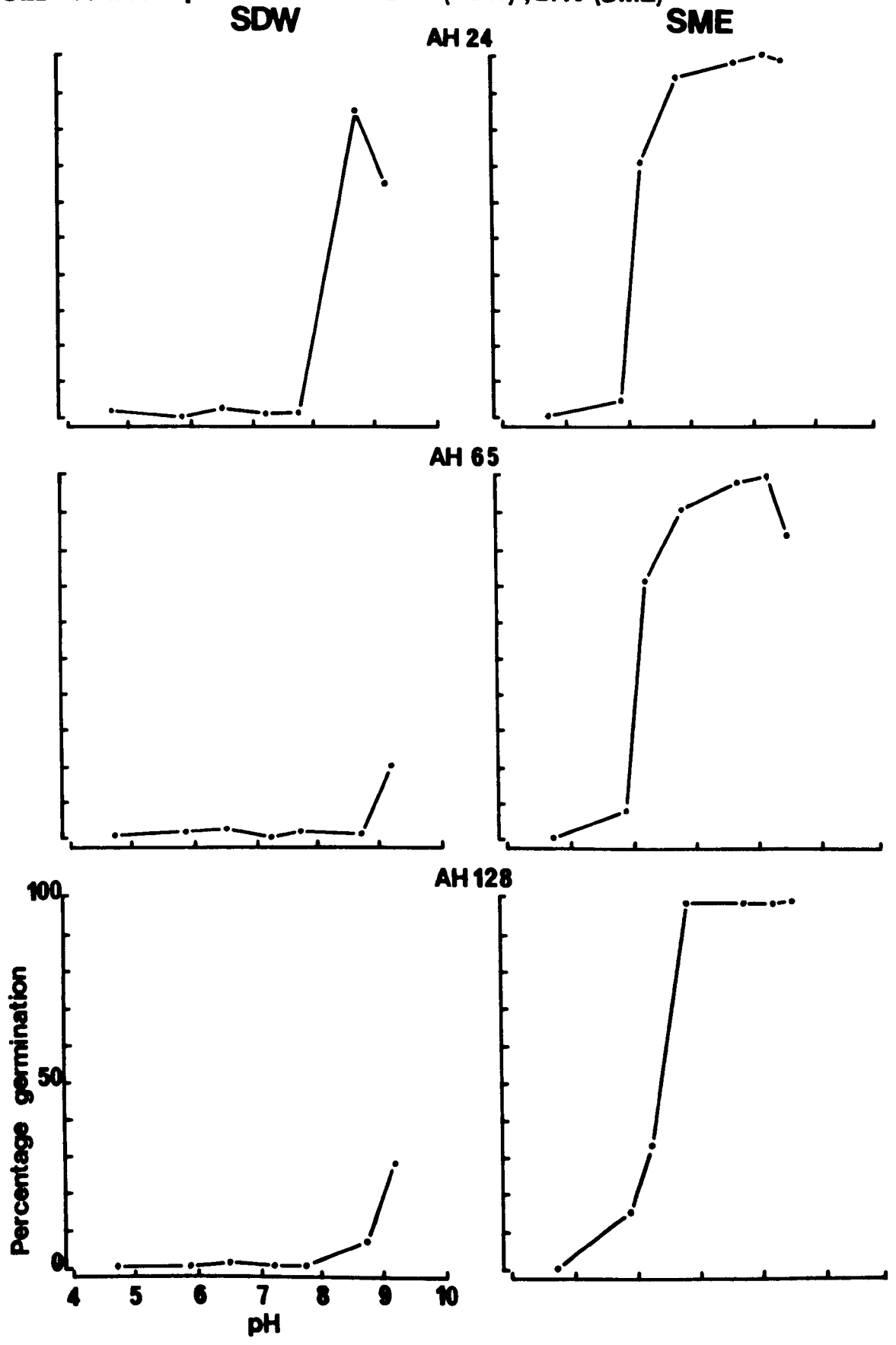


Figure 11

Effect of pH on the germination of **Pyrenochaeta lycopersici** ***conidia: Di-sodium hydrogen orthophosphate/ sodium dihydrogen orthophosphate.***

SED between pH and isolate=1.37 (SDW), 1.34 (SME)

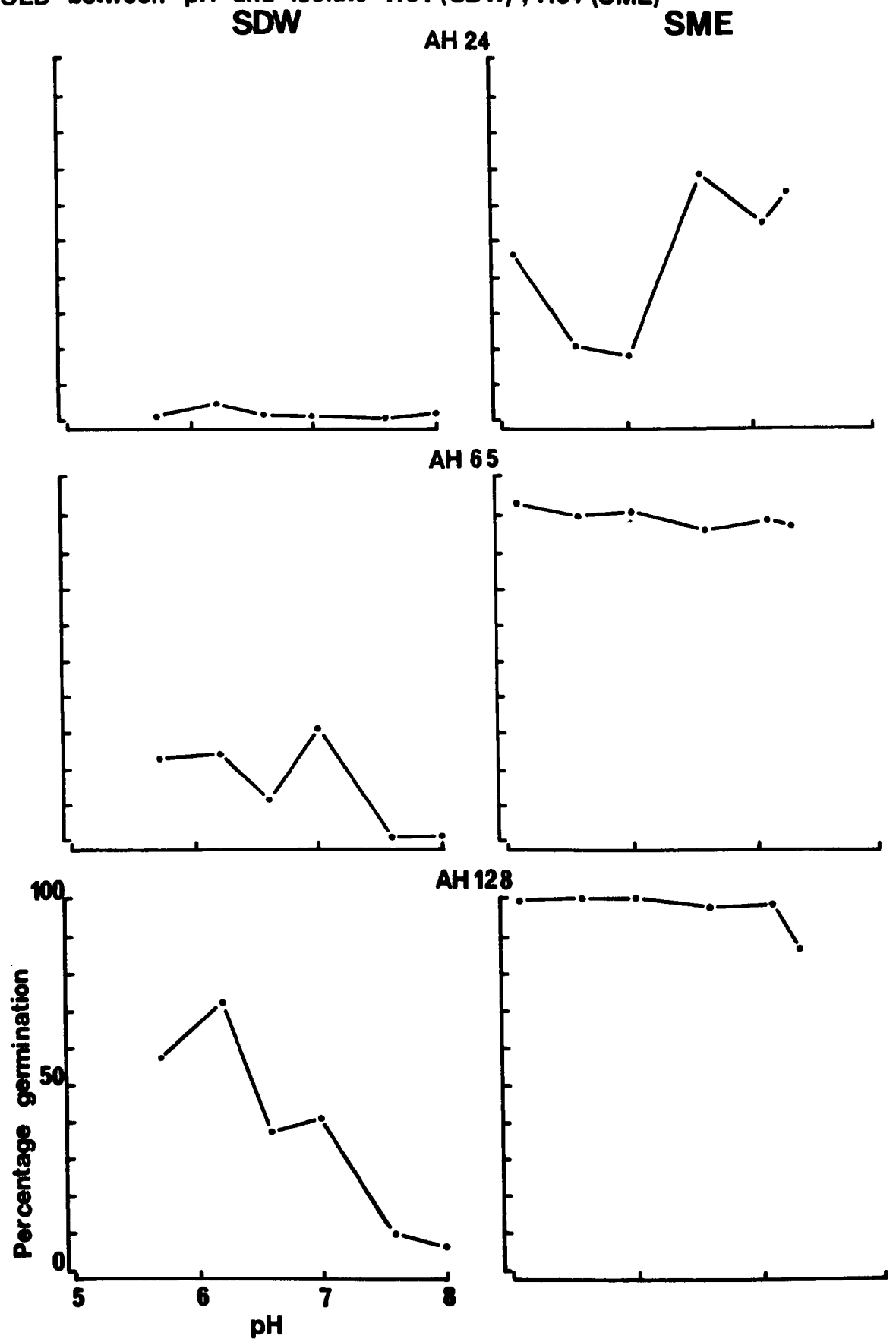


Figure 12

Effect of pH on the germination of **Pyrenochaeta lycopersici** ***conidia: Boric acid/ sodium hydroxide buffer.***

SED between pH and isolate= 2.13 (SDW), 1.80 (SME).

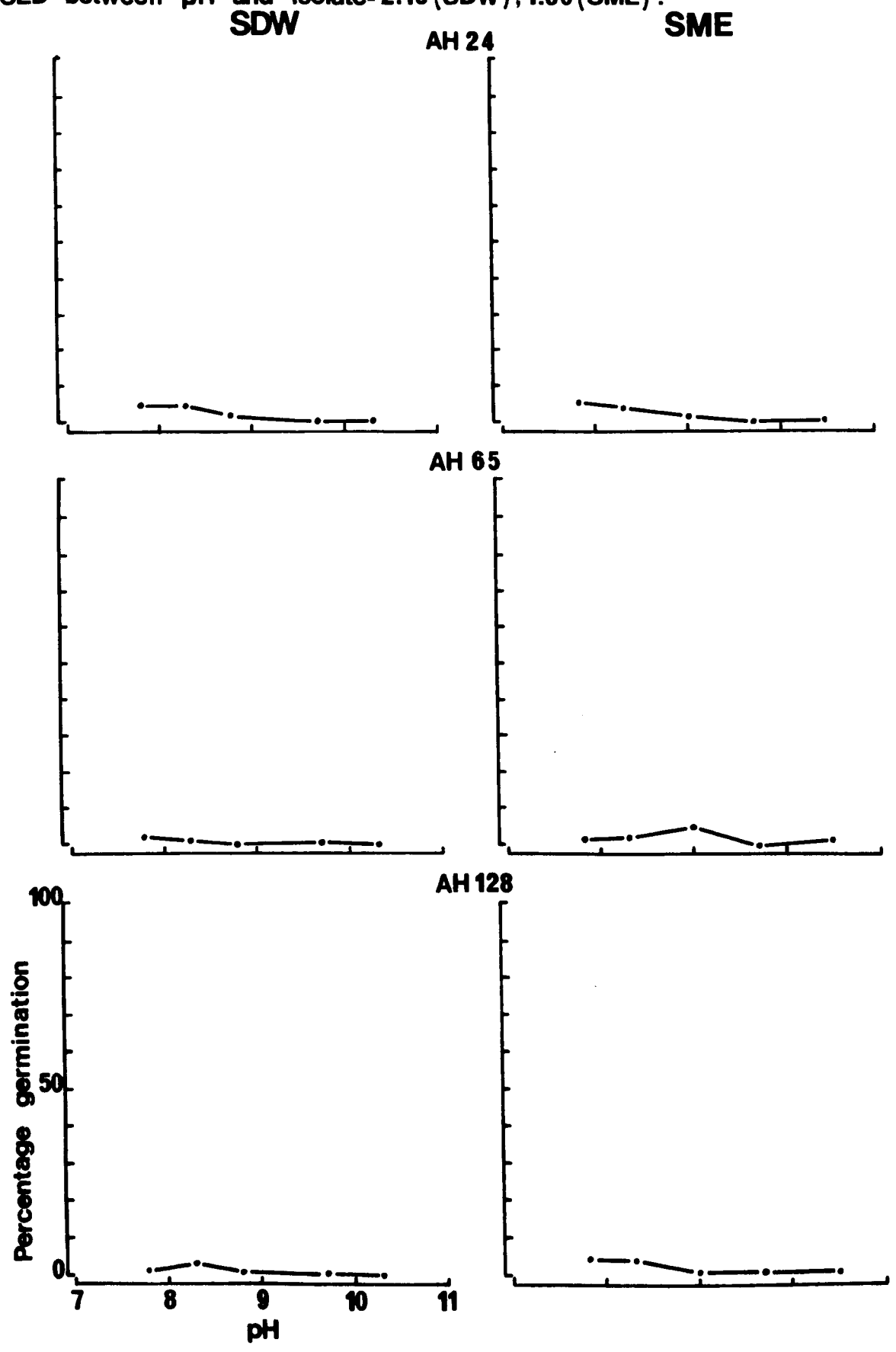


Figure 13

Effect of pH on the germination of* Pyrenochaeta lycopersici *conidia: Sodium carbamate / sodium hydrogen carbamate buffer.

SED between pH and isolate - 2.10 (SDW) , 1.60 (SME).

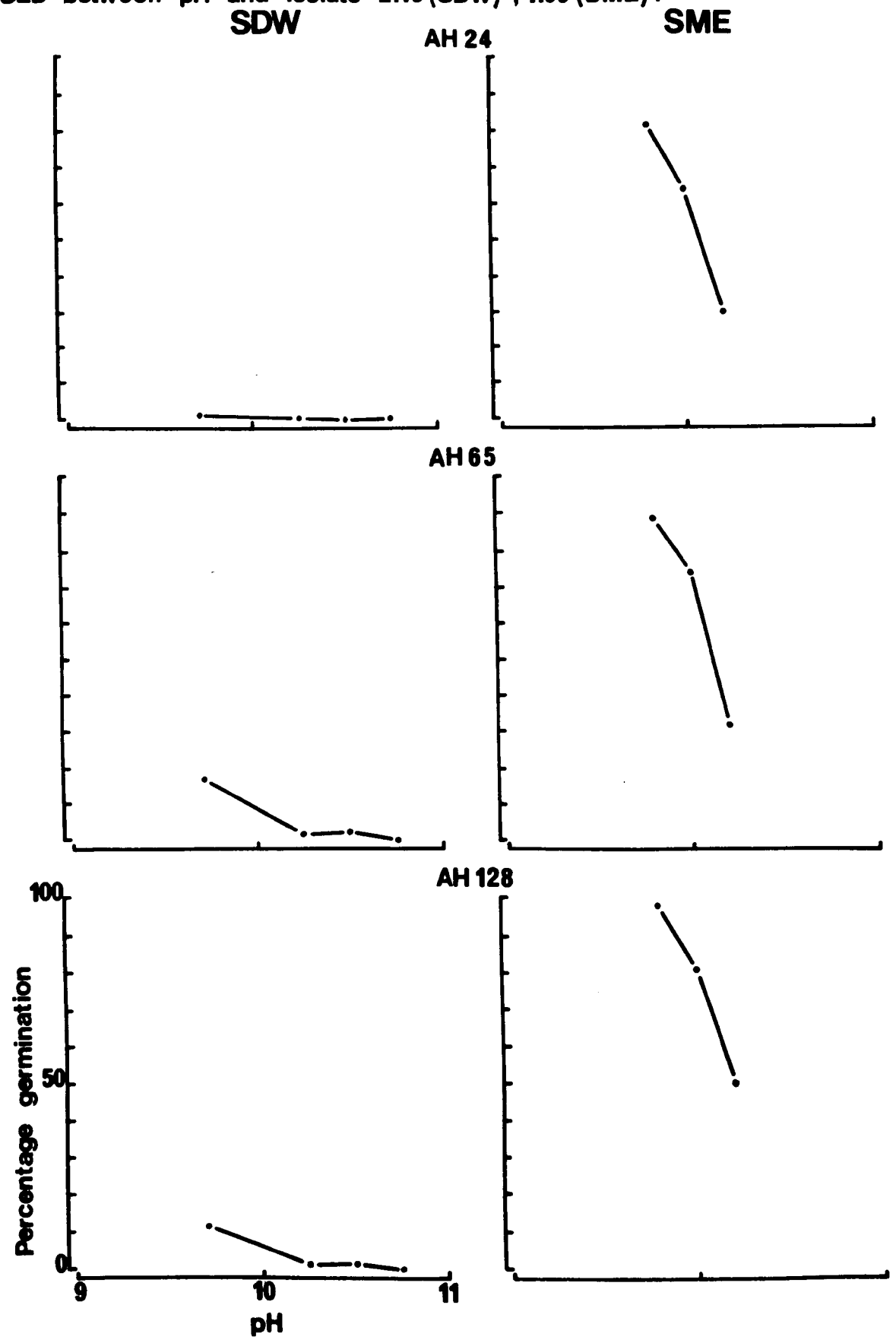


Figure 14

Effect of different buffers on the germination of* Pyrenochaeta lycopersici *conidia.

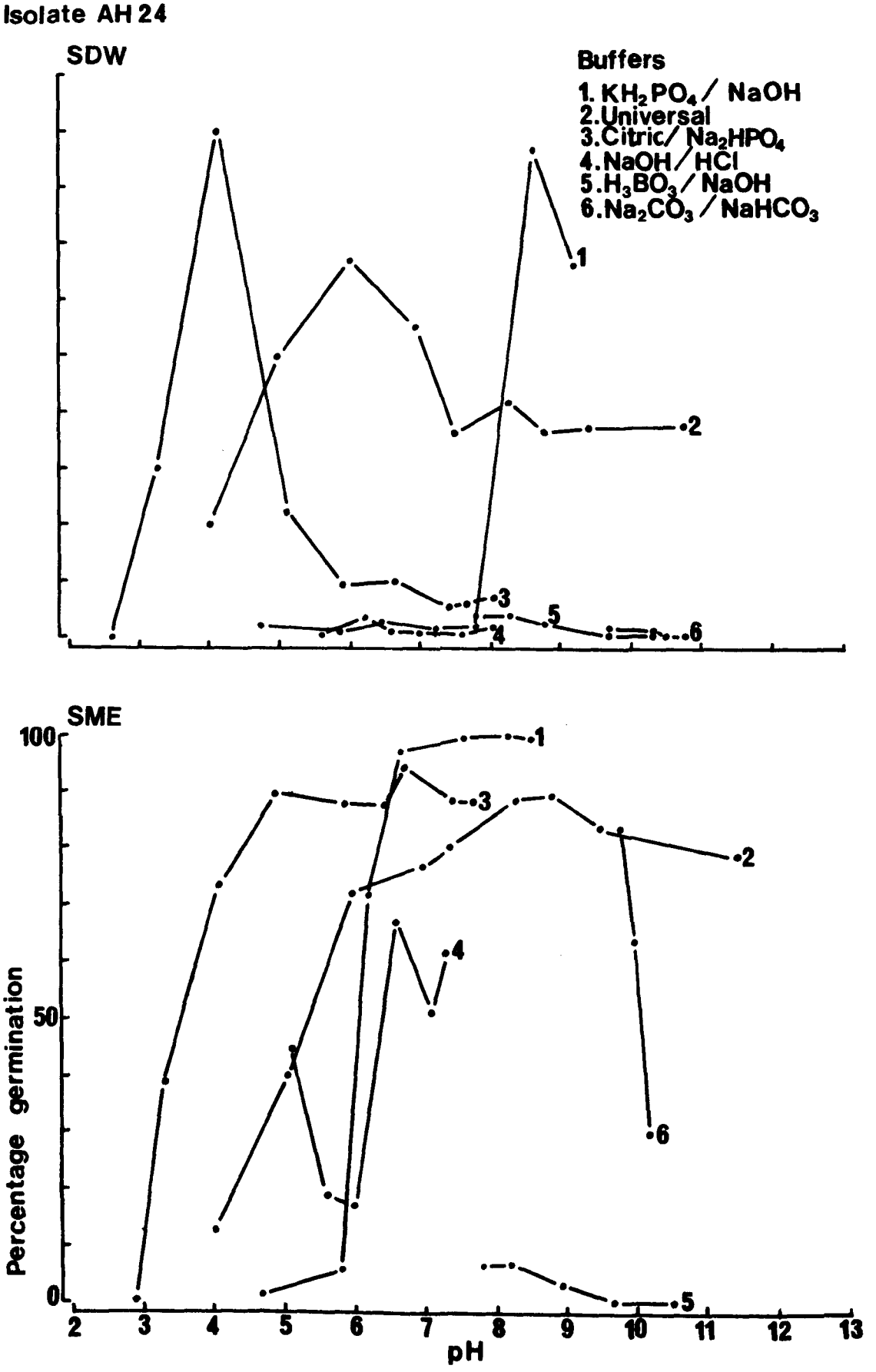


Table 32

Effect of light on the germination of **Pyrenochaeta lycopersici** *conidia*

Light treatment	Percentage conidial germination					
	AH 24		AH 65		AH 128	
	SDW	SME	SDW	SME	SDW	SME
WL full	81.0	99.4	79.2	100.0	85.0	100.0
BL full	94.4	100.0	85.2	99.0	87.0	98.6
WL 16 on/8 off	77.4	100.0	82.0	100.0	85.6	100.0
BL 16 on/8 off	79.6	97.0	77.0	99.2	87.4	100.0
WL 8 off/16 on	93.8	100.0	80.6	98.8	83.4	100.0
BL 8 off/16 on	90.8	100.0	83.6	98.2	81.4	94.4
Dark	89.2	100.0	80.0	99.6	85.0	100.0

SED between light treatment, isolate and nutrients = 1.14.

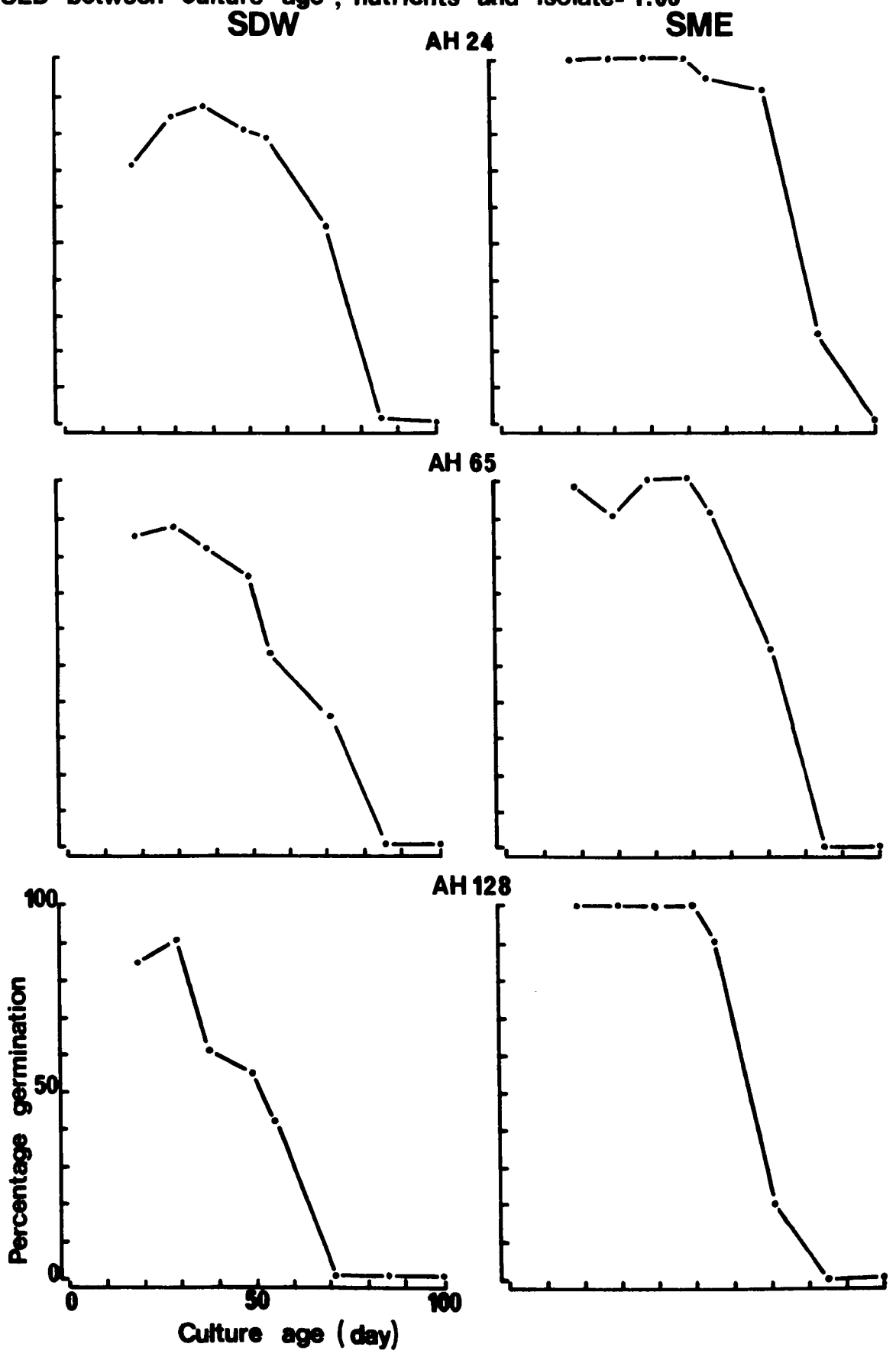
WL White light

BL Black light

x/y photoperiod (h)

Figure 15

***Effect of culture age on the germination of* Pyrenochaeta lycopersici *conidia* (*unwashed*).**



increased the level of germination in conidia taken from cultures aged up to 80 days. Variability in germination between isolates was observed. For each isolate, germination levels of washed conidia in SDW were significantly lower than those of unwashed conidia (Figure 16). Resuspension of washed conidia in a dilute cirrus extract gave similar germination levels to unwashed conidia (Figure 17). Addition of external nutrients reduced the effects of SDW and cirrus extract on conidial germination.

(f) Effect of the optical brightener Calcofluor White M2R

Conidial germination, in SDW, decreased as the concentration of the brightener increased (Figure 18). Germination, in the presence of external nutrients, was higher than that observed in SDW and not significantly affected by the brightener at concentrations $< 500 \mu g\ ml^{-1}$.

Blue-green and ultra-violet light were equally effective in inducing fluorescence (Table 33). Fluorescence from conidia incubated in SDW was greater than that observed from conidia incubated in SME. After one weeks incubation, at 5°C, fluorescence from conidia tended to decrease, in the absence of germination.

(g) Observations on conidial germination

Conidial swelling

Conidial width and length increased with the incubation period (Figure 19). Conidia increased in width by 95% (SDW) and 90% (SME) before germ tube emergence (12h). After 18h incubation,

Figure 16

Effect of culture age and conidial washing on the germination of* Pyrenochaeta lycopersici *conidia.

SED between culture age , nutrients and isolate = 1.03 (unwashed conidia) , 0.93 (washed conidia).

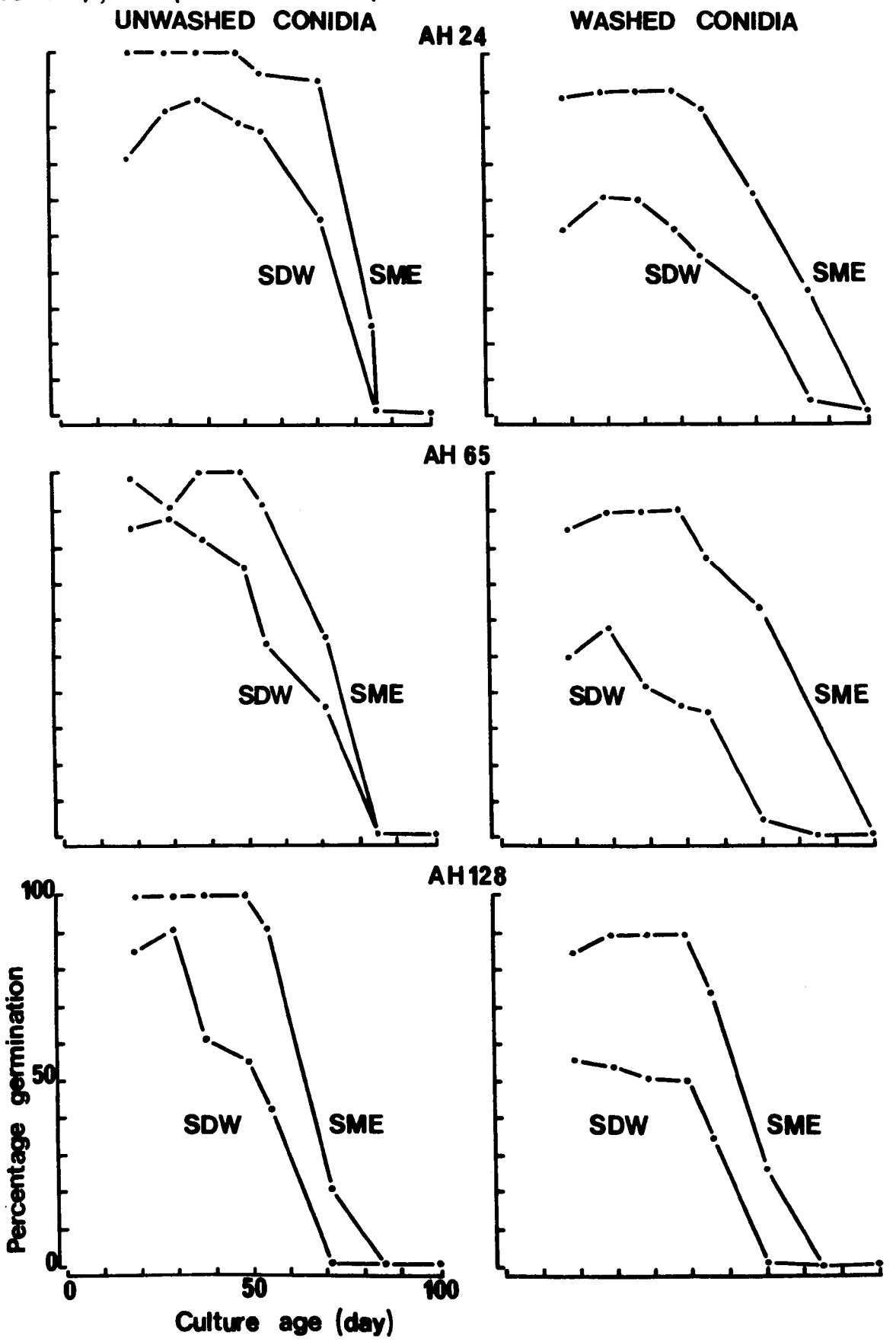


Figure 17

Effect of culture age and cirrus extract on the germination of* Pyrenochaeta lycopersici *conidia.

SED between culture age , nutrients and isolate=1.03 (unwashed conidia) , 0.93 (washed conidia).

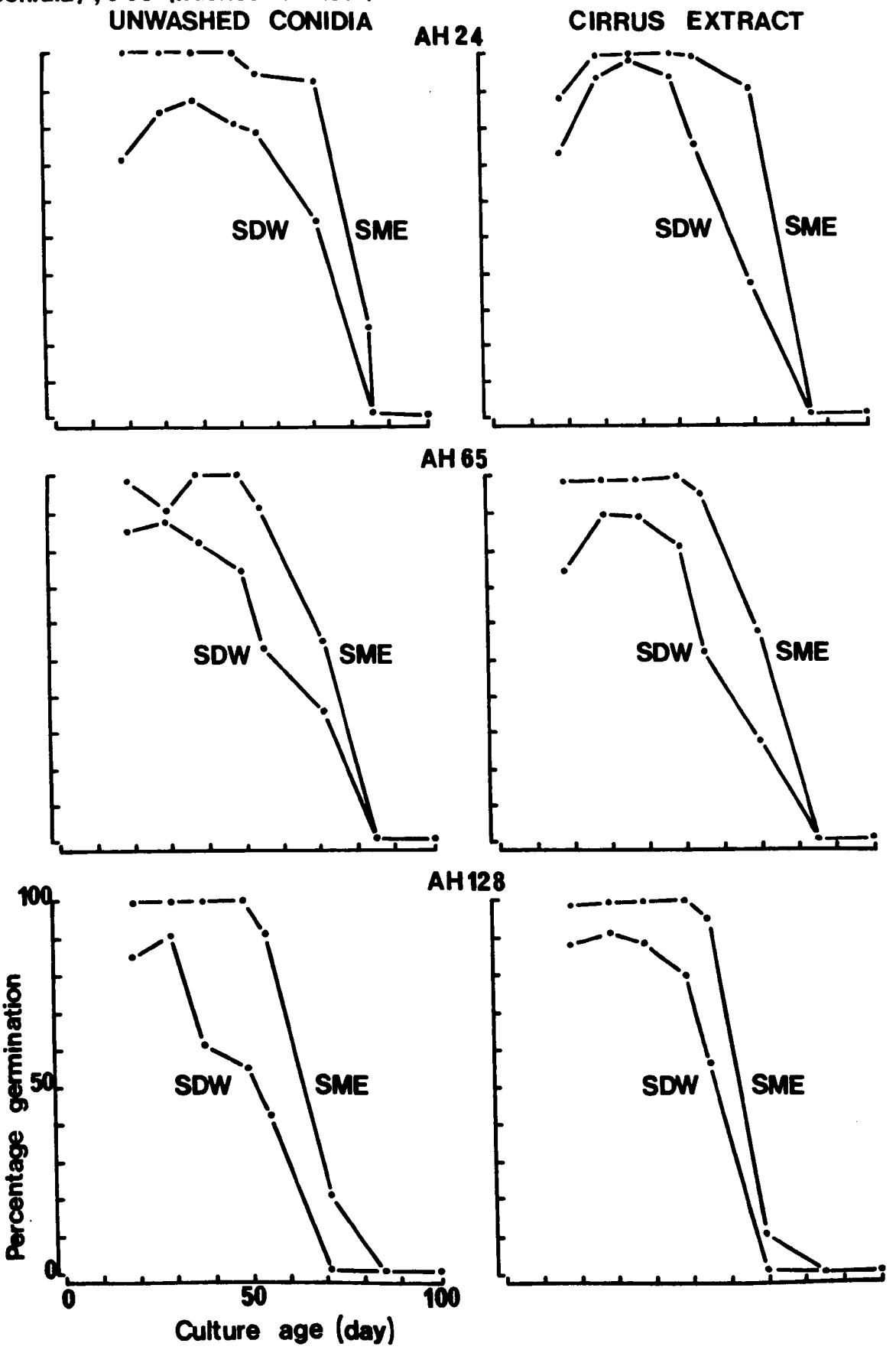


Figure 18

Effect of the optical brightener Calcofluor White M2R on the germination of* Pyrenochaeta lycopersici *conidia.

SED between brightener concentration, isolate and nutrients=0.811

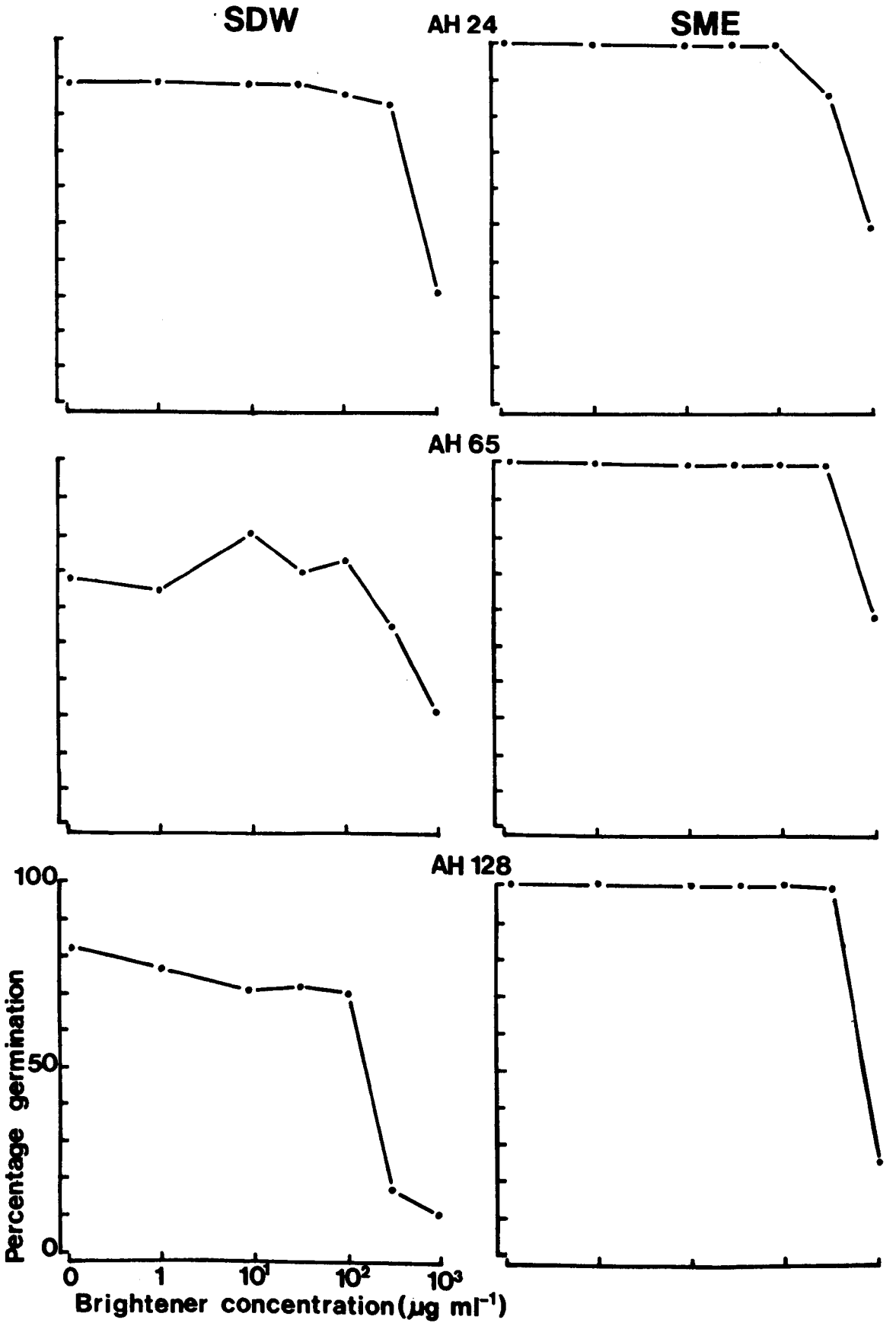


Table 33

Quality of fluorescence, stimulated by blue-green and ultra-violet light, from conidia of Pyrenochaeta lycopersici *treated with Calcofluor White M2R*

Isolate and CW concentration ($\mu g\ ml^{-1}$)	Quality of fluorescence from conidia*							
	Sterile distilled water				Sterile malt extract			
	Blue Green		Ultra-violet		Blue Green		Ultra-violet	
	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week
AH24								
0	-	-	-	-	-	-	-	-
1	+	+	+	+	0	0	0	0
10	++	++	++	+++	0	0	0	0
50	++	++	++	++	+	0	+	0
100	+++	+++	+++	+++	+	0	++	0
500	+++	++	+++	+++	++	0	++	0
1000	+++	++	+++	++	++	0	++	0
AH65								
0	-	-	-	-	-	-	-	-
1	+	+	+	+	0	0	0	0
10	+	+	+	++	0	0	0	0
50	+	++	++	++	0	0	0	0
100	++	+++	++	+++	+	0	++	0
500	+++	+++	+++	+++	++	0	++	0
1000	+++	+++	+++	+++	++	0	++	0
AH128								
0	-	-	-	-	-	-	-	-
1	+	+	+	+	0	0	0	0
10	+	+	+	+	0	0	0	0
50	+	++	+	++	0	0	+	0
100	++	++	++	++	+	0	+	0
500	+++	++	+++	++	++	0	++	0
1000	+++	++	+++	++	++	0	++	0

*Conidia incubated in SDW or SME for one day and/or one week at 5°C.

0 = No fluorescence

\+ = Faint fluorescence

++ = Good fluorescence

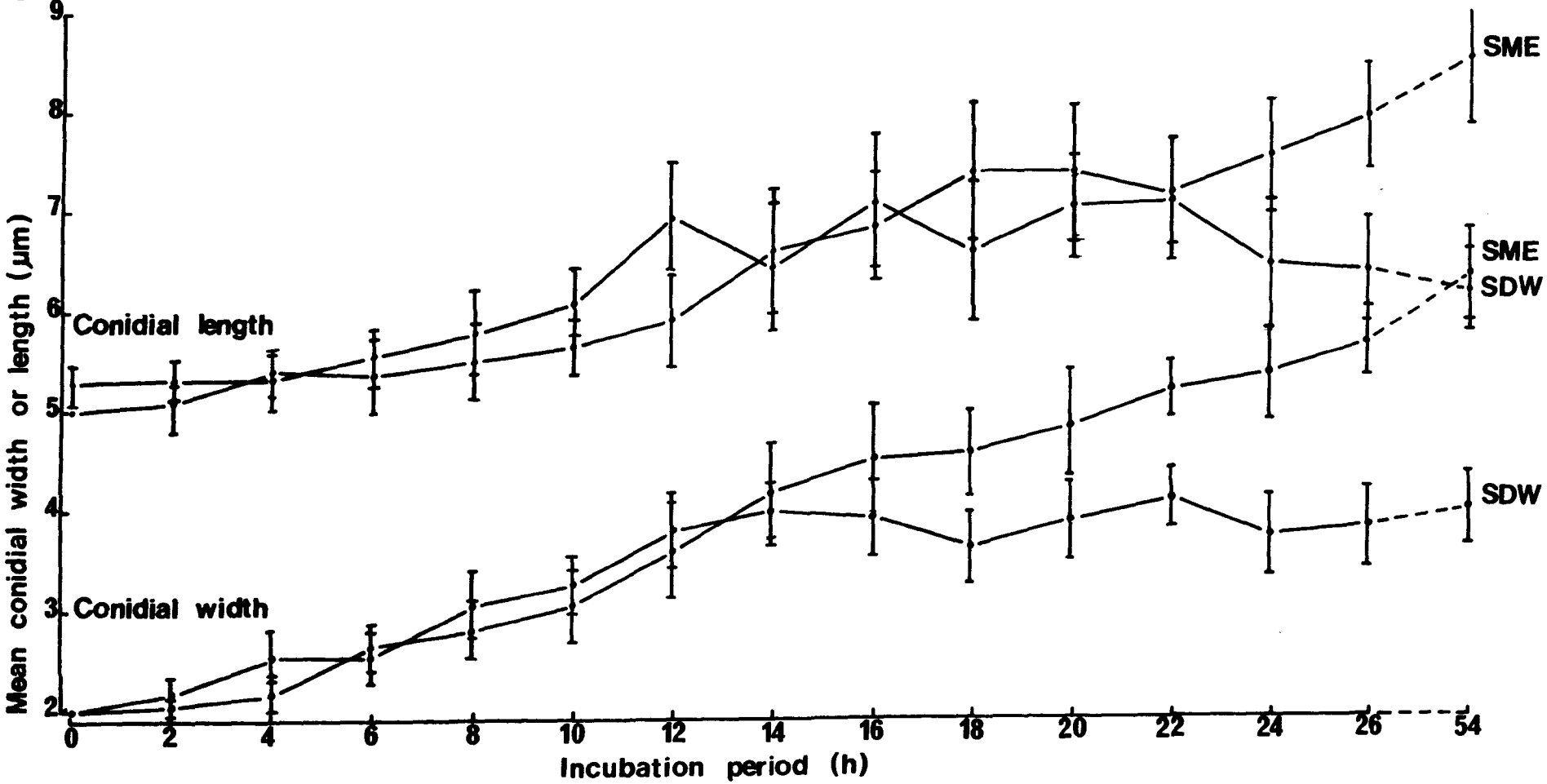
+++ = Strong fluorescence

CW Calcoflour White M2R

Figure 19

Effect of incubation period and nutrients on the width and length of* Pyrenochaeta lycopersici *conidia.

(95% confidence limits displayed).



conidia suspended in SME were significantly wider than those in SDW. Increases in conidial length were lower and more variable than increases in conidial width.

General observations

Ungerminated conidia had average dimensions (5.26 x 1.72 μm) falling within the range reported by Schneider and Gerlach (1966). All conidia were single celled and contained one or more oil droplets (Plate 7). Prior to germination, many conidia became swollen (Plate 8) and occasionally two celled. Germination normally gave rise to one germ tube (Plates 9 and 10). Production of dual germ tubes occurred erratically at all temperatures forming from the ends or one end and a side of the conidium (Plate 11). Two celled conidia did not necessarily give rise to two germ tubes. Germ tubes could branch immediately after emergence. Conidia, within the pycnidium or cirrus tendril, tended to remain ungerminated.

1.5 *Pathogenicity of* **Pyrenochaeta lycopersici** *conidia*

(a) Conidial pathogenicity in sterilised compost

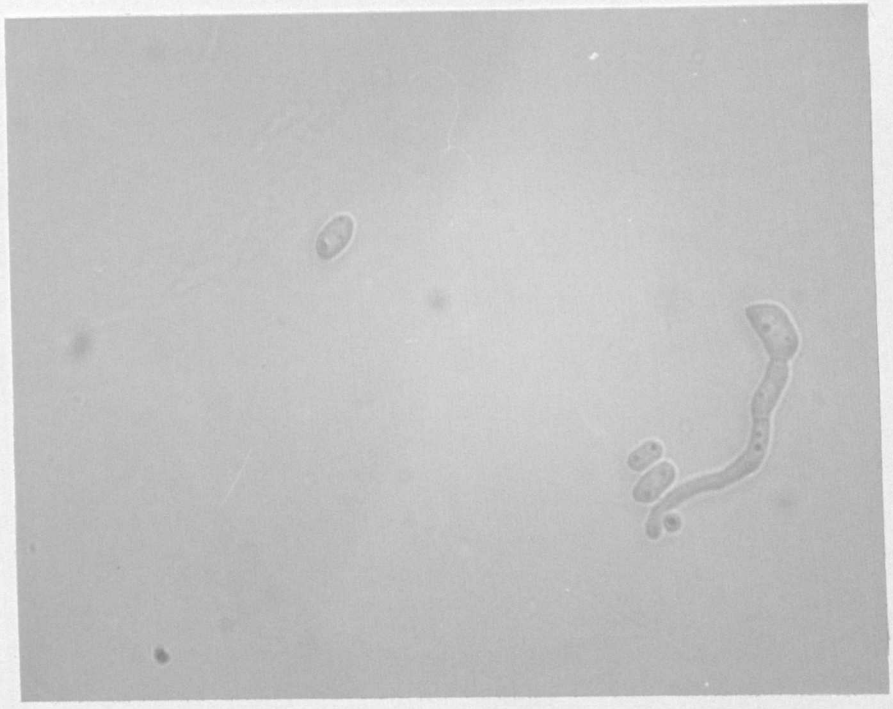
Root lesions were formed on tomato seedlings grown in sterile compost infested with *P.lycopersici* conidia (Figure 20). Four weeks after sowing, lesions developed on the roots of seedlings grown in composts containing conidial dilutions greater than 5 x 10^1 conidia per ml. Grey sterile fungi were isolated from all but the 5 x 10^1 conidia per ml dilution and control treatments. Greater lesion numbers were observed in a repeat experiment (Figure 20) and after four weeks growth lesions were

Plate 7

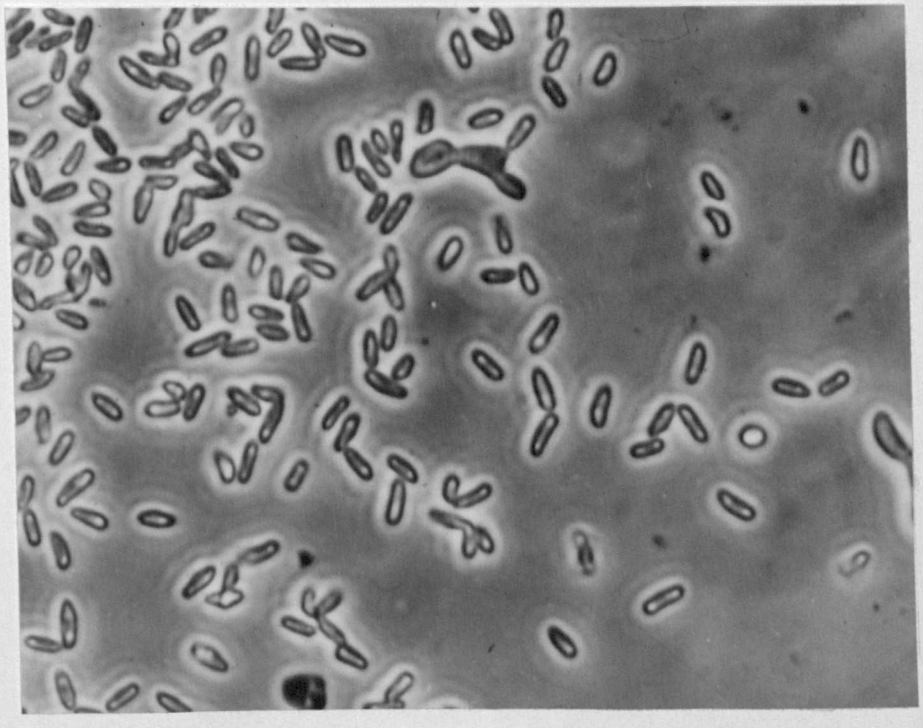
Plate 8

Plate 7 *Conidia of* Pyrenochaeta lycopersici *exhibiting characteristic oil droplets*

Plate 8 *Unswollen and swollen conidia of* Pyrenochaeta lycopersici



25μm



25μm

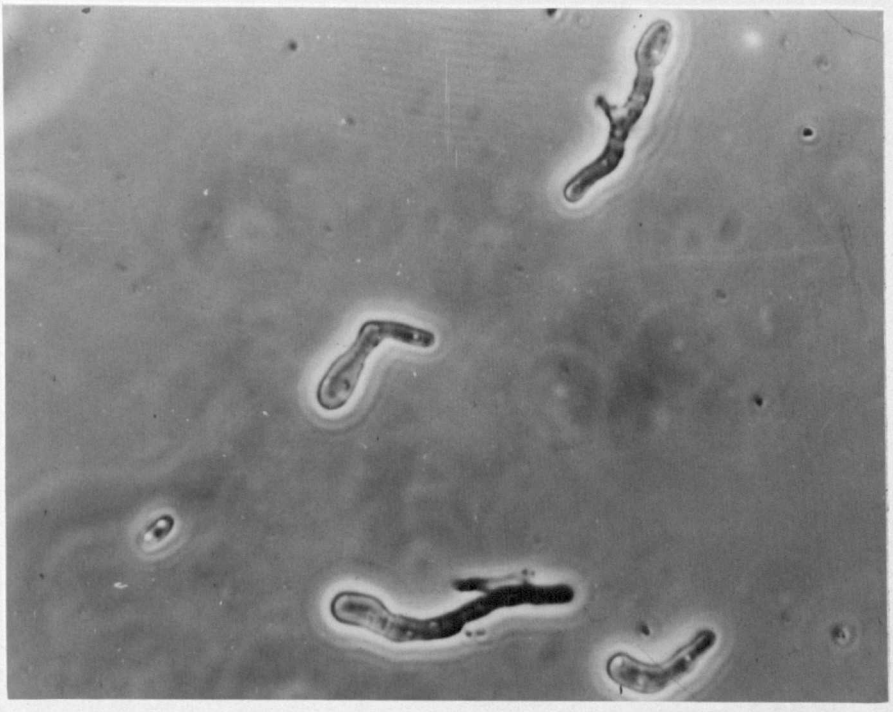
Phase positive

Plate 9

Plate 10

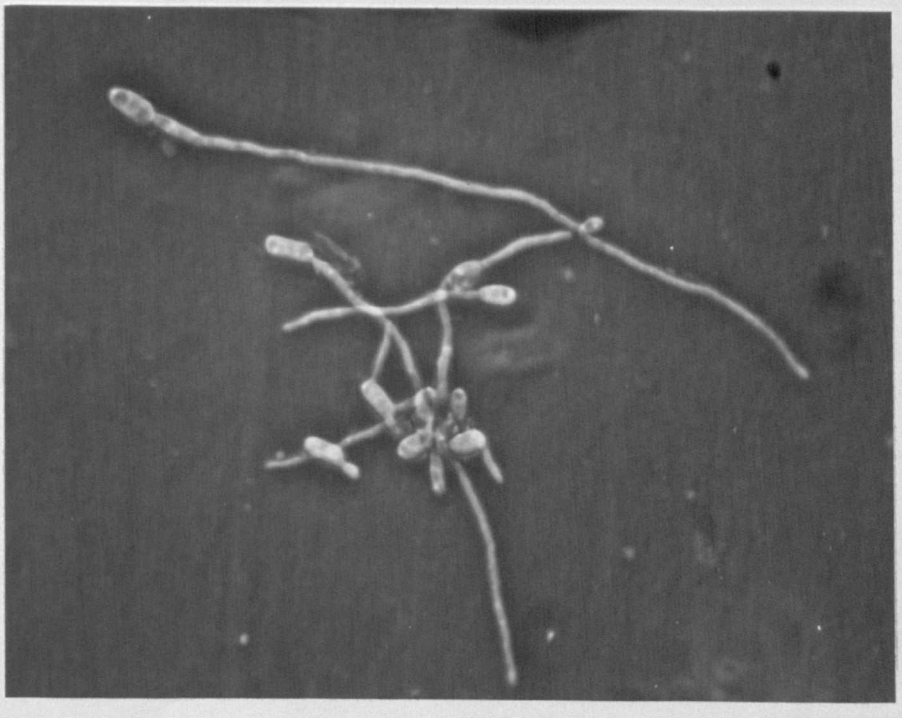
Plate 9
Plate 10

Germination of Pyrenochaeta lycopersici
conidia in sterile malt extract



25µm

Phase positive



25µm

Phase positive

Plate 11

Plate 11

Production of dual germ tubes by Pyrenochaeta lycopersici *conidia.*

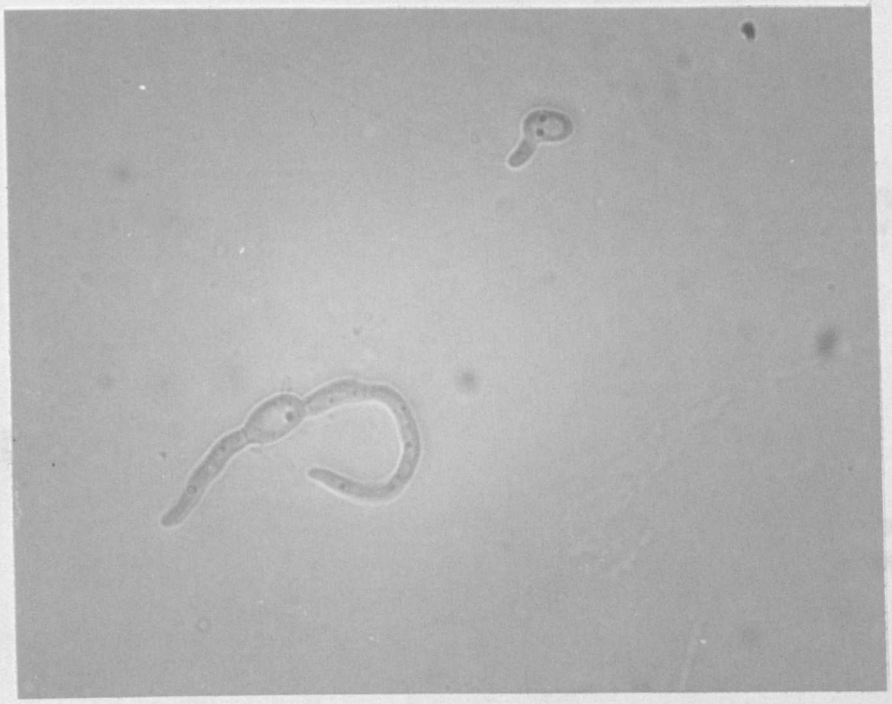
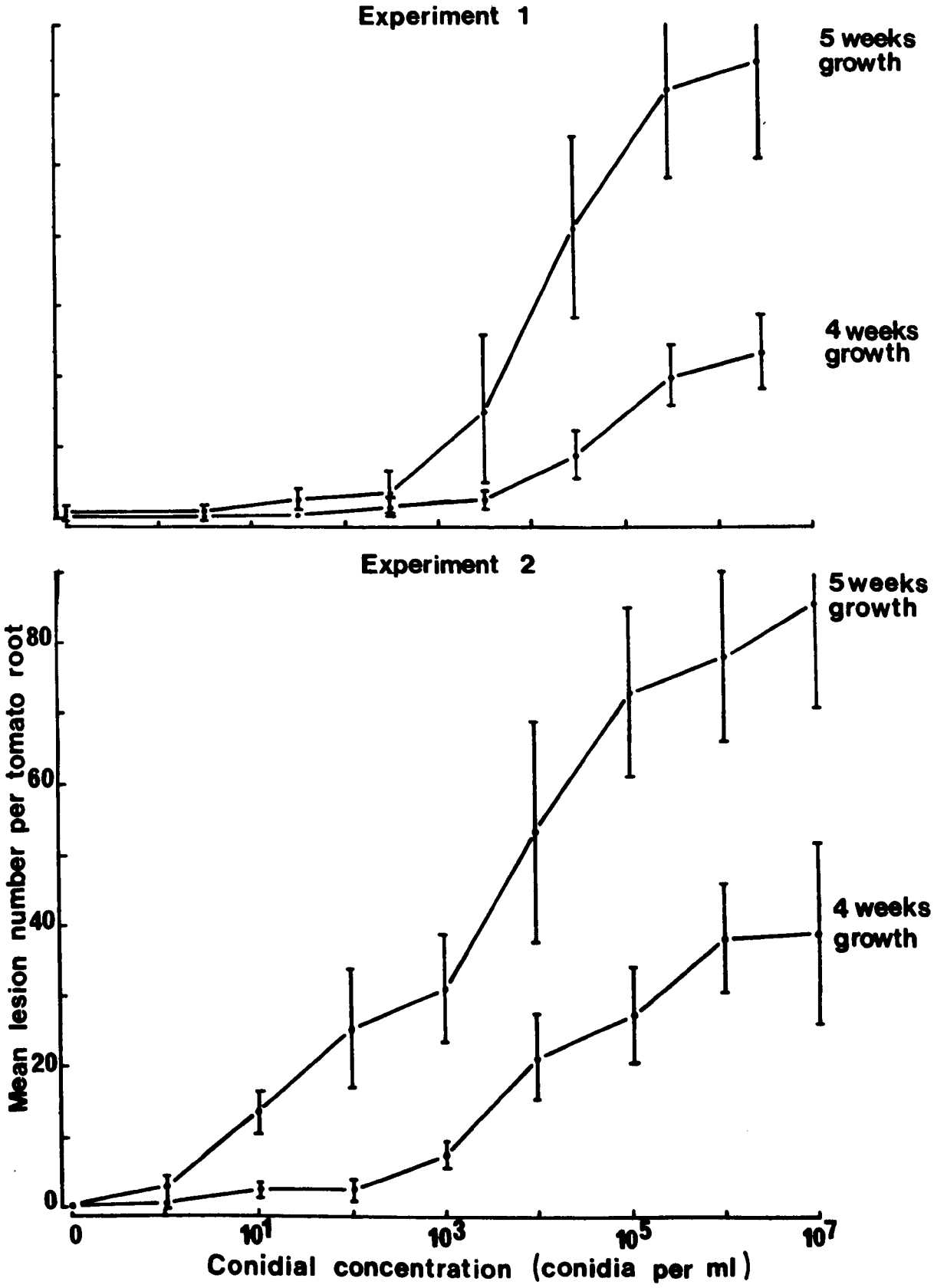

10µm

Figure 20

Number of root lesions produced on tomato plants grown for four and five weeks in sterilised compost infested with* Pyrenochaeta lycopersici *conidia.

(95% Confidence limits displayed)



observed in all treatments. In the second experiment GSF were isolated in treatments greater than 10 conidia per ml. *Pyrenochaeta lycopersici* was identified as the causal pathogen.

(b) Conidial pathogenicity in unsterilised garden soil

Root lesions were observed on tomato seedlings grown in unsterilised garden soil infested with *P.lycopersici* conidia. After five weeks lesions developed on the roots of seedlings grown in soil containing conidial dilutions greater than 5 x 10^2 conidia per ml (Figure 21). After six weeks an increase in lesion numbers per root was observed and GSF were isolated from treatments greater than the 5 x 10^2 conidia per ml dilution. *Pyrenochaeta lycopersici* was identified as the causal pathogen.

(c) Conidial pathogenicity in a tomato crop grown under commercial conditions

In 1983 symptoms of BRR were observed in all treatments and at all sampling dates (Table 34). Levels of BRR were initially low in control plots, however symptoms developed as the season progressed. In treatments 2(+,+) and 3(-,+) a high level of BRR was observed throughout the season. No symptoms of corkiness were apparent at seven weeks although corkiness had developed on many plants in all treatments 15 weeks after transplanting. A "background" level of infection was detected in G.C.R.I. field and glasshouse soils and symptoms of corkiness were particularly apparent in these treatments. Stem base lesions were observed in all treatments except the control. Root size and the amount of fine roots was lower in all treatments except the control. Yields from tomatoes grown

Figure 21

Number of root lesions produced on tomato plants grown for four, five and six weeks in garden soil infested with **Pyrenochaeta lycopersici** ***conidia.***

(95%Confidence limits displayed).

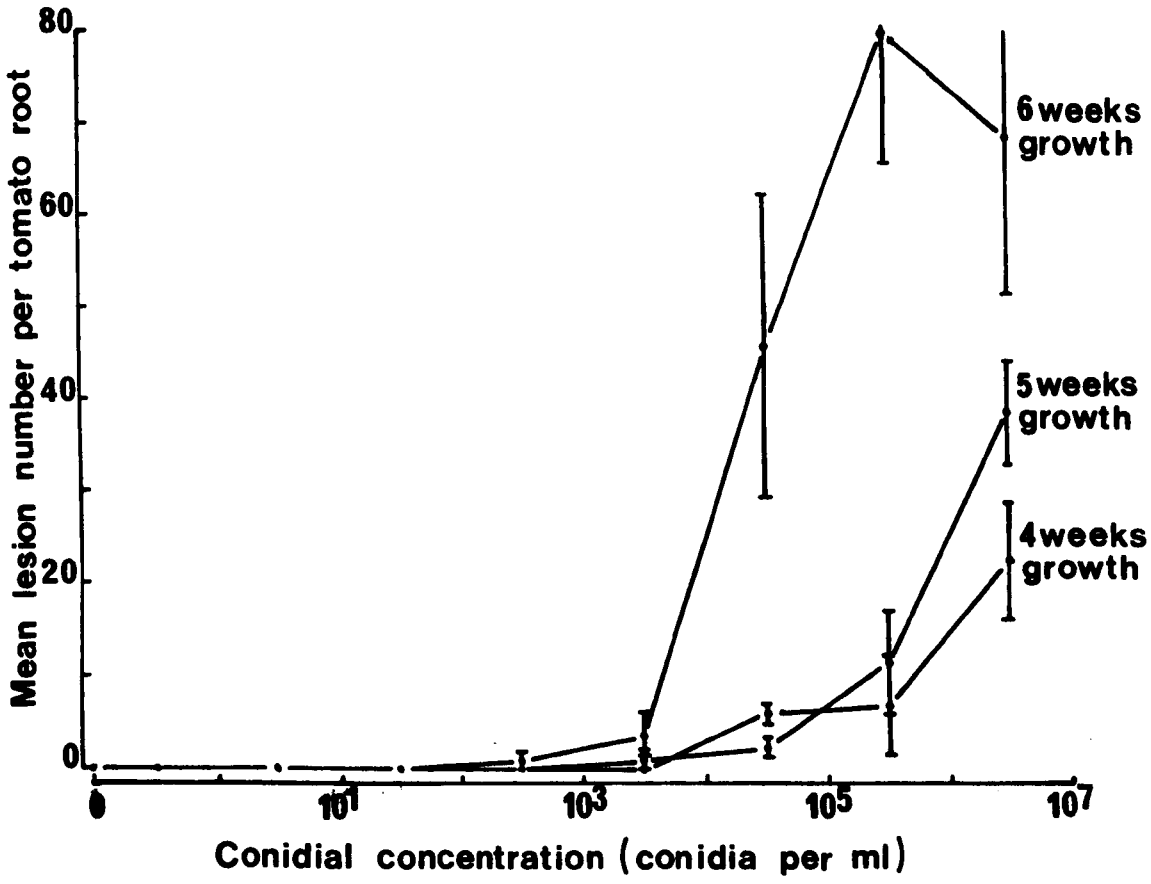


Table 34

Pathogenicity of Pyrenochaeta lycopersici *conidia in a tomato crop grown under commercial conditions. I: tomato root symptoms 7, 15 and 21 weeks after transplanting into non-infested and conidial infested soils during 1983*

Treatment / Weeks after trans-planting	Root Symptoms							
	Brown Root Rot*			Corkiness*			Root* Size	Quantity* fine roots
	7	15	21	7	15	21	21	21
1 (control)	0.4(0.6)	1.8(0.8)	1.4(0.6)	0.0(0.0)	1.8(1.6)	1.2(1.1)	2.6 (0.6)	2.6(0.9)
2 (+,+)	2.8(0.5)	1.4(0.6)	2.6(0.6)	0.0(0.0)	0.4(0.6)	1.4(0.6)	2.0 (1.0)	2.2(0.5)
3 (-,+)	2.8(1.8)	2.0(1.0)	1.6(0.6)	0.0(0.0)	1.6(1.3)	1.0(0.7)	1.8 (0.8)	1.6(0.6)
4F(+,+)	2.8(0.5)	1.3(0.5)	1.8(0.5)	0.0(0.0)	1.3(1.9)	1.3(1.3)	1.75(0.5)	2.0(0.8)
4G(+,+)	2.8(0.5)	1.5(0.6)	3.0(0.0)	0.0(0.0)	2.3(1.0)	2.3(2.1)	2.3 (1.0)	2.5(0.6)
4F(control)	3.0(0.0)	3.0(0.0)	2.0(0.0)	0.0(0.0)	0.0(0.0)	1.0(0.0)	2.0 (0.0)	2.0(0.0)
4G(control)	3.0(0.0)	3.0(0.0)	2.5(0.7)	0.0(0.0)	2.0(1.4)	3.5(0.7)	2.5 (0.7)	2.5(0.7)

* assessed using arbitrary indexes (Table 9) (S.D.).

F = Field soil

G = Glasshouse soil

First number in parenthesis represents + or - conidial inoculum in the propagation compost

Second number in parenthesis represents + or - conidial inoculum in the plot compost

in conidial infested compost were lower than those from the control, however no significant differences between treatments were observed (Table 35).

In 1984 a similar pattern of symptom development was observed (Table 36) although the level of disease was generally lower than that observed in 1983. Lower disease levels were incurred in the control treatment. The level of BRR on tomatoes grown in compost infested with conidia in 1983 (treatment 2) was similar to that observed on tomatoes grown in compost infested with conidia in 1984 (treatments 3 and 4). Root size in treatment 2 was significantly lower than that observed in the control and treatments 3 and 4. The mean number of trusses, fruit set and flowering per plant were significantly lower in this treatment, seven weeks after transplanting, whilst data from the control and treatments 3 and 4 were similar (Table 37).

Occasional pycnidia were observed on BRR infested tomato roots incubated for six to ten weeks under black light, daylight or dark conditions at room temperature.

1.6 *Behaviour of* **Pyrenochaeta lycopersici** *conidia in soil*

(a) Survival of conidial inoculum in sterilised compost and unsterilised garden soil

Lesions were observed on plants grown in conidial infested compost and garden soil, stored for varying time periods, at room temperature, in the dark (Figures 22 and 23). As the storage period increased the number of lesions per root tended

Table 35

Pathogenicity of **Pyrenochaeta lycopersici** *conidia in a tomato crop grown under commercial conditions. II: Accumulated yields from tomato plants transplanted into non-infested and conidial infested composts during 1983.*

Treatment	Accumulated yield (g)*	Mean accumulated yield, (g) (S.D.)
1 (control)	6262, 6590, 7100, 7701, 9333, 9412	7733 (1360.2)
2 (+,+)	5077, 6528, 6623, 6837, 7143, 7878	6681 (924.1)
3 (-,+)	5660, 6509, 7003, 7284, 7428, 7542	6904 (712.7)

* Yield data collected between 22.7.83 and 4.10.83

First number in parenthesis represents + or - conidial inoculum in the propagation compost.

Second number in parenthesis represents + or - conidial inoculum in the plot compost.

Table 36

Pathogenicity of Pyrenochaeta lycopersici *conidia in a tomato crop grown under commercial conditions.III: tomato root symptoms 7, 12 and 18 weeks after transplanting into non-infested and conidial infested soils during 1984.*

Treatment	Root symptoms						Root* size	Quantity* fine roots
	Brown root rot*			Corkiness*				
Weeks after transplanting	7	12	18	7	12	18		
1 (control)	0.00 (0.00)	0.00 (0.00)	1.09 (0.30)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.45 (0.69)	2.91 (0.54)
2 (-,+)	1.55 (0.69)	1.64 (0.81)	1.91 (0.54)	0.00 (0.00)	0.00 (0.00)	0.09 (0.30)	1.27 (0.47)	0.91 (0.83)
3 (+,+)	1.46 (0.52)	2.36 (0.81)	1.45 (0.52)	0.00 (0.00)	0.00 (0.00)	0.27 (0.47)	1.82 (0.41)	2.09 (1.45)
4 (-,+)	0.42 (0.52)	2.36 (0.67)	2.00 (0.89)	0.00 (0.00)	0.00 (0.00)	0.82 (0.98)	1.73 (0.47)	1.36 (0.92)

* assessed using arbitrary indexes (Table 9) (S.D.).

First number in parenthesis represents + or - conidial inoculum in propagation compost.

Second number in parenthesis represents + or - conidial inoculum in plot compost.

Table 37

Pathogenicity of **Pyrenochaeta lycopersici** *conidia in a tomato crop grown under commercial conditions. IV: number of trusses formed and fruit set on tomatoes seven weeks after transplanting into non-infested and conidial infested composts during 1984.*

Treatment	Mean number of * fruit per plant (S.D.)	Mean number of* flowers per plant (S.D.)	Mean number of* trusses per plant (S.D.)
1 (control)	45.6 (9.4)	74.5 (15.4)	5.9 (0.7)
2 (-,+)	26.3 (6.7)	36.0 (8.9)	3.9 (0.7)
3 (+,+)	47.5 (2.7)	77.8 (14.9)	6.1 (0.8)
4 (-,+)	46.9 (11.9)	77.0 (16.3)	6.2 (0.6)

* Mean of 11 plants per treatment.

First number in parenthesis represents + or - conidial inoculum in the propagation compost.

Second number in parenthesis represents + or - conidial inoculum in the plot compost.

Figure 22

Effect of storage on the inoculum potential of sterilised compost infested with* Pyrenochaeta lycopersici *conidia.

(95%confidence limits displayed)

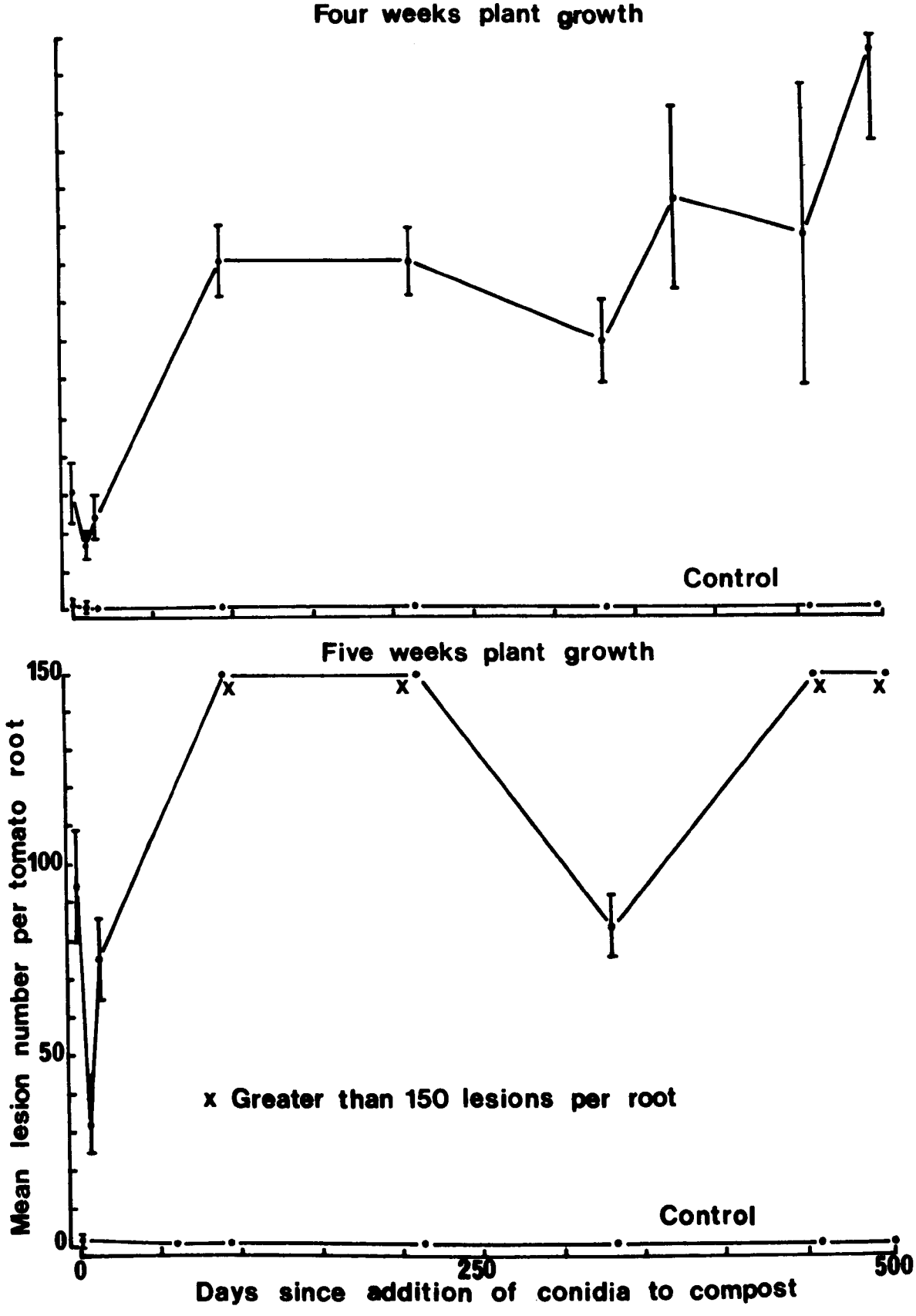
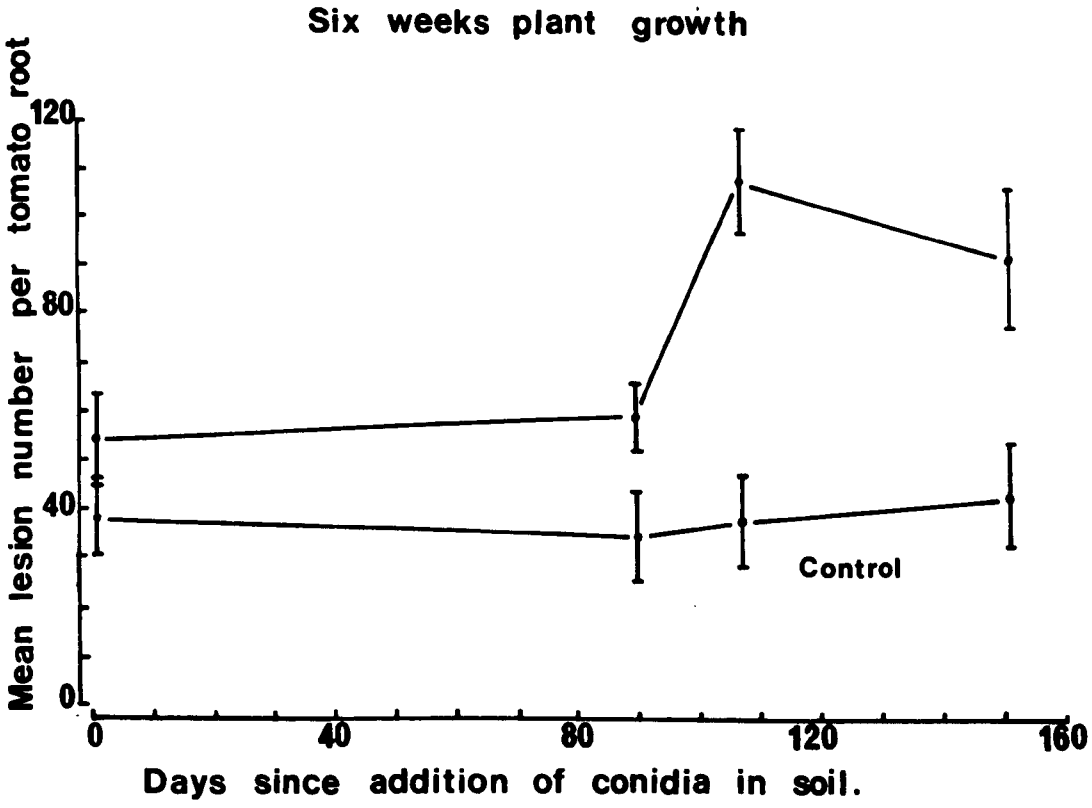


Figure 23

Effect of storage on the inoculum potential of garden soil infested with* Pyrenochaeta lycopersici *conidia.

(95% confidence limits displayed).



to increase. Greater lesion numbers were observed on the roots of tomatoes grown in conidial infested sterilised compost than in conidial infested garden soil. *Pyrenochaeta lycopersici* was identified as the causal pathogen. No fungi were isolated from the occasional lesions observed on tomato plants grown in the control compost. *Penicillium* and *Trichoderma* species were occasionally isolated from the lesions observed on tomato plants grown in uninfested garden soil (control).

(b) Germination and subsequent growth of *Pyrenochaeta lycopersici* conidia in various substrates

Conidial germination on cellophane

After one days incubation, conidial germination on cellophane was higher in control and sterilised compost treatments than in other substrates (Figure 24). After four days, little difference in germination was observed between control, sand, sterilised and unsterilised compost treatments. Soil infested with BRR and pH adjusted peat treatments had significantly lower germination levels (Figure 25). Conidia incubated in unsterilised peat exhibited almost complete inhibition of germination. However, conidia incubated in this treatment for three days and then placed at 22°C (control treatment) gave 100% germination within 24h. pH values of peat treatments were more acidic than those of other substrates (Table 38).

The "type" and quantity of mycelium and sclerotia varied between treatments (Table 39). A "frond" type mycelium (Plate 12), wider and containing more oil droplets than "normal" mycelium (Plate 13), developed after three to seven days

Figure 24

Germination of* Pyrenochaeta lycopersici *conidia on cellophane incubated in various substrates.

SED between incubation period and treatment = 1.95

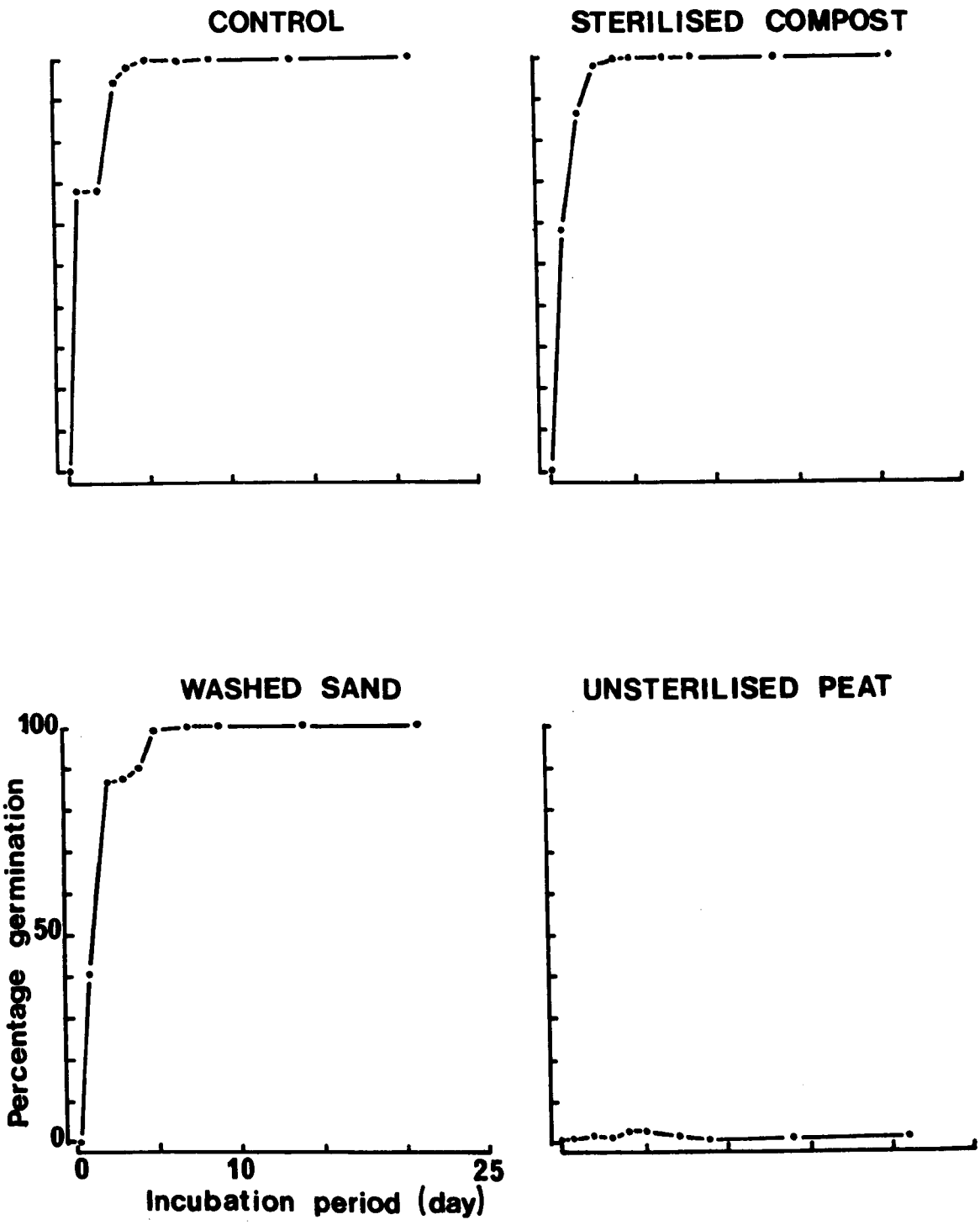


Figure 25

Germination of* Pyrenochaeta lycopersici *conidia on cellophane incubated in various substrates.

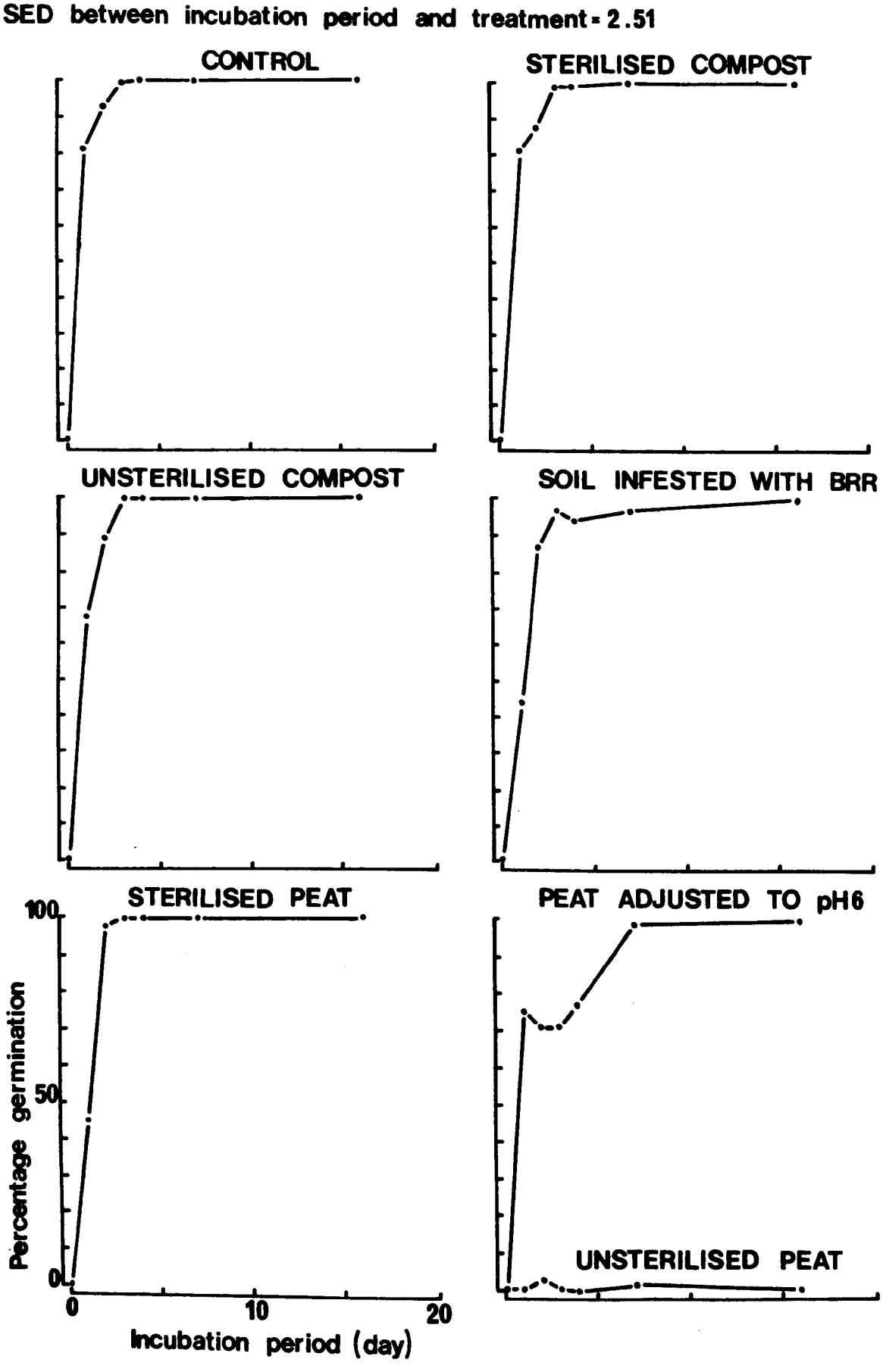


Table 38

pH of substrates used in experiment 1 and 2.

Experiment 1	pH*	Experiment 2	pH*
Sterilised compost	5.9	Sterilised compost	6.2
Washed sand	5.3	Unsterilised compost	6.1
Unsterilised peat	3.9	BRR infested soil	6.6
		Unsterilised peat	3.9
		Unsterilised peat, pH 6.0	5.5
		Sterilised peat	4.3

* measured after three days incubation.

Table 39

The behaviour of Pyrenochaeta lycopersici *conidia incubated in various substrates*

Treatment	Observation			
	Conidia	Normal mycelium	Fronded mycelium	Sclerotia
Control 22°C	100% germination after 4 days	Not extensive. Many oil droplets	Present after 7 days. Not extensive	Initials present 16 days. Some mature after 20 days. Not abundant
Sterilised compost	100% germination after 4 days	Extensive. Few oil droplets	Present after 3 days. Extensive, many oil droplets. Frond cushions present after 15 days	Initials formed after 5 days. Mature after 7 days. Abundant
Unsterilised compost	100% germination after 3 days	Extensive. Varying width, few oil droplets	Present after 3 days. Not extensive	Initials formed after 5 days. Mature after 7 days
Sand	100% germination after 7 days	Not extensive. Few oil droplets	Present after 4 days. Little branching. Many oil droplets. Thick hyphal wall	No sclerotia formed
BRR infested	100% germination after 16 days	Not extensive	Not extensive	Sclerotia initials after 16 days
Unsterilised peat	<3% germination after 16 days	No longer than conidial length	None	No sclerotia formed
Sterilised peat	100% germination after 3 days	Not extensive. Contorted, lobed wide, vacuolated. Few oil droplets.	None	No sclerotia formed
Unsterilised peat, pH 6.0	100% germination after 16 days	Not extensive. Wide, oil droplets	None	No sclerotia formed

Plate 12

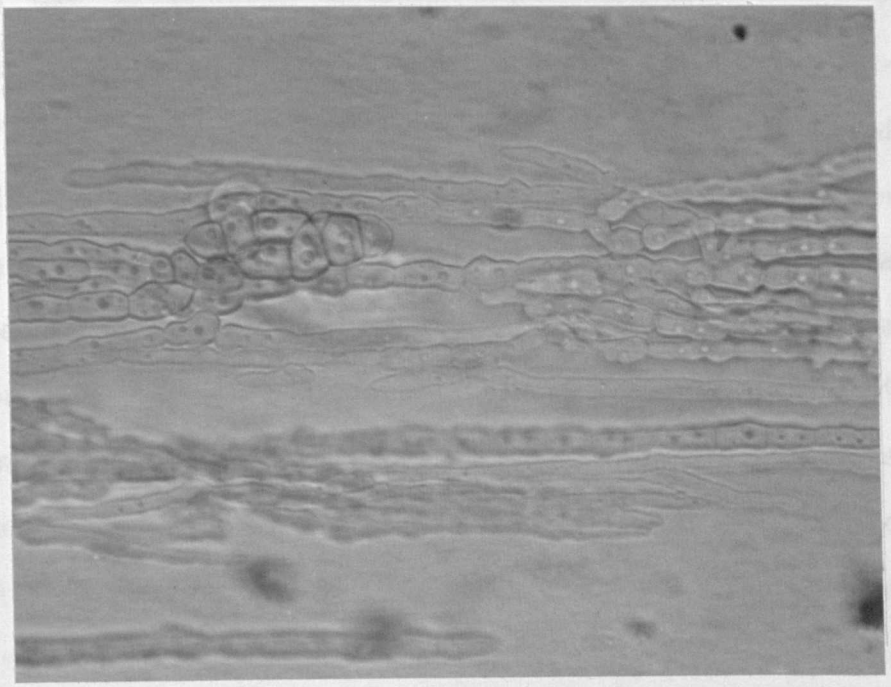
Plate 13

Plate 12

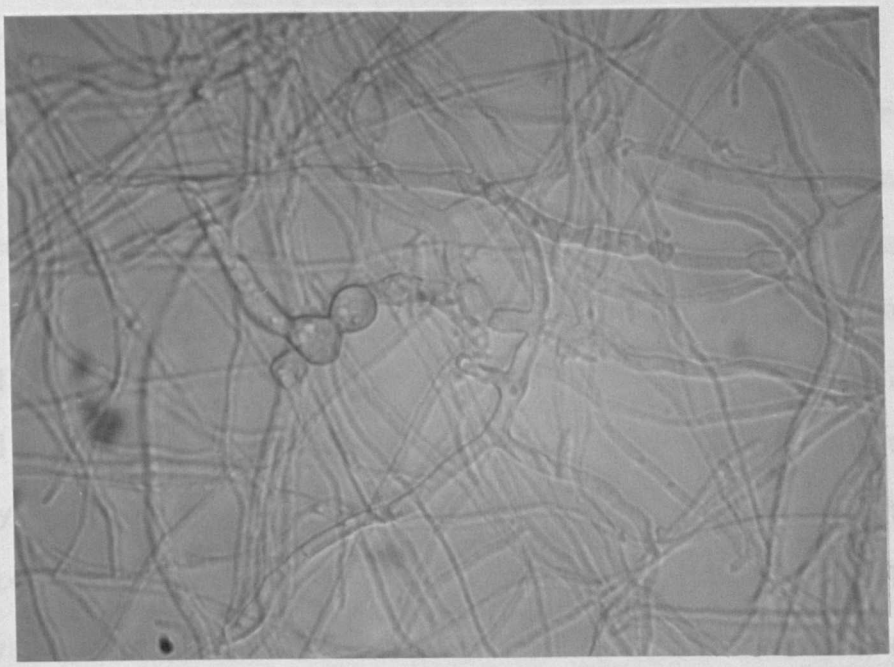
Production of frond mycelium from the conidia of Pyrenochaeta lycopersici *germinating on cellophane in sterilised compost*

Plate 13

Production of normal mycelium from the conidia of Pyrenochaeta lycopersici *germinating on cellophane in sterilised compost*



20µm



20µm

incubation in all treatments except peat soils. The frond mycelium was extensively branched and possessed a heavily convoluted cell wall. Occasionally frond mycelium developed into a "cushion" (Plate 14). Sclerotial initials were observed, after five to 16 days incubation, as a progressive budding and pigmentation of the cells, giving rise to mature sclerotia two to three days later. Prior to sclerotia initiation, hyphal cells broadened, vacuolated and darkened. Mature sclerotia showed no internal differentiation but were bounded by a layer of dense cells forming a rind (Plate 15). The majority of sclerotia were formed on the frond type mycelium, especially on hyphal cushions. Sclerotia matured most rapidly in compost treatments where they formed within seven days. No sclerotia formed in sand or peat treatments. Sclerotia formation in the control was slow and sparse.

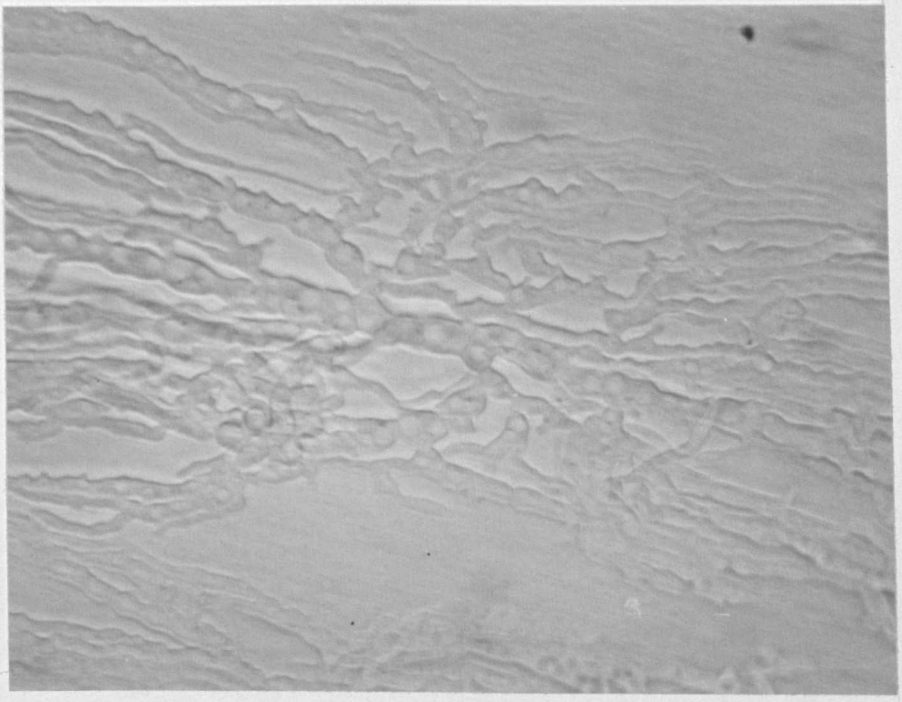
Conidial germination on polycarbonate membranes

After one days incubation, conidia incubated in sterilised compost and at 22°C (control) had swollen to form spherical structures at least double the size of original conidia. A few conidia (< 10%) had produced germ tubes. No mycelium was observed. Conidia incubated in unsterilised garden soil remained unswollen; no germination was observed. After two to four days incubation germinated conidia were observed in both substrates although levels were low (< 15%) in garden soil. Extensive mycelium (Plates 16 and 17) and the formation of sclerotia were observed within five and seven days respectively in compost and control treatments. Numbers of mature sclerotia (Plates 18 and 19), detected after nine days incubation in

Plate 14

Plate 14

Formation of a mycelial cushion from conidia of Pyrenochaeta lycopersici *germinating on cellophane in sterilised compost*

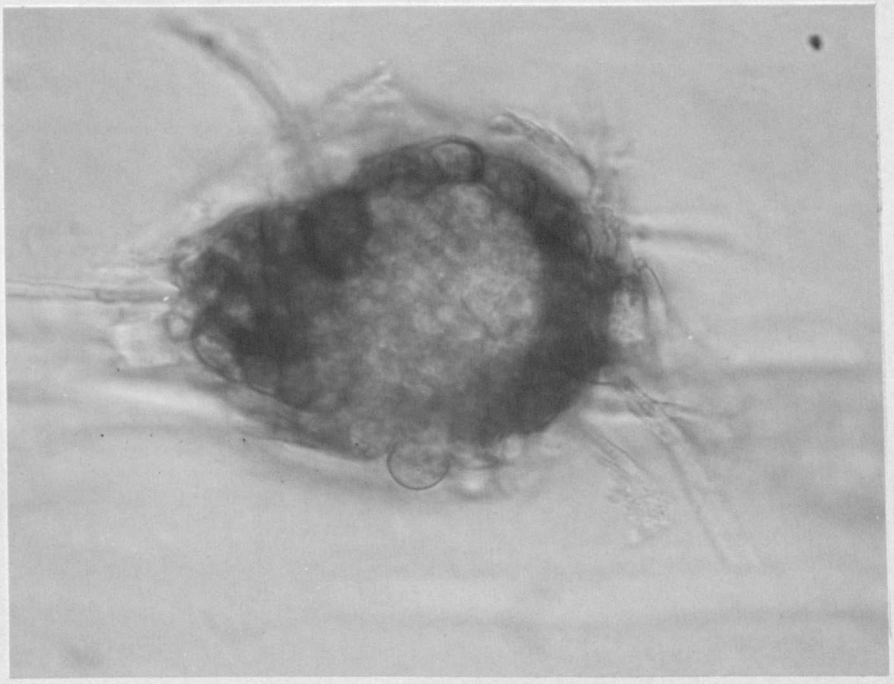


20μm

Plate 15

Plate 15

Formation of micro-sclerotia from the mycelium arising from the germination of Pyrenochaeta lycopersici *conidia incubated on cellophane in sterilised compost*



10µm

Plate 16

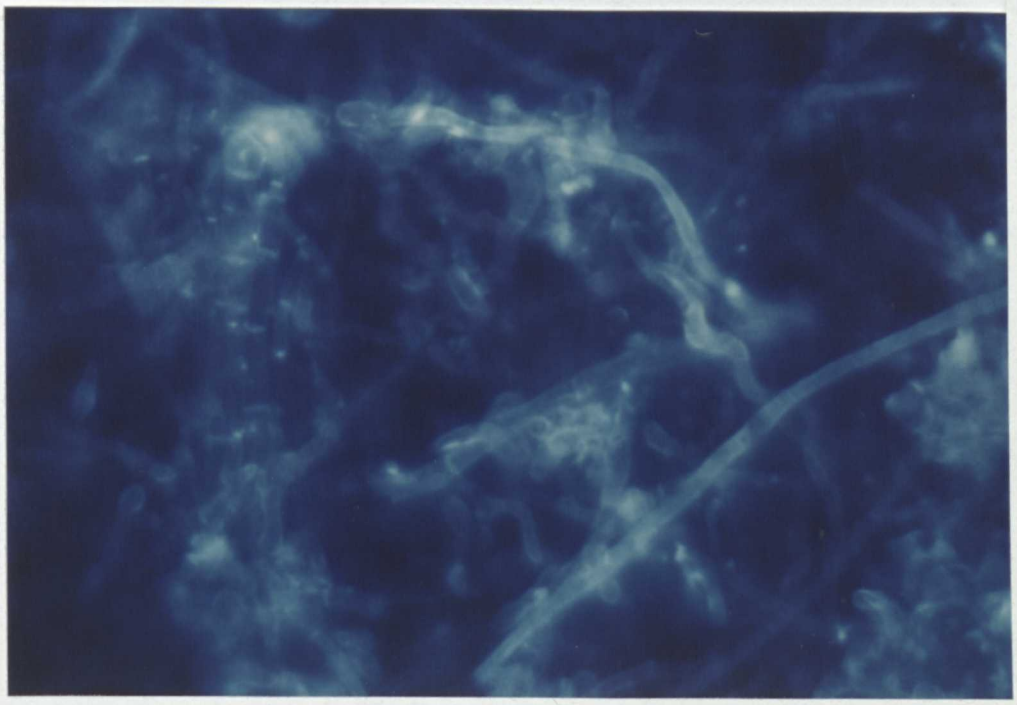
Plate 17

Plate 16

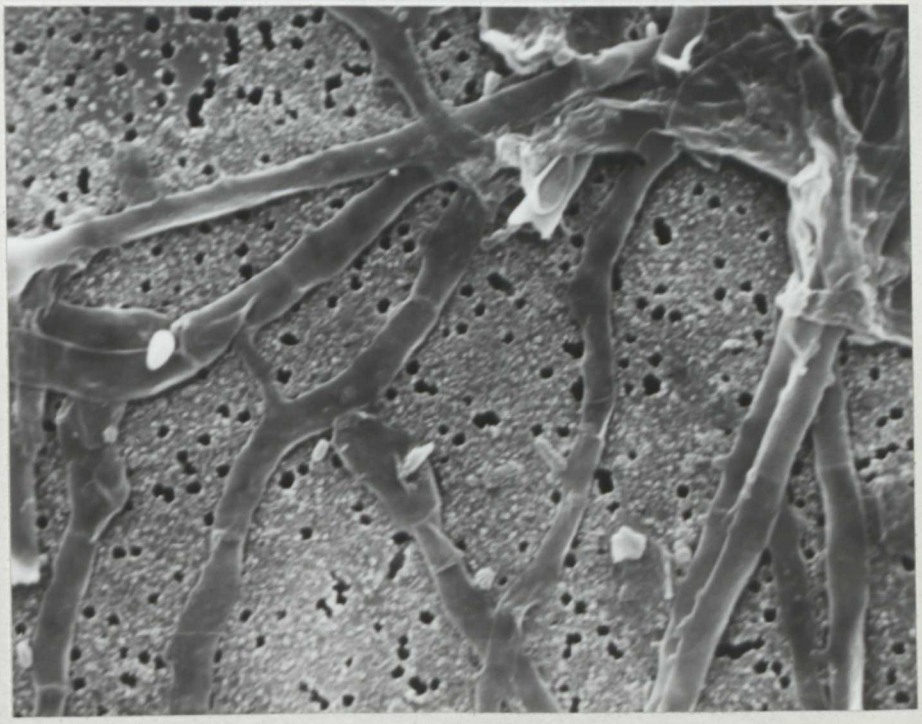
Formation of mycelium from the conidia of Pyrenochaeta lycopersici *incubated on polycarbonate membrane in sterilised compost: fluorescence stimulated by U.V. light* ; conidia treated with optical brightener.

Plate 17

Formation of mycelium from the conidia of Pyrenochaeta lycopersici *incubated on polycarbonate membrane in sterilised compost: scanning electron microscope*



15µm



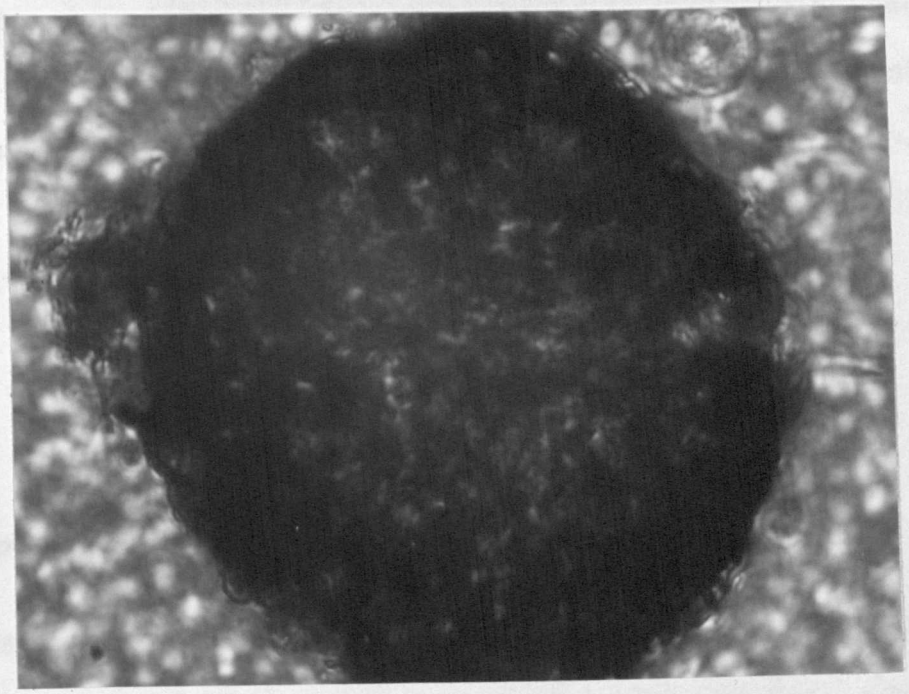
5µm

Plate 18

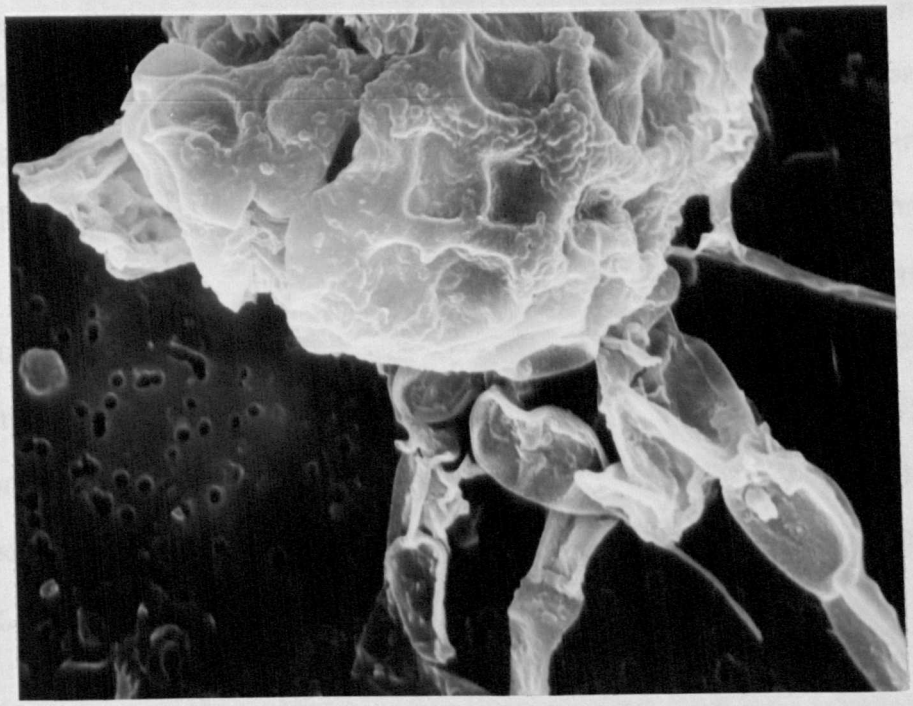
Plate 19

Plate 18
Plate 19

Formation of micro-sclerotia from the mycelium arising from the germination of Pyrenochaeta lycopersici *conidia incubated on polycarbonate membrane in sterilised compost*



5µm



5µm

Scanning electron microscope

compost, were fewer than those formed on cellophane in the same treatment. No sclerotia were detected in garden soil and "frond" mycelium was not observed in any treatments. The pH of the compost and the garden soil was 6.4 and 7.0 respectively. Conidia (untreated and brightener treated) gave > 95% germination when incubated in SME for 24h at 22°C.

SECTION TWO *BIOLOGICAL CONTROL OF* PYRENOCHAETA LYCOPERSICI

2.1 *Development of the screening technique*

A direct relationship between the concentration of soil infested with BRR and root lesion numbers was observed in all treatments (Table 40). Harvesting seedlings after two weeks growth gave lower lesion numbers per root and less variability within each treatment; however the relationship between lesion number and BRR concentration was less obvious than that after four weeks growth. Root weight differed significantly between soil dilutions; maximum reduction in root weight occured at the lowest dilutions of soil infested with BRR (Table 40) and contributed to the within treatment variability. Within each treatment, lesion numbers were greater on roots with relatively high root dry weights. A large variation in lesion numbers was observed between treatments containing five or three seedlings per pot.

Inclusion of root dry weight, shoot dry weight and shoot height (Treatment 4) individually or collectively as covariates, in analysis of variance tests, reduced the within treatment variability (Table 41).

Table 40

Effect of seed number, replication, growth period, and root weight on lesion numbers on tomato plants grown in dilutions of soil infested with BRR

Treatment	No. seed per pot	No. replicate pots per dilution	Growth period (wks)	Percentage soil infested with BRR	Number lesions per tomato root			
					Without RW covariate[A]		With RW covariate[A]	
					Mean	SED*	Mean	SED*
1	5	3	4	25	91.7	14.0	91.5	11.09
				50	84.7		86.1	
				75	128.8		119.6	
				100	118.8		122.8	
2	3	3	4	25	90.2	17.6	95.2	16.4
				50	128.7		127.3	
				75	137.7		132.4	
				100	144.2		145.9	
3	1	10	4	25	106.3	15.4	117.1	11.6
				50	124.9		127.6	
				75	155.7		136.2	
				100	125.9		131.9	
4	1	20	4	25	53.6	9.0	56.4	6.2
				50	71.1		74.2	
				75	83.9		84.7	
				100	92.8		86.0	
5	1	20	2	25	1.9	2.8	1.9	2.8
				50	2.8		2.8	
				75	6.5		6.5	
				100	9.4		9.4	

A Analysis of covariance test.

* SED between concentrations of soil infested with BRR

RW Root dry weight

Table 41

Effect of root dry weight, shoot dry weight and shoot height on lesion numbers on tomato plants grown in dilutions of soil infested with brown root rot.

Percentage soil infested with BRR	Mean number of lesions per tomato root						
	Without covariates	With RW covariate[A]	With SW covariate[A]	With SH covariate[A]	With RW + SW covariate[A]	With RW + SH covariate[A]	With RW, SW + SH covariate[A]
12.5	10.7	4.1	1.9	1.5	3.0	0.8	1.9
25.0	18.5	16.5	15.6	12.4	16.1	13.5	13.2
50.0	21.0	23.9	23.7	22.5	24.1	23.8	23.3
75.0	36.9	35.7	38.9	39.8	36.3	37.8	37.2
100.0	62.2	69.1	69.2	73.1	69.8	73.4	73.7
SED*	15.0	12.7	13.5	12.9	12.7	12.2	12.1

A Analysis of covariance test

* SED between concentrations of soil infested with BRR

RW Root dry weight

SW Shoot dry weight

SH Shoot height

2.2 *Screening potential antagonists against brown root rot (BRR)*

Eight fungal, nine bacterial and seven actinomycete isolates together with the commercial product Biovegetal were tested for their ability to reduce lesion numbers on tomato plants grown in soil infested with BRR (Table 42). Lesion numbers were reduced below control levels when the bacterial isolates E6, BK1, B10, RV3, 40B, 53B, 23C and 50B (Figure 26) and the actinomycete isolates 61, 34C, 39C and 2C (Figure 27) were applied as root dip/soil drenches. Lesion numbers were also reduced when fungi were applied as wheatbran cultures however differences were not significant when compared to the wheatbran controls (Figure 28). The wheatbran controls significantly decreased the root dry weight of tomato plants whereas fungal/wheatbran mixtures produced similar root dry weights to the uninoculated controls. The bacteria 52B, E6 and B10 significantly increased the root dry weight of tomato seedlings when compared to the controls. The product Biovegetal, at 2g and 10g per litre of soil, significantly decreased lesion numbers (Figure 29) when compared to the controls, although no significant differences in root dry weights were observed. Seasonal variations in lesion numbers and root dry weights were observed between experiments.

2.3 *Measurement of brown root rot (BRR) disease levels in soils naturally infested with* **Pyrenochaeta lycopersici**

A direct relationship (R = 0.741) was observed between the number of lesions per root on seedlings, grown in soil samples from commercial glasshouses, and the subsequent disease levels

Table 42

Effect of potential antagonists on the number of root lesions and root dry weight of tomato plants grown in soil infested with brown root rot (BRR).

Experiment 1

Potential antagonist	Mean number of root[A] lesions per plant	Mean dry root weight (mg)
Control	19.1	3.40
Wheatbran	17.3	2.70
AH119	18.0	3.50
AH113	12.4	4.10
AH101	15.9	4.25
TRC10	17.1	3.15
SED*	2.8	0.52

Experiment 2

Potential antagonist	Mean number of root[A] lesions per plant	Mean dry root weight (mg)
Control	69.9	40.65
Wheatbran	45.3	9.05
616	50.0	14.60
TRC26	30.8	27.40
TRC28	48.4	17.35
AH85	72.5	31.30
SED*	10.2	3.43

Experiment 3

Potential antagonist	Mean number of root[A] lesions per plant	Mean dry root weight (mg)
Control	34.6	8.55
52B	30.7	11.05
E6	24.1	11.60
BK1	23.1	8.10
B10	17.7	10.65
RV3	21.6	10.15
SED*	4.28	1.03

Table 42 continued

Experiment 4

Potential antagonist	Mean number of root[A] lesions per plant	Mean dry root weight (mg)
Control	61.6	28.40
40B	47.6	33.40
53B	44.1	31.10
23C	31.2	29.90
50B	23.7	32.30
SED*	6.0	3.84

Experiment 5

Potential antagonist	Mean number of root[A] lesions per plant	Mean dry root weight (mg)
Control	61.3	18.25
61	44.5	15.70
34C	47.1	16.40
39C	42.6	18.35
2C	36.6	17.50
SED*	4.4	2.30

Experiment 6

Potential antagonist	Mean number of root[A] lesions per plant	Mean dry root weight (mg)
Control	44.0	41.90
LW069	39.3	52.00
LW445	33.8	53.30
LW047	34.7	44.20
SED*	6.8	5.93

Table 42 continued

Experiment 7

Potential antagonist	Mean number of root[A] lesions per plant	Mean dry root weight (mg)
Control	159.5	60.00
2g Compost	130.3	57.30
10g Compost	148.5	53.70
20g Compost	134.0	49.40
2g Biovegetal	106.3	54.90
10g Biovegetal	104.3	55.20
20g Biovegetal	111.5	48.90
SED*	11.96	6.59

* SED between mean numbers of root lesions per plant or mean root dry weights.

A Mean number of root lesions per plant with root dry weight included during analysis of covariance.

Figure 26

Effect of potential bacterial antagonists on the number of root lesions and root dry weight of tomato plants grown in soil infested with brown root rot.

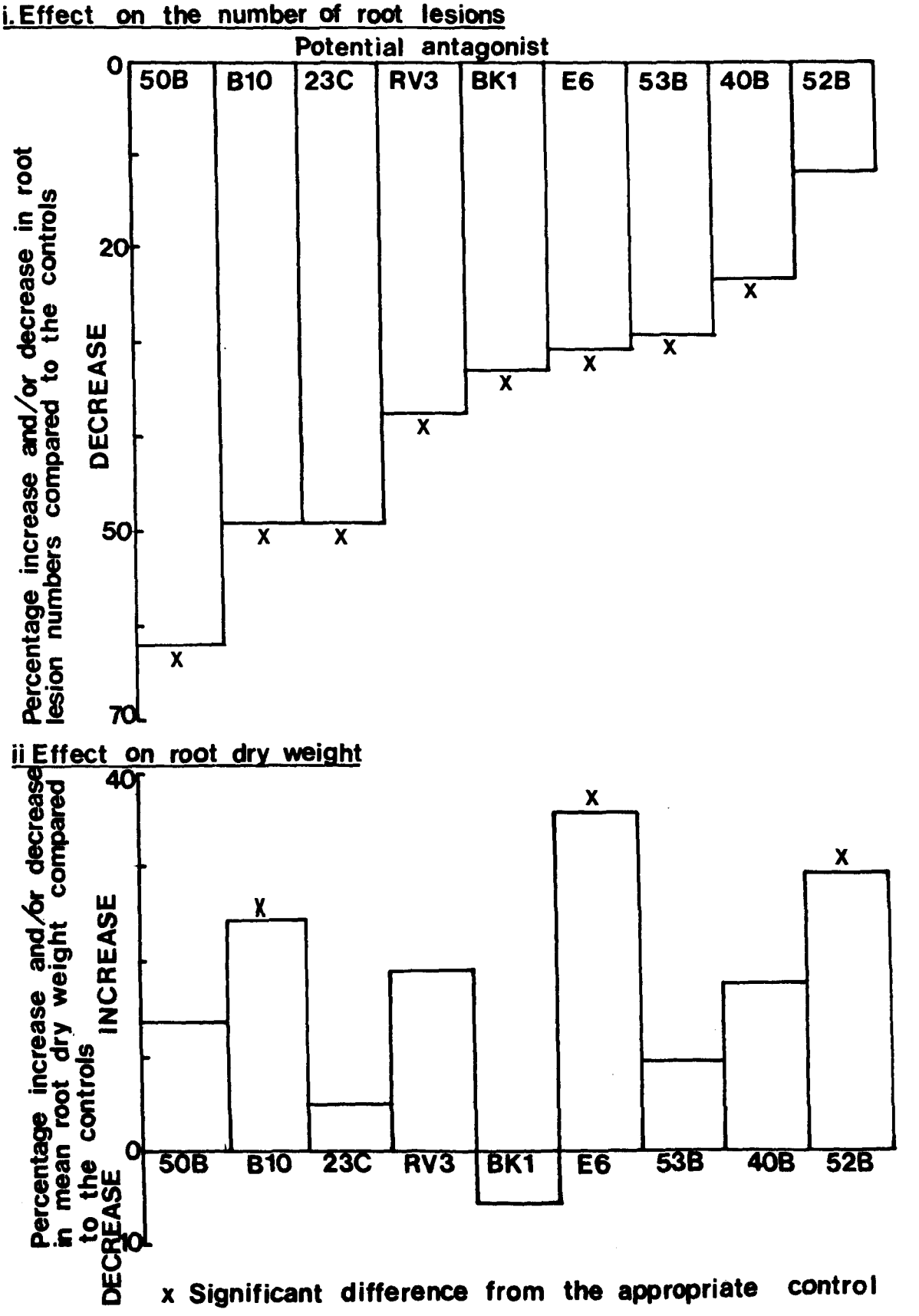
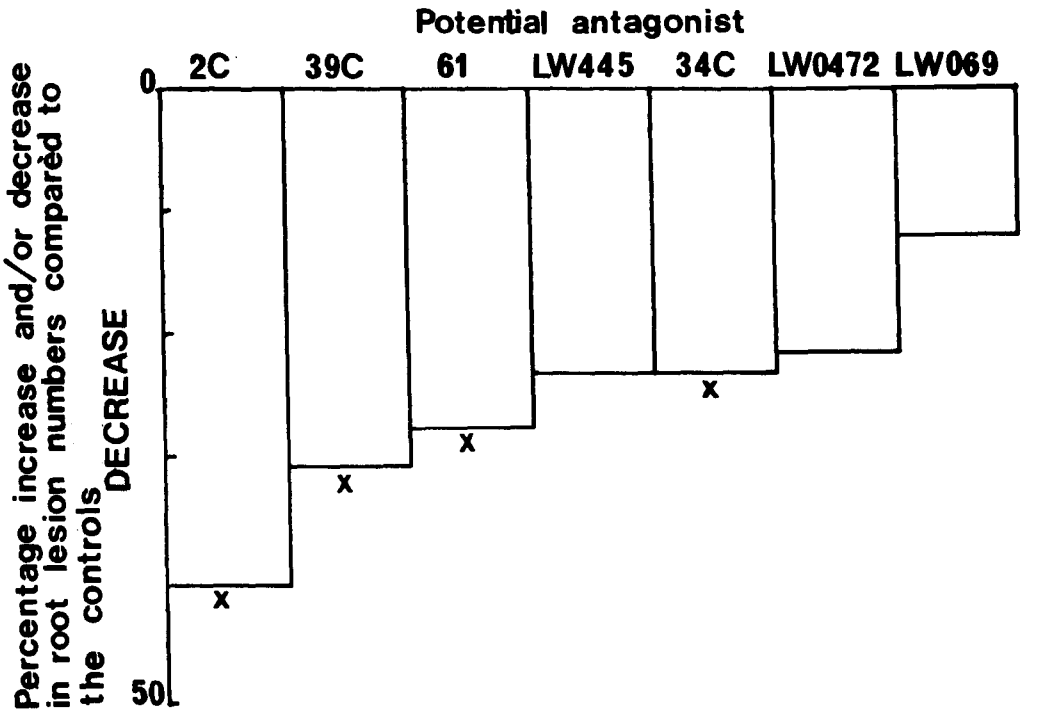


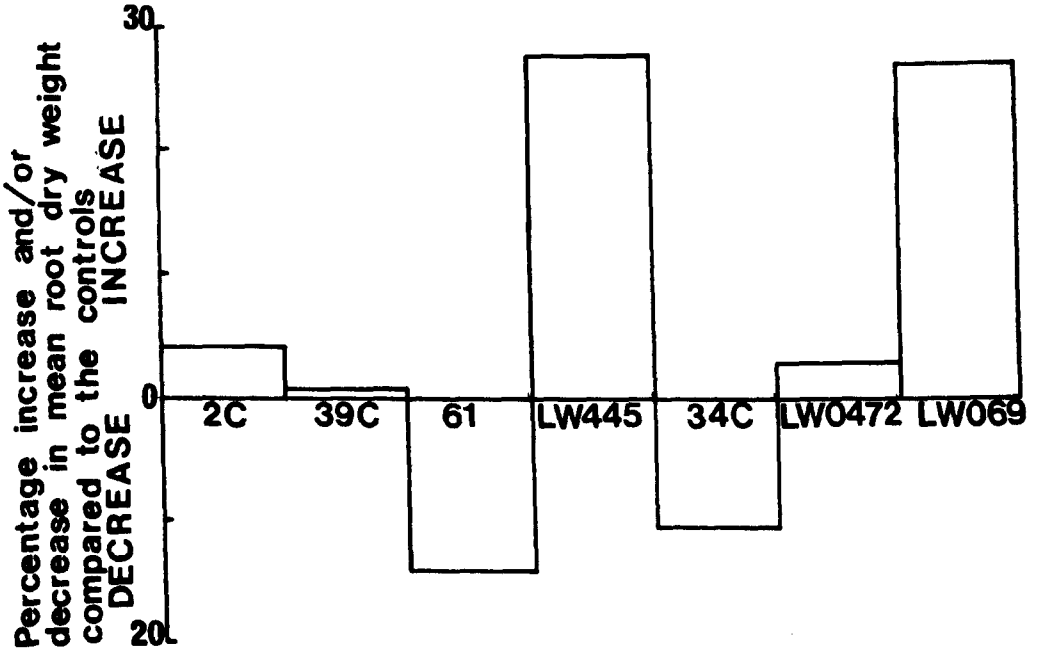
Figure 27

Effect of potential actinomycete antagonists on the number of root lesions and root dry weight of tomato plants grown in soil infested with brown root rot.

i. Effect on the number of root lesions



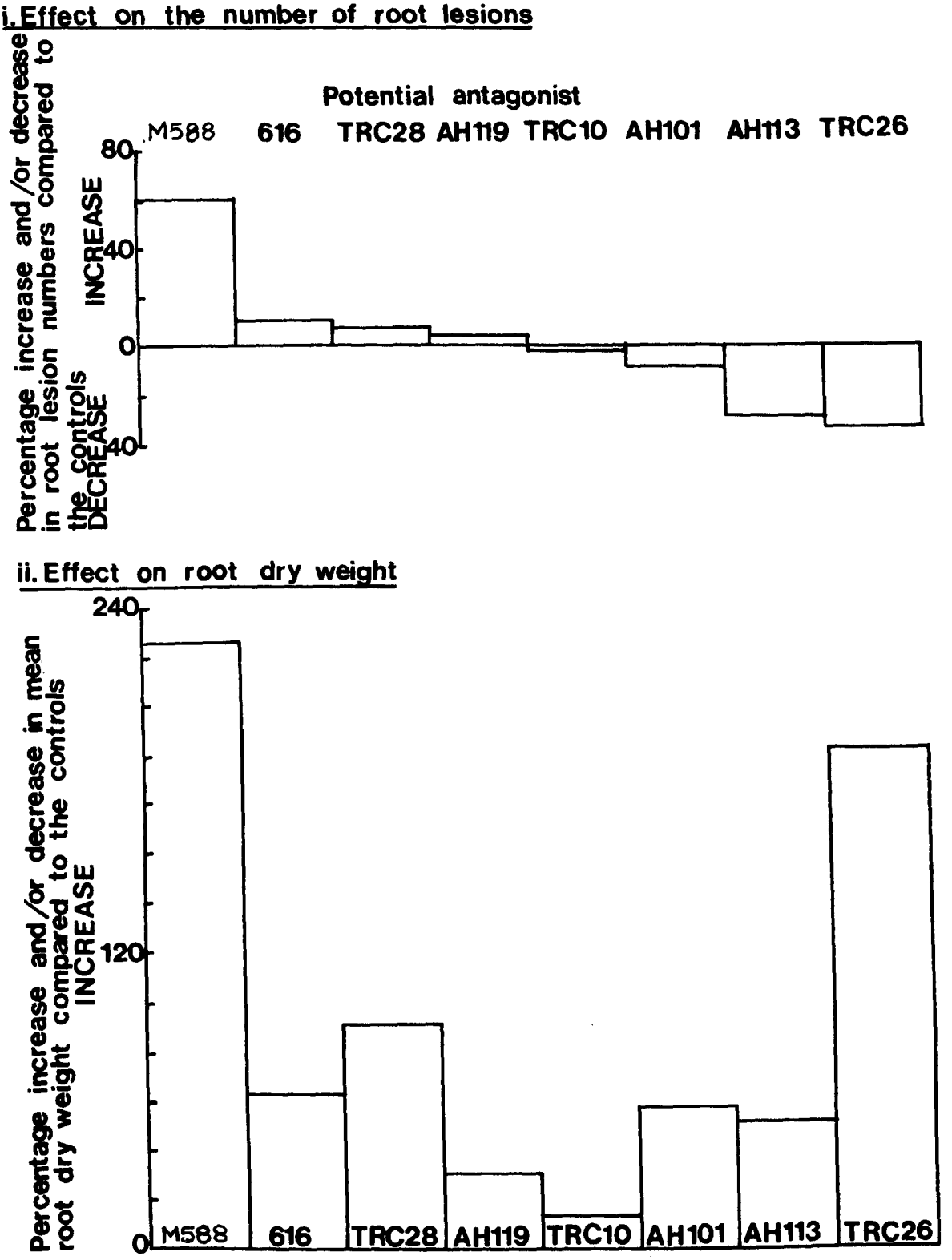
ii. Effect on root dry weight



x Significant difference from the appropriate control

Figure 28

Effect of potential fungal antagonists on the number of root lesions and root dry weight of tomato plants grown in soil infested with brown root rot.

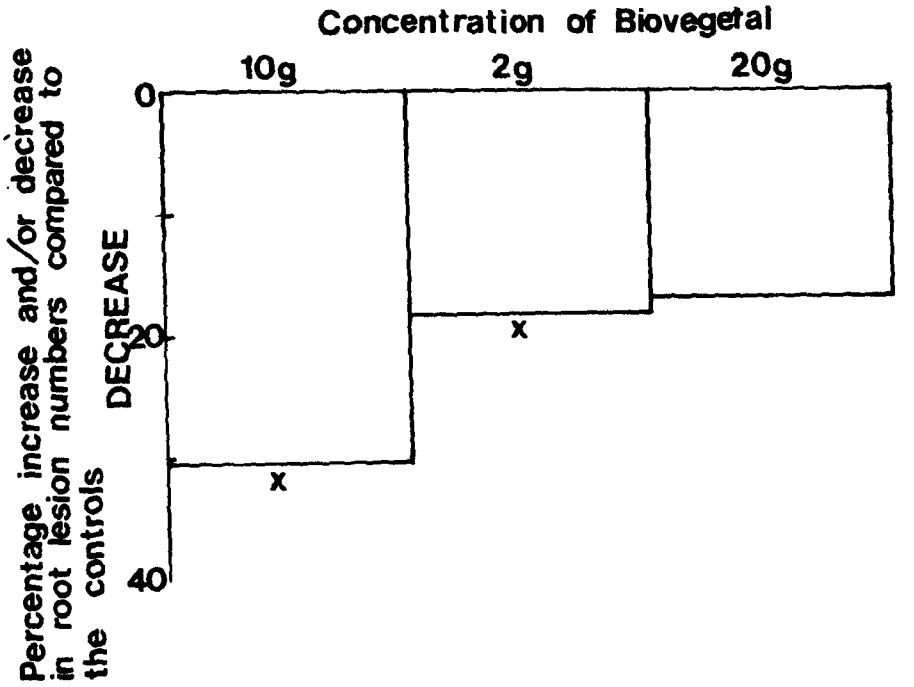


x Signicant difference from the appropriate control

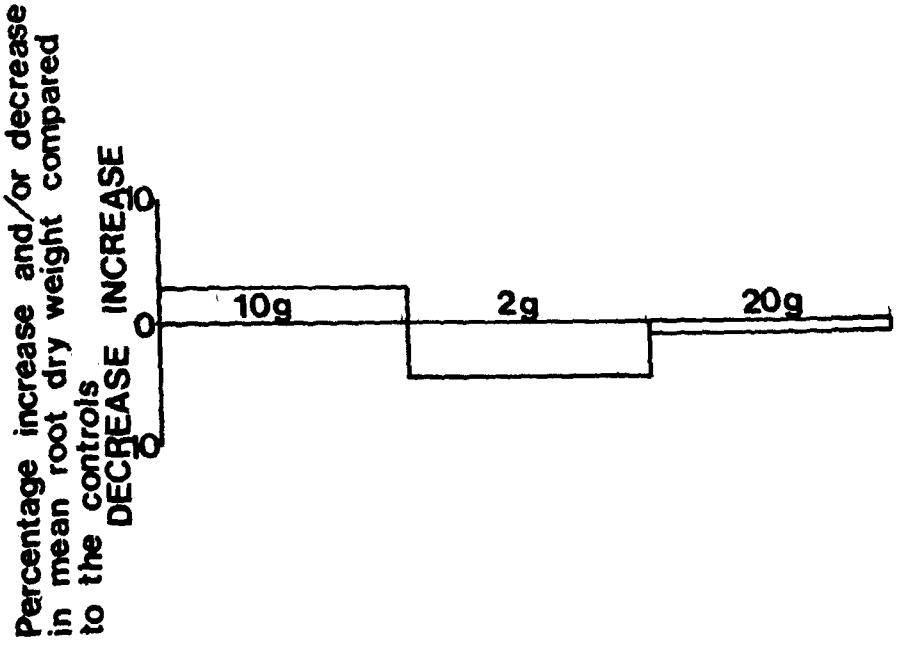
Figure 29

Effect of Biovegetal on the number of root lesions and root dry weight of tomato plants grown in soil infested with brown root rot.

i. Effect on the number of root lesions



ii. Effect on root dry weight



x Significant difference from the appropriate control

on tomato plants eight weeks after transplanting into these soils (Table 43, Figure 30):

$$y = 2.36 + 0.30x$$ (significant correlation at 0.05% probability level)

x = number of lesions per root on tomato seedlings

y = percentage BRR on tomato plants.

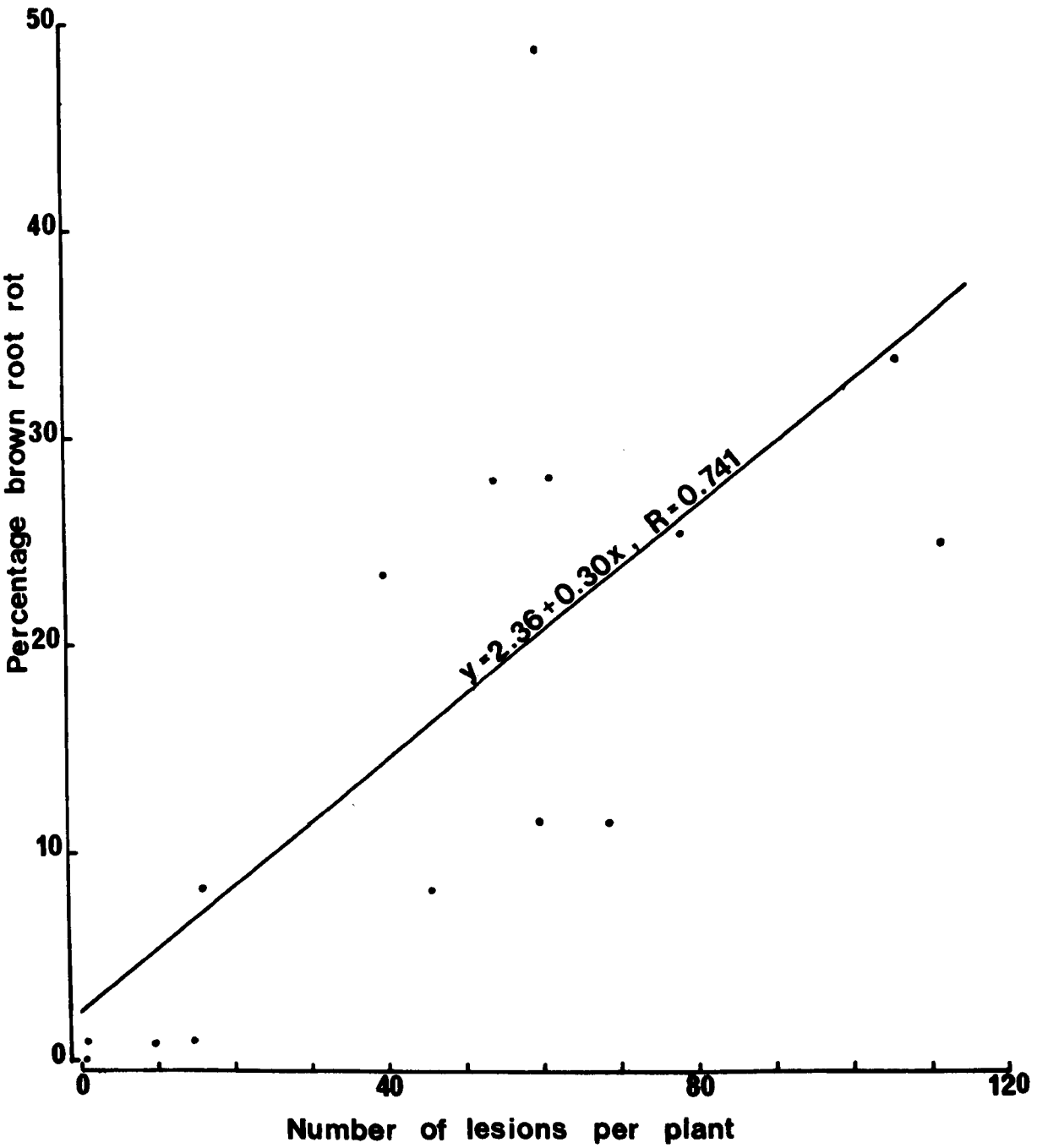
Table 43

Level of brown root rot (BRR) disease detected with the BRR assay and subsequently determined on tomato plants transplanted into the glasshouse.

Soil	Level of brown root rot (BRR)			
	BRR Assay			Glasshouse
	Mean number of root lesions per plant (S.D.)	Mean root dry weight (g) (S.D.)	Mean number of root lesions per milligram root dry weight	Percentage BRR
1983				
Hesketh Bank	0.01(0.31)	0.0067(0.0018)	0.002	0.50
Cotton End	15.90(6.41)	0.0111(0.0034)	1.430	8.30
Sandridge	37.80(12.83)	0.0135(0.0051)	2.800	23.30
Gustard Wood	61.55(29.98)	0.0131(0.0035)	4.720	28.20
Cheshunt	14.60(3.36)	0.0071(0.0017)	2.060	0.50
Sharpenhoe	69.10(19.51)	0.0112(0.0036)	6.190	11.50
1984				
Cotton End	9.75(11.73)	0.1400(0.0390)	0.690	0.75
Sandridge	45.90(25.25)	0.0175(0.0075)	2.620	8.40
Gustard Wood	106.50(45.64)	0.0869(0.0325)	1.230	34.00
Wright	59.95(26.65)	0.0107(0.0057)	5.600	11.50
Howarth	54.00(36.03)	0.0118(0.0084)	4.590	28.00
Ball	60.25(32.94)	0.0139(0.0068)	4.350	46.30
Pickovant	112.00(48.84)	0.0324(0.0176)	3.460	27.60
Hoddesdon	0.15(0.37)	0.0226(0.0119)	0.010	0.00
Kimpton	78.35(35.64)	0.0674(0.0160)	1.160	25.30

Figure 30

Relationship between the level of brown root rot (BRR) disease detected with the BRR assay and subsequently determined on tomato plants transplanted into the glasshouse.



CHAPTER 4. DISCUSSION

Difficulties in culturing and identifying *P.lycopersici* have made many plant pathologists unwilling to attempt isolation of the pathogen. This has undoubtedly led to the misidentification of the cause of BRR symptoms in the past. An isolation procedure was developed based upon a semi-selective media which incorporated both fungicides and antibiotics. Initially a range of fungicides were screened; particular emphasis was placed on finding chemicals which differentiated between *P.lycopersici* and *C.coccodes* which frequently occur together in tomato roots showing BRR symptoms. The MBC generating fungicides were effective in reducing the growth of *C.coccodes* but also proved inhibitory to *P.lycopersici*; this activity of benzimidazole fungicides against *P.lycopersici in vitro* confirms the observations of Ebben (1974) and Jeves and Smith (1980). Farley (1972), whilst developing a selective media for the isolation of *C.coccodes* from soil, noted that combinations of PCNB and Nystatin reduced the growth of this pathogen. A similar effect was demonstrated against the fungus in this study, the mixtures having relatively little effect on the growth of *P.lycopersici*. However, the incorporation of PCNB/Nystatin into agar was less effective than the addition of Bayleton (at 100μg ml^{-1}) in suppressing the growth of *C.coccodes*. Bayleton was shown to cause relatively low inhibition of *P.lycopersici* at concentrations in agar which were sufficient to markedly inhibit the development of *C.coccodes*. Antibiotics, at a range of concentrations and in various combinations, had little effect on the growth of *P.lycopersici* or *C.coccodes*. Penicillin (P), Streptomycin (S) and tetracycline (T) (which inhibits both Gram positive and Gram negative bacteria) reduced bacterial contamination

on isolation plates.

The efficiency of the chemical mixture chosen for the selective media (Penicillin, Streptomycin and Tetracycline plus Bayleton, all at 100µg ml^{-1}) was confirmed using both pure cultures of *P.lycopersici* and *C.coccodes* and during its use in this study and by others (Polley, pers. comm., Talley, pers. comm.) to make isolations from diseased tomato root systems. In practice it has been found that GSF can be successfully isolated from the roots of diseased seedlings or young plants using only the antibiotic combination of the selective medium (PST medium). However the full medium (PSTBay) is necessary for reliable isolation of GSF from the heavily suberised root systems of older plants. The presence of Bayleton in the medium also effectively suppresses the growth of *C.campanula* confirming the observation by Glover (pers. comm.) that the fungicide, when incorporated into PDA at 10 to 50µg ml^{-1}, inhibited the growth of this fungus.

Cultures of GSF and *P.lycopersici* isolated onto PDA, PST and PSTBay were similar in appearance, growth, pathogenicity and degree of sporulation; differences between isolates due to the use of different isolation media is unlikely. It may be argued however that isolation from BRR diseased root systems selects only pathogenic strains of the fungus and strains with saprophytic abilities, should they occur, are not detected.

Symptoms of *P.lycopersici*, *C.coccodes* and *C.campanula* on tomato roots can be confused. For example: the bronze-brown lesions of BRR are similar in appearance and colour to the rust-brown lesions of CRR (Plate 20), although infection by *C.campanula* frequently produces an

Plate 20

Plate 20

Tomato root systems displaying symptoms of Calyptella *root rot (CRR, left root system) and brown root rot (BRR, right root system).*

overall red appearance to the root system not observed with *P.lycopersici*. Also CRR is now considered to be largely responsible for the cortical shredding of roots often thought to be caused by BRR in the past (Clark, pers. comm.). Reluctance to isolate from infected root systems has probably led to the misidentification of CRR as BRR and may account for the infrequent reports of CRR disease. The causal agent of root disease symptoms can only be confirmed after isolation into pure culture and the use of the semi-selective media developed in this study should help in understanding the association between *P.lycopersici*, *C.coccodes* and *C.campanula*.

In order to characterise GSF cultures it was necessary to determine the pathogenicity of isolates. All GSF isolates tested proved pathogenic to tomato roots however a wide variation in pathogenicity between isolates was observed. Termohlen (1962) and Last and Ebben (1966) observed similar variations in the pathogenicity of GSF isolates whilst Manning and Vardaro (1974) observed only 23 of 32 isolates to be pathogenic to tomato seedlings. It is considered that the pathogenicity levels observed in this study fell within the natural range of pathogenic variability for *P.lycopersici*. A natural variation in pathogenicity within a range of isolates is not uncommon in plant pathogens (Burnett, 1976), however culture and storage conditions can influence the behaviour of isolates. Jenkins (pers. comm.) observed that the pathogenicity of GSF isolates declined when maintained on agar for long time periods whilst Termohlen (1962) observed the pathogenicity of a non micro-sclerotia forming GSF isolate to decline greatly if stored on agar or in soil at 23°C; storage at +2°C and/or -35°C prevented loss of pathogenicity. However Ebben (1974) observed isolates stored under mineral oil at

room temperature to be pathogenic after ten years and McGrath and Campbell (1983) maintained isolates for two years on V8A by conidial transfer, these remained sporogenic and pathogenic. In this study no effect of storage on the pathogenicity of GSF cultures was found. No correlation was observed between pycnidial and/or sclerotial production and pathogenicity confirming the observations of Termohlen (1962) and Gerlach and Schneider (1964) who were unable to detect differences in virulence between sterile, pycnidium and sclerotium producing isolates.

In this study variations in root lesion size produced by *P.lycopersici* isolates *in vitro* were observed. This variation may have been due to fluctuations in the humidity within each Petri dish. Clerjeau and Conus (1973) reduced this source of variation between GSF isolates by placing discs of wet sponge under the filter papers in each Petri dish. Although the *in vitro* technique provided a rapid method of determining the pathogenicity of *P.lycopersici* isolates the conditions bear little resemblance to those found in soil and it is unlikely that a true estimate of pathogenicity is obtained. The *in vivo* technique is considered to be a more realistic method for testing the pathogenicity of isolates.

The effect of light, temperature and medium on sporulation by *P.lycopersici* isolates was studied. In view of the comprehensive work by McGrath and Campbell (1981 and 1983) investigations into the medium requirements for sporulation were restricted to V8-Juice agar preparations and media prepared from the vegetable constituents of V8-Juice.Leach (1971) recommends the use of black light fluorescent lamps, emitting near-U.V. radiation to induce sporulation in the *Fungi Imperfecti*. A certain proportion of black light seems

necessary to induce pycnidial production in the *P.lycopersici* isolates used in this study. This contrasts with the work of McGrath and Campbell (1983) who observed little difference in sporulation by *P.lycopersici* cultures following exposure to near-U.V. or cool white illumination. However the quantity of pycnidia produced from either light source was lower than that observed under black light during this investigation. Differences in the levels of sporulation observed in this work and in that of McGrath and Campbell (1983) may be a result of the use of different light sources. Black fluorescent lamps (Philips TL80N), as used in this study, emit a proportion of visible light in addition to near-U.V. radiation i.e. 310 to 425nm (Table 6). Irradiation from a combination of black light and cool white fluorescent lamps was most favourable for pycnidial production suggesting that the wavelengths 380 to 425nm are necessary for high levels of sporulation. Black light fluorescent lamps (type BLB, General Electric Company) as used by McGrath and Campbell (1983) emit little visible light, consisting mainly of near-U.V. radiation, 300 to 380nm (Leach, 1971), possibly accounting for the reduced sporulation levels. No pycnidia were produced under "dark" conditions. Marsh *et al.* (1959) observed sporulation in many *Fungi Imperfecti* to be suppressed when grown in complete darkness. When light is excluded from growing colonies, oxidation levels may fall below the minimum requirement for sporulation (Leach, 1962) and consequently such fungi remain sterile until transferred to light conditions.

McGrath and Campbell (1983) amended V8A with calcium carbonate ($CaCO_3$) to control the pH of the medium, however they found *P.lycopersici* still raised the pH of V8A to levels not conducive to

sporulation. In this study, pycnidial production was significantly higher on V8A and home-made V8A (unamended with $CaCO_3$) than on V8A prepared according to McGrath and Campbell (1983) (amended with $CaCO_3$). Temperature requirements for sporulation in *P.lycopersici*, recorded in this study were similar to those reported by McGrath and Campbell (1981 and 1983). They found a temperature range of 20 to 24°C to be optimum for pycnidial production; sporulation was totally inhibited at temperatures >28°C. During this investigation certain isolates e.g. AH65 did not sporulate under black light at ambient room temperature, however temperatures exceeding 26°C may have occurred in this treatment. Exposure to black light at a constant temperature (22°C) enabled many GSF isolates unable to sporulate at room temperature to produce pycnidia. As temperature increased there was a change in the position of pycnidial production within the colony: more pycnidia were produced towards the edge of the Petri dish as the temperature increased from 15 to 26°C. This could have been due to the effect of temperature on mycelial spread, the optimum colony region for sporulation at each temperature falling into different agar zones depending on the growth rate of the fungus.

An inherent variability in pycnidial production on V8A was observed in this study. In addition to the natural variation between *P.lycopersici* isolates variation in sporulation could result from differences between batches of V8-Juice. V8-Juice contains approximately 80% tomato juice (Anderson, pers. comm.) but the other seven vegetable constituents may well vary in proportion with the season. When individual vegetable agars were tested it was found that an increase in vegetable extract tended to produce an increase in sporulation; celery agar produced a marked increase in pycnidial

production. This vegetable accumulates high concentrations of nitrogen (Kinsbury, 1967) which may have stimulated sporulation. However sporulation was reduced when the vegetable extract of parsley and carrot was increased. Celery, carrot and parsley are members of the Umbelliferae producing furanocoumarins (Neilson, 1970) which react with near-U.V.radiation to form an activated toxic state. Concentrated parsley and carrot agars may have contained toxic levels of furanocoumarins accounting for the reduced pycnidial production. However the furanocoumarins present in celery are only found in diseased or damaged tissue; celery agar was prepared from healthy tissue and probably contained negligible quantities of these chemicals.

Pycnidial and conidial sizes were similar to those of the type description of *P.lycopersici* (Schneider and Gerlach, 1966) although a great variability in pycnidial production, size and numbers of setae between isolates was noted. However Schneider and Gerlach (1966) and Sutton (pers. comm.) mention that pycnidial characters vary considerably from strain to strain and state that no taxonomic importance should be attached to such variation. A similar variability in pycnidial production between isolates was observed by Manning and Vardaro (1974). There was little variation in the dimensions of the conidia of isolates used in this study.

The ability to produce pycnidia in agar culture provided an opportunity to study the requirements for conidial germination. Marked differences in the level of germination, *in vitro*, between isolates was recorded, however for each isolate certain general trends were observed.

The rate and level of spore germination in fungi is influenced by temperature. Germination of *P.lycopersici* conidia was not observed at temperatures greater than 28°C whereas temperatures below 15°C slowed down their metabolism. The temperature range over which conidia germinated tended to be similar to that for mycelial growth. Temperature optima for mycelial growth and germination were also compatible but approximately 2 to 4°C above the optimum for sporulation. Germination levels greater than 90% were only obtained in the presence of nutrients and the temperature range over which conidia germinated was also greater when conidia were incubated in SME. A similar observation was made for *C.atramentarium* by Griffiths and Campbell (1973) who observed greater germination levels, especially at high temperatures, when spores were incubated in glucose solution rather than in distilled water.

Experiments revealed that the type of buffer solution, external nutrients and the strain of *P.lycopersici* modified the germination response to any given pH level. Callaghan (1974) observed that conventional buffers e.g. phosphate and sodium bicarbonate - carbonate mixtures unduly influenced germination in *Basidiobolus ranarum* Eidam. Phosphate and citric buffers were toxic to species of *Marasmius* (Lilly and Barnett, 1951). Boric acid buffer solutions were particularly unfavourable for the germination of *P.lycopersici* conidia. In fungi, the spore germination response to hydrogen ion concentration is generally similar to that for mycelial growth and sporulation. Termohlen (1962) noted the optimum growth for *P.lycopersici* occurred at pH 4.7 and declined markedly above pH 7.0. McGrath and Campbell (1983) observed pycnidial production in *P.lycopersici* to be optimum at approximately pH 5.5 although

difficulties were observed in buffering the agar; the fungus rapidly raised the pH of the medium to a level not conducive for sporulation. However in this study conidia of *P.lycopersici* preferred less acidic conditions for optimum germination.

Different light regimes tested did not significantly affect conidial germination. However sufficient light to stimulate germination may have been present during the preparation of conidial suspensions and as no true dark treatment was included, these results must be treated with caution.

The age of conidia influenced the level of germination. Poor germination in young cultures (18 days) may have been due to insufficient endogenous reserves or an immaturity of conidia. Low germination levels in ageing conidia may have been due to a depletion in nutrients and/or the onset of dormancy, either exogenous or constitutive. Exogenous dormancy, that is "the condition wherein development is delayed because of unfavourable chemical or physical circumstances in the environment" (Sussman, 1965), may have been imposed by a depletion in exogenous and/or endogenous substances. Addition of external nutrients to unwashed, washed and cirrus extract treated conidia enhanced germination above the levels observed in the absence of external nutrients. Shirashi *et al.* (1970) observed similar results with *Botrytis cinerea* Fr. when the ability of aged conidia to germinate was enhanced by the addition of several saccharides. Udebo and Madelin (1974) demonstrated that malt extract virtually eliminated reductions in germination due to the ageing of *Botryodiplodia theobramae* Pat. conidia. Another explanation for the reduced germination of conidia from ageing cultures may have been due to the onset of constitutive dormancy, that is "the condition wherein

development is delayed owing to an innate property of the dormant stage, such as a barrier to the penetration of nutrients, a metabolic block, or the production of a self-inhibitor" (Sussman, 1965). After approximately 25 days, conidia are exuded from the pycnidium as a spore tendril or cirrus. Conidia, when maintained in the tendril, on the external surface of the pycnidium, have much reduced levels of germination. Spore germination in many fungi is influenced by materials present in the cirrus (Griffiths and Peverett, 1980), however conidia of *P.lycopersici*, washed in SDW and resuspended in a dilute cirrus extract had only slight differences in germination compared to unwashed conidia. The inability to detect inhibitors in the cirrus extract may have been due to an excessive dilution of the initial extract. Although material in the cirrus of *P.lycopersici* could affect conidial germination and longevity its importance on conidia released from the pycnidium is likely to be negligible.

The ability of *P.lycopersici* conidia to cause brown lesions on tomato plants grown in compost and garden soil has been demonstrated. This is the first report of the pathogenicity of *P.lycopersici* conidia in soil. Introduction of conidia into sterilised compost provided the pathogen with an opportunity to attack tomato seedlings in conditions of reduced microbial competition. In unsterilised garden soil *P.lycopersici* conidia would have to compete with a natural soil microflora and microfauna for nutrients and space (Baker and Cook, 1974). This could explain the lower disease levels seen in the garden soil when compared to sterilised compost. Differences in lesion numbers between the two experiments assessing conidial pathogenicity in sterilised compost may have been due to variations in temperature. Temperatures under the light frames were not

controlled and ranged from 15 to 25°C with large daily and seasonal fluctuations. Termohlen (1962) observed BRR disease to decline as soil temperatures rose above 18°C; the optimum range for infection occurred between 15 and 20°C with decreased infection levels at 12, 28 and 34°C. The ability of *P.lycopersici* conidia to cause BRR symptoms on tomato plants grown under commercial glasshouse conditions was also demonstrated for the first time. Symptom appearance and development from conidial inoculum was similar to that observed on commercial glasshouse tomato crops; tomatoes grown in soil infested with conidia developed brown, corky and stem base lesions, had reduced root sizes and less small fibrous roots, all common symptoms of BRR (Ebben, 1974). Symptoms were also observed in G.C.R.I. glasshouse and field soils in which a high "background" level of BRR was recorded. In 1983 the design of experimental plots led to the cross contamination of soil between plots during overhead watering and probably accounted for the disease recorded in control soils. However after seven weeks growth, disease levels in conidial infested plots were considerably higher than those in controls. Special attention to the irrigation system in 1984 reduced the amount of cross contamination. The presence of BRR symptoms on tomatoes grown, in 1984, in compost infested with conidia in 1983 indicates a survival of *P.lycopersici* propagules.

Conidial infested sterilised compost and garden soil stored in the laboratory for 501 and 151 days respectively retained and increased their inoculum potentials. This increase may have been due to the formation of propagules, from the original conidial inoculum, which were capable of surviving and maintaining their pathogenicity in soil. The formation of these propagules would be a gradual process

and could account for the observed increase in the inoculum potential of the soils. The fate of conidia in soils, in the absence of tomato plants, was further investigated using the modified cellophane envelope technique, a method frequently employed in soil fungistasis studies (e.g. Dobbs and Hinson, 1953). Germination of *P.lycopersici* conidia in composts and glasshouse soil was similar to that of the control however the rate of germination was slower than that observed in SME. Reduced germination in washed sand may have been due to the lowered nutrient status of the substrate when compared to other soil treatments. These results contrast with those for chlamydospores of *P.lycopersici*. Khalil (1973) observed the germination of *P.lycopersici* chlamydospores to be inhibited when incubated, on cellophane, on the surface of glasshouse soils. Addition of arginine stimulated germination of the chlamydospores. It is posible that the rate and level of germination observed in soils was an artefact of the technique; the cellophane may have acted as a nutrient source allowing conidia to germinate. However Dobbs and Hinson (1953) observed the germination of fungal spores to be completely inhibited when boiled, autoclaved cellulose film was used as the spore bearing substrate. In their experiments addition of nutrients (e.g. glucose or asparagin) nullified the inhibition of germination suggesting that any nutrients present in the cellophane were insufficient to support germination and reduce fungistatic effects. Conidia of *P.lycopersici* were able to germinate on polycarbonate membranes buried in sterilised compost and garden soil. Polycarbonate is an inert material regarded as totally free of potential nutrients and conidia germinated to form extensive mycelium when buried on this substrate in sterilised compost. Germination and growth was slower and limited in garden soil possibly due to microbial competition and/or the

reduced nutrient status of the substrate. Compared to the control the rate and level of germination observed in the compost and garden soil was slow and some conidia remained ungerminated, which may indicate that a degree of fungistasis had occurred.

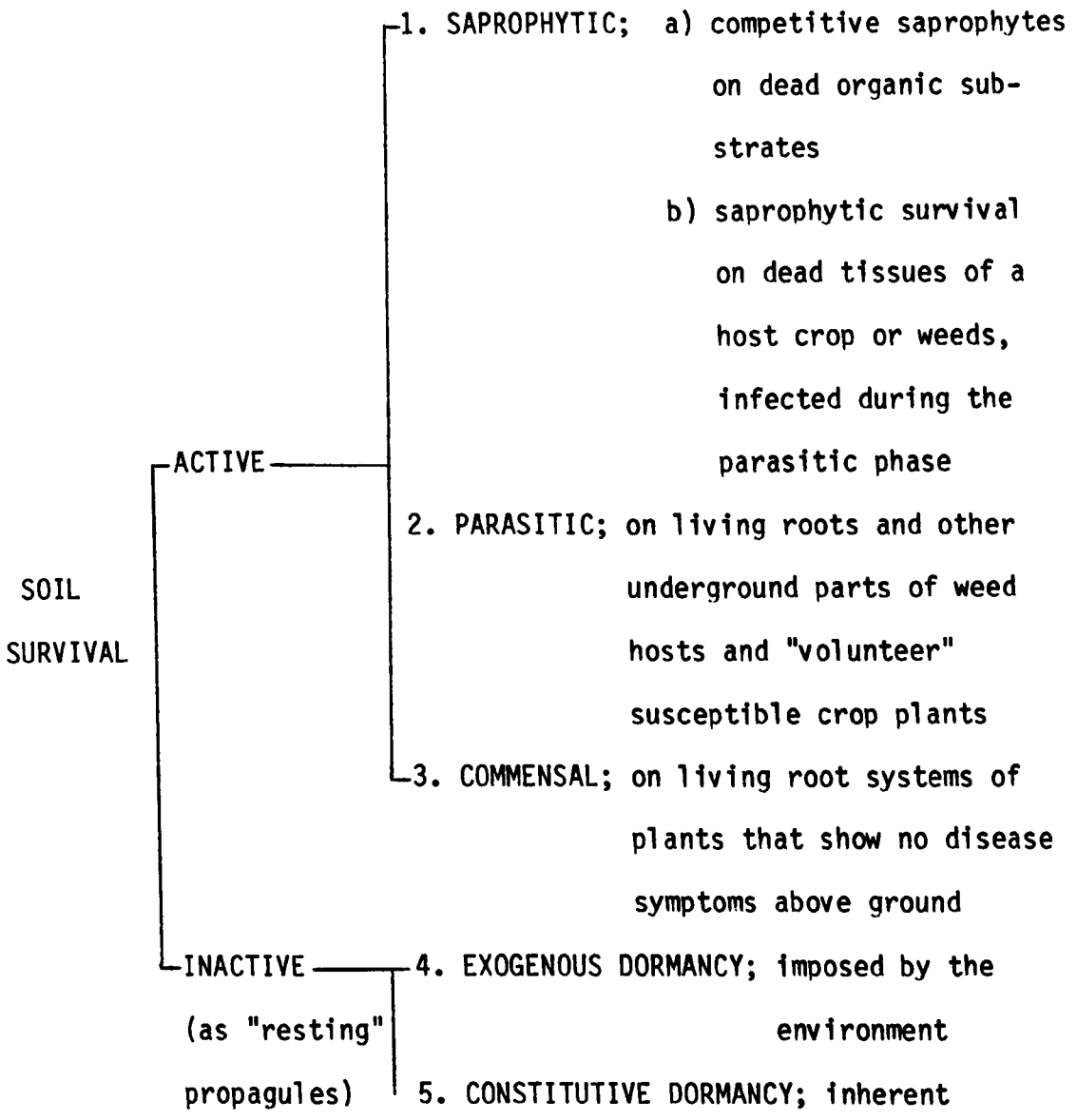
Marked inhibition of germination was observed when *P.lycopersici* conidia were incubated on cellophane in peat. Conidia turned brown and populations of bacteria were observed around the conidia after several days incubation. Tahvonen (1982a and 1982b) observed the ability of Sphagnum peat to suppress various plant pathogens. In addition to bacteria, he observed *Penicillium* spp., *Trichoderma viride* S.F. Gray and *Streptomyces* spp. to be the dominant micro-organisms isolated from the peat. These micro-organisms are known to be antagonists and producers of antibiotics in soil (Baker and Cook, 1974) and may account for the reduced germination of *P.lycopersici* conidia in peat; sterilisation of the peat permitted conidia to germinate suggesting that the inhibition may have been of biological origin. It is also possible that lignin monomers, known components of soils rich in organic matter, may account at least in part for the inhibition of conidial germination in peat. The release of volatile aromatic compounds, resulting from lignin degradation, may give rise to fungistatic and/or fungitoxic effects. Lingappa and Lockwood (1962) observed that some aromatic compounds, related to degradation products of lignin, inhibited the germination and growth of three test fungi. Germination of *P.lycopersici* conidia in sterilised peat may have been due to the removal of volatile factors and elimination of lignin decomposing micro-organisms. Another possible reason for the inhibition of conidial germination in peat may have been the acidic nature of the substrate. It has been shown that regardless of

the presence of nutrients and type of buffer solution, *P.lycopersici* conidia were strongly inhibited from germinating at low pH levels. Garrett (1970) observed that acid soil solutions may reduce spore germination. However, Schüepp and Green (1968) correlated the level of fungistatic activity directly to soil pH, inhibition being higher in alkaline than acidic soils. The increased germination observed in acidic soils was attributed either to a direct effect of pH on the fungus, especially at low nutrient levels, or to an indirect effect through suppression of bacterial and actinomycete activity. Schüepp and Green (1968) also observed that autoclaving soil removed the inhibitory effect at all soil pH levels tested. Autoclaving peat eliminated the inhibitory effect of peat on the germination of *P.lycopersici* conidia. Conidia incubated in peat for three days were able to germinate after incubation under the control conditions for 24 hours. This suggests that the factors inhibiting conidial germination were fungistatic and not fungitoxic.

The development of *P.lycopersici* and the process of sclerotial formation on cellophane buried in compost was similar to, but occurred more rapidly than that observed on agar and plant roots by White and Scott (1973) and Ball (1977). Mature micro-sclerotia of *P.lycopersici* were observed after seven days incubation in sterilised and unsterilised composts. White (1975) observed micro-sclerotia of *P.lycopersici* after four weeks incubation on 2% malt extract agar whilst White and Scott (1973) observed their formation in tomato roots inoculated with a mycelial/micro-sclerotial suspension after twelve weeks. Sclerotial development from conidia buried, on polycarbonate membranes, in compost was slower and more sparse than that on cellophane possibly due to the reduced nutrient status of the

substrate. Mature sclerotia were seen after nine to 15 days incubation.

Soil-borne plant pathogens can use a variety of different strategies to survive unfavourable periods. These have been summarised by Park (1965) and Garrett (1970):



It is generally considered that *P.lycopersici* survives and overwinters, in the absence of the growing crop host, as micro-sclerotia. These are formed in the host tissue during the process of infection (White and Scott, 1973) and are particularly common in areas of corky

root at the end of the season. A proportion of the micro-sclerotia remain in the root debris once the tomato crop has been removed. These propagules are highly resistant to dessication and dry heat (White, 1975) and those deep in the soil will be able to survive soil sterilisation to cause infection in the following tomato crop. It is also possible that the pathogen may survive unfavourable periods actively as a parasite on alternative hosts. The disease has been recorded on other glasshouse crops and common weeds, providing a means of survival for the pathogen in the absence of the tomato crop. Menzies (1973) occasionally isolated *P.lycopersici* from the roots of lettuce which exhibited no symptoms of BRR; in such a case *P.lycopersici* may form part of the microbial population inhabiting the root surface region of healthy plants. However, it is doubtful that *P.lycopersici* survives saprophytically either as a mycelial network in host tissue or as a competitive saprophyte colonising dead organic substrates. Besri (1970) investigated the saprophytic survival of *P.lycopersici* and concluded that the pathogen had no competitive saprophytic ability and should be classed as a root-inhabiting fungus as defined by Garrett (1970).

An additional method of survival may involve the formation of resting structures from conidia released from pycnidia which have formed on diseased root systems. However pycnidia have rarely been observed on roots showing BRR symptoms; it may be possible that conditions in glasshouse grown tomato crops are unfavourable for the production of large quantities of pycnidia. For example physical conditions such as light, temperature and soil moisture may limit the occurrence, position and quantity of pycnidia formed. Another explanation for the infrequent reports of sporulation in tomato crops may be the mis-

identification of pycnidia and conidia for propagules of other fungi. The micro-sclerotia and acervuli of *C.coccodes* are often setose (Mordue, 1967) and of a similar size to pycnidia of *P.lycopersici* (Plates 21 and 22). Black dot of tomatoes can be easily identified, by eye, by the presence of *C.coccodes* micro-sclerotia however closer examination of infected root areas may reveal the presence of *P.lycopersici* pycnidia (Plates 23 and 24). A number of characteristics distinguish *P.lycopersici* pycnidia from black dot sclerotia. Firstly micro-sclerotia of *C.coccodes* usually only possess two to six setae, distributed evenly over the propagule surface, whilst *P.lycopersici* pycnidia may have up to 15 shorter setae bunched around the pycnidial ostiole. Secondly the setae of *C.coccodes* micro-sclerotia are delicate and easily broken off when the propagules are removed from the root system for further examination. Observations from this study suggest that microscopical examination of BRR symptoms (x3 magnification) may reveal the presence of *P.lycopersici* pycnidia. Micro-sclerotia of *C.coccodes* can develop into acervuli (Blakeman and Hornby, 1966) containing biguttulate conidia similar in appearance but larger (16 to 24x2.5 to 4.5µm) than *P.lycopersici* conidia. Conidia of *P.lycopersici* could easily be mistaken for conidia of *C.coccodes*, especially if those of *P.lycopersici* have swollen prior to germination. Pycnidia of *Didymella lycopersici* Klebahn also fall within the same range of dimensions as those of *P.lycopersici*, although they do not possess setae. Pycnidia of *D.lycopersici* may form in dark brown sunken cankers, which develop at soil level on tomato stems (Holliday and Punithalingam, 1970), and pycnidia of *P.lycopersici*, formed on BRR stem base lesions, could be misidentified as those of *D.lycopersici*. More importantly conidia of *P.lycopersici* could be misidentified as *D.lycopersici* conidia which

Plate 21

Plate 22

Plate 21 *Micro-sclerotia of* Colletotrichum coccodes *on potato dextrose agar.*

Plate 22 *Pycnidia of* Pyrenochaeta lycopersici *on V8-Juice agar*

450µm

450µm

Plate 23

Plate 24

Plate 23 *Micro-sclerotia of* Colletotrichum coccodes *on a diseased tomato root system*

Plate 24 *Pycnidia of* Pyrenochaeta lycopersici on a diseased tomato root system

450µm

450µm

are often found in infected organic material in the soil. Microscopical examination of stem base lesions on tomato plants would differentiate between the two diseases. Infection by *C.campanula* is similar to that of *P.lycopersici* and symptoms of the two diseases are easily confused. The lack of detection of pycnidia of *P.lycopersici* may be due to the misidentification of CRR symptoms for BRR.

Pycnidia of *P.lycopersici* have occasionally been reported on the roots of glasshouse grown tomato crops in New Zealand (Newhook, pers. comm.). However, examination of photographs, provided by Newhook, revealed the pycnidia to be very similar in shape and size to *C.coccodes* micro-sclerotia. Wallace (1982) reported pycnidia of *Pyrenochaeta* sp. (*sic*) within the cortex of decaying tomato root systems. Pycnidia, observed after eight to twelve weeks decay, lay in the root cavity with their ostioles piercing the root surface. During this study, occasional pycnidia were observed within the cortex of tomato plants grown in soil infested with BRR. Their appearance on the surface of roots infected from conidial inoculum can be induced by incubating the roots for approximately six weeks. The ability of *Pyrenochaeta* sp. and *P.lycopersici* to produce pycnidia on rotting tomato roots suggests that sporulation by *P.lycopersici* may occur on root debris remaining in the soil once a tomato crop has been removed.

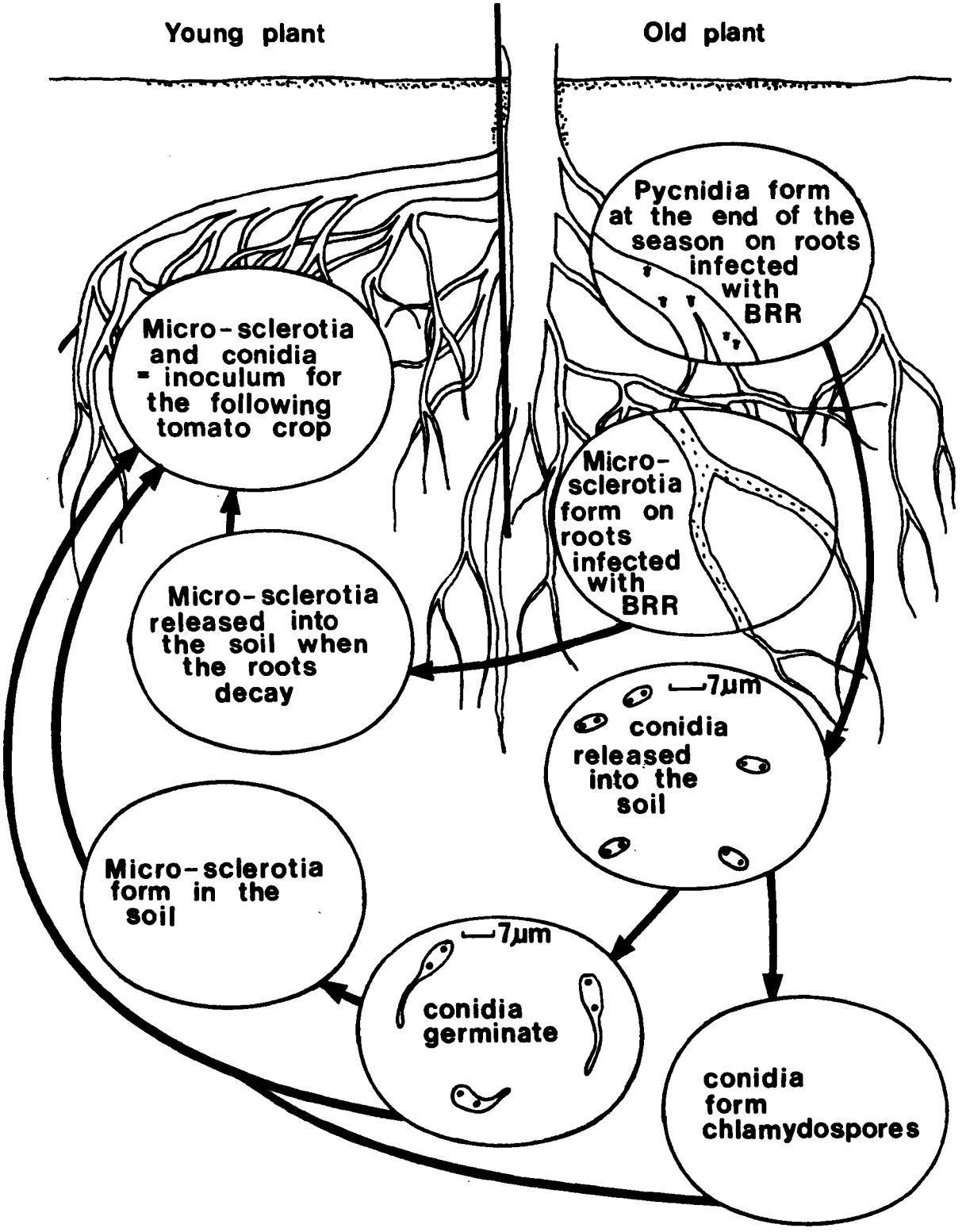
If pycnidia do form on infected roots, under natural conditions, then conidia of *P.lycopersici* may play a role in the survival and dispersal of the pathogen. Conidia, released from pycnidia, may percolate to the lower soil levels where they may remain as spores, convert to chlamydospores (survival spores) or germinate to form mycelium and subsequently micro-sclerotia. When food supplies are

depleted and/or growth conditions are adverse, existing hyphae may be modified to form chlamydospores; these structures may also originate from the modification of conidia. Chlamydospore formation has been reported for *P.lycopersici* (Khalil, 1973) and is a common phenomenon in *Phoma* species e.g. *Phoma glomerata* (Corda) Wr. and Hochapf. and *Phoma prunicola* (Opiz) Wr. and Hochapf. where chlamydospores are thought to be involved in the survival of the fungi. Little evidence of chlamydospore production by *P.lycopersici* was seen in this study, however swollen ungerminated conidia were observed on polycarbonate membranes incubated in compost and garden soil; these may have been the initial stages of chlamydospore formation. Such spores may be held in a state of dormancy until fresh nutrient reserves and/or host root exudates stimulate their germination. Under favourable conditions it seems possible that conidia may germinate in soil and subsequently form micro-sclerotia. Micro-sclerotia were observed on the mycelium resulting from the germination of conidia incubated, on cellophane and polycarbonate membrane, in soil. However attempts to detect micro-sclerotia of *P.lycopersici* in compost and garden soil infested with conidia and stored at room temperature have failed. This may have been due to the minute size of the survival propagule and the inadequacy of the separation techniques used.

Soil sterilisation is rarely effective below 40cm whilst *P.lycopersici* has been detected at depths greater than 50cm (Bakhariev, 1982). Survival propagules, formed from conidia penetrating the lower soil levels may escape the effects of soil sterilisation and provide a source of inoculum for subsequent tomato crops (Figure 31). Conidia would also provide a means of dispersal for the pathogen spreading the disease throughout the glasshouse and

Figure 31

Possible role of* Pyrenochaeta lycopersici *conidia in the brown root rot disease of tomatoes.



producing a further source of BRR inoculum. Micro-sclerotia and/or chlamydospores formed from conidial inoculum would not necessarily be associated with plant debris. This would be an advantage in terms of survival and dispersal of the pathogen, each structure acting as a single infective propagule.

The use of selected antagonists for the control of soil-borne plant pathogens is based on the hypothesis that these micro-organisms, when introduced into the soil, can act directly on the behaviour of the target pathogens. There are two general approaches used in the search for potential antagonists of soil-borne plant pathogens; *in vitro* using techniques such as dual inoculations of antagonist and pathogen on agar plates (Broadbent *et al.*, 1971; Reddi and Rao, 1971) and *in vivo* where organisms are added to pathogen infested soils (Marois *et al.*, 1981; Reinecke and Fokkema, 1981). In practice when an antagonist is added to soil its activity will depend upon its interactions with both the microflora and fauna and the physical conditions prevailing in the soil. These effects cannot be duplicated in *in vitro* studies and an antagonist selected from such studies would not necessarily have the ability to adapt to the soil ecosystem and control the plant pathogen within its natural habitat. Baker and Cook (1974) and Linderman *et al.* (1983) suggested that conditions necessary for antagonists to function are not usually provided in *in vitro* tests and often the incorrect organisms are selected for further study. They urged that plants should be used as primary indicators for screening potential antagonists. *In vitro* assays should be conducted secondarily to gain evidence in support of plant assay results. Assays should be conducted in soils where the organisms will ultimately function and,

if possible, soils naturally infested with the pathogen should be chosen.

The BRR assay system developed in this study was designed with the aim of reducing some of the problems of *in vitro* antagonist screens. Soils, naturally infested with BRR were used throughout this study to provide a mixed natural *P.lycopersici* inoculum with its complementary microflora. Soils were not sieved as such treatment would involve the removal of root debris, thought to be an important source of BRR inoculum. Controls were used in each screening batch to overcome the problem of variability in pathogen levels. It was found that a major source of variability in the BRR assay was related to the number of seedlings grown in the assay pots. Reducing the number of tomato seed from five to one per pot, in pots containing soil infested with BRR, decreased the variability within each treatment. Siemer and Vaughan (1971) also observed large variations in disease whilst using a bioassay developed to assess *P.terrestris* inoculum levels in soil. The large fluctuations were thought due to the spread of the fungus from one test plant to another when 20 onion seed per pot were sown in infested soil. Using the same host and pathogen results obtained with one seed per pot were more consistent and subject to less variability (Pfleger and Vaughan, 1971). Root dry weight, shoot dry weight and shoot height tended to decrease with increases in inoculum density and these contributed to the variability within each treatment. Inclusion of these three parameters collectively, in analysis of covariance tests, gave the greatest reduction in overall variability within the experiment. Measurement and inclusion of shoot height, shoot dry weight and root dry weight was however excessively time consuming. It was found that inclusion of only root

dry weight during analysis provided sufficient reduction in the variability within each treatment to make statistical analysis feasible.

Using the BRR assay micro-organisms were screened for their ability to reduce the level of BRR on tomato seedlings. The organisms chosen for tests were selected from species with known antagonism and/or disease control. Various fungal, bacterial and actinomycete isolates were found to be potential antagonists of *P.lycopersici*.

Lesion numbers were reduced when *Streptomyces* species were applied to tomato seedlings growing in soil infested with BRR. Streptomycetes are potentially effective antagonists of plant pathogenic fungi, especially in environments too dry for the growth of other micro-organisms. Tahvonen (1982b) reported that treatment of cauliflower seed with a *Streptomyces* sp. (*sic*) isolated from peat, controlled damping off by *Alternaria brassicicola* (Schew.) and *Rhizoctonia solani* Kuhn.. Root rot and damping off of sugar beet by *Pythium debaryanum* Hesse was also reduced after application of the *Streptomyces* species. The Streptomycete isolate obtained from Tahvonen (i.e. isolate 61) gave good control of BRR in this study. Other species of *Streptomyces* found by Solberg (pers. comm.) to be antagonistic to *P.lycopersici* in rockwool also reduced the number of root lesions on tomatoes grown in soil infested with BRR. *Streptomyces* are known producers of antibiotics and the reduced disease levels produced by certain Streptomycete isolates in this study may have been due to the production of antifungal antibiotics inhibitory to *P.lycopersici*.

Species of bacteria have also been shown to reduce BRR disease

levels. In addition to disease control an increase in root dry weight was recorded by the majority of isolates tested. Baker and Cook (1974) considered bacteria to have great potential as biological control agents as they constituted the most numerous group of organisms in the soil and root rhizosphere. They are able to multiply rapidly in response to nutrients and are capable of being active over a broad range of soil conditions. Their ability to colonise large areas of the rhizosphere suggests that they would be important antagonists of pathogens, like *P.lycopersici*, which attack roots by multiple infection. In this study a number of bacteria belonging to the *Pseudomonas fluorescens-putida* group were shown to reduce BRR disease levels. Isolates of *P.fluorescens-putida* have been shown to promote plant growth and increase yields by displacement of deleterious elements of the natural root microflora (Burr *et al.*, 1978; Suslow *et al.*, 1979). These plant growth promoting rhizobacteria (PGPR) also have potential for controlling major diseases such as take-all (Weller and Cook, 1983), flax wilt (Kloepper *et al.*, 1980) and Dutch elm disease (Scheffer, 1983). One suggested mechanism of antagonism for these bacteria is iron deprivation by ferric iron complex chelates, or siderophores, which are synthesised in the ferric iron limiting conditions normally existing in soil (Kloepper *et al.*, 1980). Siderophores produced by PGPR confer an advantage to the bacteria in competing for iron. Other rhizosphere micro-organisms that produce less siderophores or siderophores with less affinity for ferric iron cannot obtain sufficient iron for their growth. Disease control achieved by the PGPR used in this investigation may have been due to the production of siderophores, creating an iron limiting environment unfavourable for the growth of *P.lycopersici*. Biological control by bacteria

critically hinges on the suitability of the soil environment. The inability of some bacteria to reduce BRR infection and/or increase root growth may have been due to the prevalence of unsuitable soil environmental conditions restricting the multiplication and establishment of the micro-organism. Soil water potential is known to influence the effectiveness of fluorescent pseudomonads, applied to seed and seed tubers (Schippers, 1983), and under dry conditions other rhizo-bacteria may compete with PGPR, colonising the root surface and reducing their efficiency.

Incorporation of fungi into soil, infested with BRR, reduced the level of disease on tomato seedlings. However this reduction was not significant when compared to the control achieved by uninoculated wheatbran treatments. The substrate used for the augmentation of antagonists can profoundly affect their efficacy (Papavizas and Lewis, 1981a) and the wheatbran used in this study may have provided an unsuitable nutrient base for the growth of potential antagonists. However, wheatbran has been used successfully to produce inoculum of *Trichoderma* species (Henis *et al.*, 1978; Hadar *et al.*, 1979). In this study introduction of uninoculated wheatbran may have provided a substrate for the growth of resident soil micro-organisms, including any natural antagonists. However it was also found that the uninoculated wheatbran significantly reduced the root dry weight of tomato plants grown in BRR infested soils. Papavizas and Lewis (1981a) criticised the scarcity of methods for mass culturing and delivering antagonists to the soil and suggested that standard growth media and specific food bases for antagonists should be developed. This would facilitate the commercial development of formulations and enhance the effectiveness of antagonists. Further investigation into

the correct combination of wheatbran and fungal antagonist may improve the disease control observed in this study.

The soil assay was developed as a preliminary screen and it is envisaged that any micro-organisms selected from it would be further tested at commercially acceptable application rates. Ideally antagonists should not only be selected from the BRR assay by their ability to reduce disease levels but also on their ability to increase root weight. Further screening would involve the testing of potential antagonists in small scale glasshouse trials. It would be possible to apply antagonists to the peat blocking compost and record any subsequent growth stimulation. Assessments of disease control would, ideally, be recorded eight to ten weeks after transplanting into unsterilised glasshouse soil; at this growth stage the level of BRR disease is directly related to total yield losses (Ebben, 1974). However if the screening was to be carried out on a commercial glasshouse site then only end of season disease assessments may be possible. Many of the mechanisms of biological control achieved by antagonists are described in more detail by Baker and Cook (1974).

At present there are no examples of biocontrol of glasshouse crop pathogens in commercial use; this contrasts with the management of protected crop pests where biological control is well established. For example the parasite *Encarsia formosa* Gahan gives good control of whitefly whilst the phytoseid predator *Phytoseiulus persimilis* Athias -Henroit is commonly used in glasshouses to control *Tetranychus urticae* Koch, the red spider mite. Strains of the fungus *Verticillium lecanii* (Zimm.) Viegas are marketed as "Vertalec", for the control of glasshouse aphids, and also as "Mycotal", for whitefly control. One glasshouse crop disease that has been considered as a

target for biological control is *Phomopsis sclerotioides* van Kesteren, black root rot of cucumber. Ebben and Spencer (1978) used *Penicillium lilacinum* Thom. to control *P.sclerotiodes* in pot tests; disease control achieved by the introduction of shake cultures of *P.lilacinum* into black root rot infested compost, was only short-term and did not result in increased yields. However, Moody and Gindrat (1977) were more succesful with *G.roseum*; they found that this antagonist rotted sclerotia of *P.sclerotioides*, significantly reducing the incidence of black root rot. Species of the genus *Trichoderma* are reported to be efficient antagonists on a wide range of plant pathogens (Cook and Baker, 1983) however their use in the glasshouse has yet to be established.

It can be argued that the application of antagonists for the control of plant pathogens has its greatest potential in commercial glasshouse operations. The degree of environmental manipulation in a glasshouse provides an ideal opportunity for the use of antagonists against plant pathogens. Abiotic conditions, such as temperature and humidity are easily controlled and could be manipulated to some degree to suit the requirements of introduced antagonists. Husbandry methods used in the glasshouse also lend themselves to the introduction of antagonistic micro-organisms for the control of plant diseases, in particular soil-borne pathogens. Antagonists of soil-borne plant pathogens could be introduced via (a) the planting material (b) the seed bed compost and/or peat blocking compost and (c) the glasshouse soil.

Seed treatments with biocontrol agents provide an opportunity for the establishment of antagonists on the host early in the life of the plant. Cook and Baker (1983) suggest that such application provides

an economical and effective method of biological control and may ultimately prove to be the most successful form of control with introduced antagonists. Antagonists may also be established on cuttings of ornamentals by dipping the cutting in a suspension of the antagonist. Examples of disease control achieved by the introduction of antagonists onto seed and transplant cuttings are given by Baker and Cook (1974) and Cook and Baker (1983).

Linderman *et al.*, (1983) considered the placing of antagonists into the infection court before roots are colonised by competing micro-organisms to be an important consideration in antagonist introduction. It is normal commercial procedure to raise protected crop plants in seed beds and/or peat blocks prior to planting out in the glasshouse; such practices would provide an ideal opportunity to establish the antagonist in the rhizosphere before the host is confronted by the pathogen. Augmentation of sterilised soil and peat composts, with potential biocontrol agents, during early seedling cultivation may enable antagonists to colonise the root surface and protect the plant from disease. Biological control in such a manner is usually due to direct competition either for space or nutrients. Baker and Cook (1983) suggest that "beneficial micro-organisms that are strong competitors for one or more nutrients on the root surface and that are also to inhibit pathogens directly by production of antibiotics should provide the best or most consistent root protection and hence produce the greatest plant-growth responses". Antagonists applied to composts should be capable of rapid multiplication and establishment in the rhizosphere. Establishment of antagonists as primary invaders of the root surface may then preclude root pathogens from colonising the rhizosphere. Any aggressive,

root-colonising antagonists, given the advantage of being the first to colonise the rhizosphere may also protect the root from invasion by plant pathogens by limiting the nutrient supply available to other micro-organisms. A root-growth response for tomatoes was observed after augmentation of the peat blocking compost with fungal and bacterial isolates (Appendix 1). However in order to maintain the growth advantages incurred by the introduction of micro-organisms to the peat blocks, augmentation of the glasshouse soil may also be necessary.

Since natural soils seems able to resist attempts to alter their microbial balance by the introduction of antagonists (Garrett, 1970) the soil environment must be modified to stimulate the growth of indigenous antagonists or provide suitable conditions for the augmentation and establishment of pure cultures of antagonists. Such soil environmental conditions are easily produced in the glasshouse by soil sterilisation. The biological vacuum, left by soil sterilisation provides an opportunity to manipulate the soil microbial population, either by enhancing resident antagonists of plant pathogens or by introducing known potential antagonists. The recolonisation of a sterilised soil is accomplished initially by those micro-organisms having the shortest response time and the fastest growth rate; *Trichoderma* and *Pseudomonas* species are usually among the first fungal and bacterial species to recolonise fumigated soil (Bollen, 1974; Cook and Baker, 1983). Certain species of *Trichoderma* and *Pseudomonas* are also known for their antagonistic ability and an adequate delay between soil sterilisation and planting of the crop may provide an opportunity for such antagonists to recolonise the soil and control disease. Many attempts to control

soil-borne plant pathogens by direct microbial augmentation of the soil have failed. Papavizas and Lewis (1981a) suggest that the failure may be due to the presence of barriers, such as fungistasis, in natural soil. These would reduce the effectiveness and curtail the establishment and survival of introduced antagonists. For example, fungal antagonists introduced into soil, in the absence of a utilisable organic substrate may be lysed or revert to a dormant resting stage. A further disadvantage of direct augmentation of soils is the preparation of large quantities of bulky inoculum. In contrast, establishment of the antagonists on the seed and/or seedling would require less inoculum.

With respect to brown root rot, it may be possible to achieve efficient control of the disease using an integrated disease control system. Such an approach would include not only augmentation of antagonists but also make use of resistant cultivars and cultural techniques which could be easily incorporated into a tomato growing system. Initially it may be necessary to reduce the inoculum potential of a glasshouse soil by soil sterilisation. Once a low incidence of BRR has been established, other disease management techniques could help maintain the pathogen at levels sufficiently low as not to cause economic losses. Preliminary studies have shown (Appendix 1) that the introduction of antagonists into peat blocking compost provides a suitable means of initiating the biological control system. Bacteria and actinomycetes are easily applied with the water required to form the peat into blocks. However preparation of fungal inoculum is tedious and bulky and bacterial/actinomycete antagonists may be preferred due to the rapidity and efficiency of producing suitable quantities of inoculum. Increasing the size of

the peat block and hence the quantity of compost surrounding the young tomato plants has been shown to reduce the level of BRR (Last and Ebben, 1966). Such cultural treatment increases early yields and the number of healthy roots and when used in an integrated disease management approach may also encourage the establishment and growth of antagonists in the rhizosphere. In order to maintain any growth advantage and/or disease control achieved by the antagonists in the peat block it may be necessary to continue application of antagonists in the glasshouse. The disadvantages of applying antagonists to the glasshouse soil have been discussed. However it may be possible to apply biological control agents via a drip irrigation system. This approach would be most suitable for bacteria and actinomycetes and has certain advantages:

1. Antagonists are introduced directly into the rhizosphere, the area where disease control is to be achieved.
2. The amount of inoculum required is reduced and wastage is kept to a minimum. The inoculum is not broad-cast over the whole glasshouse but concentrated in the areas where required i.e. the plant root system.
3. Antagonists may be applied as required and in varying quantities. Augmentation is not restricted to one application as would broad-cast glasshouse preparations applied prior to transplanting.
4. Inoculum of bacteria and actinomycetes is easily and rapidly produced in vast quantities. Commercial preparations could be easily formulated.
5. Inoculum can be applied simply. Few man hours are required to prepare and introduce the inoculum.
6. The method ought to be inexpensive.

The use of resistant varieties would play an important role in this integrated disease management approach. Such varieties would be tolerant of low BRR levels. Consideration must also be given to the use of insecticides and fungicides for the control of pests and other plant pathogens; it is possible that certain chemicals could effect the efficiency of antagonists used to suppress BRR attack. Biotypes of *T.harzianum*, resistant to various fungicides, have been developed; some of these were more efficient than the wild strains in suppressing damping-off of cotton caused by *R.solani* and pea seed rot caused by *Pythium ultimum* Trow (Papavizas and Lewis, 1981b). In the future it may be possible to develop new biotypes of antagonists which would give enhanced biocontrol of BRR and also be compatible with fungicides and fumigants used to control other pests and diseases.

One advantage of this integrated control programme would be a reduction in the frequency of soil sterilisation. Glasshouse soils are usually sterilised annually however if such treatment could be reduced to a biannual sterilisation then a saving of approximately £3500 per hectare would be achieved. Repeated use of antagonists may increase the effectiveness of the biological control and further reduce the need for soil sterilisation. The efficiency of antagonists and the necessity for sterilisation could be monitored using the BRR assay technique.

Cook and Baker (1983) consider the biological control of plant pathogens in the glasshouse as an "unexploited opportunity". They attribute the slow development of biological control in glasshouse crops to the necessity for near-perfect disease control required in high value crops. This may be true, with respect to certain foliar

pathogens, however many soil-borne plant pathogens could possibly be controlled biologically with little or no detrimental effect on the cosmetic appearance of the produce. Yields from plants used in an integrated disease management system may be lower than those grown under normal commercial practices, however it is probable that such lost revenue would be offset by the reduced costs of biannual sterilisation. Integration of biological control with cultural, chemical and sanitation treatments has potential for stabilising and improving disease control of root pathogens of glasshouse crops.

The development of the BRR assay allowed the possibility of predicting BRR disease levels in glasshouse soils. Prediction of disease is concerned with the expression of the inoculum potential of a pathogen in a given substrate. The inoculum potential may thus be defined as the measure of the threat a particular pathogen poses to a particular crop at any one time. Mitchell (1979) considers the only valid measurement of the inoculum potential of a pathogen as the number of successful infections obtained, in optimum environmental conditions, on a susceptible host. Bouhot (1979) suggested a number of criteria which should be fulfilled during its estimation:

1. selection of a host species susceptible to the parasite
2. use of plants at their most sensitive growth period
3. apply soil, naturally infested with the pathogen, to the most sensitive part of the plant
4. standardise environmental conditions so that the inoculum potential constantly induces maximum disease
5. quantify the technique by progressive dilution of the soil sample
6. determine optimum conditions for the highest selectivity,

sensitivity and rapidity of the technique.

The BRR assay fulfills many of these criteria. As far as possible optimum conditons were used and therefore the inoculum potential of each soil sample should be indicative of the absolute inoculum potential. In the BRR assay the pathogen was studied in its natural state and environment. Its activity was measured in relation to the total natural microflora and the estimated inoculum potential should be a true value of the pathogens capacity to infect its host in the glasshouse.

A relationship between the BRR inoculum potential of glasshouse soil samples, as determined by the BRR assay, and the level of disease in subsequent tomato crops was established. Unsterilised soils exhibiting high inoculum potentials in the BRR assay (measured as lesion numbers per root) also gave a high incidence of BRR on tomatoes assessed eight weeks after transplanting into the glasshouse. A relationship between the number of lesions per root and root dry weight was also observed; for any given soil measurement of lesion numbers per gram of root dry weight reduced the variation between plants during the assessment of the soil's inoculum potential. Inclusion of root dry weight during the measurement of inoculum potential of different soils assumes that the association between lesion numbers and root dry weight is similar for all soil types. However results indicate that this relationship may vary between soils; disease levels measured with the BRR assay and assessed eight weeks after transplanting were <u>not</u> related when the inoculum potentials of <u>different</u> soils were expressed as lesion numbers per gram root dry weight. This was particularly apparent for

soils with low levels of BRR i.e. those producing 0 to 10 lesions per root, when inclusion of root weight tended to increase the variability during measurement of the inoculum potential. However measurement of root weight does have a value in assessing the ability of potential antagonists to control BRR. The screening programme involves the use of one soil, naturally infested with BRR, and hence an association between lesion numbers per root and root dry weight can be established; measurement of root weight also provides valuable information on the ability of potential antagonists to increase plant growth. The relationship between disease levels detected using the BRR assay and those observed in a tomato crop could be used to assess the potential disease control which could be acheived by the antagonist in the glasshouse (Appendix 2).

Ebben (1974) suggested that a relationship existed between levels of BRR detected eight weeks after transplanting and total yield losses. Yield losses were less significantly related to BRR when the disease was assessed at 16 weeks and had no relation to the percentage BRR apparent at the end of the season. Polley (pers. comm.) confirmed the relationship between the level of BRR, eight weeks after transplanting and final yield losses. Thus the soil assay provides a method of predicting both the level of BRR in a glasshouse and the yield losses which could occur in that season. However the measurement of *P.lycopersici* infection in the glasshouse was based on assessments of root browning eight weeks after planting and the effects of root loss were ignored. The lack of root size measurements can lead to inaccuracies in assessments of BRR disease (Last *et al.*, 1969). For example, equal amounts of BRR disease can, at one instance represent 15% BRR and shortly afterwards 33%, the

increase being attributable to the loss of healthy distal roots without an appreciable increase in the absolute amounts of colonised root. In one soil (Cheshunt) a low incidence (<1%) of BRR was recorded eight weeks after transplanting. The soil assay predicted a higher incidence of soil infestation. Resampling 12 weeks after transplanting recorded 23% level of BRR, the rapid increase in disease possibly due to root loss.

The BRR assay measures the inoculum potential of *P.lycopersici* in soils infested with the pathogen. The disease appears early in the season and the majority of recognisable lesions formed on four week old tomato seedlings are *P.lycopersici*. Occasionally *C.coccodes* and *C.campanula* were isolated from these lesions, however both pathogens tend to appear later in the season (Last *et al.*, 1966; Clark *et al.*, 1983). Assessment of BRR, eight weeks after transplanting could however occasionally include symptoms produced by black dot and *Calyptella* root rot. Detection of these two diseases in the glasshouse, at eight weeks, but not in the BRR assay may, in some instances, affect the relationship between BRR assay and glasshouse disease assessments.

The influence of other pathogens is only one of a number of factors which could affect the prediction system. Soil conditions, sowing date, variety, climate may also influence the level of BRR detected in a glasshouse in any one year. For example, higher levels of BRR may be tolerated by a tomato crop where soil conditions allow compensation for root loss by encouraging new root formation (Ebben, 1971a). It would be necessary therefore to carry out BRR assay tests, disease assessment and yield loss studies on many tomato varieties, grown in a range of soil types, at different sites in

order to establish the critical soil infestation levels for a range of soil conditions.

From this study two areas have emerged which would benefit from further research. Firstly an investigation into the interaction between the fungi associated with tomato roots and secondly attention to the screening and application of antagonists for the control of glasshouse soil-borne diseases.

The biology and control of selected tomato root pathogens has frequently been investigated in isolation however there are very few studies of the interactions of these pathogens both with each other and with the root rhizosphere microbial population. In particular the assocation between *P.lycopersici*, *C.coccodes* and *C.campanula* would appear to be complex and studies are required to ellucidate the relationship between the pathogens and the symptoms they produce.

Continued screening of micro-organisms for their ability to control BRR should present a number of potential antagonists for secondary testing in glasshouse experiments. During the search for antagonists of BRR emphasis was placed on the use of a "natural assay" rather than *in vitro* culture studies and any further research in this area should continue to make use of the developed BRR assay. The use of more than one micro-organism may well prove valuable; ideally a "soup" of antagonists and plant growth promoting microbes providing adequate disease control with an additional increase in plant growth should be sought.

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Appendix 1

A preliminary trial testing the potential of antagonists for the control of brown root rot (BRR) in the glasshouse

Introduction

Preliminary investigations were undertaken to test various bacterial and fungal isolates for their ability to reduce BRR disease levels and increase the growth of tomatoes in commercial glasshouse crops. Crops were grown under organic conditions and the restraints of commercial practices placed restrictions on replication within the experiments.

Methods

The crop was organically grown at Pilling, Lancashire (Mr. D. Blair). Tomato seedlings were raised in seed compost (Fisons, Universal) and transferred 14 days later to peat blocks (Fisons selected sedge peat); 80l peat compost plus 0.68Kg Humber organic fertiliser, 0.11Kg Dolomite, 0.11Kg calcified seaweed, 0.11Kg dried blood and 0.056Kg bone meal. After approximately 28 days plants were transferred to an unheated glasshouse in which the soil had recently been steam sterilised. The crop recieved no fungicide or insecticide applications.

Experiment 1

Fungal and bacterial inoculum was prepared (Chapter 2, Section 1.1) and incorporated into the peat blocking compost as follows:

1. Control; 7.5l uninoculated wheatbran + 3.75l TW

2. Fungal inoculum; isolates AH119, AH101, AH113, TRC10 and M588 (1500ml, by volume, of each isolate, mixed).

3. Fungal and bacterial inoculum; fungal isolates and rates as for treatment 2, bacterial isolates; E6, BK1, B10 and RV3 (300ml of each isolate, mixed and made up to 3.75l with TW).

per 160l peat blocking compost

For details of isolates see Table 13.

Test plants (variety Abunda) were transferred into the peat blocks (160 plants per treatment) and transplanted into the glasshouse 28 days later. End of season assessments of percentage BRR, percentage corkiness and root size, using arbitrary indexes (Table 12), were recorded. Isolations were made from lesions to check the identity of the causal pathogen.

Experiment 2

Investigations were concentrated on the effects of potential antagonists on plant growth in peat blocks.

Fungal and bacterial inoculum was prepared (Chapter 2, Section 1.1) and incorporated into the peat blocking compost as follows:

1. Control; no augmentation

2. Control; uninoculated wheatbran + 3.75l TW

3. Fungal inoculum; isolates AH119, AH101, AH113 and TRC10 (1500ml, by volume of each isolate, mixed).

4. Bacterial inoculum; isolates E6, BK1, B10 and RV3 (300ml of each isolate, mixed and made up to 3.75l with TW).

5. Fungal and bacterial inoculum; prepared as for treatments 3 and 4.

} per 160l peat blocking compost

For details of isolates see Table 13.

Test plants (variety Ostona) were transferred into the peat blocks (80 plants per treatment). Shoot height, stem dry weight, leaf dry weight and leaf area of ten plants per treatment were measured after 28 days and the remaining replicates transplanted out in the glasshouse.

Results

Experiment 1

After four weeks growth in peat blocks plants grown in the presence of fungi and/or fungi plus bacteria were taller and had an increased leaf area compared to plants grown in the control treatment. No assessments of plant growth and disease levels were made at this stage. End of season assessments demonstrated that plants grown in the presence of potential antagonists had increased root sizes and reduced BRR disease

when compared with the control plants (Table 44).

Experiment 2

After 28 days growth in peat blocks plants grown in the presence of potential antagonists showed significant increases in shoot height, shoot dry weight, leaf dry weight and leaf area when compared to control plants (Table 45). Plants from treatment 5 (fungal and bacterial inoculum) tended to show the greatest improvement in growth. Apart from mean leaf area no significant differences in plant growth were observed between fungal and bacterial antagonists when applied as separate treatments (treatments 3 and 4). The formation of the first truss was considerably advanced by the presence of antagonists. However, plant growth advantages were eventually lost after the tomatoes were transferred into the glasshouse and the season progressed.

Discussion

These preliminary experiments indicate that the addition of microorganisms into the peat blocking compost can stimulate plant growth and reduce BRR attack.

An important advantage of increased plant growth at the beginning of the season is earlier fruit picking and hence an improvement in early crop yields. Sportelli *et al.* (1983) observed similar benefits when various fungal diseases of glasshouse tomato crops were controlled with *T. viride*. Early flowering and picking and a reduction in the severity of symptoms caused by *Fusarium oxysporum* Schlecht. ex Fries

Table 44

Testing of potential antagonists for the control of brown root rot (BRR) in the glasshouse. I: Experiment 1, end of season tomato root symptoms on plants grown in peat blocks with and without the presence of potential antagonists.

Treatment	Mean brown root rot* (S.D.)	Mean corkiness* (S.D.)	Mean root size* (S.D.)
Control	3.0 (0.0)	1.8 (0.9)	1.8 (0.4)
Fungi	1.5 (0.6)	0.9 (0.8)	2.5 (0.5)
Fungi & Bacteria	1.4 (0.5)	1.2 (0.4)	2.3 (0.6)

*Assessed using arbitrary indexes (Table 12)

Table 45

Testing of potential antagonists for the control of brown root rot (BRR) in the glasshouse. II: Experiment 2, growth measurements on plants grown in peat blocks with and without the presence of potential antagonists.

Treatment	Mean Height (cm)	Mean stem dry weight (g)	Mean leaf dry weight (g)	Mean leaf area (cm^2)
Control	32.5 (1.5)	0.44 (0.01)	0.98 (0.14)	300.0 (40.0)
Control (amended)	38.4 (2.4)	0.45 (0.02)	0.87 (0.19)	275.0 (80.0)
Fungi	39.0 (1.1)	0.53 (0.03)*	1.04 (0.12)	420.0 (40.0)*
Bacteria	40.3 (1.8)	0.57 (0.01)*	1.18 (0.18)	540.0 (70.0)*
Fungi & Bacteria	55.3 (2.0)*	0.81 (0.12)	1.50 (0.29)*	330.0 (30.0)

95% confidence limits displayed in parenthesis

*treatment significantly different from the amended control.

f. sp. *lycopersici* (Sacc.) Snyder and Hansen, *Verticillium dahliae* Klebahn and *R. solani* were observed when the antagonist was applied to glasshouse soils. In these preliminary investigations end of season assessments demonstrated that root size was increased and percentage BRR decreased when tomato plants were grown in the presence of potential antagonists. However, the benefit of reduced disease levels did not appear to be associated with an increase in fruit yield. In order to maintain the growth advantages incurred by the introduction of the micro-organisms to the peat blocks, augmentation of the glasshouse soil with potential antagonists would also be necessary.

Appendix 2

Possible use of the BRR assay to predict the degree of disease control and consequent yield increases achieved by potential antagonists applied to glasshouse tomato crops.

A relationship between the number of lesions per root on seedlings grown in soil samples from commercial glasshouses and the subsequent level of disease on tomatoes eight weeks after transplanting into the soils has been established:

$$y = 2.36 + 0.30x \quad \text{(Chapter 3, Section 2.3)}$$

x^+ = number of lesions per root, measured in the BRR assay

y^* = percentage BRR, observed in the glasshouse

Theoretically the relationship could be used to extrapolate the disease control achieved by antagonists in the BRR assay (i.e. x^*) to that which might be achieved by their introduction into the glasshouse (i.e. calculation of y^+). Using the above formula potential antagonists could reduce glasshouse disease levels of BRR by 13% below the controls (Table 46).

Polley (pers.comm.) observed a 0.35% decrease in the total yield of glasshouse tomato crops for every 1% increase in BRR. Using this relationship an estimate of the total yield of tomatoes grown in the presence of antagonists could also be calculated; potential antagonists could reduce yield losses by up to 5% (Table 46).

Prediction of disease control and yield increases using the above formulae rely on the ability of the potential antagonists to maintain their effectiveness, in the glasshouse at a similar rate to that

Table 46

Predicted disease control and yield increases achieved by potential antagonists introduced into a glasshouse tomato crop.

Potential * Antagonist	Predicted level of [A] BRR (%)	Predicted yield increases[B] above the control (%)
Control	12.74	-
E6	9.59	1.10
BK1	9.29	1.21
B10	7.67	1.77
RV3	8.84	1.37
Control	20.84	-
40B	16.64	1.47
53B	15.59	1.83
23C	11.72	3.19
50B	9.47	3.97
Control	20.75	-
61	15.71	1.77
34C	16.49	1.49
39C	15.14	1.97
2C	13.34	2.59
Control	41.45	-
2g Biovegetal	34.25	2.52
Control	46.91	-
10g Biovegetal	33.65	4.64

* Potential antagonists which significantly reduced disease levels in the laboratory

A Predicted from the formula $y = 2.36 + 0.3x$ (with RW covariate)

B Predicted from the relationship that for every 1% increase in brown root rot there is an equivalent 0.35% decrease in yield (Polley, pers.comm.)

achieved in the BRR assay. This is unlikely but the methods may give an indication of the upper limits of disease control which could be achieved by an antagonist in the glasshouse.